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Research Journal



Introduction

We are proud to present the fifth edition of our annual student research publication. The Nikola Tesla STEM Research Journal enables STEM students to share their research with the academic and business communities. The investigations included in this edition demonstrate both the increasing depth and diversity of our studies. In addition, we have included a list of the awards and honors earned by Tesla STEM students this year.

Our thanks to the Tesla STEM staff for ensuring all students are afforded every opportunity to showcase their knowledge and talents and to our students, for their dedication to STEM Literacy. Many thanks to the dedicated student researchers and mentors, your long hours and hard work made this publication possible.

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Biomedical Engineering



3D Segmented Tongue Prosthesis Construction for Total Glossectomy Patients

Katherine Bo, Davina Lau, Michelle Yeh

Abstract

Currently, there are only separate tongue prostheses available that serve the purpose for either swallowing or speech. Our objective is to build one single prosthetic tongue that will help improve both speech impairment and dysphagia in a patient who has had a total glossectomy. In order to construct our prosthetic, we 3D printed a tongue that was segmented into three parts and connected with magnetic clasps and small rings made out of wire. After the construction of our prosthetic was finished, we compared the prosthetic tongue to a normal human tongue by performing an appearance test and mobility test. In comparison to the human tongue, our tongue prosthetic is different in color, texture, and size. Despite these differences, the prosthetic tongue had a similar shape of an actual human tongue and an almost similar angle of mobility when the tongue moves up, down, and sideways. With our findings, our construction of the prosthetic tongue could potentially be a more effective treatment than the current separate tongue prostheses because patients would not experience the inconvenience of constantly switching between two tongue prostheses.

The number of oral cancer patients in the nation

has surged to 7,800 in 2015, up from around 2,100 in 1975, according to estimates by the Japan Society of Oral Oncology. A glossectomy, one of the solutions to treating oral cancer is the removal of the whole or parts of the tongue, which is done surgically. Patients with a glossectomy will experience complications that could affect their ability to speak, communicate, creating an impact on the quality of life and daily comfort. Examples of these complications include dysphagia and speech disorders. Dysphagia is a condition in which a patient faces difficulty and pain when swallowing. People with dysphagia may either be completely unable to swallow or have trouble safely swallowing liquids, foods, or saliva. This makes it difficult for people with dysphagia take in enough calories and fluids to nourish the body which could lead to additional serious medical problems. Speech disorders are a type of communication disorder in which a patient is not able to produce speech sounds incorrectly or is completely unable to speak or understand speech. Tongue prostheses have been developed to help address these problems.

Currently, the most effective tongue prostheses are single piece tongues, where patients are required to switch out the specific type of tongue they are

using in order to alternate between swallowing and speaking. Our goal is to create a single piece tongue prosthetic that is beneficial towards swallowing and speech so that patients would not experience the inconvenience of constantly switching between two tongue prostheses.

Functionality/Originality

The functional goal of our prosthetic device is to create a prosthetic tongue in order to improve impaired speech and dysphagia (swallowing disorder) in patients with a glossectomy. Our prosthetic tongue will enhance speech by shaping the oral cavity and creating a closer contact between the prosthetic tongue and the palate. For swallowing and chewing, the prosthetic tongue will guide food between the dental arches and then to force the bolus into the oropharynx. Our prosthetic will be segmented into three parts which will act as the tip, the middle, and the back of the tongue. Two magnetic claps are glued to each flat side of the segments and are connected by small rings made of wire.

Currently there are only separate tongue prostheses available that serve the purpose for either swallowing or speech. Our objective is to build one single prosthetic tongue that will help improve both speech impairment and dysphagia in a patient who has had a glossectomy, which would make our prosthetic different from all others. This also makes our prosthetic more cost-effective than other tongue prostheses because creating one tongue prosthetic that fixes

speech impairment and trouble in swallowing will take fewer materials than making two prosthetics that each functions in either speech or swallowing. Not only will it be more cost-effective, but it would also be less time-consuming to produce. Our prosthetic is segmented into three different parts and is connected with rings in order for the prosthetic to move like a normal human tongue. While many other tongue prostheses are made of silicon, ours is created with plastic from 3-D printing which is lightweight compared to silicon and is more accessible.

Results

After construction of our tongue prosthetic was finished, we compared the appearance and angle of mobility between the tongue prosthetic and a normal human tongue. In comparison to the human tongue, our tongue prosthetic is different in color, texture, and size. Our prosthetic was printed in black colored plastic and has a smoother texture while a normal human tongue is pink and a lot bumpier. The dimensions of our prosthetic tongue is also greater than the actual size of the human tongue. Since our prosthetic was 3D printed, our tongue is solid and will not feel like an actual human tongue. Despite these limitations, the prosthetic tongue had a similar shape of an actual human tongue and an angle of mobility when the tongue moves up, down, and sideways. In the future, we could improve our tongue prosthetic by making the prosthetic similar to the dimensions of an actual human tongue so that the prosthetic

could actually fit in a mouth of a patient with a total glossectomy. We would also add wires which would be attached to the lower jaw and the sides of the tongue in order for a patient who is wearing this prosthetic to have a proper and comfort fit. Since our prosthetic tongue is more cost-effective and requires materials that are more accessible, our finished prosthetic tongue could potentially be a solution for patients with glossectomy in third world countries.



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<http://doi.org/10.7860/JCDR/2016/15868.7184>

This literature review goes through each type of tongue prosthesis in greater detail including their function, how the prosthetic works, significant features of the prosthetic, and the limitations of each type of prosthetic. This source also gives background information on what a glossectomy is, and how swallowing and speech works. This review built our understanding on the science and anatomy of the tongue which helped us think of an idea of a prosthetic.

2. Logemann, J. A. (2014). Critical Factors in the Oral Control Needed for Chewing and Swallowing. *Journal of Texture Studies*, 45(3), 173–179.

<http://doi.org/10.1111/jtxs.12053>

This review article presents the results of a series of studies that examine the role of each of the stages

of the oropharyngeal swallow, as well as going over the critical factors in the oral control needed for chewing and swallowing. This source created a better understanding on the process of chewing and swallowing and helped us figure out which features are the most important for our tongue prosthetic.

3. Moore, D. J. (1972). Glossectomy rehabilitation by mandibular tongue prosthesis. *The Journal of Prosthetic Dentistry*, 28(4), 429-433. doi:10.1016/0022-3913(72)90245-4

This source evaluated the effectiveness of prosthetic rehabilitation on voice, speech, and swallowing in patients undergoing glossectomy by performing a systematic literature review and meta-analysis of individual cases. This literature review gave us a better understanding on information about glossectomy and helped us figure out what our prosthetic will look like and how it will function.

4. Tonake, T. (2016, February 1). Japanese team invents movable tongue prosthesis to enable speech for cancer victims. Retrieved January 26, 2018, from <https://www.japantimes.co.jp/news/2016/02/01/national/science-health/japanese-team-invents-movable-tongue-prosthesis-enable-speech-cancer-victims/#.WmrMPK6nGUk>

This article goes in detail about dentistry researchers at Okayama University, who developed a product that could be the world's first movable tongue prosthesis to help oral cancer patients who have partially lost the ability to speak. This source helped us figure out how to make our prosthetic beneficial for articulation speech.

5. Yoon, J., Lee, S., Lee, J., & Oh, N. (2013, October 30). Prosthetic rehabilitation for a glossectomy patient - a clinical report. *The Korean Academy of Prosthodontics*. Retrieved January 15, 2017, from <https://doi.org/10.4047/jkap.2013.51.4.347>

This scientific article goes into detail on a tongue prosthetics works in patients with a total glossectomy and the significant features of the tongue prosthesis which makes it more beneficial for patients with total glossectomy. This source helped us design our prosthetic that will benefit patients with total glossectomy.

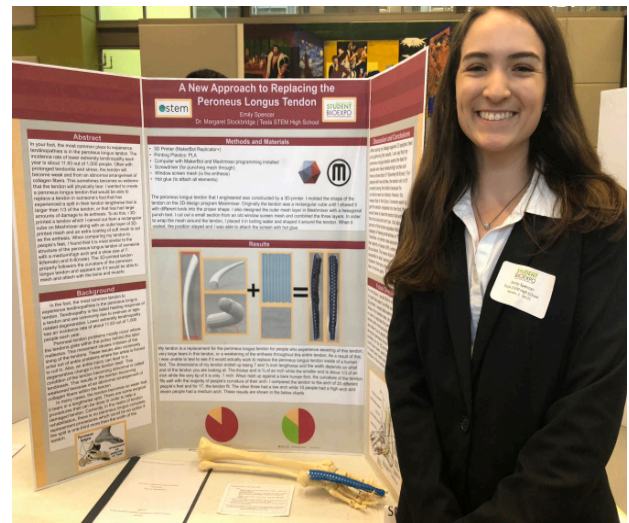
A New Approach to Replacing the Peroneus Longus Tendon

Emily R. Spencer

Abstract

In your foot, the most common place to experience tendinopathies is in the peroneus longus tendon.

The incidence rate of lower extremity tendinopathy each year is about 11.83 out of 1,000 people. Often with prolonged tendonitis and stress, the tendon will become weak and from an abnormal arrangement of collagen fibers. This sometimes becomes so extreme that the tendon will physically tear. I wanted to create a peroneus longus tendon that would be able to replace a tendon in someone's foot that has experienced a split in their tendon lengthwise that is larger than 1/3 of the tendon, or has had large amounts of damage to its enthesis. To do this, I 3D-printed a tendon which I carved out from a rectangular cube on Meshmixer along with an outer layer of 3D-printed mesh and an extra coating of soft mesh to act as the enthesis. When comparing my tendon to people's feet, I found that it is most similar to the structure of the peroneus longus tendon of someone with a medium/high arch and a shoe size of 7-9(female) and 6-8(male). The 3D-printed tendon properly follows the curvature of the peroneus longus tendon and appears as if it would be able to mesh and attach with the bone and muscle.



Keywords: Peroneus longus tendon, tendinopathies, 3D-printed, mesh

Background Research

In the lower half of the body, the Achilles tendon is the most commonly tendon that experiences tendinopathies. However, in the foot, the most common tendon to experience tendinopathies is the peroneus longus tendon. Tendinopathy is the failed healing response of a tendon and are commonly due to overuse or age-related degeneration⁵. Lower extremity tendinopathy has an incidence rate of about 11.83 out of 1,000 people each year.

Peroneal tendon problems mostly occur where the tendons glide within the pulley behind the later malleolus. This movement causes irritation of the lining of the tendons. These issues also commonly arise out of ankle problems where the ankle is forced to roll in. Also, an ankle injury can lead to a

degenerative change in the tendon itself. This condition of the tendon becoming abnormal is called tendinosis¹. This results in the tendon becoming weakened because of an abnormal arrangement of collagen fibers within the tendon. In many cases, the tendon becomes so weak that it tears in a lengthwise split. There are some surgical procedures that can be done in order to help a damaged tendon. These include tendon release where the irritated lining of the tendon is removed, debridement where irritated tissue inside of the tendon is removed, and tendon repair where when a tendon is split down the length and can be repaired with sutures if the split is smaller than one-third of the width of the tendon⁸.

The peroneus longus tendon lies in the groove of the cuboid bone in the foot. It then extends along the outer side of the foot until it reaches its insertion point at the medial cuneiform and the first metatarsal bone. In other words, it lines behind and below the peroneus brevis tendon and angles forward under the sole of the foot where it connects to the bottom of the main bone of the big toe (the first metatarsal bone). The peroneus longus tendon is order to glide along the inferior surface of the retrotrochlear process. This smoothness also allows for the tendon to sit properly along the peroneus brevis tendon⁷. The shape of this tendon is concave and tunnel like.

Doctors have been able to repair ripped tendons through surgery, with the most common type of including penocutaneous techniques which include

three types of tendon repair: primary, delayed, or secondary. Primary repair of a sudden injury is typically done within 24 hours of the tare, while delayed primary care is done a few days after the tare, but there is still an open wound present. Secondary repairs may occur two to five weeks after the injury and can include tendon grafts where tendons from other areas of the body are inserted in the place of the damaged tendon, a penocutaneous surgery, and other types of complex surgical procedures⁴. Currently, in the realm of tendon rehabilitation, there is no peroneus longus complete replacement procedures which could be an option if the split is one-third more than the width of the tendon.

Another very interesting form of tendon repair during a surgery is a pen like device that doctors are able to utilize to fill in missing tissue, tendon, and bone areas. The pen is, put simply, a portable 3D printer that allows for simple corrections and fills to repair small areas of damage. Researchers in Australia pioneered this solution in 2013, however their invention hasn't been widely spread across the market in the United States⁶.

Methods

The peroneus longus tendon that I created was 3D printed using a Replicator+ printer by Makerbot. The materials I needed to gather for this project included a 3D printer, which was supplied to me through my school, a proper program to model a 3D print on, the 3D printing plastic: PLA, along with a screwdriver,

window screen mesh, and a hot glue gun. In order to design my 3D print, I did research focusing on the dimensions and mold of the “average” peroneus longus tendon.

After gathering this information, I constructed a 3D model of the tendon in Meshmixer, a program dedicated to creating and molding objects to be suitable to 3D printing. The tendon originally started off as a basic cylinder but with the shaving and curving tools provided, I was able to properly shape my tendon. The bottom end of the tendon, the one that attaches to the medial cuneiform at the first metatarsal bone, is a small, circular shape allowing for the proper connection. The top part of the tendon is a flattened cylindrical shape with a bit of a rounded point. This shape works best for attaching the tendon to the head and proximal two third of the fibula; this is where the peroneus longus tendon originates from.

Entheses are the insertion sites, osteotendinous junctions, and osteoligamentous junctions that allow tendons to attach muscle to bone. The enthesis is virtually a gradient tissue that is classified into four distinct zones. Each of these zones has varying cellular compositions, mechanical properties, and functions in order to properly facilitate joint movement². Quite often, the enthesis is damaged or tendon insertions fail. These things would result in even more surgical intervention to recreate the natural organization of the enthesis in order to prop-

erly connect the tendon to the bone. There is also a portion known as the enthesis organ which is a collection of tissues adjacent to the enthesis itself which functions as stress dissipation from any pressures put onto the tendon³.

My 3D-printed tendon has three parts: the actual tendon, the enthesis, and another layer of thicker mesh that is able to withstand force-generating muscles in order to properly support the tendon. The enthesis is made out of the thin mesh wire from a window screen that only covers the outside of the thicker mesh on the side that connects the tendon to the bone. The hexagonal structure of the mesh allows for easy connection to the muscle through the holes and strong shape. When printing the outer mesh layer, I had to use supports on the print because each layer of filament wouldn't cool in time and get dragged around the printing platform. I used a 7% fill for the rafts and had to punch out each raft from below the hexagonal figure of the mesh. To do this I used a screwdriver. In order to connect all of the parts of my tendon, I used either boiling water or hot glue. To connect the hexagonal mesh to my tendon, I submerged the plastic in boiling water so that it would become flexible. Then I quickly shaped it around the tendon and held it there until it hardened. This allowed the mesh to stay attached to the tendon. For the mesh from the screen, I had to use hot glue in order to glue the 7x3/4 inch piece to the hard mesh on the underside where connection to bone would

occur.

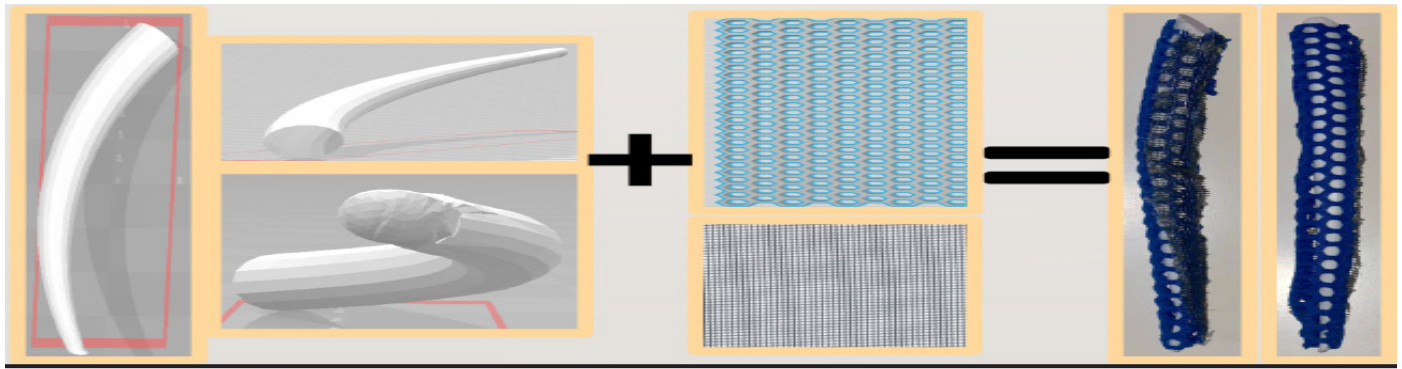
Results

My tendon is a replacement for the peroneus longus tendon for people who experience severing of this tendon, very large tears in this tendon, or a weakening of the enthesis throughout this entire tendon. As a result of this, I was unable to test to see if it would actually work to replace the peroneus longus tendon inside of a human foot. The dimensions of my tendon ended up being 7 and $\frac{3}{4}$ inch lengthwise and the width depends on what end of the tendon you are looking at. The thicker end is $\frac{3}{4}$ of an inch while the smaller end is about $\frac{1}{3}$ of an inch while the very tip of it is only .1 inch. When held up against a bare human foot, the curvature of the tendon fits well with the majority of people's curvature of their arch. I compared the tendon to the arch of 20 different people's feet and 17 of them had the tendon fit. 10 of those people had a medium arch, 7 of them had a high arch, and 3 had a low arch. For everyone that had a low arch, the tendon did not wrap properly because the curvature was too intense. The other three had much larger feet than the others and therefore the curvature of the tendon occurred too early. From all of these comparisons I have concluded that the tendon would be a best fit for those with a shoe size 7-9 (female) and 5-7 (male) and for those who have a high or medium arch.

One issue that I ran into while creating my tendon occurred when I was trying to print my mesh. For

some reason, the 3D-printer I was using was unable to print the honeycomb mesh without peeling it off of the base. This means that the PLA did not adhere properly to the base after it had been printed and as a result no proper shape would be made. After attempting this with different print settings and on different printers, I put supports on the print. I did not want to do this originally because I knew it would cover up the holes in the mesh. After the print was finished, I had to take a screwdriver and knock out all of the supports that were in-between each section of the mesh. This was not optimal and resulted in part of my mesh breaking.

For the future, I would like to make a new type of mesh that wasn't so thick and could be easily 3D-printed. I would also want to make the shape of my tendon less circular and more flat in order to replicate the peroneus longus tendon with more accuracy. This flatness would also allow it so it would rest most comfortably along the peroneus brevis tendon.



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Creating a Functional, Rehabilitating Hand Exoskeleton for Stroke Survivors

Rashida Hakim, Vaishnavi Phadnis

Abstract

Our project attempted to create a prosthetic device which provides passive extension assistance for the many patients with extensor weakness or flexor hypertonia in the hands following a stroke. Some goals for this device would be that it would be lightweight and customizable due to the use of 3D printed components and would serve simultaneously rehabilitating and practical functions. In the short term, the extension assistance provided by the elastic in our device will allow patients to grip objects of various sizes in their daily life. In the long term, use of the device will strengthen the extensor muscles and reduce hypertonia to eventually eliminate the need for the device altogether. We successfully 3D printed and assembled a prototype of this device and tested it using force probes to compare the extension assistance provided to the normal biomechanics of the human hand and found they followed the same general pattern of curvature. This design shows promise for stroke survivors with hand function impairments once aspects such as comfort and aesthetic value are further refined.

Background

Stroke is the leading cause of serious, long-term disability in the United States. According to the

Centers for Disease Control and Prevention, approximately 795,000 people suffer a stroke annually and 38% of stroke survivors report major difficulty with hand function three months post-stroke. The most common hand function impairment among stroke survivors is the fingers being stuck in a permanent grasp position due to flexor hypertonia and weakness in finger extensors (Duncan, Bode, Lai & Perera, 2003). Stroke survivors with this impairment are unable to pick up and manipulate objects, tasks which are a vital to many daily life activities. However, this impairment can be overcome, and repetitive use of the affected limb in physical therapy can help stroke survivors regain hand function (Butefisch, Hummelsheim, Denzler, & Mauritz, 1995). Therefore, our goal is to create a prosthetic hand exoskeleton that will encourage repetitive use of an impaired hand as well as providing functional extension assistance for daily life activities.

We decided on a passive arm rehabilitation device because devices powered by electric or pneumatic actuators are usually prohibitively heavy and bulky. The Saeboflex is an example of a commercially available and lightweight passive and rehabilitation device that has been successful in assisting with opening the grasp of patients (Farrell, Hoffman,

Snyder, Giuliani, & Bohannon, 2007). This device uses elastic cables attached to the distal ends of the finger to provide assistance with extension. However, since the force applied by the cable increases with finger flexion, the device limits range of motion to the point where the patient can only grasp objects that are 3-4 inches in diameter. To resolve this problem, a team developed the Hand Spring Operated Movement Enabler (HandSOME), a passive hand rehabilitation device that uses elastic cables in a 4-bar linkage to provide increasing extension assistance with increasing finger extension angle. This leads to the patient having the larger range of motion required to grasp varying sizes of objects (Brokaw, Black, Holley, & Lum, 2011). However, this device has a few flaws. Unlike the Saeboflex, it joins the four fingers into one linked arrangement, meaning that pinch grasp for independent fingers cannot be rehabilitated. In addition, it is quite heavy which means that patients have to support their impaired hand with their other hand during use – meaning patients cannot practice two-handed motions.

Functionality and Originality

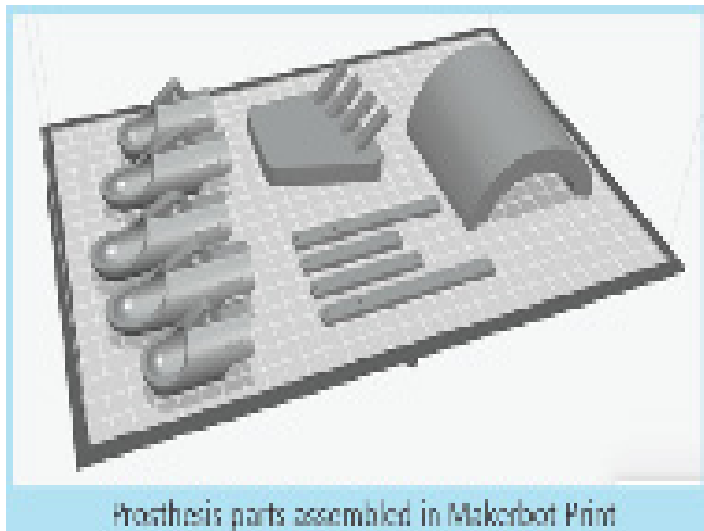
Our design combines elements from the Saeboflex and the HandSOME to create a lightweight, accessible, and functional hand exoskeleton for stroke survivors. It provides increasing extension assistance with increased extension angle like the HandSOME while also being lightweight with multiple grip styles like the Saeboflex. This means that the device

is useful for both rehabilitation purposes and for functional activities like grasping and manipulating objects. With our device, stroke survivors can be engaging in physical therapy for their hand as they go about their daily lives.

Our device facilitates patients in doing a pinch-pad grasp, which is a movement where the thumb and forefingers are pressed together and is used to pick up objects. To do this, our prosthetic provides assistance to the extensor digitorum muscle which extends the metacarpophalangeal joints. One important feature of our device is the increasing torque that the elastic cords apply to the metacarpophalangeal joints as the hand opens. There is no extension torque applied by the elastic cord when the hand is in a fully flexed position so a patient can firmly grip a small object. However, as soon as the patient's flexor muscles relax slightly and the angle of extension increases, the elastic cord applies a torque on the joint to provide assistance to the weakened extensor muscles. This allows a patient with flexor hypertonia and extensor weakness to have a normal range of motion for a pinch-pad grasp.

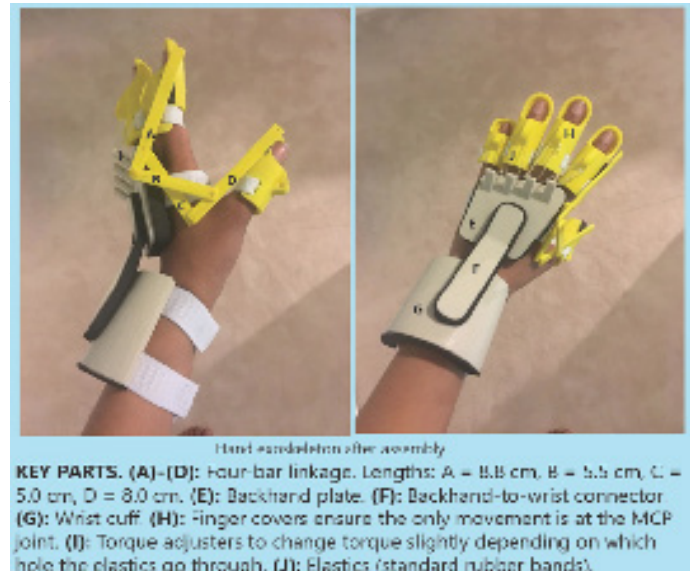
Another unique feature of our device is how lightweight and customizable it is. If a patient has extreme flexor hypertonia and needs additional extension assistance, multiple elastic cords can be run in parallel to provide more torque. Also, since our prosthetic is mainly comprised of 3D printed plastic parts (Figure 1), the design can be adjusted to a spe-

cific patient's size and symptoms using a computer modeling tool. Then it can be printed individually without expensive injection molding or welding: manufacturing methods that are only cost-effective for high volume production runs. The PVA plastic used for 3D printing also makes our device lightweight compared to most metal exoskeletons. Lightness is an especially important consideration because stroke survivors often suffer from proximal arm weakness, which means a heavy exoskeleton would not be functional for daily use. Neither the Saeboflex nor the HandSOME are 3D printed designs, so our device is the first to allow stroke survivors this level of customization and accessibility in their prosthetic hand exoskeleton.



tying elastic and Velcro straps to the prosthetic, and attaching the stretch cabling that provides the extension assistance to the digitorum muscles in the fingers (Figure 2). We plan on writing a description of the assembly process and creating a complete list of materials for patients who wish to create and use this device independently.

In order to test the model, we used force probes attached to the distal ends of the fingers to measure the extension assistance our prosthetic provides for various extension angles and compared to the torque



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Results

We successfully printed our prosthetic device after designing it in Fusion 360. Once the device was printed, finishing was done: including affixing foam to surfaces that come in contact with the hand,

Table: Force vs. Palm-Finger Angle

Angle between palm and fingers (degrees)	Force (N): normal hand without prosthesis	Force (N): normal hand with prosthesis (fingers held limp)
90	98	109
100	106	114
110	108	120
120	114	120
130	124	128
140	113	132
150	95	110
160	75	88
170	47	29
180	33	19

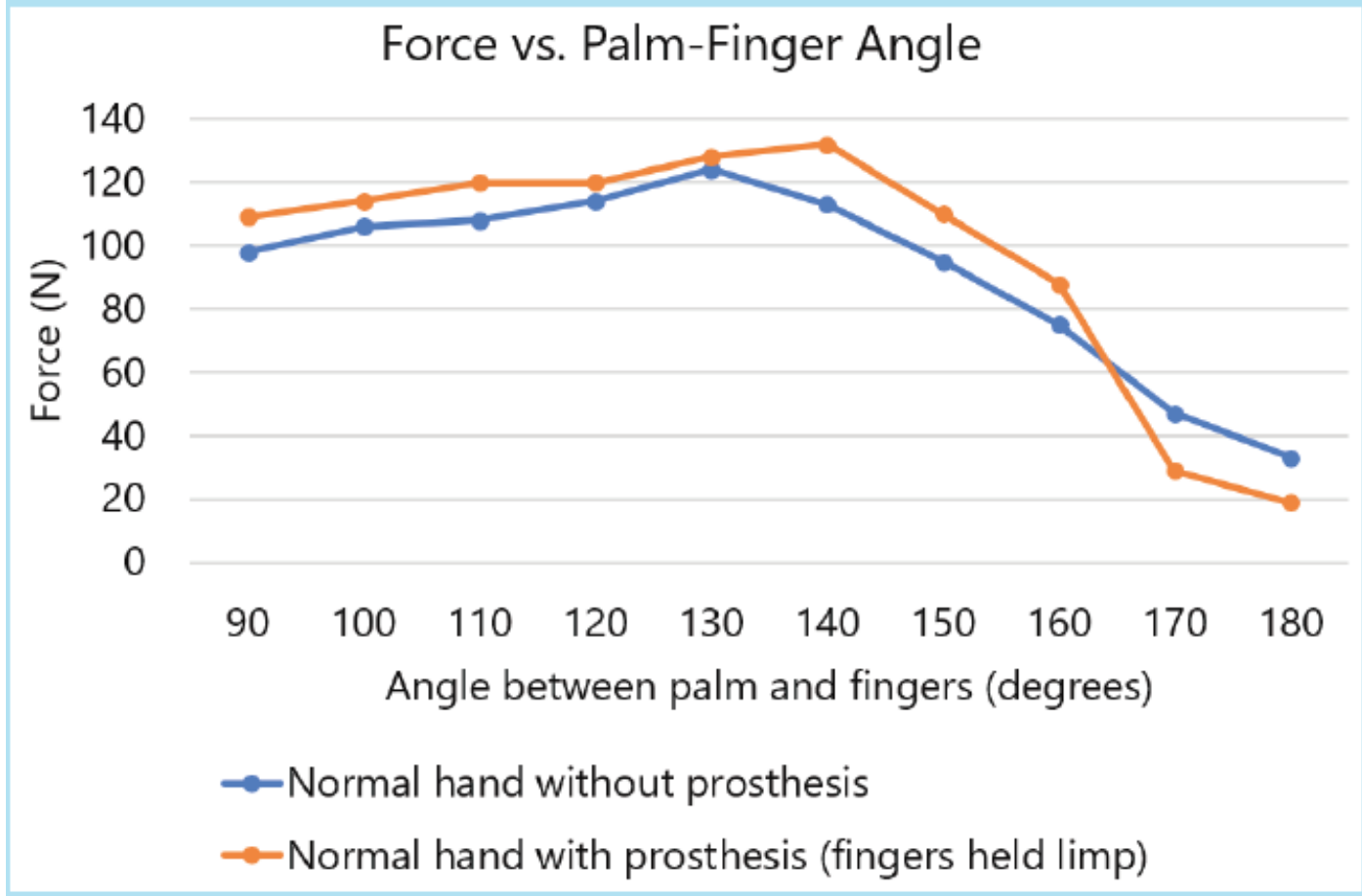
Some challenges we noticed with preliminary qualitative testing of the prosthetic is that the elastic cables seem to have too high a k-constant and not stretch easily enough to allow finger flexion. This problem could be resolved by using stretchier cables. This issue inspires a further level of customization for our prosthetic which is the elasticity of the cables. We plan to create a table matching k-constants for the cable to the level of weakness in the hand so that rehabilitation of the hand can progress as the muscles become stronger.

Another current challenge is that the 4-bar linkage between the thumb and forefinger seems flimsy and

doesn't provide enough force to lift the thumb consistently. This issue should be resolved in the next prototype by increasing the thickness of the linkage pieces and 3D printing the pivots between them rather than affixing the pivots afterwards.

Despite these challenges, the device was easy to custom fit and appears to provide noticeable extension assistance to the non-impaired hand of our initial test subject. Overall, our design creates another option for stroke survivors with impaired hand function by combining the adjustable extension assistance of a rehabilitation device with the practicality of a functional prosthetic.

Figure: Force vs. Palm-Finger Angle



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Designing and 3D Printing an External Fixator Clamp

Dane Sisko

Abstract

The goal of this project was to research prices and designs of current clamps for external fixators, and then make a 3D printable external fixator clamp. The reason for this was to lower the price of external fixators for the consumer, make them readily available for hospitals, and to reduce the waste of fixator parts. Two options were explored, one using a consumer grade 3D printer using PLA filament, which would mean a lower durability than a normal clamp, and an industrial printer using titanium powder as filament, which would mean an increased durability but a greater startup cost. The outcome of this was a simply designed, 3D printable external fixator clamp that costs a fraction of the price that normal clamp price when printed with PLA filament, and a fraction of the price and same durability when using the titanium alternative.

Introduction

This project's goal was to reduce the price of current external fixator clamps by designing a clamp that could 3D printed at hospitals, replace current clamps being used, and reduce the price of current clamps. On the parts list that looked at the average cost for clamps was around \$600-900 per clamp, and for each external fixator an average of five are used. This meant that around, according to an anal-

ysis on usage and associated cost for an urban level I trauma center, \$5900 is spent on parts per external fixator, and the majority of this cost comes from the highest priced item, the clamps. From the same study it shown that the cost of external fixator from components was \$670,805 per year, the high price largely due to the parts being used as single use parts. The project's goal is to lower the price of external fixators, and the solution to the price is through the 3D printing of fixator clamps. It is important to lower these types of cost when possible because if you were to reduce these costs by even 20% or 30% then there would be a lot of money being able to be put towards other medical services every year. Through my own research I have not been able to find any current research pertaining to the 3D printing or reduction of price for external fixators besides one article focusing on the 3D printing of a part, not a clamp, that is used for lower body fixators.

Methods

The final design of the 3D printed fixator clamp was inspired by cheese borough clamps, commonly found in scaffolding, because it was a simple design that could serve the same purpose of the clamps used for external fixators. The model was created Sketch-up, and then printed the file through the Makerbot application. The model was printed using a Replica-

tor +, and used up 12.13 grams of filament. This model would be a good alternative for the current clamps being used because the price of the printer used is around 2500\$ and the price of filament is around 20\$ per kilogram. This means that the price per clamp and clamps used per fixator would be dramatically decreased.

Results

Overall the clamp works as it is supposed to and is able to resemble an external fixator when clamped onto wooden rods, same size as titanium rods, as place holders. One problem that is potentially important is the durability of the model itself because of the PLA filament. The solution to this problem to this would be to have a more expensive startup and running cost that this model used but would be quickly be cheaper over time than the current design being used for clamps. This would be to use an industrial 3D printer, that is able to print with metal powders, costing around \$50,000 and use titanium powder which costs around \$700 per kilogram . This would solve the durability problem and the clamps would be made with the same material as the current clamps being used.

Key features to highlight on the model:

Simple: Simple design that only uses the 3D printed model, nuts, and bolts in order to make.

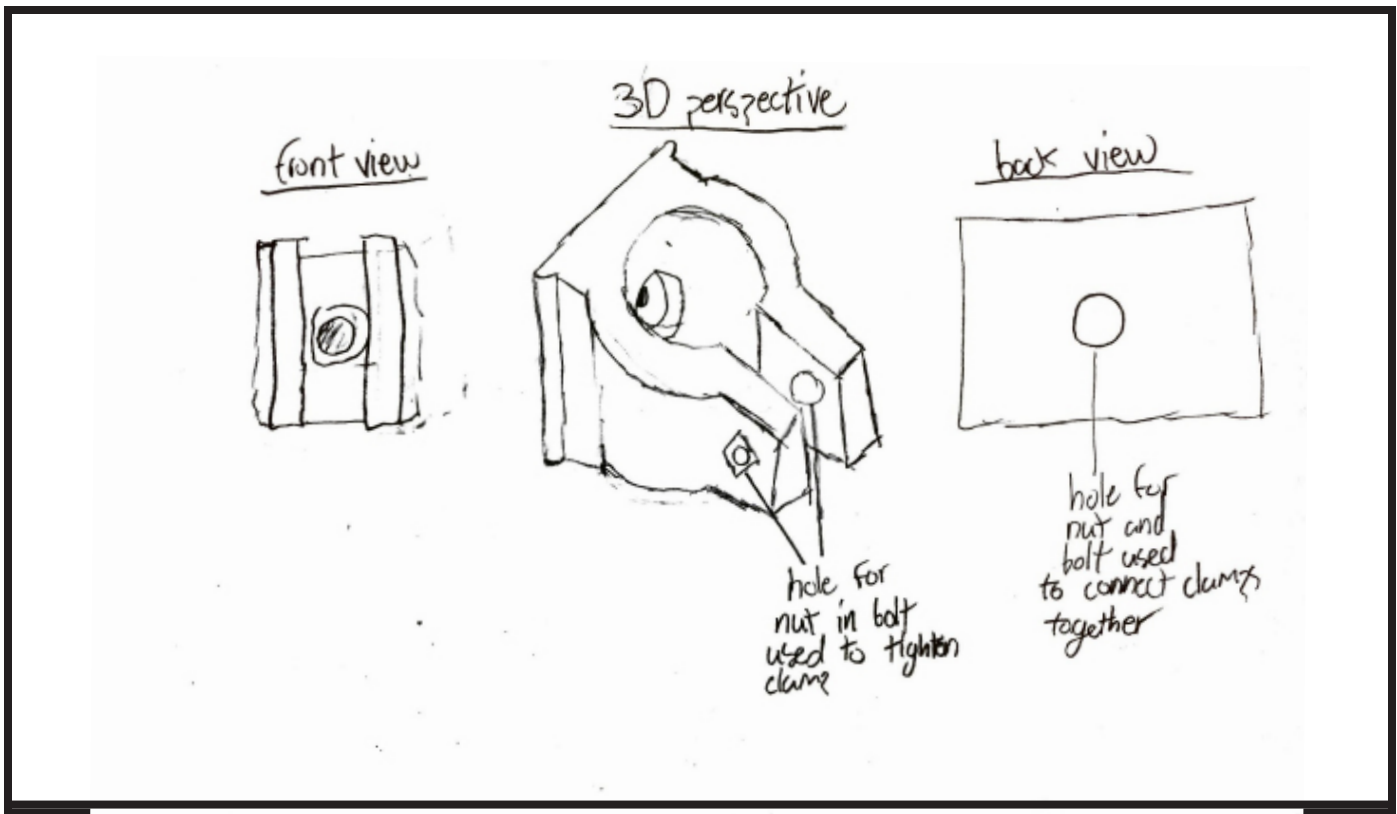
Cheap: The design only takes about 12.13g grams of filament per clamp which means that a kilogram of spool along with a 3D printer can make around 80

clamps per spool. Using this process, this means that each clamp only costs 25 cents to make, and if you were to use a industrial printer and print with titanium powder, around \$700 a kilogram, then the cost would end up being around \$8.75 per clamp.

