ABSTRACT

The Effect of Penetratin and Silica Capping Agents on Silver Nanoparticle Cellular Uptake in a Caco-2 cell line

Taylor E. Goldammer

Thesis Director: Dr. Erica Bruce

Understanding the biochemical pathway of nanoparticle uptake in human cells has important implications for nanomedicine and occupational health risk assessments. The mechanism of NP uptake in cells remains largely unknown, and studying the pathway of NPs may lead to improvements in nanotechnology and a better understanding of the risks involved with occupational exposure. The hypothesis was that silver nanoparticle uptake is dependent upon its capping agent. The goal was to evaluate the uptake kinetics of two different capping agents on AgNPs and the extent of NP uptake in a Caco-2 cell line. This was done by measuring and comparing the NP mass in the free media, adsorbed to the cell surface, and taken up into the cells. The Caco-2 cells were exposed to AgNPs for 24 hours, the NPs with penetratin and silica capping agents had an 11.54 and 0.97 mean percent of cellular uptake, respectively. AgNPs with a penetratin capping agent had a significantly greater amount of cellular uptake than AgNPs with a silica capping agent, which can be explained by the charge and hydrophobicity of the capping agent. This study is significant because this data helps to understand the kinetics and extent of AgNP uptake in human colorectal adenocarcinoma cells. If we are able to learn the pathway of NPs in cells, then this information can be applied to develop new cancer treatments involving nanotechnology and assess the human health risk of environmental exposures to NPs from sources such as soil and air pollution.
APPROVED BY DIRECTOR OF HONORS THESIS:

________________________________________________

Dr. Erica Bruce, Department of Environmental Science

APPROVED BY THE HONORS PROGRAM:

________________________________________________

Dr. Andrew Wisely, Director

DATE: ____________________
THE EFFECT OF PENETRATIN AND SILICA CAPPING AGENTS ON SILVER
NANOPARTICLE CELLULAR UPTAKE IN A CACO-2 CELL LINE

A Thesis Submitted to the Faculty of
Baylor University
In Partial Fulfillment of the Requirements for the
Honors Program

By
Taylor E. Goldammer

Waco, Texas
May 2013
# TABLE OF CONTENTS

List of Figures iii

List of Tables iv

Chapter One: Introduction and Literature Review 1
   Nanoparticle Properties 1
   Nanomedicine 2
   Occupational Exposure 3
   Hypothesis 5
   Significance of Research 5

Chapter Two: Methods 7
   Cell Culturing 7
   ICP-MS 11

Chapter Three: Results 13

Chapter Four: Discussion 16

Appendix 19

Bibliography 20
LIST OF FIGURES

Figure 1. 50 nm Silica-Coated Silver Nanoparticles 13

Figure 2. Silver Nanoparticle Uptake in Caco-2 cells after 24 hours 15
LIST OF TABLES

Table 1. Cell Uptake Data for Penetratin-Coated Silver Nanoparticles 14

Table 2. Cell Uptake Data for Silica-Coated Silver Nanoparticles 14

Table A.1. Mass of Silver in 9 Samples with Penetratin-Coated AgNPs 19

Table A.2. Mass of Silver in 9 Samples with Silica-Coated AgNPs 19
CHAPTER ONE
Introduction and Literature Review

Understanding the biochemical pathway of NP uptake in human cells has important implications for nanomedicine and occupational health risk assessments. The mechanism of NP uptake in cells remains largely unknown, and studying the pathway of NPs may lead to improvements in nanotechnology and to a better understanding of the risks involved in occupational exposures to NPs.

Nanoparticle Properties

There are many characteristics of NPs that make them desirable for medical applications but also raise concerns about NP cytotoxicity. The composition, size, shape, surface charge, and the capping agent on the NP are all contributing factors to its biochemical pathway.

NPs are unique because of their size which is typically 100 nanometers or less. They are the same size as biological molecules and structures living inside of cells,¹ and this property makes NPs applicable to nanomedicine. NPs are able to cross biological barriers and fit through pores more easily because of their small size. Furthermore, the comparatively small size of NPs to other biological molecules also means that they have a higher surface/volume ratio.¹ NPs participate in chemical interactions because of their large surface area. The importance of size is exemplified by looking at the difference between elemental metal silver particles in the body and AgNPs. The size of the particle greatly affects how the substance interacts with a biological system. The toxicity of a substance is determined by its size; however, even though NPs are small they may still
have a toxic effect due to their biological activity. The toxicity of NPs is also determined by the concentration of NP exposure.

