Optimization of Poly(Lactic-Coglycolic Acid) Drug Encapsulated Magnetic Nanocarriers and Effect of Magnetic Configuration on Nanocarrier Physical Dispersity in 3-D Transparent Resin of Human Cochlea for Enhanced Therapeutic Response to Inner Ear Disorders

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Abstract

In order to optimize magnetic drug delivery localized to the inner ear, this research project was divided into 3 parts. First, the nanocarriers were optimized using variable protocols and emulsion system synthesis that allowed for the modification of solvent, drug type, and polymeric surfactant. The configuration of some concentrations with other chemical types, for example, the prednisolone acetate to chloroform concentrations above 2.2 results in high drug loading efficiency. The second focuses on a computer automated aggregation kinetics analyzer that measured the rate of chain aggregation and disaggregation, which plays an important role in determining how or if a nanoparticle solution will be able to be flushed out of the cochlea with ease. Therefore these nanoparticles were upheld to high physiochemical standards that would ensure the greatest drug effect on the desired area. The third subsection focuses on the effect of magnet height on the physical dispersity of the solution in the specific geometric shape of the localized region (the cochlea). The cochlea was generated on Autodesk Inventor using specific calculations of the lengths and areas of the Scala Tympani and Scala Vestibuli canals. Assessing physical dispersity against magnet height showed higher particle intensity at one given location farther from the basal area of the entrance of the Scala Vestibuli. By using chemical systems of synthesis, physiochemical tests for evaluation of nanoparticle, and magnet configuration for greatest therapeutic effect on hair cells, this research report can be viewed as both as data supporting specific configurations leading to optimization of the process, and as a precursor to magnetic drug delivery that can be localized in many regions.

Introduction

Cancer is the uncontrollable growth of cells. So far, there has been effective medications that are able to arrest cells in G0 (with multiple consequences), yet no device or method to control the medication so that it may only attack tumor cells, and not healthy cells. Cancer therapy through the use of chemotherapeutic drugs is found to have both positive and negative consequences. Less than approximately 5% of therapeutic drugs are able to reach the tumor (or disease location), and results in toxic waste in the cell as well as severe complications that could result in more cancers (1). By attacking healthy peripheral cells, the patient must undergo side effects such as the loss of hair, fatigue, death, and other complications specific to type of disease. Killing healthy cells creates an exponential growth pattern that is only started in a certain time period measured after the proliferation of the first thousands of cells. This prevents doctors from using initial high doses or strong chemotherapy that could actually cure the cancer. In this case, some patients are unable to receive full treatment due to the immense side effects and lack of strength of the body during the lag period, and denies them the full prospects that chemotherapy may offer. While this specific project doesn't provide localization of magnetic drug delivery to cancerous tissue, it does provide (a) a method of relieving symptoms like hearing loss due to chemotherapy localized in prostate cancer, and (b) a model to which magnetic nanoparticles may be synthesized and tested for localization of other damaged tissue.

In other cases doctors are unable to treat the disease because of anatomical and physiological barriers such as ones brought upon diseases like sudden sensorineural hearing loss

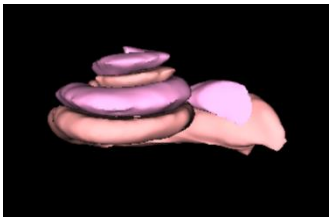


Figure 1, (8)

(SSNHL), Ménière's, and tinnitus. In order to fully understand why the research is needed, one

must have a basic understanding of the cochlea and its function. The cochlea houses millions of hair cells (nerves) that allow for sound transmission to the brain. These hair cells are responsible for receiving and transmitting specific frequencies of sounds (determined by location of hair cell throughout the cochlea) by vibrating from the force that longitudinal waves make inside the cochlea. In cochlear damage, these hair cells can be torn off, bent or broken, due to sound waves high in amplitude (9,10).

The aforementioned diseases have anatomical barriers such as the blood-labyrinth barrier and inability to inject to the ruptured organ (the cochlea). Currently, in order to provide some treatment to the inner ear, a doctor must use local anesthesia to inject drugs through the outer and middle ear (rupturing several organs), and even then only 1 out of every 10 million particles will reach base of the cochlea. The cochlea (Figure 1) exhibits a spiral geometric shape with 2 ovular openings (the scala vestibuli and scala tympani) for fluid and sound waves to enter and exit.

