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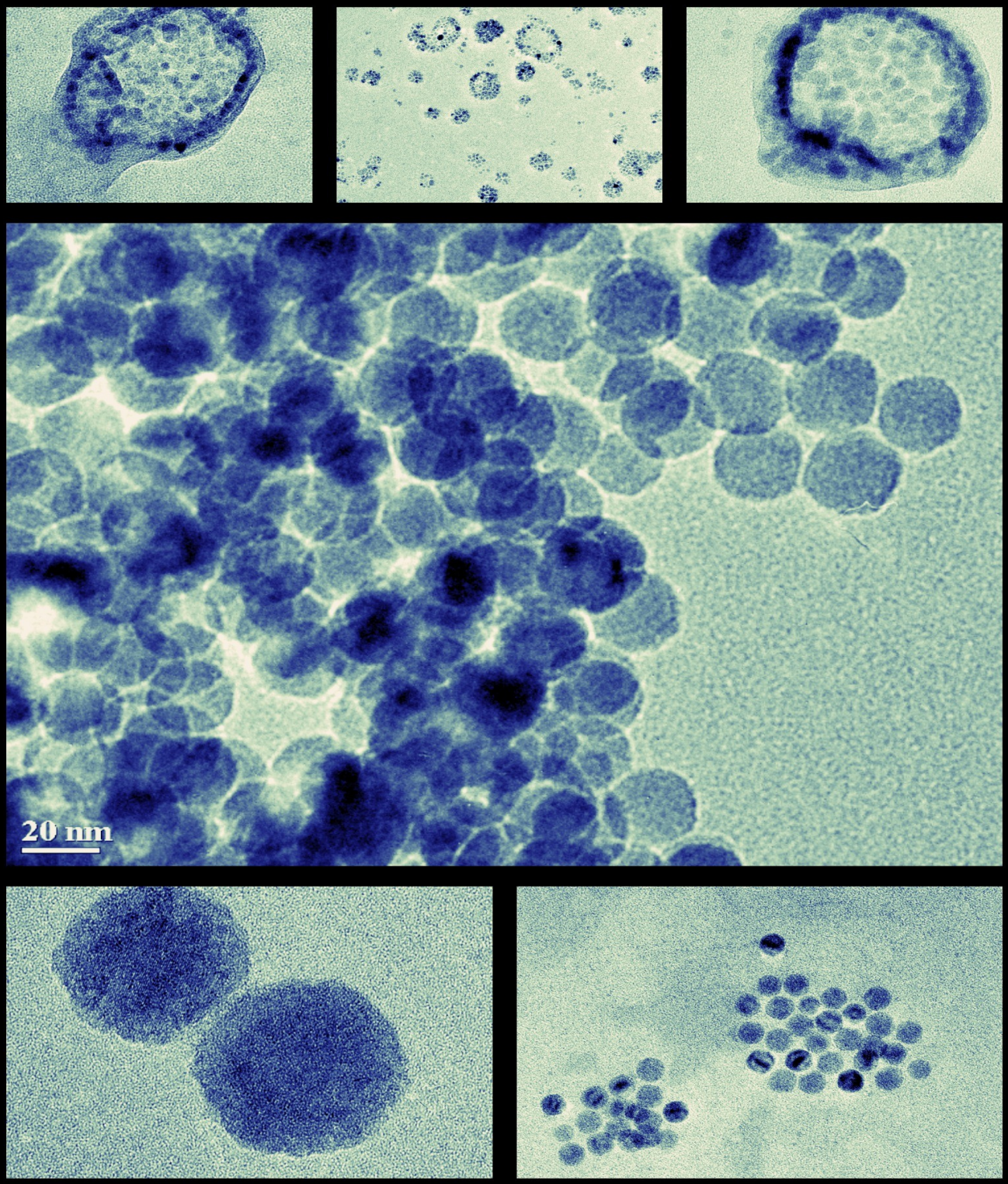
The Journal of Science and Health at the University of Alabama



May 2013 Volume 10

From The Editor

Dear Readers,

I am proud to announce that this is our tenth publication of *JOSHUA*. It is indeed an achievement that this journal has reached the close of its first decade and I am very proud to bring you this fine collection of scientific writing.

Research is certainly the backbone of science and I am extremely proud of our contributors. As a scientific researcher myself, I can attest to the countless hours spent in the lab documenting every observation and bit of data. These men and women are dedicated to the scientific community as a whole and to their respective institutions. I would like to be the first to say "Bravo".

When I became editor of *JOSHUA*, I wanted to ensure that I would not only continue the excellent legacy that had previously been established but I wanted to enhance the scope of this journal. My desire to reach outside our esteemed assemblage and gather scientific articles from other universities was meant as a means to forge strong bonds and collaborate with our colleagues. My inspiration is the continued growth of our journal into a comprehensive format that would impact you, the reader, by allowing you the opportunity to experience cutting edge undergraduate research. In doing so, my hope is to encourage young scientists to explore novel avenues of research.

It has been my distinct pleasure to work with such an amazingly talented group of researchers. And I wish to thank them for their contributions. I would also like to extend a special thank-you to Dr. Guy Caldwell, our faculty advisor. In addition, I want to thank the members of my editorial staff for their untiring efforts and dedication to this publication.

I sincerely hope that we have provided you with a most intriguing and educational read.

Sincerely,
Jonathan R. Belanich
Editor in Chief

About the Cover

The TEM images, taken by Amanda Glover on the TEM in the Bevill building at the UA campus, show proof of concept for many of our nanoparticle experiments. The images of the particles alone give a size distribution as well as an idea of uniformity. The images of micelles show that particles can be entrapped inside of polymeric micelles. A similar idea goes for the micelles with drugs and magnetite loaded.

Image credit: Amanda Glover

Questions should be directed to joshua.alabama@gmail.com, and more information and all archived volumes are available at bama.ua.edu/~JOSHUA.

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Magnetic Particles with a Polycaprolactone Coating and preparation of Magnetic Micelles for Drug Delivery

M. Adam Beg[1], Amanda L. Glover[1], Jacqueline A. Nikles, Ph.D.[2], David E. Nikles, Ph.D.[1]

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Uniform, monodisperse, magnetic nanocrystals were created in a two-step synthesis giving an average size of 24 nanometers. 3-Aminopropyltrimethoxysilane was bound to the surface of single-crystal magnetite nanoparticles, giving a particle surface bristling with primary amine groups. A tin-catalyzed ring-opening polymerization of ε-caprolactone was initiated from the surface bound amines to give particles coated with covalently bound polycaprolactone. FT-IR and X-ray photoelectron spectra confirmed the presence of the polymer on the particle surface. The coated particles were incorporated into magnetic micelles made from poly(ethylene glycol-b-caprolactone) diblock copolymers. These particles were trapped in the semi-crystalline core of the micelles. The micelles were characterized by dynamic light scattering to determine their hydrodynamic radius. TEM imaging showed the average size for the micelle core and the number of magnetic particles in the core.

Introduction

Cancer is the second leading cause of death in the United States, causing 23% of the deaths recorded in 2009, a total volume of 567,628 deaths. Cancer is the leading cause of death in people aged 40 -79. In 2013, a total of 1,660,290 new cancer cases and 580,350 cancer deaths are projected to occur [4]. The three common methods of treating cancer are surgery, chemotherapy, and radiation. These methods have now been in practice for decades. Both chemotherapy and radiation harm normal somatic cells and can cause damage to critical tissues. When used in conjunction, the danger is even more apparent [3].

To prevent this damage, a more specific targeting of chemotherapeutic drugs is necessary. The objective of this project is to create a nanoscale vector for targeted cancer drug delivery. The method is to synthesize magnetic nanoparticles and to modify the surface chemistry of these particles. These modifications would allow for polymerization from the particle surface and would increase the ability to crystallize these particles with a chemotherapeutic agent inside a polymer micelle. The application of a magnetic pulse would theoretically allow for release of the drug at specific sites in the body.

The large-scale synthesis of monodisperse nanoparticles is possible with varying degrees of size control. The nanocrystals were synthesized through a thermal decomposition of a metal-oleate complex. Size can be influenced by solvent choice as well as reaction time [2]. The surfaces of these particles have been modified to adsorb polyethylene glycol to the surfaces as well as similar copolymers to change the way they are treated within the body [1]. The effect of polyethylene glycol (PEG) has been shown to delay and prevent opsonization and subsequent removal of nanoparticles and nanodevices by the body's immune response [5]. It was predicted that these properties could be used to create a magnetically loaded micelle for future uses in cancer drug delivery.

Methods

Iron Oleate Synthesis [2]

The preparation of the metal-oleate complex was completed by reacting iron(III) chloride with sodium oleate. Iron(III) chloride (10.8 g) and sodium oleate (36.5 g) were added to a round-bottom flask and dissolved in a mixture of ethanol (80 mL), hexane (140 mL), and distilled water (60 mL). The solution was heated in an oil bath at 70 ˚C for a period of 4

hours. After being cooled to room temperature, the organic layer containing the iron-oleate complex was washed three times with distilled water in a separatory funnel. The hexane was evaporated off and the complex was allowed to dry in a crystallization disk.

Magnetite Nanoparticle Synthesis [2]

Nanoparticles were synthesized from the thermal decomposition of the iron-oleate complex in solution with oleic acid. The iron-oleate complex (27 g) was added into a three-neck round-bottom flask with oleic acid (5.4 g) and dissolved in octadecene (200 g). The reaction was magnetically stirred and heated under nitrogen. The reaction was kept at reflux (320 ˚C) for a period of 30 minutes. The reaction was allowed to cool, and then was precipitated with ethanol and centrifugation to give a viscous black solution.

3-Aminopropyltrimethoxysilane Attachment

An exchange of 3-aminopropyltrimethoxysilane (APTMS) for oleic acid on the surface of the particles occurred at room temperature in solution. The magnetite solution (20 g) was dispersed in toluene (100 mL). APTMS (20 mL) was added, and the solution was magnetically stirred under nitrogen in a round-bottom flask. After 72 hours, the solution was precipitated with acetone and centrifugation, then reconstituted in toluene and precipitated with acetone three more times.

Polycaprolactone Surface Polymerization

APTMS-coated nanoparticles (1 g) were added to a three-neck round bottom flask with oleylamine (36 µL), mesistylene (8 mL), distilled caprolactone (8 mL), and 6 drops of a $Sn(Oct)_2$ catalyst. The flask was sonicated for 15 minutes, then a stirbar was added and the reaction mixture was heated to 110 ˚C while stirring under nitrogen. After 36 hours the heat source was removed and the reaction was allowed to cool. Dichloromethane was added to the black solution to reduce the viscosity. The solution was precipitated with ethanol and centrifugation.

Creation of Magnetic Micelles

Magnetic micelles were prepared by adding a solution of the diblock copolymer polyethylene glycol -b-caprolactone (10.0 mg) and iron oxide nanoparticles (1.0 mg) in tetrahydrofuran, THF, (filtered through 0.2 µm syringe filter) to ultrapure water dropwise with probe sonication. The THF was then allowed to evaporate overnight and the resulting solution was filtered through a 0.45 µm syringe filter and diluted to a known final volume of 10 mL with ultrapure water.

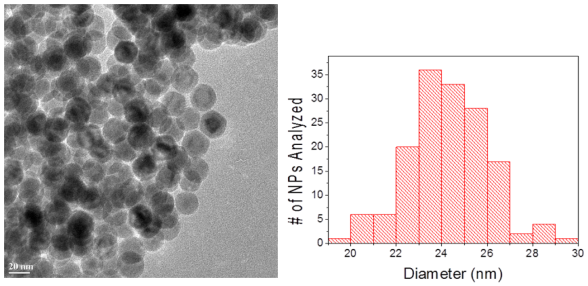

Figure 1. TEM of the magnetite particles with a size distribution chart.

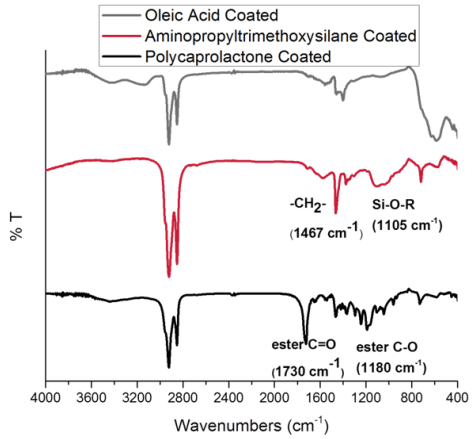

Figure 2. Infrared spectra of the magnetite particles with three different surfactants.

Results

The iron-oleate complex produced between 26 and 28 g of product. The magnetite reaction was scaled down appropriately. The reaction produced 500 mL of particles in solution. Transmission electron microscopy was used to image these particles, showing a mean size of around 24 nm (Fig. 1). The APTMS reaction gave a yield of 1.5 g of black particles, IR spectra comparison between the original magnetite and the APTMs nanoparticles confirmed the replacement (Fig. 2). Polymerization was quantified

by atomic absorption showing 4% iron content. The presence of the polymer was also confirmed by IR spectroscopy (Fig. 2). The magnetite was loaded into the micelles, and dialysis calculations gave an average of 30% loading. TEM imaged the magnetite within the micelles (Fig. 3).

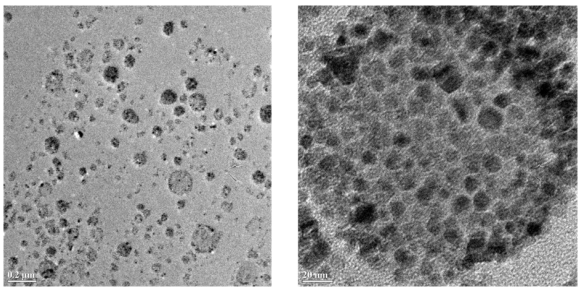

Figure 3. TEM of the magnetic micells show more than one micelle in a variety of sizes and a close up of one micelle showing multiple nanoparticles within the core of each micelle.

Discussion and Conclusion

The reaction to create magnetite is both reproducible and adjustable. Size control can be achieved with the use of different solvents and heat times. The reduction of the aliphatic carbon hydrogen bonding in the IR spectra as well as the disappearance of the oxygen indicates that the APTMS did replace oleic acid at the particle surface. Polycaprolactone can be polymerized from the particle surface, and these particles can be readily dispersed into copolymer micelles. Current experiments demonstrating drug loading and magnetic heating will show the viability of this concept for cancer treatment. A successful cancer treatment system such as this would have high drug loading with little to no drug release until induced by magnetic heating. In the pursuit of this goal, many variables must be adjusted to provide the ideal delivery vector.

A cancer drug delivery system could have multiple uses in cancer treatment. In addition to a targeted treatment for tumors, the magnetic properties of such a system could be used to detect malignant cancers through magnetic resonance imaging (MRI). With the metastasis of cancerous tumors currently leading to a steep decline in survival chances for patients, a way to clearly detect the spread of tumors should be very beneficial to cancer treatment. In addition, this type of drug delivery would reduce the sickness associated with chemotherapy, and the risks associated with surgery. Though cancer treatment research is far from the finish line, the progress is promising.

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Acknowledgments

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About the Author

Adam Beg is a Junior at the University of Alabama from Tuscaloosa, AL. He is double majoring in Chemistry and Biology with a minor in Computer-Based Honors. He has worked with Dr. Nikles for six years, and will continue for his final year of undergraduate studies. Adam is an officer of Gamma Sigma Epsilon, the chemistry honor society.

Characterizing the Magnetic Heating of Nanoparticles using Meltable Solids

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Iron oxide nanoparticles have potential applications in a magnetically-triggered drug delivery system for improved cancer therapy. To develop more accurate heat transfer and diffusion models for drug release, it is important to characterize the heating of these nanoparticles. High frequency alternating current (AC) magnetic fields were mapped to determine the field strengths and frequencies that could be generated and used for magnetic heating. Heating experiments were conducted on magnetic nanoparticles (MNPs) imbedded in polycaprolactone and other solids to estimate local temperatures and determine the specific absorption rate (SAR) of maghemite nanoparticles when placed in a magnetic field, which was applied by a magnetic induction coil. The effect of parameters such as concentration and field strength on the SAR was examined, and different methods of SAR calculation were compared.

Introduction

The adverse side effects associated with the use of chemotherapy drugs are well documented [1]. To combat these complications, a nanoscale, targeted, magnetically-triggered drug delivery system is being developed. In this system, diblock-copolymer micelles composed of hydrophobic polycaprolactone, (-PCL-), and hydrophilic polyethylene glycol (-PEG-) subunits are self-assembled around magnetic nanoparticles and chemotherapy drug molecules in solution (Figure 1). By applying a magnetic field to the micelles, the crystalline PCL core can be melted, thus releasing a specified dose of chemotherapy drug directly to cancerous cells [2]. Additionally, the melting point of the PCL nanocrystals in the micelle core corresponds with the temperature range for effective hyperthermia, whereby heat applied to a tumor site serves to kill cancerous cells [3]. Thus, this system would serve to provide combination chemotherapy/hyperthermia therapy to cancer patients.

In addition to the ability to apply chemotherapy and hyperthermia therapy to cancer cells, targeting ligands placed on the micelle are designed to improve the localization of the therapy after injection (which reduces the harmful effect on healthy cells and the affiliated side effects) [4]. The rapid angiogenesis associated with cancerous formation often leads to an increased permeability of the tissue's microvascular system [5]. This enhanced permeation and retention effect in combination with the addition of a targeting ligand to the micelles results in improved targeting effectiveness in the delivery of chemotherapy drugs to cancerous tissue.

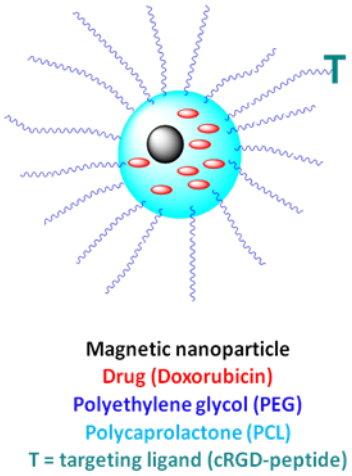

Figure 1. Magnetic micelle for targeted chemotherapy drug delivery.

This paper is focused on understanding the heat that can be generated by the MNPs placed inside the micelle. By characterizing the nanoparticles that will be utilized in this targeted drug delivery system, more accurate heat transfer and diffusion models can

be developed that will aid in better predictions of how drug release is activated. An understanding of the heating properties of the MNPs is imperative in micelle design, as this will provide insight into the number of nanoparticles needed in each micelle to activate drug release by melting the PCL core, as well as the quantity of MNPs necessary to provide adequate hyperthermia to the surrounding tissue.

Methods and Materials

Magnetic field mapping

Prior to performing heating experiments, the magnetic fields generated by the magnetic induction coils were mapped using a field probe specifically designed for high frequency coils (AMF Life Systems, Rochester, MI). Calculations were performed to determine the generated magnetic field strength as a function of the voltage supplied to the coil for several axial positions from the top to the bottom of the coil (Figure 2).

