Cellular Transport of Functionalized Gold Nanoparticles

A Major Qualifying Project

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By

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Abstract

The biomedical industry is faced with the constant challenge of creating treatments to help patients that are cost-effective and easy to synthesize and administer. Recent advances in nanotechnology have sparked research in the use of nano-scale particles for medical applications. Gold nanoparticles have become a popular choice of vector in drug delivery research, however, the mechanism of cellular uptake of these particles is still unknown. By attaching fluorescent functional groups to gold nanoparticles, and observing how these nanoparticles transport through lipid bilayers, which mimic cell membranes, I have gained a better understanding of the cellular transport properties.

Acknowledgements

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Executive Summary

This report contains the introductory work to my Master's thesis. I am studying how functionalized gold nanoparticles transport through cell membranes. To reduce the unknowns and complexities of using living cells, I am synthesizing "model" cells that mimic the properties of cell membranes. This report contains the process I used to create model cells, and the results of these experiments. To gather data for this experiment, I am functionalizing the gold nanoparticles with fluorescent tags and collecting images using a confocal fluorescent microscope. In this report, I describe the functionalization procedure and the results and difficulties of the experiment. Finally, I discuss the work I will conduct as I progress to my thesis.

Research in the use of nanotechnology for medical applications is becoming increasing popular. There are some exciting possibilities of nanotechnology being used for cancer therapy, drug delivery, and imaging. For example, it may be possible to attach chemotherapy drugs to nanoparticles, and selectively target the cancerous cells without damaging the surrounding healthy cells. Although many applications are being studied, it is still unknown how nanoparticles transport through cell membranes. Although some studies have shown the mechanism to be somewhat driven by endocytosis, this process doesn't not completely describe the phenomenon. With a better understanding of the cellular uptake mechanism, it could be possible for researches to create effective medical treatments using nanotechnology.

To create the "model" cells, I used POPC, a lipid found in eukaryotic cell membranes. By dissolving this lipid in a solution of chloroform and methanol, and shaking the solution, I created structures that self-assembled in to cell-like shapes. I am functionalizing the gold nanoparticles with FITC, a fluorescent tag that can be seen on a fluorescent microscope. The nanoparticles I am using have amine groups on the surface, and the functionalization procedure I am using will covalently bond the FITC molecules to the amine groups. I then introduced the nanoparticles in solution to the cells, which have been adhered to a glass microscope slide and rehydrated with water. The data collecting I will be conducting as part of

my Master's thesis will involve using the fluorescent microscope to count the nanoparticles inside and outside of the cells, and use the ImageJ software package to plot the concentration of nanoparticles as a function of time.

The most successful aspect of my work thus far is the synthesis of the model cell membranes. I can readily create cells that are relatively uniform in size and shape, and are well distributed. They are also on the same order in size as human cells and are well suited for using as a stand-in for living cells. I have also completed a majority of the functionalization procedure for the nanoparticles. The problems I encountered will be resolved shortly in to my Master's work. I have also worked with both cross-polarizing and fluorescent microscopy techniques, which will allow me to collect data for my thesis. The work I have completed during my MQP has set a majority of the procedural work needed for my graduate studies.

1 Introduction

1.1 Nanotechnology in Medicine

Although medical technology is rapidly advancing, scientists are constantly searching for drugs that will be more effective, cheaper to produce, and treat more diseases. For example, despite having found medicines that can treat cancerous cells, specifically targeting the cancer cells without harming surrounding cells has proven quite difficult. Nanoscience, the study of particles on the scale of 10^{-9} meters in size, is a quickly growing field of research. Particles of that size have unique properties that larger particles lack, and researchers are currently exploring the possibility of using these particles for medical applications. By attaching drugs to nanoparticles, it could be possible to deliver medicine directly to cells and sites of action. Although applications of these particles are being intently studied, it is still unknown how nanoparticles transport through cell membranes.

1.2 Determining the Uptake Mechanism of Gold Nanoparticles

Recent studies have shown that the effectiveness of a drug is determined by the dose and duration of the drug at the cellular site of action. To create drugs using nanotechnology that will act effectively, it is important to understand the mechanism that drives cellular transport. The objective of this project is to better understand what the driving force of cellular uptake of gold nanoparticles is.

1.3 Using Fluorescent Microscopy to Observe Transport of Nanoparticles Through Model Cells

To address this problem, I have created "model" cell membranes that mimic the properties of living cells without the complexities of a living environment. By creating model cells, I will be able to isolate the physics of the system without needing to worry about extra variables

such as proteins embedded in the surface of the cell, or an additional cellular skeleton. I have also attached fluorescent tags to gold nanoparticles that will allow me to view the nanoparticles using a fluorescent microscope. By viewing the fluorescence of these particles, I will be able to count the particles inside and outside of the cells, and observe how the concentrations change with time and particle size. I can then further alter parameters such as concentration and environment to better understand the uptake driving mechanism.