NPs can be manipulated by adding a capping agent, functional group, or a polymer in order to give them an affinity to certain molecules. NPs can be coated with nanoshells of silver or other metals to give the particle desirable characteristics. For example, nanoshells of gold can convert light into heat to release drugs to tissues in cancer treatment.\(^2\) Furthermore, the antimicrobial properties of AgNPs are important in medicine. In nanotechnology, researchers are looking at these NPs to inhibit the growth of microorganisms that cause disease.

\textit{Nanomedicine}

Nanomedicine is a new field in science dating back to the late 1960s.\(^1\) There has been a recent explosion of nanotechnology in diagnostic applications, nanodrugs, and regenerative medicine. Nanodrugs can target active molecules and interact with diseased tissues because of the small size of NPs (100 nanometers or less). NPs can deliver agents to particular regions, tissues, or cells. Therefore, nanomedicine offers specificity in delivering drugs. Regenerative medicine involves the development of biocompatible materials to support cell growth in cell therapy.\(^1\) Nanomedicine is a growing field and continues to utilize AgNPs in biomedical applications because of their antimicrobial properties. For example, AgNPs are added to wound dressings, surgical instruments, and bone substitute materials.\(^3\) AgNPs have been found to help treat infections on wounds and burns caused by multi-resistant bacteria and fungi.

Another use of nanotechnology has been drug delivery in cancer therapy. It is believed that drug treatments can be improved and more efficiently delivered to
biological targets with nanotechnology. One solution for the concern of cancer drug toxicity is to couple a NP with a ligand in order to deliver genes such as Raf more selectively to cause local apoptosis in tumors. Nanomaterials are created for diagnostic purposes to provide a more accurate diagnosis by looking at a disease from a molecular scale. NPs are designed to recognize a target with a surface ligand when they are used for drug therapy, but these same interactions can be used to bind biomarkers for diagnostic tests.

Nanomedicine offers promising options for diagnosing and treating disease in the future. Since NPs can be capped with different substances, NPs will be created with affinities to different molecules and medicine will be personalized for patients. In the future, researchers want to incorporate complex, multivalent nanoparticles, so it is important to find out how NPs behave once injected into humans. While these new treatment options are exciting and offer promising results, the biggest concern with nanotechnology is the unknown health hazards associated with NPs. Without a risk assessment, it is difficult to get new drug therapies with NPs approved by regulatory agencies. Without knowing the exact biochemical pathway of NPs, researchers fear that the small size of NPs will disrupt vital cellular activity because they are the same size as organelles.

Occupational Exposure

Due to the rapid growth of nanotechnology, AgNPs can be found in electronics, clothing, the food industry, sunscreens, and medical devices. Nanocrystals are found in products such as silver burn cream, shoes, and athletic equipment. They have been
increasingly used in industry as amendments to textiles, cosmetics, sprays, plastics, and paints. Since the use of NPs in consumer products is increasing this means there is an increased release of NPs into the environment. Therefore, it is becoming necessary to find out if manufacturing these products is harmful to humans in the work place.

The toxicity of NPs in the environment and in the workplace is not fully understood. In order to develop a human health risk assessment for occupational diseases, it is important to understand the biochemical pathways and kinetics of NPs in contact with human cells. We need to assess the toxicity of NPs and predict their human health risk. A common way that people are exposed to NPs is by inhalation. Manufactured NPs are breathed in which is a concern because we do not know the significance of exposure via inhalation of antimicrobial AgNPs. Inhalation of NPs occurs as a result of airborne particles and it has been associated with lung cancer. Alveoli which are thin, single layered cells in the lungs are especially susceptible to NP exposure because their large surface area makes them less protected than the lung airways. Public health professionals fear that NP exposure may lead to pulmonary and cardiovascular disease.