Important idea: While my model is not the most elaborate or the best design for a clamp, it is an important example of what should be done with external fixators. It shows that there is a much cheaper alternative to the current external fixator clamps being used today.

Conclusion and Future steps

Currently the design of the model work well with the use of a nut and bolt, and is able to hold a properly sized wooden pole as a placeholder for a pin. The current design accomplished the main goal of this project by lowering the price of current external fixator clamps. A future step that would be important to complete is to properly scale the design of the model to fit the current pins used for external fixators. Another future step would be to create different clamp designs, one that can hold two pins near each other for example. The reason for this is because there are several types of clamps that could be made inside Sketchup and I believe that making these would further improve the design aspect of the project.



Annotated bibliography

Chaus, G. W., Dukes, C., Hak, D. J., Mauffrey, C., & Mark, E. (2014, October). Analysis of usage and associated cost of external fixators at an urban level I trauma centre. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/24845407>

This article was useful for my project because it showed the annual costs of external fixation parts, the cost per fixator, and that most of the parts being used were being treated as single-use. This gave me a better perspective on what I wanted to accomplish with my project.

MacKenzie, E. J., Hoyt, D. B., Sacra, J. C., Jurkovich, G. J., Carlini, A. R., Teitelbaum, S. D., & Teter, J. R. (2003, March 26). National inventory of hospital trauma centers. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/12672768>

This article gave me a look at how many level I trauma centers there are in the US, and the importance of this was that it gave me the ability to roughly scale up the costs for fixators based off the analysis of an urban level I trauma center.

HandyTools

Brandon Taylor Stumpel

Author Note

This report was prepared for Biomedical Engineering taught by Dr. Margaret Stockbridge and will be used in the Northwest Association for Biomedical Research at Shoreline Community College on May 18, 2018.

Abstract

Every year, there are thousands of men and women across the planet who are severely injured during their job, specifically in the manufacturing and construction industries. For some of these individuals, their lives are abruptly changed through the result of an amputation of a limb. This tends to lead to the individual having a difficult time performing tasks as they used to, especially when it comes to their work. For those who have lost a hand, the dominant one particularly, and work in construction or with machinery, being able to continue working seems doubtful. Our product, HandyTools, was made for the intended purpose of giving these types of individuals a way to continue working as normally as possible. The inspiration for our design originated from electric power tools, having accompanied a rechargeable battery, interchangeable tips, and a trigger into our prosthetic. The hope for the success of this project is to create a light-weight prosthetic that attaches to the end of the forearm and allows the

individual to perform various tasks involving a variety of interchangeable tools such as drills, screwdrivers, and wrenches. Our future ambitions involve removing the trigger all together, as well as the need of using both arms, and using nerve impulses to turn the power tool in the prosthetic.

HandyTools

For this project, there was one main source that inspired us to create a hand prosthesis. “Year One of [the Occupational Safety and Health Administration]’s Severe Injury Reporting Program: An Impact Evaluation” written by David Michaels, PHD, MPH and published on March 17, 2016 became our source of motivation as it allowed us to picture how many individuals are severely injured on the job and how many of those people ended up with necessary amputations. In their first full year collecting data, OSHA received 10,388 incidents of severe work-related injuries, resulting in 2,644 amputations – most for the arms and hands. It is a staggering number by itself, yet it does not take into account everyone that could possibly be impacted. OSHA is a U.S. Government agency and is not based in all fifty states. Only twenty-six states have OSHA-approved State Plans, and not every injury or amputation is reported from those states. This leaves twenty-four other states that don’t report their data, which means the

number of amputations is likely to be even larger. As stated before, OSHA is a U.S. Government agency, which means that in this report, the rest of the world is not considered. All of these potential unknowns added together could mean we are looking at tens of thousands of men and women amputated every year from work-related injuries alone. The thought of being able to develop a prosthetic that may allow these individuals to keep their jobs or hopefully live a life where they are able to still do projects or work by themselves, is something fulfilling to our team and a motivator for us. The report confirmed to us the work we were planning on doing was going to be impactful.

The next step was to begin brainstorming potential ideas for the design and functionality of our hand prosthetic. We wanted to take into account how manufacturing and construction work led to the most amputations so developing a prosthetic that would allow these people to keep working in these fields would have the largest impact. This led us to the types of tools often used in these kind of industries, and we started at the essentials: Drills, Screwdrivers, and Wrenches. We then began thinking in what way could we incorporate these tools into a hand prosthetic? We agreed that an electric power tool/drill was something that was very easy to use and parts at the tip could be interchangeable. Thus, we began to design how we could make a power tool into a prosthetic.

Background Research

In recent years, the technology for prosthetics has been slowly improving. Researchers are finding more biologically friendly materials and are making designs that allow people to have more functionality (Piazza, 2017). Our project looks to take this one step further by designing a hand prosthetic, with the intended purpose of helping individuals achieve mechanical tasks with various tools.

Without a hand, especially a dominant one, doing tasks such as using a screwdriver or turning a bolt becomes increasingly difficult. Our device will allow individuals to be able to again use these tools, allowing them to tackle household project as if they still had their hand. We always took into consideration individuals whose livelihood depends on a prosthetic like this, such as construction workers or mechanics. These people could have been injured during their job, but if they want to stay in that profession, or because it's the only job they have ever known, they shouldn't be limited to what they can and cannot do. As stated earlier, we are looking to improve these individual's quality of life.

By allowing these individuals the opportunity to use their prosthetic as a tool, we believe this will increase their psyche and improve quality of life. A common, unfortunate occurrence for amputated individuals is a feeling of helplessness. They are not able to do tasks as they did previously, and sometimes they are not able to do some tasks at all again.

Individuals who have had to amputate their hand experience this problem often, which is why we are looking to develop a prosthetic that can help them perform mechanical tasks again.

Functionality and Originality

Our prosthetic, HandyTools, is a hand prosthetic intended for individuals who would like the availability of using mechanical tools without assistance. The prosthetic is installed with a rechargeable electric motor that rotates a slot either clockwise or counter clockwise, for tightening and loosening respectively. The individual will then be able to use various attachments (screws, drills, socket, etc.) to slip into the slot, like a drill bit, and the motor will turn the attachment depending on the desired action. This allows for hand amputees to do various tasks by themselves that require mechanical tools, and thus, increase quality of life as these individuals will be less limited in the things they can do.

Currently, few hand prosthesis have been manufactured that are intended for tool use. Many have been developed for holding/picking up objects with some even showcasing picking up items like screwdrivers. However, as of our current research, no hand prosthetic has been developed specifically for the use of tools. Our prosthetic does not have a physical hand, but is rather a nub with its powered motor and multiple attachments, meaning that it is not intended to be an everyday prosthetic. Instead, it acts as any other electric power tool (like a drill), except the tool

and prosthetic are one entity.

In our designs, inspiration comes from electric drills. As one would switch in various drill bits depending on the size they need, for our prosthetic, different tools can be used for different tasks and varied sizes. The end of the prosthetic will spin like the chuck of an electric drill, and the torque selection/speed adjustments will be closer to the forearm. The battery, while ideally would be built inside the prosthetic, will instead be at the bottom for multiple reasons. Firstly, with the battery at the bottom rather than side or top, it allows for more visibility and accessibility to switching out attachments. Next, with it outside, it makes switching out batteries easier than opening up the prosthetic and changing from the inside. Finally, with the heat produced by the battery, we feel it would be safer and more comfortable if it was farther away from sensitive skin.

Results

We were able to finally print a successful model after a bit of trial and error with the 3D printers. The first model we had for the prosthetic was problematic in that it would cause the printer to jam due to the overflow of plastic at some point in the printing process. After trying out a few printers with that design, we eventually conceded and worked on a new design, which had the same functions as our old one, but would not make any mistakes in the modeling or printing process. Eventually we had to use a personal 3D printer instead of the ones offered

to us at the school because the ones at school were not fixed correctly. When we did print it, we printed it on a smaller bed, so we had to downsize it a bit to get it printed right, but eventually we plan on scaling it to the correct size. The final product is currently lacking the motor and other electrical components that will be used to make it function. This is primarily due to the size of the current print not fitting with other parts to finish the prosthetic. In the end, the prosthetic printed well and looks good for the next stage of printing and creating the working version soon.

Future Ambitions

For future development, there are many new ideas and changes we would like to implement to our prosthetic.

The main purpose for HandyTools is functionality, and the ability to let hand amputees live a more normal life in that they are able to still do work and projects that require physical tools. This means that as we make updates, we want to add more tools that can be interchanged at the tip besides the standard drill, screwdriver, and wrench functions. Examples of this would be auger bits, hammers, and possibly even a saw, however for that we would also need to incorporate the ability for the saw to oscillate back and forth.

The next aspect that we might focus on is aesthetics. Because we wanted our prosthetic to be lightweight and functional, our prosthetic could be

classified as bulky and not as aesthetically pleasing as some hand prosthetics on the market. This would mean replacing the rectangular casing with something more rounded that may resemble more of the end of the forearm. This would also allow access to tighter spots the individual is trying to reach with their prosthetic. Right now, the battery is an external attachment that comes down from the main body. In the future, finding a way to make a slot for the battery to slide into, rather than be on the outside, we not only make the prosthetic look smoother, but also remove excess bulk.

Our biggest ambition involves removing the necessary two-handed use of the prosthetic. As it is now, the prosthetic is essentially a power tool that attaches to the end of the amputated forearm. The individual uses the arm with the prosthetic to aim and control where the prosthetic lines up, and then uses the other hand to pull the trigger to start the rotation and to change the torque. Somehow, we would like to make the prosthetic completely usable with the amputated arm and not have to use the remaining hand. Our idea for this is using current breakthrough prosthetic technology that is using neural signals to control prosthetics. People are able to think of closing their hands and sensors will sense these signals coming from the brain and cause the prosthetic to act in that motion through coding. It is a lofty idea, but if somehow we were able to connect neural signals to rotate the power tool, it may allow individuals to have

more access to reaching or doing certain projects.

Conclusion

The HandyTools prosthetic is a work-in-progress. We found that we had difficulty creating a main body that was not large or clunky that also would be able to house all the necessary electrical components. We also noticed that movement was restricted when trying to aim the prosthetic towards an item such as wood when using the drill. Because the prosthetic is a two-handed device, stabilizing the item in which is being interacted with is a bit more difficult. Also,

because the prosthetic is straight and not flexible, the ability to move the power tool in a slight direction where a wrist would act is not possible.

However, we do believe that this is a step in the right direction. We are trying to give individuals the ability to do tasks by themselves again, increasing their quality of living. In the future, we would like to make adjustments to turn HandyTools into an all-purpose tool that looks as excellent as it functions.

Annotated Bibliography

C. Piazza et al., “*The SoftHand Pro-H: A Hybrid Body-Controlled, Electrically Powered Hand Prosthesis for Daily Living and Working*,” in *IEEE Robotics & Automation Magazine*, vol. 24, no. 4, pp. 87-101, Dec. 2017. doi: 10.1109/MRA.2017.2751662

In this article, the researchers have looked at the possibility of applying SoftHand technology, one of the closest pieces of technology to mimicking human touch, to hand prosthetics suitable for daily work activities. They have specified that some of the requirements this prosthetic should have include high grip power, grasp versatility, durability, resistance to water, temperature, dust, and low costs. The prosthetic isn't meant to be used for gestures or aesthetics, rather to be used as a useful tool. The article references body powered prosthetics and multiactivation-modalities prostheses (which are battery powered), and how creating a hybrid between the two may create the best prosthetic for work/daily tasks. Our group will heavily refer to this article as we too are looking to build a tool prosthetic for hand amputees that will likely be battery powered.

K. Baun., M. Lang., T. Ryan., N. Kearns., “*Case Comparison of Electric Digits and 3D Printed Body Powered Hand Prosthesis*,” in *Journal of Hand Therapy*, vol. 31, no. 1, pp. 142-143, Mar. 2018. doi: 10.1016/j.jht.2017.11.003

The researchers in this article have recognized how the fast rise of 3D printing may start to rapidly dominate medical tools. Apparently, 3D printing has been gaining growing interest by patients and companies alike as they are cheaper to manufacture and are faster to make. The article looked to compare new 3D printed body powered hand prosthetics to the conventional electric prosthetics. One downside that was found

with the 3D models were that they were not as durable as conventional prosthetics, which is to be expected as the material isn't as sturdy or of quality. They also sometimes fail to perform basic prosthetic principles in that they can be stiff and less alterable. This information is useful to our group and project in that the first version of our prosthetic is 3D printed. We will then have to decide whether continuing 3D prints and its cheaper costs outweigh the possible durability and quality issues that may arise from them.

Harris, Jordan William, "*Optimization of Prosthetic Hands: Utilizing Modularity to Improve Grip Force, Grasp, and Versatility*" (2017). UNLV Theses, Dissertations, Professional Papers, and Capstones. 3079.

The researchers here believe that current upper limb prosthetics that are commercially available are outdated and unsatisfactory, even though there are many that exist. Because of the limitations of these single-minded prosthetics some people have to either have multiple prosthetics, or have decided that they are not worth it, discarding them completely. This is why these researchers have decided to create a new hand prosthetic, one that will apparently be a well-rounded tool and be the only prosthetic a person needs. They wanted to lower costs, and speed up manufacturing, so they believed that a 3D model, which they stated was durable, would be the best route to go. However, while testing, the researchers did notice that although they were looking to make a single, all-rounded product, the research group demonstrated that an interchangeable system could increase hand customizability. This is good for our project because we are looking to make a prosthetic that the individual can interchange with a different prosthetic that may have more aesthetics or hand movability. Our prosthetic is meant to have the function of construction/home improvement, and be effective, not pretty.

M. P. Mounika., B. S. S. Phanisankar., M. Manoj., "*Design & Analysis of Prosthetic Hand with EMG Technology in 3-D Printing Machine*," in International Journal of Current Engineering and Technology, vol. 7, no. 1, Feb. 2017. doi: 10.1016/j.jht.2017.11.003

This article focuses on a design for a prosthetic hand that is 3D printed with electromyography (EMG) technology. The researchers have emphasized that one of the main requirements for a prosthetic, specifically an artificial arm/hand, is its functionality. Their goal was to create a cost-effective prosthetic using batteries, EMG technology, and that would provide the closest resemblance to a human hand as possible. While we are wanting our prosthetic to be a bit more mechanical, in that we will not be using EMG, we have still viewed this design with its battery placement, type of battery, and the advantages of 3D printing. We also found that later in the paper, the group of researchers were looking to create molds for interchangeable fingers on the prosthetic hand, which could later be developed to be interchangeable tips such as screwdrivers,

laser pointers, and bottle openers. This was almost exactly what we want to do with our prosthetic, just on a smaller scale, with its interchangeable parts.

Cherelle, P., Grosu, V., Cestari, M., Vanderborght, B., Lefeber, D. (2016) The AMP-Foot 3, new generation propulsive prosthetic feet with explosive motion characteristics: design and validation. *Biomedical Engineering OnLine*, 15(Suppl 3): 145

The researchers in this study developed a prosthetic called the Ankle Mimicking Prosthetic (AMP-) Foot 2, meant to improve upon current prosthetics for amputees by mimicking the feeling of walking by giving the necessary push-off forces. The AMP-Foot 2 is capable of giving accurate forces by using limited power. They used an explosive elastic actuator that delivers full ankle torques, around 120 Nm, and power, around 250 W. The researchers are working on creating the AMP-Foot 3 that will be able to provide a 75 kg amputee with the necessary propulsive forces. We want to use this article to see how the researchers used the actuator and batteries to help their prosthetic's functionality. We want to do something similar with our prosthetic and a motor.

Imagine Tomorrow 2018: First Place

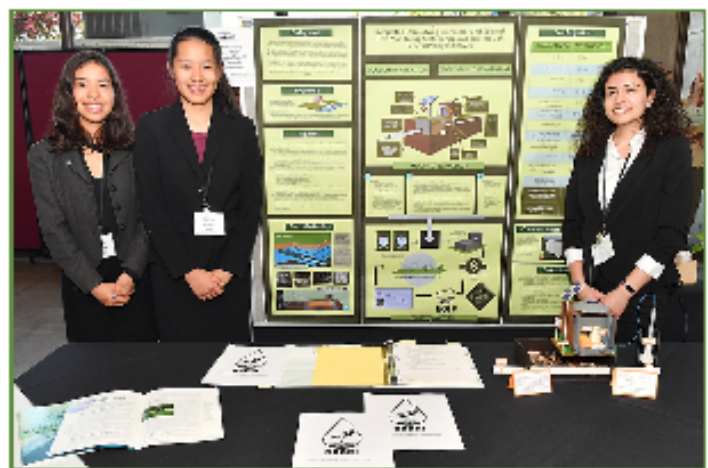
Iron Food, Water, and Energy Challenge, Multi-Approach



Aditi Joshi, Tanni Srikanth, Vidhi Jain, Lahari Nidadavolu, Roshni Srikanth

Imagine Tomorrow 2018: Second Place

Iron Food, Water and Energy Challenge, Design Approach



Anusha Srivastava, Esther Wang, Rachel Alwan

Engineering

Imagine Tomorrow 2018: First Place
Boeing Aerospace Challenge, Design Approach



Anna Whiteside, Daniel Shalko, Ayushi Desai, Rhea Shinde, Ayan Gupta

The Application of Infrared Thermography to Architecture to Identify Heat-Emitting Building Materials

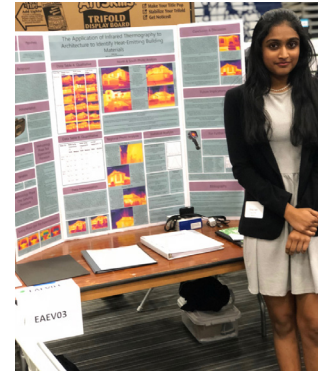
Ritika K. Iyer

Author Note

This research was supported by Dr. Adam Arabian of Seattle Pacific University, WA.

Abstract

There are 120 million single-family homes in the United States which account for 40 percent of the United States' annual carbon dioxide emissions. Of these households, 32 percent of homeowners report using an age-old heating system, operating at minimal efficiency, which ultimately causes major heat loss in the home through the windows. This investigation focused on recognizing the biggest area of heat loss in a single-family home, which turned out to be the windows, disproving the hypothesis that the biggest area of heat loss would be the walls due to their large surface area potentially causing more cumulative heat loss. The investigation was carried out using the Forward Looking Infrared Radiometer (FLIR) One for iOS Thermal Imaging Camera to take infrared images of certain areas, such as the walls and windows, of two nearly identical single family homes to quantify the largest amount of heat lost (dependent variable) and to specify the building material needed to retain the heat (independent variable). Three trials of the investigation were done during the day to produce high-quality images for



in-depth analysis to show that cement-reinforced areas let out 20 percent more heat than stone-reinforce areas. Heat loss is a major issue facing architecture today and research in this field allows for architects to analyze and implement efficient and economical house designs for future.

Keywords: Infrared Thermography, heat loss

Introduction

There are more than 120 million residential homes in the United States which contribute to close to 40 percent of the United States' annual carbon dioxide emissions (Environmental and Energy Study Institute). About 32 percent of these households report using age-old home heating equipment, which do not operate at full efficiency (U.S. Department of Energy). Furthermore, 48 percent of energy consumption in U.S. homes was for space heating and cooling (U.S. Energy Information Administration). Infrared thermography is a cutting-edge method of testing quantifiable aspects of heated areas, such as

net heat loss from a building. An infrared camera is a non-contact (does not touch the subject being tested) device that identifies infrared energy, or radiating heat, and produces a thermal image on its display camera.

Research in the field of infrared thermography is important since it helps identify room for improvement in heat-holding capabilities of cement, stone, brick, wood, glass, and any other building materials. The energy that comes from the combustion of fossil fuels that are used to heat a room are being wasted when the building's design and materials are flawed. The depletion of these fossil fuels ultimately contributes to global warming. Infrared thermography, if applied to single-family residences, can make residents aware of the areas of the areas of significant heat loss so that they may work to improve insulation, design, and efficiency of their heating systems.

The numeric data collected from the infrared camera, such as temperatures of certain areas, can then be used to perform calculations and quantify the amount of heat lost from a building. The qualitative data, the images from the infrared camera, may be used to visually depict the calculations. Infrared thermography can help solve issues of heat loss from a building, a significant problem facing architecture today. Certain types of building materials retain heat better than others, so by using infrared cameras, the areas of heat loss are identifiable and there is a clear idea as to where a building design can be

improved.

Conclusion

Infrared thermography, a cutting-edge method of testing quantifiable aspects of heated areas, such as net heat loss from a building, is carried out through an infrared camera, a non-contact device that identifies infrared energy, or radiating heat, and produces a thermal image on its display camera. The infrared camera used for this experiment was the FLIR (Forward Looking Infrared Radiometer) One for iOS Thermal Imaging Camera which connected to the FLIR One app on an iOS-capable smartphone. The camera was used to take thirty infrared photos of two identical (on two levels) single-family homes in Sammamish, Washington, and the app was used to add "points" to identify temperatures of specific areas of the home at certain instances. These images were taken and analyzed to identify the area of the home with the greatest amount of heat loss and the specific building material necessary to retain the heat.

Three trials of the experiment were conducted for reliability. The numerical values of radiating heat (in degrees Fahrenheit) were averaged per area (walls and windows) for each of the two home sites. These values were then taken and put in lists for statistical analysis tests such as Chi Square. This investigation focused on recognizing the biggest area of heat loss in a single-family home, which turned out to be the windows (approximately 7 degrees Fahrenheit

warmer than walls), disproving the hypothesis that the biggest area of heat loss would be the walls due to their large surface area potentially causing more cumulative heat loss. Furthermore, the building material that efficiently retained internal heat was cement. The cement-planked exterior of Home Site A proved to have a significantly lower radiating temperature than the wood and stone-planked exterior of Home Site B by 10 degrees Fahrenheit.

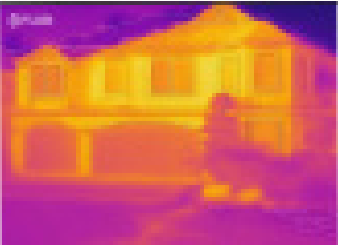
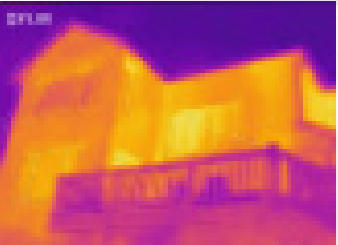
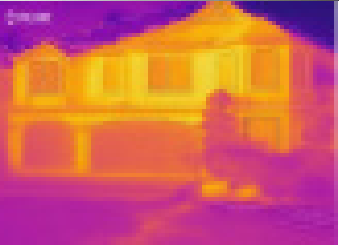
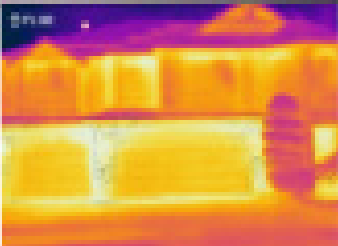
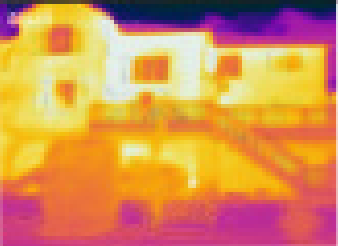
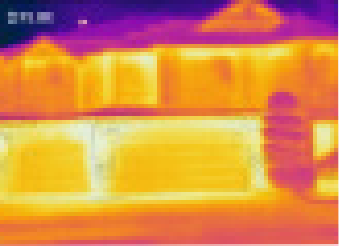
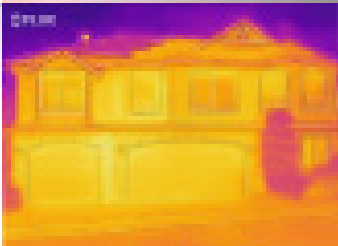
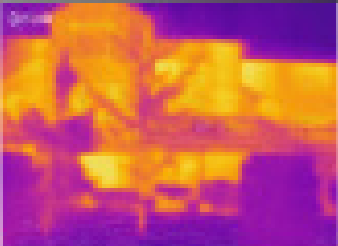
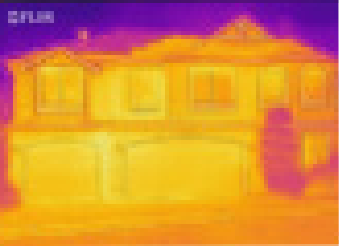
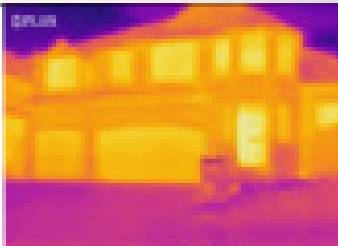
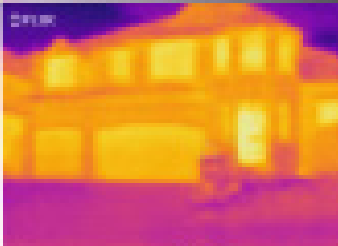
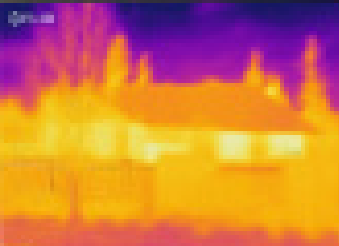
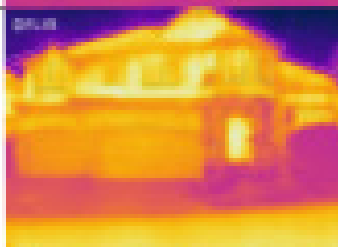
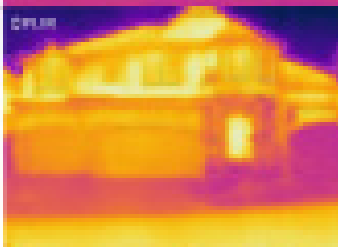
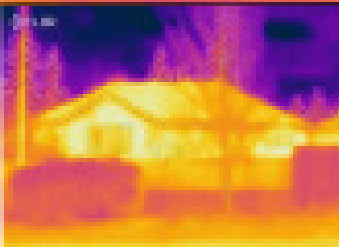
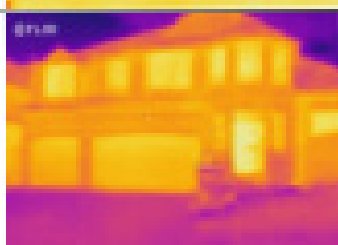
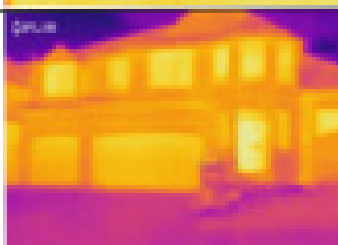
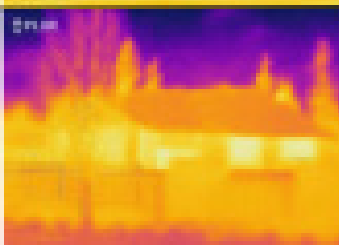
The findings of this experiment were statistically significant, with a two-tailed p-value of 0.0178. An unpaired t-test statistical analysis was also done to get a t-value of 2.5617. Both values ensure the statistical reliability of the data. To sum up, the biggest area of heat loss in a single-family home is through the windows and an efficient building material that retains the heat is a cement-planked exterior.

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Data Table A

Qualitative Data Table

Home Site	Outside Home Images	North Facing Images	South Facing Images
Home Site A			
			
			
Home Site B			
			
			

Environmental Science



Consumer Device for Measuring Mercury Level in Fish

Victoria Alkin, Kushal Kedia, Jadrian Png, Thalia Tsai

Problem

The consumption of mercury through contaminated fish has become a problem of large proportions. Much of the time, people are unaware of how much they are ingesting and how much they can consume before reaching harmful levels. Consuming mercury, especially in large amounts, can lead to a number of health problems, and also to birth defects if consumed by a pregnant woman.

Mercury itself occurs naturally in the environment in the forms of plants and animals. It is also produced anthropogenically from human industrial activity such as coal-fired plants, waste incineration, and cement production. Recent estimates, according to the EPA, have found that annual global mercury emissions from both natural and anthropogenic sources are in the range of 5,000 to 8,000 metric tons per year.

From the air, mercury eventually diffuses into bodies of water. Once there, methylmercury (MeHg^+) is formed from inorganic mercury (Hg^{2+}) by aquatic bacteria. Because of methylmercury's high affinity to sulfhydryl groups in proteins, this heavy metal (in both methylmercury and inorganic mercury form) is rapidly incorporated into the food chain through zooplankton as they filter water to feed on algae. Within the food chain, mercury bioaccumulates in aquatic

organisms,

and biomagnifies from one trophic level to the next. This means that with each subsequent level of the food chain, the organisms contain more and more mercury (See Figures 1 and 2). Hence, fish, which usually occupy the last levels of aquatic food chains, can have quite levels of mercury. When humans eat mercury-contaminated fish, this mercury is transferred into the human body.

This consumption of mercury can have very harmful effects. Mercury-related health problems include long-lasting neurological and developmental deficits, damage to central and peripheral nervous systems, muscle weakness and lack of coordination, issues with the immune and digestive systems, loss of peripheral vision, tremors, headaches, insomnia, impairment of speech, hearing, and walking, and problems with the lungs, kidneys, skin, and eyes. With high quantities of mercury, consumers can experience very severe, and often permanent, health effects (See Figure 3).

Consumption of mercury can also lead to birth defects if consumed by a pregnant woman. Infants in the womb can be exposed to methylmercury when their mothers eat fish that contain it, and this exposure can adversely affect unborn infants' growing brains and nervous systems. These children may

experience problems with cognitive thinking, memory, attention, language, fine motor skills, and visual spatial skills. It has been estimated by the EPA that more than 75,000 newborns in the United States each year may have increased risk of learning disabilities associated with in-utero exposure to methylmercury.

Almost all of the people in the world have trace amounts of methylmercury in their tissues, which reflects how prevalent mercury is in the environment. In a 2015 report done by NOAA, it was found that the average American consumes about 15.5 pounds of fish and shellfish per year, and much of this contains mercury, some of it with quite high levels. According to the NRDC, eating contaminated fish is the number one cause of mercury exposure in America. However, the negative effects of consuming mercury-contaminated fish could be avoided, if consumers could easily check the concentration of mercury in their fish, and make an educated decision as to whether or not it is safe to eat.

Chosen Solution

Our proposed solution is an easy-to-use and accurate portable tool that can measure the concentration of mercury in a fish sample, showing a user whether the fish is safe to consume or not. This device would consist of a probe made from a synthetic fluorogenic polymer that undergoes a reaction and changes color upon contact with mercury, and a box with a UV light source, a small hole on the top to insert the probe, and a slit through which to view the probe.

(See Figures 4, 5, 6, and 7) A user would have to first stick the probe into the fish sample and leave it there for 20 minutes in order to allow the reaction to take place. Then, the user would remove the probe, insert it into the slot in the box, and turn on the UV light. The probe will then emit blue light of various intensity depending on the amount of mercury present in the fish sample. If there is little to no mercury present, the color will be a light blue, if there is a medium quantity the blue will be of a medium shade, and if there are large amounts of mercury the probe will glow a dark blue shade. This corresponds directly to the safety of the fish sample – the darker the blue, the less safe it is to consume. There will be a key on the front of the box (see Figure 8) linking each shade of blue to the range of mercury concentrations (in ppm) that the fish sample may contain, and the relative safety of consuming it.

The polymer used in the probe is a sulfur-containing chromogenic fluorogenic modified polymer called JG25 (see Figure 9). It has been used in multiple studies previously, and one of its main characteristics is its reactivity with mercury. The main functional group of the polymer which is responsible for its reactivity is sulfhydryl, to which both Hg_2^+ and $MeHg^+$, or mercury and methylmercury, bind naturally. When it binds, it produces a quenching effect on the initial fluorescence of the core structure by a photoinduced electron transfer (PET) (a transfer of electrons from one substance to another due to

the presence of light) when exposed to UV light (at around 366 nm in wavelength). This means that it dulls the glow of the polymer, and is what causes the polymer's glow to become darker and deeper in color. This JG25 polymer is synthesized by photochemical initiated radical polymerization of the hydrophilic monomers 2HEA (2-hydroxyethyl acrylate) and PGM (peptidoglycan), cross-linking agent EGDMA (connects parts of the polymer through covalent bonds), and JG10 ($[\text{Cu}(\text{NCCH}_3)_4]^+$) (see Figure 10). The co-monomer molar ratio of 2HEA/PGM/EGDMA is 95/5/5, respectively. For the photochemical initiated reaction, 2,2-Dimethoxy-2-phenylacetophenone (DMPA) is used as the photochemical initiator (meaning that it commences the polymerization). Once synthesized, the JG25 polymer is a light orange-yellow color. The JG25 polymer is synthesized as a film-shaped functional membrane, which is then bonded to a polymeric hydrophilic matrix (the JG25 polymer coats the inner matrix) to form a probe that can be inserted into a fish sample to detect levels of mercury within the fish.

To use the device, a user must first insert the JG25 polymeric probe into the fish sample. Once the probe is lodged in the sample, if there is mercury present, either in Hg^{2+} or MeHg^+ form, it will begin to bond to the probe, causing it to undergo a quenching chemical reaction. The extent to which the reaction occurs is directly proportional to the concentration of mercury present in the sample. However, there

is a limit of detection, or an asymptotic maximum for the levels of mercury that can be detected – for Hg^{2+} , the probe cannot detect concentrations higher than 6.6×10^{-6} M, and for MeHg^+ , concentrations higher than 1.5×10^{-6} M cannot be detected. If concentrations higher than these values are present in the sample, the probe will max out and display the values of the upper limit. However, this is fine, since these upper limits are well above any safe concentration of mercury, so if such high levels are displayed, a user can positively assume that the fish is unsafe, without needing to check if the exact levels are even higher. The reaction typically occurs fully within 15 minutes, but it is advised to leave the probe lodged in the fish sample for approximately 20 minutes to ensure accurate results. After 20 minutes with the probe secured in the fish sample have passed, the user can remove the probe, place it in the box, and turn on the UV lights. With exposure to UV light, the probe should immediately fluoresce, and the color of its glow can be gauged through the slit in the front side of the box and compared to the key next to the slit to determine the concentration of mercury. The key will also display whether each level is relatively safe or unsafe to consume. This portable, low-cost, and reliable tool can provide a quick, efficient, and accurate representation of the concentration of mercury in a fish sample. Thus, it can prevent mercury-related health problems (such as long-lasting neurological and developmental deficits,

and issues with the immune system, digestive system, lungs, kidneys, skin, and eyes) and birth defects by allowing people to know whether a fish is safe to consume or not.

Alternative Solutions

There are very few tests for detecting mercury in fish, and none that are as quick, accurate, and cost-effective as our device. The alternative methods were found to be time-consuming, complex, inefficient, costly, and inconvenient to transport, unlike our solution that utilizes the JG25 fluorogenic polymer.

The first alternative solution is the gold nanoparticles solution developed by the researches at Northwestern University in Chicago, IL. This test uses functionalized gold nanoparticles “hairs” attached to a piece of glass and covered in a thin film. The sample of fish or water is then placed on the sensor. Any mercury or other ionized metals that come into contact with the “nanohairs” will be trapped by the functionalized gold, creating a circuit. The glass plate is then inserted into a voltage measuring device that measures the voltage between the electrodes. Higher heavy metal concentrations will lead to higher voltage measurements. This new sensor is very accurate, and is able to get measurements nearly identical to measurements by the USGS taken from the same samples. Furthermore, the samples used in the research were taken from mosquito fish from the everglades. These fish are at the bottom of the food

chain and would have some of the lowest measurable mercury levels due to the lack of bioaccumulation. These levels would be as low as or lower than concentrations found in the water of the everglades. The sensors cost less than \$10 to fabricate, but the voltage meter used to analyze the samples costs a few hundred dollars, thus making this solution too expensive for an average consumer to use.