1.4 This Report

This report is a midway report to what will be my Master's thesis. In this report, I have included the procedures and methodology I have used thus far, which set the groundwork for my graduate research. I have also included background information on research in this field conducted thus far and details about the microscopy techniques I am using. Finally, I discuss the work I will be continuing and the methods I will be using to collect and analyze data.

2 Literature Review

2.1 Usage of Nanotechnology in Biomedical Science

The biomedical industry faces the constant challenge of creating treatments that will not only help patients, but that are cost-effective and easy to synthesize and administer[1]. The use of nanotechnology is expected to revolutionize the biomedical industry. "Nano," meaning one billionth, defines the length scale (10^{-9} meters) of the systems being studied in nanoscience[2]. Such small systems are of interest because at this length scale, properties such as melting point, optical properties, and electrical properties change. Scientists are beginning to exploit these properties to control matter on the nanometer scale. In the medical field, one of the most important applications of nanotechnology is controlled drug release systems, which offer numerous advantages over conventional medical treatments[3].

2.1.1 Drug Delivery Systems

Some of the many possible biomedical applications of nanotechnology include making it possible to improve the delivery of poorly water-soluble drugs, targeting specific cells or tissues, delivering larger drug molecules directly to sights of action, and allowing for visualization of drug delivery in real time[4]. One of the main problems with current cancer therapies is that they lack the ability to target cancerous cells without also harming surrounding healthy cells[5]. Carbon nanotubes have been studied as a possible vector that could be loaded with drugs to target cancer cells and treat them. The bonds to the surface of the nanoparticle can be either covalent or non-covalent. Covalent bonds are much stronger, and the bound molecule will remain attached to the nanoparticle in most conditions. Non-covalent bonds are less stable bonds, which are susceptible to environmental factors. This can be useful to release drugs at a target location with an environment that will cause the bond to break. According to a recent study, the efficacy of a drug is based on the dose and duration of the drug at the intracellular site of action[6]. With the use of nanotechnology, it could be

possible to control both dose and duration at the cellular level, and create extremely effective drugs.

2.1.2 Toxicity

Nanotechnology holds great promise for biomedical uses, however, little is known about potential short and long-term effects of nanomaterials on humans and the environment[7]. A study conducted on mice showed that airborne single-walled carbon nanotubes (SWNTs) in low concentrations triggered the formation of granulomas[8]. However, in a different study with gold nanoparticles, they were shown to be fairly non-toxic to a line of leukemia cells[7]. In this study, gold nanoparticles of several sizes and with various surface modifiers were studied. Although some of the precursors of the nanoparticles showed toxic effects, the nanoparticles themselves seemed to have little effect on the heath of the cells. In comparison to other nanoparticles, gold nanoparticles seem to be less toxic, and would likely be an appropriate choice for medical applications.

2.2 Gold Nanoparticles

Gold nanoparticles are not a new concept. In fact, medieval glass workers unknowingly created gold nanoparticles when they made red stained glass by mixing gold chloride in molten glass[9]. More closely related to modern chemistry, Michael Faraday was the first scientist to create gold colloids by vibrating particular fluids on an elastic surface[10]. Although gold nanoparticles are colloids, the properties at the nano scale differ greatly from their larger bulk gold counterparts[11].

2.2.1 Properties of Gold Nanoparticles

Physicists predicted that nanoparticles between 1-10 nm in diameter would display electronic properties that would behave according to quantum mechanics[12]. As it turns out, the properties behave neither as those of molecular compounds or bulk gold, but are strongly

dependent on particle size, shape, and concentration. Optically, light interacts with free electrons near the colloidal nanoparticles causing resonant oscillations known as surface plasmons[13]. For nanoparticles around 30 nm in size, the surface plasmon resonance causes light in the blue-green spectrum to be absorbed, and red light to be reflected. This yields the rich red color as seen in Figure (2.1). As the particle size increases toward the bulk gold limit, the color becomes clearer as the light reflected is in the infrared spectrum. Optical properties for different applications can be obtained by varying the size or shape of the nanoparticles.

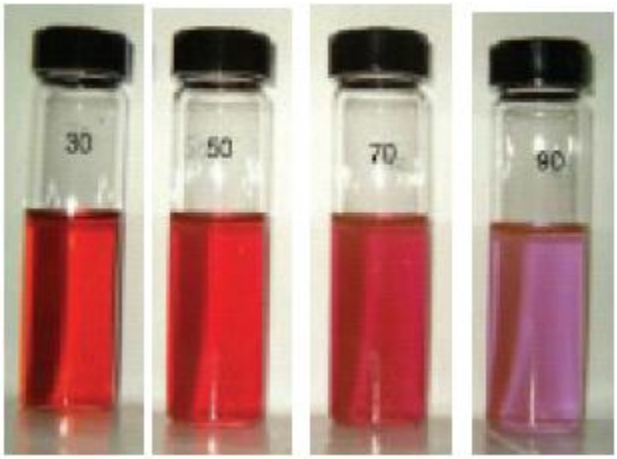


Figure 2.1: Color of nanoparticle solutions for different particle sizes[14]