For proper magnetic injection into the cochlea the magnetic configuration of magnets, and the field strength, density, and flux (of those respective magnets) must be optimized for the disease location. This paper explores some of the specifics needed for nanoparticle injection across the first spiral of the cochlea, and distribution of nanoparticle throughout the spiral. In order to determine dispersion mechanisms of MNP's throughout the basal area and throughout the cochlea, specified dimensions of the cochlea (2, 3) were used to generate a 3-D model in Autodesk Inventor, and print using transparent resin. Magnets were placed at variable heights in order to determine intensity of Chitosan-Prednisolone-Fluorescence Loaded Magnetic Nanoparticles at different locations in the cochlea. A fluorescence microscope imaged the cochlea, and a program on Matlab determined quantifiable intensity plots.

In order to ensure maximum therapeutic effect, properly stabilized magnetic nanoparticles must first be synthesized to ensure no aggregations (and thereby lower chances of inflammation), while at the same time maintaining high standards of multiple physiochemical properties such as zeta potential, mean particle diameter, and the Polydispersity index. In order to ensure sterility and proper degradation, the external carrier, poly-lactic-coglycolic-acid (PLGA), was used. PLGA is a widely used in drug carrier applications because of its low toxicity and ability to hydrolyze in the Krebs Cycle and be released as harmless waste (Carbon Dioxide, Water). This paper explores; (1) the variable concentration of solvents, drug, and variable protocol to effectively obtain lowered mean particle diameter and aggregation levels (this increases permeability for absorbance into cellular membrane), (2) computer programs used to quantify aggregation kinetics like rate of chain formation and degradation under magnetic field by binary imaging programing in Matlab simulations, and (3) variance of particle physical intensity over distance with variable magnet height. Producing a viable particle loaded with enough drug that is capable of permeating the membrane is necessary in order to penetrate the barriers present not only in the middle/inner ear, but also around the body. This research focuses on localized drug delivery, but the principles proved by the paper such as increased efficiency of ferromagnetic liquid and biocompatibility in the human body show immense prospects in optimizing drug delivery in the eye, brain, and other anatomically difficult to reach locations. In order to create a truly revolutionary method of treating cancer and other anatomically difficult to reach diseases, it is imperative to first optimize the drug delivery process.

II. Materials and Methods

This project consists of three subprojects that overall contribute to the optimization of Magnetic Nanocarriers, and therefore will be divided as such. The first, optimization of the nanoparticle, was synthesized using primarily a single water phase/oil phase emulsion system with a variable centrifugation protocol and the end of the procedure. First, (as shown in figure 2) a solution was generated by mixing a concentration of Magnetic Cores (Ferrous Oxide), Poly (Lactic Co-Glycolic Acid) polymer, and a therapeutic drug (one lowers effects of sudden hearing loss, swelling, pain, and other ear related symptoms) such as methyl/prednisolone/acetate, prednisone acetate, and oflaxacin. This solution is then thoroughly mixed with the oil phase, with a concentration of solvent such as DCM, or Chloroform.

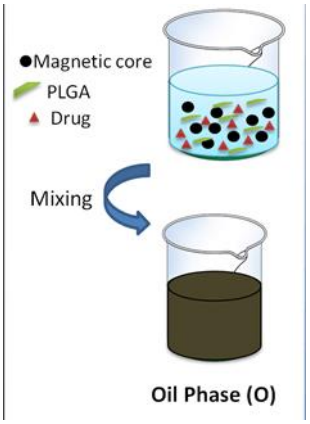
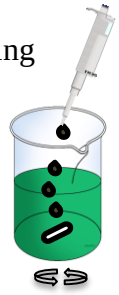


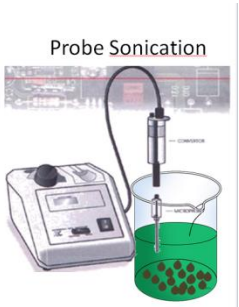
Figure 2 (4)

Next, a water phase with small concentrations of polymeric surfactant molecules like Poly (Vinyl Alcohol), and pluronics was stirred using a magnetic stirrer to induce lowered aggregation levels. The new and mixed oil phase was then added to the new existing water phase (Figure 3), and sonicated for 15 to 20 minutes (Figure 4).



Water Phase (W)
(2% PVA (or) Pluronics)

Figure 3, (5)



re 4, (6)

The resulting sample is then evaporated for 5 hours and then centrifuged at a standard RPM of 3,000 revolutions per minute. The resulting pellet is removed and lyophilized for stabilization under room temperatures. In a variation to the protocol, the sample was centrifuged at 3,000 RPM, then the supernatant was filtered out and the resulting pellet was centrifuged at 6,000 RPM, and this process would be repeated in 3,000 RPM increments until 15,000 RPM was reached.

After variable configuration of chemicals and centrifugation, the samples were tested for physiochemical properties such as a lowered mean particle diameter and polydispersity index, and a higher magnitude of zeta potential in the negative direction using a Zetasizer Nanoscale measurement device, and High Performance Liquid Chromatography was used to determine drug encapsulation efficiency.