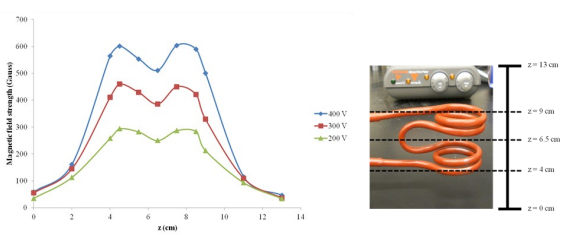

Figure 2. Left: magnetic field map for petri-dish coil as a function of z position. Right: illustration of z positions in relation to coil.

Investigating maximum local temperature

There were two challenges to measuring temperature during heating experiments. For one, the magnetic field interferes with most thermocouples and thermometers, so either an infrared camera (IR Thermacam, FLIR Technologies, Portland, Oregon) or an optical probe (Optotemp Fluorescent Probe, Ocean Optics, Dunedin, FL) were used to measure temperatures. Second, temperature measurement on a nanometer scale is very challenging, and the measured values represent the bulk temperature of the solution or material being heated (rather than the nanoparticles themselves). Because the temperatures of nanoparticles may be significantly higher than the surrounding solution, an experiment was designed to indirectly measure the temperatures generated on the surface of maghemite (γ-Fe_2O_3) nanoparticles in the magnetic field. For this experiment, composite samples were produced that contained both maghemite nanoparticles and various organic compounds with known melting points (Table 1).

Compound	Melting point
Polycaprolactone (-PCL-) 2000	41-51°C
Parafilm	~60 °C
Dimethyl isophthalate	66-68 °C
Naphthalene	79-80 °C
Adipic acid	~152 °C
Camphor	179.75 °C

Table 1. Organic compounds utilized in local MNP temperature experiments.

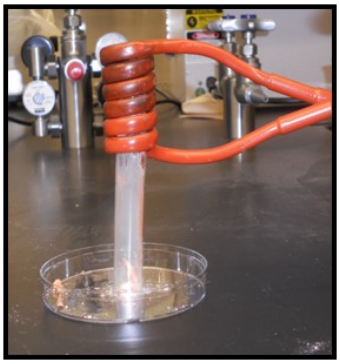

Figure 3. Melting time experiment using adipic acid/ maghemite nanoparticle sample in 6-turn test-tube coil.

Figure 4. Heating slope experiment setup. IR thermacam is focused on sample from above.

It was hypothesized that the melting of the composite sample in the magnetic field could serve as an indication that the local temperature of the nanoparticles was reaching the melting point of the solid, even if the IR thermacam reading indicated that

the sample surface had not reached that temperature. The final experimental setup consisted of the mixed PCL/MNP sample being solidified in a test tube and then placed into the induction coils. This test tube design allowed for the use of crystalline or brittle solids and enabled easy mixing of the nanoparticles in the molten compound. Visual confirmation of melting could be observed for each sample, indicating that the surface temperature of the nanoparticles exceeded the melting point of the substance and providing quantitative evidence that the power generated within the nanoparticles was large enough to overcome the heat of fusion (melting) for each substance.

Melting time vs. heating slope technique: estimating specific absorption rate

A series of experiments were designed to test methods of estimating the SAR of the maghemite nanoparticles being considered for use in the targeted drug delivery system. The SAR serves as a measurement of the power dissipated by the nanoparticles in the AC magnetic field, thus providing a standardized way to compare the effectiveness of different varieties of nanoparticles. In this set of experiments, a ceramic saddle was imbedded into each composite sample of PCL and maghemite nanopowder that was tested.

The coils were used to apply a magnetic field to each inverted sample, and the time elapsed before the saddle fell out of the sample was recorded, providing an estimate of the time it took for the entire polymer sample to melt. This melting time was then used in the following equations, providing an estimate of the SAR (W/g) for the nanoparticles in that particular field:

$$\Delta U = m_{comp}\Delta H_f + m_{comp}c_p(T_{melting} - T_{initial})$$

Equation 1

$$rate\ of\ energy\ input = \frac{\Delta U}{t_{melt}}$$

Equation 2

$$SAR = \frac{rate\ of\ energy\ input}{m_{MNPs}}$$

Equation 3

Where ΔU is the internal energy change (J), m_{comp} is the mass (g) of the composite's main component (in this case PCL), ΔH_f is the heat of fusion for PCL (J/g), C_p is the heat capacity of PCL $(\frac{J}{g*K})$, $T_{melting}$ is the melting point of PCL (ºC), $T_{initial}$ is the initial temperature of the sample (ºC), t_{melt} is the time (s) that it takes for the entire sample to melt, and m_{MNPs} is the mass (g) of nanoparticles included in the sample.

The resulting SAR estimate was compared against SAR values calculated using the heating slope method for PCL/MNP composites (Figure 5) according to the following equation:

$$SAR = \frac{m_{soln}c_p\left(\frac{dT}{dt}\right)}{m_{MNPs}}$$

Equation 4

Where m_{soln} is the mass of the total solution and $\frac{dT}{dt}$ is the slope of the temperature versus time plot immediately following the melting of the composite sample.

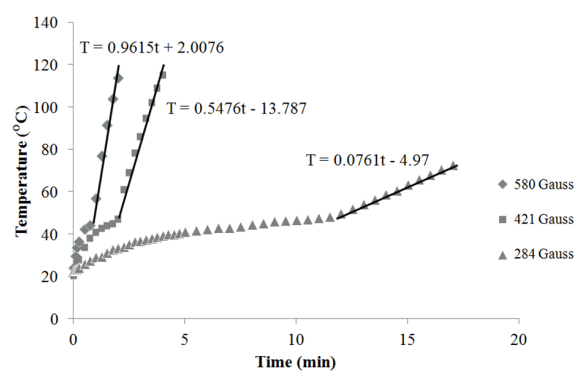

Figure 5. Heating curve for 10 mg/mL maghemite in PCL 2000 subjected to magnetic field applied by petri-dish coil. A linear fit was applied to each curve post-melting, and the resulting slope was used in SAR calculations.

Results and Discussion

Samples containing two weight percent maghemite nanoparticles were successfully melted for each of the tested compounds. The highest melting compound, adipic acid, which melts at a temperature of approximately 152 ^{0}C, entered the liquid phase in a 590 Gauss magnetic field within five minutes. Thus, these initial studies indicate that the surface temperature of these nanoparticles can reach at least 152 ^{0}C in our applied magnetic field.

In the SAR estimation experiments, the melting time and heating slope calculation methods were found to yield SAR values on the same order of magnitude (Table 2). Certain trends were noted upon changing the parameters of the experiment: the power absorbed by the nanoparticles increased with increasing field strength, and stayed relatively constant with changing nanoparticle concentration and differing calculation methods. In addition, we found that the initial slope method could be applied to the nearly linear temperature versus time plot acquired immediately following the melting of a sample, rather than the exponential curve found upon first heating the solid sample.

Sample Concentration (mg/mL)	Magnetic Field Strength (Gauss)	Calculation Method	SAR (W/g nanopowder)
1	590	Melting time	167-183
		Heating slope	121
10	284	Heating slope	10.8
	421		77.9
	590		137
20	590	Melting time	118-169
10	590	Melting time	163
20	590		134
40	590		147

Table 2: Representative SAR data for heating of maghemite-PCL nanocomposites.

Conclusion

The melting time method of estimating SAR was found to be comparable (same order of magnitude) with the SAR found by the heating slope method. It was found that the heating slope method could be applied to the temperature versus time slope immediately post-melting, allowing for a linear rather than exponential fit to the data. These results support the use of several relatively simple methods of comparing the heating effectiveness of various nanoparticles; however, we found the heating slope method to provide the most readily reproducible results. The investigation of these calculation methods serves to help determine the best candidate for applications in a nanoscale, magnetically-triggered drug delivery system.

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About the Author

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Diblock Copolymer Coated Magnetite Nanoparticles as Targeted, Magnetically Triggered Drug Delivery Devices for Cancer Therapy

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Magnetite nanoparticles (24 nm diameter) with a semi-crystalline diblock copolymer corona were studied for application to a new anticancer drug delivery system. The magnetic nanoparticles were synthesized through the decompositon of iron(III) oleate in refluxing 1-octadecene. To create the corona, the alcohol group at the terminus of the polycaprolactone block of a poly(ethylene glycol-caprolactone) diblock copolymer was reacted with 3-isocyanatopropyltrimethoxysilane to give a silane coupling agent bound to the polymer by a urethane linkage. When these silane-terminated polymers bind to and coat the surface of 24 nm diameter magnetite nanoparticles, they can form a corona and crystallize, which allows for the entrapment of anticancer drug in the center. A sharp peak at 1725 cm^{-1} in the IR spectrum for the polymer coated particles indicated the polycaprolactone block was crystalline. Dynamic light scattering of these particles dispersed in water showed the average hydrodynamic radius was 28 nm. Use of a drug analog showed that the particles successfully encapsulate drug in the hydrophobic polycaprolactone interior, and that the amount encapsulated is directly proportional to the concentration of the diblock copolymer coated magnetic nanoparticles present.

Introduction

Medusa Particles are the drug delivery devices of a nanoscale, targeted, magnetically triggered drug delivery system for cancer chemotherapy. This new cancer treatment mechanism will allow for improved treatment of cancer while reducing the side effects experienced by patients. The system will function by having a drug trapped in a particle that can be delivered to a targeted cancerous site (through attachment of a ligand) where the drug will be released from the semi-crystalline particle after application of a radio frequency ac magnetic field causes the crystalline corona to weaken and release its hydrophobic contents from the amorphous interior. This semi-crystalline delivery particle, the Medusa Particle, consists of a magnetic nanoparticle core, which is attached to a crystalline polycaprolactone shell by polyethylene glycol and a silane linker, to a yield a poly(ethylene glycol-*b*-caprolactone) diblock copolymer. The magnetic nanoparticles used have been determined to have a radius of 24 nm; dynamic light scattering has shown that attachment of the diblock copolymer increases the hydrodynamic radius to 28 nm (Figure 1), confirming attachment of the polymer to the nanoparticle. This was further proven by characterizations by ^{1}H NMR and IR. The crystallinity of the poly(ethylene glycol) block was indicated by a peak at 1725 cm^{-1} in the IR spectrum [9]. The semi-crystallinity of these particles is significant because the amorphous interior allows for the encasement of non-polar anticancer drug (such as Doxorubicin), but the crystalline exterior provides the structure for entrapping the drug to prevent leakage of the drug into the bloodstream before its intended release at the cancerous site. The hydrophilic polyethylene glyocol allows for the dispersal of the Medusa Particles into the bloodstream while the hydrophobic polycaprolactone keeps the anticancer drug encapsulated.

Micelles with a magnetic core are currently employed in medical diagnostic imaging and are being investigated for drug delivery [9]. However, the concentration of nanoparticles in each micelle cannot be controlled during micellar assembly. By creating a particle that has a set volume (it depends only on the radius of a single micelle and its attached diblock copolymers) the amount of drug released by each particle can be more precisely known than if a micelle were used. Additionally, micelles use multiple nanoparticles as the core, whereas Medusa Particles have only one nanoparticle as the core. Use of Medusa Particles will reduce the amount of ferrous oxide needed for the treatment which will reduce the dosage of the nanoparticles needed for drug delivery.

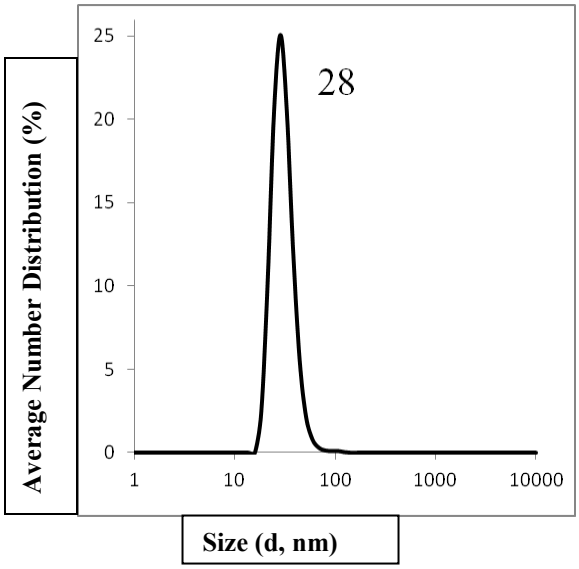

Figure 1. Dynamic light scattering distribution of the Medusa Particles, showing an average hydrodynamic radius of 28 nm.

The intended release mechanism will employ a radio frequency ac magnetic field, which will potentially operate in a manner similar to an MRI. The patient will be injected with the Medusa Particles, which are loaded with anticancer drug and have cyclic RGD peptide groups conjugated to the terminus. The cyclic RGG will bind selectively with cancer cell integrins; once the Medusa Particles have attached to the cancer cells, the ac magnetic field will be used to cause the magnetic nanoparticles to oscillate, which will warm the particles. This heat will cause the crystalline structure to melt, which will release the anticancer drug directly to the site of cancerous cells. This mechanism has many advantages, but the most significant is that it would release an exact amount of drug (the volume of drug contained per particle would be calculated from the particles' volume and substantiated by drug release studies) directly to the site of cancerous cells. The side effects experienced from current cancer treatment mechanisms are attributed to the indiscriminate destruction of cells by cancer drugs, so that healthy cells are destroyed along with cancerous cells during treatment.

This is especially of concern for chemotherapy treatments that use cell cycle non-specific antitumor antibiotics, such as Doxorubicin. These cell-cycle non-specific drugs can induce apoptosis through interference with DNA replication in both healthy cells and cancerous cells, so attachment and release only at the site of cancerous cells would reduce the destruction of healthy cells [2,5,8]. The ability to control the site of delivery and the exact mechanism of delivery would allow for smaller drug dosages to be used in the patient (as the drug would only interact with cancerous cells and no longer with healthy cells), which would reduce the side effects experienced by patients, as well as the cost to patient and insurer. Additionally, there have been recent shortages of certain anticancer drugs within the United States; reducing the necessary amount of cancer therapeutic agent would ameliorate this situation.

Experimental Methods and Materials

The 24 nm magnetic nanoparticles that form the center of the Medusa Particles were synthesized through the thermal decomposition of iron(III) oleate in refluxing 1-octadecene using a literature procedure (Shouheng Sun, et al). To create the silane terminated diblock copolymer, 5 grams of 20:1 poly(ethylene glycol-*b*-caprolactone) diblock copolymer (molecular weight 2000 amu) was reacted with 0.75 milliliters of 3-isocyanatopropyltrimethoxysilane at the alcohol terminus in a solution of 10 milliliters of dimethylformamide and 0.08 milliliters of a tin catalyst (dibutyltin dilaureate). This reaction was carried out under nitrogen gas at 80°C for six hours, and enabled the silane coupling agent to bind to the diblock copolymer. The silane terminated diblock copolymer (2.5 grams) was then attached to the surface of 24 nm diameter magnetite nanoparticles (4 grams) in 50 milliliters of toluene while stirring under nitrogen at room temperature for eight days, producing the final Medusa Particles. The Medusa Particles were then precipitated with hexane and isolated by filtration. The resultant particles were dialyzed in a 1:1 tetrahydrofuran and pure water solution to remove unbound polymer.

To verify the ability of these particles to encapsulate drug in an aqueous solution, pyrene was used as a drug analog for fluorescence. The Medusa Particles were loaded with a pyrene solution; three different concentrations of the particles (0.1 g/L, 0.5 g/L, and 1.0 g/L) were dispersed in minimal amounts of tetrahydrofuran before addition to 10.00 mL of pyrene with a finger probe. The tetrahydrofuran was allowed to evaporate overnight and the solution was then diluted back to 10.00 mL with the pyrene solution. The fluorescence was then measured, and the intensities were compared at an emission wavelength of 390 nm for the two excitation wavelengths of 333 and 338 nm.

Results

The av-360 ^{1}H NMR spectrum provided conclusive evidence that the silane attachment to the diblock copolymer was successful, as shown in Figure 2. A Jasco FT/IR-4100 was used to confirm the crystallinity of the diblock copolymer corona, as indicated by the peak at 1725 cm^{-1} shown in Figure 3. A tunneling electron microscopy image, in Figure 4, shows the Medusa Particles.

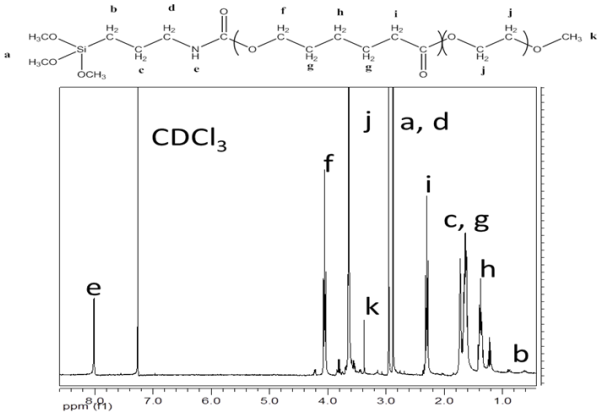

Figure 2. ^{1}H NMR of the silane terminated diblock copolymer.

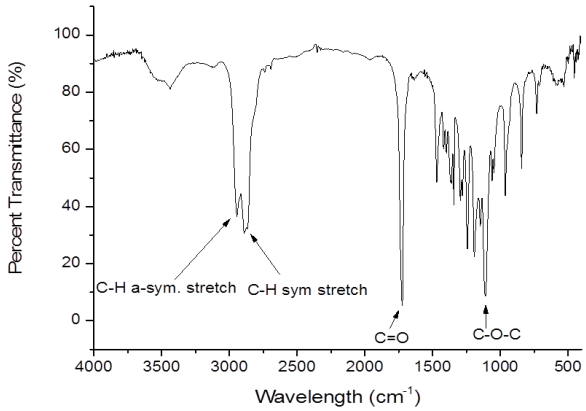

Figure 3. Infrared spectrum of the 24 nm magnetite nanoparticles with covalently bound diblock copolymer indicates the crystallinity of the diblock colymer's corona with a carbonyl peak at 1725 cm^{-1}.

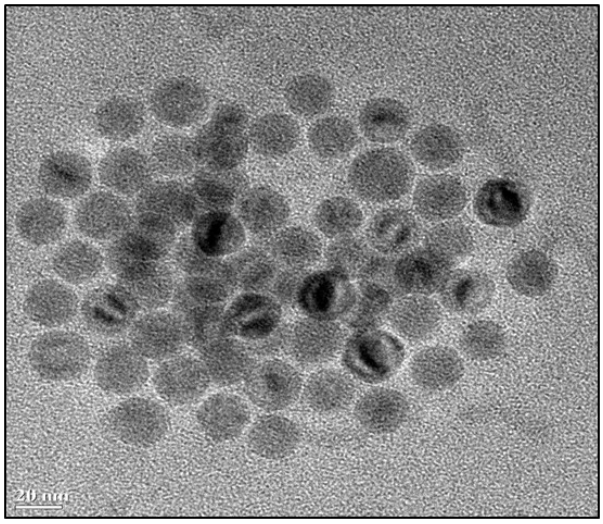

Figure 4. A tunneling electron microscopy image of the Medusa Particles.