2.2.2 Synthesis and Functionalization of Gold Nanoparticles

The Brust-Schiffrin method of synthesizing gold nanoparticles, published in 1994, had a considerable impact on the field of nanotechnology[12]. This method allowed for synthesis of thermally stable and air-stable gold nanoparticles, and also controlled dispersity and size for the first time. To create colloidal gold particles, chloroauric acid ($HAuCl_4$) reacts with tetraoctylammonium bromide in a solution of toluene and sodium borohydride. The $HAuCl_4$ disassociates, and the gold particles aggregate. Depending on the chemical used to stabilize the nanoparticles, various functional groups can be formed on the surface of the particles. For example, various gold complexes such as gold(I) amine complexes have been used in

synthesis of amine-stabilized gold nanoparticles, which have NH_2 groups on the surface, as can be seen in Figure (2.2). Additional functional groups can be bonded with the functional groups formed on the surface of the nanoparticles.

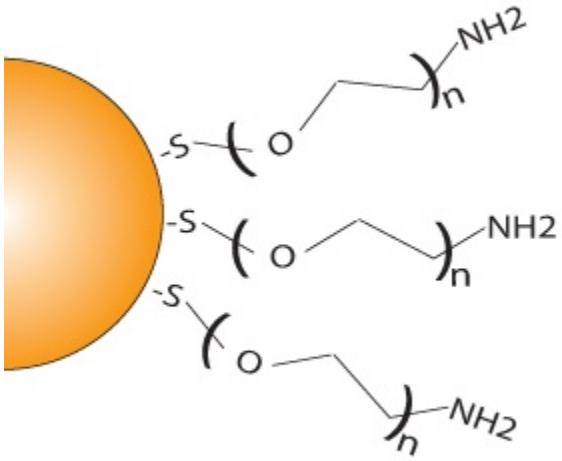
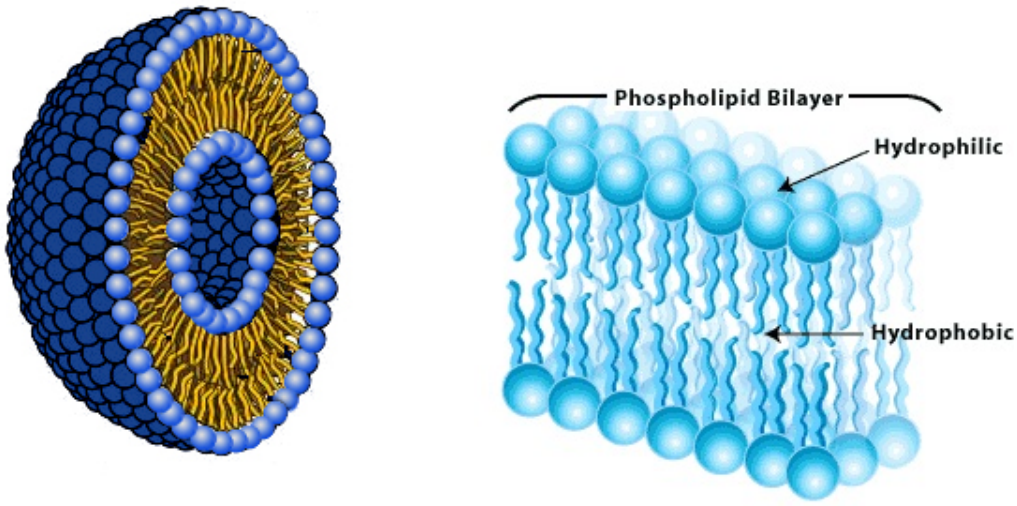


Figure 2.2: Amine-functionalized Nanoparticle[15]

2.3 Lipid Bilayers

A lipid bilayer is composed of lipids with hydrophobic tails, which drive the formation of the bilayer[16]. In aqueous solutions, the lipids form micelles, which eliminate unfavorable contacts between water, and the hydrophobic tails. A suspension of phospholipids can form liposomes, which are closed vesicles bounded by a single bilayer, as can be seen in Figure (2.3). These liposomes can serve as a model of a biological membrane.



(a) Liposome[17] (b) Lipid bilayer[18]

Figure 2.3: Structure of Liposomes

2.4 Microscopy

2.4.1 Cross-Polarizing Microscopy

Polarized light is randomly oriented light that has been filtered such that the remaining light waves all oscillate in the same plane[19]. In cross-polarizing microscopy, light passes through a first polarizing filter, and is then blocked by a second filter oriented at a right angle to the first. Polarizing microscopes use polarized light to enhance the contrast of images obtained with birefringent (doubly refracting) materials[20]. The light from the source passes through a polarizer before passing through the birefringent specimen, and then is passed through a second polarizer, known as an analyzer, which recombines the light rays, as shown in Figure (2.4).