The nanoparticles were then tested visually under a 20x magnification on the Zeiss opmi 1-fce microscope with a Hamamtsu ORCA flash 4.0 mounted camera. The nanoparticles were prepared at a 3 mg/mL solution and injected onto a slide. A 0.4 Tesla Magnet was placed approximately at 1.2 centimeters from the slide cover itself, and the resulting particle kinetics was documented before, during, and after placement of the magnet using the Optix recording software. These standardized videos were then inputted into a Matlab program that utilized binary image programming to analyze the rate of aggregation and disaggregation as the magnet was placed in and out of effect. The program took the black and white images, and filled in the white edges. By doing so, it was able to add all the white pixels, and divide by the number of separate white edged objects to get the average. This program was then verified by overlaying the black and white images on the non-binary videos for accuracy.

In order to fully understand the aggregation kinetics of the magnetic drug delivery to the inner ear, its anatomy and structure must fully be understood for proper magnetic injection to occur. The drug must be injected so that the magnets are able to reach to the inner ear, primarily the cochlea for a therapeutic response. A 3-D model of the cochlea (Figure 5) , accurate to scaled dimensions (2,3) was generated using Autodesk Inventor 2014, and printed using Transparent Resin from the Tissue Engineering Laboratory at the Fischell Department of Engineering. This model was then used to test 150 nm

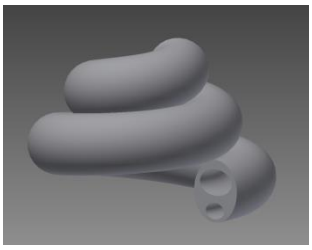


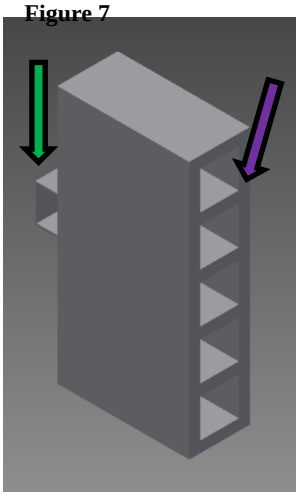
Figure 5

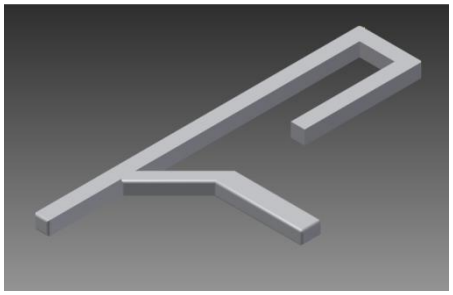
Chitosan (surfactant)-Prednisolone (Drug)-Fluorescent-Chemicell (Manufacturer) particles by injecting them through the entrance of the Scala Vestibuli and determining the physical dispersity of the nanoparticles as a function of magnet height placement. The canals were first washed with a Phosphate Buffered Saline solution to mimic the electrolyte solution of the inner ear. The magnet was placed at heights of 0 cm and 1 cm for 20 minutes, and at a distance of 2 centimeters from the cochlea model (accurate to the distance it would be placed if in effect on a rat's ear). Because of its ability to pull over long distances, the magnet would be placed on the opposite side to injection. The results were viewed using a fluorescent filter, and analyzed by plotting intensity of fluorescence against physical distance of the cochlea (respective to the height used).

For stabilization and insurance of proper magnetic injection into the inner ear, a medical device was designed using Autodesk Inventor that was able to use the force of the jaws to hold in place magnets at the proper distance from the inner ear. The device was designed so that the part that holds the magnets could be moved in the horizontal direction to account for variable face structure. A biting piece (Figure 6) was designed in such a way that the person would be biting on the attachment labeled by the red arrow, and the magnetic holder (Figure 7) would be able to slide on the extrusion labeled by the blue arrow. The magnets (currently designed for .1 Tesla) would be placed on the slots designated by the purple arrow. The sliding device attached to the back of Figure 7, labeled by the green arrow, would slide across the extrusion labeled by the blue arrow.

Figure 6

Figure 7





III. Results

Optimization of magnetic drug delivery requires hundreds of adjustments to the concentrations of solvent, drug, surfactant, and protocol in order to achieve high standards of physiochemical properties. Instead of simply centrifuging the Oflaxacin encapsulated Dichloromethane (DCM) and Chloroform Solvent nanoparticles at 3,000 RPM, the sample was centrifuged at increments of 3,000 RPM (supernatant was taken out after each cycle), and the Polydispersity Index and Mean Particle Diameter was measured at the end of each cycle. A chi square test analyzing difference (Figure 8) found that the 386.466 at 3,000 RPM is greatly statistically different because a calculated value of 102.95 is 26.8 times greater than the standard chi square value of 3.84 (at p=0.05, and degrees of freedom at 1).