Proton nuclear magnetic resonance, Fourier Transform infrared spectroscopy, and tunneling electron microscopy were used to verify that the particles had been created. Fluorimetry provided proof that the particles are capable of encapsulating drug in the hydrophobic interior. Figures 5 and 6 show the intensities of the different concentrations of Medusa Particles at excitation wavelengths of 333 and 338 nm; Figure 7 gives a graph that shows the definitive results. For pyrene in a hydrophilic region, the ratio of the intensities of fluorescence at 338 and 333 (I_{338}/I_{333}) will be less than one. For pyrene in a hydrophobic region, this ratio will be greater than two. As can be seen from the graph, the pyrene fluorescence data indicates that pyrene is able to be trapped in the hydrophobic polycaprolactone core of the Medusa Particles. Figure 8 shows that the amount of pyrene trapped in Medusa Particles is directly proportional to the concentration of Medusa Particles used.

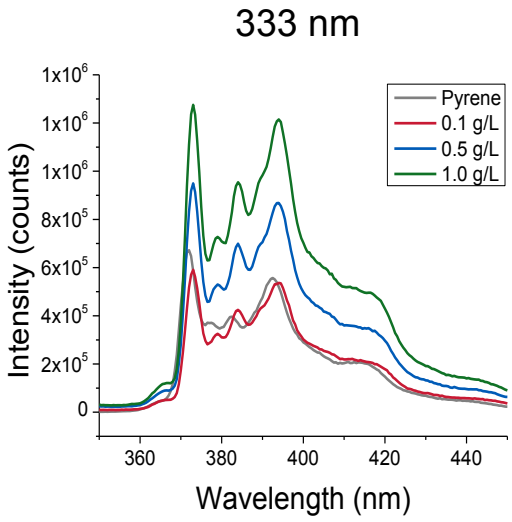

Figure 5. The fluorescence spectra for three concentrations of the Medusa Particles and a pyrene solution for an excitation wavelength of 333 nm.

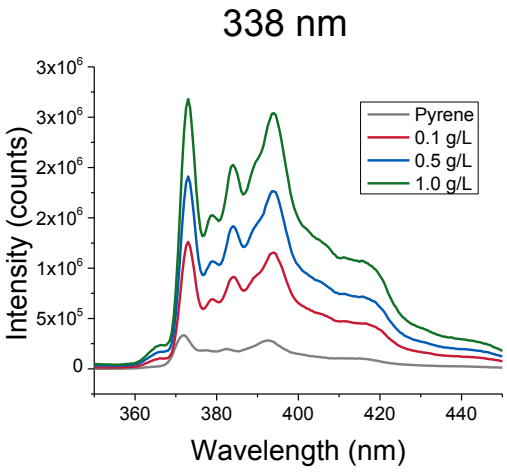

Figure 6. The fluorescence spectra for three concentrations of Medusa Particles and pyrene at an excitation wavelength of 338 nm.

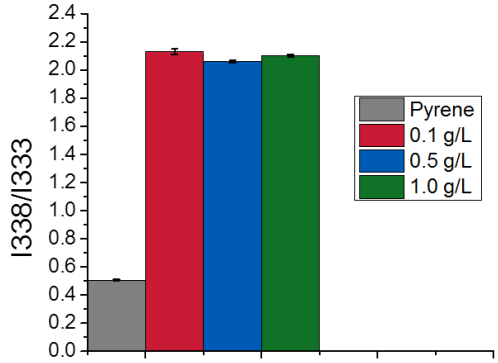

Figure 7. A comparison of the ratio of the intensities of the fluorescence spectra at excitation wavelengths of 333 and 338 nm. For $I_{338}/I_{333} < 1$, the pyrene is in a hydrophilic region. For $I_{338}/I_{333} > 2$, the pyrene is in a hydrophobic region.

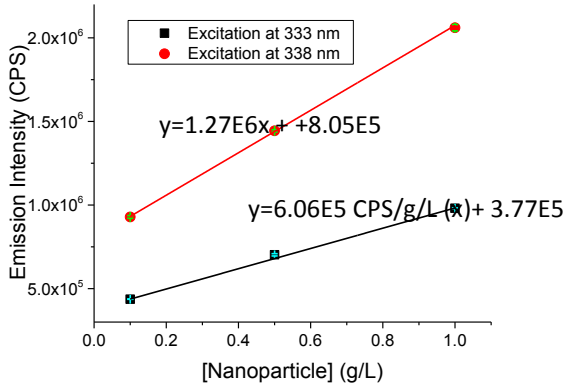

Figure 8. The fluorescence intensity is directly proportional to the concentration of Medusa Particles in the pyrene solution.

Discussion

The characterization techniques investigated have confirmed that diblock copolymers can be attached to the surface of a magnetic nanoparticle to produce Medusa Particles. Medusa Particles have shown an ability to encapsulate a cancer drug analog and to form a crystalline shell in solution. These particles trap drug proportionally to the concentration of Medusa Particles used in solution, proving the viability of these particles to function as a drug delivery

system.

Magnetic nanoparticles have been proven to be safely removed from the body by the liver, and are currently used in imaging procedures [1,10]. The size of the particles is significant, as particles less than 10 nm in diameter are readily absorbed by blood vessels and quickly expelled from the body. Particles larger than 10 nm are able to remain in the blood stream long enough to travel to the cancerous site, but are removed from the body before any toxicity effects (such as acculumation of damaging oxidants that can cause destruction of cellular proteins, enzymes, lipids, and nucleic acids) can take place [7]. These particles are assumed to be safe for human use also because of the ease of dispersal of Medusa particles into tetrahydrofuran. Disperal in tetrahydrofuran is indicative of the particles' safe dispersion into blood. Since they disperse so readily without falling out of solution, it is assumed that these particles do not aggregate in the blood stream and do not pose a risk of causing thrombosis. This will be verified by animal studies.

While this cancer therapy mechanism has the potential to be applied to various types of cancers, it is currently being used with prostate and breast cancer cells because they share the same RGD peptide [4]. The prostate and breast cancerous cells can be injected into laboratory mice and the drug can be administered through the bloodstream, and its effectiveness can be studied. A collaborative research group at The University of Alabama in Birmingham has investigated the attachment of the targeting ligands for this RGD peptide to the diblock copolymer so that targeted drug delivery studies with this mechanism can be carried out. Should this cancer therapy mechanism be proven to be safe and effective, it will revolutionize cancer therapy treatment and make significant contributions to the care and comfort of cancer patients by increasing the effectiveness of treatment and decreasing the side effects that are encountered with cancer treatments today. These new nanodevices promise to provide the oncologist with unprecedented spatial and temporal control of chemotherapy.

Further Areas of Exploration

The Medusa Particles have been created and characterized and are now being investigated for drug delivery efficiency with pyrene as a drug analog before investigation with Doxorubicin will be carried out Heating studies will next be conducted to find the optimal heating range and magnetic field parameters, which willshow the necessary frequencies and timing needed for the ac magnetic field to melt the particles and release drug. A mechanism for drug release in the heating studies has been established.

In addition to the use of Medusa Particles in drug delivery, they may also have application in medical diagnostic imaging. Magnetic nanoparticles are currently being used in various imaging procedures, so Medusa Particles have the potential to enhance MRI imaging by loading dyes that would give greater images of affected body parts when the particles are bound to the cells [3]. Loading the particles with MRI contrast dyes will indicate the feasibility of use of these particles as imaging agents.

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Morgan Whitaker is a junior at The University of Alabama. She is originally from Scottsdale, Arizona, and is studying Biochemistry with minors in Computer-Based Honors and Spanish. She has been researching in Dr. David Nikles' lab in the chemistry department for two years now, and in April 2013 she presented her research at The American Chemical Society National Meeting in New Orleans, Louisiana. Morgan also volunteers in local medical clinics in both Scottsdale and Tuscaloosa, and hopes to attend medical school.

Inositol Phosphate Metabolism: Genes and Their Products That Contribute to Neural Tube Defect Phenotype and Rescue in Mammals

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Neural tube defects (NTDs) are a class of birth defect characterized by malformation of the neural tube during embryonic development. Consequently, this may result in serious impairment of the central nervous system–brain and spinal cord. One well-known example is spina bifida, a condition characterized by an incomplete closure of the neural tube during embryogenesis. Importantly, folic acid administration to women during pregnancy has been demonstrated to greatly reduce the incidence of such NTDs in their children. However, a subset of these defects does not appear amenable to prevention by folic acid administration. Thus, supplemental compounds have been proposed to aid in disease alleviation. One such compound is inositol, an important vitamin involved in various bodily activities. In at least one mouse strain, the curly tail strain, inositol administration to pregnant mice has been shown to reduce the prevalence of NTDs in their offspring. Based on this observation, human trials are now underway. Currently, the PONTI (prevention of neural tube defects by inositol) study invites women who have had a pregnancy affected by a NTD to participate in a clinical assessment of the therapeutic efficacy of inositol administration. In this light, a review of the genes (and gene products) involved with both inositol metabolism and disease manifestation seems appropriate. To date, at least four such genes have been identified: Inpp5e, Itpk1, Pip5k1c, and Ipk2. Additionally, several gene products have been shown to mediate inositol's therapeutic effect in the curly tail mouse strain: PKCβ1, PKCγ, and PKCζ.

Introduction:

Inositol is a six-carbon polyalcohol carbohydrate often capable of rescuing mammalian embryos from neural tube defects (NTDs), which are a class of birth defect resulting from improper neurulation (the process by which the brain and spinal cord are formed) during embryogenesis. Failure of this process is responsible for the incomplete closure of the neural tube seen in spina bifida patients and, in the case of cranial neurulation failure, exencephaly [7]. Among humans, the prevalence of NTDs is approximately 1 per 1000 births in the United States [14]. It has been known since the 1991 publication of the Medical Research Council Vitamin Study concerning the prevention of NTDs that a large majority of human embryos can be rescued from the NTD phenotype by administration of folic acid concomitant with development—approximately 70%. The remaining 30%, however, are resistant to folic acid supplementation [18]. Within this latter portion, a subset of folate-resistant NTDs may be receptive to rescue upon inositol administration, as evidenced by experiments with mouse models.

Currently, the PONTI (prevention of neural tube defects by inositol) study invites participation from women who have had a previous pregnancy affected by a NTD (and who wish to become pregnant again) in order to assess the therapeutic efficacy of inositol administration in the prevention of these disorders [5].

Due to the complicated, multifactorial process responsible for human neural tube closure, it has been difficult to isolate genes within human populations solely responsible for NTDs. However, many mouse models displaying a NTD phenotype exist—exceeding 240 models as of 2010 [9]. Among these mouse strains, several are known to be receptive to exogenous inositol treatment during embryogenesis, resulting in inositol-mediated NTD rescue. For example, the curly tail (ct) mouse line is prone to developing spina bifida with incidence between 40-60% but can be rescued from this defect with high success by administration of exogenous inositol [4]. In order for inositol administration to avert defect, however, there

must be adequate function of the Grhl3 gene [8]. Additionally, within other mouse strains, several other genes – and gene products – have been shown to be involved with either neural tube defect development or prevention, raising the possibility that homologous human genes involved in neurulation disorders can be located.

Although the number of genes involved with mouse NTDs tentatively equals 244, the number of genes directly involved with both inositol metabolism and NTD development is currently numbered at four [9, 15]. By means of gene mutation, four genes directly involved with inositol metabolism have been shown to play a role in embryonic neural tube closure. Specifically, those genes include: Inpp5e, Itpk1, Pip5k1c, and Ipk2. These genes have been subjected to mutation with the consequence of increased susceptibility to NTDs, primarily exencephaly and spina bifida. Additionally, several gene products are known to mediate inositol's NTD preventative effect in the curly tail mouse strain: PKCβ1, PKCγ, and PKCζ. The genes responsible for generating these protein kinases may not been directly involved in mediating inositol's ability to prevent neural tube defects, but their products have be shown to be essential to proper neural tube closure when inositol is administered exogenously during embryogenesis [3]. Similarly, at least one gene has been posited as being necessary for NTD rescue by inositol: Grhl3. Deletion or mutation of this gene results in NTD in every circumstance.

Itpk1

Encoded by a gene of the same name, Itpk1 (1,3,4-trisphosphate 5/6-kinase) is an important kinase involved in many cellular processes. Itpk1 is also responsible for mediating production of higher phosphorylated inositol forms in mammalian cells; these include IP5, IP6, IP7, IP8, and inositol pyrophosphates. Specifically, Itpk1 is integral to conversion of I(1,3,4,5,6)P5 to IP6 (table 1). Besides being a component in several proteins (adenosine deaminase, arrestin, and auxin), IP6 is also known to be involved in mRNA export, transcriptional regulation, Dbp5 ATPase activity, binding of clathrin assembly proteins, disrupting clathrin cage assembly, inhibiting serine and threonine kinases, stimulation of other kinases such as casein kinase-2, endocytosis, and nonhomologous DNA doubled-stranded break end joining– among others [11]. Notably, IP6 absence during mouse embryogenesis results in early embryo termination, implying a critical role for this inositol phosphate in development.

By means of β-galactosidase containing gene trap cassette insertion into the second intron of the Itpk1 gene, mouse mutants with reduced Itpk1 expression were created [18]. Upon examination of homozygous gene trap mutants, increased incidence of exencephaly and spina bifida-type NTDs were observed. The total NTD occurrence was found to be greatest between embryonic day 9.5 and 12.5, with overall prevalence being approximately 23%. In contrast, a group of wild-type mice controlled for genetic background were examined and displayed no incidence of NTD.

INPP5E

Inositol polyphosphate 5-phosphatases (INPP5E) are lipid phosphatases that mediate the removal of the D-5 phosphate from the inositol ring of inositol phosphates and phosphoinositides. To date, ten such 5-ptases have been identified in mice. Their assorted roles include: regulation of actin polymerization, endocytosis, haemopoietic (formation of blood cell components) cell proliferation, insulin signaling, neurite elongation, neuronal differentiation and function, synaptic vesicle recycling, and vesicular trafficking [10,12]. In addition, the 5-ptase INPP5E is integral to primary cilium function—a structure which extends from the surface of inactive differentiated cells and engages in the processing of extracellular signals. Specifically, this is accomplished by regulating both PI3K, a kinase family responsible for phosphorylating the 3 position on the inositol ring, and ciliary growth factor.

The presence of INPP5e in mammalian cells is critical during embryogenesis. Deleterious mutation of INPP5e is known to cause Joubert syndrome in humans and mutant mice lacking this 5-ptase display high incidence of anophthalmos, hexadactyly, cystic kidneys, and various skeletal deformities [2]. In reference to neural tube defects, mice embryos homozygous for Inpp5e deletion display a 30% incidence of NTD (anencephaly and exencephaly) by GD 15.5. Underscoring the importance of INPP5e, all homozy-

gous null mice suffer either embryonic or postnatal death [10].

PIP5K1c

Phosphatidylinositol-4-phosphate-5-kinase I (PIP5KI) is a protein ubiquitous in eukaryotic cells. Its many isoforms serve various functions within the mammalian cell—some distinct and others common. For example, PIP5KIα is known to mediate extracellular keratinocyte differentiation while PIP5KIγ is has been shown to be involved with cell membrane anchoring to the cytoskeleton in megakaryocytes [17,19]. Additionally, α, β, and γ PIP5KI isoforms all phosphorylate phosphatidylinositol 4 phosphate, generating PI(4,5)P_2 as a corollary. Upon hydrolysis, biphospholipase C, PI(4,5)P_2, produces important secondary messengers involved in neural tube closure (figure 1). Consequently, deletion of the PIP5KIγ isoform by gene mutation results in reduced PI(4,5)P_2 formation—by approximately 40%—and, therefore, an increase in the number of mice displaying the NTD phenotype. Upon PIP5KIγ deletion, mice suffer incomplete neural tube closure in the head region (exencephaly) exclusively, while caudal neural cord closure continues unimpeded. Specifically, the penetrance of exencephaly in PIP5KI$\gamma^{-/-}$ mice is 100%. Underscoring the importance of PIP5KIγ to embryonic development, mice homozygous for its deletion die in preponderance by gestational day 11.5 [16].

IPK2

Ipk2 (inositol polyphosphate kinase 2) is a protein kinase that phosphorylates key intermediates in the synthesis of IP_6 (figure 1). By phosphorylating the D-6, D-3, and/or D-5 positions on these intermediates, higher phosphorylated IPs are produced—the result being proper neural tube closure in ct mice. In the mammalian cell, there are two proposed pathways for the generation of IP_6 and other higher phosphorylated IPs, both starting from Ins(1,4,5)P_3. The first pathway is known to exist in yeast (Saccharomyces cerevisiae), fruit flies (Drosophila melanogaster), and the common brown rat (Rattus norvegicus). Beginning with Ins(1,4,5)P_3, Ipk2 sequentially phosphorlyates IP substrate from IP_4 to IP_5 then followed by IP_6 generation by Ipk1. The second hypothetical pathway not only involves sequential phosphorylation by Ipk2 and Ipk1 but also phosphorylation of Ins(1,4,5)P_3 by a IP_3 3-kinase and dephosphorylation of Ins(1,3,4,5)P_4 by IP_5 phosphatase. In addition, Ins(1,3,4)P_3 is phosphorylated by Ins(1,3,4)P_3 5/6 kinase (Itpk1).

Ipk2 deletion by homologous recombination results in the removal of 90% of IP_6 production. Mice homozygous for deletion terminate during embryogenesis, with lethality occurring around GD 9.5 for Ipk2 deletion. Before developmental cessation, these mice display wide ranging defects: absent somite formation, abnormally small allantois that fails to fuse with the chorion (resulting in defective nutritional connection with the mother), open anterior neural tube, and kinking of the neural tube. Heterozygous mice, however, are discernibly free of defect. In their case, it can be presumed that adequate levels of higher phosphorylated IPs are produced, accentuating Ipk2's (and resultant IP synthesis) importance in mammalian development. It is still an open question as to whether Ipk2 loss results in these embryonic mutant phenotypes directly or rather through the loss of downstream products [3].

What Are Some of the Gene Products Regulating Inositol-Mediated NTD Rescue?

PKCβ1, PKCγ, PKCζ

Protein kinase c (PKC) represents a family of serine/threonine kinases sometimes categorized by their dependence upon Ca^{2+} and diacylglycerol (DAG) for functionality. Conventional PKCs (βI and γ) require the presence of both while atypical PKCs (ζ) require neither. The roles of PKC in the mammalian body range from transcription regulation and cell growth to, importantly, signal transduction. It is important to note, however, that direct inactivation of the genes coding for these PKC isoforms does not result in neural tube defect. Instead, it is suggested that exogenous inositol administration adverts the spina bifida phenotype in curly tail mice by allowing inositol to promote cell cycle stimulation in the hindgut developing curly tail embryos. In order for this to occur, specific PKC isoforms must be present, namely PKCβI, γ, and ζ.