Another solution is a Scripps mercury detection kit that was developed by the Scripps Research Institute which involves a long and complicated process to break down a sample of the fish, extract the mercury, and measure its level. The process begins with the user placing a piece of the fish in a tube with acid and enzyme to digest it for a few hours, then placing a resin coated stick in the tube that binds to the mercury. This stick is then placed in another tube of acid which strips the mercury, to which an indicator is added which changes color to show mercury presence. This process is time-consuming and complicated, and requires careful attention from the user, as well as handling of various chemicals. The use of our GJ25 fluorogenic polymers is much faster, since the entire process can be completed in as little as 20 minutes, and simpler for consumers.

The final candidate for a detection solution is a method that uses fluorescent DNA probes and silver ions, and was developed by Li Deng and Ronghua Yang of Hunan University in China who were seeking a more convenient approach. They sought

a more convenient approach because separating methylmercury from other forms of mercury, which usually requires methods such as gas chromatography or high-performance liquid chromatography, can be time-consuming and tedious. The tool, developed by the researchers from China, was able to detect methylmercury quickly, sensitively, and selectively in a laboratory, along with accurately measure and record the amount of methylmercury present. Inside of the tool for the fluorescent probes, the team designed carboxyfluorescein dye which has a glow that is enhanced when bound to methylmercury. The team then used silver ions that sop up ionic mercury into Hg-Ag clusters which bind to the DNA strands and extinguish the remaining fluorescence of any unbound DNA strands. The intensity of the glow then offers a way to measure methylmercury accurately even with ionic mercury present. Even in samples containing 50 times more ionic mercury than methylmercury and ions of other metallic elements, the probes were still able to accurately measure the methylmercury concentrations. However, despite the DNA probe's accuracy, it is not as portable and easy to use as a colorimetric meter, which is what the JG25 fluorogenic polymer is based off of. Along with that, the DNA Probe faces a significant limitation for its selectivity of methylmercury, which may not be enough for some samples while the JG25 probe does not.

Scenario of Real-Life Applications

In order to make this a reality, there are several steps that must be taken. First, a working device must be made. Our prototype is fully functional other than the lack of the real JG25 polymer, so the next step would be to synthesize the JG25 polymer in a laboratory and create a probe that is made up of the JG25 polymer, and can therefore detect mercury in fish.

The next step would be intensive testing of the device. Since this is a health and safety-related tool, it is critical that it is always accurate and that its measurements provide a factual representation of the concentration of mercury in a fish. If the device were to work incorrectly and come up with a false negative, displaying that a fish has little to no mercury when in reality it has high quantities, this could have the dire consequences of users consuming fish with unsafe amounts of mercury, creating health problems for themselves, and potentially their child in the case of a pregnant mother. A false positive would have less severe consequences, but if the device were to display that a fish has a high concentration of mercury when it does not, this could result in a user losing money by throwing away an expensive fish that they would have otherwise safely consumed. So, it will be vital to conduct extensive testing of the device before making it available for commercial use.

One method to test this device would be to create artificial samples with known quantities of mercury (by infusing them with mercury of various amounts),

and then testing the mercury quantities with the device. The device can be deemed accurate if it always displays the correct range of mercury concentrations. The device's longevity would also have to be tested (whether or not it retains its accuracy after numerous uses). This could be done by testing devices on numerous samples, and making sure that the accuracy of its measurements does not decrease with the number of uses. If the device prototype passes all the trials of product testing, it can be deemed accurate and safe for consumer usage.

The next step would be to make this device available for commercial use. This would require large-scale production of the device and consumer packaging and sale of the device. Further screening of the device in subsequent years would also be necessary to make sure the device continues to work properly.

Once this device is in commercial use, it can have tremendous positive effects. By giving people the ability to check the concentration of mercury in fish, it can prevent people from eating fish unsafe for consumption, thus drastically decreasing the number of mercury-related health problems, such as brain damage, muscle weakness, speech and hearing impairment, loss of peripheral vision, digestive and immune system issues, and problems with the lungs, kidneys, skin, and eyes. This can also greatly decrease the number of mercury-related birth defects, as pregnant mothers will know whether or not a fish is safe to consume, and will be able to prevent health problems in their child.

This device also has one more possible application - an accompanying crowdsourcing phone application that users can download free of charge that can keep them informed as to the typical mercury levels of fish of certain types, from certain stores, etc. It would work as such: users, when using the tool to determine the mercury concentration of their own fish sample, can enter into the application the fish type, mercury levels, store bought, location, and other information. All of this information would then be compiled, calculating the average mercury concentration for each store and fish type inputted. Then, when choosing fish to purchase, users can enter the application and look at the profile of each fish type at each store. (See Figure 11) If a certain fish at a certain store tends to have very high mercury levels, users will know not to buy that fish type at that particular store. Also, if a store has generally high mercury levels in their fish, users will be forewarned to not buy their fish there. This will have two major effects. First of all, it will keep shoppers informed as to where to buy fish with relatively low mercury contents, and where not to shop for certain fish types. This will decrease instances in which a shopper buys a fish only to find that its mercury concentration is high and it is unsafe to consume. Secondly, this will push store owners and managers to sell fish with low mercury contents, since they will know that this information is now being kept track of and available to the public.

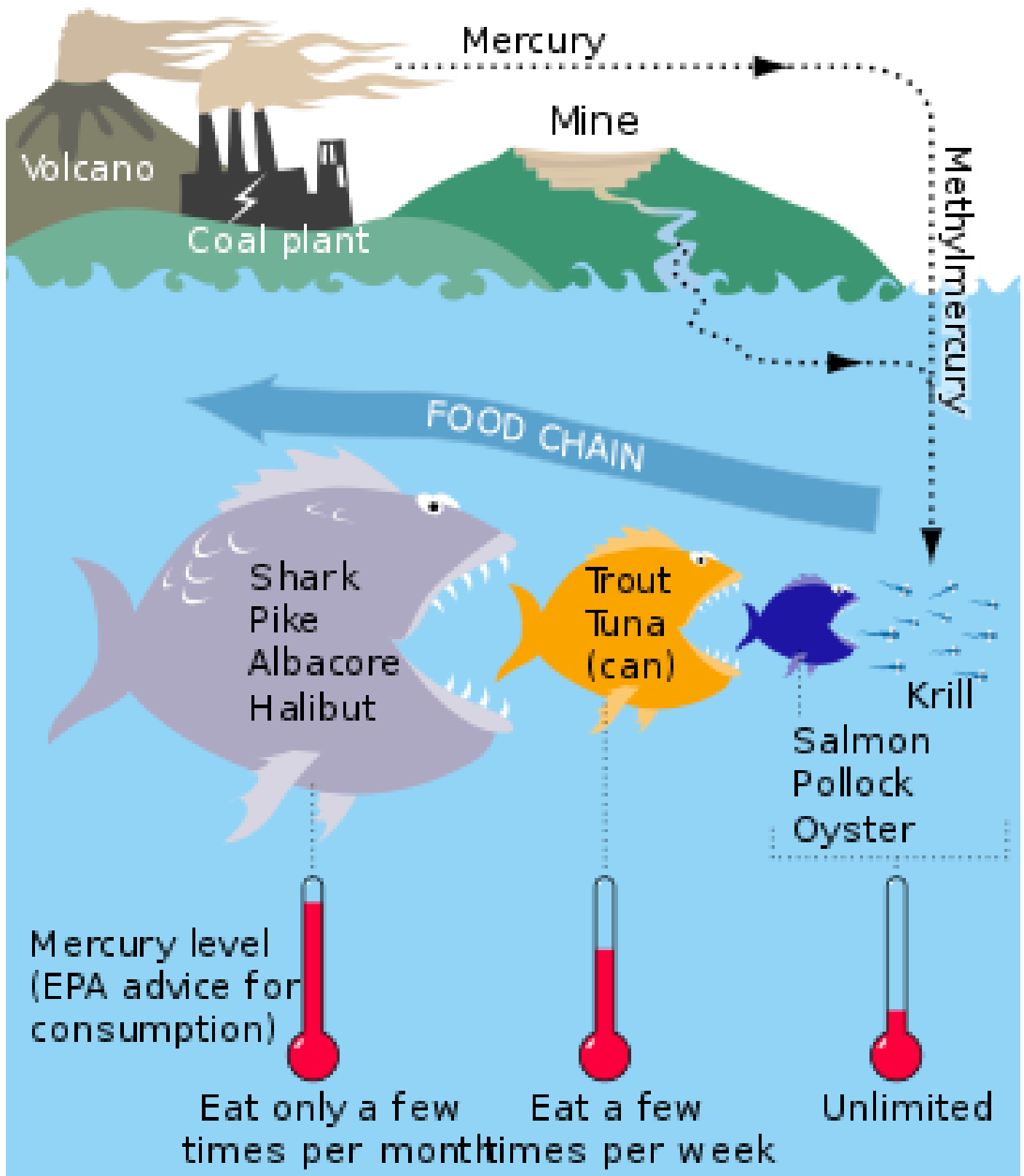


Figure 1 - Bioaccumulation of Mercury in the Aquatic Ecosystem

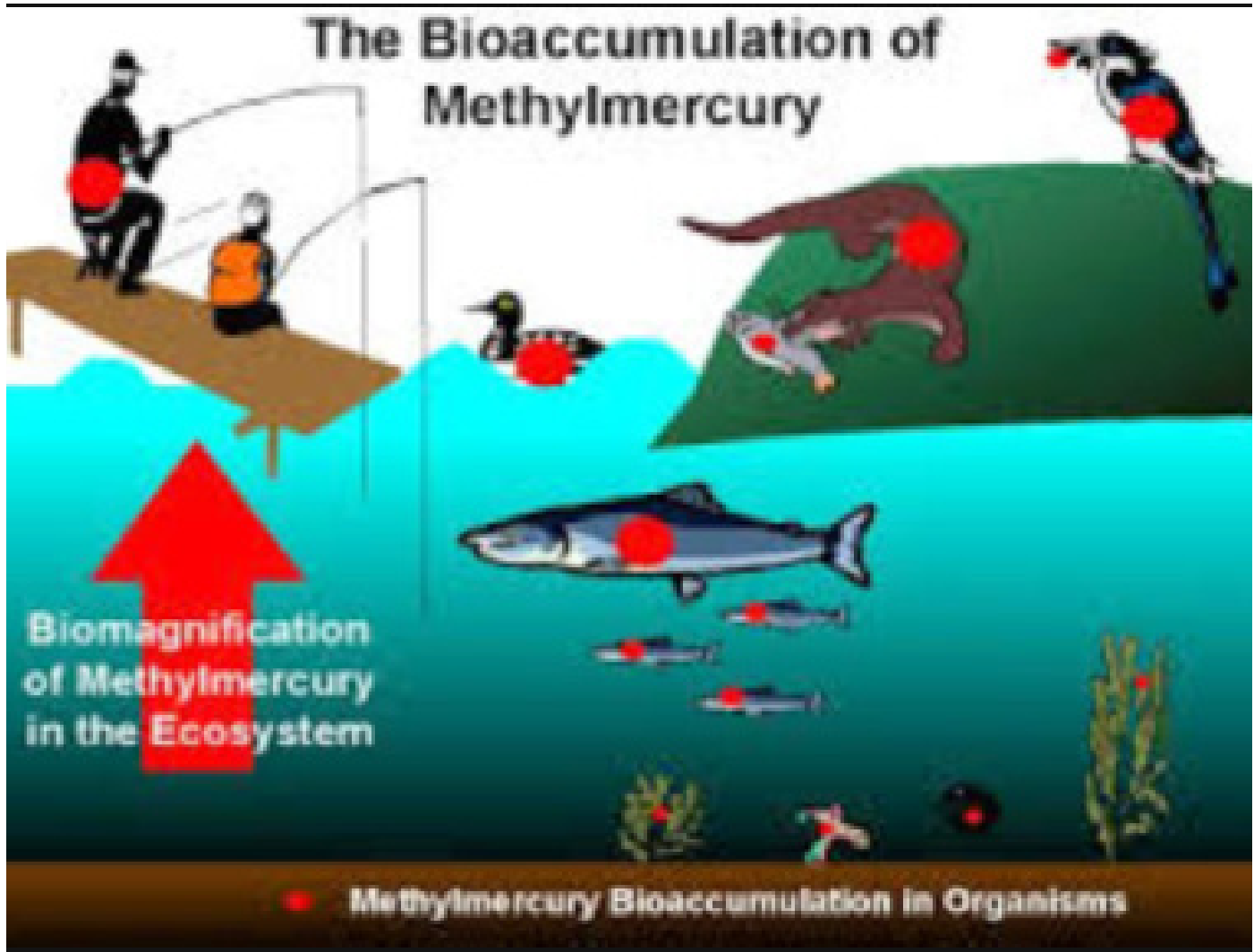


Figure 2 - Bioaccumulation of Mercury in the Aquatic Ecosystem

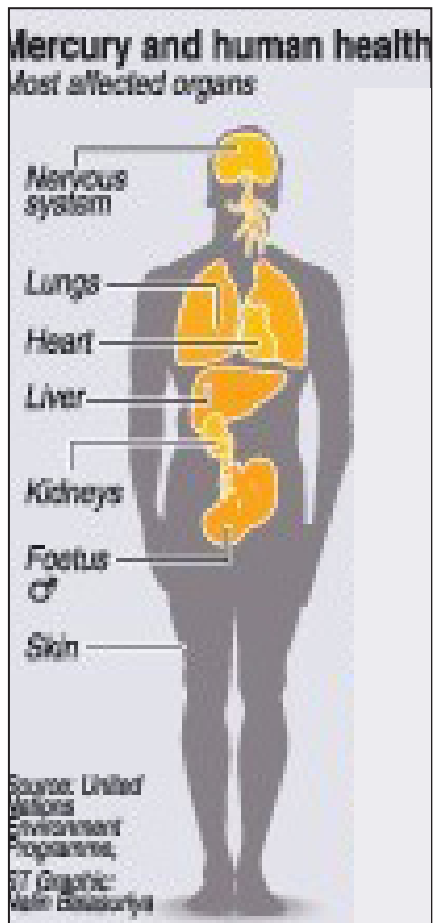


Figure 3 - Affected Organs

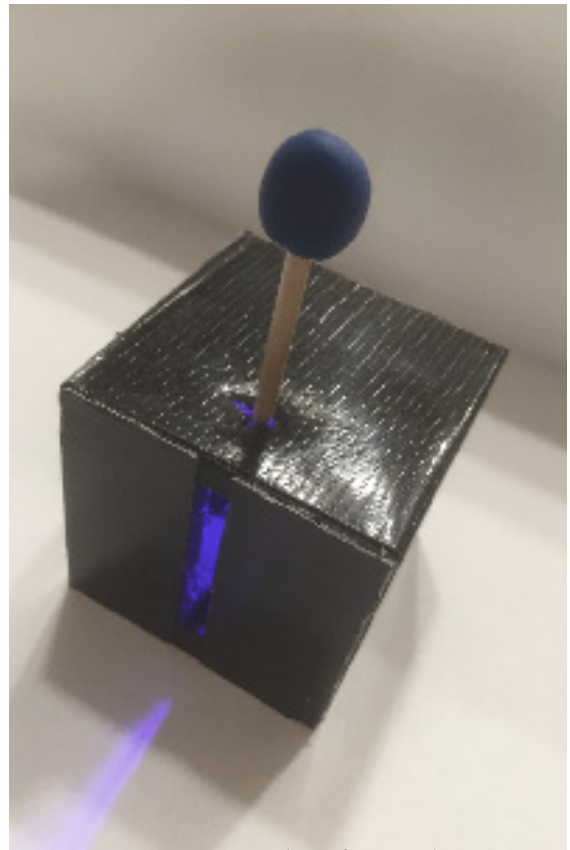


Figure 4 - Prototype (Angle View)

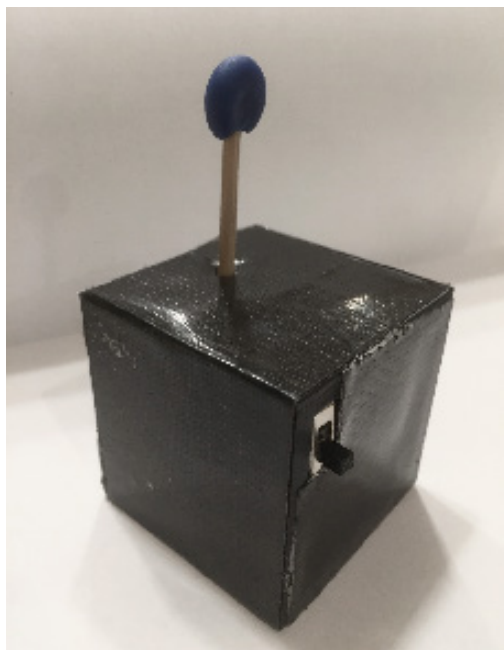


Figure 5 - Prototype (Back View)

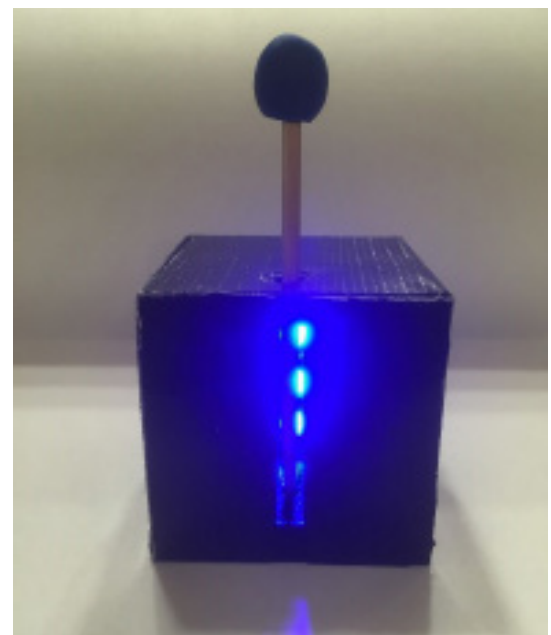


Figure 6 - Prototype (Front View)

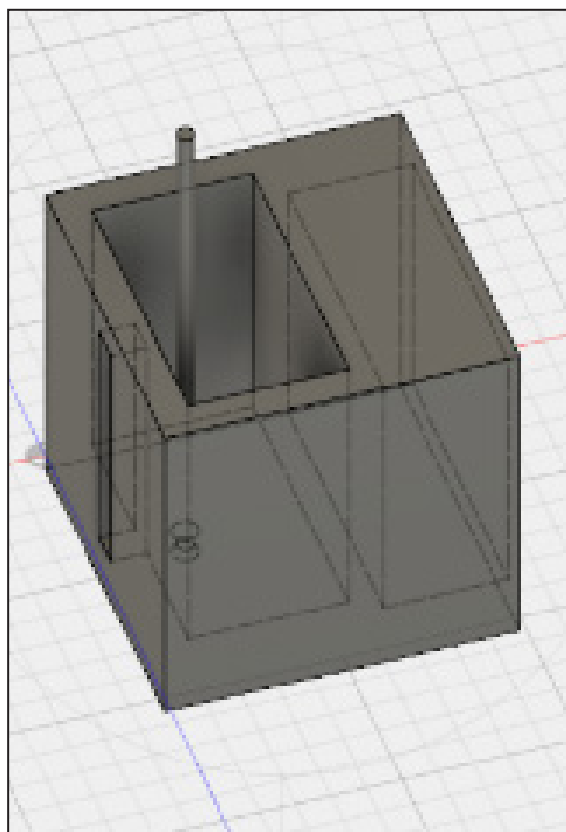


Figure 7 - CAD 3D Model

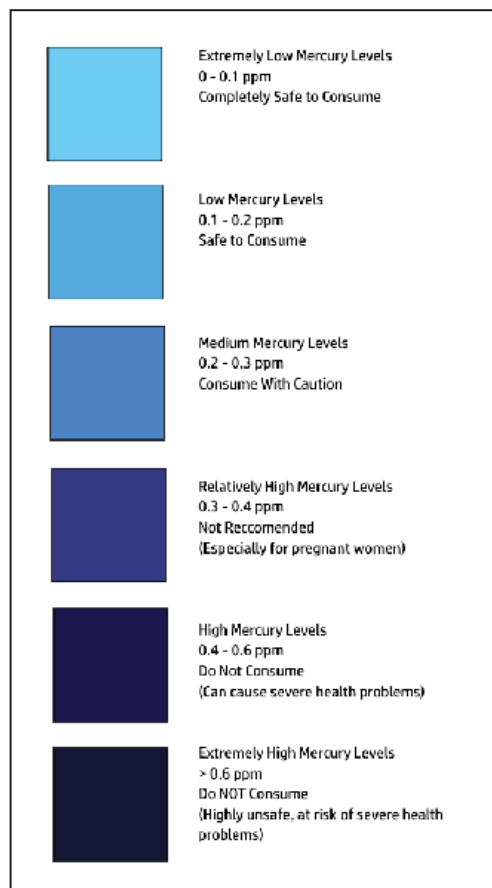


Figure 8 - Key (fluorescence corresponding to mercury levels)

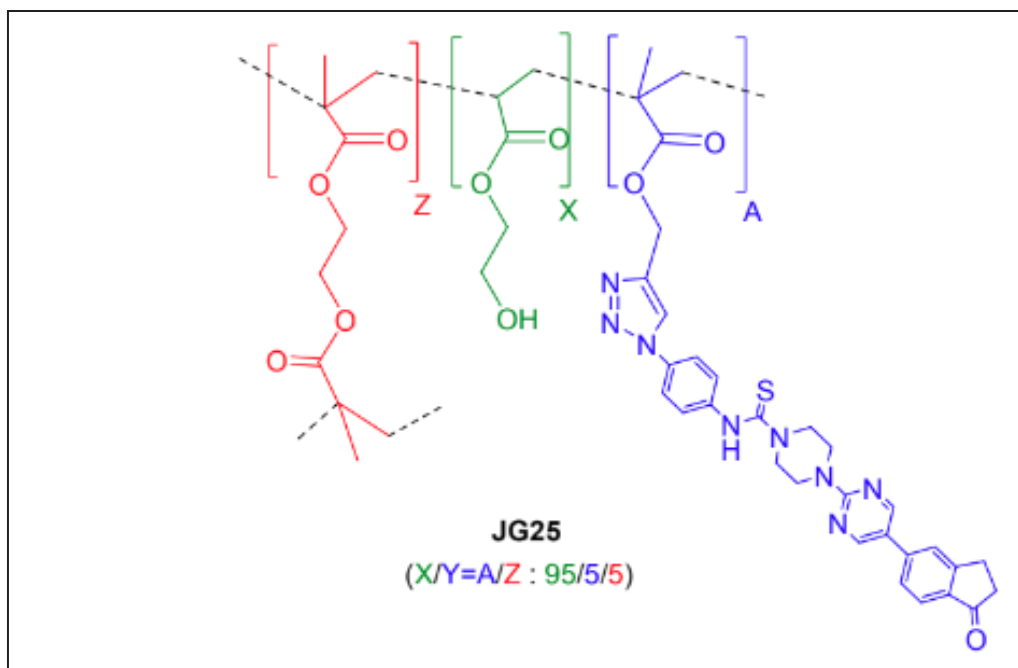


Figure 9 - JG25 Polymer

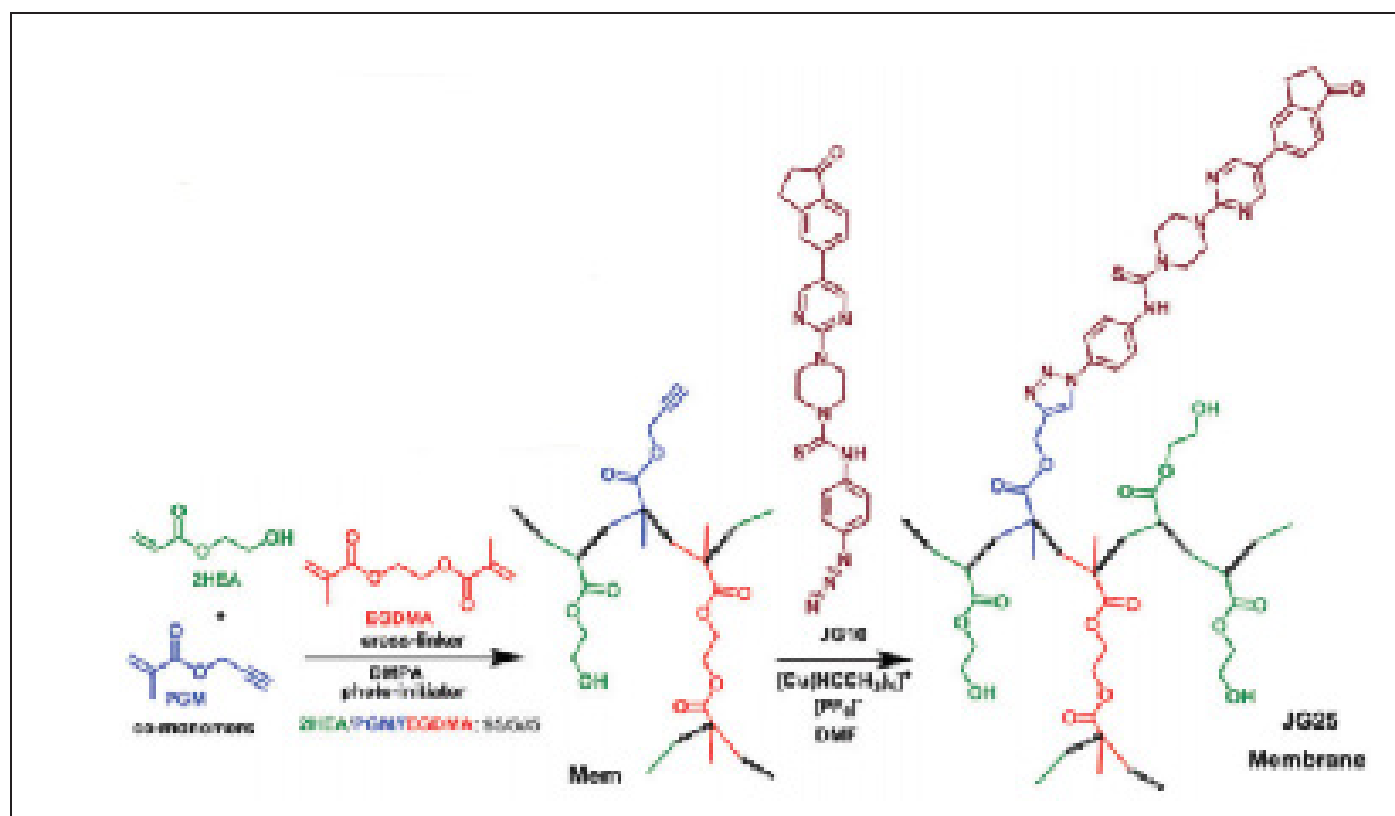


Figure 10 - Synthesis of JG25 Polymer

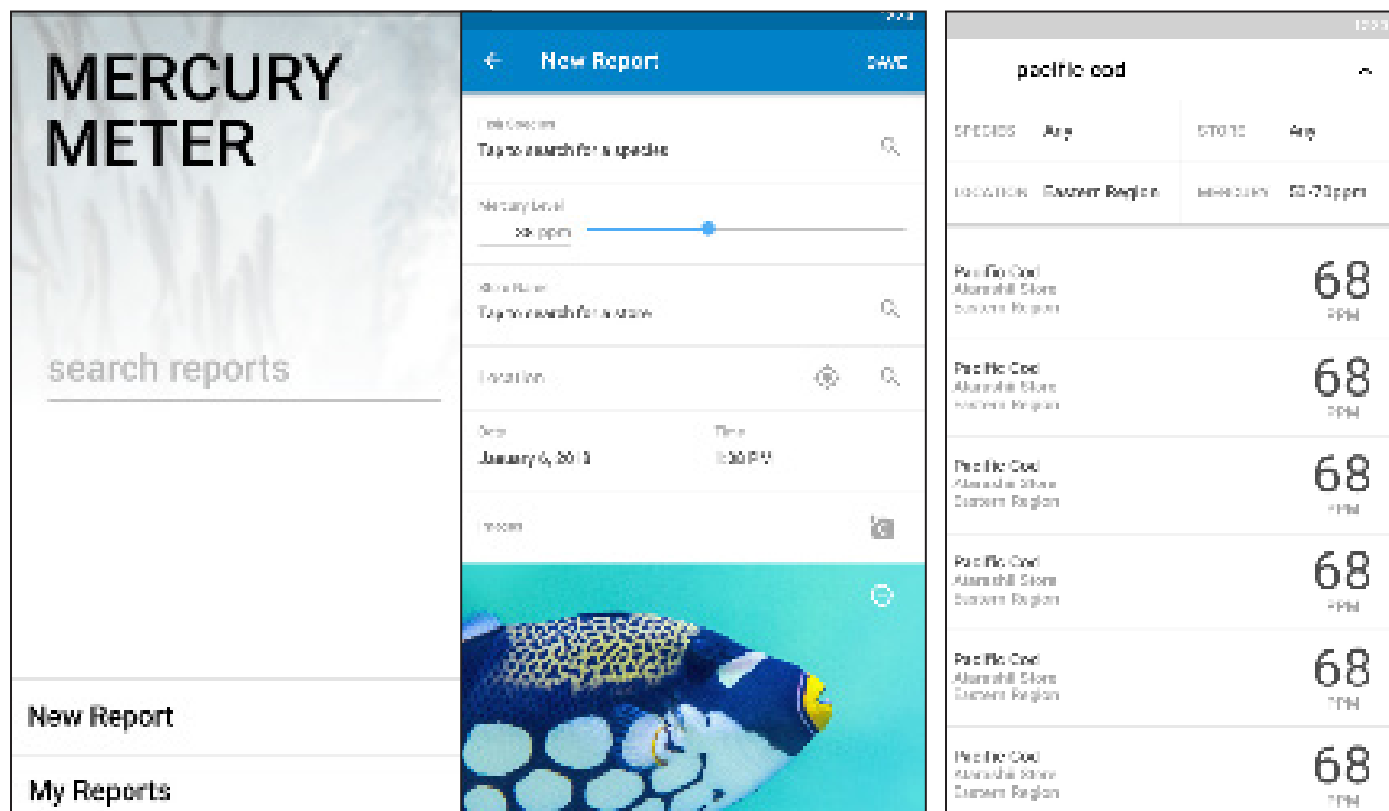


Figure 11 - MercuryMeter application mock up

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Medicine



Gene Expression Levels of Carboplatin Sensitive and Resistant Ovarian Cancer Tumors and Its Implications on Precision Medicine

Amrita Narasimhan

Abstract

In 2017, there were an estimated 22,440 new cases of ovarian cancer, over half of which resulted in deaths. Early and effective treatment with frontline chemotherapeutics like Carboplatin increase chances of survival by 63.6%. Genes which are differentially expressed between sensitive and resistant tumors can provide insight on a patient's condition and how to most effectively treat ovarian tumors. Using a public dataset from NIH, gene expression levels between the Carboplatin sensitive and resistant tumors were compared to find experimental significance by dividing the average gene expression level in the resistant tumors by the average gene expression level in the sensitive tumors. Genes with a greater than two-fold difference were identified and were found to be NBN (Nibrin), CPNE3 (Copine III), and RHOBTB2. Analysis of variance in R was used to compare patients to find those with statistically significant gene expression values. Significant p-values were seen when comparing patients 1 and 6, whose cancer had metastasized. The p-value was 4.06×10^{-11} for patients 4 and 1, 1.5×10^{-9} for patients 5 and 1, 1.73×10^{-7} for patients 6 and 1, 2.98×10^{-9} for patients 6 and 2, and 1.89×10^{-10} for patients 6 and 3. By analyzing multiple different datasets, a panel of genes which

predict causes for low survival such as metastasis or drug resistance can be identified. This research would contribute to refining personalized and precision medicine through the development of novel therapeutics which target the problems posed by the genes identified, and would improve the efficiency of ovarian cancer treatment.

Background

For 2017, it has been estimated by the American Cancer Society that around 22,440 women will receive a new diagnosis of ovarian cancer, and around 14,080 women will die from it. Unfortunately, ovarian cancer is difficult to diagnose due to its lack of symptoms until later stages, as well as the lack of an effective screening test. Even after it has been diagnosed, it is not certain that the cancer will respond to the chemotherapy drug prescribed. A potential solution to this problem is to incorporate precision medicine. Precision medicine is a type of medical care in which treatment is customized for group of people. Using precision medicine will ensure that the disease will effectively respond to the treatment which has been administered. This research seeks to investigate whether differentially expressed genes of carboplatin sensitive and resistant ovarian carcinomas can give an insight into potential genes and

factors which contribute to carboplatin resistance, and whether those genes can lead to improved outcomes for precision medicine. This was done using a dataset found in the NIH repository.

Ovarian cancer begins in the ovaries, which are the reproductive glands found in females. Cancer occurs when cell replication malfunctions, and cells do not undergo apoptosis as required. This causes the abnormal cells to continue division, eventually growing out of control and outnumbering the normal cells, causing tumors. The cancer cells can then spread to other parts of the body (known as metastasis). This is especially easy in ovarian cancer given the ovary's proximity to other organs. Due to a lack of symptoms in early stage ovarian cancer, it is normally only caught in its later stages. By that point, it may be too late for treatment. That is why it is critical for ovarian cancer to be treated as soon as possible, with medicine that will work.

Carboplatin is a chemotherapy drug used to treat ovarian cancer. It kills cancer cells by creating DNA lesions in the form of bulky adducts, which prevent polymerase from continuing DNA replication. This in turn signals to the cell's apoptosis mechanisms. Carboplatin resistance occurs when the apoptosis mechanisms don't work, which may happen due to factors such as mutations in the apoptosis pathways.

By studying gene expression levels, we can get an understanding of the malfunctioning biological mechanisms, as well as what leads to those malfunc-

tioning biological mechanisms, which can help medical providers give effective treatments. In addition, we can ensure that those treatments will work with people who have similar gene expression levels

Methods

Data Collection

Data was collected from <https://www.ncbi.nlm.nih.gov/>. In the drop down menu to the left of the search bar, GEO DataSets was selected. "Ovarian Cancer" was typed in the search bar, data set labeled Carboplatin sensitive and resistant ovarian carcinoma was located, and GEO Profiles was clicked. On the right side of the page, Download profile data was selected. The open button was selected and saved as "Carboplatin Data". An Excel spreadsheet was created (spreadsheet 1). In spreadsheet one, the tab labeled Data was selected. From Text/CSV was chosen, and the file Carboplatin Data was imported.

Data Analysis

Dropdown menu labeled Data Type Detection was Located and Based on entire Dataset was loaded. A second Excel spreadsheet was created (spreadsheet 2). A data table was made with categories for Gene ID, each of the three samples for every patient, average gene expression level, and standard deviation such as the one below.

Gene ID -- Patient -1 -1 -- Patient -1 -2 -- Patient -1 -3 -- Average -- Stdev

In spreadsheet 1, under column 21 (labeled Gene Symbol), all the genes were copied and pasted into

data table made in spreadsheet 2 under the Gene ID column. The gene data for every patient was moved to spreadsheet 2, starting with Patient -1-1 and continuing until Patient -6-3. The average gene expression levels for the three samples for patient one through six were calculated. This was done by clicking on the box under the Average column corresponding to samples that the average was being taken of. The Formulas tab at the top of the page was clicked, followed by Insert Function. Under box labeled Select a function, AVERAGE, then OK was selected. In the box labeled Number 1, the ID of the boxes which contain the data the average was being taken of (ex. F21:F23) was typed in, then the OK button was clicked. The standard deviation of the gene expression levels for each patient were calculated by following the steps described for calculating the average gene expression level, except with STDEV selected instead of AVERAGE. Once the average and standard deviation had been calculated for all 6 patients, a bar graph was created out of the average expression levels and the genes, in order to compare expression levels of specific genes between Carboplatin sensitive and resistant tumors. To do this, all data for a specific patient (Gene ID, expression levels from all three samples, and average) was selected. The tab labeled insert was selected, then the drop down menu labeled Insert Column or Bar graph next to Recommended Charts was selected. The first option available was clicked. The graph was right

clicked, then the Select Data option was chosen. The boxes next to the patient sample numbers were unchecked, leaving only the box next to average checked. The ok button was clicked, creating a bar graph for the data for all six patients. Error bars were added to the graph by clicking on the chart, then the tab labeled Design. Add Chart Element was chosen. The cursor was placed over Error Bars, and Standard Error was chosen. Error bars for all six bar graphs were created. The fold levels for the expression data for each gene were measured by dividing the average expression level for patient four (Carboplatin resistant) by average expression level for patients one, two and three (Carboplatin sensitive). This was repeated for patient five and patient six. All data was placed in new data table, such as the one below

Gene ID -- 4,1 -- 4,2 -- 4,3 -- 5,1 --
5,2 -- 5,3 -- 6,1 -- 6,2 -- 6,3

All data in new data table was selected, and Conditional Formatting was applied. The cursor was placed on Highlight Cells Rules, and Greater Than... was chosen. In the box that appeared, 2.0 was typed in under Format Cells that are "GREATER THAN:". Cells which had been highlighted were viewed, and genes or patients which had a high fold level were noted.