Scientists are concerned about NP exposure because their size and surface area allows them to easily interact with larger particles and have potentially toxic effects. The most sensitive parts of the body to NP exposure from the environment are the lungs from breathing, skin through absorption, and GI tract from ingestion. Researchers have shown that exposure to antimicrobial AgNPs by inhalation or ingestion causes silver to accumulate in the brains of rodents. Other studies have shown AgNP exposure has led to an inflammatory response, oxidative stress, genotoxicity, and cytotoxicity in humans.
as well as the accumulation of silver in the liver.\textsuperscript{10, 11} Studies have shown that AgNPs may cause DNA damage and apoptosis in human cells if they are inhaled, ingested, or injected.\textsuperscript{6} These studies make it evident that the toxicity of AgNPs needs to be further evaluated, especially with their increasing popularity in medicine and consumer products.

\textit{Hypothesis}

The hypothesis states that silver nanoparticle uptake is dependent upon the capping agent on the NP. The goal was to evaluate the uptake kinetics of two different capping agents on AgNPs and the extent of NP uptake in a Caco-2 cell line. This was done by measuring and comparing the NP mass in the free media, adsorbed to the cell surface, and taken up into cells.

\textit{Significance of Research}

This study is significant because this data will help to understand the kinetics and the extent of AgNP uptake in human colorectal adenocarcinoma cells. A human Caco-2 cell line is used in order to simulate how NPs would act inside the body. The main sources of NP exposure are medicine, industry, and the environment. This study helps us determine the toxicity of NPs in order to conduct a human health risk assessment for ingested NPs. If we are able to learn the pathway of NPs in cells, then this information can be applied to prevent exposures and develop cancer treatments involving nanotechnology.

With the explosion of nanotechnology in medicine and in industry there comes a concern for the long term effect of NPs on human health. Scientists and professionals in the public health field need to know the risk of NP exposure in order to determine if the
benefits of nanotechnology outweigh possible health problems. The large surface area of NPs gives them greater surface energy and a potential for greater reactivity.² Known adverse effects from NP exposure include enhanced endocytosis and inflammatory responses.² NPs are believed to remain in the body for years and metal NPs are known to cause chromosomal fragmentation, DNA strand breaking, point mutations, oxidative DNA adducts and alternation in gene expression.⁵ It is also relevant to discover the threshold concentration of AgNPs that is hazardous to humans. By examining the biochemical pathway of NPs in human Caco-2 cells, this experiment is significant because it will help assess the risk associated with AgNP exposure in humans and it will advance our knowledge of AgNP toxicity.
CHAPTER TWO

Methods

The two types of AgNPs used in this experiment had a core size of 50 nm and were coated with either penetratin or silica. The silica-capped AgNPs were obtained from Nanocomposix and had a silica shell that was 23 nm thick. The penetratin-capped AgNPs were obtained from Particular GmbH, Hannover, Germany. Cell culturing was used to create samples that were digested by acid and heat in order to convert the silver to silver ions. ICP-MS was used for the quantitative analysis to detect the silver ions. Therefore, the ICP-MS was used to detect AgNP uptake in human colorectal adenocarcinoma cells.

Cell Culturing

Caco-2 cells were cultured for this experiment and this involved starting the cells, feeding the cells, and splitting the cells. To start the cells, since they were frozen at 1 million/vial, 19 mL of media was added to a T-75 flask and hot water was added to a beaker. A vial of cells was obtained from the -196 °C liquid nitrogen dewar and the procedure was done quickly at this point to minimize the amount of time the cells were exposed to DMSO in the freezing media. The flask was labeled with the passage number 26 and as Caco-2 cells. The vial of cells was completely immersed in the beaker with hot running tap water. After the cells were thawed, the outside of the vial was dried and wiped with a paper towel sprayed with alcohol. The vial was uncapped and its contents were added to the cell culture flask. The contents were mixed with the micropipette to dilute the DMSO as much as possible. The flask was placed in the incubator for two hours. Then the cells were checked to see if they had been plated on the bottom of the
flask. After the cells were plated, the media with diluted DMSO was removed and 20 mL of new media was added to the T-75 flask.

Feeding the cells involved warming the media in the 37 °C water bath for 30 minutes, putting the flasks and media in the hood, and flaring the closed lids, opened lids, and necks of the flasks. Then the media was aspirated with a Pasteur pipet and suction hose. If there was a large amount of debris, PBS with Ca\(^{2+}\) and Mg\(^{2+}\) was added to the flask and rinsed out. Then 8 mL of fresh media was added to T-25 flasks or 20 mL of fresh media was added to T-75 flasks. The confluence was checked daily and the media was changed every 48 hours. If the confluence was above 70% then the media was changed every 24 hours, and if the confluence was low then only half of the media was replaced.