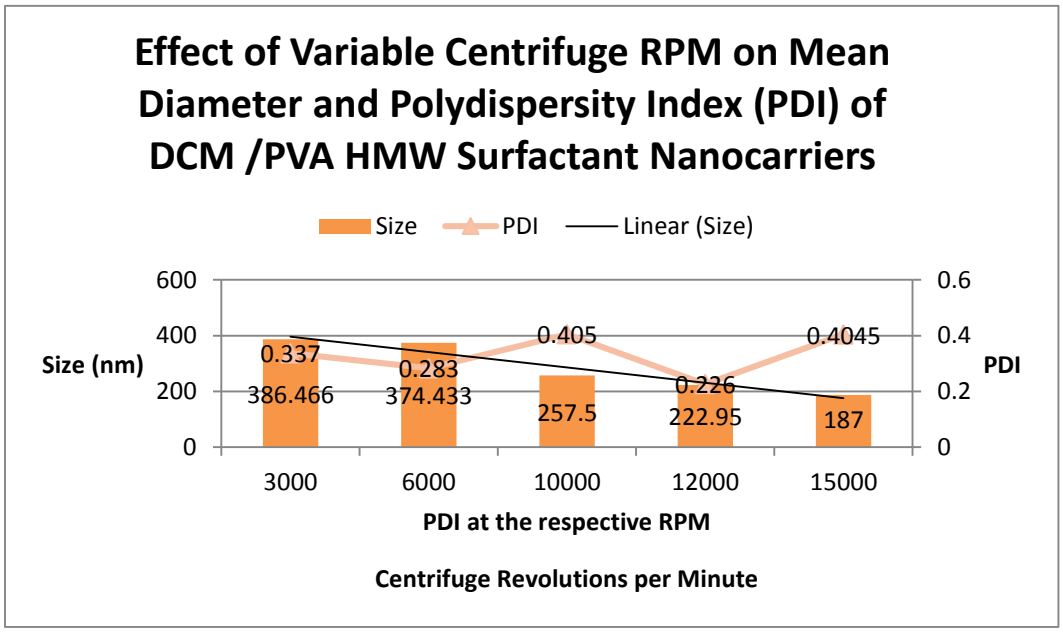


Figure 8

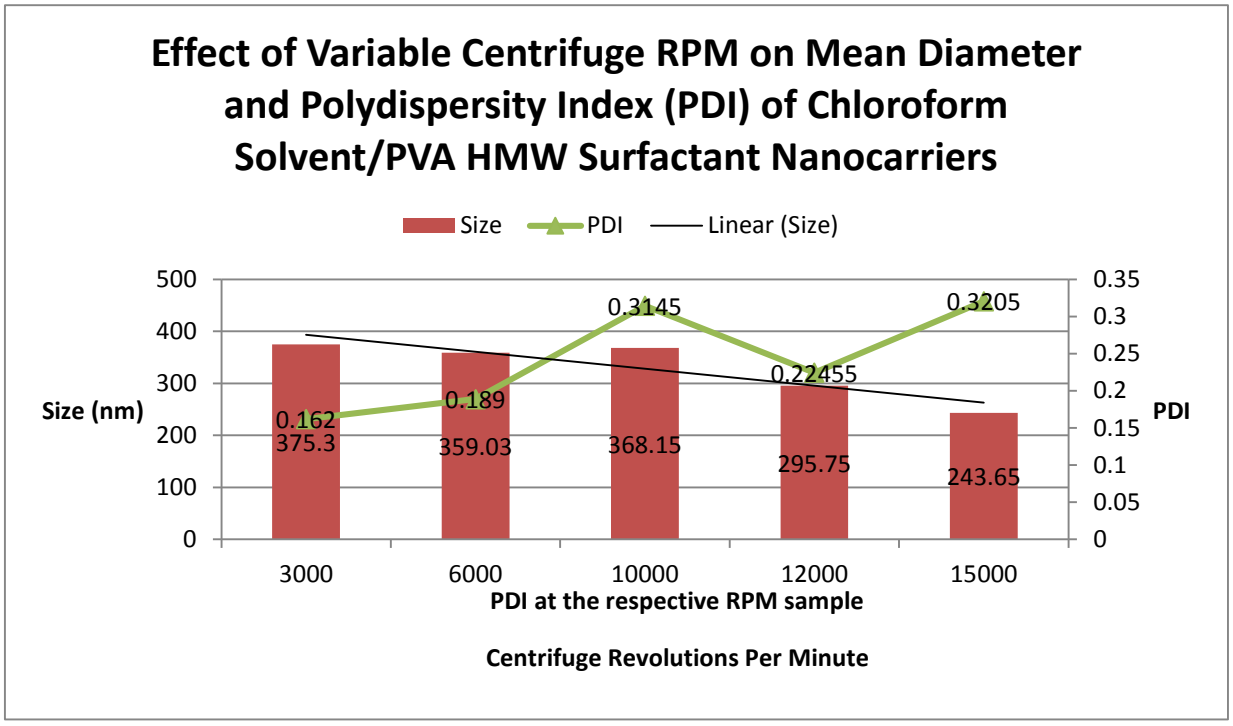


Figure 9

The same downwards linear trend was observed for Chloroform Solvent nanoparticles (Figure 9). Although mean particle diameter shows lowered size, the Polydispersity Index of both samples were deemed not statistically different because the DCM chi square calculated value of 0.012 is less than the standard value of 3.84 (at p=.05, degrees of freedom at 1), and the Chloroform chi square calculated value of .154 is less than the standard value of 3.84 (at p=.05, degrees of freedom at 1). Figure 10 shows the percent difference of the 15,000 RPM solution, to the 3,000 RPM solution, respective to solvent type.