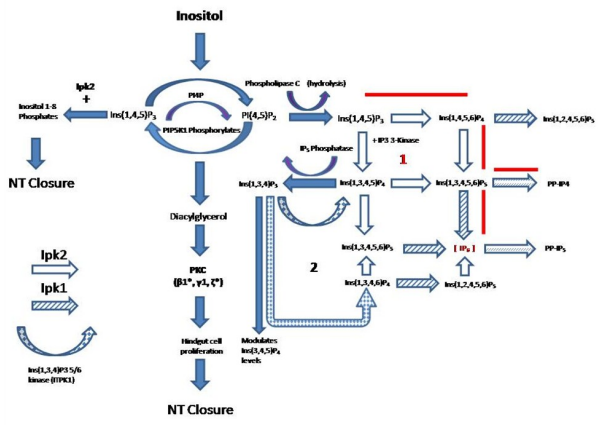

Figure 1 Adapted from [4,6,7]. After glucose is phosphorylated into G-6-P, MIP synthase catalyzes the synthesis of free inositol from an inositol phosphate [1]. From that point, inositol metabolism results in the generation of higher phosphorylated IPs and neural tube closure. PKC isoforms also participate in neural tube closure. Two potential pathways for the generation of IP_6 are represented, with path one being highlighted with red bars. Hollow arrows represent the action of Ipk2, stripped arrows represent the action of Ipk1, and the two checkered arrows represent the action of ITPK1.

Rescue of the curly tail mouse line from NTD may be accomplished by administration of exogenous inositol concomitant with early embryogenesis. The basis behind inositol's ability to mediate this rescue lies in its ability to correct defective hindgut cell proliferation—the embryonic abnormality resulting in spina bifida. However, successful prevention of the spina bifida phenotype requires the presence of three particular PKC isoforms: βI, γ, and ζ. PKC isoforms βI, γ, and ζ do not directly arbitrate neural tube close. Their stage-dependent presence, however, is what imbues inositol with this ability. As evidence, posterior neuropore (PNP) closure–a defect in which leads to spina bifida– is significantly reduced in ct mice with inositol present but treated with peptide inhibitors to these isoforms. Mice treated with inhibitors to other PKC isoforms showed no significant lengthening of the PNP in the presence of inositol. In the absence of inositol, however, no PKC isoform was able to affect PNP closure. Together, this indicates that PKC isoforms βI, γ, and ζ are required for inositol to abrogate NTD but do not directly mediate PNP closure. Furthermore, PKCβI and PKCγ are indispensible to proper neural tube closure whereas PKCζ is partially necessary—affecting PNP length less dramatically in the presence of inositol and a corresponding PKC inhibitor [6].

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Is There a Gene That Mediates Inositol's Ability to Rescue Mice From NTD?

Grhl3^{ct}

Grhl3^{ct} (grainy head like three) is a closely related homolog to the evolutionarily conserved Grh gene found in *Drosophila.* Both genes have critical morphological and developmental functions in developing embryos. Grh, for example, arbitrates dorsal/ventral and terminating patterning in fruit flies. Furthermore, disruptions in Grh function result in the formation of a dorsal hole located where the two regions of the dorsal epidermis unsuccessfully contact and fuse. As a result, it is proposed that this genetically-mediated process may serve as an archetype for vertebrate epithelial fusions, namely neural tube close in the case of curly tail mice. Grhl3^{ct}, on the other hand, is pivotal to proper neurulation in developing ct mice. As evidence, all Grhl3$^{ct-/-}$embryos invariably suffer various morphological abnormalities ranging from absent vertebral arch formation and splayed spinal processes to exencephaly and spina bifida (failure of the neural tube to close). Upon closer examination, mutant mice homozygous for Grhl3^{ct} deletion presented with both neural fold elevation failure and stagnantly convex neural epithelium [14].

Ordinarily, curly tail mice tend to present with folate-resistance NTDs that are susceptible to embryonic rescue upon exogenous inositol administration. **Contrariwise,** Grhl3$^{ct-/-}$ mice are both folate and inositol resistant—all present with the spina bifida phenotype [8]. This could possibly indicate that inositol rescue of ct mice from NTD is dependent upon the Grhl3 gene. A more complete mechanism of action can be theorized in several ways. First, it is possible that an unknown gene dependent upon Grhl3 presence could mediate inositol function. Secondly, MAP kinases, which phosphorylate Grhl3 factors, could be involved in some way. Thirdly, PKC may somehow be involved [14]. Fourthly, Grhl3 could be transcribed at an increased level in ct mice upon inositol introduction, perhaps then mediating neural tube closure. Interestingly, it has been observed that increased Grhl3 expression leads to spina bifida rescue in the curly tail mouse model [8].

Discussion

The development of mammalian neural tube defects is a multi-faceted process. In some cases, it is likely that one or more genes or their products are responsible, likely due to defect or absence. In most cases, folate administration concomitant with early embryogenesis can prevent defects in neural tube closure or development. In a number of folate-resistant cases, inositol administration may accomplish this instead. Targeted deletion of genes within several mouse models have illustrated key roles that particular genes play in NTD development or rescue and inositol phosphate metabolism. In some instances, mice normally susceptible to rescue by inositol administration become resistant upon gene disruption, as is the case with Grhl3. Similarly, for inositol mediation of NTD rescue, the gene product PKC is required. Selective deletion of the PKC isoforms βI, γ, and ζ result in an abrogation of rescue ability by inositol, implying that these products mediate its affect somehow. In other cases, gene disruption results in both NTD and impaired inositol metabolic processes. Selective mutation of Inpp5e concludes with altered PtdIns ratios, severely impaired PtdIns(3,4,5)P_3 and moderately impaired PtdIns(4,5)P_2 activity. Disruption of Itpk1 and Ipk2 both result in impaired IP_6 production, among other effects. Finally, elimination of Pip5k1c culminates in reduced PI(4,5)P_2 production (table 1).

Together, these results demonstrate key roles for inositol and its metabolic products (including inositol phosphate) in neural tube closure in the mouse, suggesting analogous roles in other mammals such as *Homo sapiens*.

It is likely that the list of genes and products presented in this paper is likely not a total listing of the number that actually exists. Future research will be focused on identifying additional genes and products that mediated proper embryonic neurulation. One future endeavor that researchers may pursue lies with

attempting to correlate the function other 5-ptases that hydrolyze IPs with NTDs. Furthermore, future researchers may also focus their studies on identifying additional genes that correlate with NTDs. Perhaps those genes will be closely related to those already known to play a role in inositol mediation of NTDs. For example, another grainy-head-like gene has been demonstrated to be involved with neural tube defects. Specifically, targeted deletion of Grhl2^{ct} results in failed closure of the rostral end of the forebrain resulting in exencephaly [13]. Also, considering Ipk1's role in the synthesis of IP_6 (figure 1), future research may focus on its role in mammalian neural cord defects. It has been noted that curly tail mice appear unable to down-regulate myo-inositol-1-phosphate synthase (MIPS) production when compared to their straight tail littermates [1]. It remains a possibility that constitutive production of inositol phosphates plays a role in NTD development, as opposed to lack of production via gene disruption. In any case, considering the complicated and intertwining role of genetics and environment, it is likely that no one gene is responsible for the majority of NTDs in other mammals, including humans. Many of the processes involving neural tube defect development remain unknown, leaving many aspects yet explored.

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About the Author

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The Use of a Drosophila Laminin A Mutant as a Model for Gestational Diabetes

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Mutations in the Laminin A (LanA) gene have shown significant metabolic effects on Drosophila melanogaster adults; these include changes in TAG storage and body weight. Since these phenotypes correlate to the development of diabetes, this finding led us to our present study. We aim to model gestational diabetes in Drosophila using a previously implicated LanA mutant. In humans, gestational diabetes mellitus (GDM) is characterized by high blood glucose and triglyceride levels in the mothers, as well as the mothers giving birth to larger babies. Therefore, we measured the following phenotypes; total glucose concentration, total triglyceride concentration, egg volume, and pupae weight. The results showed that the mutant laid larger eggs than the wildtype, which correlates to what is seen in humans. However, the mutant flies had significantly lower glucose concentrations, lipid concentrations, and pupae weight than the wildtype flies. A second focus of the experiment was the effect of dietary perturbations on the phenotypes. The specialty diets consisted of 6% sugar, 12% sugar, and 1.5% fat. The 6% sugar and 1.5% fat diet caused the most variance in glucose concentration, lipid concentration, and pupae weight in the mutant fly compared to the wildtype. Additionally, we found that the age of the mother dramatically affects egg volume in the mutant. 22 day old mothers laid significantly larger eggs than 8 day old mothers. These findings support the hypothesis that the LanA gene maybe playing a role in the GDM pathway.

Introduction

In previous studies, mutations in the *Laminin A (LanA)* gene have shown significant metabolic effects in *Drosophila* melanogaster adults, such as changes in TAG storage and body weight [1]. These phenotypes which serve as biomarkers for diabetes led us to our present study. We aim to model gestational diabetes using a previously implicated *Drosophila LanA* mutant.

In humans, gestational diabetes mellitus (GDM) is characterized by high blood glucose and TAG levels in mothers, as well as mothers giving birth to larger babies. Therefore, we measured the following phenotypes in the mutant fly compared to the wildtype; total glucose concentration, total triglyceride concentration, egg volume, and pupae weight. The experiment also analyzed the effects of dietary perturbations on the phenotypes. The diets used were normal, 6% sugar, 12% sugar, and 1.5% fat.

Methods

Genetic lines used

The mutant line BG 01389 was created by the Berkeley Drosophila Genome Disruption Project .The mutation is a single transposable insertion in an otherwise isogenic Canton-S background, causing a hypomorphic mutation in the LanA gene. Canton-S is the isogenic wildtype that was used as the control in the study.

Experimental diets used

The normal diet consists of cornmeal, yeast, agar, and molasses which provides 4% sucrose. This diet is what all laboratory flies have been maintained on for many generations. Three experimental diets were used. The 6% sugar diet consists of the normal diet supplemented with a 2% increase in sucrose (molasses). The 12% sugar diet consists of the normal/ base diet supplemented with an 8% increase sucrose (molasses). Lastly, the fat diet consists of the normal diet supplemented with 1.5% coconut oil.

Hemolymph/ glucose concentration

1st instar larvae were collected from laying chambers and placed on different diets. Larvae were allowed to grow and feed on diets until the 3rd instar larval stage. 3rd instar larvae were harvested from food, starved on water agar plates for 3 hours, and sacrificed in liquid nitrogen. For adult fly collection,

1st instar larvae were harvested from laying chambers and placed on different diets. Larvae were allowed to grow and feed on diets for 15, 20, and 30 days. Adults were harvested from food, an estheticized, and sexed. Only adult females were placed in microtubes (10 per tube) and flash frozen in (l) N_2. Samples were processed using the Sigma Aldrich Glucose Assay Kit. A spectrophotometer measured the absorbance, and concentrations were estimated from the absorbances using a standard curve.

Lipids concentration

3rd instar larvae samples were collected the same way as in the glucose protocol. Samples were processed using the Sigma Aldrich Triglyceride Assay Kit. A spectrophotometer measured the absorbance, and concentrations were estimated from the absorbances using a standard curve.

Pupae weight

1st instar larvae were harvested from laying chambers and placed on diets. When larvae developed into dark pupae, pupae were collected and stored in Ringer's solution at -20 C. Pupae were weighed one at a time on a precision balance.

Egg Size

For the maternal generation, 1st instar larvae were collected and placed on special diets. These flies were allowed to grow into adults on their respective diets for 12 days. These were the mothers. The mothers were put into laying chambers and allowed to lay overnight. The next morning, their eggs were collected and plated on a microscope slide (25 eggs per diet). Eggs were photographed using a digital camera mounted to a compound light microscope. The length and width of eggs were measured using the Motic software program on a computer. Egg volume was estimated using the formula for the volume of an ellipsoid, $V= 4/3 * \pi * width^2 * length$.

Results

Mutant larvae showed no significant difference in glucose levels from the wildtype; however the mutant adults had significantly lower glucose levels (figure 1a). Also, mutant larvae were found to have significantly lower lipids than the wildtype (figure 1b), and accordingly female mutant pupae weighed significantly less than wildtype pupae (figure 1c). Lastly, the mutant flies laid significantly larger eggs than wildtype.

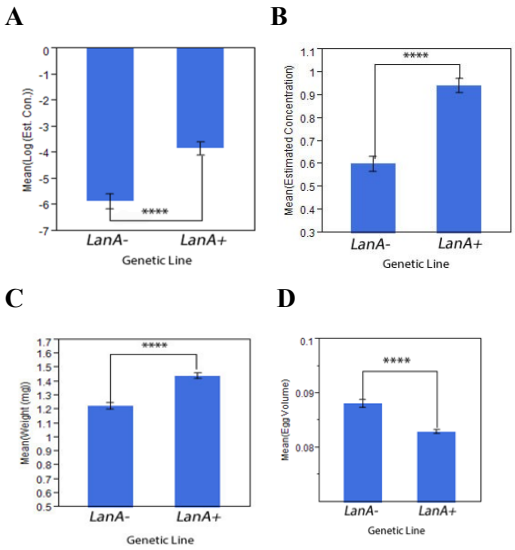

Figure 1: Genetic effects on the GDM phenotypes; a.) glucose b.) lipids c.) pupae weight d.) egg volume

Dietary Effects

The diets that caused the most variance in glucose concentrations in the mutant line from the wildtype were; normal, 12% sugar, and 6% sugar diets (figure 2a). The fat and normal diet caused the greatest differences in egg volume between the two genetic lines (figure 2b). Fat, 6% sugar, and normal diets all caused significant differences in lipid concentrations between the two genetic lines (figure 2c). Lastly, the fat, 6% sugar, and 12% sugar diets all caused a significant difference in pupae weight in the two genetic lines (figure 2d).

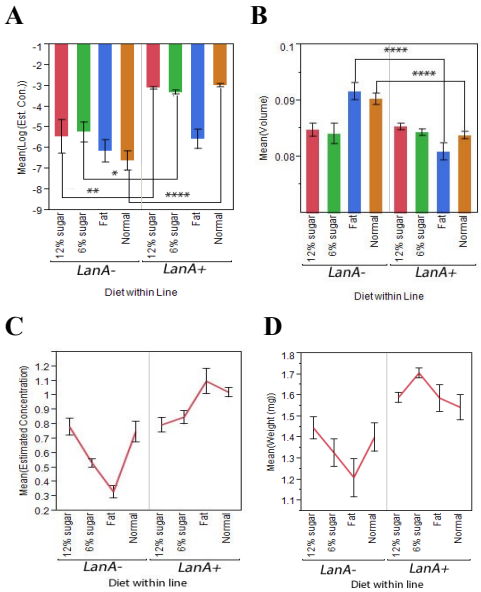

Figure 2: *Diet effects on GDM phenotypes; a.) glucose b.) egg volume c.) lipids d.) pupae weight. Diets that caused the most significant differences are indicated by asterisks.*

Age Effects

With increasing age, the mutants laid significantly larger eggs whereas the wildtype did not (figure 3a). Additionally, it was found that glucose levels rose with increasing age in both genetic lines (figure 3b).

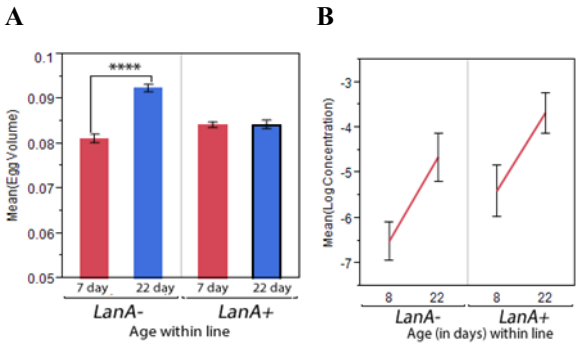

Figure 3: Age effects on two phenotypes; a.) egg volume b.) glucose concentration. Significant differences are represented by asterisks.

Conclusion

The *LanA* mutant had profound differences in metabolic phenotypes from the wildtype. The phenotypic effects of this mutation however do not correlate to what is seen in humans. Gestational diabetic mothers have higher glucose, TAG levels, and tend to weigh more. The mutant flies showed lower glucose, TAG levels, and the pupae weighed less than the wildtype. However, egg volume was the exception. It does correlate to what is seen in humans, because GDM occurs more in older women, and can cause the baby to be born abnormally large.

From all the diets tested, the fat and 6% sugar diets had the greatest effect on the majority of the phenotypes. Egg volume, larval lipids, and pupae weight, were significantly affected by the fat diet; whereas glucose, larval lipids, and pupae weight, were significantly affected by the 6% sugar diet.
It appears that this mutation shows protection against GDM . This implies that the gene may still be playing a role in the GDM pathway.

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About the Author

Joana is from Madison, AL. She is a Junior, double majoring in Spanish and Biology. She has been working in Dr. Laura Reed's laboratory for two years. During her time in the lab, she has participated in the Howard Hughes Medical Research internship. She was awarded the McWane Undergraduate Research Fellowship. Joana presented her findings in the form of a poster at the 59th annual Drosophila Research Conference in Washington D.C. She has also participated twice in the Undergraduate Research symposium.

Population Data Analysis for Flattened Musk Turtles (*Sternotherus depressus*) in the Upper Black Warrior River Basin

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Sternotherus depressus, or the Flattened Musk Turtle (FMT), is a federally threatened species of turtle that lives in the rocky streams of the upper Black Warrior River basin in central Alabama. Beginning in the summer of 2012, FMTs were collected from several tributaries of the Black Warrior River. Standard environmental data was collected at each trapping site, and the turtles were measured, sexed, tagged, and released back into their habitat. In this project, the obtained environmental data were analyzed using statistical methods in order to determine where exactly the FMT population is currently located and what environmental factors (such as air temperature, water temperature, water pH, and dissolved oxygen) most greatly affect the presence of FMT populations. GPS data was also collected at each site, and this data was used to create maps of areas where turtles were caught. These maps were compared to maps made using data from a 1983 FMT study by Ernst et. al [1]. Comparison of these maps has shown that the FMT's range has become greatly reduced since surveys conducted in the early 1980s. When the environmental data were analyzed, it was found that only one of the variables was very slightly statistically significant when compared to the number of turtles that were caught. In the future, these statistical tests will be compared with tests from the Ernst study. This comparison should shed some light on how the FMT's habitat has changed since 1983, and if this change is due to human activities, natural processes, or temporary anthropogenic damage to river ecosystems.

Introduction

The Black Warrior River Basin in north-central Alabama drains through 15 counties, covers 6,392 square miles, and contains extensive coal deposits. The land in the area is used for numerous agriculture, strip mining, and forestry activities [2]. "Pollution caused by coal mining near the Black Warrior River has landed the river on the annual list of America's Most Endangered Rivers" [3]. The pollution in this river system has far-reaching effects on flora and fauna in the area. Some species are more sensitive to this habitat degradation than others, and pollution in the water could cause endemic populations to decline.

Sternotherus depressus (the Flattened Musk Turtle; FMT) is a small aquatic turtle related to the Loggerhead Musk Turtle, *Sternotherus minor*, and native to the Black Warrior River Basin in central Alabama. It was first described by Tinkle and Webb [4]. The FMT has a slightly dorso-ventrally flattened carapace, which allows it to hide and live in cracks between rocks that other turtle species would find inaccessible [5]. Due to habitat degradation within its range, the FMT population has declined and the species has been placed on the "Federally Threatened Species" list [6]. This decline could be due to the FMT's sensitivity to pollution in the water, or it could be due to a die-off of the species' main food source - freshwater mussels and clams [7] - caused by pollution or habitat alteration.