Splitting the cells involved putting the media, PBS-Free (free of Ca\(^{2+}\) or Mg\(^{2+}\)), and 2x Trypsin in the water bath and preparing a labeled 15 mL centrifuge tube, cell counter vial, and new flasks in the hood for each flask of cells that were split. The closed lids, opened lids, and necks of the flasks and bottles were flamed. The media was aspirated off with a Pasteur pipet and suction hose. Then the flask was rinsed with 8 mL PBS-Free for T-25 flasks or 20 mL for T-75 flasks and they sat for 1 minute and were rocked to rinse the cells. Then the PBS-free was aspirated off and replaced with 2 mL 2x Trypsin for T-25 flasks and 3 mL for T-75 flasks. The flask was flamed, recapped, and rocked with the trypsin solution for 2 minutes. Then the flask was whacked to detach the cells and checked under the microscope. Trypsin should not be in contact with the cells more than 5 minutes or it will lyse the cells, so the flask was quickly returned to the hood, flamed, uncapped, and 8 mL of media was added to T-25 flasks and 7 mL of media to T-
75 flasks. A pipet was used to mix and disperse the cells then the 10 mL in the flask was transferred to a 15 mL centrifuge tube. A 20 μL aliquot was placed in the coulter counter vial with 20 mL of isotonic saline solution. The vial was capped and inverted then the cells were counted 3 times with a baseline of blank, and the number of cells in the pellet was found. The 15 mL centrifuge tube was flamed and capped then centrifuged at 3000 rpm for 3 minutes. The 15 mL centrifuge tube was carefully put back in the hood and the lid and neck were flamed, the new flasks were flamed and uncapped, and the media and trypsin from the tube were aspirated. It was important not to aspirate any of the pellet.

The amount of media to add to the centrifuge tube was calculated in order to have a concentration of 1 M of cells per mL in each flask. Once the cells were resuspended in the centrifuge tube and fresh media was added to the new flasks (18 mL for T-75 and 6 mL for T-25), 1 mL of the cell suspension was added to each new flask and they were rocked to disperse the cells evenly. Finally, the flasks were placed in the incubator.

The Caco-2 cells were seeded in the flasks, dosed with AgNPs with either penetratin or silica capping agents, and the samples were prepared for the experiment. A total of 8 T-25 flasks were labeled with information about the cell type, passage number, flask number, seeding date, freezing date, and initials. 3 flasks were exposed to AgNPs with penetratin capping agents for 24 hours, 3 of the flasks were exposed to AgNPs with silica capping agents for 24 hours, and 2 flasks were controls.

When the cells were ready to be dosed with 50 nm AgNPs, the vial of NPs was put in a sonicator for 10-15 minutes. The final concentration of AgNPs in the media of each flask was 3 ppm and the mass of AgNPs added to each flask was 24 μg. The AgNPs coated with silica came in a stock solution of 1 mg/mL and the AgNPs coated with
penetratin came in a stock solution of 130 µg/mL. After removing the T-25 flasks containing 8 mL of media from the incubator the necks and lids were flamed under the hood. Then 3 of the flasks were dosed with 24 µL of the silica-capped AgNP stock solution and 3 of the flasks were dosed with 0.18 mL of the penetratin-capped AgNP stock solution. The lids were flamed and put back on the flasks, the flasks were gently rocked back and forth, and they were put into the incubator. The 2 control flasks were not dosed with AgNPs. It was important to work efficiently and minimize the time the AgNPs were exposed to light because they are UV sensitive.

In order to prepare for sampling, eight 50 mL centrifuge tubes needed to be labeled for each sampling group. A total of 24 centrifuge tubes were needed: 9 for AgNPs with penetratin, 9 for AgNPs with silica, and 6 for the controls. There were 3 sampling groups for each flask: the group that measured the amount of AgNPs in the free media, the group that measured the amount of AgNPs adsorbed to the cell surface, and the group that measured the amount of AgNPs taken up into the interior of the cell. The sampling tubes for each group were placed according to their corresponding flasks on a rack. The label on each tube included information on the coating type and size of the AgNP, time of exposure to NPs, date of the sampling, objective, and the flask number. PBS-free was warmed in a 37 °C water bath for 30-45 minutes, the bottle was flushed with 100% ethanol, and the bottle was wiped out with a tissue. 67-70% nitric acid, cell lytic lysis buffer, foil, and a small sized cell scraper was obtained for the experiment.