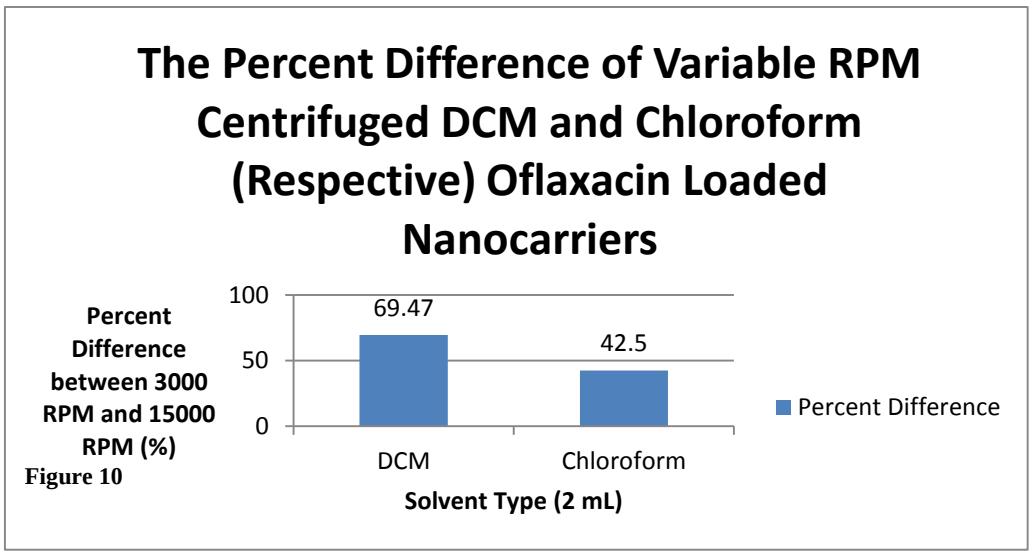


Figure 10

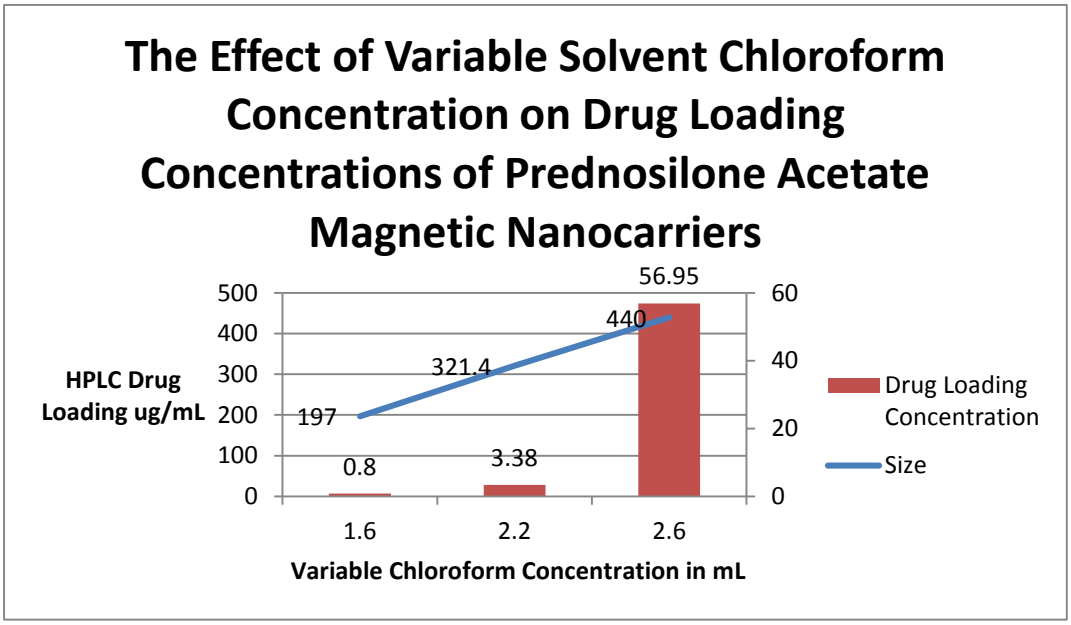


Figure 11

The difference in the change in size of nanocarriers is greater in the DCM solvent by 27.2 because of higher miscibility with the given configuration of drug and other chemical. The solvent plays a large role in solubility and miscibility of drug, and thereby can predict high encapsulation rates. Figure 11 shows the increasing trend of Prednisolone Acetate drug loading as a function of increasing Chloroform concentrations, and its side effect of mean particle

diameter. This exponential-like increase in drug loading as chloroform concentration increases by increments of 0.4 mL, predicts high therapeutic response to the cochlea. By creating high levels of solubility we allow more drugs to be loaded and thereby more overall efficiency.

Binary image programming was utilized to determine nanoparticle aggregation kinetics like the rate at which paramagnetic (or ferromagnetic) particles form and disintegrate chains. Figure 12 shows random Brownian motion of the "OZB1(3)" particles, whereas figure 13 shows individual particles coming together due to enhancement of paramagnetic properties under a magnetic field. Figure 14 exemplifies the degradation nature of magnetic chains necessary for proper fluid removal from the cochlea.

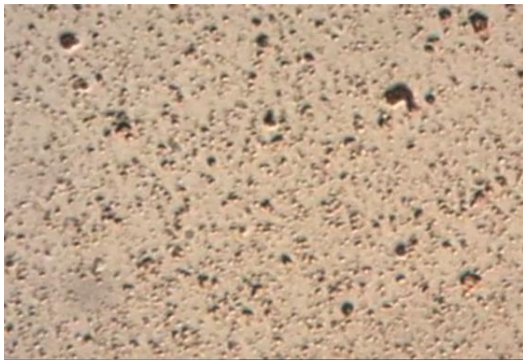


Figure 12

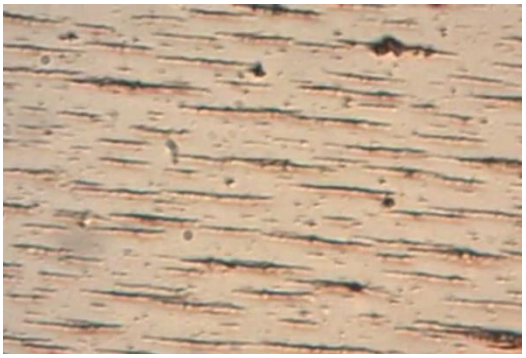


Figure 13

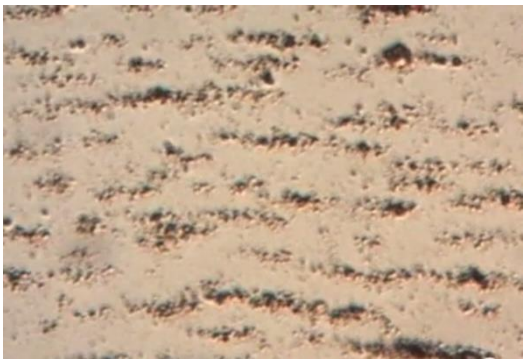


Figure 14

Figure 15 shows how the high magnetic susceptibility (higher ferrous oxide particles) of OZ Bioscience Magnetic Particles allow for rapid chain formation growth and, degradation (equally

important). The chain length before the influence of magnetic field is at 1.1 micrometers, and right before the magnetic field is removed, the chains exhibit lengths up to 3.4 micrometers, resulting in a net change of 2.3 micrometers. Therefore the average rate of chain aggregation from 5 seconds to 60 seconds is .0418 micrometers per second, and the rate of chain degradation from 60 seconds to 70 seconds is approximately -0.18 micrometers per second.