In the 1970s and 1980s, Ernst et al. conducted an extensive capture-tag-release operation on *S. depressus* in order to define the total range of the species [1]. The range was defined as a region within the Black Warrior River Basin about 2,345 square miles in area. In this study, many of Ernst et al.'s historic trapping sites were visited, in order to determine whether those areas still host a healthy population of *S. depressus*.

The purpose of this study was to determine whether the FMT range has changed significantly since the Ernst et al. study, and if so, to determine where the range is currently located and what environmental factors have the most effect on the health of the FMT population.

Methods and Materials

Turtle Sampling

In order to collect the data required for this project, FMTs had to be collected from sites in their population range that were previously reported by Ernst et al. and Mount et al. [1,8]. Because this species is federally threatened, permits had to be obtained in order to collect them, and each captured turtle had

to be returned to its original habitat. Ideal FMT habitats are shallow, rocky streams with a healthy population of freshwater mussels, clams, and snails for the FMTs to eat [5,7], and most of the sites utilized contained areas matching this description. Netted traps of rectangular shape with one-directional openings on either side were set in streams and lakes throughout the upper Black Warrior River Basin. These traps were baited with either canned sardines or canned cat food (any brand, as long as it had a very strong smell). Generally, between 10 to 20 traps were set at each site at arbitrary intervals upstream and downstream of the starting location, in rocky areas within the various streams that seemed to be ideal FMT habitat. The traps were marked with orange flagging tape to ensure collection the next day. Many of the sites that were utilized corresponded to sites used during the Ernst et al. study [1]. Traps were set in the early evening and collected early the next morning.

Data Collection

At each trapping site, GPS data was collected so the FMT range could be mapped. Environmental data was also collected using a water quality meter (YIS Professional Plus, Yellow Springs, OH, USA) in order to determine which factors have most greatly affected FMT population levels and range. The environmental data points collected were air temperature, water temperature, water pH, dissolved oxygen content of the water expressed as both mg/L and as a percentage, and the CF (conductivity factor) value in mm Hg and C-uS/cm. Each data point was collected in the morning as traps were collected and in the evening as traps were set, to account for daily / hourly fluctuations during the trapping period.

The CF value is the conductivity factor of the water. It is a measure of the conductivity of the water sample, dependent on the dissolved salts in the solution. This value can be used to determine water quality, as many dissolved salts have a high probability of being pollutants. However, materials such as naturally -occurring ions and minerals from soil and rock can be dissolved in the water, and these non-hazardous materials will affect the CF value of the water. Therefore, the CF value has inconclusive causation and cannot be used to determine what kinds of ions are dissolved in the water, but it is helpful in determining whether more extensive water-quality tests need to be performed.

Location Data and Statistical Analysis

The GPS data collected at each site was entered into Google Earth in order to map the current range of the FMT population. The trapping area was determined by calculating the area enclosed by all the trapping sites, and the FMT range was determined by calculating the area enclosed by the locations where FMTs were caught. Then, GPS data from the Ernst study was also entered into Google Earth and subjected to the same analysis. The two FMT ranges (Ernst in 1983 and the current from 2012) were then compared against each other to determine whether the FMT range had changed and/or diminished since the Ernst study [1].

The environmental data collected at each site was organized into a Microsoft Excel spreadsheet based on data type. To determine which of the variables most greatly affected the number of turtles caught, average values were calculated for each variable, along with the correlation coefficient to the average number of turtles caught. Then, *t*-tests were performed on each variable. A *t*-test is a statistical assessment of two populations that examines whether the two populations are different. Sites where turtles had been caught were counted as one population, and sites where turtles had not been caught were another. This statistical breakdown will be expanded upon in later analyses of the data.

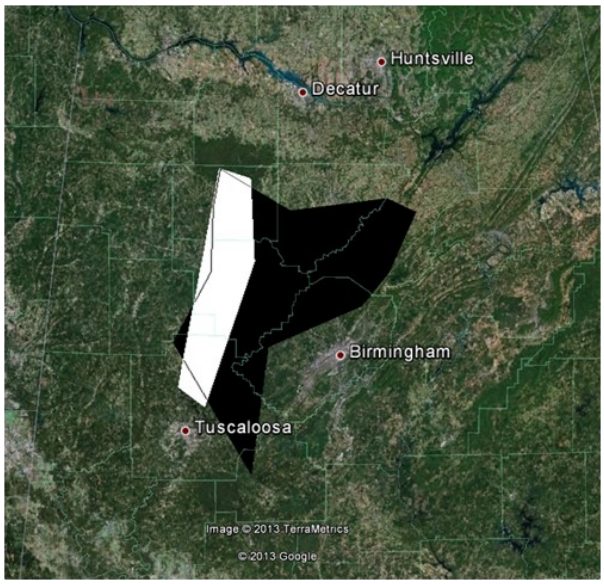
Results

Range Comparison and Population Density

The current range was determined to have an approximate area of 918.4 square miles, while the range determined from the 1983 Ernst study had an area of 2345.3 square miles. In the 1983 study, FMTs were caught in about 86% of the trapping area. In the current study, turtles were caught in only about 33% of the trapping area. The current FMT range is about 31.2% of the size of the original FMT range as described by Ernst [1]. These data show a decrease in the size of the FMT range. Figure 1 shows the comparison of the 1983 Ernst range (black) with the current range (white). The westernmost portion of the original range, consisting of the Sipsey Fork; Mulbery Fork (below the confluence with the Sipsey Fork); and the North River drainage, is the only area where FMTs still live.

The population density, which is the total number of turtles per unit area, for the Ernst study was calculated to be 0.25 turtles per square mile. In contrast, the population density in 2012 was calculated to be 0.07 turtles per square mile. This shows a 28% decrease in population density since 1983. This means that, on average, Ernst caught many more FMTs per site than the current study, and it could indicate a decline in FMT population over time.

Figure 1: Comparison of the current FMT range



(white) with the FMT range defined by Ernst et al. (black)

Statistical Analysis Results

The correlation coefficient between two data sets measures the relationship between the two variables. The closeness of the correlation coefficient to ±1 indicates the closeness to a linear relationship [9]. A perfect positive correlation, meaning that as the value of the environmental factor increases the number of turtles caught also increases, is equal to one. A perfect negative correlation, meaning that as the value of the environmental factor increases, the number of turtles caught decreases, is equal to negative one. A correlation coefficient of zero indicates that the two variables in question are not linearly related.

The *p*-value of a *t*-test indicates the probability that the distribution of environmental conditions where FMTs were caught or not caught is different. A *p*-value at or below 0.05 indicates that there is a 0.95 or greater probability that differences in environmental conditions where turtles were trapped or not trapped is different. If the *p*-value is over 0.05, the probability that there is no difference in conditions is high, while the probability that there is a difference in conditions is low.

Results of the statistical analysis of the environmental data are summarized in Table 1.

Variable	*Correlation Coefficient*	*P-Value*
Number of Traps	0.1147	0.7243
AM Air Temperature	0.1661	0.3449
PM Air Temperature	0.2441	0.1316
AM Water Temperature	0.3013	0.0518
PM Water Temperature	0.0171	0.8292
AM Water pH	-0.0614	0.2907
PM Water pH	-0.0953	0.6290
AM DO%	-0.1896	0.9255
PM DO%	-0.1999	0.6564
AM CF	-0.0629	0.7274
PM CF	0.1330	0.0500

Table 1: Summary of environmental data analysis from correlation calculations and t-tests.

Discussion

Summary of Results

After analyzing the relevant data, it has been determined that both the FMT range and population density have decreased since the Ernst et al. study [1]. There could be many reasons for this decline, such as over-capture by humans, pollution contributing to a die-off of the FMT food source, pollution directly affecting the FMTs themselves, or changes in natural environmental factors. Increased siltation caused by agriculture and mining in streams where FMTs are found could also be degrading their habitat by filling in the cracks that the turtles use to escape from predators.

The *p*-value of the p.m. CF seems to be statistically significant (it is equal to 0.05). This could mean that the conductivity factor of the water affects the population of FMTs in the area. However, the a.m. CF *p*-value (0.7274) very strongly shows that there is no difference in the CF where FMTs were caught and where they were not caught, so the p.m. CF value probably indicates an error in the data. As discussed earlier, more water-quality tests would have to be performed in order to determine whether the CF values are significant. Also, the *p*-value of the a.m. water temperature is almost significant (0.0518, barely over 0.05). Since turtles are exothermic, it would make more sense to have a greater trapping success at areas where the water temperature was warmer in the morning (indicating a warmer nighttime temperature). The warmer nighttime temperature would cause the FMTs to be more active, and that would possibly correspond to a greater trapping success.

None of the other environmental factors that were analyzed seem to have any significant effect on the number of FMTs that were collected. There are several reasons why this could be the case. Either the other environmental factors do not have an effect on the FMT population, or the many sites where no turtles were caught skewed the calculated values and caused a large error in the results. Also, historic pollution could have caused a die-off in the FMT population or their food sources. This historic pollution and possible resultant die-offs would not be reflected in current water conditions, but would affect the current population of FMTs. Possible water pollutants that were not measured include fecal coliform, nitrogen or phosphorus pollution from agriculture, and toxic runoff from mining activities. Levels of these pollutants could have an effect on the FMT population, but they would have to be measured at each trapping site in order to determine their effect.

Current and Future Work

Currently, the environmental data from the 1983 Ernst study is being analyzed in the same way as the 2012 data. When this analysis is completed, the two data sets will be compared in order to determine whether there have been any significant changes in the environment since 1983, and if so, which environmental variable has changed enough to cause the observed changes in the FMT population.

To determine whether the sites where no FMTs were caught skewed the results and caused a large error, those sites will be left out of a secondary statistical analysis. The results of this analysis will be compared with the preliminary results to determine any significant change in the results.

Importance and Implications

The Black Warrior River Basin is a major water source for a large part of Alabama, including the major metropolitan areas of Birmingham and Tuscaloosa. Pollutants in area waterways could be very dangerous not only for wild animals and plants, but also for humans. Establishing which factors are causing this turtle species to decline could help determine what needs to be done in order to return the watershed to a more pristine state.

It is also possible that the FMT population decline could have an effect on other species in the area that either depend on the FMT as a food source or as a host organism. If FMTs were to become extinct, there could be an effect on the larger ecosystem of the Black Warrior River Basin, which could cause other species to decline or become overly prolific.

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Growing Old: The Negative effects of Ageism and Inequality on the Health of the Elderly

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In a community with a steadily increasing elderly population, the health concerns of seniors can no longer be ignored as they have a major impact on health care spending and the quality of life of a growing portion of the population. The increase in chronic conditions associated with this population does not occur in isolation but is part of a cultural environment and is impacted by both stereotypes and self-stereotypes within that particular culture. This review analyzes some of the current literature pertaining to the effects of these cultural factors on the health status and quality of life of the elderly. When combined, this research shows that seniors are not only impacted by the stereotypes formed by others but also by self-stereotypes that tend to change over time. The variations in social inequality across cultures reveal the impact that opinions about aging have on the health status of the elderly, both in a positive and negative direction, and that these culture-based stereotypes, in turn, effect the social infrastructure in place that would further increase seniors' quality of life.

Background

According to a report titled "The State of Aging and Health in America 2007" conducted by the CDC, the cost of caring for elderly persons is three to five times more than the cost of healthcare for the younger generations. This is due mostly to the fact that 80% of persons over the age of 65 have a chronic condition, based on data from the same study.[1] .A combination of anthropological and psychological research into the impact of ageism, cognitive dissonance, and stereotypes about the elderly may debunk the sense of inevitability of this phenomenon. Investigation into sociocultural factors related to degeneration would benefit both the seniors suffering the reduction in quality of life as well as the persons funding the healthcare systems as these factors are simpler to either mitigate or even eliminate than the biological and anatomical ones. As part of medical intervention programs focus on preventative treatment programs for both chronic and acute conditions, it would be beneficial to work towards adjustments to cultural perceptions of aging and the elderly as they produce a buffering effect to many health concerns.

In a country so focused on equality and fair treatment, some might find it strange that this sense of equal citizenship is not translated through the lifespan of one individual but rather rises and then falls again with age. This inequality is more implicit than other forms of inequality and is represented by the decrease in medical attention given to the elderly, the spending costs on their living conditions, and the amount of focus they receive in nutrition-management programs. For example, the government food stamp program's patronage is composed only of approximately 9% of seniors, despite their much larger proportion amongst the general population. Government nutrition programs in general reach less than 10% of all at-risk seniors, which, according to Nancy Wellman, is a reflection of both ageism and a more strict poverty line applied to older generations.[2].While demographic information is helpful in making generalizations about a population, ageism occurs when those demographic data are used indiscriminately to apply to an individual without taking into account that individual's life history, social connections, and physical environment, instead diminishing his personhood to a state that is solely dependent upon his age.[3] Yet judgments based on age are overwhelmingly common in many societies that value the progressive and reproductive capabilities of youth to the detriment of the elderly.

Cross-Cultural Comparisons

As the number of older citizens grows in many countries, the effects of age discrimination can no longer be ignored. China and the US, two countries with some of the fastest growing populations of seniors, both face similar challenges of caring for these individuals in a climate that no longer fully values them as equals. Baozhen Luo examines the cultural backgrounds of these two very different nations as they impact the magnitude and repercussions of ageism.[4] In his sample of college students, he found that American students used a higher standard age to classify a person as "old" than the Chinese students, though both groups

gave an age higher than the official retirement age for either country. Baozhen also found, however, that Chinese students spent more time on average with their grandparents than did American students, yet Americans were more like to interact with non-kin seniors. Furthermore, Chinese students showed a greater discrimination towards the elderly, despite the typical image of China as a highly collectivist culture. In both societies, those individuals with the most interaction with the elderly had a more positive attitude towards them. While the causes of these disparities are up for debate, the effect on the elderly is reflected through both social and governmental institutions. According to Robert Butler, institutional framework in place to help the elderly improve their health, economic standings, and quality of life are greatly hindered by the perceptions and stereotypes held by the general community.[5]

Butler's statement can be displayed by examining the differences in community support services (CSS) between two countries with differing treatment styles of their elderly populations. This relationship is examined by Susan Collins and her two co-authors by comparing the public policy styles of two countries both typically considered "western" nations, The U.S. and Sweden.[6] The main piece of legislation within the U.S. that directly affects the quality of life of senior citizens can be found in Title 1 of the Older Americans Act which dictates the basic rights of the elderly that must be protected through public funding. This funding is typically the responsibility of local, non-profit organizations or private corporations. According to Collins, the general involvement of the U.S. government in the lives of seniors is a secondary role that displays the cultural view of elders as disconnected from the center of the community. Sweden's policy, on the other hand, reflects a drive to maintain a person's independence and to encourage his continued involvement in the social infrastructure by promoting policies that improve his ability to perform the basic activities of daily life. In Sweden, the government takes a primary role in the care of its elderly, elevating these persons from the "secondary citizenship" role they possess in the U.S. Further research is required to compare the direct impacts on health by these two differing policies and levels of equality, but applications of anthropological thought about the effects of social stratification and Richard Wilknson's work on relative deprivation allow the assumption that the country with the smallest disparity in social advantage would have the "healthiest" population.[7]

The Impact of Culture on Health

Stereotypes and community opinion of the elderly do play a role in the health of this stigmatized population, though the direct cause is not fully understood. The effect of the brain on the body is still not fully understood. Sydney Greenfield, in his book about religious healing in Brazil, witnesses the abilities of faith healers to perform invasive procedures on their patients without any reports of pain and with full recovery by the patients despite no use of anesthesia or antiseptics and attributes this to the incredible ability of the mind to somaticize suggestions and beliefs.[8] Further effect of the mind on the body was found by Richard Davidson, working with the Laboratory for Affective Neuroscience, during a study that examined the connection between training in mindful-meditation and the body's immune response to the influenza vaccine through a controlled, randomized study over an 8 week period.[9] While the exact cause of the correlation requires further investigation, the relationship between negative perceptions of aging and the elderly and the health status of those respective individuals has been documented using several parameters and controls.

The idea of "ageism" is still not fully accepted within the general population, meaning that most stereotypes of the elderly function beneath the conscious thoughts of those that hold them. For this reason, it serves as a difficult barrier to overcome, despite the impact that its removal would have on quality of life for every person fortunate enough to reach an advanced age. Unique to age-related stereotypes is that they steadily develop into self-stereotypes and it is these self-stereotypes that, according to Becca Levy, have an impact on mental and biological functioning.[10] Levy found that older persons primed with words associated with a negative stereotype of the elderly performed worse on memory tests than those primed with positively associated words. It should further be considered that the positive primers also seemed to be correlated with an increase in cognitive performance, when compared to the unprimed control group. Considering these primers were presented without the conscious awareness of the participants, it leads to a conclusion that the impact of these stereotypes also occurs with the same lack of awareness.

Levy expanded the study to include the effects of these stereotypes on physical functioning, to show that they have an even more direct impact on health. Her results reveal that those primed with a negative stereotype not only suffered greater cardiac stress during a strenuous physical action, but that they also had an increase in vascular activity *before* the assigned task. The build-up of this effect over the 23 years studied by Levy's other work may have led to

reduced life expectancy of those with a more negative opinion of themselves as an elderly person, by as much as 7.5 years. Fortunately, while negative stereotypes are easily absorbed into self-perceptions, so, too, do positive stereotypes make an impact on the subconscious of the person and, therefore, affect their health statuses in the same manner. To examine this effect, Levy conducted another longitudinal study that investigates the effects of positive expectations over an 18 year period when other predicted variables are controlled.[11] This particular study found that those with a more positive perception of aging reported better functional health throughout the years of the study when compared to the baseline levels measured 18 years earlier. When the covariate of social isolation was added to the equation, the correlation became even stronger. Self-perceptions of aging, when compared to other factors often measured in longitudinal health studies, proved to be a greater predictor of functional health over a period of time than both race and socioeconomic status, though it was not as strong as age and overall functional health.

While Levy's work shows the impact of self-stereotypes on physiological and psychological functioning, attitudes and perceptions of the self, no matter how ingrained they have become into the subconscious, are in a steady process of adaptation and change. Pnina Ron conducted a study in Israel to investigate this change in attitudes and how it might impact the lives of elderly persons[12]. 75% of all elderly persons in her study had changed their perceptions on aging either from positive to negative or vice versa. She explains the change of positive to negative perceptions as a reflection of the "youth-centered nature" of a western society that values utility and productiveness where older persons are no longer contributing and may instead be viewed as a burden on the present. This change was found to be related to a decline in perceived health status by those individuals with a negative perception of aging. Cause and effect, however, cannot be determined using these data. At this point, no speculation can be made as to if the change was made due to a decline in health or if the decline was a "nocebo" effect (a term coined by Robert Hahn to describe the physiological effect of expectations of a negative outcome) caused by the embodiment of the negative stereotype of older persons as degenerating and burdened by excessive chronic illness[13].