Statistical Analysis

A one way analysis of variance was performed in R to determine validity of the differentially expressed genes.

Further methods

Once genes of interest were chosen, genes were researched to determine function. Patients of interest were chose as well, and the paper the dataset was based on was referenced to determine abnormalities in patients with irregular gene expression levels.

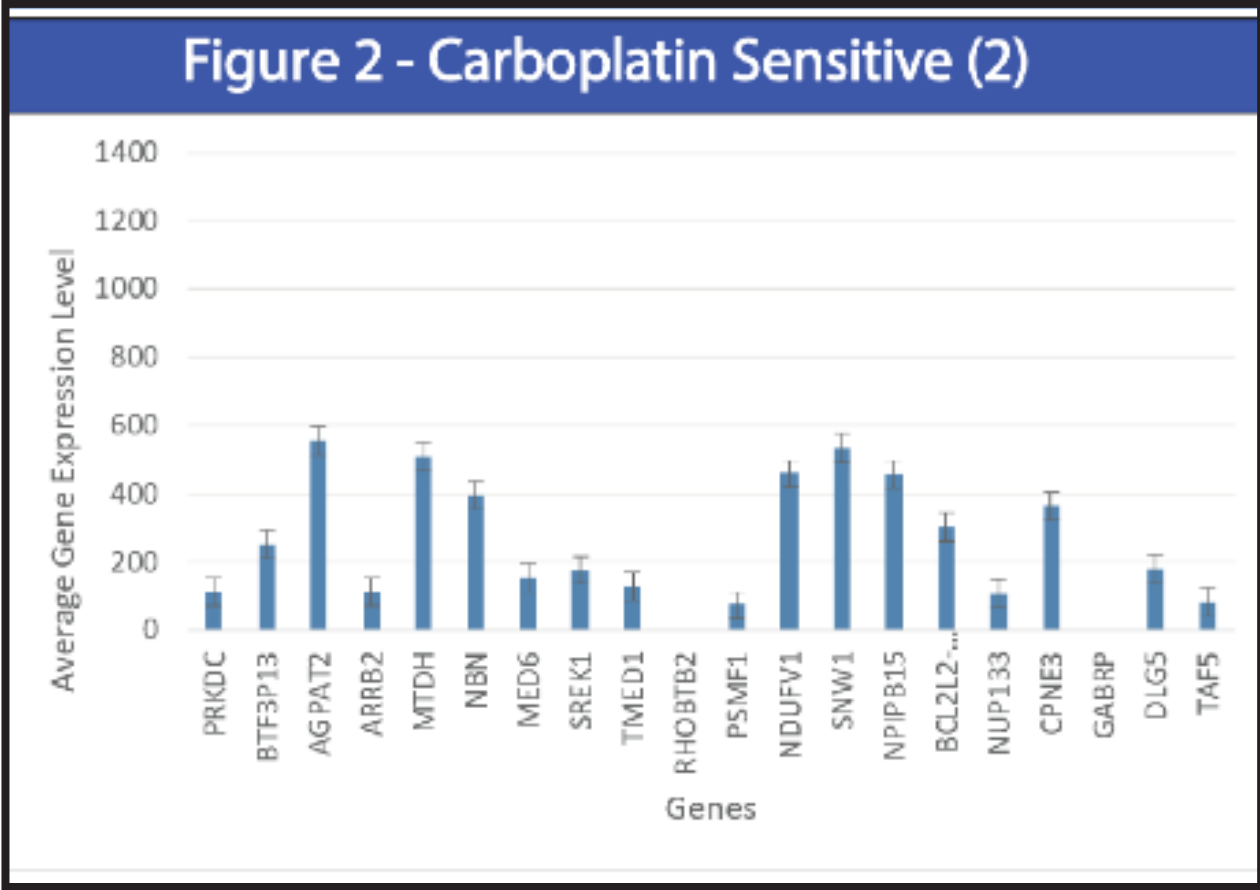
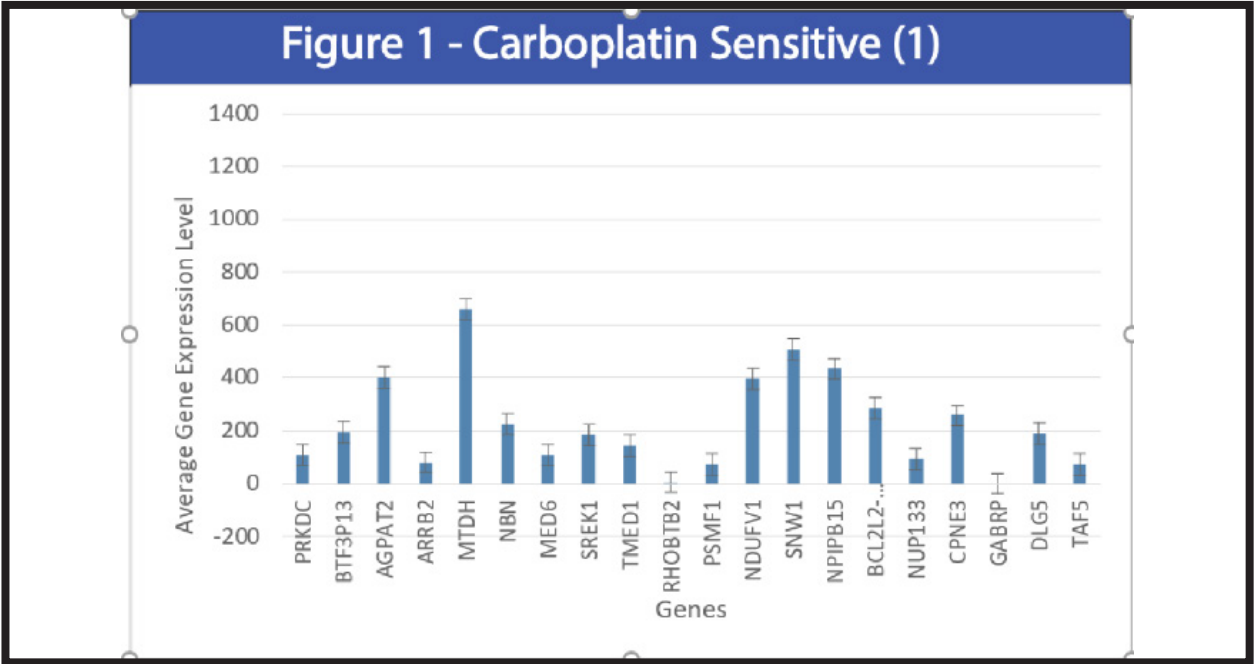


Figure 3 - Carboplatin Sensitive (3)

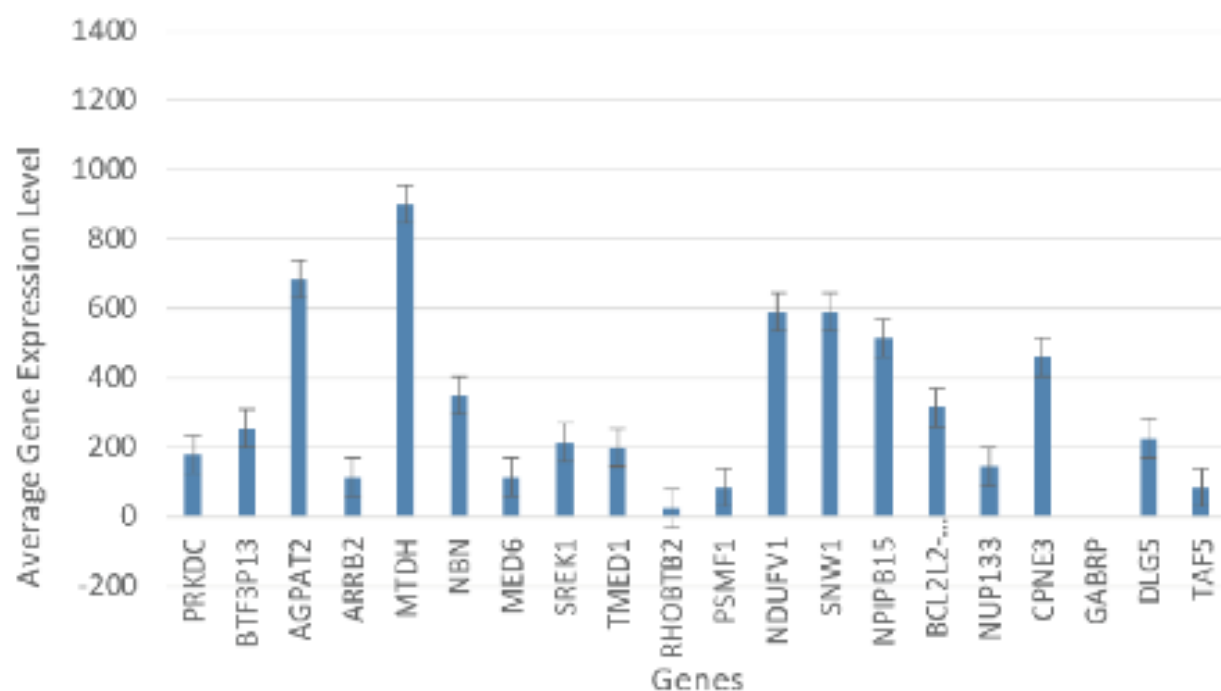


Figure 4 - Carboplatin Resistant (1)

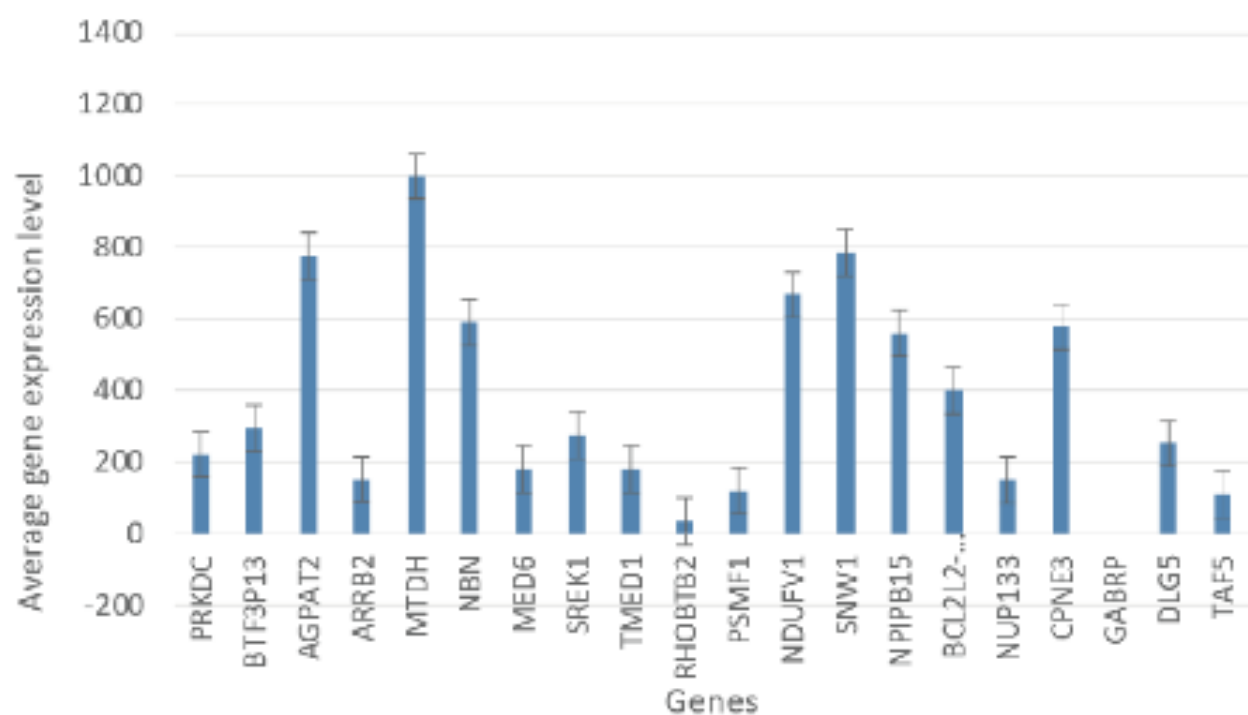


Figure 5 - Carboplatin Resistant (2)

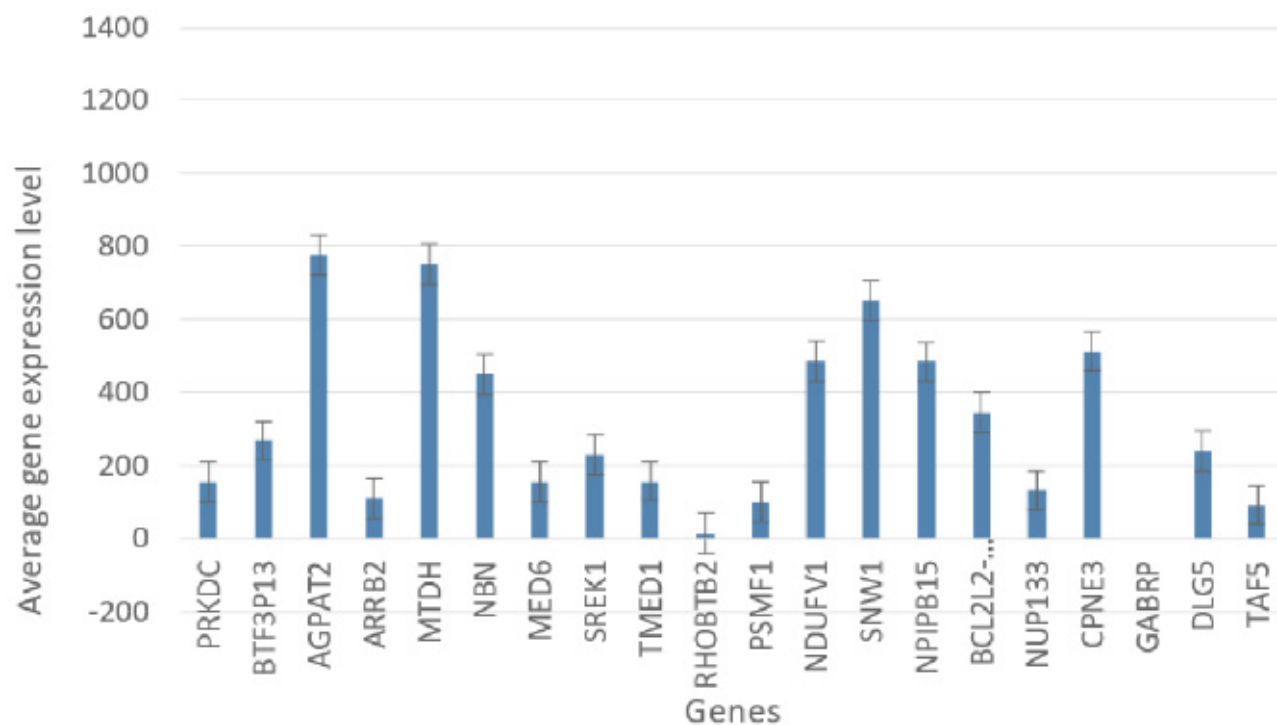


Figure 6 - Carboplatin Resistant (3)

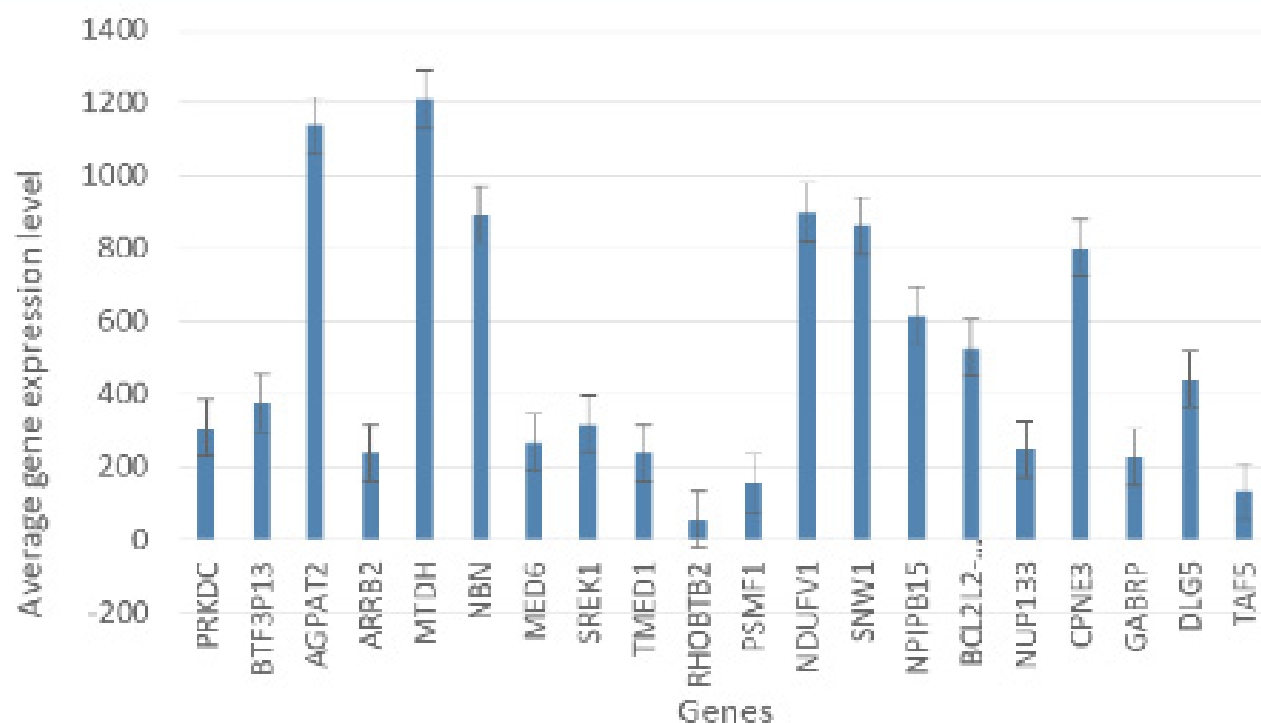


Table 2 - Gene Expression Fold Levels

Gene ID	4,1	4,2	4,3	5,1	5,2	5,3	6,1	6,2	6,3
PRKDC	0	1.921855	1.2401066	1.4305703	1.339144	0.864104	2.84211	2.660474	1.716712
BTF3P13	1.5190925	1.1683234	1.1549559	1.3748954	1.057422	1.045324	1.925628	1.480987	1.464042
AGPAT2	1.9323205	1.3911005	1.1281492	1.9351416	1.393131	1.129796	2.841735	2.045799	1.659094
ARRB2	1.8806085	1.3478682	1.3369339	1.35359	0.970144	0.962274	2.954601	2.117619	2.100441
MTDH	1.5160701	1.9565378	1.1062345	1.1393252	1.470336	0.831334	1.837055	2.37078	1.340449
NBN	2.6411914	1.4981843	1.6932072	2.0169892	1.144113	1.293046	3.997604	2.267593	2.562772
MED6	1.6779689	1.1588419	1.5538701	1.4338023	0.990215	1.327762	2.482232	1.714284	2.298652
SREK1	1.4818581	1.5368026	1.261651	1.2521634	1.298591	1.066089	1.712898	1.776409	1.458358
TMED1	1.2462097	1.3887771	0.8898859	1.0944766	1.219686	0.781537	1.650741	1.839588	1.178752
RHOBTB2	6.4725301	0	1.2880557	3.0131699	0	0.599631	11.06909	0	2.202786
PSMF1	1.6478957	1.5671151	1.3912667	1.3834562	1.315639	1.168009	2.157246	2.051497	1.821295
NDUFV1	1.690687	1.451788	1.1347047	1.2273054	1.053884	0.823706	2.279338	1.957261	1.529778
SNW1	1.5500914	1.4686701	1.3299864	1.2807425	1.213469	1.098884	1.703065	1.613608	1.461238
NPIP15	1.2868424	1.2269181	1.0875283	1.1125326	1.060725	0.940217	1.419693	1.353583	1.199803
BCL2L2-PA	1.4073068	1.3154737	1.2703943	1.2164533	1.137074	1.098108	1.856557	1.735409	1.675939
NUP133	1.5841991	1.3683371	1.0303611	1.4156839	1.222784	0.920759	2.620604	2.263522	1.704437
CPNE3	2.237967	1.5875568	1.2580406	1.9819639	1.405955	1.114132	3.104749	2.20243	1.745289
GABRP	0	0	0	0	0	0	0	0	0
DLG5	1.3320304	1.390227	1.110553	1.2632966	1.31849	1.053248	2.323726	2.42525	1.937359
TAF5	1.4639108	1.2863668	1.2547155	1.2376689	1.087564	1.060804	1.782056	1.565927	1.527397

P-values from One- Way Analysis Of Variance (ANOVA) of Gene Expression Level

	Patient 4	Patient 5	Patient 6
Patient 1	4.06e-11 ***	1.5e-09 ***	1.73e-07 ***
Patient 2			2.98e-09 ***
Patient 3			1.89e-10 ***
Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Based off the highlighted cells from the Gene Expression Fold Level table, it can be seen that the experimentally significant genes are CPNE3 (Copine III), NBN (Nibrim), and RHOBTB2. It was also determined that the patients who had statistically significant gene expression values ($p < 0.05$) were patients one and six. Upon further research, it was found that patients one and six had both experienced metastasis (to the liver and omentum, respectively). This shows that looking at whether a patient has metastasized, or looking at the overexpression of the genes CPNE3, NBN, and RHOBTB2 can be potential indicators of possible carboplatin resistance. The ANOVA was performed to ensure that the differing gene expression values for

patients one and six were indeed significant. With p values close to 0, it was determined that the gene expression values were statistically different enough to draw valid conclusions from them.

Discussion

The determination of experimentally significant genes and statistically significant patients in this dataset can be applied to other patients as well. The limitation of this experiment is that not much can be determined using the three significant genes which were found. However, if this experiment were to be repeated again with multiple datasets such as this one, they can be analyzed to determine a panel of many genes. The expression levels of those genes can be closely monitored in patients and can be used as markers for drug resistance or metastasis. Since platinum drugs are used to treat many other cancers apart from ovarian cancer, the information these genes can provide are not limited to just ovarian cancer.

The genes found through this study can be used in the development of novel medications for personalized and precision medicine. By developing therapeutics which specifically target the problems identified by the expression levels of particular genes (in this case drug resistance or metastasis), it will provide information on what drugs will not work for treating the patient, or whether or not the treatment currently being used will need to be changed.

Conclusion

Potential factors of carboplatin resistance are the overexpression of NBN, CPNE3, and RHOBTB2, as well as metastasis. Knowing this can help pave the way to more effective uses of personalized and precision medicine.

Acknowledgements

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Improving the Production of the Anti-Cancer Drug Paclitaxel Through Biosynthesis in Bacteria

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Author Note: Funding from the Fred Hutchinson Cancer Research Center in Seattle, Washington.

Abstract

Taxol is one of the most commonly used chemotherapy drugs to treat breast cancer, and by the y Improving the Production of the Anti-Cancer Drug Paclitaxel

Through Biosynthesis in Bacteria. Improving the Production of the Anti-Cancer Drug Paclitaxel

Through Biosynthesis in Bacteria. ear 2000, it had reached an annual sale of 2.1 billion dollars per year. Currently, it is produced either by extraction from the Pacific Yew tree, which is ecologically damaging, or through chemosynthetic procedures, which is economically ineffective. The average annual treatment for a patient without insurance in the United States is \$80,000, while the average annual salary is \$44,000. We decided to biosynthetically create enzymes that are crucial for the production of Taxol in hopes to eventually create a cheaper way to produce the drug, or to understand the enzymes well enough to manipulate them and produce a different drug. The goal was to be able to produce these certain enzymes in e-coli. The bacteria did in the end produce these enzymes. In the two trials in which the expression of the gene were tested, both times the gene was

expressed and the protein was more than 98% pure.

Introduction

The number of new cancer deaths are anticipated to increase to 22 million in the span of the next two decades. Taxol is one of the leading chemotherapy medications. An annual dose of Taxol with a biweekly regiment costs about 80,000 USD. The average annual salary for a US worker is 44,000 USD. This drug is unaffordable, and its source is continuously depleting. Paclitaxel is derived from the Pacific Yew Tree, a species only found in the Northwest. The derivation of the drug is ineffective and inefficient, so we pursued biosynthetic production of it.

Taxol has been found in many different types of the yew trees over the years, but it is most commonly extracted from the Pacific Yew Tree found in Northwest America. Typically, Yew trees grow very slowly, they grow around 6 to 45 feet tall, and must be over 100 years old in order to harvest the right amount of paclitaxel. To yield Taxol, you must clear-cut Yew trees large enough or else you may not get anything at all. The problem with this is that the species of tree was already an endangered species before paclitaxel was approved by FDA in 1992. Now that the demand for Taxol has increased, more trees have been used and cut down for this process.

This is a huge problem for our environment; certain animal's habitats are taken over, and in the end this entire process disrupts the whole biosphere.

It takes roughly 6 100-year-old Pacific Yew trees to treat one patient, meaning that the amount of taxol now available is enough to treat fewer than 1,000 patients per year and this only decreases as we continue the treatment. People have tried replanting the trees after the extractions, but since it must grow for 100 years and the demand for Taxol is only increasing, it isn't an efficient source.

Background of Paclitaxel

Paclitaxel is an anti-cancer chemotherapy drug, a taxane that functions as a "antimicrotubular agent. It is also called by the brand name "Taxol ©" and "Onxal ©". Paclitaxel is used for the treatment of breast, ovarian, lung, bladder, prostate, melanoma, esophageal, as well as other types of solid tumor cancers. It has been known to be one of the top anti-cancer drugs in the United States since 1992.

Cancer is abnormally fast-growing cells, or the stimulation of the process of cell division. During the process of mitosis in normal cell division, there are certain structures that are utilized in maintaining the overall shape of the cell, which are broken down in order for the insides to replicate. Taxol is the drug that stabilizes the microtubules, or the structures that maintain the shape, and keep it from breaking down which prevents the accurate duplication of the cell. Therefore, it kills tumors before they have a chance

to metastasize.

Taxol belongs to a family of drugs called plant alkaloids. Taxanes, a type of plant alkaloid, are derived from the bark of the Pacific Yew tree. It is derived through the processes of chemosynthesis and manual extraction today.

Taxol was originally discovered in the 1960s by Arthur Barclay, who worked as a botanist for the US Department of Agriculture. During the increase of the spread of cancer, the demand for a cure became increasingly prevalent. Arthur Barclay worked under contract to the US National Cancer Institute in order to screen many plants for a potential cure for cancer. In the process, he discovered the Pacific Yew tree from which Taxol is derived. Taxol was selected for commercial development in 1977 and was first tested in patients in 1984.

Background on Chemosynthesis

The chemosynthetic production of taxol is taking individual substrates and functional groups and binding them in order to create the final product of paclitaxel. This process occurs through the additions of enzymes, which create the bonds necessary to attach certain functional groups to the base substrate. In paclitaxel, the base substrate is the compound geranyl-geranyl diphosphate.

Biosynthesis

The idea was to create a more efficient biosynthetic pathway to carry out the processes necessary by the chemosynthetic pathway or manual extraction

and produce the final product of paclitaxel. Biosynthesis is a multi-step process of taking individual parts, or substrates and using enzymes and cofactors in order to create larger products in living organisms. The conversion of the production of paclitaxel from a chemosynthetic perspective to a biosynthetic would greatly increase the yield of the drug, as the cofactors would all be produced in the living organism.

Reasoning behind the choice of DBTNBT and BAPT

BAPT and DBTNBT are the proteins we picked from the biosynthetic pathway. We looked at a few other proteins from the pathway, but we saw that DBTNBT and BAPT were more unique since they have not been researched very much. Both enzymes are in a part of the pathway where they join together two separate parts and therefore are ideal places to study because these would be places to alter the enzymes to get a different final chemotherapy drug.

Hypothesis

If we produce our proteins DBTNBT and BAPT in *E. coli*, then the overall production cost for Taxol will decrease because the most expensive step of the biosynthetic pathway is to stimulate the production of the certain enzymes that are responsible for creating the final product.

Question

Can we make an *e-coli* bacterium produce an enzyme that is used in the Taxol biosynthetic pathway

in order to make it more efficient and less expensive?

Final Vision

Being able to produce large quantities of Taxol through the biosynthetic platform. Producing all of the enzymes required for additions on the base protein, geranyl-geranyl diphosphate in *e-coli* is the first step towards producing the chemotherapy drug biosynthetically. The enzymes that we chose have not been worked with before and are crucial final steps in the process.

Background Research on Cancer

Cancer

Cancer is the uncontrolled amount of cell growth of abnormal cells in the body. Normal cells go through the process of mitosis, and split into many different cells that have specific bodily functions. New cells are made to also replace the older and damaged ones that way there is a constant number of cells in the body. This process is called apoptosis.

Cancer occurs when the normal process of replacing cells stops working, old cells don't die, and new cells are constantly being formed into abnormal cells. The extra cells build up inside the body, and form extra tissue creating tumors. When the cancer spreads, they call this "advanced cancer". The cells keep growing consistently, and form in different parts, in the end destroying the entire system. This process is also known as metastasis.

The most common areas in the body where cancer

touches are the brain, liver, lungs, breast and lymph nodes. If the cancer is not caught in an early stage, it can only get worse over time, and in the end corrupts the entire body.

Microtubules and their job in cell division

Microtubules function through their position as a part of the “mitotic spindle”. The mitotic spindle is a macromolecular structure that is used in the process of mitosis to pull away the sister chromosomes to form daughter cells with identical DNA as the parent cell. The function of the mitotic spindle is to properly align the chromosomes at the mitotic plate and then pull them apart into two daughter cells. This is only possible if the tension of the microtubules is correct, as if it is not, the chromosomes will not properly align at the plate. Taxol interferes with the tubulin and causes the tension in the microtubules to differ and therefore remain in the G₁ phase too long, which activates a checkpoint that leads straight to apoptosis.

Procedure

1. Sequencing and codon optimization on both protein sequences of BAPT and DBTNBT
 - Use NCBI in order to get the protein sequencing from the optimal tree
 - Transfer the protein sequence into nucleotides utilizing BLAST
 - Codon optimize for e-coli using Idt (integrated DNA technology)

- o Codon optimization is the process of changing the final base(or wobbly base) in order to create the most ideal production of the enzymes in the K-12 strain of e-coli.

2. Ran a PCR on the DNA
 - First PCR reaction: Denature, Anneal, Extend
 - o Denature: Process of splitting the double stranded helix of DNA into 2 single stranded at 95 degrees
 - o Anneal: Add primer (polymerase) from 5' to 3' on either side. Add the his tag when in the process, so it can be determined on which side to attach the DNA to the plasmid. Done at 55 to 64 degrees Celsius.
 - o Extend: Since the process of PCR is done in order to amplify DNA, the polymerase duplicates each respective nucleotide to increase the total amount of DNA available. Done at 60 degrees Celsius.
 - o Maintain reaction at 4 degrees Celsius for the rest of the reaction
3. Ran a gel electrophoresis on the PCR DNA
 - Create the gel through agarose buffer microwaved
 - Dye DNA with buffer so the strains will show up when the gel is run
 - Add TAE on top of the gel box
 - Load ladder on each side of the gel
 - Load each of the samples

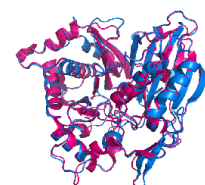
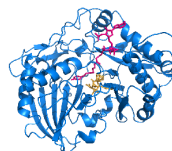
- Set voltage at 80A and run for 30 minutes
- 4. DNA gel extraction
 - Take previously run gel into the microscopy room
 - Turn on blue light and wear orange goggles to contrast blue light
 - Use a razor blade to cut out each band of DNA and place in its respective tube
 - Take a picture of the remaining gel using the x-ray machine
 - Weigh the samples and mark on the top of the tube in grams
 - Use the ZymoClean procedure to extract the DNA from the gel
- 5. PCR #2
 - Same three steps: Denature, Anneal, Extend
- 6. Digest
 - DNA in tubes
 - o Add alkaline phosphate into the tubes
 - o ½ microliters of each of the primers
 - Put in 30-degree room for 30 minutes
 - Gibson reaction
- 1. Assembles DNA in a correct fashion, using three enzymes: exonuclease generates long overhangs, polymerase fills in the gaps of the annealed single strand regions, and DNA ligase seals the nicks of the annealed and filled-in gaps
- 2. Use the machine for 30 minutes
- 7. Transformation
 - Incubate and plate on agar plates with LB

broth, ampicillin and carbenicillin and let it sit and grow

- 8. Sequence Verification
 - Send to Fred Hutch gene verification
- 9. Sonication and IPTG
 - Put in the sonicator with lysis buffer in order to lyse the cells open
 - Use IPTG, which is a molecular reagent that is a molecular mimic of allolactose, a lactose metabolite that triggers transcription of the lac operon, which causes the activation of the polymerase going across the DNA.
- 10. Protein Gel Electrophoresis
 - Prep by running through column and adding loading buffer, load all washes and elutions
 - Run at 160 volts for 20 minutes
 - Stain with coomassie blue and destain with the destaining buffer.

Conclusion

The question that was investigated was whether crucial enzymes in the biosynthetic pathway could be produced in e-coli. It was hypothesized that they could be produced, and when the experiments were performed, it was concluded that the results matched the hypothesis. The proteins were shown to be more than 98% pure, and showed up both times that we ran our gels.



<ul style="list-style-type: none"> - 1, 2, 20, 50, 200, 1000 microliter pipettes - Microcentrifuge - 1.5 microliter microcentrifuge tubes - Waste bins - Pipette tips (different for each volume) - Deionized water - TAE buffer - 2% agarose gels (for gel electrophoresis) - <u>Accuprime</u> reaction mix - Primer mix - Template DNA - <u>AccuPrime</u> DNA polymerase - Autoclaved water - Spin Columns 	<ul style="list-style-type: none"> - Water bath - Tube heater - Warm room - Cold room - X-ray camera for imaging of gels - Razor blades - Microscopy room - Orange goggles to counter blue light - <u>ZymoClean</u> DNA recovery kit - Microwave - Freezer - Bacteria plates - Cell spreaders - <u>Lazy susan</u> for spreading cells on plates - Measuring scale - PCR tubes
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Future Research Plans

Produce all of the enzymes in the bacteria, and eventually use e-coli as a tiny factory for the production of Taxol, therefore making it more efficient and cheaper to buy.

Take the protein produced by the e-coli and use the purified protein to take a crystal structure. Manipulate the structure of the protein in order to change the final structure of Taxol, and maybe create a more effective chemotherapy drug.

Problems with Research

1. Taxol immunity has been shown to have been developed in cells over time, which could cause for the drug to be ineffective.
2. One of our proteins ended up being insoluble, and this is a problem if we want to crystallize and take a crystal structure in the future.