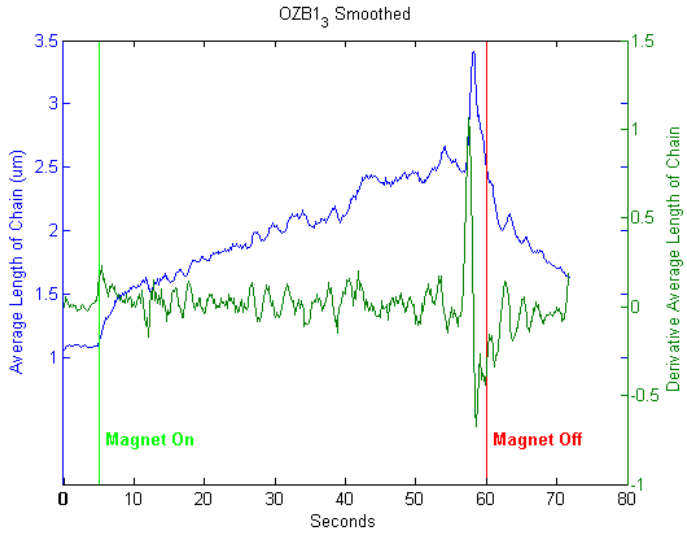


Figure 15

This control group was varied against a DCM Solvent/Prednisolone Acetate Magnetic Nanocarrier whose HPLC results showed the highest drug loading efficiency, at 82.26 micrograms per milliliter. Figure 16 shows the average rate of chain formation (of experimental group) from 6 seconds to 21 seconds at approximately 0.0267 micrometers per second when a magnetic field of 0.1 Tesla is applied. When a 0.4 Tesla field is applied, Figure 17 shows the change in length (of experimental group) from 6 to 22 seconds as 1.2 micrometers, and thereby the average rate of chain formation is at 0.075 micrometers per second, and the average rate of chain degradation is -0.25 micrometers per second.

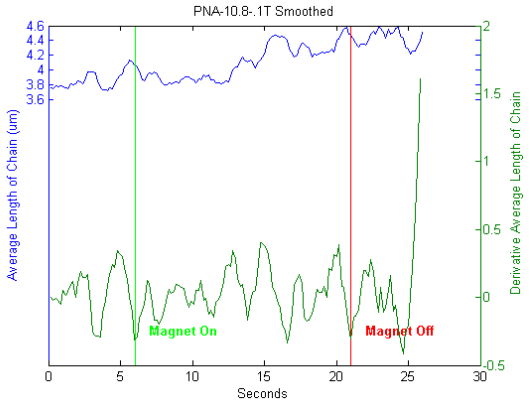


Figure 16

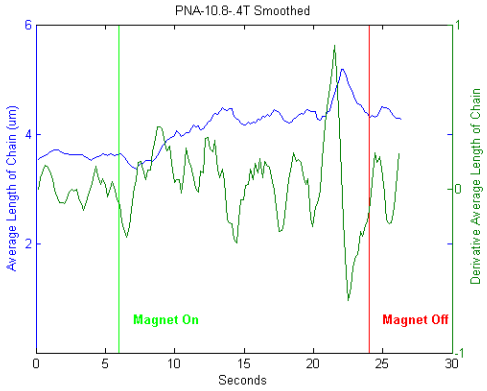


Figure 17

A fluorescence tagged, Chitosan magnetic nanoparticle was then injected into the opening of the cochlea model. Figure 18 shows the physical dispersity of the fluorescence when the magnet was placed at different heights. The ultimate goal is to magnetically inject the highest magnitude of particles to the farthest distance in the cochlea. From the data we can conclude that using a 1 cm configuration, the highest percentage of particles at 25 % were located at the 2 centimeter location whereas in the 0 cm configuration, the highest percentage of particles at 24% was located at the entrance of the basal layer. Unfortunately, in the 1 cm configuration, the percentage of particles after the maximum marker location decreases significantly at 8 % from 2-3, 2 % from 3-4, 2 % from 4-5, and 5 % from 5-6, thereby an average decreasing rate of 4.25 % per centimeter. The 0 cm configuration shows percentage decrease after the maximum at 3 % from 1-2, 1 % from 2-3, 0% from 3-4, 9% from 4-5, 2% from 5-6, thereby an average decreasing rate of 3.2 % per centimeter. The importance of extreme intensity of particles at a position farther than the maximum of another configuration renders viable optimization results.