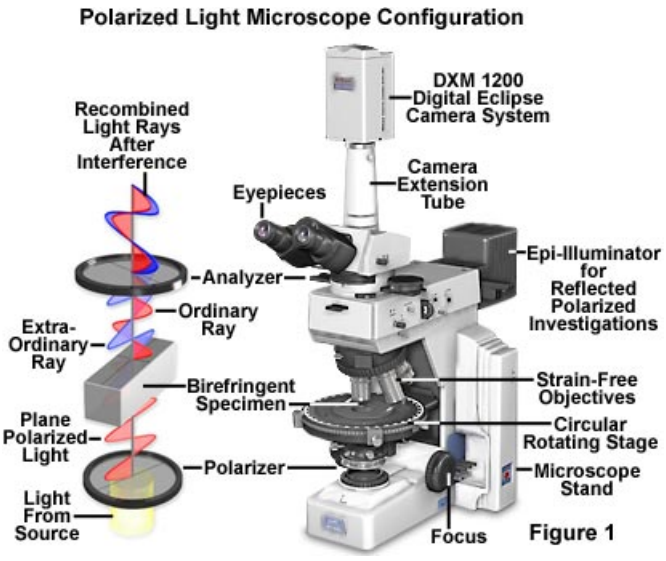


Figure 2.4: Cross-polarizing microscope[20]

The contrast in the image occurs when the birefringent specimen produces two wave components of different velocities that, when recombine, interfere constructively and destructively. The contrast-enhancing properties of polarizing microscopy can provide detailed information about the structure and composition of materials.

2.4.2 Confocal Fluorescent Microscopy

Fluorescent microscopy works on the principles of fluorescence, which means that when an object is irradiated with light at a specific wavelength, the electrons in the object will be excited and emit light at a certain detectable wavelength[21]. A properly configured microscope will show only the fluorescent structures that will then be superimposed with a dark background image. Confocal fluorescent microscopy provides several advantages over conventional microscopy as the focal plane of the microscope can be moved[22]. Because of this, the microscopes have a shallow depth of field and can collect images from thick specimens without out-of-focus interference. To remove the out-of-focus aspect of the image, the light emitted from the focal plane passes through a pinhole aperture, and the remaining light is excluded, as shown in Figure (2.5).

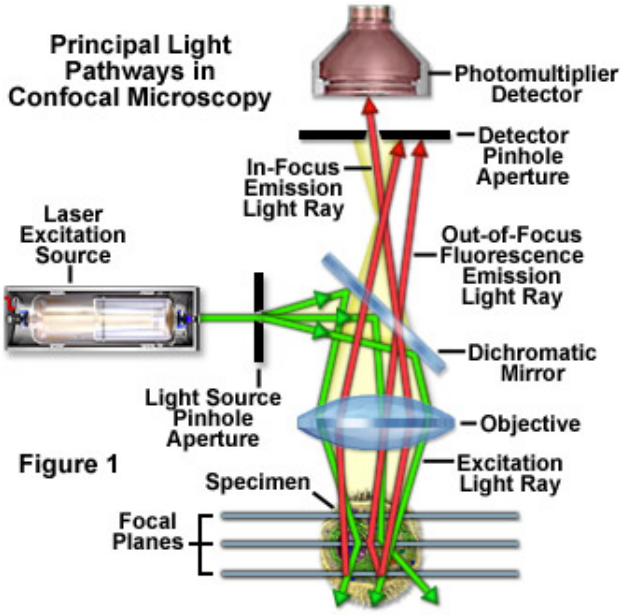


Figure 2.5: Confocal microscope[22]

3 Methods

3.1 Vesicle Preparation

To isolate the exact physical properties of gold nanoparticles interacting with cell membranes, I decided to use "model" cell membranes, which will mimic the properties of an actual cell membrane without the complexities of living cells. To create these lipid vesicles, I used 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC), obtained from Sigma-Aldrich (refer to Appendix A for full description of materials). POPC is a lipid that occurs naturally in eukaryotic cell membranes, and is an appropriate substitute that will mimic many properties of a human cell.

3.1.1 Creating Lipid Solution

The most crucial step in the processes for creating the model cells is the preparation of the lipid solution. The POPC must be carefully massed in a glass vial. A 2:1 solution of chloroform to methanol is added to this. The ratio I found to work best is 2mg of POPC to 1mL of solution. The sample must then be vigorously shaken for about 30 seconds. This shaking process breaks the lipid structures up, allowing them to reform in cell-like shapes at appropriate sizes.

3.1.2 Slide Preparation

After preparing the lipid solution, I placed a 10μL drop on to a well cleaned microscope slide. The solution must then be dried on a hot plate at 40°C. This causes the lipid structures to adhere to the slide. The vesicles can then be re-hydrated with deionized water. This allows the cell-like structures to reform.