One final variable important to the quality of life in older adults is the extent of social integration vs isolation. A common occurrence amongst the elderly is a steady decline in social network size with a greater dependence on a few close relationships. While conducting a six year study of a group of seniors age 65+, Oliver Huxhold and colleagues found that those with higher amounts social engagement reported either a maintenance of or increase in life satisfaction. In accordance with anthropological thought on the social "buffering" system against both disease and illness, those seniors considered the least isolated reported a much better quality of life.[14] Of course, this correlation is more circular than a direct, linear form of cause and effect. Those seniors with a larger support network are buffered from the deleterious effects of isolation and inequality. The healthiest seniors, those with the smallest decrease in the ability to perform the basic activities of daily life, are then able to participate more in community activities and to create a stronger social network. For this reason, efforts to "defeat" ageism would allow more opportunities for seniors to become integrated into the extensive social networks around them and to thus improve their quality of life even further.

Concluding Thoughts

The senior population is growing, and further research is required to improve the quality of life of this already disadvantaged group. Research should investigate further links to physiological disease and negative stereotypes of the elderly to find ways to help mitigate the rising health costs for this population. Also, research should look into cultural biases as a potential cause for disease as those considered to be "old" conform to the view of their population as ridden with chronic conditions. Potential research questions include cultural opinions on the inevitability of degenerative diseases like Alzheimer, dementia, and osteoporosis, differences in ageism both inter and intraculturally to classify the discrimination as either implicit or explicit and to discern the exact repercussions of these cultural ideals, and an investigation of cultural consonance amongst the elderly to potentially discover a correlation between an inability to perform the expected roles of a senior citizen and the somatization of those inabilities in the form of disease and illness. The results of the studies discussed throughout this paper and the answers to these research questions would benefit the millions of people quickly approaching the age range to officially be considered "old," which is why this particular field is growing so rapidly and gaining ground as a solid line of investigation.

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Maternal Child Health Care on Pine Ridge Indian Reservation: Explaining Current Disparities and Efforts for Improvement

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The purpose of this study was to evaluate opportunities and barriers to high-quality, culturally-appropriate maternal and child health (MCH) care for Oglala Lakota Sioux living on Pine Ridge Indian reservation. 21, in person, semi-structured interviews were conducted to assess MCH care on the reservation. 6 employees of the Indian Health Service and 14 women seeking pre- or post-natal care were interviewed. Data were qualitatively analyzed using content analysis. The interviews resulted in defining the role of the midwife and revealed barriers to health care. Strengths of the MCH program on the reservation were the hospital breastfeeding initiative and improved prenatal diet habits. Opportunities for improvement are centering programs, cultural revitalization, housing, water and sanitation, and specialty care through the IHS. The largest barrier to prenatal care was transportation so a bus system or ride-share program could be implemented. Also, the IHS could consider enacting greater incentives for specialty care providers.
Key Words: Midwifery, Indian Health Service, Pine Ridge Indian Reservation, Access to Prenatal Care

Introduction

The U.S. spends $86 billion annually on maternity and newborn services, which is more than any country in the world, but it ranks below 40 other countries in maternity health services [1]. Between two and three women die every day while giving birth in the United States [2]. Maternal mortality rates in 1987 were 6.6 deaths per 100,000 live births, this rate doubled in 2006 to 13.3 deaths per 100,000 [3]. Maternity services and care are unevenly distributed across racial groups, ethnic groups, income levels and regions of the country.

Within the United States, the disparities are worse amongst American Indians. This population has some of the highest disparities in health in the United States, especially in maternal health [4]. Current research shows five primary contributors that lead to the disparities: limited accessed to health facilities, poor access to health insurance, insufficient federal funding, quality of care issues, disproportionate poverty and poor education [5]. American Indians have more adverse maternal risk factors, including being unmarried and under 18 years of age, than whites and Hispanics [6].

In a case study of Washington State, the Department of Health and Human Services reported that American Indian women are the poorest and most likely to be enrolled in Medicaid than any other racial/ethnic group, with 79.2% of them enrolled [7]. It was also found that American Indian women were most likely to have high blood pressure, most likely to get late or no prenatal care, least likely to be married, and most likely to be abused by a partner before or during pregnancy [8].

While one in four women do not receive adequate prenatal care in the first trimester, the number rises to one in three for American Indian women [9]. The maternal mortality rate for American Indians is 16.9 deaths per 100,000 live births, higher than the national rate of 13.3 deaths [10]. The infant mortality rate of American Indian children is 1.7 times higher than the non-Hispanic white rate at 8.3 per 1,000 live births [11]. The rate of preterm birth for American Indians is 13.6% of all live births, the second highest rate for racial/ethnic groups in America [12]. The low birth weight rate is 7.4% for infants born to American Indian mothers, while their Sudden Infant Death Syndrome (SIDS) rate is the highest of any racial/ethnic group [13].

Early breastfeeding rate for American Indian women is 47%, below the national average of 62% women early breastfeeding [14]. The rates of fetal alcohol spectrum disorders (FASD), including fetal alcohol syndrome, for American Indian populations are some of the highest rates in the nation with some areas reporting 2.5 per 1,000 live births, far exceeding the national rate range of 0.2 to 1.0 per 1,000 live births [15]. These statistics show a lack of prenatal and postpartum care for American Indian women.

These numbers represent the whole American Indian population, but certain reservations have worse disparities based on their location, poverty and political and historical situation. A report conducted in 2004 by the Office of the General Counsel, U.S. Commission on Civil Rights focused on American Indian health care disparities. The authors stated that “the existence of glaring disparities across a wide range of health status, outcome, and service indicators, combined with the manner in which the disparities mirror patterns of his-

torical discrimination, makes a convincing argument that the current situation is in fact discriminatory" [16]. Pine Ridge Indian reservation of the Oglala Lakota Sioux people in South Dakota is a notorious example of governmental discrimination. It is the site of Wounded Knee in 1890 and the American Indian Movement's 71-day siege resulting in FBI violence towards American Indians on Pine Ridge. The effects of these events and years of oppression are clearly seen in their health disparities, especially maternal child health.

Pine Ridge is the second poorest county in the country. At the same size as Connecticut, Pine Ridge has little infrastructure, small housing developments, only one hospital and two small health clinics for a population of over 40,000. A history of broken treaties and battles with the United States government has led to a defeated population with a poverty rate of 80%, unemployment estimated at 85%, a median yearly income of $3,500 and a life expectancy average of 52 years for women and 45 years for men [17].

This tumultuous history has led to harsh health disparities for the Lakota people. The population has 8 times the national average of diabetes and twice the rate of heart disease. Rampant alcoholism affects 80% of the population and the suicide rate is twice the national average. In the MCH realm, cervical cancer rate is 5 times the national average, 1 in 4 infants is born with Fetal Alcohol Spectrum Disorder (FASD) and infant mortality is estimated at 3 to 5 times higher than the national rate [18].

In a South Dakota Department of Health's Perinatal Health Risk Assessment Report from 2009, there is no mention of the disparities had by the Lakota people. The report is 77 pages long and briefly mentions the work of the Indian Health Service (IHS) but there is no attention given to the MCH disparities of those on Pine Ridge [19]. A general health report of South Dakota in 2010 reported 7.04 infant death rates per 1,000 live births and American Indian births were the majority of overall South Dakota births with 25.6% of births [20]. Every day in 2010, there was an average of 2.2 low birth weight babies born, 0.7 births to teenagers and 0.2 infant and fetal deaths [21]. There is little legitimate data surrounding the health and lifestyles of the Lakota people. For instance, the population is estimated at 40,000 people but in reality, the population is probably much higher. The lack of housing has pushed multi-generations into a single house, some trailers supporting over 20 people, yet this is not accounted for during census surveillance. In addition, the IHS produces maternal child health data from their hospital and clinics but this does not account for the services some Lakota women seek off of the reservation or when they choose not to seek health care at all. The health disparities faced by the Lakota people on Pine Ridge, specifically the women and children, must be addressed with additional research so that culturally sensitive and evidence-based approaches can be used to improve maternal and child health care.

Methods

Background and setting.

The project was conducted on Pine Ridge Indian reservation in South Dakota within the Pine Ridge Service Unit, including the Pine Ridge Hospital and Kyle Health Clinic. The sample population was the Oglala Lakota Sioux American Indians of Pine Ridge Indian reservation. Six employees of the IHS were interviewed to understand the initiatives of IHS at the national level and more specifically in the Pine Ridge Service Unit. All patients interviewed were Lakota. None of the employees of the IHS interviewed were of tribal descent. The hospital and clinic on the reservation are part of the IHS, which is a branch of the Department of Health and Human Services charged with the health care and well-being of American Indians.

The interview was semi-structured, with questions relating to prenatal care, access to care, postpartum care, breastfeeding initiatives, community health work and culturally competent care that respects Lakota culture and beliefs during health care delivery. The interviews were intended to be formative and hypothesis generating, rather than hypothesis testing. All interviews were audio recorded with a Smart Pen, and handwritten case notes were also gathered.

This study was approved by the Human Subjects' Institutional Review Board (IRB) at Davidson College. Approval was also obtained from the IHS IRB, located in Aberdeen, SD (the regional headquarters). The IHS IRB also required approval from the Oglala Lakota Sioux tribal government and Service Unit approval of the hospitals and clinics on Pine Ridge. After receiving all approvals for human subjects' research, the project was completed in the summer of 2012.

Recruitment of Indian Health Service employees.

The employees of IHS were contacted via email before interviews took place. The four interviews of IHS midwives took place on the Pine Ridge Indian reservation in the hospital or clinic. In addition, an interview was conducted at the IHS headquarters in Rockville, Maryland with a senior headquarters staff member to ensure the interview guide was informed

by the overall goals, programs and initiatives of the IHS. There was also an interview conducted with an Aberdeen Area senior staff member on Rosebud Indian Reservation, a neighboring reservation of Pine Ridge, where he was temporarily working.

The employee interviews at Pine Ridge Hospital and Kyle Health Clinic were all with midwives. These interviews were taken in their closed offices or the clinic break room with the door shut. Written informed consent was received by all interviewees and their anonymity was maintained. Each participant received a copy of the signed consent form.

Recruitment of patients seeking care at the Indian Health Service.

Women were recruited to participate in this study if they received care from the midwives in the Pine Ridge Service Unit. The women had to be currently pregnant or have been pregnant within the last 5 years. They had to be at least 18 years of age and they were voluntary participants recruited by posting flyers in the midwife clinic. The midwives also helped in the recruitment by speaking to the patients after their appointment, informed them of the research being conducted and asked if they would like to participate. There was a $15 incentive for the women to participate. The informed consent was read aloud to the participant to ensure they understood the goals, risks, and benefits of participating in the interview. All women who agreed to participate gave written informed consent. The interviews were conducted in the break room in the midwifery clinic with the door shut and a sign on the door that an interview was in progress.

Evaluation and analysis.

The audio-recorded interviews were transcribed and then analyzed for key themes related to the overall goals of the project: barriers to access to care including geography and transportation, appointment attendance, culturally competent care, prenatal habits and efforts for improvement including breastfeeding initiatives, midwifery style of care and government initiatives on the reservation. The responses of both the employees of the IHS and patients were considered to analyze the effectiveness of midwifery care and programs set in place by the IHS as attempts to ameliorate the maternal child health disparities on the reservation. Special attention was given to convergent and divergent themes across the patient and employee populations. Interviews concluded when saturation occurred, when response became redundant, and no new information was provided with subsequent interviews. A summary of key findings will be shared with the members of the tribal government and Pine Ridge Service Unit.

Results

*The role of the midwife at Pine Ridge***.**

One midwife described their role as midwives in the IHS as to "provide comprehensive women's health care…in a holistic, culturally appropriate setting." Another midwife described that midwives have been accepted by the Lakotas since they started working with IHS. She stated "it has been a perfect match for women's health and Native families, at least on Pine Ridge."

Working in an understaffed and underfunded environment, the midwives still see themselves as a "positive force" for providing maternal and child health care to Oglala Lakota Sioux women and families. Two midwives in the Pine Ridge Service Unit described that a "healthy community is based on a healthy mother and healthy children," which is a belief that fuels their passion and commitment to working on the reservation. They also explain the difficulties and challenges they face working in an impoverished area and in a government organization with a lot of "red tape." "You're hanging on trying to be on top of your game and you don't have the support, you just go do your job…it's a real tough place," admits one midwife at Pine Ridge Hospital. One midwife described her four year employment at the hospital as "treading water" and that there have been "times when it's just me and one other nurse midwife…So I'm doing practice of what five midwives should be doing."

Barriers to maternal health care among Lakota women.

The Lakota women, as one midwife said, "want to have healthy babies, they want prenatal care, they come in for follow up" but it is the barriers to access to care that result in poor maternal and infant health disparities. Barriers include geography and transportation, patients' lack of prioritization of health care, access to health care providers and continuity of care, and historical discrimination of American Indians.

*Geography and transportation***.**

Pine Ridge Indian Reservation is the size of Connecticut but the only places for prenatal care and access to obstetrics and gynecology services is at the Pine Ridge hospital and Kyle Health Clinic. Women must travel far distances in order to receive care. Most women interviewed had to travel 30 minutes by car to

get to their appointments. However, "transportation is a gigantic issue…people don't have cars" describes a midwife.

Patients cited lack of cars for transportation to appointments as a major barrier to health care access. One patient said she "usually hitch hikes to get up here or I ask one of my relatives to see if they can give me a ride." When those options don't work out, she walks into town, which is 35 miles from her home. Another patient cited difficulty managing appointments with jobs available on the reservation that had little schedule flexibility so it was "kind of challenging for me to make it to my appointments. So it was go to work and make money or go to your appointments. So it's kind of hard to choose." One patient reported that "rides are starting to get hard to find" and she is dependent on family members and neighbors for rides to her appointments. A midwife described the transportation barrier as "if they have one car in their household that car needs to be used to go to work and if someone else has an appointment for a pap smear, work of course is going to supersede an appointment…we have a high no show rate for appointments."

Another barrier discussed was the weather conditions on the reservations. Patients worried about walking to the hospital in the heat of the summer and their inability to travel in the winter because of below freezing temperatures. An Aberdeen area staff member admits "the winters here can be very hard so we have to really maximize the use of resources such as home visiting services like public health nursing and try to really have our outlying clinics in the highest capabilities possible."

Patients' lack of prioritization of health care.

One midwife described this high no show rate for appointments because of different priorities. The 10th of every month is pay day "so they go to Rapid City to shop and they'll just bail on their appointments, it's just not as important. Food is more important than a prenatal visit." Another reason for a high no-show rate for appointments is the Lakota cultural value of putting family and community before oneself. One midwife described it as "the Lakota value of valuing your community…in the Lakota perspective you look out for your whole family clan and if someone needs you, you may put aside what you needed to do that day to help that person and if that means missing your pap smear appointment, well that happens."

In addition, the prenatal care rates vary from regions on the reservation. One midwife reported that "Pine Ridge has, according to our stats, the highest rate of no prenatal care and substance abuse in pregnancy." This is largest, most densely populated town on the reservation and the hospital is located right in the middle. The midwife surmised that the patient's thought process is that "they see the hospital and say it is there and I'm fine. If I have a problem, I can go there." Some patients, therefore, do not value preventative care and only go to the hospital for emergencies. In this case, they do not go to regular prenatal care appointments but will go only if they have a noticeable problem.

Access to health care providers & continuity of care.

Low staff numbers and male OBGYNs also present a barrier to care. One midwife described a contract OBGYN who didn't understand the culture and, to the patients, was chauvinistic and "they didn't understand [him] and they weren't going to come back." Many patients explained their discomfort with male providers. When the contract OBGYNs are male, then it is "a little more uncomfortable," notes one patient. Another patient declared "I don't like other guys touching me," reflecting the frustration with male OBGYNs and a barrier to access, because many women refuse to be seen by a male doctor for OBGYN services. One of the midwives described seeing a white male "who is not familiar with the culture…is very invasive for a lot of them."

The low retention rate for IHS employees results in difficulties sustaining a solid practice. One midwife lamented "I've been here four years and in those years we've had ten permanent nurse midwives fill those five positions." Another midwife reflects on the hospital being staffed at 60%: "that's very low, so you can imagine trying to a run a clinic at 60% staff. It's just not possible so people are short-staffed."

Furthermore, a lack of resources leaves the providers unable to provide services to high risk pregnancies. Diabetic women have to "deliver in Rapid City because we don't have the nursery care needed for babies born to diabetic moms. We just don't." The IHS is "funded at 50%," a midwife recounts, "and it's a population that gets sick at a younger age…so people come for services and we can't afford those services so we send them out for additional services. You can see that can really upset people who live here…A health care system that cannot meet their health care needs. That's based on funding."

A major problem discussed by all midwives was the lack of staff and especially lack of constant, long-term staff. An Aberdeen area staff member describes the loan repayment program as helpful in attracting contract doctors and health care providers. "The IHS scholarship and loan repayment used as an incentive to actually recruit people to come and work

on the reservation in our IHS facilities." In addition, the "rural OB nurse residency program, which serves as the mechanism to train nurses to take care of people in rural and remote areas" has been good at training nurses to provide on reservations.

However, the IHS hires these contract workers who only work for a certain amount of time. "We have contract OBGYNs," a midwife explained, "they change every two weeks. They are not committed to the community because they are contract docs. They have no investment." While the contract system does bring in providers, it is difficult for the midwives and other providers who work there long term. One midwife explains that "even when we get a doc we still do it with contract docs. The problem with the contract docs is they set up a plan and then two weeks later the plan changed with the next doc."

Historical discrimination of American Indians.

The challenges also extend beyond any particular reservation to the historical discrimination experienced by American Indians. An IHS headquarters staff member explains American Indian culture as life revolving "around the mother and the grandmother" and that there has been "high regard for the rule of the mother and the women and really the sanctity of child-rearing." However, the "genocide and apartheid and unbelievable oppressive discrimination in every possible way" that American Indians have faced because of "the almost genocidal policies that have been pursued by a federal government" have resulted in a defeated and impoverished population. A practicing midwife on the reservation ties these problems to health: "The women are very healthy and our birth outcomes are great but there are so many social issues that interfere with health."

Within the American Indian community, there is a general distrust of government programs, especially with the Indian Health Service. "There is a long history," a midwife describes, "of stories of the Indian Health Service experimenting on people or breaching their confidentiality." Another midwife discussed "the system in IHS is somewhere people have felt so marginalized and so degraded and not listened to."

Many patients interviewed were shy to discuss their feelings towards the Indian Health Service, perhaps because of the fear that the interviewer would report their stories. However, one patient spoke of her unfortunate experiences with the outpatient clinic of Pine Ridge Hospital. "They read the x-ray on my ankle wrong," she said, "They said it wasn't broke but it was and I needed screws." She had to go off of the reservation to receive care. She also mentioned her "aunties don't like coming here" because they lost her baby. Another woman who came to Pine Ridge for prenatal appointments said that "some people say they probably practice on us" and that the doctors are "foreigners that you can't really understand." There is a general distrust for the IHS on the reservation among the Lakotas. However, only one patient had anything negative to say about the midwife clinic on Pine Ridge, citing their aunt's negative experiences of the IHS midwives "losing her baby." Overall, the midwives are trusted and respected as providers whereas the rest of the hospital is seen with distrust and sometimes fear and anger.

Strengths of the Maternal and Child Health Care Programs on Pine Ridge Indian Reservation.

Baby-Friendly Hospital Breastfeeding Initiative.