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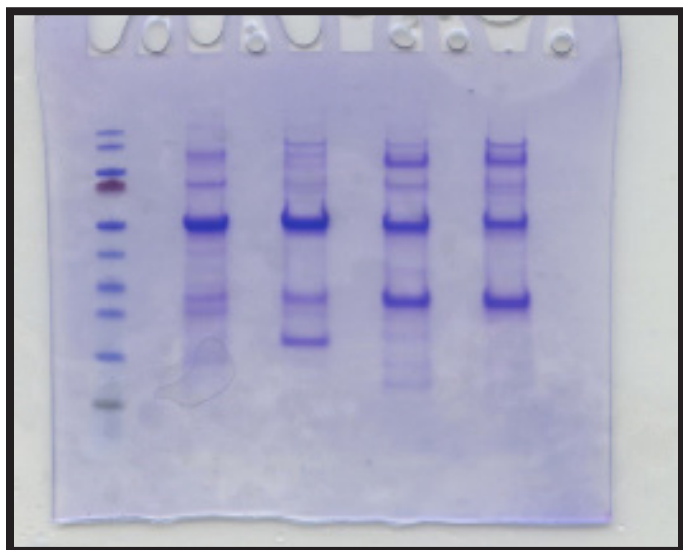


Figure 1

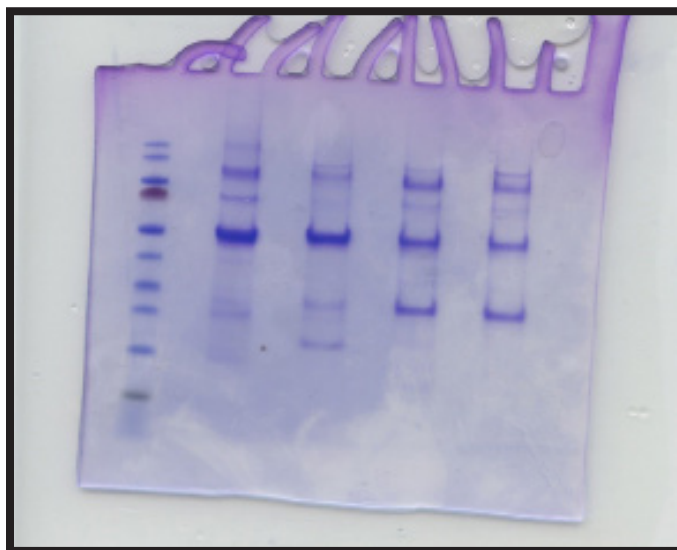


Figure 2

Figures 1 and 2. These are our two gels from our Protein Gel Electrophoresis, and display all four proteins, C-His DBTNBT, N-His DBTNBT, C-His BAPT, N-His BAPT

Infectious Disease Research - Hepatitis

Aivy Tran

I. INTRODUCTION

Although many viruses can cause hepatitis, hepatitis as the main manifestation of viral infection in humans is caused by 5 specific virus species: hepatitis A, B, C, D, and E. These viruses each cause acute hepatitis. However, only the hepatitis B and C viruses cause chronic infection. Chronic infection increases the risk of a person developing cirrhosis, liver failure or liver cancer. The following research paper will specifically focus on the hepatitis B.

II. CAUSE AND EPIDEMIOLOGY

HBV which is derived from the Hepadnaviridae family and the Orthohepadnavirus genus. The hepatitis B virus is a small, circular, and partially double-stranded enveloped DNA genome. The hepatocytes are the primary site for replication of HBV and humans are the only known supply for human HBV genotypes. Therefore, a mode of transport of hepatitis is from person to person. Although, HBV genotypes do exist in some primates. The pathogen HBV is relatively stable in heat as it remains infectious for at least one week in the environment. Thus, the pathogen is highly infectious. The hepatitis B virus contains an outer envelope and an inner core. The outer envelope of the virus is composed of a surface protein called the hepatitis B surface antigen (HBsAg). HBsAg can be detected by a simple blood

test and a positive test result indicates a person is infected with the hepatitis B virus. The inner core of the virus is a protein shell referred to as the hepatitis B core antigen (HBcAg). This antigen contains the hepatitis B virus DNA and enzymes used in viral replication. The port entry of the hepatitis B virus enters the host liver cell and is transported into the nucleus of the liver cell. Then, the viral DNA is transformed into a covalently closed circular DNA (cccDNA), which serves as a template for viral replication. This creation of a new HBV is packaged and leaves the liver cell, with the stable cccDNA remaining in the nucleus where it can integrate into the DNA of the host liver cell.

A. Risk Factors

HBV is transmitted through exposure to blood and bodily fluids. As a connection to other disorders, HBV is the primary cause of liver cancer, which is the second leading cause of cancer deaths. Autoimmune hepatitis is liver inflammations that occurs when the body's immune system turns against liver cells. It is a chronic disease in which the body's immune system attacks the normal liver cells and causes inflammation and liver damage. Untreated autoimmune hepatitis can lead to scarring of the liver (cirrhosis) and eventually to liver failure. When diagnosed early, autoimmune hepatitis can be con-

trolled by drugs to suppress the immune system. A myriad of environmental triggers, autoimmunity, and genetic predisposition can lead to autoimmune hepatitis. However, about 70 percent of people with type I autoimmune hepatitis are female. HBV can occur from direct contact with infected blood, unprotected sex, use of illegal drugs, and/or contaminated needles. The hepatitis B virus can infect infants, children, teens, and adults. It is not a genetic disease it is an infectious disease that is transmitted through blood. Although everyone may be at risk for a hepatitis B infection during their lifetime, there are groups of people who are at higher risk because of where they were born, their occupation or life choices. The following are high risk groups susceptible for hepatitis B: individuals born in countries where hepatitis B is common (Asia, Africa, South America, Pacific Islands, Eastern Europe, and the Middle East), health care providers/emergency responders, men who have sex with men, and illicit drug users. There is an increased genetic predisposition when infants born to mothers who are positive for the core antigens of hepatitis B (both HBsAg and HBeAg). These infants with both antigens are at a higher risk of acquiring the infection than those born to HBsAg-positive mothers who have lost the HBeAg antigen. Infants born to mothers with HBV infection and a high viral replication are at the highest risk for perinatal transmission of hepatitis. However, C-sections reduce perinatal transmission from HB-

sAg-positive women to the infants.

B. Symptoms

Hepatitis B is referred to as the "silent infection" because most people do not have any symptoms when they are first infected. Testing is the only way to know if you are truly infected because most healthy adults do not experience any symptoms when they are first infected with HBV. The common symptoms of the hepatitis B infection are fever, fatigue, loss of appetite, mild nausea/vomiting, abdominal pain, light-colored stools, and dark-tea colored urine. Due to these symptoms, many will ignore the symptoms and attribute it to the flu.

Although, 1 percent of those infected will develop fulminant hepatitis which can be fatal and result in liver failure and death. These serious symptoms include severe nausea and vomiting, jaundice (yellow skin and eyes), and a swollen stomach. The stages of acute hepatitis B include an incubation period for 75 days on average. However, it may vary from 30 to 180 days. The liver injury during infection is mediated by the liver host cell's immune response. Initial infection may show no symptoms, but acute inflammation may occur. The rate of chronic HBV infection is related to the age of the infection, occurring in 80 to 90 percent of infants infected through vaginal delivery. Hepatitis differs from other diseases because while most diseases have only one pathogen, hepatitis has various types of pathogens. These are ranging from A, B, C, D, and E and each

have a slightly different mode of transmission and prevention plan. For example, hepatitis A virus is transmitted through contaminated food and water and proper sanitation is the most effective method of prevention. Other types of pathogens for hepatitis like HCV are transmitted through infected blood, unprotected sex, and contaminated needles, but there is no vaccine as a preventative in the same way the hepatitis B virus has a vaccine. What makes hepatitis infectious disease unique is each hepatitis virus is different, but they all share a target - the liver.

III. DIAGNOSIS

Clinically, it is impossible to differentiate hepatitis B infection from hepatitis caused by other viral agents. Hence, confirmation of the diagnosis is essential to treatment. Access to affordable hepatitis testing is limited and approximately only 9 percent of HBV infections have been diagnosed serologically. This means 91 percent of HBV patients have not been diagnosed through bodily fluids like blood and saliva. The guidelines for HBV testing is recommended as the use of a single serological in vitro diagnostic test (IVD). The testing is like the influenza detection test, but the antibody being detected is different, the HBsAg antibody is being tested here. Either a laboratory-based immunoassay, a chemiluminescence immunoassay or a rapid diagnostic test (RDT) will be used. For the influenza RDT, the likelihood of a false positive was exponentially high. However, hepatitis B RDTs vary considerably

amongst brands and have displayed low sensitivity in HIV-infected patients. The pooled sensitivity of RDTs amongst HIV-positive patients was merely 72.3 percent.

The physical appearances of the hepatitis B virus diagnosis are a blood test that can be done at your local doctor's office or health clinic. Only one sample of blood is needed for the test but the "Hepatitis B Panel" includes 3 parts. All three tests are required to diagnose an infection. The primary panel tests the hepatitis B surface antigen (HBsAg). This test can detect the actual presence of the HBV in your blood. If a person tests "positive", then the second panel will be tested to determine if this is an acute or chronic HBV infection. A positive test result may mean you are infected and can spread the HBV virus to others. The second-panel tests if you have antibodies for HBV, a positive test result means you are "immune" and protected against HBV and cannot be infected. The third panel is to test the hepatitis B core antibody. A "positive" HBcAb indicates a past or current HBV infection. If the final panel is positive, the test requires you to talk to your healthcare provider for a complete explanation of your hepatitis B status.

A. Abbreviations and Acronyms

Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV), and Hepatitis E virus (HEV). Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody

(anti-HBs or HBsAb), and Hepatitis B core antibody (anti-HBc or HBcAb)

B. PROGNOSIS

The mortality rate of hepatitis C is much more severe than that of HBV or any of the other virus types of hepatitis. According to CBS News, “Approximately 3.5 million Americans are currently living with hepatitis C and about half are unaware of their infection”. In 2013, over half of hepatitis C-related deaths occurred in people ages 55 to 64. In hepatitis B, an acute infection only stays in the system for around one to three months. However, 1 in 20 affected adults will have a chronic infection that stays in the body for 6 months or more. According to the News of Medical Life Sciences, “90 percent of infants who are infected as newborns go on to develop chronic infection”. About 20 percent of people with a long-term infection will develop liver disease and will have scarring/cirrhosis of the liver. About 10 percent of the initial 20 will develop liver cancer.

Those living with chronic hepatitis B infection may or may not require drug treatment. Although, there are many preventative measures to protect the liver to improve their health. Patients should check with their pharmacist to avoid over-the-counter drugs like acetaminophen or non-hepatitis B prescription drugs before taking them because many of these drugs are processed through the liver. Those with hepatitis B should get the hepatitis A vaccine to protect themselves from another virus attacking the

liver.

IV. TREATMENT

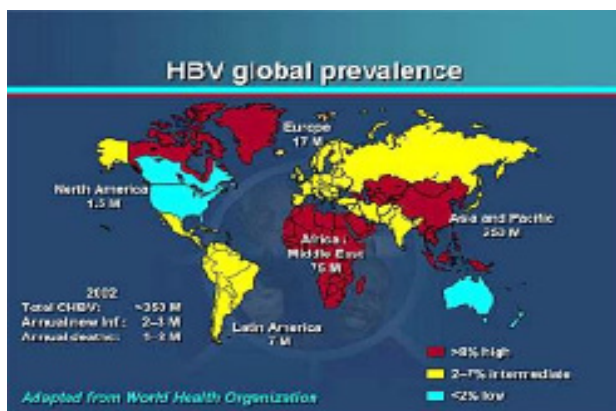
There is no specific treatment for acute hepatitis B disease. Clinical management is based on supportive therapy and symptom relief. This includes providing adequate nutrition and replacement of fluid loss through vomiting and elimination of fecal matter. The purpose of antiviral therapy for chronic HBV is to reduce the rate of mortality due to progressive liver disease. Antiviral drugs with a high barrier to resistance (either tenofovir or entecavir) were recommended as the preferred first-line treatment to avoid the deleterious effects of drug resistance. Treatment of chronic HBV infection has been shown to prevent or delay the progression to cirrhosis, reduce the incidence of hepatocellular carcinoma (HCC), and improve survival through long-term viral suppression, but is not considered a cure. Evidence shows that antiviral treatment in the third trimester of pregnancy can lower viral load in women, and decrease perinatal HBV transmission from mothers with a very high viral load. This method is not ideal and it seems that prevention is key.

A. Prevalence

Hepatitis B is a global public health threat and the world’s most common serious liver infection. It is up to 100 times more infectious than the HIV/AIDS virus. HBV is also the primary cause of liver cancer, which is the 2nd leading cause of cancer deaths in the world. One million people die each year from

hepatitis B and related diseases. The rise of HBV is up to 40,000 Americans will become newly infected each year. In 2014, there were an estimated 19,200 new hepatitis B virus infection in the United States. However, the official number of reported Hepatitis B cases is much lower. Acute Hepatitis B in the United States has declined by approximately 82 percent since 1991. At that time, routine Hepatitis B vaccination of children was implemented and has dramatically decreased the rates of the disease in the United States, particularly among children. The geographic distribution of hepatitis indicates that the transmission risk is 70-100 percent in Asia and 40 percent in Africa. The reason for the spread was Individuals born to parents who have emigrated from countries where hepatitis B is common. Additionally, HBV spread because Children adopted from countries where hepatitis B is common entered in America and other first world countries. Lastly, adoptive families of children from countries where hepatitis B is common spread the disease. The map that denotes the prevalence will be in the figures and tables section below.

B. Figures for Prevalence



On a pandemic scale, the pandemic scale index was developed by the Centers for Disease Control and Prevention (CDC) as a new pandemic influenza planning tool for use by states, communities, businesses and schools, as part of a drive to provide more specific community-level prevention measures.

The case fatality ratio of hepatitis has stayed within the 3 to 4 range on a 5-range scale because 450,000 to 1,800,000 people have been affected.

V. FREQUENCY

Safe and effective vaccines against hepatitis B have been available since 1982. The active substance in the hepatitis B vaccine is the viral surface protein HBsAg. The primary 3-dose hepatitis B vaccination series is conventionally recommended for infants plasma-derived hepatitis B vaccine of 1,578 uninfected persons aged 6 months to 50 years prevented all clinically apparent and chronic HBV infection for at least 30 years. The Global Advisory Committee on Vaccine Safety has confirmed the excellent safety profile of hepatitis B vaccine.

Figure 1.: This is a global map of the HBV global prevalence. It includes the annual infection rate as well as the annual death rate. Currently, the average death rate is at 1 to 2 million and the death rate is so saturated because of its presence in 3rd world countries.

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Viability of Urinary Polyamines as a Preliminary Test for Cancer

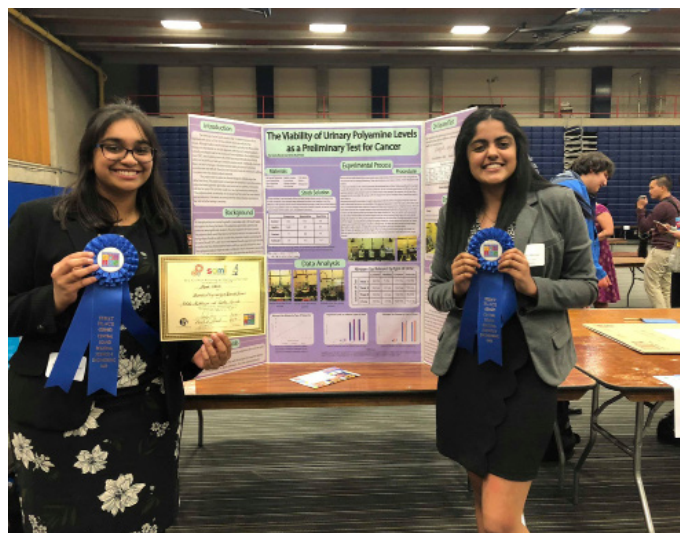
Ishika Mukherjee and Eesha Murali

Author Note

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Abstract

Currently, cancer survivors must go through invasive and painful tests in order to confirm that they are still in remission. This project sought to explore an alternate diagnosis method using urine polyamine levels rather than blood. To make the urine samples, synthetic urine was modified by adding respective amounts of different polyamines to represent samples from healthy subjects, as well as patients with ovarian (OCa) and testicular (TCa) cancer. The urine was then mixed with nitrous acid and the volume of the product nitrogen gas product was measured. The OCa sample released 12.87 mL more nitrogen than the healthy sample, and the TCa sample released 12.53 mL more than the healthy. This suggests that this test can visibly differentiate between urine samples from healthy/noncancerous subjects and urine from OCa and TCa patients. With further confirmation through experimentation using actual patient urine samples, this test offers a replacement for modern day complete blood count panels and



can eliminate the need for painful blood draws and inconvenient trips to the lab for low-risk cancer survivors.

Keywords: nitrous acid, spermidine, spermine, putrescine, polyamine

Viability of Urinary Polyamines as a Preliminary Test for Cancer

The American Cancer Society projects that over 1.7 million Americans will be diagnosed with cancer in 2018. Of these, 609,640 Americans will die of the disease. Although modern cancer treatments are often successful, the efficacy of the therapy critically depends on an early diagnosis, both in new and in relapsing patients. Currently, oncologists look at immunoglobulins, the complete blood count (CBC), and circulating tumor cells to diagnose cancer. All of these require the collection of blood, which can be painful and can take up to a week to return results.

Additionally, the repetitive blood draws can lead to damage in the blood vessels, making the process increasingly uncomfortable and difficult. These tests are inconvenient and add unnecessary suffering to a patient who has already endured treatment.

In 1971, Dr. Diane Russell first showed that polyamines were a viable way of testing for cancer, which supported the theory that levels of three polyamines (spermidine, spermine, and putrescine) were significantly elevated in the urine of patients with various types of cancers. Polyamine levels dropped to normal post-remission and again would rise during relapse. Russell concluded that polyamines could be a promising test for cancer (Russell, Levy, Schimpff, & Hawk, 1971). All three of these polyamines are essential to growth in mammalian cells. They interact with negatively charged biomolecules and are involved in various cellular processes, including cell replication and tumorigenesis (Mandal, Mandal, Johansson, Orjalo, & Park, 2012). Later, labs in Israel (Horn, Beal, Walach, Lubich, Spiegel, & Marton, 1982) and Tokyo (Sato, Kase, Tajima, Sawamura, & Matsushima, 2001) repeated Russell's experiment and confirmed that these three polyamine levels could be an accurate indicator for cancer. These polyamines play an important role in cell division, which is increased in cancer cells relative to healthy tissues.

Putrescine, spermine, and spermidine are all either primary or secondary aliphatic amines. A chemical

reaction with nitrous acid can be used to indicate the type of amine present in solution. By using this reaction in urine samples, the product (nitrogen gas for primary amines, insoluble nitrosamine oil for secondary amines) can be measured to calculate the concentration of polyamine present in the sample. These levels are elevated in various types of cancers (Russell et al., 1971). In this proof of principle project, we used simulated urine samples that are made to imitate aliphatic polyamine levels in ovarian and testicular cancer as well as a healthy urine sample.

This project seeks to explore an alternate diagnosis method using three urinary polyamine levels (spermine, spermidine, and putrescine) as a painless, noninvasive indicator for cancer. As an alternative to blood draws, this urine test would serve as a high-sensitivity screening test. If polyamine levels are elevated, an oncologist can perform more invasive and accurate follow-up tests for a final diagnosis. If levels are normal, then the chance of cancer recurrence is low, and no further testing is necessary. We hypothesized that the TCa (testicular cancer) and OCa (ovarian cancer) samples would have higher nitrogen release than the healthy samples.

Methods and Materials

Synthesis of Artificial Urine Samples

Obtaining true clinical samples of human urine proved to be difficult due to high school student status. To work around this, urine samples were synthesized in order to test whether or not the dif-

ference in the amount of reaction product would be large enough to be an accurate indication of cancer.

First, synthetic urine was obtained from Carolina Scientific which contained an accurate representation of the sodium, ions, and proteins in real urine. To this, putrescine, spermidine, and spermine were added to simulate different levels of polyamines with different cancers and healthy patients following the levels discovered in Russell et al.'s and Sato et al.'s studies. The stock solutions were made with the ratios shown in Table 1.

1.0M sulfuric acid, 1.0M NaNO₂ solution, buret clamps, Erlenmeyer flasks, stir pills, and stir plates were also obtained for this experiment.

Because of the health hazard, the urine samples were made under a fume hood with masks and gloves to ensure there was no chance of inhalation. These stock solutions were stored in closed bottles at 40 C until needed.

To make each sample, 20 mL of each polyamine was measured into a 250mL Erlenmeyer flask. For example, to make the synthetic OCa sample, 20 mL of ovarian putrescine, 20 mL of ovarian spermi-

dine, and 20 mL of ovarian spermine solutions were mixed to make one whole OCa urine sample. The magnetic stir pill was then dropped into the urine flask. A syringe with a stopper was kept on standby where it could later be easily reached. A diagram of the setup is as shown in Figure 2a and 2b.

Setting Up Nitrous Acid Test (NAT)

Because nitrous acid is highly unstable, it needed to be prepared directly before integration into the urine and under a fume hood to prevent any inhalation of the gaseous byproduct. To form the acid, 1.0 M sulfuric acid and 1.0 M sodium nitrite were incubated at -200C until they reached a temperature of 00C. 30 mL of each chemical were then measured into separate beakers using separate graduated cylinders; all precautions were taken to make sure the chemicals did not mix before they were intended to. Then, the sulfuric acid was poured into the sodium nitrite beaker and swirled slightly until the solution turned a faint blue color and started fizzing. This solution was then poured quickly but carefully into the urine flask. The flask was quickly closed with the stopper, ensuring that the syringe was tightly screwed on. The lip where the flask met the rub-

Table 1
Ratios of Polyamines in Simulated Synthetic Urine

	Putrescine	Spermidine	Spermine
Control	0	0	0
Healthy	0.05	0.1	0.1
Testicular	1.3	0.2	0.5
Ovarian	1.1	0.9	1.3

Note. All measurements are in mg / 50mL. For convenience, the Healthy Putrescine levels were doubled (0.1 mg dissolved in 100mL) rather than having to measure out 0.05 mg. These levels were calculated using numbers primarily from Russell et al. (1971).

ber stopper was covered in duct tape to result in an airtight seal. This flask was then placed inside of a large beaker filled with ice and on top of a stir plate, the syringe held in place by a Bunsen clamp. The setup is shown in Figure 1a and 1b.

After 72 hours, the samples were checked again. The mL of displacement in the syringe was noted as shown in Figure 1c. The flask was opened under the fume hood and excess chemical dumped into a waste receptacle for proper disposal.



Figure 1 a



Figure 1 b

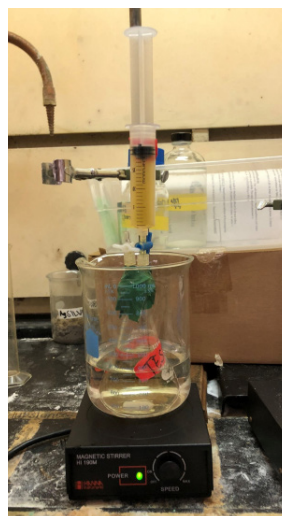


Figure 1 c

Figure 1. Setup and labeling of Erlenmeyer flasks (A), overall experimental setup showing how syringes were connected to flask and Bunsen clamps (B), diagram showing what is being measured (C).

Results

After running three trials of each urine sample and three trials of a control sample (no polyamines present and measured solely the gas released by the decaying nitrous acid), the data showed that the OCa and TCa samples released much more nitrogen gas than did the healthy sample. Statistical analysis using chi square test showed that the levels measured in both OCa and TCa samples were significantly higher than the level in healthy sample (Fig 2). Also, because the control trials did not present with any displacement in the syringe, it could be concluded that no gas from the decomposing acid itself contributed to the gas measured and all gas was produced in a reaction of the nitrous acid and polyamines (Fig 2).

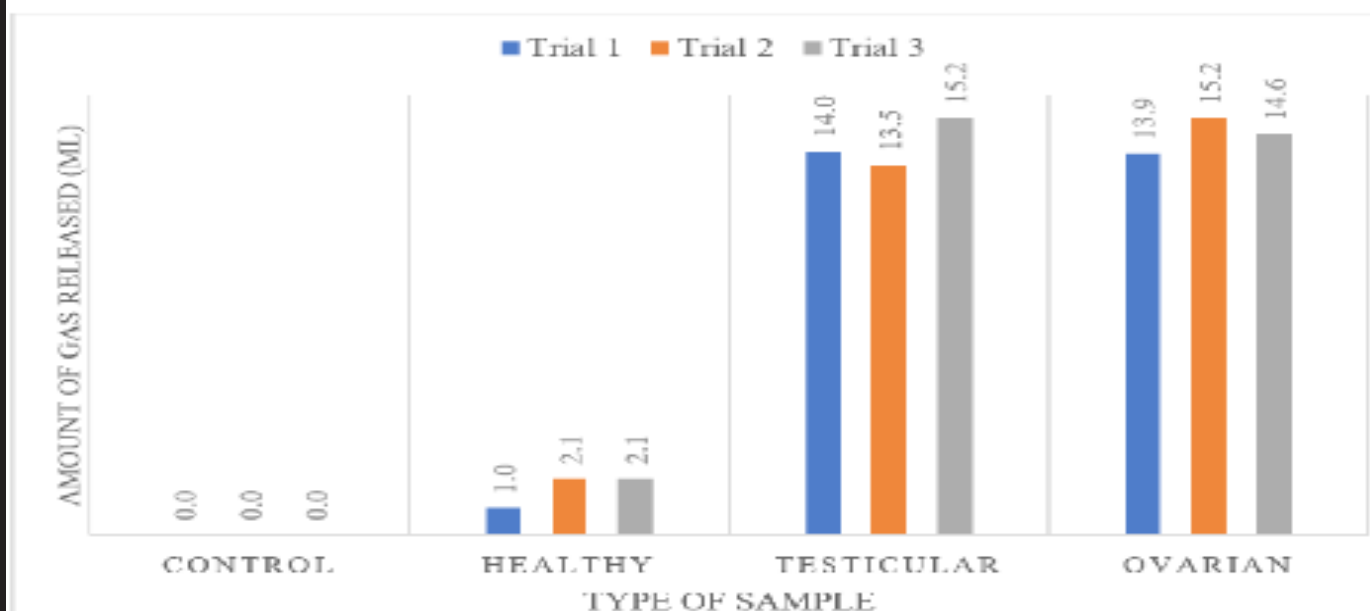
Data Analysis

Chi Squared Test

Data collected through the NAT was analyzed for statistical significance through the Chi-Squared Test. Through this test the categorical data from

Figure 2

Nitrogen Gas Released by Type of Urine



Note. Bar graph of total gas collected after nitrous acid test with simulated control, healthy, TCa and OCa samples (n=3).

the cancer tests were analyzed. The null hypothesis stated that after a polyamine nitrous acid primary and secondary chemical test, there will be no difference in the nitrogen gas levels between healthy and cancerous urine. The expected value according to the null hypothesis would be the average volume of the nitrogen gas in the healthy urine which was 1.7mL. The observed was the average volume of gas in the ovarian and testicular urine (14.57 and 14.23 respectively). After performing the test, the chi-squared value received was 189.8. The degree of freedom was determined by the number of categories (2) subtracted by 1 therefore being 1. According to the Chi-Squared table, with the p-value of 0.01, the critical number is 6.64. As the Chi-Squared value is greater

than 6.64 the data rejects the null hypothesis and is therefore statistically significant.

Correlation Coefficient

After the data was deemed statistically significant, the strength of the correlation between elevated polyamine level and increased gas product was calculated with the R-value. A strong correlation was found ($R = 0.989$ for putrescine, 0.725 for spermidine, and 0.998 for spermine) as shown in Figure 2. This strong relationship shows that the NAT could be efficiently used to detect an elevated level of polyamines in a sample, indicating that other cancers with elevated polyamines can also be detected with the NAT.

Table 2

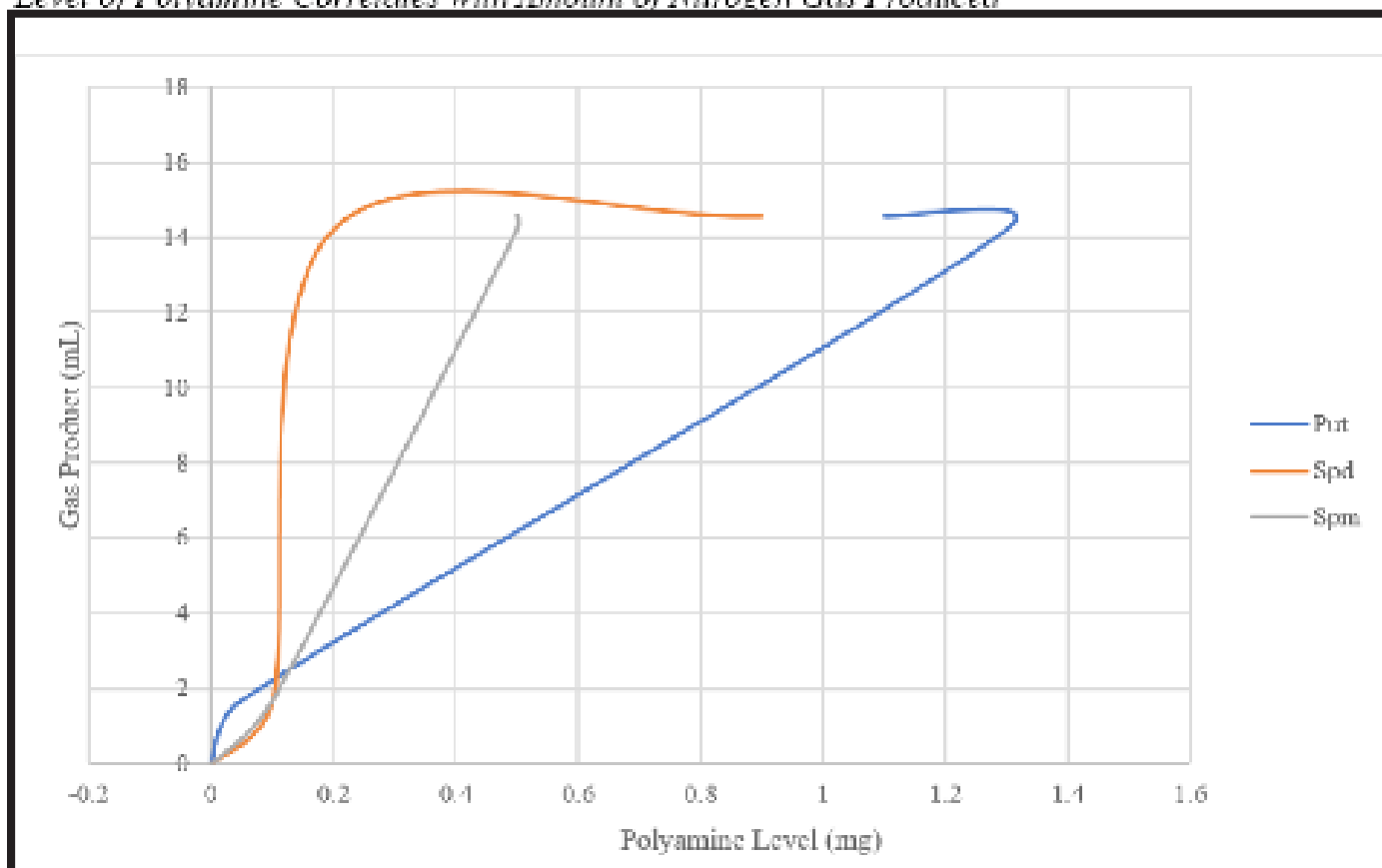
Degrees of Freedom

p	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.32	11.34	13.28	15.09	16.81	18.28	20.09

Chi-Squared table showing critical values based on p-values and degrees of freedom. Adapted from [Urry \(2014\)](#).

Figure 2

Level of Polyamine Correlates with Amount of Nitrogen Gas Produced



Note. The level of nitrogen gas strongly correlates with the amount of polyamine in solution. R= 0.989 for putrescine, 0.725 for spermidine, and 0.998 for spermine.

Discussion

The current results show that the nitrous acid method can successfully detect the differences in polyamines present in various urine samples. However, there are some limitations to the procedure that was used for this study. First and most importantly, the NAT was not conducted using real OCa/TCa patient or healthy volunteer samples. The simulated urine had polyamine levels that were calculated by averaging trials already run at other institutions and have been previously reported (REF). The levels that were tested in this study were accurate, but do not account for any natural variations that may exist in human samples. In a clinical application, not everyone will have the same polyamine levels and will have other environmental/genetic factors that are expected to affect those levels. There is no way to test this accurately without obtaining real samples because such variation cannot be artificially simulated or represented. Also, the test was only tried on OCa and TCa samples, so it is unknown if the NAT can indicate the presence of other types of cancers.

Needless to state that further validation of the NAT would require testing a large number of actual human urine samples. Samples would be obtained from healthy, non-cancerous people as well as patients with various phases of OCa and TCa. Additionally, this method should be tested with samples of other types of cancers in various stages before it is considered for clinical use. It should also be tested with

people who have other medical conditions to see if the NAT would have any false positives for conditions other than cancer.

Additionally, although the NAT is faster than current CBC panels, it still does take three days to get results. This could be solved by finding a factor that would increase the rate of the reaction. Since the acid must be cold, increasing the heat of the reaction would not be a viable option. Increasing pressure, concentration, or adding a catalyst may be worth considering when trying to speed up the reaction.

However, these results still have huge implications for the future of cancer detection. This test can provide a visual representation of the spermidine, spermine, and putrescine levels in the urine. Since it has been proven that polyamine levels dropped during remission and rose again during relapse (Russell et al., 1971), the NAT could be performed on patients in various stages of cancer and in remission to monitor for signs of recurring cancer. Doctors should look for any large increases in the nitrogen gas released, which would likely indicate cancer. This test could also be routinely performed on high-risk patients, such as women with a significantly higher chance of developing ovarian cancer due to the BRCA1/BRCA2 mutation, to monitor their condition and call for more accurate (and often invasive) diagnostic tests at the first sign of elevated polyamine levels. It eliminates the need for painful blood draws and reduces the need for commutes to hospitals which may

be far away for some patients. Instead, this test could collect the urine and be performed at a local clinic, especially in rural or underdeveloped areas, where a regular blood draws and pathological evaluations may not be possible due to lack of appropriate laboratory equipment or skilled personnel. Additionally, the strong R-values suggest that uses for the NAT could be expanded from just indicating OCa and TCa to indicating the presence of any cancer that results in an increase in urinary polyamines.

Further Research

As reported by multiple groups in the past, the increase in levels of spermidine, spermine, and putres-

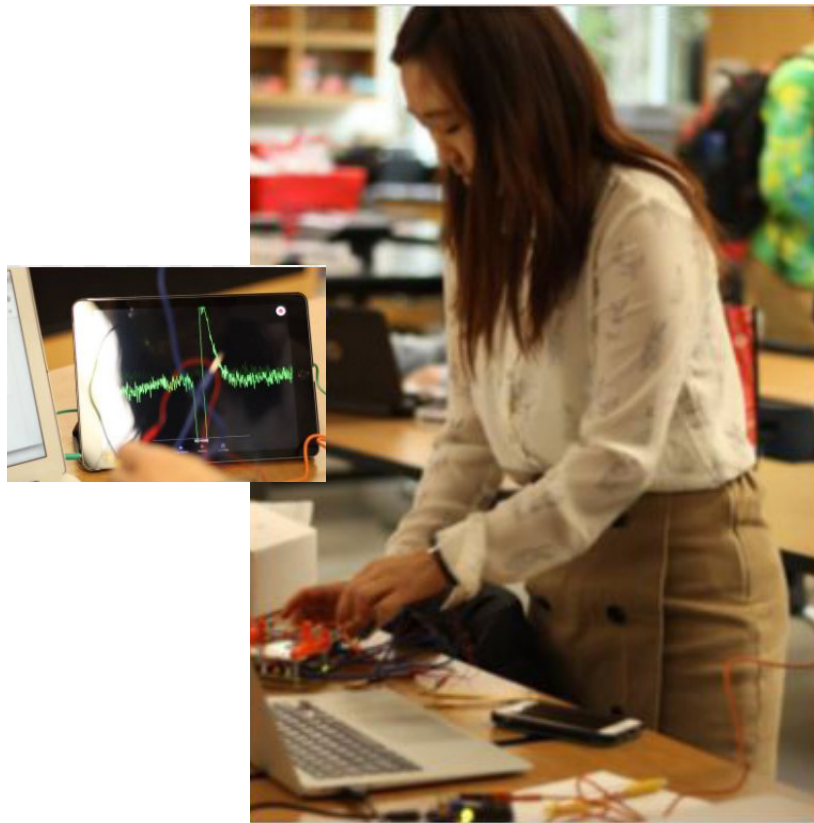
cine do indicate cancer. However, the tests for these polyamines have been done with mass spectrometry and liquid chromatography. The procedure used in this study can significantly reduce expenses, technician skill and time needed to use the tests that already exist today. The materials are relatively cheap and do not require much special equipment, which could mean that the test may be more readily available in smaller clinics and communities rather than just in hospitals in urban areas. This test can be further developed into an easy to use kit which would require very little equipment or skill to use and could possibly be used as an at-home test for cancer.

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Neurosciences



Determining Developmental Dyscalculia in 9th – 12th Grade Students Using Numerical Stroop Task Scores & Academic Achievement

Swarali Parikh

Hypothesis

If students score low on the Numerical Stroop task and their academic achievement is low, then they have developmental dyscalculia.

Numerical Stroop Task

The Numerical Stroop Task is designed to show a student's ability in mathematics. There are two conditions that are tested in the Numerical Stroop Task: physical size and numerical value. The first condition is the physical size of the digits where the individual is to name the number with the bigger physical size (i.e. 5 8). The size congruity effect is where the individual responds fast to the congruent (i.e. 5 8) over the incongruent (i.e. 5 8). The second condition is comparing the numerical values where the individual is to name the digit with the greater numeric values regardless of the physical size of the digit. Facilitation occurs when the individual responds faster to the congruent pair (i.e. 5 8). Interference occurs when the individual responds faster to the incongruent pair (i.e. 5 8).