IV. Discussion

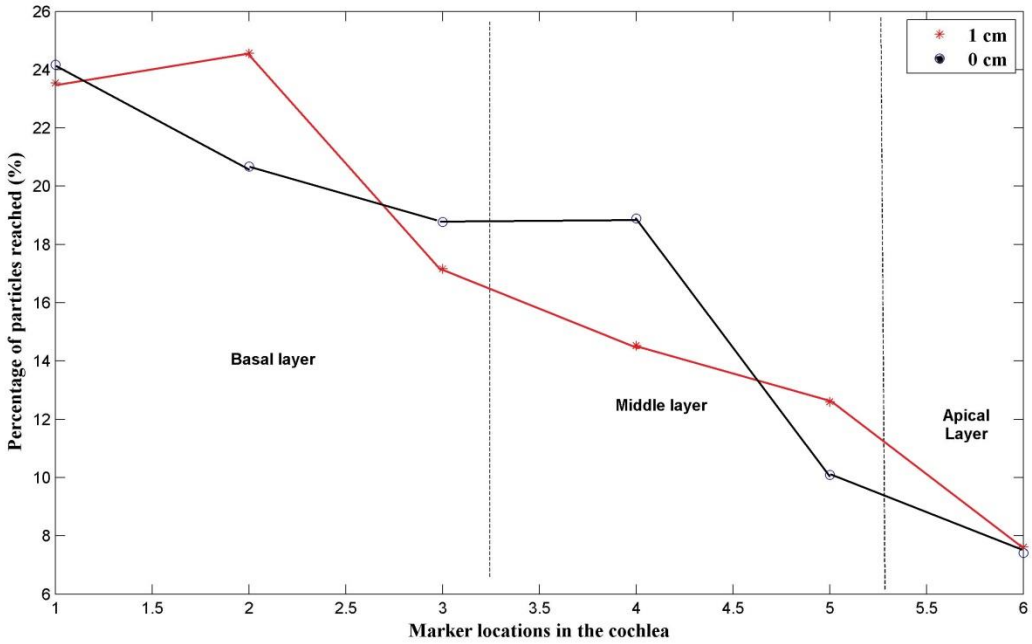


Figure 18, (7)

The magnetic nanoparticles exhibited high physiochemical properties when centrifuged (making sure supernatant was being taken out after each cycle), at increments of 3,000 RPM up till 15,000 RPM. The lowered size values obtained show prominence as particles with low mean particle diameter have a higher chance of penetration across the middle ear membrane. An unfortunate consequence of this procedure is that the yield due to this procedure is extremely low, and therefore to produce the same quality particles, it would take more initial solution. As centrifugation RPM increased, the PDI increased, thereby indicating an unfortunate decrease in homogeneity. The increase in PDI was greater in Chloroform than in DCM, and the percent difference in DCM when measuring size was also greater, rendering DCM a better solvent for PVA surfactant-Oflaxacin encapsulated nanoparticles. Another key component of particle synthesis is the drug loading efficiency. Particles with Prednisolone Acetate integrated into the Chloroform solvent at concentrations higher than 2.2 mL showed one of the highest efficiency values. The same drug, integrated into a DCM solvent at the same concentration rendered significantly higher drug loading efficiency and aggregation kinetics (when compared to standard OZ Bioscience Particles). The increasing concentrations of solvent allowed for higher drug encapsulation due to greater ability of the prednisolone acetate molecule to become miscible in the solvent. Chain aggregation was higher when influenced by the 0.4 Tesla, as expected, concluding that susceptibility saturates at a point higher than 0.4 Tesla, and that there is potential for higher intensity of nanoparticle solution dispersity when a high magnetic field is used. A consequence of this is that when using a greater density of magnetic field, the magnet itself tends to be bigger, which causes the valuation of the magnetic effects on the particle to be inaccurate since they are not being affected at or near the origin of the magnet. The rates at which chains aggregate, and disaggregate are at higher values than the control group, predicting

higher magnetic susceptibility values, and less time required for nanoparticles to remain in the ear. High disaggregation rates are valued because in order for the fluid to be properly dispensed from the cochlea after drug action, it must be in a state in which enzymes may break it down, or immune system may engulf it through use of macrophages and possibly Cytotoxic and/or a B-Cell immune response (more research is needed on this topic).

Magnetic configuration at 1 cm shows highly prominent results because it allows for more magnetic nanoparticles to reach a variance of hair cells. As more nanoparticles travel throughout the cochlea, there is a higher chance of hair cell treatment, and thereby increased hearing. This primarily occurred because the magnetic field density originates at a higher point in the x-axis, causing nanoparticles to rise to that location as particles tend to move from lower density points to higher density points. The magnetic configuration experiment renders valuable results, but it is limited because it only determines physical dispersity on a model basis. In order to fully implement this on a mouse model, or human model, one would have to determine magnetic density values at the x and y point of the location of the cochlea in a human skull to achieve the same results.

V. Future Research

In order to deem magnetic drug delivery safe to the inner ear, one must study how the nanosolution would be cleared from the inner ear. In order to do this, a mouse magnetically injected with a nanoparticle solution must be studied over a long period of time to determine if there is an immune response. Blood tests can report abnormal levels of macrophages, B-cells, cytotoxic cells, or histamine. If any of these is reported, further research must be done to determine if the effect of innate or specific inflammation causes negative symptoms, and if these symptoms are negatively greater than the positive results of drug action.

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