3.1.3 Cross-Polarizing Microscopy

I used cross-polarizing microscopy to observe the structure of the vesicles. The cells I had synthesized were stained with diI, a red dye that adheres to lipids. This allowed me to view the cells both with a bright-field view and under cross-polarizing conditions. One of the next steps I will be conducting is to remove the dye, as it interferes with the fluorescent microscopy. Because the cells will no longer be stained, the cross-polarizing microscope will allow me to image the cells by viewing the birefringence of the bilayer. This technique will show how the lipid structures are arranged and give me information about the surface properties and makeup of the cells.

3.2 Functionalization of Gold Nanoparticles

To functionalize a nanoparticle means to attach some sort of molecule to the surface of the nanoparticle. The nanoparticles I am using are functionalized with amine groups on the surface. I am further functionalizing them with Fluorescein isothiocyanate isomer I (FITC). This additional functional group will allow me to view the nanoparticles with a fluorescent microscope, as the FITC molecules will fluoresce when excited by the proper wavelength laser. To attach the FITC molecules to the nanoparticles, I am following a procedure similar to one found in an Advanced Functional Materials (AFM) journal article[23].

3.2.1 Creating the Buffer Solution

A Britton-Robinson buffer solution is used to maintain the pH of the solution as the fluorescent tags bond to the amine groups on the gold nanoparticles. This solution is made up of equal parts 0.04M boric acid, 0.04M phosphoric acid, and 0.04M acetic acid. Then, the solution is titrated with 0.1M sodium hydroxide, until a pH of 9.0 is reached. To do this, I first created dilutions of each of the reactants to obtain the proper molarities. I then began adding the sodium hydroxide to the acids, and checking frequently with pH strips until I reached the correct pH.

3.2.2 Introducing the Nanoparticles

In the procedure from the AFM article uses 100 mg of nanoparticles that are then dispersed in 20 mL of the buffer solution. The gold nanoparticles I am using are expensive, and come in much smaller quantities, so the procedure has to be adjusted. The nanoparticles are in solution with water, so mass of the nanoparticles in solution must be determined by the weight concentration of each solution. Properties of each nanoparticle size are given in Table (3.1).

Nanoparticle size (nm)	Weight Concentration (mg/ml)	Surface Area (nm^2)	Molar Mass (g/mol)
20	2.66	1.26×10^3	4.68×10^7
40	2.33	5.03×10^3	3.91×10^8
60	2.15	1.13×10^4	1.32×10^9
80	2.03	2.01×10^4	3.13×10^9

Table 3.1: Properties of gold nanoparticles

Using the weight concentrations of the type of nanoparticle I was using, I calculated the amount of buffer solution needed for 0.05 mL of nanoparticle solution. These two solutions were then mixed with a solution with the fluorescent tag. The FITC tag used in this procedure is in a powder form. To create the solution, the powder is massed in a glass vial, and dissolved with methanol at a concentration of 0.25 mg/mL. This solution is added in a 1:20 FITC/buffer solution ratio. The final solution is then stirred over night.

3.2.3 Washing Procedure

To remove the unreacted dyes from the solution, I conducted a series of dilutions using a centrifuge. I began by adding 1.0mM NaOH to the nanoparticle solution, and centrifuging until the solution separated, and I could remove the liquid sitting on top of the nanoparticles. I repeated this twice, and then twice more using 1.0mM HCl. Then I repeated the same procedure twice using purified, deionized water, and added 0.5 mL of water to the final solution. To prepare the sample for observation on the fluorescent microscope, I added a

10μL drop to a slide prepared with rehydrated vesicles. I then sealed the slide with a cover slip and incubated the sample at 37° to mimic the temperature of a human body until observation.

3.2.4 Fluorescent Microscopy

Using the Leica SP5 Point Scanning Confocal at Gateway Park, I was able to take fluorescent images of my sample. The FITC tag has an excitation wavelength of 492 nm, so I used the argon laser on the microscope to excite the sample. Because the microscope is confocal, I was able to adjust the focal plane and observe the sample directly on the glass slide and throughout the sample. The data collection I will be conducting for my Master's thesis with this microscope is described in the Future Work section.

4 Results and Discussion

4.1 Results

4.1.1 Vesicle Preparation

My first attempt used 5 mg of POPC to 0.5 mL of solution. The results of this are shown in Figure (4.1).