The Baby--Friendly Hospital Initiative is, according to an Aberdeen area staff member, a program "co-administered by the World Health Organization (WHO) and UNICEF and it is based upon the 10 steps to successful breastfeeding and through those 10 steps, hospitals and health care environments transform their care delivery models into one that is conducive to infant bonding and appropriate feeding." The implementation of this initiative has resulted in "breastfeeding initiation rates and exclusivity rates going up" along with "dramatic increases in staff knowledge and in their commitment to empower people to make healthy choices." While this is being implemented in all IHS facilities, midwives at Pine Ridge reported that because midwifery inherently promotes breastfeeding, the initiative "really hasn't changed our practice except that we are more mindful of talking about the benefits of breastfeeding with each mom at each visit."

A midwife at the Pine Ridge Service Unit explained that there should be focus on the "role of the nurse in labor and delivery…if the nurse is really gung-ho on breastfeeding and really encouraging then that's a really a good thing for the mom. If she's not really willing to get in there and help her and show her, then I think the mom is going to give up and take up bottle feeding."

The patients interviewed overwhelmingly knew that breastfeeding was important and good for their baby, but that did not necessarily mean that they breastfed for a long time. One patient recognized the importance of breastfeeding describing that she "noticed the difference from breastfed and bottle-fed babies. Breastfed babies are kind of more smarter I would say. She started talking when she was 2 and everyone was amazed, walking early and riding bike

early. So this baby I will breastfeed too!" Another patient also saw the benefits of breastfeeding among her own children: "I breastfed all my kids and they all have good teeth. My other little girl she used the bottle and it rotted out her teeth and she had to get those pulled out." In addition, women saw breastfeeding as a "very good connection between the mother and child and good food source," citing what the midwives had told her.

Prenatal habits.

The women interviewed were part of the group of women who do receive prenatal care and so their prenatal habits are better than those not receiving care. They reported that with habits such as smoking and drinking, they quit once they found out they were pregnant.

A tangible improvement in prenatal habits of Lakota women was the increase of water drinking. The midwives have "been encouraging more women to drink more water instead of soda" during prenatal appointments and there is a public effort with water-promoting posters placed in the waiting room and around the clinic. "I'm seeing a lot more women come in with water bottles...it's a small thing," reports one midwife. Another midwife reported "I have seen them go from carrying in soda pops and they're carrying water bottles. That is huge! That is the greatest change I have seen. I've seen it. That is, to me, that is a change…I see them in the community drinking water, it's tremendous." Six patients reported the encouragement to drink water from the midwives. One patient described the midwife suggestion "to drink a lot water, at least three big tall water bottles, make sure to drink a lot of water." Another patient said "they encourage a lot of water, especially with it being the hot summer months."

The qualitative interviews informed the reasons for high maternal and infant health disparities on Pine Ridge Indian reservation: social, political, economic, and geographic challenges were obvious barriers to access to care. The interviews also highlighted some important and positive initiatives to ensure good health outcomes among mothers and babies. The IHS employees and patients, though, introduced and suggested ideas for improvements and programs to reach more patients and provide culturally-competent care.

Opportunities for Improvement

Centering.

"This is the first thing IHS has done for us that truly fits with our culture"

Centering is a "midwifery invented prenatal approach," explained an IHS employee, and it is in the process of being implemented on Pine Ridge Indian Reservation and has had success in other American Indian communities. Centering is group prenatal care with women on similar gestational tracks that includes prenatal education and check-ups for an entire group of women in hopes of sparking feedback and conversation among them. This group prenatal care allows for a woman to come in and be seen by a midwife "very briefly for information that has to be shared privately and that can be in a corner of the room" and then they join a group of women who are at the same point in their gestation. This was started by a midwife who worked in a federally-funded health center and was "tired of delivering the same education over and over again," explains an IHS headquarter staff member, and so she combined the prenatal appointments in a group. The midwife introduces a topic of conversation and the patients continue the discussion and "topics can go from benign nutritional to very intense domestic violence topics. That's the beauty." There is strong research that shows centering reduces preterm birth and low birth weights. It is the "gold standard" of prenatal care, an IHS headquarters employee explains.

On Pine Ridge, one midwife has a grant that will construct a new space in a building in order to have centering. One midwife explains her idea of centering with IHS on Pine Ridge: "If you think of traditional prenatal care, I'm sure they brought the community. Someone helping the women make clothes the baby's going to wear, breastfeeding, and traditional herbs. That is the kind of centering I'm anticipating here." Her vision for centering on Pine Ridge includes mixing "experienced moms and having young moms learn from these older moms."

Patients interviewed also desired some sort of group classes. One patient suggested "counseling for the women because they've…been through so much traumatizing things in their own childhood and growing up to where they don't have enough self-respect to take care of their bodies." This patient importantly portrays a key aspect of centering which is group cultivation of self-respect during pregnancy and teaching and learning ways to make the mother and her baby healthier. She further explained her idea as "a club for pregnant women to come together and encourage each other to stay healthy, to give each other support…something like that would help." Another patient expressed her wish that "there was more parenting around here like parenting classes" which would be a major part in a centering program. In addition, one midwife added a suggestion for the program of a more culturally competent practice of "offering

hospitality at your classes" such as a meal, which is very "culturally appropriate" for the Oglala Lakota Sioux.

Most of the patients interviewed mentioned dissatisfaction with the health of mothers and babies on the reservation and desired more programs for their health services. The employees of the IHS also desired to add new programs and improve health care delivery but because of constant "administration changes and staffing becomes shorter," implementing new programs is difficult.

Cultural revitalization.

One midwife saw the systemic problem coming from the impoverished lives on the reservation and she thought cultural revitalization would improve health disparities for the Lakotas on Pine Ridge and help raise them out of poverty culture. One midwife asserts that "the Lakota culture is very focused on maternal child health and well-being and holistic care. But, I think you really have to separate poverty culture and reservation culture versus Lakota culture…the culture is not what the poverty is. Poverty supersedes, it's a survival mechanism." Adding to this idea, another midwife suggested "encouraging them to really embrace their culture which has really great values. They've just gotten kind of lost along the way," she posits. There has been a big push on the reservation for a cultural revitalization because of centuries of oppressive policies from the American government to ban their cultural practices. This suggests that the previous values of matriarchy and respect for the mother could be reinstated and maternal and child health would become a priority on the reservation.

One patient, when asked of traditional Lakota views on pregnancy, relayed a traditional story of pregnancy for the Lakota people. Women were "considered in general really sacred as being the bringer of life. They were cherished. And I think that needs to come back because we're not cherished as much anymore. There is this ceremony called the Sundance. When I was little I heard that the women bring life to the earth so the men honor that and suffer through the Sundance, as showing to pray for the people but also to help endure the pain…It came hand in hand, the Sundance for the men and the birth of the child for the women." She suggests recognizing this fundamental cultural value as a way to improve maternal and child health on the reservation.

Housing, water and sanitation.

Another midwife suggested improved housing, water and sanitation initiatives to improve maternal child health. If given a sum of money to improve the health of Lakota woman and families, she said "I would put it in the community. I would build housing. I would build some sort of infrastructure to support the people." She also pointed out that "several women have no running water. How do you keep infection down when the insides of their homes have no running water in tubs? How do you care for your private areas when you need a tub bath?" This lack of housing, water and sanitation perpetuates the maternal and child health disparities and they must be combated in order to improve the health of the community.

Specialty care. A

ll of the IHS employees interviewed wanted specialty care providers in IHS hospitals in order to improve maternal and child health care. They want a maternal fetal medicine specialist on a weekly basis because of their high risk patients. Because of the unreliability of contract doctors, the midwives would like to have specialty care at least once a week at the hospital because they are unable to handle high-risk pregnancies as midwives. One of the midwives wanted a permanent, female OBGYN who "likes to do surgery and isn't scared of high risk." This would ensure the health of her patients, especially the high percentage of high risk patients, and bring her peace of mind to "know that my patient is getting the best care I can provide for her." An IHS headquarters staff member asserted that "patients need more access to specialty care."

Discussion

The findings from this study reinforce earlier studies that show American Indian women suffer social and economic disadvantages that result in higher health disparities than other racial/ethnic groups, such as poverty, poor education and limited access to health facilities [22]. The interviews also uncovered several important findings unique to the Lakota women and Pine Ridge Indian reservation. The effects of these social and economic disadvantages are seen in the health of the women and children of Pine Ridge Indian Reservation, such as 25% of infants born with FASD and an infant mortality estimated at up to 5 times higher than the national rate [23].

There are some limitations to the study. The 20 overall interviews, 6 provider and 14 patient interviews, is a small sample size. However, the 20 qualitative interviews were also a strong point of the study. The individual, focused and in-depth interviews resulted in qualitative data that explains the current disparities on Pine Ridge reservation and initiatives of the IHS to improve these disparities. The providers and patients delved into issues and topics addressing

the realities, challenges, and opportunities on the reservation for maternal and child health which adds new information to the existing literature. This study also provides quality and effective ideas for improvement from the providers who work first hand with the barriers to access to care. The patient feedback provided the population's needs and desires in health care. The IHS is charged with serving this population and could take this patient feedback to inform effective and quality policies. The patient interviews offer critiques of the current health services and suggestions to provide culturally competent, effective and quality care. Collectively, the qualitative interviews (while limited in size) provide richness in depth and ultimately led to community-driven solutions to address maternal and child health disparities that exist on Pine Ridge.

This study recognizes the unique challenges of providing maternal and child health care on Pine Ridge Indian reservation. The interviews of patients and providers offered experiences and opinions from the givers and receivers of care. The high poverty and unemployment and lack of housing and development on the reservation add to the barriers of access to health care for pregnant Lakota women and their children. These barriers must be addressed in order to improve disparities. The major complaint for access to care was lack of transportation, so a bus system or ride share program on the reservation could be implemented to improve prenatal and postnatal care. In addition, the IHS could consider enacting greater incentives for specialty care providers and OBGYNs to work long term at Pine Ridge hospital. They could also re-envision their contractual loan repayment programs to ensure longer stays that would lead to greater investments in the community. Because of high number of morbidities suffered by Lakota women such as diabetes and high blood pressure, there are a large number of high-risk pregnancies that need services from an OBGYN or specialty care provider, such as maternal fetal medicine. These additions would benefit both the patients of Pine Ridge hospital as well as the midwives in their attempts and desire to provide quality health care to the population.

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Ayahuasca Shamanism in the Peruvian Amazon: Contemporary Indigenous Knowledge, Bioscience, and Intellectual Property

Andrew Huckins-Noss

This paper focuses on the various medicinal, spiritual, and ceremonial uses of the Ayahuasca vine by indigenous shamans, healers, and people in the Peruvian Amazon, specifically in Iquitos, Peru. In addition to the medicinal and spiritual uses of the Ayahuasca vine, I introduce the concept of intellectual property rights (IPR) and consider some of the difficulties in establishing indigenous ownership over knowledge, as well as some of the concerns that arise in the decontextualization and simultaneous recontextualization of ayahuasca in the international drug development market.

Key terms: Ayahuasca, shamanism, Peruvian Amazon, indigenous medico-botanical knowledge, biopiracy, intellectual property rights (IPR), epistemology, U.S. patent system, Risk-Access-Benefit Sharing (RABS), institutional actors.

Introduction

What are the consequences and implications of recent efforts to internationalize medicinal use of the Ayahuasca vine, an important plant in indigenous knowledge systems of the Amazon? What happens to indigenous medico-botanical knowledge itself when it travels and becomes recontextualized? What happens to the people and the place from which this knowledge was extracted? Whose knowledge is this and how does one claim ownership over the plant and over information about its spiritual and medicinal uses? These are some of the main questions that arise in the debate over ayahuasca and its internationalization; patents and intellectual property are sought out by biomedical scientific researchers looking to isolate and reproduce the compounds from plants in indigenous medico-botanical healing systems. Thus, contemporary indigenous medico-botanical knowledge—in all of its complexity, nuance, context, and relationship to the land—comes to interface with the realm of biomedical sciences, which have an invasive approach and seek to extract the plant and the knowledge from its social, cultural, historical, and epistemic contexts.

In this paper my aim is threefold: First, I will describe the ayahuasca vine, noting its various medicinal and spiritual uses by shamans in the Peruvian Amazon. While many different indigenous groups in the Amazon use ayahuasca in ritual and spiritual ceremonies, in this paper I will focus on the indigenous healers and users of ayahuasca in the urban slum of Belén on the eastern side of the city of Iquitos, Peru. This allows me to focus in on the complexities of one group and their relation with the ayahuasca vine, while reading more broadly to gain an understanding of regional variations and the generalities that may exist across groups. Second, I will present some of the main ethical issues that have come to the fore as a result of biopiracy and the actions of biomedical researchers and entrepreneurs who seek to locate and extract indigenous medico-botanical knowledge. Often the aim is to isolate compounds and reproduce them in a laboratory setting, later obtaining a patent and rights to the distribution and flow of use of the product. Third, I will attempt to destabilize some of the common assumptions about ownership and intellectual property, considering the extent to which knowledge can be owned, if at all, and the difficulties that arise in trying to force a Western policy on a sacred plant that has been used in medical and spiritual contexts for hundreds of years by trained shamans and knowers. In addition to answering the central questions raised at the beginning of the paper, I seek to introduce and develop the following argument:

The concept of intellectual property and the U.S. patent system are ideological constructs that exist within a Western-epistemic domain. As such, they do not do justice to protection, ownership, and concerns of authority over knowledge that exists within some indigenous healing systems, such as that of Ayahuasca shamanism in the Peruvian Amazon. This is a complex healing system with variants among indigenous groups throughout the Amazonian rainforest; it involves an entire epistemic, methodological, and ontological framework that is intricately enmeshed with the land and people and presents ethical and functional concerns when it is extracted and recontextualized by biomedical entrepreneurs. Social scientists that examine the disconnect between sacred knowl-

edge and Western policy can help to untangle the different threads of understanding that exist in these two realms. What remains clear is that we must adopt new ideologies and frameworks for thinking about the protection of indigenous medico-botanical knowledge that involve the people they seek to protect and rest upon the methodological and epistemic concerns of that group.

My research engages a number of sources including ethnographic texts by anthropologists who document their experiences with ayahuasca shamanism through participant observation (literally taking the various preparations of the ayahuasca vine, either in tea or as part of a concentrated tincture) and structured/semi-structured interviews that sought to obtain the knowers' perspectives on the vine, as well as some of the views of the indigenous peoples in the village that use the vine routinely for spiritual and medicinal uses. The texts that most centrally inform my analysis here are "A Hallucinogenic Tea, Laced With Controversy: Ayahuasca in the Amazon and the United States" by Marlene Dobkin de Rios and Roger Rumrrill, from which I draw upon their ethnographic work in the Peruvian Amazon as well as their analysis of current issues in the scientization and recontextualization of ayahuasca as a patent drug; "Singing to the Plants: Mestizo Shamanism in the Upper Amazon" by Stephen Beyer, from which I draw upon his analysis of shamanism as a complex and multi-layered system of knowledge; "Antipodes of the Mind: Charting the Phenomenology of the Ayahuasca Experience" by Benny Shanon, which I utilize in order to help explicate the various visions and hallucinogenic manifestations that have been recorded; "Contesting Space and Time: Intellectual Property Rights and the Indigenous Knowledge Systems Research in South African Universities" by Mogomme Masogoa; and "Beyond Patents: The Culture of Native Healing and the Limitations of the Patent System as Protective Mechanism for Indigenous Knowledge on the Medical Use of Plants," by Ikechi Mgbeoji. Additional critical essays and shorter works that I draw from in this paper are listed in the Bibliography.

Part I: The Plant and its Contex

Iquitos, Belén, and indigenous healers:
The Peruvian city of Iquitos is located approximately 2,300 miles inland from the mouth of the Amazon River. Belèn is an urban slum near the eastern border of the city and is a community of approximately 12,000 people where indigenous healers, known locally as *sacerdotes* and *curanderos*, may be found with knowledge of the ayahuasca vine and its various spiritual and ritual uses. The city is home to many farmers' markets and the majority of its residents engage in agricultural work.

Map of Iquitos, Peru (Belénn is on the east side of the city) (Left); Map of South America with Peru highlighted in Red (Right).

Ayahuasca Tea: Preparations, Properties, and Components:

Ayahuasca is a drink that is made from the boiled stems of the ayauasca vine, *Banisteriopsis caapi*. Most often, Ayahuasca is prepared with other plants in addition to the ayahuasca vine, but sometimes the ayahuasca vine is used alone. The three most common admixtures are known as the compañero plants—such as the shrub chacruna, *Psychotria viridis*; a shrub closely related to chacruna known as sameruca, *Psychotria carthaginensis*; and a plant known as ocoyage, *Diploterys cabreran*a. The ayahuasca vine itself is woody, light brown in color, and weaves its way around tropical flora of regions of the Amazonian rainforest in Peru, Colombia, Ecuador, Brazil, Bolivia, and Venezuela. It has a striking double-helix pattern that resembles a strand of DNA. In addition to these plants, as many as 55 other different plant species may be added to the mixture; however, the final product is still called ayahuasca and it invariably contains at least some amount of the boiled stems of the ayahuasca vine [1].

Chemically, the ayahuasca vine works in the body in a similar manner to other hallucinogenic substances, such as LSD, peyote, psilocybin, etc. Harmine is one of the main alkaloids contained within the ayahuasca vine and it acts on the body as a selective Monoamine Oxidase A (MAO-A). The pharmacological action from here becomes more complex, and goes beyond the scope of this paper, which seeks to understand the uses and appropriation of ayahuasca in indigenous knowledge and biomedical sciences; however, what is most important is that MAO-A is an enzyme that de-aminates various chemicals in the brain that are integral to mood, appetite, and balance:

norepinephrine, serotonin, dopamine, and epinephrine to name a few. This complex chemical interaction is only part of what accounts for the intensity of an ayahuasca experience; the setting, context, and unique genetic make-up of a person determine the rest.

Left—the ayhhuasca vine in the Amazonian rainforest; Right—the ayahuasca vine along with other plants to be included in the preparation of tea. This particular picture shows ayahuasca vine and charuna leaves.

Potential Harms and Health Risks of Taking Ayahuasca:

Repeated use of ayahuasca can potentially raise blood pressure, as cardiovascular stimulation is often an effect of the substance. Additionally, there are a number of drug interactions to take note of: " Within the ayahuasca plant, chemicals called beta-carbolines have the potential to interact dangerously with other substances such as amphetamines and MDMA (ecstasy), as well as antidepressive and antianxiety medicines such as Zoloft, Paxil, Prozac, Luvox, Lexapro, and Effexor to name a few"[9].

Diet Taboos:

As a result of the purgative and diuretic properties of ayahuasca, preparation before the experience involves dieting—primarily consuming bland foods low in salts, oils, and fats. As Beyer draws to our attention, the term "la dieta," used with respect to the food restrictions and beliefs followed by many indigenous ayahuasca shamans, is much more comprehensive than its equivalent in English. In addition to food restrictions followed by healers in the days preceding a healing ceremony, "la dieta' encompasses social isolation and sexual abstinence. Other prohibitions may include avoiding the sun by keeping oneself indoors and trying not to be seen by strangers. Spicy foods may also be avoided in the ayahuasca diet [1] As one can see from the limitations, the healer's diet is bland to prepare for the intense purgation involved in taking ayahuasca and learning its ways as a form of medico-botanical knowledge. Knowers have been in contact with and actively using and learning about ayahuasca for years. Thus, "la dieta" becomes essential in maintaining health and strength through the violent purge that the body endures with each ingestion of ayahuasca. Bland foods, such as rice and beans, constitute the bulk of the shamans' diet in the Peruvian Amazon.