Developmental Dyscalculia

The comorbidity rate of Developmental Dyscalculia (DD) and Attention Deficit Hyperactivity Disorder (ADHD) is 25% in children. The prevalence

of developmental dyscalculia is five to six percent in the school-aged population and is as common in girls as in boys. Developmental Dyscalculia is where children have difficulty solving simple arithmetic operations (i.e. simple, single-digit addition, subtraction, multiplication, and division problems) as well as recalling or remembering numerical facts (i.e. percentages). If not treated early enough, then these students will struggle to perform everyday tasks like trouble counting change, reading the time, etc.

Procedure

1. Make the Numerical Stroop Task using A. Heine's et. al. conditions
2. Acquire high school students to take the Numerical Stroop Task
3. Have Human Consent Forms signed by students and parents
4. Schedule time with students to administer the Numerical Stroop Task
5. Record the following in a data table in Microsoft Excel
 - a. Grade Percentage the students has in their current math class
 - b. Time Taken on Stroop Task Scores in seconds

c. Numerical Stroop Task Scores

6. Graph the data on a graph

7. Use Correlational Analysis to analyze data

using this equation:

$$R^2 = 1 - \left(\frac{\sum_i (y_i - f_i)^2}{\sum_i (y_i - \bar{y})^2} \right)$$

Data

The results show that there is a correlation between the presence of DD and low academic achievement. As the Stroop Scores get high so are the student's grades which means it is a direct correlation. The R-squared value is 0.37 which means there is a correlation, but not a strong one. (Figure 1). There is also an inverse correlation between time taken on the Stroop Task and academic achievement as the R-squared value is 0.35 (Figure 2). Lastly, in Figure 3, the graph proves there is no correlation between time taken on the Stroop Task and the Stroop Task Scores themselves. Figure 3 strength-

ens the fact that DD has to do with the intelligence of the student than with any other factors and proves the following statement is a misconception: if the student spends more time on the Stroop Task, they would have high scores.

Conclusion

If given the opportunity to be replicated, the subject population would be much larger as 9 students simply isn't a big enough sample size for there to be a significant correlation to be seen. Another factor that would be altered is the grade level; the new procedure would call for second grade students because that is when students start learning the preliminary math concepts. A potential solution to DD once it has been diagnosed in an individual, is to place the child in a special education program guiding him/her to help him/her understand the preliminary mathematical concepts better. If not treated upon, children and high school students will carry this developmental disorder into their adolescent and adult life which can have negative effects in their everyday life.

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Student Roll #	Math Grade Level	Letter Grade	Grade %	Time on Stroop Task (secs)	Stroop Task Scores	% Accurate
F1	7	A	92.56	128.73	128	100
F2	9	B-	80.16	118.42	128	100
SO1	7	S	36	168.84	114	89.0625
SO2	8	P	66.03	172.66	126	98.4375
J1	9	C	76.64	71.43	127	99.21875
J2	9	NC	41.51	123.89	128	100
J3	9	P	61.74	129.48	128	100
SE1	13	A	94.17	66.57	128	100
SE2	8	P	68.54	131.56	128	100

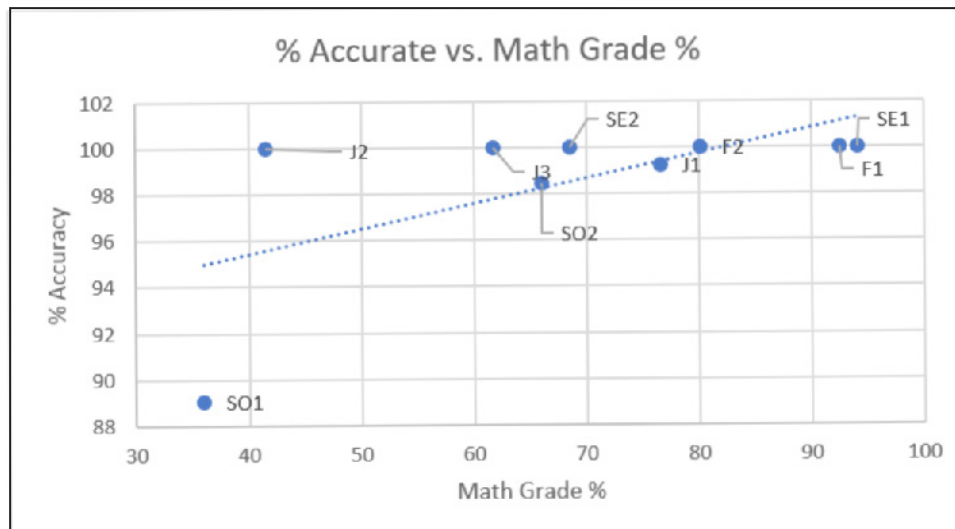


Figure 1: We can see some correlation between increasing accuracy on the Stroop Task and math grade % increases. R2 value is 0.37.

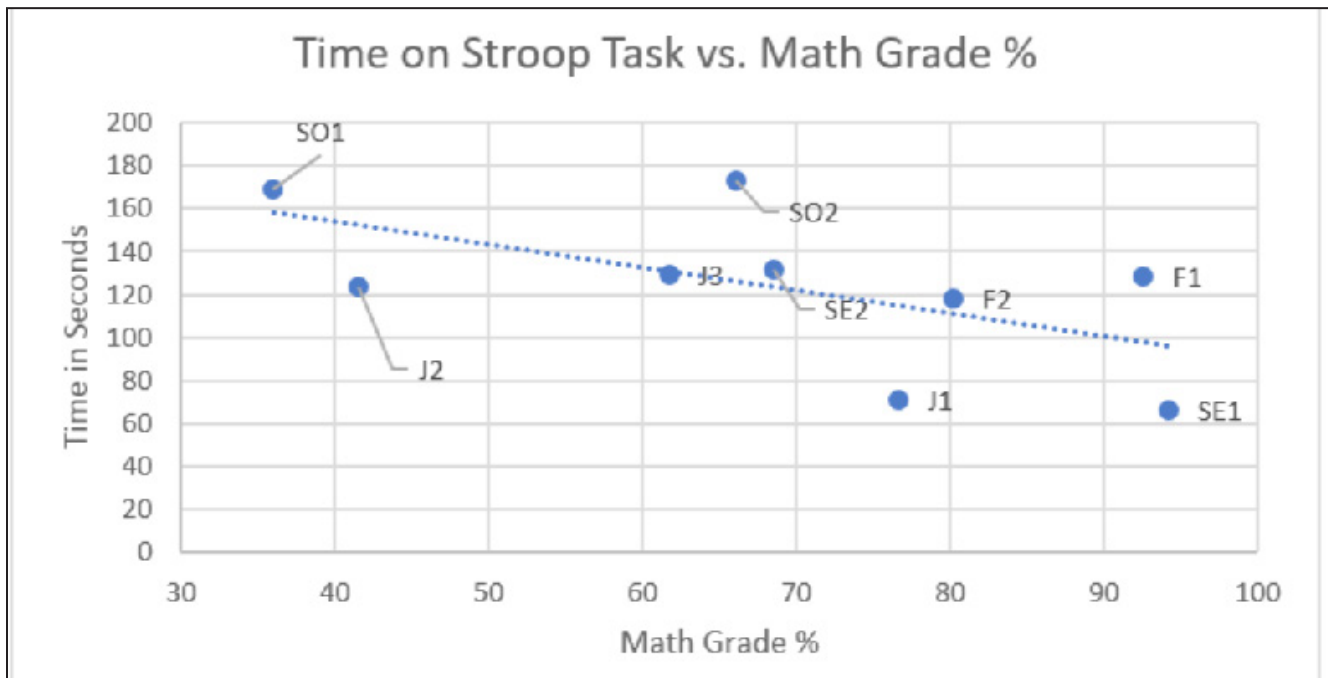


Figure 2: We can see some correlation between decreasing time to finish the Stroop Task and math grade increases. R^2 value is 0.35

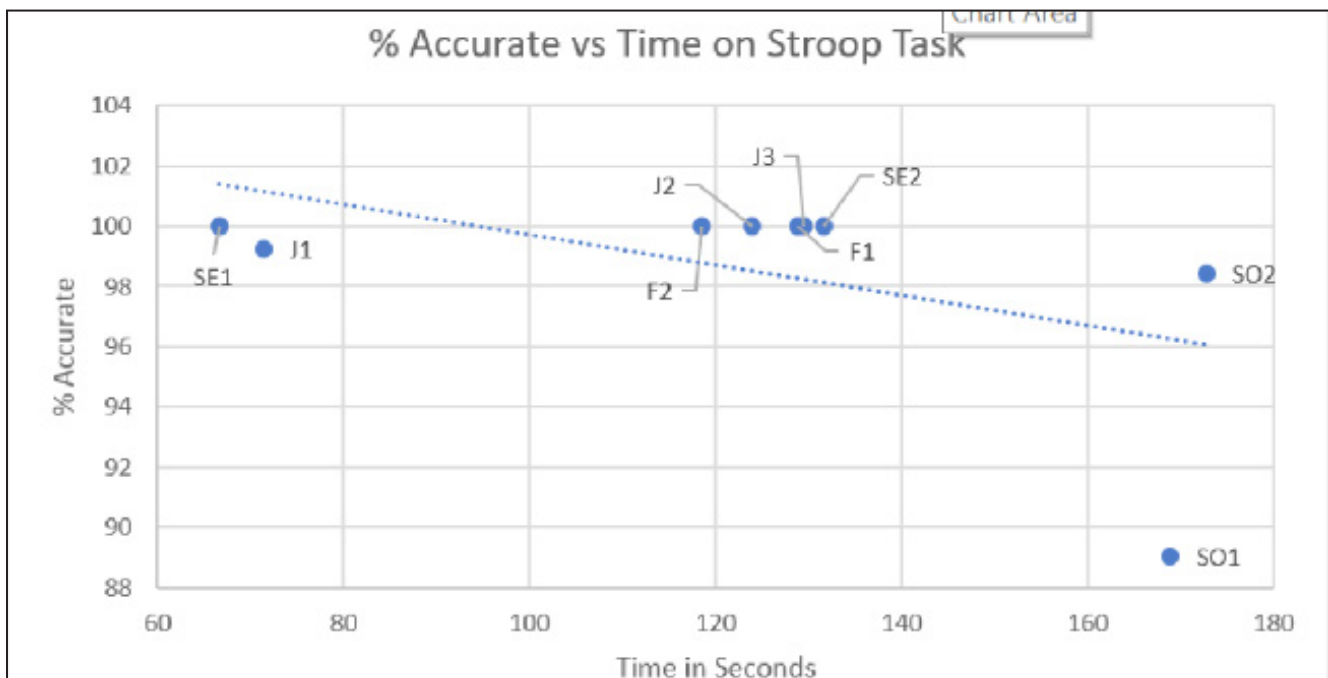


Figure 3: No correlation exists between increasing time taken for the Stroop Task and the increasing accuracy of the test.

Determining the Effects of Psychological States on High Schoolers' performance in Chess

Eric Yin and Toma Itagaki

Competition is prevalent in many people's lives and individuals are often faced with underdog situations. This experiment investigates how an underdog or favored situation can affect a person's mental state and their cognitive performance. Using chess as the platform, this experiment assessed the relationship between performance, and blood pressure levels (stress). After a set of preliminary matches to determine each participant's skill level, each participant played 3 games against a computer of equal skill level. Participants were informed about facing a player of greater, equal, and less skill level (despite the fact it is always the same skill level) to see if their self-confidence has any effects on how they perform. There was a 0.01 alpha value (t-test) for 29% of participants showing a strong correlation between increased cognition and increased blood pressure. Furthermore, when participants were told they were facing a computer of easier skill level, they performed 13% and 120% better compared to participants that were told they were facing computers of higher and equal skill level respectively. By further understanding how blood pressure and a person's self-confidence can affect cognitive performance we can further the field of industrial organiza-

tional psychology.

The ADAA clinically diagnosed 18% of the nation's population with anxiety already making it a factor in daily life. However, some professionals speculate that 85% of the world's population are affected by some form of low self-esteem. Prior statistics indicate that there is a significant impact of cortisol on a person's athletic performance, as losers consistently show an average of 35% increase in cortisol levels (Filaire). Similar studies on hockey teams have indicated that team's score lower on cortisol tests and win 14% more games while playing in their home venue (Carré). However, there are many existing confounding variables. Theoretically speaking, mental processes affect muscle memory and athletic talent less than it would affect a mental game. Similarly, these experiments do not take into account the varying skill level of each player, and that poor performance linked to low self-confidence is likely to be predetermined by a large gap in skill within athletic competitions. Research shows that greater self-confidence and less somatic anxiety are linked with enhanced physical performance, yet there has been no research to investigate the effects on cognitive performance (Anghelache, Filaire).

This project sets to investigate the relationships between self-confidence, stress levels, and cognitive performance in a simulated chess competition with opponents of different skill levels.

Methods

Participants

Participants included 7 high school volunteers (7 males), none of which had extensive chess experience. These participants were gathered at around 12:00 PM each day for 8 days to play preliminary and experimental matches.

Materials

Systolic blood pressure levels were taken through an Omron Blood pressure monitor. Before each match (regardless of whether it was preliminary or experimental), blood pressure was taken. After each match blood pressure was taken. The change in blood pressure from before the game to after the game was also calculated and recorded. Chess was played through an application called Chess.com and opponent levels were determined by the Chess.com rating of computer ratings that ranged from 1 to 10 (10 being hardest). Each participant played level four as their first preliminary match. Cognitive performance or performance of the chess game was calculated using an algorithm that computed whether the outcome of the match, and the values of the remaining chess pieces. Each chess piece has a unique point value (9 Queen, 5 Rooks, 3 Bishops, 3 Knights, 1 Pawns) and the values of the player's remaining

pieces were added and divided by ten (to scale to the win/loss point) and the opponent's pieces were added and divided by ten as well. The final score consisted of a point if the player won or a negative point if the player lost, plus the player's scaled piece values minus the opponent's scaled piece values.

Procedure

Before starting any measurements or chess games, each participant signed a human consent form with parent acknowledgement. Since each participant would be participating in experimental trials, there was no need to assign participants in to two groups.

For 5 days, each participant showed up around 11:30 PM and took initial blood pressure levels before starting a game. After reading the participants' blood pressure, confederates handed them a laptop with the chess application open (computer level was hidden) and told the participant what level computer they were facing. After the match, the confederate took the participants' blood pressure levels. If the player had won their match, the confederate would assign the player to a higher computer level and vice versa to measure the player's baseline or "equal" matched computer level.

After the preliminary placement matches were finished, the players would arrive without being told there was anything different. The confederate would take blood pressure the same as done during the initial five days and the game would not look different. For the next three days, the participant played

the same computer level (determined by the placement matches) but each day, the confederate would deceive the player into thinking than he is playing an opponent of a higher, equal or lower skill level than they actually are. This effectively creates a scenario where perform is altered primarily due to the change in mental perception of the situation rather than the skill level of the opponent.

Although there is a small sample size, statistically speaking, the data is relatively significant. There has been shown to be a significant change between matches (Equal, easy, and hard) despite the fact

that they were facing the same CPU level the entire time. Based on our sample data, 100% of participants played their highest scoring match when told they were facing easier or harder opponents. Participants split into two differing groups, those who performed well against an “easier” opponent and those who performed well against a “harder” opponent (view table 1). This split in differences is examined through our T-values in Table 2, indicating the significance of the change between each value. We can see that the high performance all 7 players boasted during the deceptive matches are outlandish, containing T-values of 0.01, 0.01, 0.1, and 0.025 (view table 2).

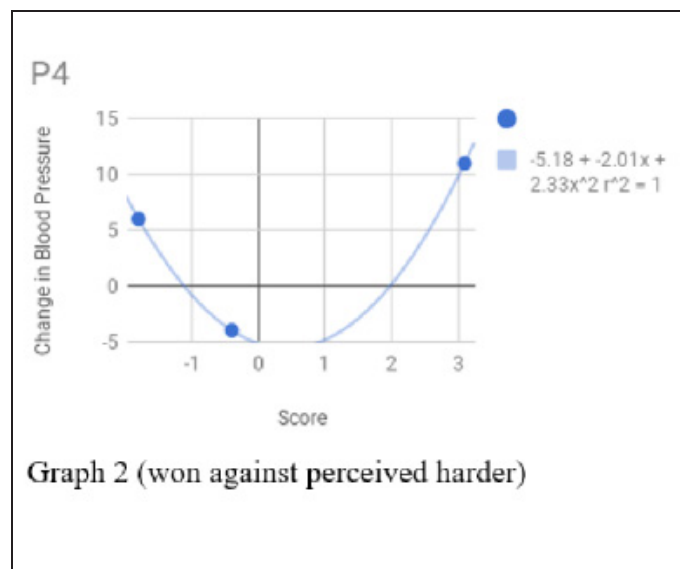
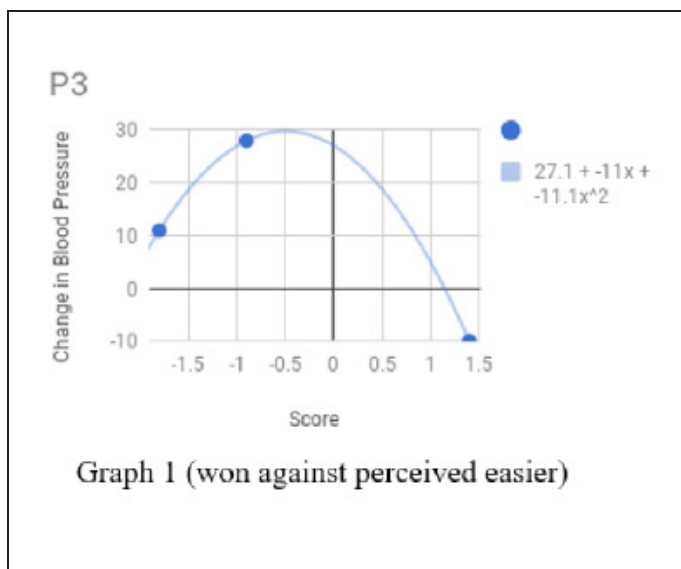
Results

Scores	P1	P2	P3	P4	P5	P7
Equal	-1.5	-1.7	-1.8	-0.4	-2.2	2.5
Easier	1.6	3.7	2.4	-1.8	-1.3	4.2
Harder	-1.1	-0.1	-0.9	3.1	3.2	2.8
Blood Pressure						
Equal	10	5	11	-4	13	-30
Easier	-16	-33	-10	6	2	2
Harder	5	6	28	11	-26	-11

Table 1

TTest charts	Easier vs Equal	Easier vs Harder	Harder vs Equal	Harder vs Easier
Mean Difference	3.6	2.8	4.45	4.7
Sum of Squares	7.46	3.22	1.805	0.08
Standard Deviation	1.576916823	1.036018018	1.343502884	0.282842713
Standard Error of Mean	0.788458412	0.518009009	0.95	0.2
T - Value	4.565871766	5.405311398	4.684210526	23.5
Significance (alpha value)	0.01	0.01	0.1	0.025

Table 2



Beyond the significance in difference between the scores of each participant, individuals appeared to have a negative or positive parabolic curve when viewing their scores. Viewing graph 1, we can note the positive parabolic function in their own blood pressure v. score graph. The opposite can be seen in graph 2 for those who won against perceived harder opponents.

Discussion

The data observed above strongly indicates the representation of 2 distinct personalities. Those who perform well against perceived “easier” opponents and those who performed better against perceived “harder” opponents. This is most prominently shown through our T-table test where we are able to state with high significance that their good performances against perceived easier and perceived harder opponents are outlandish (view table 1). Because this odd data occurred multiple times, we can safely assume that this represents two distinct personalities. Based on this one can conclude that a person’s perception

and self-confidence during the match has a profound effect on their cognitive performance. These two personalities manifest themselves even further when we look at the parabolic shape of their score v. change in blood pressure graph. The two differing parabolic patterns (positive and negative) similarly show the divide in personality types and the kind of trends we can expect to see from these two different personalities. As such our investigation originally in the effects of environmental stressors on cognitive performance revealed rather that differing personalities react differently under the effects of stress.

To answer our research question, there was no definitive correlation between all participants’ scores in the three experimental situations. Our hypothesis, if a participant is put into the underdog situation, their performance will decrease because it will cause an increase in blood pressure and if a participant is put in the favored situation, their performance will increase because they will have left fluctuation in blood pressure, was not fully supported by our data.

The data showed some participants losing during the underdog situation and winning in the favored situation, but this observation was not statistically significant. Instead, we were able to find success in statistically proving an increase in blood pressure after a mentally rigorous task and found an interesting split of personalities in the participants. 6/7 participants won very hard against one only to lose significantly against the other two scenarios. As such we theorize that almost everyone performs significantly better when in a easier or harder environment (depending on which match was won). We believe that setting up an environment where 1/2 of the class performs better as an underdog and 1/2 class performs better as a favored individual would promote the most academic success.

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Incorporation of Self-Esteem Enhancement Strategies in Social Media, their Effects on Adolescents Suffering from Depression.

Kyra C. Bethune

Abstract

Within the past 10 years of the “Digital Age”, or the age of widespread increase in media usage, the percentage of adolescents who have reported experiencing frequent drops in their level of self-esteem has nearly doubled, correlating with increasing reports of depression levels in teens. The hypothesis of this experiment was that if positive appearance-based comments online are used as a self-esteem enhancement strategy (independent variable), then the measured self-esteem that an adolescent suffering from depression feels will increase (dependent variable), but at a smaller value than what an adolescent that does not suffer from depression experiences because it takes longer for those with low self-esteem to improve their perceived self-worth, as they do still experience self-doubt. Adolescents between 10 and 19 took a quantifiable depression inventory and submitted a photo of themselves, while a social media environment was simulated by having comments about the participant’s appearance be posted online, and data was collected before and after being exposed to the online comments using self-reported reports on the participants’ self-esteem and an electroencephalogram to record spikes in brain activ-

ity (a response that indicates a positive emotional response). The results will be statistically analyzed with using a t-test to determine if the data is statistically significant. If hypothesis proves correct, these results could be used to help educate teens about the important impact that social media can have in the worldwide fight against teen depression.

Keywords: adolescents, self-esteem, depression, body-image.

When surveyed, 70% of girls ages 15-17 reported that they would avoid simple daily activities, like going to school, when they felt self-conscious about their looks (Slater, et al., 2015). Yet, that is not the worst extent of the increase in low self-esteem problems occurring throughout the nation: 44% of surveyed high school girls and 15% of high school girls were seen to be attempting to lose weight because they weren’t satisfied with the way they looked.

Self-esteem is considered to be a person’s own evaluations of themselves (of their self-worth), either positive or negative, to the extent of being considered useful and capable to society. Various factors are seen to impact self-esteem, including self-objectification, social comparison, and body image. It has also been found that self-esteem has a negative correlation with depression ($r=-3.1$) and anxiety ($r=-3.0$)

(Manna, G., 2016). This links all factors associated with self-esteem to mental disorders that are becoming more commonly reported.

Additionally, the Electroencephalogram serves as a testing method of monitoring possible electrical abnormalities in the brain or abnormalities in brain wave frequency.

The electroencephalogram has widely been used as a source for investigating depression disorders throughout the last decade. It has been found (Jaworska et al. 2012) that there are significant differences in the resting state of someone who has depression and a healthy patient. This is because of the discovery of alpha wave asymmetry in the frontal lobe for those suffering from depression. Other differences in healthy patients and depressed patients were found as well: increased alpha wave, delta, and theta wave activity was found in healthier patients than in depressed patients, suggesting that those with depression have deficits in brain wave activity.

Social networking sites are websites that allow users to communicate online by posting photo statuses, by sharing comments, or by submitting information. In the past decade the number of social media users has doubled (Armenaki, et al., 2015), and the age of exposure to such sites has dramatically lowered. This has allowed those still in the adolescent stage of cognitive development to become increasingly socially dependent on media sites, which ultimately start to function to shape their sense of self-worth.

The ability of someone to post a picture of themselves online can change the way they perceive their own self-worth, as body image is a contributing factor to self-esteem. Body image is not just determined by what you think of your appearance, but what others think as well. This project investigates how appearance-based compliments online influence the self-esteem levels of an adolescent suffering from depression. It was hypothesized that If we use positive appearance-based comments online as a self-esteem enhancement strategy, then the measured self-esteem that an adolescent suffering from depression feels will increase, but at a smaller value than the increase in self-esteem in an adolescent that does not suffer from depression because it takes longer for those with low self-esteem to improve their perceived self-worth, as they do still experience self-doubt.

Methods

Literature Review

The outcomes of self-objectification have been leading to significantly higher rates of depression, body shame, and eating disorder in women from ages 12-16 worldwide. Many studies have proved as evidence for the Self-Objectification Theory, which suggests that exposure to objectification from others can lead to women and girls to see themselves as objects based on their appearance. The research article by Amy Slater and Marika Tiggemann looked to investigate potential factors that would confirm the

Self-Objectification Theory as well as contribute to self-surveillance, body shame, and eating disorders. Surveying females between the ages of 12 and 16, participants were surveyed on their daily media exposure (hours per day), extracurricular activities (either appearance-based or not) (hours per week), and frequency of positive or negative comments online (0-2 scale). Self-Objectification was measured by the net amount of appearance-based values; self-surveillance was measured by providing how many times a day the participants surveil themselves; body shame was measured on a 1-7 scale of self-hate based on not meeting body standards; eating disorders were measured by a 1-6 scale of how scared a person was to gain weight.

Results found that media exposure and negative appearance-related comments online were positively correlated (r -value of 3.5) with self-objectification, self-surveillance, body shame, and eating disorders. Positive comments were positively correlated with self-objectification and self-surveillance. Extracurricular activities had a negative correlation with all 4 possible surveyed results. These results imply that even though positive comments are meant to be complimentary, they can still negatively impact a person's mental health. The experiment regarded in this research paper's topic also uses positive appearance-related online comments as a way to investigate its impacts, however, this experiment measures the significance of mood elevation with those who

suffer from a psychological condition and with those who do not.

Self-evaluation, or self-esteem, may be seen as a crucial part to our social well-being, but it is still associated with a broad range of mental disorders and social problems. Recently, empirical studies have shown that subjective well-being has a high correlation with high self-esteem, which is the most dominant predictor of happiness. Positive self-esteem has been associated with mental well-being, adjustment, happiness, success, satisfaction, as well as successful medical recoveries.

Negative self-esteem was discovered among researchers (Mann, M., Hosman, C. M., et al. 2004) as a result of a child's upbringing, seeing negative self-worth as an outcome of low maternal upbringing, child maltreatment, and a maternal history of depression. Negative and positive feelings of self-worth are also interpreted as a result of cognitive inferential processes (the act of seeing your own qualities as poor in comparison to someone else). This diminishing of self-appreciation and creation of self-defeating attitudes plays a significant role in the development of a variety of mental disorders. Correlational studies are consistently showing a strong negative correlation between self-esteem and depression. Self-esteem is not only a factor of mental health disorders like depression, anxiety, eating disorders, and social dysfunctional, but it can also be considered a consequence of these disorders as well.

Self-esteem enhancement strategies are slowly being included into schools to help prevent any potential mental health-related consequences of low self-worth. These strategies relate to the central experiment of this research paper, as it is looking for possible effective ways to incorporate methods of improving self-esteem in adolescents suffering from depression.

Participants

Adolescents (16 girls, 14 boys, ages 10-19, mean age of 16.5 years) were recruited through flyers posted in Kirkland, WA and Redmond, WA. Participants were not compensated for their participation. There were 30 total participants. All participants were chosen using a random number generator of all of those who responded to the flyer, and 2/3 of the total responses were ultimately chosen. Participants were emailed with a copy of a consent to participate form which informed participants of safety risks of the experiment and the purpose of the experiment, asking for their signature as well as parental signatures as well. Participants were also asked to send a photo of themselves that they felt they looked good in with the assurance that their photo would be kept private from the public. Dates were sent to inform the participants of when they would be scheduled to participate in the experiment.

Procedure.

A social media environment was simulated using an online Instagram account that's accessibility was

kept Private to the public, so those who are not a part of regulating the experiment could not see any photos submitted. All photos sent by participants for the use of the experimenters were posted on this online social media simulation. 6 separate private accounts were also formed with incoherent usernames (example: 84nf3904j) to prevent any bias to genders in the acceptance of compliments. Each of the 6 accounts was formed for the purpose of posting appearance-related compliments in regards to the participant with the function of attempting to improve their self-esteem levels (see Figure 1).

The participants were asked before engaging in the experiment to take the Mental Health America Depression Inventory to quantify their level of depression they were experiencing. Their results were not shown to them, as that could effect their mood following taking the depression inventory. Their results were used to determine what data group the participants were to be sorted in: Minimal Depression (a score of 1-5) or Depression (a score of 6 or above).

Participants' levels of self-esteem were measured in 2 ways: self-reported data and EEG recorded data. For both methods, data was collected before and after the participants were exposed to the online appearance-based compliments.

Self-reported levels of self-esteem were recorded by asking for the participant's amount of self-esteem (self-pride) they are feeling on a scale of 1 to 10.

This was done before and after they were exposed to the appearance-related compliments. The data was analyzed using a 2-sample t-test to compare the collected data. Data analysis looked to compare if there was a significant increase in self-reported self-esteem after being exposed to online compliments. Data analysis also looked to compare if there was a significant difference in the mean change in self-esteem that was recorded in participants who were experiencing depression versus those who had minimal depression.

The neurological response of the participants to the appearance-related comments were recorded by using the Backyard Brains Heart and Brain SpikeShield EEG machine. The location of the electrode used was in the F3 location of the possible locations, as this is where the emotions are processed in the Frontal Cortex in the Brain. A baseline EEG was taken for 15 seconds before the participant was exposed to the online compliments, and an EEG was also taken as the participants were reading the compliments for 15 seconds. Using the SpikeRecorder app, the recordings of the EEG could be analyzed by using the Spike Analysis function to count the total number of spikes in alpha wave activity that were found outside of the average spike frequency. The data analysis occurred by using a 2-Sample t-test to compare the changes in mean activity in alpha wave frequency. The data analysis looked to compare if there was a significant increase in alpha-wave spikes

before and after exposed to the online compliments. Data analysis also looked to compare if there was a significant difference in the mean change in self-esteem (before and after exposed to the appearance-related compliments) that was recorded in participants who were experiencing depression versus those who had minimal depression.

Results

Using a t-test for the mean number of alpha spikes in participants with minimal depression, it was found that there was a significant increase in the difference of mean alpha spikes after and before exposed to these compliments. See table 1 for results of experiment. The mean number of spikes before exposed was 50.25, the standard deviation was 2.769 with an n (number of participants) of 14. The mean number of spikes after exposed was 74.062, the standard deviation was 3.991 and the n (number of participants) was 14. Doing the calculation of $((74.062 - 50.25) - 0) / ((3.991)^2/14 + (2.769)^2/14)^{(1/2)}$, we get a t value of 6.9835 which gives a p-value of less than .0001, implying that there is a statistical significance in these results.

Using a t-test for the mean number of alpha spikes in participants with depression, it was found that there was a significant increase in the difference of mean alpha spikes after and before exposed to these compliments. See table 2 for results of experiment. The mean number of spikes before exposed was 46.571, the standard deviation was 5.747 with an n

(number of participants) of 16. The mean number of spikes after exposed was 61.07, the standard deviation was 5.663 and the n (number of participants) was 16. Doing the calculation of $((61.07-46.571)-0)/((5.663)^2/16+(5.747)^2/16)^{(1/2)}$, we get a t value of 6.7425 which gives a p-value of less than .0001, implying that there is a statistical significance in these results.

Using a t-test for the mean number the level of self-esteem in participants with minimal depression, it was found that there was a significant increase in the difference of mean level of self-esteem a participant felt after and before exposed to these compliments. See table 1 for results of experiment. The mean level of self-esteem before exposed was 7.5, the standard deviation was .894 with an n (number of participants) of 14. The mean level of self-esteem after exposed was 8.812, the standard deviation was .75 and the n (number of participants) was 14. Doing the calculation of $((8.812-7.5)-0)/((.75)^2/14+ (.894)^2/14)^{(1/2)}$, we get a t value of 4.4978 which gives a p-value of .0002, implying that there is a statistical significance in these results.

Using a t-test for the mean number the level of self-esteem in participants with depression, it was found that there was a significant increase in the difference of mean level of self-esteem a participant felt after and before exposed to these compliments. See table 2 for results of experiment. The mean level of self-esteem before exposed was 5.887, the stan-

dard deviation was .663 with an n (number of participants) of 16. The mean level of self-esteem after exposed was 7.143, the standard deviation was 1.35 and the n (number of participants) was 16. Doing the calculation of $((7.143-5.887)-0)/((1.35)^2/16+ (.663)^2/16)^{(1/2)}$, we get a t value of 3.796 which gives a p-value of .0014, implying that there is a statistical significance in these results.

Using a t-test for the comparison in the difference in the increase in self-esteem in participants with depression versus those without depression, it was found that there was not a significantly greater increase in the levels of self-esteem a participant with minimal depression versus those with depression. The mean increase in self-esteem of those with minimal depression was 1.135, the standard deviation was 1.169 with an n (number of participants) of 16. The mean increase in self-esteem of those with depression was 1.285, the standard deviation was 1.39 and the n (number of participants) was 14. Doing the calculation of $((1.135-1.285)-0)/((1.169)^2/16+(1.39)^2/14)^{(1/2)}$, we get a t value of -0.3173 which gives a p-value of .7536, implying that there is not a statistical significance in these results.

Using a t-test for the comparison in the difference in the increase alpha spikes in participants with depression versus those without depression, it was found that there was a significantly greater increase in the levels of alpha spikes a participant with minimal depression versus those with depression. The

mean increase in alpha spikes of those with minimal depression was 23.812, the standard deviation was 3.27 with an n (number of participants) of 16. The mean increase in alpha spikes of those with depression 15.071, the standard deviation was 3.852 and the n (number of participants) was 14. Doing the calculation of $((23.812-15.071)-0)/((3.27)^2/16+(3.852)^2/14)^{(1/2)}$, we get a t value of -0.3173 which gives a p-value of .7536, implying that there is a statistical significance in these results.

Discussion

The purpose of this study was to determine how positive appearance-based comments online can affect the self-esteem of an adolescent suffering from depression.

It was found that there was a significant increase in the levels of self-reported self-pride (self-esteem) for an adolescent both suffering from depression and an adolescent who has minimal depression after being exposed to appearance-related compliments. This indicates that participants were able to identify their positive reaction to the compliments, ultimately being aware of the change in their self-perception. This data helps support the part of the hypothesis that predicts that appearance-related compliments will help improve levels of self-esteem.

However, examining the results for the difference in the increase in self-esteem in participants with depression versus those without depression, it was found that there was not a significantly greater in-

crease in the levels of self-esteem a participant with minimal depression versus those with depression. This indicates that for both groups of participants, they reported that they experienced the relatively same level of increase in their self-esteem after being exposed to appearance-related compliments. This could be because of the possible unsatisfactory scale that was used to rate self-esteem, or possibly because of the unknowingness of their own emotional change after being exposed to a stimulus. This data goes against the predicted result that there would be a difference between the results of those who have minimal depression versus those who suffer from depression.

It was found that there was a significant increase in the levels of alpha wave spikes for an adolescent both suffering from depression and an adolescent who has minimal depression after being exposed to appearance-related compliments. This indicates that the participant's biological response to the online compliments ensures the changes in their positive emotions, corresponding to how they perceive their body image, showing that this positively impacts their self-esteem. This data helps support the part of the hypothesis that predicts that appearance-related compliments will help improve levels of self-esteem.

Examining the results for the difference in the increase in alpha wave spikes in participants with depression versus those without depression, it was found that there was a significantly greater increase

in the levels of self-esteem a participant with minimal depression versus those with depression. This indicates that participants with minimal depression experienced a greater amount of increase in spikes in alpha waves (increase in their emotional response) after being exposed to appearance-related compliments. This could be because it takes longer for those with low self-esteem to improve their perceived self-worth, as they do still experience self-doubt, so those suffering from depression would not experience a greater increase in positive reactions, which would influence their self-esteem. It could also be because it has been proven that the amount of alpha level activity is lower in those who have depression as well, and lower activity relating to alpha wave frequency. This information helps prove the hypothesis as biologically accurate, which over self-reported data, is to be considered as more accurate.

Conclusion

The hypothesis of this experiment was proved to be partially correct, with the exception of there not being a significantly greater increase in the levels of self-reported self-esteem in a participant with minimal depression versus those with depression. It was found that positive appearance-based comments on social media can serve as a significant method of improving levels of self-esteem. This increase in levels of self-esteem can be because the increase in positive emotions (supported by the statistical significance of the biological and self-reported data) as a reaction to how others perceive the way the participant looks can influence factors that determine levels of self-esteem, like body image. This discovery in the study can ultimately be used to help educate adolescents on the important impact that social media can have in the worldwide fight against depression.