There are three sampling groups for each flask and sampling occurred after 24 hours. After 24 hours, the flasks were removed from the incubator and gently rocked back and forth several times. The first sampling group measured the amount of free NPs
in the media by using an automatic pipette to suck out all the media from the flask and putting the media into a labeled tube. The second sampling group measured the amount of adsorbed NPs on the cell surface by adding 8 mL of PBS-free to each flask and gently rocking the flask back and forth three times. An automatic pipette was used to remove the media and PBS-free mixture from each flask and it was placed in a labeled tube. The third sampling group measured the cell uptake by adding 250 µL of cell lytic lysis buffer to each flask. Each flask was rocked back and forth a couple times. There was enough buffer in the flask to coat the bottom. A timer was set for 15 minutes, and after 15 minutes the entire bottom of the flask was scraped with a cell scraper. An automatic pipette was used to remove the liquid out of the flask and it was put in a labeled tube. 8 mL of PBS-free was added to the flask to wash off any remaining cells, and then this was repeated two more times. The tubes were covered tightly with foil because the AgNPs are UV sensitive. 50 µL of nitric acid was added to the first sampling group tubes, 125 µL was added to the second sampling group tubes, and 125 µL was added to the third sampling group tubes. The tubes were stored on a rack and placed under the hood.

**ICP-MS**

The quantitative analysis was completed using ICP-MS which required digestion of the samples. In order to prepare the samples for digestion, each tube was gently shaken and the liquid in the tube was poured into a labeled 75 mL glass vessel. Sampling group 1 tubes were rinsed twice with 2.5 mL of media, and sampling groups 2 and 3 were rinsed twice with 2.5 mL of PBS-free. 15 µL of 1000 ppm Rh stock solution was added, then 5 mL of 67-70% nitric acid was added, and finally 1 or 2 pieces of boiling stones were added to each glass vessel. The vessels were capped and put in the Scientific AIM500
Block Digestion System digestion machine. About 15-18 sample vessels were arranged randomly in the digestion machine and space was left between the vessels. The samples were heated at 100 °C for the first 90 minutes then the temperature was increased by 2 °C every minute until the digestion machine reached 130 °C. Once the temperature reached 130 °C the samples were kept at this temperature for 6 hours.

After digestion, Milli-Q water was added to each vessel until each vessel had a total of 75 mL of liquid, then each glass vessel was inverted 10 times. 15 mL plastic tubes were obtained and labeled with an assigned sampling group. 4 mL of liquid from the vessel was added to the corresponding 15 mL plastic tube with a new pipette. Milli-Q water was added to each 15 mL plastic tube until each tube contained a total of 12 mL of liquid, and then each tube was inverted 10 times. The Rh concentration in each tube was 50 ppb.

The standards were prepared which included blanks with 50 ppb Rh and varying amounts of silver from a silver ion standard solution of 100 ppm: no silver, 1 ppb silver, 10 ppb silver, 50 ppb silver, and 100 ppb silver. Next the samples were analyzed on the ICP-MS, which is a PerkinElmer SCIEX ICP Mass Spectrometer ELAN 9000 that uses ultrapure Argon as a carrier gas. The gas and cooling system were opened and the Elan software was started. After waiting 5 minutes, the daily performance test was run with the setup solution. A run list was created and the samples were analyzed using the calibration curve built by the ICP-MS that corrects the data. After the ICP-MS analysis, the samples were put away, the cooling system was closed, and the gas was turned off.
CHAPTER THREE

Results

In this experiment, there were a total of 8 flasks with Caco-2 cells. There were 2 control flasks without AgNPs, 3 flasks injected with 0.18 mL of penetratin-coated AgNP stock solution, and 3 flasks injected with 24 μL of silica-coated AgNP stock solution. The 50 nm silica-coated AgNPs are pictured in Figure 1. Sampling was completed 24 hours after the AgNPs were injected. Samples were separated into three groups: free AgNPs in the media, AgNPs adsorbed to the cell membrane, and AgNPs that were taken into the cell. These sampling groups correspond to the labels media, membrane, and uptake. After analyzing the samples by running the ICP-MS, the mass of silver in the three sampling groups for each flask was obtained. The percent of AgNPs free in media, adsorbed to the cell membrane, and that underwent cell uptake was calculated by dividing the mass of silver in each sampling group by the total mass of silver recovered for the flask. The mean percent of silver in each sampling group was calculated for the triplicate data for both penetratin-coated and silica-coated AgNPs.