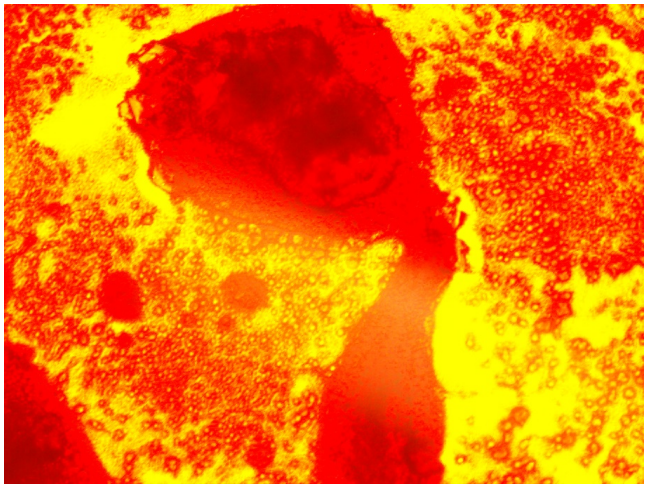
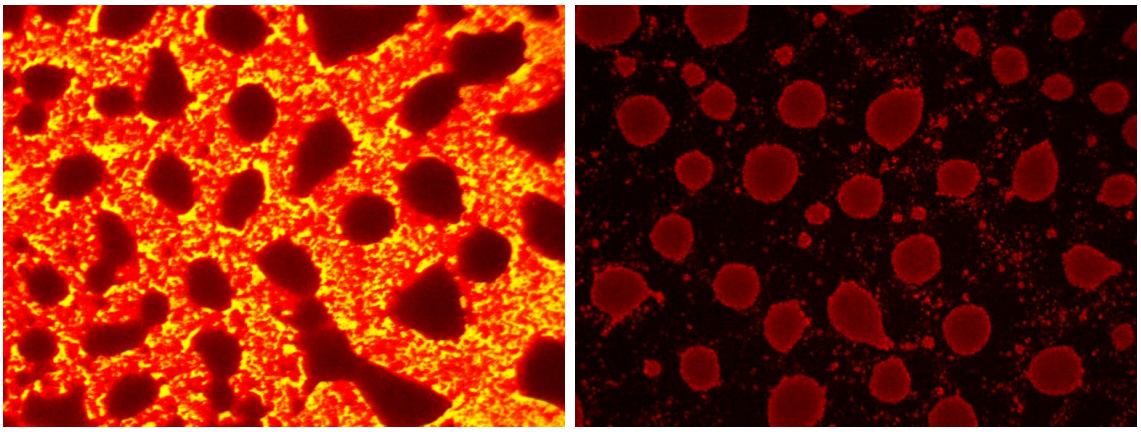


Figure 4.1: 10mg/mL POPC, 200x magnification

In this image, the lipid, stained with 3,3'-Dihexyloxacarbocyanine Iodide (diI), did not assemble into cell-like structures. This was because the concentration of the POPC was too high, and layers of vesicles were formed on top of each other. I determined that a more suitable concentration of POPC was 2mg/mL of solution. Another important factor in the creation of the vesicles is the shaking procedure. I discovered that a sample that had previously been successful did not yield cell-like structures when later reused. This is because I had not re-shaken the sample. The vigorous shaking causes the lipid structures to break, and reform in more uniform cell shapes. Figure (4.2) shows a comparison of shaking techniques.



(a) 2mg/mL POPC, 200x, less shaken (b) 2mg/mL POPC, 200x, more shaken

Figure 4.2: Comparison of shaking techniques

In Figure (4.2a), the sample had been shaken enough to break the lipid structures in to mostly separate shapes, however, this figure shows structures that have not completely separated and are oblong in shape. The same sample was shaken more vigorously, and Figure (4.2b) shows this resulted in individual cell-like structures that are more uniform in size and shape.

4.1.2 Gold Nanoparticles

Following the procedure above for the functionalization of the gold nanoparticles, I created a sample using the 60 nm nanoparticles. After introducing a drop of this solution to a slide containing the vesicles, I sealed the solution with a cover slip and let the nanoparticles interact with the vesicles for 8 hours at a temperature of 37°C. I then observed the sample on the fluorescent microscope, the results of which can be seen in Figure (4.3).