Spiritual and Medicinal Uses:

The ayahuasca vine has been a sacred plant in many indigenous cultures of the Amazon for nearly 8,000 years, allowing perceived communication with spirits of the dead and spirits of the earth [1].The stems of the plant can be pounded together and boiled in water to make a tea known as ayahuasca—this is a Quechua word meaning "spirit vine", "vine of the souls", or "vine of the dead". The vine has many different names throughout various indigenous groups of the Amazon. For instance, in Colombia the ayahuasca tea is referred to as yajé and contains a mixture of the ayahuasca vine as well as complementary herbal additives [5].

Ayahuasca preparation is often elaborate and varies from region to region; however, the process that I describe in the following paragraphs is that of the shamans in Belén of the Peruvian Amazon. First, the vines are obtained in the rainforest, usually among low-lying shrubs and are cut into foot-long pieces. The portions of the vine are then brought back and boiled in water, along with several other herbal admixtures, such as Psychotria viridis (chacruna). Once the preparation is boiled and cooled, the resulting liquid is ayahuasca tea that is ready for consumption and medicinal or spiritual use

Left—the boiling process involved in the preparation of ayahuasca tea in the Peruvian Amazon; Right—a small cup containing the boiled ayahusca tea ready for consumption

Mapping the Phenomenology of the Ayahuasca Experience:

The phenomenology of the ayahuasca experience is complex and varied; individuals' experiences

with drugs are highly subjective and dependent upon variables specific to that person's genetics. Despite these factors, certain aspects of the ayahuasca experience seem common to a majority of people, which I now will describe and map out. The first sensation that ayahuasca produces is disgust. Sometimes, those who take it will even vomit or produce expressions of revolt directly after drinking the mixture. Within the first few minutes, the aspect of force is experienced: "…when the 'force strikes, usually around forty minutes after consumption, many are prone to vomit. It is a vomit like no other—drinkers often feel that they are pouring out the depths of both their body and their soul" [8].

Additionally, there are other aspects common to the experiences of many drinkers, such as the general and unspecific feeling that one's body and perception are different during inebriation. Some describe it as if a cloud was within their bodies, or that some force over which they have no control was moving their body. Usually the harshest effects of ayahuasca inebriation occur during the first 90 minutes following the ingestion. Throughout this time, and mounting in intensity leading up to 90 minutes, visions can be extremely intense, often frightening. The next period is often more manageable for people, and generally lasts for 2 hours. In this stage, drinkers may truly come to understand and like the ayahuasca experience. It is in this period that a person may begin to become more introspective, "…presenting with moments of exhilaration and great wonderment" [8]. The final stage of the ayahuasca experience is the mellowest and is sometimes accompanied by feelings of profound relaxation, serenity, and extreme peace of mind. It is during this stage that people also generally feel a great "…love for fellow men and women and deep affinity to nature and to all existence" [8]. Although the inebriation lasts 4-6 hours, the sense of revelation and inner peace may remain with the drinkers' spirits through the following day, described as an afterglow.

There are a number of other important aspects of ayahuasca visions that should be mentioned here: First, the term beautification articulates that under the inebriation of ayahuasca colors may seem brighter or more saturated. Additionally, people sometimes perceive that there is an all-encompassing harmony within the landscape around them and may even see objects fuse together into one. Meaningfulness is another important and often-reported feature of the ayahuasca experience, in which things seem to have profound meaning and significance. It is with this type of appreciation that new understandings and realizations are reached [8].

An artistic rendering, by Pablo Amaringo, a Peruvian artist known for his use of vibrant and contrasting colors, of visions most commonly produced through ayahuasca inebriation. Participants often report snake-like imagery and objects which resemble the ayahuasca vine itself but in a larger and transformed manner. This particular rendering of what one might possibly see during an ayahuasca experience shows the extent to which colors are brighter and various objects seem to blend into one another

In addition to the spiritual enlightenment and interconnectivity that may come as a result of taking ayahuasca tea preparations, the vine has profound purgative and diuretic effects that can be particularly useful to some people in areas where intestinal worms are more common due to unclean drinking and bathing water. For example, in "Singing to the Plants: Mestizo Shamanism in the Upper Amazon", Beyer argues that the ayahuasca experience is simultaneously mental and irreducibly physical. The body is the instrument through which the shaman evokes certain spiritual changes and realizations in patients which are accompanied by very intense bodily excretion: "nausea vomiting, sucking, gagging, belching, blowing, coughing up, spitting out,; perfume, tobacco smoke, rattling, whispering, whistling, blowing, singing, the taste of tobacco and ayahuasca, the imagery and ritual of the body, conflict, mess" [1].Here, Beyer communicates the extent to which the ayahuasca experience is profoundly metaphysical, and puts the mind in touch with the body such that purgative properties of the ayahuasca accompany, and in a sense, complement the often immensely profound spiritual and ideological revelations that take place on the emotional and sensory levels.

The mechanisms and principles that underlie the use of ayahuasca as a medico-botanical plant with

curative capacity are fairly simple: the intense purgation rids the body of any illness or unclean spirits, which is accompanied by intensely profound spiritual and intellectual enlightenment and auditory/visual hallucinations. Despite this seemingly straightforward underlying principle, shamanic use of the ayahuasca vine is complex and varied. For centuries, practitioners of indigenous knowledge have used the vine to heal as well as to bewitch enemies. "The visions that accompany the tea help the shamans do battle with the enemies from other tribes, as well as predict the future." [1] Further, social anthropologist Stephen Hughes Jones points specifically to the ambivalent nature of the shaman in Amazonia; shamans may use their power for good or evil. Anthropologist Mary Douglas calls this "…the theory of the unity of knowledge—that those who can cure can kill" [1]. This type of knowledge construction is an important part of ayahuasca shamanism among indigenous people in the Amazon, as it acknowledges the power and responsibility that come with being a healer.

As with any healing system, there are various systems of belief held by practitioners and patients, which legitimize the medical practices and give them relevance within specific cultural and epistemic contexts. Various forms of medico-botanical knowledge are not different and involve complex systems of belief and power hierarchies of knowing and believing, where knower, healer, patient, and community are all interwoven in a nexus of shared belief and mutual legitimization. French anthropologist and sociologist Claude Levi Strauss developed the theoretical concept that there are three basic layers, or levels, of belief within any type of ethnomedical healing system. First, the healer must believe in him or herself and the power and knowledge that s/he holds regarding the medicine, the illness of the patient, and their overall confidence in the methods that they have practiced and perfected through years of apprenticeship type training; second, the patient must believe in the power and knowledge of the healer to heal; and third, the community, or larger general culture in which the healer is practicing, must acknowledge the legitimacy of the healer by believing collectively in the methods practiced. These three layers of belief are mutually reinforcing, co-functional, and sustain the healing practice within the community. For instance, in "The Sorcerer and His Magic" Levi-Strauss describes a situation in which a Zuni boy is accused of sorcery, but in the process comes to feel a sense of power and trust in himself through belief by the community that he possesses supernatural powers:

> At times [the boy's] face became radiant with satisfaction at his power over listeners…The girl recovers after he performs his curing ritual. The boy's experiences during the extraordinary ordeal become elaborated and structured. Little more is needed than for the innocent boy finally to confess to the possession of supernatural [healing] powers already acknowledged by the group [10].

The three layers of belief are clearly expressed in this narrative, where we see the power of shamanic constructions of knowledge and the interplay between patient, healer and a community of believers.

In ayahuasca ceremonies, the healing is communal, with many people (sometimes up to 30) taking the tea at one time, and frequently the healer takes the mixture as well; however, his or her job is often quite complex and he or she may decide to take only a small bit of the substance to accompany patients on the journey while retaining certain faculties in order to make use of the knowledge and "perform" the healing ritual. In the ethnographic text "Singing to the Plants: A Guide to Mestizo Shamanism in the Upper Amazon," Stephen Beyer draws our attention to the extent to which shamanism and indigenous medico-botanical healing systems are often performative and involve a number of aspects which establish the knower as legitimate and worthy of performing the healing ritual. For instance, Beyer notes "Historian Ronald Hitton puts it this way: all shamans are performing artists. If shamanism is partly a craft and partly a spiritual vocation, it is also an aspect of theater…" [1].Further, Beyer delves into some of the complexities and conflicts of interest that can potentially arise within the shaman-patient relationship: "The shaman's task is to constitute all the participants into an active presence with which a dynamic relation can be created. The shaman keeps the audience active and interested by providing enigmatic commands and riddles, mysteries that engage the audience" [1]. Thus, the shaman must negotiate a persona within the ceremony such that he

or she is simultaneously performing and participating alongside those drinking the brew.

In the image that follows, depicting the beginning of an ayahuasca experience of the Amazon, it is clear that the healer-patient relationship is of chief importance; however, the shaman maintains a clear distance and sense of removal from the participants in order to negotiate the performance and draw upon a wide range of knowledge and skills. A seeming paradox emerges, wherein the shaman must guide and help participants through the ayahuasca experience, but at the same time must maintain distance, ambiguity, and mystery.

Part II: The Internationalization of Ayahuasca

What happens to a sacred plant that is extracted from its context and made transposable to consumers across the world? What types of ethical concerns emerge with regard to protection of knowledge? Beginning in the late 1950's and early 1960's, botanists and anthropologists began exploring and recording experiences with ayahuasca, soon raising the question of whether it could be commercialized, in turn bringing up ethical issues of knowledge protection, ownership, access, and rights. Power is an essential concept to introduce into this discussion, insofar as indigenous rights to knowledge and epistemologies are subject to the power differential that privileges Western bioscience. For instance, in his article " Contesting Space and Time: Intellectual Property Rights and the Indigenous Knowledge Systems Research in South African Universities" Mogomme Masoga discusses Intellectual Property Rights and Indigenous Knowledge Systems in South Africa, arguing, "When one looks at the IPR (Intellectual Property Rights) and IKS (Indigenous Knowledge Systems) Research – the question of power remains, and probably will always. The current IKS Research in South Africa vividly displays a lack of space. The indigenous epistemology world has been invaded and occupied apparently without any ethical consideration" [3]. Here, Masoga draws our attention to the importance of considering power inequalities in the conversations (or a lack of conversations) that take place between bioprospectors in Western bioscience and indigenous healers within shamanic medical systems. Power garnered by Western bioscience, he argues, allows for the invasion of the indigenous epistemology world. Further, Masoga contends:

In short, there is need for local critical mind space (cf. mapping local knowledge) in looking at the IPR and the Indigenous epistemologies research. The glossary to be used in this process should be locally oriented – directed and controlled by custodians of local knowledge and wisdom. Further, there is a need to suspend, for a while, dominant language formulations and to allow local perspectives or voices to become dominant in the debate on IPR and IKS research. As indicated earlier on, it is not helpful to use dominant discourses in protecting local discourses. [3]

This helps elucidate the extent to which protection of indigenous knowledge must have a locally oriented glossary and be constructed within the epistemic context of the indigenous people, in order to take fully into account how they conceptualize risk, access, and benefit sharing.

This conception of locally determined policies that acknowledge the epistemic concerns of the group resonates with Linda Tuhiwai Smith's text "Decolonizing Methodologies: Research and Indigenous Peoples". She argues for a revamping of research methodologies that ask questions in a different way and approach issues within the epistemic and methodological framework of the indigenous group of knowers. For instance, near the conclusion of the text Smith argues "Engaging in a discussion about research as an indigenous issue has been about finding a voice, or a way of voicing concerns, fears, desires, aspirations, needs, and questions as they relate to research. When indigenous peoples become the researchers and not merely the researched, the activity of research is transformed"[7]. In a similar manner, the approach to protection and ownership of indigenous medico-botanical knowledge (such as that which I have documented in this paper through the various uses of ayahuasca in the Amazon) must take into account local meanings of ownership and incorporate them into policy that protects indigenous plant knowledge in a way that is relevant to the knowers ,producers, and users of this knowledge.

In his article "Insurgents at the Gates? Patents, Biopiracy and the (re)Emergence of Indigenous Peo-

ples' Knowledge in Contemporary International Law" Ikechi Mgbeoji articulates the importance of analyzing the key institutional actors within the realm of international law that deals with the appropriation of indigenous knowledge but privileges the white, European mindset: "However, in recent times, this attitude, as reflected in both international and domestic law, has witnessed a slight change. Modern international law instruments such as the Convention on Biological Diversity (CBD) now recognize the empirical and scientific character of indigenous peoples' knowledge. Similarly, some states have begun to enact domestic laws protecting traditional knowledge" [4]. Other key Institutional actors include: (TRIPS), Trade-Related Aspects of Intellectual Property Rights, which seeks to develop effective intellectual property rights that do not serve as barriers to international trade. Risk, Access, Benefit Sharing (RABS) is another important consideration in the construction and implementation of international policy regarding the use of medico-botanical knowledge; this determines how knowledge is protected and what access people have to it, as well as where benefits (monetary or other) go. The issue, however remains the same: Intellectual Property Rights must be articulated by the indigenous people whose knowledge it attempts to protect. As scholar Vandana Shiva attests "the globalization of Western intellectual property rights will inevitably diminish the world's biodiversity because Western intellectual property regimes place no value on the communal knowledge of indigenous societies" [10].

In addition to this invasion of indigenous epistemic space and continual epistemic violence—wherein indigenous ways of knowing were discredited and disavowed—the structure, language, and underlying concerns of IPR are currently unable to accommodate the complexities of establishing ownership and authority on a sacred plant that is part of an indigenous healing system. For example in "Beyond Patents: The Culture of Native Healing and the Limitations of the Patent System as a Protective Mechanism for Indigenous People on the Medicinal Use of Plants" by Ikechi Mgbeoji, it becomes clear that there are numerous dimensions and complexities to consider in attempting to use a Western derived patent system in the protection of indigenous plant knowledge. The main thrust of Mgbeoji's argument in this text is: the patent system, a form of intellectual property rights (IPR), is "theoretically and operationally incapable of accommodating the peculiar demands of native healers" [4]. In other words, the epistemic context in which concerns are raised and addressed through the patent system does not resonate with indigenous forms of protection/ownership of medico-botanical knowledge. He goes on to offer his advice for coordinating better, more effective mechanisms of protection for indigenous medico-botanical knowledge:

> Tinkering with dominant intellectual property regimes perpetuates the colonial mindset that indigenous peoples did not have autochthonous and effective legal regimes for the propagation, transfer, sharing, and alienation of knowledge. The better view, in my opinion, is to revitalize pre-existing rules and sanctions by which traditional knowledge of the uses of plants by native healers were protected [4].

Here, Mgbeoji advocates for the revitalization of pre-existing systems of protection of indigenous knowledge, which simultaneously listens to the voices of the indigenous people and allows them to ask the questions that matter to them.

In considering the internationalization of the ayahuasca vine, various differences in approach arise that are worth considering in the context of differing epistemologies/methodologies among biomedical scientists and indigenous healers. First, it becomes clear that indigenous plant knowledge systems and biosciences "know" the plant differently. For instance, while indigenous knowledge systems often focus more on additiveness and multiplicity—looking particularly at the multiple uses and synergistic effects of plants—the biosciences tend toward reductiveness and the isolation of various compounds that can then be produced synthetically in a laboratory setting [12]. However, this type of approach advances its own ideological presuppositions, as Professor Chidi Oguamanam notes in Mgbeoji's text: "the emphasis on active ingredients…advances not only the Western scientific culture but also advocates 'mercantilism' and 'extractivism', with which Western science and its intellectual property allies have besieged indigenous knowledge systems" [4]. Here, we see the extent to which the fundamental approaches to obtaining effective medicine differ in indigenous knowledge systems versus biomedical ones.

The concept of "Drug Tourism" is one that has gained relevance in recent decades and is an example of some of the more potentially dangerous ramifications that may result from the decontextualization or extraction of indigenous knowledge from its social, cultural, and physical environment, and subsequent transposition of knowledge in a new context for recreational consumption. Consumers from developed nations, primarily in the West, have sought to arrange special vacations and experiences in which they experiment with indigenous uses of medicinal and hallucinogenic plant substances. This concept finds reso-

nance in the text "A Hallucinogenic Tea Laced With Controversy," in which Dobkin de Rios and Rumrrill report: "Unscrupulous practitioners who exploit the tourists abound, and they are conscious of the farce they perpetrate. In Amazonian cities, middle-class men become instant traditional healers without undergoing an apprenticeship period…They give tourists mixtures of 12 or more different psychedelic plants to help them mystically become embedded in the universe"[5]. What is perhaps more alarming is that some seduce and abuse patients under the influence of the drug, or obtain other forms of personal power and control over those who are intoxicated. Another interesting point that Dobkin de Rios and Rumrrill raise concerns the role that anthropologists have played in inadvertently drawing attention to substances and their various spiritual and medicinal uses, as a result of studying the plants in ceremonial contexts and using ethnographic methods to document their own experiences and the experiences of others:

> In the process of examining esoteric drug rituals, the anthropologist and other social scientists must take responsibility, at some level, for the outcome of their work. There has been an increase in drug-related tourism…by unsuspecting men and women who are seeking help for their psychological problems [5].

This is an example of a darker side of globalization, in which indigenous knowledge becomes commodified, decontextualized, and, in a certain sense, abused.

Conclusion

To conclude, this paper has asked and attempted to answer a number of central questions circulating through the Intellectual Property/ayahuasca debate. This paper has also put forth and defended an argument: the concept of intellectual property and the U.S. patent system exist within a Euro-Western episteme. As such, they do not do justice to protection, ownership, and concerns of authority over knowledge that exist within some indigenous healing systems, such as Ayahuasca Shamanism in the Peruvian Amazon. Additionally, IPR does not take into account communal ownership of knowledge within indigenous healing systems, and, as a result, cannot accommodate the nuance and complexity of plant knowledge in indigenous healing systems.

What remains clear is that social scientists and those involved in development studies, as well as scientific/biomedical researchers, must adopt new ideologies and frameworks for thinking about the protection of indigenous medico-botanical knowledge. Intellectual Property Rights cannot be stretched, strained, or truncated in a way that will aptly fit the extreme variety and specificity of indigenous knowledge systems and notions of protection of medico-botanical plant knowledge. These regimes must involve the people whose knowledge they seek to protect and shall use a local glossary and epistemic context, in which relevant questions are asked and effective policy regarding protectionism is developed and put into action. Still more questions may be asked If we are to approach a better model for the protection of indigenous medico-botanical knowledge: Is it possible to develop an intellectual property type regime that is sensitive to the communal aspects of knowledge in some indigenous cultures, where information is shared and transferred through apprenticeships and close relations? If medico-botanical plant knowledge is extracted, reduced, and made transposable to a wider public through bioscience and laboratory production, is the original cultural context from which the knowledge came at all degraded or changed? How do we account for power inequalities across cultures in the context of intellectual property rights?

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