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Table 2:

Participants with Depression (Score 6 or Above)

Depression Score	Self-Reported Pride Before Exposed to Comments (d)	Self-Reported Pride After Exposed to Comments (a)	Difference In self-reported self-pride (a-d)	Baseline # of recorded spikes in alpha wave frequency (b)	Post-Experimental # of recorded spikes in alpha wave frequency (e)	Difference in # of spikes (post-baseline) (r)
11	6	7	1	52	65	13
9	6	6	0	40	59	19
9	7	10	3	39	61	22
13	5	6	1	40	55	15
8	6	8	2	54	67	13
17	6	6	0	46	66	20
8	7	8	1	44	60	14
12	5	5	0	49	68	19
15	6	9	3	42	59	17
16	6	7	1	57	69	12
7	5	7	2	39	48	9
10	6	7	1	46	59	13
13	5	8	3	51	61	10
9	6	6	0	43	58	15
	x-bar: 5.887 S _x : .663 N: 14	x-bar: 7.143 S _x : 1.35 N: 14	x-bar: 1.285 S _x : 1.39 N: 14	x-bar: 46.571 S _x : 5.747 n: 14	x-bar: 61.07 S _x : 5.663 n: 14	x-bar: 15.071 S _x : 3.852 n: 14

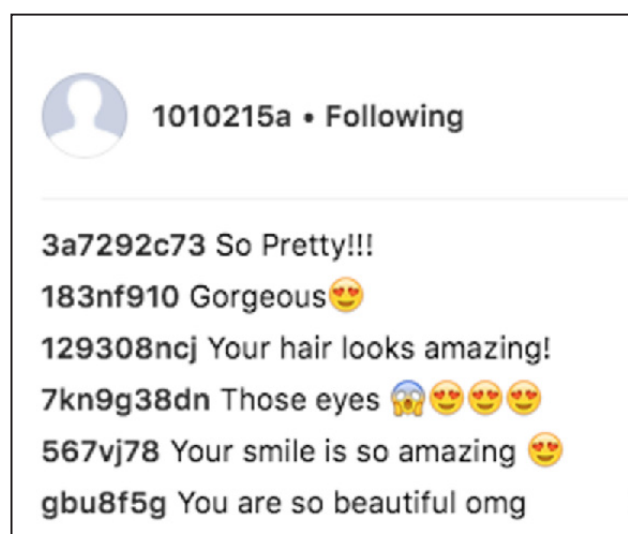


Figure 1. An example of the online simulation comments that are based on the participants' appearance

Making Self-regulation Available to All Students

Samuel Thornton

Summary

Research suggests that obtaining self-regulation skills in early childhood has been associated with successful, adaptive, healthy development throughout one's life. Conversely, those who lack sufficient self-regulation skills are at greater risk for low achievement, emotional and conduct problems and school dropout during adolescence. Unfortunately, self-regulation curriculums are almost exclusively taught exclusively to atypically developing students by occupational therapists in public school settings. The research investigated the impact of teaching an evidence based successful self-regulation curriculum (*How Does Your Engine Run?*) to elementary school educators so that they may employ this curriculum with all students. The hypothesis for this research was that the positive outcomes associated with obtaining self-regulation skills among the atypically developing students may also be beneficial for all students, regardless of their age or developmental trajectory. Results indicated that the administration of a self-regulation curriculum on a normally developing student class via a teacher is feasible.

Rationale

Research suggests that mastering behavioral aspects of self-regulation is important in early child-

hood (Matthews, J.S., Morrison, J., Ponitz, C.C. & McClellan, M.M. 2009). Possessing these skills helps children adjust to school, benefit from learning experiences, and succeed in their social interactions (Blair, 2002; Dobbbs, Doctoroff, Fisher & Arnold, 2006; Vitaro, Brendgen, Larose, & Trembley, 2005). The positive outcomes seen in early childhood are also correlated with attainment of higher education and greater success in jobs requiring organizational and interpersonal skills (Heckman, Stixrol, & Urazua, 2006; Miller et al. 2005; Muraven & Baumeister, 2000). Additionally, children who lack sufficient self-regulation skills are at greater risk for low achievement, emotional and conduct problems and school dropout during adolescence (Duncan et al., 2007, Eisenberg et al., 2000; Shaw, Gilliom, Ingoldsby, & Nagin, 2003; Vitaro et al., 2005).

One evidence-based curriculum: *How Does Your Engine Run?* The *How Does Your Engine Run?* Program (AP) for Self-Regulation (Williams, M.S. & Shellenberger, S., 1994) has been utilized internationally for more 20 years to improve self-regulation with children, teens and adults with special needs (Nalley, C., 2013; MacCobb, S., Fitzgerald, B. & Lanigan-O'Keefe, C., Irwin, N., & Mellerick, N. 2014).

Given the importance of possessing self-regulation skills and the extensive literature indicating the correlation to successful outcomes in life, the question then arises, why are self-regulation curriculums (e.g., *How Does Your Engine Run?*) not taught to all children, regardless of their developmental trajectory? Recently conducted qualitative research within one classroom indicated that it is feasible to teach the, “*How Does Your Engine Run?*” program to educators and outcomes in the classroom among students included improved self-regulation vocabulary and feeling recognition capacity (Blackwell, A.L., Yeager, D.C., Mische-Lawson, L, Bird, R.J., & Cook 2014).

The objective of this research is to investigate the impact of teaching educators and staff in an elementary school setting (K-5), an evidence-based curriculum (i.e., *How Does Your Engine Run?*) to utilize with their students day-to-day. This study is intended to understand if the positive outcomes of possessing self-regulation skills that have been demonstrated among the special needs population are also beneficial to all students regardless of their age and developmental trajectory.

Hypothesis

If teachers are taught to administer the “*How Does Your Engine Run?*” curriculum, then their students will be provided with an opportunity to learn self-regulation skills, which is positively correlated with successful development.

Methodology

Participants:

Teachers, teaching assistants, paraprofessionals, administrators and staff at Lakeview Elementary School in Kirkland, WA (a suburb of Seattle, WA) in King County. Lakeview Elementary School is a public school in the Lake Washington School district that educates children from Kindergarten up to 5th grade.

Student enrollment at Lakeview for the 2016-17 school year is 562 students with three to five classrooms per grade level. Student Composition Asian 9.4% African American 1.9% Hispanic 9.0% Native American 0.6% Caucasian 73.1% Pacific Islander 0.8% Two or More Races 5.3% Special Ed 10.5% Transitional Bilingual 9.6% Free/Reduced Lunch 15.8% Male/Female 53/47%

Recruitment:

With the help of the principal, all teachers, teaching assistants, paraprofessionals, administrators and staff were encouraged to participate in this research.

Procedures:

Survey #1: to obtain a baseline measurement.

Participants will be surveyed regarding their current understanding of self-regulation curriculum, tools and strategies. Sample questions in the pre-survey include:

- i. What do they know about self-regulation?
- ii. Did they learn about self-regulation curriculum? If so, when/where? What curriculum do they

use?

iii. Perceptions of the effectiveness of the curriculum – what works, what doesn't, what would you change?

Presentation:

30-minute brownbag presentation at the school during lunch. Sam, we will likely need to provide three options for attendance. Morning session, Lunch session, Afterschool session.

Survey #2: to obtain a measurement of comprehension of the “*How Does Your Engine Run?*” program presentation. What did they learn from my presentation of the *How Does Your Engine Run?*” program? Post -survey on their way out immediately after presentation (Did you learn anything new?)

Survey #3: to assess the impact of the implementation of the “*How Does Your Engine Run?*” curriculum with K-5 students at Lakeview Elementary (e.g., did you use the curriculum, how did you use it? when, how?) Where (e.g., classroom or playground?) Please describe a situation What was helpful, if anything? Which strategies worked, which didn't? Please describe? at didn't will, if anything?

Data Analysis:

This research will yield both qualitative and quantitative data. The qualitative data gathered from the open-ended survey questions will be analyzed to systematically extract key themes. I will use qualitative data analysis methods from methodological experts (Miles, M.B., & Huberman, A.M., 1984;



Figure 1 Diagram of How Does Your Engine Run

LeCompte, M.D., Millroy. W.L., & Preissle, J. 1992; Wolcott, H.F., 1994; Geertz, C., 1973). I will also be gather quantitative data (e.g., frequencies of responses to baseline questions, behavioral instances pre- and post- exposure to the “*How Does Your Engine Run?*”)

Results

Preliminary results indicate that teachers had little understanding of self-regulation curriculum. Among the teachers who expressed their knowledge and current usage, there was tremendous variability and it largely focused on regulating social behavior (e.g., *Kelso's Choice*) to reduce conflict among students versus self-regulation. **Kelso's Choice** is the current curriculum that is taught to educators beginning in Kindergarten in the Lake Washington School District. Follow up interviews with Occupational Therapists revealed that the “*How Does Your Engine Run?*” curriculum was utilized in their sessions with atypically developing students, which support research indicating that self-regulation curriculum is currently not being utilized with all K-5 students.

All teachers reported that self-regulation is a critical life skill to possess and that it is not currently part of the curriculum. Preliminary data on the effectiveness of the “*How Does Your Engine Run?*” curriculum in the general education classroom indicated the importance of having visual supports to utilize with students and educators also appreciated the importance of the curriculum being tailored to the grade levels they were teaching (K-2nd) is different from (3rd-6th). Final results indicated that the use of “How Does Your Engine Run?” was shown as highly effective with the students successfully using the strategies from the curriculum.

Conclusions

Although exploratory in nature, this study suggests that it is feasible to teach Elementary School educators a self-regulation curriculum to utilize in a general education classroom. It also identified an important difference between a curriculum that regulates social behavior in the classroom and a self-regulation curriculum. Further research is needed to determine the generalizability of these findings. Do these different curriculums complement each other? Would the need for regulating social conflict be less necessary if students possessed self-regulation skills (i.e., the ability to identify their emotions, the ability to generate and utilize methods to get to a calm state, and in doing so, self-regulate before issues arise).

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Observing the Relationship between Perceptions of Older Sibling and Brain Activity

Sreejita Ghose

Abstract

The purpose of this project is to determine whether the difference in age between siblings affects the amount of support the older sibling provides to the younger sibling. Previous research has shown that wide-spaced sibling pairs show more affection and spending of time with each other, so the current hypothesis is that higher amplitudes in beta waves indicate more encouragement. The nine older siblings and each of their younger sibling's each completed two different questionnaires assessing the degree of support provided by the older sibling, then the younger sibling completed an art project with their older sibling's encouragement, while the older sibling was connected to an EEG. The results revealed a negative correlation between size of age differences and younger siblings' ratings of perceived support from their older sibling with an R-value of -0.72062 , $p < .05$, which is contrary to the hypothesis. A one-way analysis of variance showed that the older siblings with the mid-range brain waves gave the lowest ratings of their level of support for their younger siblings and the older siblings with the low-range brain waves gave the highest ratings, and this is significant at the $p < .01$ level. This finding contradicts the hypothesis, because it suggests that

older siblings who think of themselves as supportive of younger siblings, showed less engagement as measured by their brain waves. This experiment illustrates the complexities of sibling relationships.

Older sibling's influence on younger sibling's

A study was done at University of North Carolina at Chapel Hill by researchers, and the results showed that people who had good social connections with their family members would live 7.5 years longer than those who had not. Also, a study done by Duane Buhrmester at University of Texas and Wyndol Furman at University of Denver showed that subjects were more affectionate with wide-spaced than narrow-spaced siblings. This means that if parents want to have more than one child, they may want to have children that are more widely-spaced apart. Also, adolescents (younger sibling) reported spending considerably less time engaging in enjoyable activities with siblings and felt slightly less warm toward siblings than did younger (younger sibling) subjects. This means that maybe siblings should try to spend more time with each other, so that can continue to when they get older. The project seeks to investigate how the older sibling perceives their support to their younger sibling vs how the younger sibling perceives their support from the older sibling. Also

measuring the amplitudes beta waves of the older sibling will help to understand whether they show support in what their sibling does.

Research questions

What perceptions of support from the older siblings do the younger sibling have? Do those perceptions match up with the brain action potential as shown by the older sibling?

Hypothesis

If the older sibling shows higher amplitude measurement in beta waves from their visual cortex in the brain, then that means that they demonstrate involvement in the activities that the younger siblings are involved in their day-to-day lives.

Methods

Participants

A Survey Monkey link was sent out to people who were interested in participating. The survey contained four questions. The reasoning behind the third question was because the older sibling had to wear a headband, since they were connected to the Heart and Brain Spiker Shield, which collected the brain waves.

I. If you are the older sibling, please select one of the choices regarding your age group. Note: If you do not fit into any of the choices, then you will not be able to participate in the experiment.

- o 8-10 years (tween)
- o 11-12 years (tween+)
- o 13-15 years (teen)

- o 16-18 years (teen+)

2. Please tell the age difference between you and your younger sibling (it must be larger than a year)
3. Do you as the older sibling have lice? (If yes, then you cannot participate)
 - o No
4. Please provide me with email, so I can give you details on where the experiment will be conducted and when to show up. There will be a Human Informed Consent Form, and you may choose to drop out of the experiment anytime you wish.

Materials

There should be questionnaires, Heart and Brain Spiker Shield, playdoh, journal to record observations, timer, and laptop.

Design and Procedure

I got two participants for every group, except for the 8-10 years group, where there was an additional third group. After there were enough participants, both the older sibling and younger sibling were given questionnaires, regarding the support of the sibling. They had to complete them separately, so that they would not influence each other's answers.

- I. The younger sibling will be given a questionnaire with the following question and rate on the 5-point Likert-type scale- (1-Strongly Disagree, 2, 3-Moderately Agree, 4, 5-Strongly Agree)
 - Do you feel like your older sibling cares about you excelling on what you do (School, extra-

curriculars, competitions, etc.)?

2. The older sibling will be given a questionnaire with the following question and rate on the 5-point Likert-type scale- (1-Strongly Disagree, 2, 3-Moderately Agree, 4, 5-Strongly Agree)

- Do you feel like you care about your younger sibling excelling on what they do (School, extracurriculars, competitions, etc.)?

After both siblings were done with completing the questionnaires, the younger sibling had a maximum of 15 minutes to complete an art project with play-doh, while the older sibling was connected to the Heart and Brain Spiker Shield. Prior to doing the art project, I told them that if they thought their project was good enough, they could participate in an art competition. This was so that the younger sibling would be more motivated to do the project plus the older sibling so he or she would be more supportive and helpful during the experiment. Using the glue bottle, I had to put some glue on the metal buttons of the headband, then the headband was placed on the head of the older sibling, with the metal buttons on the back of person's head. An electrode sticker was placed behind the ear, on the bony part. Then a thick black wire got connected to the laptop, with the other end connected to the spiker shield. The orange wire got connected to spiker shield, and the two red alligator clips connect to the headband, while the

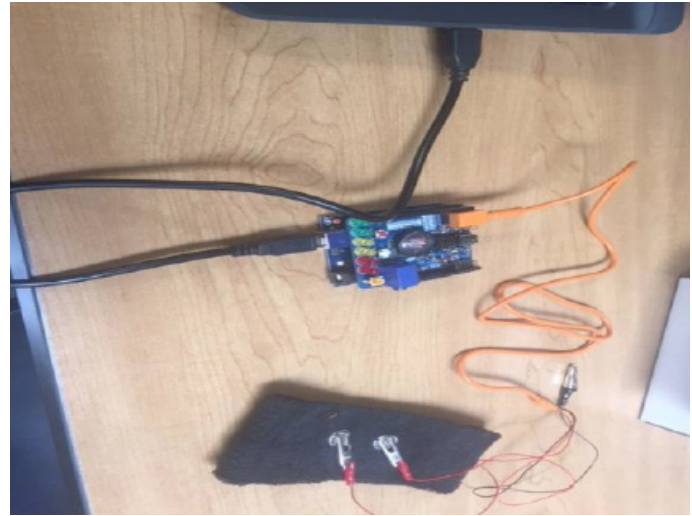
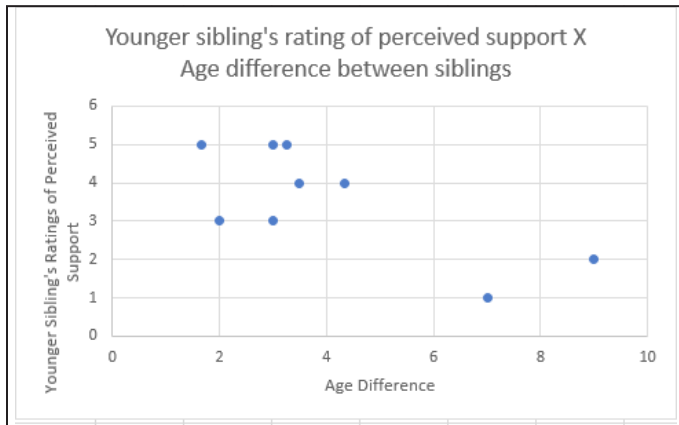


Figure 1. This is for the setup for the Heart and Brain Spiker shield to Laptop.

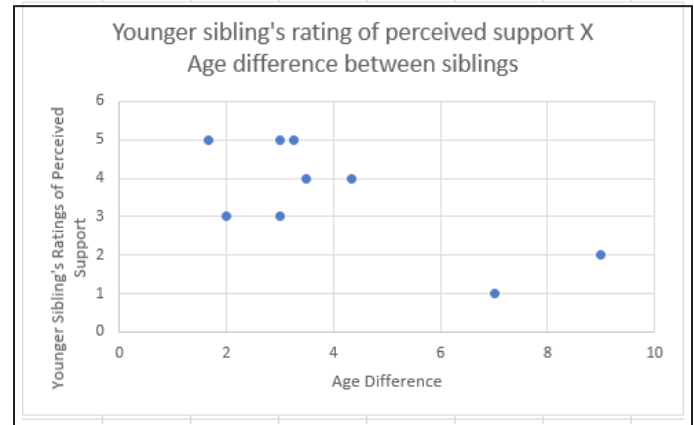
black alligator clip connects to the electrode. The brain waves were then recorded, while the younger sibling was completing the art project. I also made notes of observations as well.

Results

The results revealed a negative correlation between size of age differences and younger siblings' ratings of perceived support from their older sibling with an R-value of -0.72062 , $p < .05$. A one-way analysis of variance showed that the older siblings with the mid-range brain waves gave the lowest ratings of their level of support for their younger siblings and the older siblings with the low-range brain waves gave the highest ratings. There was an R-value of 0.655329 , $p < .05$, which is a positive correlation between the age difference and the difference of ratings. The average rating on the questionnaire by the older sibling was 4.16667 , and for the younger sibling it was 3.55556 .



Graph 1. This is for the difference in Ratings Vs. Age Difference.



Graph 2. This is for Age Difference Vs. Younger Sibling's ratings of perceived support.

Low	Middle	High	Anova: Single Factor					The older sibs with the mid-range brain waves gave the lowest ratings; the older sibs with the low-range brain waves gave the highest ratings. Significant at the $p<.01$ level.
5	4	4	Older Siblings' Brain Waves = IV; Older Siblings' Ratings = DV					
4	3.5	4	SUMMARY					
5	3	5	<u>Groups</u>	<u>Count</u>	<u>Sum</u>	<u>Average</u>	<u>Variance</u>	
			Low	3	14	4.666667	0.333333	
			Middle	3	10.5	3.5	0.25	
			High	3	13	4.333333	0.333333	
ANOVA								
Source of Vari		SS	df	MS	F	P-value	F crit	
Between		2.166667	2	1.083333	3.545455	0.096282	5.143253	
Within Gr		1.833333	6	0.305556				
Total		4	8					

Figure 2. This is the one-way analysis for the brain waves.

Discussion

The older siblings average rating was higher compared to the younger sibling, which is very natural, because the question was about the older sibling, so self-serving bias came into play. The social desirability effect was there as well, because older siblings would not want to give lower ratings of themselves, since it would make them seem like unsupportive siblings. It is true that in many sibling relationships, there can be rivalries, which is why the younger sibling gave lower ratings, and the question

was about someone else, not them. There is also the social desirability effect in the brain waves, because according to the one-way analysis of variance, older siblings with mid-range brain waves gave the lowest ratings of support, and this might be because the older siblings do give relative amounts of support to their younger sibling in their day to day lives, but they do not want to show off that value, since other people may think they are a show-off, as opposed to the older siblings who had low brain waves, but gave higher ratings of support. This may be because they

already think they are doing a decent job, or want to cover up the fact, that they really do not care about what their younger sibling is up to. These findings may also account for cultural values, since different genders and races were used. Also, more participants would have yielded better results.

Except for one group, every younger sibling spent less than 15 minutes making their art project, even though they were told that if they thought their sculpture was good enough they could enter in an art competition if they wanted to, and this may be because many of the younger siblings are not so much interested in sculpture making. Also with exception of two groups, every older sibling talked first, for example by saying things like “Make something” or “What are you going to make?”, and since I as the experimenter was there, experimenter bias did come into play, which may have caused the older siblings to ask, so the real question is, if I was not in the experiment, would they still have asked?

Conclusion

The results revealed a negative correlation between size of age differences and younger sib-

lings’ ratings of perceived support from their older sibling with an R-value of -0.72062 , $p < .05$, which is contrary to the hypothesis. This means that as the age difference increased, younger siblings gave lower ratings, which goes against the study that was done by Duane Buhrmester at University of Texas and Wyndol Furman at University of Denver. A one-way analysis of variance showed that the older siblings with the mid-range brain waves gave the lowest ratings of their level of support for their younger siblings and the older siblings with the low-range brain waves gave the highest ratings, and this is significant at the $p < .01$ level. More participants would have yielded better results for this experiment. This experiment has gone further into the complexities of sibling relationships.

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The Efficacy of a Counter-Stereotypical Scenario in Reducing Racial Implicit Bias

Christine Lee

Abstract

Although the era of explicit racism has elapsed, racial implicit bias still perpetuates in society and can be seen in the growing inequality gap between races and political movements. This project aims to find a relationship between a reduction in racial implicit bias and brain activity to research the biological nature of racial implicit bias by testing the effectiveness of a counter-stereotypical scenario. Participants were divided into a control and experimental group, where the experimental group was given a story with counter-stereotypical characters that has been shown to reduce racial implicit bias. The dependent variables were results from the Harvard Implicit Association Test (IAT), facial muscle reaction time, and brain activity. Analysis of the data has shown that administering the story reduced racial implicit bias through a reduction in facial reaction time in response to African American stimulus with a p-value of 0.08. These results suggest that an intervention that targets fear reduction may be effective in reducing racial implicit bias.

Keywords: racial implicit bias, IAT

Although explicit racism has decreased in America, subtle displays of racism remain. These vestiges of explicit racism can be seen in the fewer num-

ber of minority groups in leadership and political positions, and as a gap in socio-economic status (Augoustinos & Reynolds, 2001). Mimicry has been shown to be a strong measure of ingroup bias, an individual's tendency to favor others that are in the same ingroup as him/her (Brewer, 1979). This mimicry has been expanded to include facial mimicry. Many studies have shown that facial mimicry is a robust and reliable measure of the participant's ingroup bias (Sachisthal, 2016). As racism can be seen as an issue of implicit bias for people to favor others of the same race, facial mimicry may be applied to test the implicit bias of participants. Previous studies have shown that white participants facial muscle latency is shorter when mimicking another white participant versus a black participant (Rauchbauer, 2016).

A study by Wheeler and Fiske (2005) has shown that white participants' amygdala – a region of the brain that regulates fear and emotion – is activated when seeing a black face compared to a white face. This suggests that a racial implicit bias could be linked to greater amygdala activation in white participants. Furthermore, studies have shown that a higher ratio of theta to beta waves from electroencephalograms (EEG) are negatively correlated to in-

hibition to fearful faces (Putman et al., 2010). These findings suggest that measuring the theta to beta wave ratio may indicate an activation of the amygdala and greater fear response, suggesting that a participant's racial implicit bias is stronger. Implicit bias has primarily been measured by the implicit association test (IAT) developed by Greenwald, et al. in 1990. This test measures the difference between participants' reaction times in grouping items together. Implicit bias is thought to be unchangeable because it is a result of a person's individual experiences that have strengthened an individual's perception of the world (Blair, Ma, and Lenton, 2001). Various experiments have consequently been conducted to find the most effective intervention to reduce racial implicit bias (Lai et al., 2014). These studies found that using a counter-stereotypical exemplar was the most effective in reducing racial implicit bias, especially when there is a high level of self-involvement (Marini et al., 2012). A high level of self-involvement was shown through the participants greater reduction in implicit bias when the story used the second-person "you," compared to a low-level of self-involvement of "he" or "she." Thus, these studies suggest that using an intervention that presents a counter-stereotypical exemplar to white participants will reduce their racial implicit bias. This reduction in implicit bias can be seen through a decrease in participants' facial mimicry reaction times and an increase in theta/beta ratio. As a result, it may be predicted that if partici-

pants read a story that has a Caucasian person as the villain and an African American person as the hero, then their facial mimicry latency will decrease because their implicit bias towards African American will decrease, and their theta to beta wave ratio will increase to levels greater to those in controls because of their reduced implicit bias which reduces fear.

Method

Participants

12 adults (6 male and 6 female) ages 18-24 were recruited from the Bellevue College campus and Tesla STEM High School. They were invited to participate through flyers and emails, and were asked to come in for a one-time 30-minute session to collect data. Participants were recruited in a rolling basis and were placed randomly into the control or experimental group by a stratified random sampling algorithm that separated the participants into the control and experimental group and ensure that there was an equal balance of males and females in each group.

Procedure

Participants were given the Harvard Implicit Association Test (IAT) for race at the beginning and end of the experiment to establish a baseline of implicit racial bias (IRB). IRB was represented numerically by converting the results of the IAT as follows: strong automatic preference for European Americans = 7, moderate automatic preference for European Americans = 6, etc. Then, an electroen-

cephalogram (EEG) (Heart and Brain Spikershield from Backyard Brains) was placed in the occipital lobe of the participant. The Spike Recorder app was set to 1-100 Hz and a 60 Hz notch filter was used to reduce electrical noise. Participants were then given a scenario to read, and brain activity was collected for the time participants read the scenario. The control group read the counter-stereotypical scenario without any racial information, whereas the experimental group did. Then, an electromyogram (EMG) from Backyards Brains was used to measure the facial muscle reaction time in the zygomatic major (ZM). The EMG was two popsicle sticks with electrodes that touched the ZM of participants. Participants were shown video stimuli on a laptop of an African American and Caucasian smiling three times respectively to acquire a total of three trials for each category of stimuli. EEG and EMG data in response to facial stimuli was collected concurrently. Afterwards, participants took the IAT for race once more.

Data Analysis

IAT data was converted numerically as described in the procedure. Raw EMG data was processed to include only spikes that were above 1 Hz. The muscle reaction time was measured in milliseconds from the beginning of the recording to the first muscle action potential. EEG data was processed through code in Matlab that extracted the beta and theta power waves of the brain activity.

Results

2-sample t-tests were done to compare the average reaction times of the control and experimental group by category of racial stimuli. In response to African American stimuli, there was a t-value of 1.593 and a p-value of 0.085. In response to Caucasian stimuli, there was a t-value of 1.037 and a p-value of 0.1727. Comparing the differences in response time for individual participants, a t-value of 2.67 and a p-value of 0.015 was concluded. There was no significant change in IAT with a p-value of 0.628. In addition, a relationship between the IAT and brain activity was not found as the experimental group's r-value was 0 compared to the control group's 0.392. In addition, there was an interesting correlation between brain activity and reading the scenario. There was a strong correlation of 0.4 for the control group and weaker relationship with the experimental group with 0.371.

Discussion

Although there were some significant results, the overall conclusion of this study is inconclusive because of the lack of an appropriate sample size and discrepancies in the data analysis. (The small sample size of twelve cannot be said to be a representative sample.) One of the largest misgiving is that the EEG was not able to record brain activity in the amygdala because the available EEG machine was not advanced enough to measure such specific brain activity. Rather, the EEG measured brain activ-

ity from the occipital lobe which provides unreliable data and results. However, there were interesting relationships between the EEG and IAT data suggesting that perhaps there is a relationship between vision and implicit racial bias that could be researched in the future. There are also various confounding variables in the experiment, particularly with the IAT. The IAT measures response time to seeing a visual stimulus on the screen. Some participants may be better at testing than others, and others still may adapt from taking the test two times, thus skewing results. In addition, there may be a huge possibility for error in collecting the data as the EMG and EEG equipment were very rudimentary. There could have been errors in mistakenly analyzing artifacts as muscle potentials or certain frequencies of brain activity.

To improve the robustness and reliability of the data, more participants must be recruited to test this hypothesis. In addition, access to more accurate EEG and EMG technology would be extremely beneficial in collecting more accurate results. More research could be done in how to analyze EEG and EMG data to remove artifacts and analyze the proper muscle potentials. To solve the issue of implicit bias, further research of interventions must be done to test their efficacy. Racial implicit bias is something that appears to be evolutionarily wired into humans, thus it is more important that interventions that can

change the brain directly must be developed.

Conclusions

The data cannot support the hypothesis that a counter-stereotypical scenario reduces racial implicit bias by decreasing facial mimicry reaction times or an increase in the beta/theta ratio. The EMG data showed a p-value of 0.015 when describing the difference between changes in facial reaction times in individuals. It also showed a p-value of 0.085 when comparing the control and experimental group's facial muscle reaction time showing that there was a significant change if the alpha level is set at 0.1 instead of the scientific standard of 0.05. However, the EEG data is completely inconclusive and does not show a relationship between an increased beta/theta ratio to a decrease in racial implicit bias. This can be seen in the r-value of 5×10^{-5} , which shows a null relationship. Due to the discrepancy in data, as the EMG data suggests that the counter-stereotypical scenario may be effective while the EEG data completely rejects it, a definitely conclusion on the efficacy of a counter-stereotypical scenario cannot be made.

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Utilizing Music Therapy to Help Teenagers with Depression and Anxiety

Victoria Alkin

Rationale

Many teenagers today suffer from depression, anxiety, proneness to bad moods or lack of motivation, especially when dealing with a high workload. National Institutes of Mental Health reports that in 2015, an estimated 3 million adolescents aged 12 to 17 in the United States had at least one major depressive episode in the past year. This number represented 12.5% of the U.S. population aged 12 to 17. In addition, depression in teens has been on the rise nationally. According to National Trends in the Prevalence and Treatment of Depression in Adolescents and Young Adults study published in the journal *Pediatrics*, the 12-month prevalence of major depressive episodes (MDEs) increased from 8.7% in 2005 to 11.3% in 2014.

At the same time, studies have shown that music can have a positive effect on mood – cheering people up, decreasing anxiety, and boosting energy and motivation. For example, Individual music therapy for depression: randomized controlled trial study published in *British Journal of Psychiatry* reported that participants receiving music therapy plus standard care showed greater improvement than those receiving standard care only in depression symp-

toms (mean difference 4.65, 95% CI 0.59 to 8.70), anxiety symptoms (1.82, 95% CI 0.09 to 3.55) and general functioning (−4.58, 95% CI −8.93 to −0.24) at 3-month follow-up. Other articles and books show similar trends.

The goal of this project is to research effects of specific music attributes (such as tempo, key, pitch and lyrics) and their values on human mood, and identify attributes/values that are most effective in providing positive influence on teenagers' mood and motivation.

Research Question

Which attributes of music (such as tempo, key, pitch and lyrics) have the greatest positive effect on teenagers' mood?

Hypothesis

The music attributes that have the greatest effect on improvement of human mood are upbeat tempo (100-160bpm), a major key and uplifting lyrics. Pitch and structure have a lesser effect.



Procedure

Below is one of the questions of the survey:

- * 2. On a scale of 1 – 10 with 10 being the most positive, describe your mood:

	1 (Completely Negative)	2	3	4	5 (Neutral)	6	7	8	9	10 (Completely Positive)
Sad vs. Happy	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Stressed vs. Serene	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lethargic vs. Excited	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fatigued vs. Alert	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tense vs. Calm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Upset vs. Content	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Prev

Next

Human Participants Research

Participants: Participants were of the age range of high school (grade 9-12) and included multiple genders, racial/ethnic groups, etc. Vulnerable populations included only minors.

Recruitment: Participants were recruited from multiple high schools in the area through online advertising (e.g. a Facebook post stating the purpose of the experiment, outlining the participant experience, and inviting interested students to participate), word of mouth, and other methods.

Methods: Participants were asked to listen to several songs and fill out short questionnaires asking them to rate certain emotions on a scale of one to ten, as well as a PHQ-4 questionnaire (a non-intrusive tool for detecting anxiety and depression, broadly used for psychological assessment). Time involved for each subject was approximately 15 minutes, and only one session was necessary.

Protection of Privacy: No identifiable information was collected at any point in the experiment. All data was confidential and anonymous. The data was col-

lected through an anonymous questionnaire, which at no point instructed the participants to disclose any identifiable information, including names, phone numbers, birth dates, or email addresses.

Risk and Safety

There were no major risks to participants in this research, and virtually no physical risks. However, there was a very slight potential for psychological discomfort when filling out a questionnaire during the experiment, due to unwillingness to answer questions rating mood. To minimize this risk of discomfort, it was ensured that the experiment was entirely voluntary, and therefore if a participant didn't not feel comfortable answering a certain question, they could skip it or discontinue the experiment. The questions were also not intrusive or complicated.

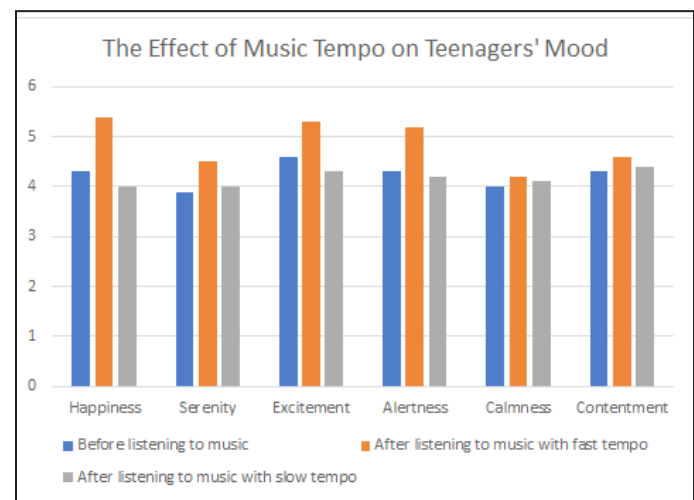
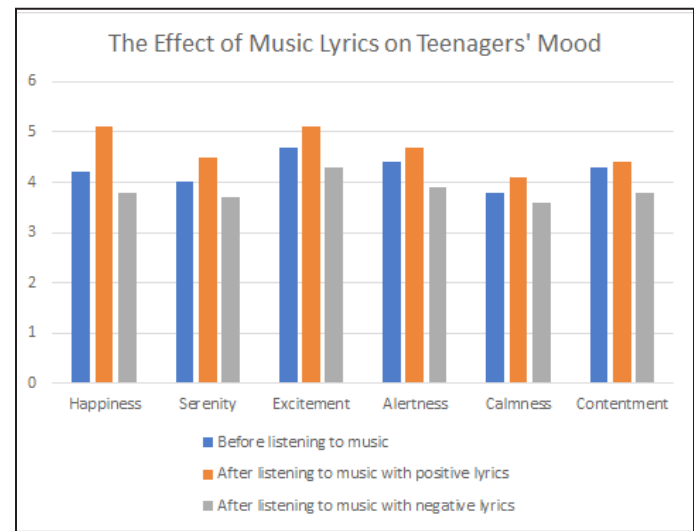
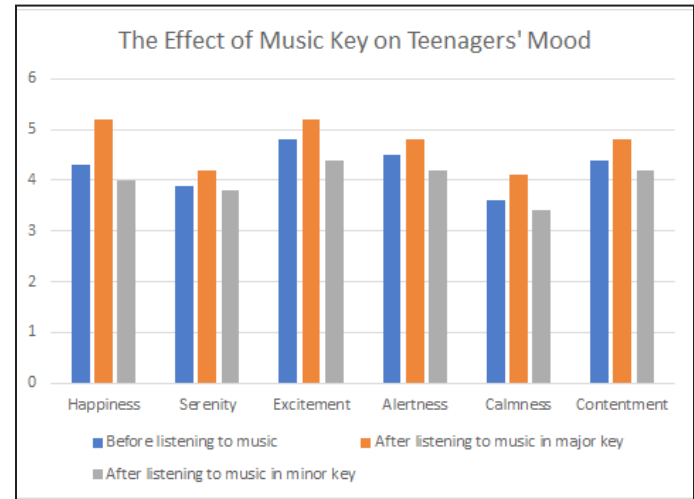
Another potential cause of discomfort was the songs themselves (e.g. loud sounds within songs, melodies that the listener finds unpleasant, etc.). To address this, songs were selected to minimize discomfort, and again, participation was entirely voluntary, so that a participant could skip a song if they wished to do so. There were no major benefits to participants (as compensation was not provided), other than the pleasant experience of listening to various songs. As for society, the experiment has many applications, including an app that utilizes music therapy to help teenagers with depression, anxiety, motivation, and mood, and a part of CBT (Cognitive Behavioral Therapy) used by specialists.