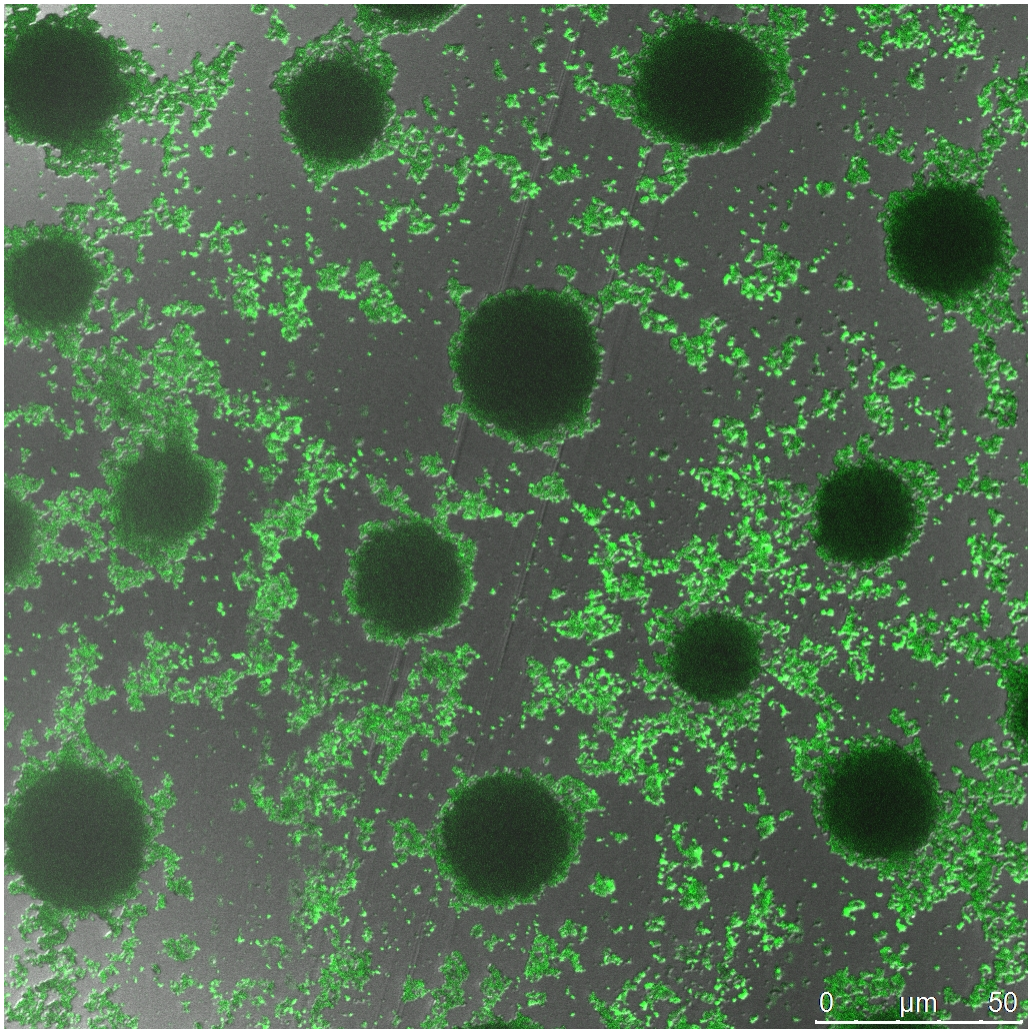


Figure 4.3: Fluorescent microscope, sample with 60 nm nanoparticles

The material fluorescing in green is actually the dye used to stain the vesicles as well as the FITC molecules left in the solution. The diI molecules fluoresce at a similar wavelength to the FITC, and thus the dye on vesicles the would mask any nanoparticles inside. In addition, there are no spots that are clearly brighter than the surrounding dye, indicating that the nanoparticles were not functionalized. However, this sample is successful, in that the vesicles are nearly spherical and similar in size. As the scale on the image indicates, the vesicles are on the order of 30 μm, which is an approximately the size of a human skin cell, and a little larger than a blood cell. This size vesicle is an appropriate analogue for medical application. Also, as the confocal microscope can change focus planes, I observed that the

remaining lipid seen around the cells was not floating freely in solution, but rather adhered to the glass surface, and therefore will not interfere with the nanoparticles in the solution.

As a control test, I created a slide that contained only the solution with the gold nanoparticles. All that appeared on this slide when looked at on the fluorescent microscope was a green haze. Because there were again no obvious bright spots, it was clear that the nanoparticles remained unfunctionalized. In addition, the washing procedure was not completely effective, as FITC molecules were still floating in solution.

4.2 Discussion

4.2.1 Possible Problems with the Buffer Solution

One possible problem with the buffer solution that could have affected my functionalization procedure is the titration. Unfortunately, a pH meter was not available while I was synthesizing this solution. Instead, I used pH strips, which do not provide the same level of accuracy as a pH meter. The pH of the solution has to be within a certain range for the reaction to occur, so if my buffer solution was outside of this range, it could contribute to the nanoparticles not being functionalized.

4.2.2 The Washing Procedure Affecting Functionalization

The most likely step in the functionalization procedure that would have caused the FITC molecules to not be attached to the nanoparticles was the washing procedure. The test tube with my first sample was destroyed by the centrifuge. Although the liquid had leaked out, an important observation was that the gold had actually accumulated on the sides of the test tube. Following this, I tried using a different centrifuge that would not destroy the test tube, and had a variable speed control so I could reduce the centrifuging speed. This did help, however, after the first several washes, the color of the solution began to change from a red color to a gray color. Solutions containing larger gold nanoparticles tend to have a grayer color, so this color change indicates that the nanoparticles were aggregating as the

solution was being centrifuged. Shortly after this color change occurred, a slight gold layer appeared on the test tube walls. If the gold particles were aggregating, not only were they not ending up in the solution added to the vesicles, but any bonds made with the FITC molecules were likely broken.