Figure 1. 50 nm Silica-Coated Silver Nanoparticles
Table 1. Cell Uptake Data for Penetratin-Coated Silver Nanoparticles

<table>
<thead>
<tr>
<th></th>
<th>Mean Percent Silver</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>86.86</td>
<td>0.1388</td>
</tr>
<tr>
<td>Membrane</td>
<td>1.58</td>
<td>0.2001</td>
</tr>
<tr>
<td>Uptake</td>
<td>11.54</td>
<td>0.0874</td>
</tr>
</tbody>
</table>

Table 1 shows the mean percent of the penetratin-coated silver nanoparticles in the free media, adsorbed to the cell membrane, and taken up into the cell and the corresponding standard error for the three trials.

Table 2. Cell Uptake Data for Silica-Coated Silver Nanoparticles

<table>
<thead>
<tr>
<th></th>
<th>Mean Percent Silver</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>96.97</td>
<td>0.1643</td>
</tr>
<tr>
<td>Membrane</td>
<td>2.07</td>
<td>0.1472</td>
</tr>
<tr>
<td>Uptake</td>
<td>0.97</td>
<td>0.0522</td>
</tr>
</tbody>
</table>

Similarly, Table 2 shows the mean percent of the silica-coated silver nanoparticles in the free media, adsorbed to the cell membrane, and taken up into the cell and the corresponding standard error for the three trials.
Figure 2. Bar graph that represents the amount of silver nanoparticles in the free media, adsorbed to the cell membrane surface, and taken up into the cells after 24 hours.

Figure 2 is a bar graph that includes the amount of AgNP cell uptake of both penetratin (in blue) and silica (in red) coated samples. The bar graph shows the mean percent of the AgNPs found in the free media, adsorbed to the cell membrane, and taken into the cells after 24 hours. The results show that there are more silica-coated AgNPs in the free media. 96.97% of the silica-coated AgNPs were in the free media while 86.86% of the penetratin-coated AgNPs were in the free media. This shows that silica-coated NPs are more likely to stay in the free media than be taken up into Caco-2 cells. Furthermore, 1.58% of the penetratin-coated AgNPs and 2.07% of the silica-coated AgNPs were adsorbed on the cell membrane. Lastly, the penetratin AgNPs had a cellular uptake of 11.54% which was significantly greater than the silica AgNP cellular uptake of 0.97%. Therefore, penetratin coated AgNPs are more readily taken up into Caco-2 cells after 24 hours than silica coated AgNPs, and the rate of cell uptake is dependent on the capping agent on the AgNP.
CHAPTER FOUR

Discussion

The results of this experiment show that AgNPs coated with penetratin had a significantly greater amount of cellular uptake than AgNPs coated with silica after 24 hours. There was a larger amount of silica-coated NPs in free media than penetratin NPs because more of the penetratin NPs had been taken up into the cells. Furthermore, there were similar amounts of silica and penetratin coated NPs adsorbed to the cell surface.

The charge and hydrophobicity of the coating could affect the cellular uptake rate of the AgNPs. The charge of the cell and NP is measured by its zeta potential. Since the cell membrane surface is hydrophobic, molecules with large charges have a harder time passing through the biological membrane. The zeta potential of the coating affects the mobility of the NP due to the electrochemical effects. The speed and direction of travel depends on the magnitude and charge of the NP compared with the cell membrane.

According to the manufacturer, the silica-coated AgNPs have a zeta potential of -28.2 mV. Through experimentation, the penetratin-coated AgNPs were found to have a zeta potential of -7.29 mV in the media. The larger amount of cellular uptake of penetratin-coated AgNPs can be explained by their more positive charge. The general trend was that more positively charged NPs have greater cellular uptake compared to NPs with a strong negative charge which had little or no absorption. This might be explained by the repulsion between the negative charge of the coated AgNP and the negatively charged interior of the cell.