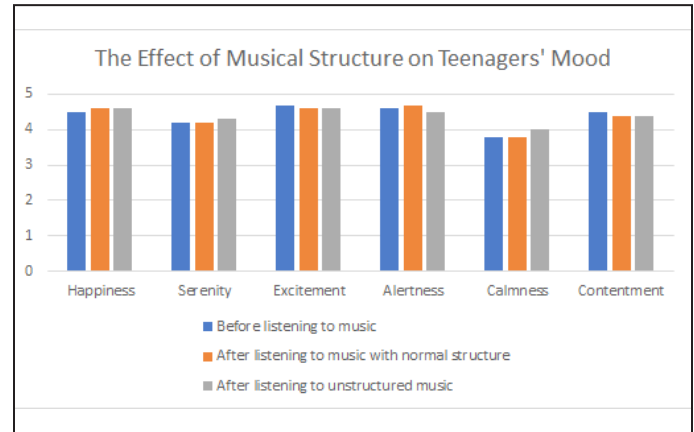
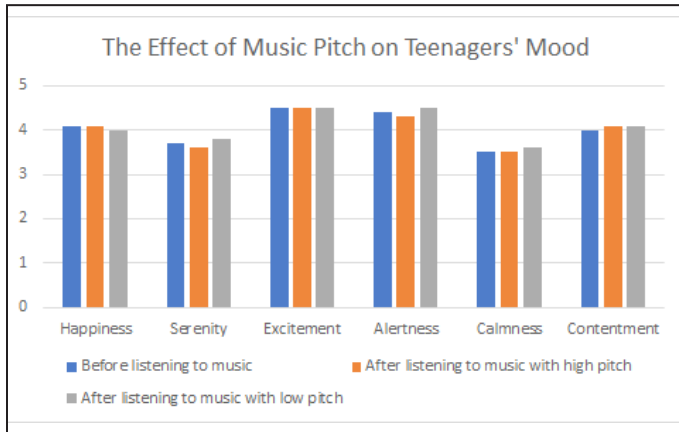
Results and Data Analysis

Results reported by survey participants were aggregated and statistical analysis was performed as described below. Table 1 below shows raw results for all participants for the effect of music in a major key on teenagers' mood.

	Sad vs. Happy				Stressed vs. Serene				Lethargic vs. Excited						
	Before Music	After Music	After - Before	Diff ²	Before Music	After Music	After - Before	Diff ²	Before Music	After Music	After - Before	Diff ²			
1	5	6	1	0.2	0.04	4	4	0	0.2	0.09	4	5	1	0.5	0.25
2	4	5	1	0.2	0.04	3	4	1	0.7	0.49	5	6	1	0.5	0.25
3	6	7	1	0.2	0.04	2	2	0	-0.3	0.09	2	2	0	-0.5	0.25
4	6	6	0	0.8	0.64	4	4	0	0.2	0.09	1	2	1	0.5	0.25
5	5	6	1	0.2	0.04	5	7	2	1.7	2.89	4	4	0	0.5	0.25
6	4	4	0	-0.8	0.64	1	2	1	0.7	0.49	5	7	2	1.5	2.25
7	4	6	2	1.2	1.44	5	4	-1	-1.2	1.69	9	9	0	-0.5	0.25
8	3	5	2	1.2	1.44	6	6	0	0.2	0.09	6	7	1	0.5	0.25
9	5	4	-1	1.8	3.24	4	5	1	0.7	0.49	4	5	1	0.5	0.25
10	4	3	-1	-1.8	3.24	3	3	0	-0.3	0.09	2	3	1	0.5	0.25
11	5	5	0	-0.8	0.64	3	3	0	-0.3	0.09	7	8	1	0.5	0.25
12	3	3	0	0.8	0.64	2	3	1	0.7	0.49	4	4	0	0.5	0.25
13	4	3	-1	-1.8	3.24	4	5	1	0.7	0.49	3	4	1	0.5	0.25
14	1	2	1	0.2	0.04	1	2	1	0.7	0.49	5	5	0	-0.5	0.25
15	2	5	3	2.2	4.84	5	5	0	-0.3	0.09	6	7	1	0.5	0.25
16	8	8	0	0.8	0.64	6	5	-1	1.3	1.69	5	4	-1	1.5	2.25
17	4	5	1	0.2	0.04	8	8	0	-0.3	0.09	7	8	1	0.5	0.25
18	5	5	0	-0.8	0.64	1	2	1	0.7	0.49	3	4	1	0.5	0.25
19	5	7	2	1.2	1.44	6	6	0	0.2	0.09	4	4	0	0.5	0.25
20	6	5	-1	1.8	3.24	4	4	0	0.2	0.09	6	7	1	0.5	0.25
21	2	4	2	1.2	1.44	3	3	0	-0.3	0.09	4	2	-2	1.5	2.25
22	1	2	1	0.2	0.04	1	1	0	-0.3	0.09	4	5	1	0.5	0.25
23	8	9	1	0.2	0.04	5	4	-1	1.3	1.69	1	2	1	0.5	0.25
24	7	6	-1	1.8	3.24	2	3	1	0.7	0.49	9	8	-1	1.5	2.25
25	5	6	1	0.2	0.04	8	8	0	-0.3	0.09	4	3	-1	0.5	0.25
26	4	4	0	-0.8	0.64	8	9	1	0.7	0.49	6	7	1	0.5	0.25
27	4	7	3	2.2	4.84	4	4	0	0.2	0.09	5	5	0	0.5	0.25
28	3	4	1	0.2	0.04	2	2	0	-0.3	0.09	3	3	0	-0.5	0.25
29	6	9	3	0.8	0.64	7	4	-3	-2.3	5.29	5	4	-1	-1.5	2.25
30	2	4	2	1.2	1.44	5	6	1	0.7	0.49	5	6	1	0.5	0.25
31	4	5	1	0.2	0.04	2	3	1	0.7	0.49	6	5	-1	1.5	2.25
32	2	3	1	0.2	0.04	4	4	0	-0.3	0.09	2	2	0	-0.5	0.25
33	4	6	2	1.2	1.44	8	9	1	0.7	0.49	5	5	0	-0.5	0.25
34	5	5	0	2.8	7.84	3	4	1	0.7	0.49	7	7	0	0.5	0.25
35	5	6	1	0.2	0.04	2	3	1	0.7	0.49	6	8	2	1.5	2.25
36	6	7	1	0.2	0.04	4	5	1	0.7	0.49	5	6	1	0.5	0.25
37	2	3	1	0.2	0.04	6	5	-1	-1.2	1.69	7	6	-1	-1.5	2.25
38	5	5	0	2.2	4.84	5	4	-1	1.3	1.69	2	2	0	0.5	0.25
39	4	6	2	1.2	1.44	4	4	0	0.2	0.09	8	7	-1	1.5	2.25
40	1	4	3	1.2	1.44	4	6	2	1.7	2.89	2	3	1	0.5	0.25
41	4	4	0	-0.8	0.64	3	5	2	1.7	2.89	5	5	0	-0.5	0.25
42	5	5	0	0.2	0.04	2	2	0	0.2	0.09	3	4	1	0.5	0.25
43	5	4	-1	-1.8	3.24	6	7	1	0.7	0.49	9	7	-2	-2.5	2.25
44	2	2	0	-0.8	0.64	4	4	0	-0.3	0.09	2	3	1	0.5	0.25
45	8	9	1	0.2	0.04	3	4	1	0.7	0.49	5	6	1	0.5	0.25
46	7	7	0	0.8	0.64	3	2	-1	1.3	1.69	7	7	0	0.5	0.25
47	4	6	2	1.2	1.44	7	7	0	-0.3	0.09	6	8	2	1.5	2.25
48	1	5	4	1.2	1.44	5	5	0	-0.3	0.09	4	5	1	0.5	0.25
49	5	7	2	1.2	1.44	2	3	1	1.3	1.69	5	7	2	1.5	2.25
50	6	6	0	0.8	0.64	3	2	-1	1.3	1.69	6	5	-1	1.5	2.25
51	5	6	1	0.2	0.04	1	2	1	0.7	0.49	5	7	2	1.5	2.25
52	4	7	3	2.2	4.84	1	1	0	-0.3	0.09	8	7	-1	0.5	0.25
53	1	2	1	0.2	0.04	5	3	-2	2.3	5.29	5	3	-2	1.5	2.25
54	2	3	1	0.2	0.04	4	4	0	0.2	0.09	4	6	2	1.5	2.25
55	5	4	-1	-1.8	3.24	5	6	1	0.7	0.49	5	6	1	0.5	0.25
56	4	5	1	0.2	0.04	2	2	0	-0.3	0.09	2	4	2	1.5	2.25
Mean:	4.304	5.07	0.768	1.86	3.929	4.18	0.268	0.79	4.804	5.2	0.394	1.65			

The following charts show aggregate results of effects of different music attributes on teenagers' mood.





Statistical Analysis

□ Statistical significance paired t - tests were performed on all collected data for each music attribute and t-values were calculated according to the

following formula
$$t = \frac{m - \mu}{s/\sqrt{n}}$$

where μ is a mean of the before listening to the music values, m is the mean of the after listening to the music values, s is the standard deviation of the differences between the two observations on each pair, and n is the number of participants. Corre-

sponding p-values (probabilities that that the results from collected data after listening to music occurred by chance) were found based on the t-distribution table and degree of freedom, where degree of freedom is $df=n-1$.

➤ Specifically, for the effect of the music in a major key on happiness:

- Variance is $s^2 = \frac{\sum_1^n diff^2}{n} = \frac{\sum_1^{56} diff^2}{56} = 1.86$

- Standard deviation is $s = \sqrt{1.86} = 1.36$

- *T-value* is computed as follows:

- $t = \frac{5.070 - 4.303}{1.36/\sqrt{56}} = 3.218$

- The degree of freedom is $df = n - 1 = 56 - 1 = 55$

- According to the t-distribution table *P-value* is 0.001, which means that there is only 0.1% probability that the results from this experiment happened by chance.

Similarly, the t- and p- values were calculated for the effect of all the music attributes on all mood characteristics such as happiness, serenity, excitement, alertness, calmness, and contentment (see Table 2 below for their p-values).

Table 2: P-values of Effects of various Music Attributes on Mood Characteristics						
	Happiness	Serenity	Excitement	Alertness	Calmness	Contentment
Music in a major key	0.001	0.01	0.003	0.002	0.008	0.008
Music in a minor key	0.01	0.06	0.001	0.005	0.08	0.001
Music with positive lyrics	0.001	0.025	0.005	0.001	0.005	0.007
Music with negative lyrics	0.005	0.008	0.001	0.05	0.008	0.01
Music with fast tempo	0.005	0.0025	0.001	0.001	0.01	0.008
Music with slow tempo	0.01	0.8	0.0025	0.03	0.08	0.06
Music with high pitch	0.2	0.2	0.1	0.25	0.25	0.1
Music with low pitch	0.1	0.25	0.2	0.1	0.2	0.2
Music with clear structure	0.1	0.1	0.2	0.2	0.2	0.1
Music without clear structure	0.1	0.3	0.1	0.25	0.1	0.5

Conclusion

Results reported by research participants demonstrate strong correlation between mood of teenagers and three music attributes: major key, fast tempo, and positive lyrics. Music in major key was shown to result mostly in positive shift of the participants' mood in all tested categories: happiness (p-value of 0.001), stress (p-value of 0.001), excitement (p-value of 0.001), alertness (p-value of 0.001), tension (p-value of 0.001) and contentment (p-value of 0.001). Similar, though slightly weaker, correlation was observed for the impact of positive lyrics and faster tempo. No strong correlation was observed between mood and other tested music attributes such as pitch and structure.

Significance and Real-Life Application

These results can be leveraged in order to develop applications that therapists who practice cognitive behavioral therapy (CBT) can give to their teenage patients in addition to other CBT tools, such as worksheets.

Potential application experience could include suggesting music aligned with tastes of the teenager undergoing therapy and with recommendations of the therapist. The same application could be also used in self-directed mode, without a therapist's direction. It can be useful for CBT in particular, due to CBT's very structured approach to addressing problems and its utilization of exercises outside of treatment sessions.

Also, unlike medications such as anti-depressants, music therapy is entirely unobtrusive, which might make families want to try this solution first before starting medication. In addition, the approach used in this research can be leveraged in other research projects focused on other music attributes.

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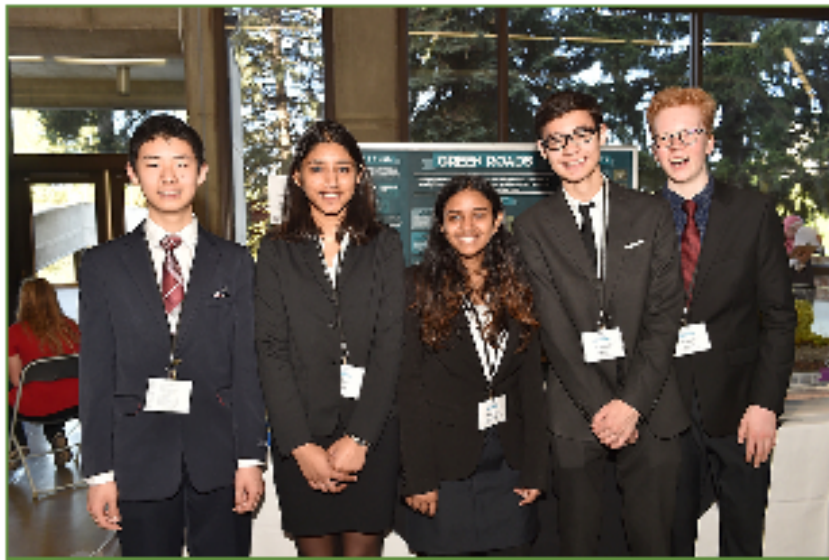
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Achievements/Awards

Imagine Tomorrow 2018: First Place

McKinstry Built Environment Challenge, Technology Approach



Maxwell Wang, Anika Chelani, Rishika Veeramachaneni, Mathias Foster, Tristan Stevens

Verizon App Challenge: Best in Region, Best in State, Best in Nation - Spring 2017

“Take Me There App”

Ayan Gupta, Rudy Banerjee, Sid Chandrasekar, Stephen Yamasaki, Rachel Oommen, Rhea Shinde and Tristan Stevens

Intel International Science and Engineering Fair (ISEF) - May 2017

NASA Special Award

1st place - Apoorv Khandelwal, *“Molecular Dynamics Simulation and Experimental Fabrication of Nanoporous Graphene Membranes for Optimal Water Permeability in Reverse Osmosis Desalination.”*

Cellular and Molecular Biology

3rd place – Vaishnavi Phadnis, *“Neurotrophin Receptor Isoform Expression Analysis in Breast Invasive Carcinoma: Potential Considerations for Targeted Therapy and Precision Medicine.”*

Computational Biology and Bioinformatics

3rd place - Neha Hulkund, *“The Application of Machine Learning Algorithms on EEG Data to Predict and Detect Epileptic Seizures.”*

SAMVID Education Foundation Special Award, Second Agni Award

Neha Hulkund, *“The Application of Machine Learning Algorithms on EEG Data to Predict and Detect Epileptic Seizures.”*

Technology Student Association (TSA) - National Top 10 High Schools - July 2017

4th place - Coding

4th place - Engineering Design

1st place - Music Production

American Association of University Women (AAUW) Awards for Outstanding Female Students in the World of STEM

Math - Alexia Friedman

Science - Lauren Kim

Technology - Christine Lee

Central Sound Regional Science and Engineering Fair (CSRSEF)

Behavioral and Social Sciences (BEHA)

1st place - Catherine Benson, *“The Effects of Changing Behavioral Tendencies Toward a Person with Schizophrenia or Psychosis NOS”*

2nd place - Claire Whiteside, *“Perceived Credibility of Political Advertisements on Facebook”*

2nd Place - Kyra Bethune, *“Incorporation of Self-Esteem Enhancement Strategies in Social Media”*

Honorable Mention - Toma Itagaki and Eric Yin, *“Determining the Effects of Psychological States on High Schooler’s Performance in Chess”*

Biochemistry, Cellular and Molecular Biology, Microbiology (CELL)

1st place - Sarah Suhy, *“Siderocalin and Its Interactions with Adipocytes”*

2nd place - Eudoxie Hudry and Nisha Thottam, *“Improving the Production of the Anti-Cancer Drug Paclitaxel Through Biosynthesis in Bacteria”*

Biomedical Engineering, Material Science (ENBM)

1st place - Ishika Mukherjee and Eesha Murali, *“Viability of Urinary Polyamine levels as a Preliminary Test for Cancer”*

Honorable Mention - Sarah Berg, *“Dielectric Elastomers in 3-D Printed Prosthetic Fingers”*

Biomedical and Health Sciences (BMED), Translational Medicine (TMED) and Chemistry (Chem)

2nd place - Sashwatha Shankar, *“Generating a Machine Learning Model to Determine the Metastatic Potential of Lung Cancer Tumors”*

Computational Biology & Bioinformatics (CBIO)

3rd place - Amrita Narasimhan, *“Applying Machine Learning Techniques for Identification of Discriminative Biomarkers”*

Earth and Environmental Sciences (ENEV)

Honorable Mention - Ritika Iyer, *“The Application of Infrared Thermography to Architecture to Identify Heat-Emitting Building Materials”*

Honorable Mention - Betty Park and Emma Drapp, *“The Study of Air Quality Conditions in Relation to Renal Transplants Through a Data Analysis Approach”*

Embedded Systems, Robotics and Intelligent Machines (ROBO)

1st place - Neha Hulkund, *“MRI Multiple Sclerosis Lesion Segmentation Using Deep Learning”*

3rd place - Jack Tribolet and Yukito Shida, *“Generating Haptic Feedback Based Upon Calculated Data and User Information”*

Engineering Mechanics (ENMC), Environmental Engineering (ENEV)

Honorable Mention - Niranjana Sahi and Aditya Balasubramanian, *“Augmented Malleability in Pipeline Joints for Improved Resistance to Seismic Ground Forces”*

Physics and Astronomy (PHYS), Mathematics (Math)

1st place - Aashna Sheth, *“The Application of Microwave Satellite to the Statistical Hurricane Intensity Prediction Scheme (SHIPS)”*

Systems Software

2nd place - Jeremy Yang, *“Pop Dungeon”*

Special Category Awards:

Tableau Award - Claire Whiteside, *“Perceived Credibility of Political Advertisements on Facebook”*

Aashna Sheth, *“The Application of Microwave Satellite to the Statistical Hurricane Intensity Prediction Scheme (SHIPS)”*

Excellence in Scientific Research (\$500) - Ritika Iyer, *“The Application of Infrared Thermography to Architecture to Identify Heat-Emitting Building Materials”*

NASA Earth Systems Science Award - Betty Park and Emma Drapp, *“The Study of Air Quality Conditions in Relation to Renal Transplants Through a Data Analysis Approach”*

Bellevue College scholarship (\$1,000) – Samuel Thornton, *“Making Self-Regulation Tools Available to All*

Students”

Association for Women Geoscientist’s Award - Aashna Sheth, “*The Application of Microwave Satellite to the Statistical Hurricane Intensity Prediction Scheme (SHIPS)*”

Intel Award, Excellence in Computer Science (\$200) - Neha Hulkund, “*MRI Multiple Sclerosis Lesion Segmentation Using Deep Learning*”

American Chemical Society (ACS) Prize - Ishika Mukherjee and Eesha Murali, “*Viability of Urinary Polyamine levels as a Preliminary Test for Cancer*”

Supernova Awards - Claire Maurer, “*Encoding Quantifiable Data for Nose-to-Finger Test with Essential Tremor*”

American Meteorological Society Award - Aashna Sheth, “*The Application of Microwave Satellite to the Statistical Hurricane Intensity Prediction Scheme (SHIPS)*”

US Metric Associations Award - Niranjana Sahi and Aditya Balasubramanian, “*Augmented Malleability in Pipeline Joints for Improved Resistance to Seismic Ground Forces*”

2018 Northlake Solo & Ensemble Festival

1st Alternate on Bassoon - Pinakin Kanade

1st Alternate on Clarinet - Ashwin Naresh

Future Business Leaders of America (FBLA) District/Region Competition

Advertising

1st place - Emma Drapp

Agribusiness

6th place - Aran Punniamoorthy

Banking and Financial Systems

4th place - Larry Wang and Fred (Frederick) Qin

Broadcast Journalism

1st place - Catherine Oei, Rayan Krishnan and Aakash Ramachandran

3rd place - Amrita Narasimhan and Sonika Tayade

Business Ethics

1st place - Salil Kanade and Prerana Kulkarni

3rd place - Audrey Tseng and Larry Wang

4th place - Catherine Oei and Ramya Kunapalli

5th place - Aashna Sheth, Ila Sharma and Amrita Narasimhan

Client Service

2nd place - Brandon Wui

4th place - Mihika Vankamamidi

Computer Problem Solving

1st place - Lawrence Atienza

5th place - Kevin Yap

Cybersecurity

4th place - Pranav Sukumar

Database Design and Application

1st place - Kevin Yap

3rd place - Tyler Warden

4th place - Rishi Kavikondala

Digital Video Production

5th place - QingHui Xie, Rishika Veeramachaneni and Anika Ghelani

Economics

6th place - Tyler Warden

Electronic Career Portfolio

2nd place - Sonika Tayade

3rd place - Victoria Alkin

5th place - Ritika Iyer

Entrepreneurship

1st place - Siddharth Shende

3rd place - Ranveer Thind, Aakash Ramachandran and Rayan Krishnan

Financial Math

3rd place - Rishika Veeramachaneni

Global Business

6th place - Shreyas Kulkarni

Graphic Design

3rd place - Aditi Ekbote and Lahari Nidadavolu

5th place - Andrew Nguyen

6th place - Akshay Murthy and Ritika Iyer

Impromptu Speaking

6th place - Claire Maurer

Insurance and Risk Management

1st place - Fred Qin

6th place - Toma Itagaki

Introduction to Business

3rd place – Jiachi (George) Zhang

4th place - Akshay Murthy

Introduction to Business Presentation

1st place - Claire Yang, An Doan and Mihika Vankamamidi

Introduction to FBLA

2nd place - Deveshi Thakur

Introduction to Public Speaking

5th place - Siddharth Shende

6th place - Amrutha Srikanth

Job Interview

4th place - Rishi Kavikondala

Management Information Systems

1st place - Maxwell Wang and Ranveer Thind

Marketing

6th place - Sashwatha Shankar and Varun Venkatesh

Organizational Leadership

5th place - Aashna Sheth

Parliamentary Procedure

1st place - Audrey Tseng, Rayan Krishnan, Fred Qin, Amulya Paramasivam and Lawrence Atienza

Publication Design

4th place - Osbert Lee, Toma Itagaki and Goutam Krishnamoorthy

Public Service Announcement

3rd place - Ranveer Thind and Aakash Ramachandran

Public Speaking

6th place - Lauren Kim,

Website Design

5th place - Pranav Sukumar, Andrew Nguyen and Jack Tribolet

Word Processing

3rd place - Ritika Iyer

5th place - Supriya Baskar

6th place - Ajay Rajasekaran, Parum Misri and Varun Wescott

Future Business Leaders of America (FBLA) State Competition

Advertising

9th place - Emma Drapp

Broadcast Journalism

3rd place - Amrita Narasimhan and Sonika Tayade

5th place - Catherine Oei, Rayan Krishnan and Aakash Ramachandran

Business Ethics

1st place - Aashna Sheth, Ila Sharma and Amrita Narasimhan

Business Financial Plan

3rd place - Rishi Kavikondala and Aashna Sheth

Business Plan

2nd place - Sonika Tayade and Sashwatha Shankar

4th place - Siddharth Shende and Caleb John

Coding and Programming

1st place - Maxwell Wang

3rd place - Pranav Sukumar

Cybersecurity

10th place - Pranav Sukumar

Database Design and Application

5th place - Rishi Kavikondala

Electronic Career Portfolio

4th place - Ritika Iyer

6th place - Sonika Tayade

8th place - Victoria Alkin

Entrepreneurship

1st place - Siddharth Shende

4th place - Ranveer Thind, Aakash Ramachandran and Rayan Krishnan

Graphic Design

1st place - Akshay Murthy and Ritika Iyer

9th place - Andrew Nguyen

Introduction to Business Presentation

5th place - Claire Yang, An Doan and Mihika Vankamamidi

Introduction to FBLA Procedures

1st place - Deveshi Thakur

Introduction to Public Speaking

8th place - Siddharth Shende

Management Information Systems

1st place - Maxwell Wang and Ranveer Thind

Marketing

1st place - Sashwatha Shankar and Varun Venkatesh

Public Service Announcement

1st place - Ranveer Thind and Aakash Ramachandran

Website Design

4th place - Pranav Sukumar, Andrew Nguyen and Jack Tribolet

Word Processing

7th place - Ritika Iyer

Chapter Report Initiative Award – Tesla STEM High School

**Health Occupation Students of America – State Leadership Conference Future Health Professionals
Washington State**

Individual Awards

Dental Science

1st place - Niranjan Sahi

Extemporaneous Writing

5th place - Christine Lee

Healthy Lifestyle

3rd place - Sreejita Ghose

Prepared Speaking

3rd place - Graciela Lagraba

Researched Persuasive Writing

2nd place - Anusha Srivastava

Veterinary Science

4th place - Katherine Chalmers

Medical Terminology

2nd place - Vaishnavi Phadnis

Nutrition (knowledge test)

4th place - Lauren Kim

Nutrition (knowledge test)

5th place - Ananya Nandula

Dental Terminology (knowledge test)

2nd place - Niranjan Sahi

Medical Assisting

5th place - Eesha Murali

Team Awards

Biomedical Debate

1st place - Anika Ghelani, Rishika Veeramachaneni, Rachel Alwan, Anusha Srivastava

Community Awareness

5th place - Aditi Subramanyam and Daphanie Chan

Health Career Display

4th place - Daphanie Chan and Nitisha Gautam

Health Education

2nd place - Ayushi Desai and Nitisha Gautam

Medical Innovation – Existing

2nd place - Suhani Arora and Jessica Singh

3rd place - Eudoxie Hudry and Nisha Thottam

5th place - Ayushi Desai and Reeteka Kudallur

Medical Innovation - Original

2nd place - Anika Ghelani, Rishika Veeramachaneni, Sunya Mohammed

3rd place - Arundhati (Diya) Basu and Sameera Balijepalli

MRC Volunteer Recognition Award

Barbara James Service Award

2nd place – due to Jessica Singh

*Top three places advance to the National Conference in Dallas, Texas June 2018

Hunt theWumpus

Team: Wump.Us – Runners Up

1. Maxwell Wong
2. Siddharth Sherde
3. Surya Gorantla
4. Tony Li
5. Arnav Sacheti
6. Tristan Stevens

Name: Spice Girls – Runners Up

1. Swarthmika Kakivaya
2. Aryo Karai
3. Archi Faugno
4. Luca Chang
5. Tiger McDaniel
6. Eric Bethune

Name: Unicorn Ninjas - 2nd Place Best Implementation

1. Rachel Alwan
2. Pamela Cheema
3. Roshni Srikanth
4. Sowmya Pratipati
5. Diya Bajju
6. Amrutha Srikanth

Imagine Tomorrow Environmental Science Competition

Challenge Topic 1: The Itron Food, Energy, and Water Challenge – Design Approach

2nd Place

Title: “*Integrated Denitrifying Bioreactor and Edge of Field Monitoring System Application Through Federal Policy Initiatives*”

Team Members: Rachel Alwan, Anusha Srivastava, Esther Wang

Challenge Topic 1: The Itron Food, Energy, and Water Challenge – Multi-Approach

1st Place

Title: “*Toilets for Education*”

Team Members: Vidhi Jain, Aditi Joshi, Lahari Nidadavolu, Roshni Srikanth, Tarini Srikanth

Challenge Topic 2: The Boeing Aerospace and Transportation Challenge – Design Approach

1st Place

Special Award for Exceptional Teamwork

Title: “*Producing Biofuels and Preventing Eutrophication Through the Application of a Passive Filtration System Utilizing Localized Algae Blooms*”

Team Members: Ayushi Desai, Ayan Gupta, Daniel Shaikh, Rhea Shinde, Anna Whiteside

Challenge Topic 3: McKinstry Built Environment Challenge – Technology Approach

1st Place

Title: “*Implementing Hyperaccumulators and Pervious Concrete to Redesign Roads for the Phytoremediation of Runoff while Drastically Reducing Civil Infrastructure Construction Costs*”

Team Members: Mathias Foster, Anika Ghelani, Tristan Stevens, Rishika Veeramachaneni, Maxwell Wang

Lexus Eco Challenge

Operation Sustain, Washington

Team: Rayan Krishnan, Anne Lee, Parth Nain, Isaac Perrin, Fred Qin and Suchi Sridhar

Pacific Model United Nations State Competition

Best Large Delegation Award – Team Award

Best Delegate:

Graham Sabin (G20)

Siddharth Shende (ECOSOC)

Outstanding Delegate:

Spring Chenjp (African Union)

Shaurya Vashisth (ECOSOC)

Honorable Mention:

Anna Whiteside (ECOSOC)

Position Paper:

Tejus Krishnan (G20)

Rishika Veeramachaneni, (Joint Crisis Committee)

National Merit Finalists

Vaishnavi Phadnis, Anne L. Lee,

Jeffrey T. Cheng, Christina L. Goto, Rashida Hakim, Natali M. Kendal-Freedman, Neha S.Nagvekar,
Frederick L. Qin, Suchi S. Sridhar, Yury Sudzilouski, Anna D. Vasyura, Brandon W. Yue

National Merit Semi Finalists

Prerana J. Kulkarni, Parth Nain

National Center for Women Information Technology (NCWIT)

Honorees

Anne Lee, Thalia Tsai, Hamsa Shankar, Aashna Sheth, Daniela Shuman, Yogitha Sunkara

Sonika Tayade, and Audrey Tseng

Northwest Association for Biomedical Research Student Biology Expo

SMART 3D Printing and Design

1st place – Vaishnavi Phadnis and Rashida Hakim

3rd place – Michelle Yeh, Davina Lau, and Katherine Bo

Honorable mentions:

Brandon Stumpel and Devin McGlynn,

Megan Kodati, and Mimi Lee

Lab Research - Honorable mention

Christina Goto and Prerana Kulkarni

Neuroscience

2nd place – Anna Gimera and Donovan Hesselroth

PTSA Reflections

Music Composition, Award of Merit - Victoria Alkin, “*Crossing the Horizon*”

Technology Student Association (TSA) - State Competition

3D Animation

*3rd place - Vivek Gopalam, Jasarjan (Arjan) Singh

4th place - Daniela Shuman, Dharini Sribalaskandarajah

Biotechnology Design

*1st place - Victoria Alkin, Kushal Kedia, Jadrian Png, Thalia Tsai

*2nd place - Olivia Blevins, Goutam Krishnamoorthy, Audrey Tseng

Coding

*3rd place - Ranveer Thind, Maxwell Wang, Aditya Balasubramanian

Engineering Design

*1st place - Osbert Lee, Niranjana Sahi, Tej Sathe, Pranav Sukumar, William Wang

*3rd place - Ila Sharma, Dharini Sribalaskandarajah, Audrey Tseng

4th place - Kushal Kedia, Aakash Ramachandran, Varun Venkatesh

5th place - Ritika Iyer, Rishi Kavikondala, Lahari Nidadavolu, Daniela Shuman, Ranveer Thind

Essays on Technology

*2nd place - Thalia Tsai

Extemporaneous Speech

5th place - Victoria Alkin

Future Technology Teacher

*1st place - Daniela Schuman

Music Production

*3rd place - Victoria Alkin

Prepared Presentation

5th place - Pranav Sukumar

Promotional Design

*1st place - Akshay Murthy

*3rd place - Betty Park

SciVis

5th place - Rishi Kavikondala, Ryan Koshy, Aakash Ramachandran, Niranjana Sahi

Software Development

4th place - Matthew Saveliev, Daniela Shuman, Thalia Tsai

STEM Careers

*1st place - Ranveer Thind

*3rd place - Sashwatha Shankar

4th place - Varun Venkatesh

VEX Robotics

*3rd place - Ayan Gupta, Ryan Koshy, Akshay Murthy, Tristan Stevens

5th place - Mathias Foster, Maxwell Wang

*Top three place finishers qualify for the National Conference in Atlanta, June 2018

Washington Aerospace Scholars

Kushal Kedia

Jack McGuigan

Washington State Science and Engineering Fair (WSSEF) – 62nd annual

Science Teacher of the Year, (outstanding science or engineering teacher) - Kate Allender

Eudoxie Hudry and Nisha Thottam, “*Improving the Production of the Anti-Cancer Drug Paclitaxel Through Biosynthesis in Bacteria*”

- 1st place in Cellular and Molecular Biology Category, Senior Division
- Society for In Vitro Biology special category award

Ishika Mukherjee and Eesha Murali, “*Viability of Urinary Polyamine levels as a Preliminary Test for Cancer*”

- 1st place in Biomedical Engineering Category, Senior Division

Rayan Krishnan, “*Utilizing Idealized Simulations to Investigate the Impacts of Carbon Emissions on Polar Vortex*”

- 1st place in Environmental Sciences Category, Senior Division
- NASA Langley Research Center special category award, invited to present at NASA Virtual Science Colloquium

Amrita Narasimhan, “*Applying Machine Learning Techniques for Identification of Discriminative Biomarkers*”

- 1st place in Computational Biology and Bioinformatics Category, Senior Division
- US Army special category award in computer and biomedical sciences

Neha Hulkund, “*MRI Multiple Sclerosis Lesion Segmentation Using Deep Learning*”

- 1st place in Embedded Systems Category, Senior Division
- INTEL Excellence in Computer Science Award, (\$250)
- Ohio Wesleyan University Outstanding Achievement in Science Scholarship (\$20,000 renewable scholarship, total of \$80,000)
- US Army special category award in computer sciences
- Wolfram Research, Inc. Mathematica Certificate of Achievement Award
- Evergreen State College one quarter tuition scholarship
- Side note: was also pulled for ISEF judging and almost won a trip to Pittsburgh

Lukas Spring, “*Examining the Potential of Smile Photography and the Importance of the Maxillary Incisors in the Identification of Disaster Victims*”

- 2nd place in the Biomedical and Health Sciences Category, Senior Division

Anjali and Akash Srivastava, “*Building an App to Improve Reading Readiness of Children*”

- 1st place in Systems Software Category, Senior Division

Perna Sheokand, “*Blue Light Effects on Working Memory of Preschoolers*”

- 1st place in Behavioral and Social Sciences Category, Senior Division

Kyra Bethune, “*Incorporation of Self-Esteem Enhancement Strategies in Social Media*”

- 1st place in Behavioral and Social Sciences Category, Senior Division
- American Psychological Association Award

Sashwatha Shankar, “*Generating a Machine Learning Model to Determine the Metastatic Potential of Lung Cancer Tumors*”

- 1st place in Biomedical and Health Sciences, Senior Division
- Society for In Vitro Biology special category award

Sarah Suhy, “*Siderocalin and Its Interactions with Adipocytes*”

- 1st place in Cellular and Molecular Biology Category, Senior Division

Megha Mattikalli, “*Advancing Diagnosis of Lung Cancer using Visual Biomarkers*”

- 1st place in Computational Biology and Bioinformatics Category, Senior Division
- U.S. Army special category biomedical sciences category

Ritika Iyer, “*The Application of Infrared Thermography to Architecture to Identify Heat-Emitting Building Materials*”

- 1st place in Environmental Sciences category, Senior Division

Pranay Muthineni, “*Making Desalination Cost Effective and more Efficient for Third World Countries Using Solar Power*”

- 1st place Environmental Engineering Category, Senior Division
- IEEE - Seattle Section, computer science special category award, \$75

Christine Lee, “*The Efficacy of Counter-Stereotypical Scenario in Reducing Racial Implicit Bias*”

- 1st place in Behavioral and Social Sciences Category, Senior Division

Jack Tribolet, “*Generating Haptic Feedback Based Upon Calculated Data and User Information*”

- 1st place in Embedded Systems Category, Senior Division

Aashna Sheth, “*The Application of Microwave Satellite to the Statistical Hurricane Intensity Prediction Scheme (SHIPS)*”

- 1st place in Mathematics Category, Senior Division
- Office of Naval Research - U.S. Army/U.S. Marine Corps award
- Mu Alpha Theta Certificate of Achievement Mathematics award, \$75
- Sigma Xi, Scientific Research Society special category award
- U.S. Army special category award in computer science
- USDA Forest Service Pacific Northwest Research Station award, \$50
- American Meteorological Society special category award

Sonika Tayade and Hamsa Shankar, “*Predictions Using National Health and Nutrition Examination Surveys*”

- 1st place in Biomedical and Health Sciences Category, Senior Division

Jeremy Yang, “*Pop Dungeon*”

- 1st place in Systems Software Category, Senior Division