5 Summary and Future Work

5.1 Summary

The work completed so far has set the groundwork for a successful Master's thesis. I have shown that I can synthesize model cells that are uniform in size in shape, and should act appropriately as stand-ins for living cells. Furthermore, I have familiarized myself with both cross-polarizing and confocal fluorescent microscopy, which I will be using to collect data. I have worked through a majority of my functionalization procedure, and have the necessary materials to create many samples to study. Although I have yet to perfect the procedure, I have several ideas to test as I begin my thesis work. With most of the procedural work in place, what remains is creating samples and data collection and analysis.

5.2 Future Work

As this research progresses, the remaining work will be focused on refining the procedures, and analyzing data collected on the fluorescent microscope. Once the procedures are finalized, I will be able to vary the variables in the system such as concentration of nanoparticles, functionalization, and environment, to observe what affects the cellular uptake of the nanoparticles.

5.2.1 Refining the Washing Procedure

The first test to try is to observe the nanoparticles after being mixed with the FITC solution, but before the washing procedure. This test will determine if the centrifuging is breaking the bonds with the FITC molecules. Although the unreacted dye will show up on the fluorescent microscope, it will only be as a green haze in the background. If the nanoparticles were successfully functionalized, they should be clearly brighter than the fluorescing solution. If the background fluorescence is uniform, and not interfering with seeing the nanoparticles, I can collect data without having to wash the nanoparticle solutions. If the FITC is bonded

to the nanoparticles, but the remaining dye interferes with data taking, I will have to adjust my washing procedure to something gentler that will allow me to wash out the unreacted dye without causing the nanoparticles to aggregate.

5.2.2 Collecting Data

To collect data, I will make a series of identical samples, and introduce the nanoparticles to the vesicles at a series of different times. By counting the nanoparticles both inside and outside of the vesicles in each sample, I will be able to plot the concentration of the nanoparticles as a function of time. The rate at which the nanoparticles enter the cells, and the equilibrium point of the system will provide insight as to how the nanoparticles enter the cells. The analysis of the images I collect from the fluorescent microscope will be conducted with ImageJ, a software package developed by the National Institutes of Health.

5.2.3 Modifying the Gold Nanoparticles

To better understand what affects the mechanisms of cellular uptake, I will begin varying parameters of the system. In addition to adjusting the concentration of the nanoparticles, modifications to the nanoparticles themselves could alter how they enter the cells. It is possible to attach several functional groups to the nanoparticles, so a possible avenue to explore would be adding hydrophobic and/or hydrophilic functional groups. The lipid bilayer has both hydrophobic and hydrophilic properties, so the addition of these functional groups would likely affect cellular uptake. If this is the case, it would indicate the the uptake process is not purely dictated by endocytosis.

5.2.4 Changing the Environment

In addition to altering the nanoparticles, changes to the environment could affect the system dynamics. Currently, the solutions containing both the cells and nanoparticles are composed primarily of water. Depending on the biological environment being studied, water may not be

the best substitute. Bovine serum albumen, for example, is often used in medical research, as it more accurately mimics a human environment. By changing the environment the vesicles are in to represent human systems, I could gain an understanding of how gold nanoparticles might act if used on a living subject.

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Appendices

A Materials

- Gold Nanoparticles: Obtained from Cytodiagnostics. Sizes of 20 nm, 40 nm, 60 nm, and 80 nm. Nanoparticles functionalized with amine groups.
- 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC): Obtained from Sigma-Aldrich. A self-assembling lipid found in eukaryotic cell membranes.
- Fluorescein isothiocyanate isomer I (FITC): Obtained from Sigma-Aldrich. A fluorescent labeling reagent. Has an excitation wavelength of 492 nm and emission wavelength of 518 nm.
- 3,3'-Dihexyloxacarbocyanine iodide (diI): Obtained from Sigma-Aldrich. A fluorescent protein labeling dye. Has an excitation wavelength of 485 nm and emission wavelength of 501 nm.
- Methanol: Obtained from Sigma-Aldrich. Anhydrous grade solvent. 99.8% assay.
- Chloroform: Obtained from Sigma-Aldrich. Anhydrous grade solvent. $\geq$ 99% assay.
- Ethanol: Obtained from Sigma-Aldrich. Anhydrous grade solvent. $\geq$ 99.5% assay.
- Acetic Acid: Obtained from Sigma-Aldrich. Volumetric grade acetic acid solution, 0.5 M.
- Boric Acid: Obtained from Sigma-Aldrich. Molecular biology grade buffer salt. $\geq$ 99.5% assay.
- Phosphoric Acid: Obtained from Sigma-Aldrich. BioReagent, 85% assay.
- Hydrochloric Acid: Obtained from Sigma-Aldrich. Acid concentrate. Concentration of 0.5 M.

- Sodium Hydroxide: Obtained from Sigma-Aldrich. Base concentrate. Concentration of 0.1 M.