The hydrophobicity of the coating is another important factor that influences cell uptake. Generally, a higher hydrophobicity of the NP surface is associated with higher
cellular uptake. Higher hydrophilicity is associated with a lower cellular uptake rate. The lipid bilayer has a hydrophobic membrane that is impermeable to most water soluble, hydrophilic molecules. Penetratin is a cell-penetrating peptide that is amphipathic. The penetratin amino acid sequence has 16 residues which may be a contributing factor to its increased cellular uptake ability. Penetratin has a high density of basic amino acids such as arginine and lysine and has hydrophobic residues such as tryptophan.\textsuperscript{13} The larger hydrophobicity of penetratin could explain why penetratin-coated AgNPs have greater cellular uptake than silica-coated AgNPs.

Other considerations that explain the results of this experiment are the size and shape of the chemical structure of the coatings on the AgNPs. Even though molecular weight has been shown to be a less important factor in cell membrane permeability, a smaller and narrower shape will more easily penetrate the lipid bilayer and cell membrane. Since penetratin contains an amino acid sequence, its narrow shape may contribute to its increased cellular uptake and explain why this peptide is able to penetrate the cell membrane with such great efficiency. When comparing sizes of AgNPs, the hydrodynamic size must be used because small water molecules interact with the surface of NPs that make them bigger than their actual size. Furthermore, the amount of exposure time between dosing the Caco-2 cells with the coated AgNPs and preparing the samples also affects the results. The NPs aggregate over time due to ionic interactions with salts. In this experiment, samples were taken 24 hours after Caco-2 cells were dosed with the AgNPs. In order to control for ionic interactions, the same experiment should be done at different sampling intervals to evaluate the cellular uptake when the Caco-2 cells have been exposed to coated AgNPs for different amounts of time.
This data elucidates the kinetics of AgNP uptake in Caco-2 cells for penetratin and silica capping agents. Since penetratin-coated AgNPs had a significantly greater amount of cellular uptake than silica-coated AgNPs, it can be inferred that penetratin-coated AgNPs are more toxic inside the body and would more readily be taken up into the cells. This information can be applied to medicine and industry in order to help people choose effective capping agents. For example, in medicine a penetratin coating may be chosen to be absorbed into certain cancer cells and in industry a silica coating may be chosen for products in order to avoid absorption that would be dangerous to human health. Concerning the long term effect of NP exposure on human health, this study suggests that the rate of cellular uptake and the toxicity is dependent upon the capping agent on the AgNP.
APPENDIX

Table A.1. ICP-MS values of the mass of silver in the 9 centrifuge tube samples corresponding to the three sampling groups taken from 3 flasks with Caco-2 cells dosed with penetratin-coated AgNPs. The masses for the three sampling groups: media, membrane, and uptake and the total mass of recovered silver in each flask are given.

<table>
<thead>
<tr>
<th></th>
<th>Mass of Silver (μg) in Flask 1</th>
<th>Mass of Silver (μg) in Flask 2</th>
<th>Mass of Silver (μg) in Flask 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>20.1</td>
<td>18.7</td>
<td>20.5</td>
</tr>
<tr>
<td>Membrane</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Uptake</td>
<td>2.7</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Total Mass of Flask</td>
<td>23.1</td>
<td>21.6</td>
<td>23.6</td>
</tr>
</tbody>
</table>

Table A.2. ICP-MS values of the mass of silver in the 9 centrifuge tube samples corresponding to the three sampling groups taken from 3 flasks with Caco-2 cells dosed with silica-coated AgNPs. The masses for the three sampling groups: media, membrane, and uptake and the total mass of recovered silver in each flask are given.

<table>
<thead>
<tr>
<th></th>
<th>Mass of Silver (μg) in Flask 1</th>
<th>Mass of Silver (μg) in Flask 2</th>
<th>Mass of Silver (μg) in Flask 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>20.3</td>
<td>21.9</td>
<td>21.4</td>
</tr>
<tr>
<td>Membrane</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Uptake</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Total Mass of Flask</td>
<td>20.9</td>
<td>22.5</td>
<td>22.2</td>
</tr>
</tbody>
</table>


