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Metal Related Nanoparticles' Physical Behaviors in Different Physiologica Environments

Muhetaer Tuerhong

METAL RELATED NANOPARTICLES' PHYSICAL BEHAVIORS IN DIFFERENT PHYSIOLOGICA ENVIRONMENTS

by

Muhetaer Tuerhong, ME

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ABSTRACT

In the past decades, the development of nanotechnology has had tremendous successes in material science. In this technology, the pertinent materials are used at the intermediate scale between individual molecules and their size in the nanometer region(1-100nm) compared to bulk materials. This nanoscale size provides a larger surface area; therefore, nanoparticles would be perfect essential components of nanotechnology. The reduced size of nanoparticles has a larger surface ratio to volume, which can modify their chemical, mechanical, structural, and electrical properties.

In this study, the main goal is to test different metal related nanoparticles, such as CuNPs (Copper nanoparticles), FeNPs (Iron nanoparticles), CuHARS (Copper high-aspect ratio structure) and, Zn (Zinc microparticles) with different biological environments. In specific biological environments, such as sterilized water, deionized water, and various cell culture media, nanoparticles will change their morphology in different degrees; also, in living cells (astrocyte and CRL rat brain glioma cells) environment these nanoparticles either damage the cells or would not harm the cells.

The hypothesis of this project is that CuHARS or CuNPs under biological conditions would degrade. In order to prove this hypothesis as valid, CuHARS with CuNPs were tested in sterilized water and cell culture media at room temperature and body temperature. The result showed that CuHARS and CuNPs will degrade in cell

culture media at room temperature and body temperature at different pace. Surprisingly, CuHARS and CuNPs in sterilized water are aggregated in different levels. Charges around the nanoparticles cause them to aggregate or evenly disperse in water, but they do not degrade. After the testing, image analysis methods were used to extrapolate nanoparticles as either aggregating, degrading, or more stable.

After testing FeNPs in astrocyte cells and CRL rat brain glioma cancer cells, the hypothesis was that FeNPs would not affect morphology of the cells. Results showed that astrocyte and CRL rat brain glioma cancer cells were not damaged and were healthy. However, other nanoparticles, such as CuNPs and, CuHARS have toxicity by nature, they harmed the normal astrocyte and CRL rat brain glioma cancer cells.

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DEDICATION

I dedicate my thesis to my beloved family and many friends. First and foremost, I wish to express a special feeling of gratitude to my wonderful loving parents, Tuerhong and Zohra, who supported my studies in the US. There is no word I can use to describe my gratitude to them; without their support I wouldn't be here today. My sister Zubaida, has always been supportive, and has always encouraged me many times when I was facing some hardships here. You have never left my side and are very special.

I also dedicate this thesis to my aunt and her husband who live in Shanghai and who have supported me from the beginning. I will always appreciate what you all have done for me, especially uncle Hasan who financially supported me my studies at Louisiana Tech. Thank you so much and much love to my uncle Hasan.

Lastly, I dedicate this work to my host family, Petersons, back in Colorado and other family friends there. You guys have always been encouraging me and show your love to me. I will never forget you guys. I love all the people who supported me, and I pray that God will bless you and your families.

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CHAPTER 1

INTRODUCTION

1.1 Metal Related Nanoparticles

In nanotechnology, nanoparticles are fundamental building blocks for various applications. Nanoparticles exist with great chemical diversity in the form of metals, metal oxides, semiconductors, polymers, carbon materials, organics and biological based compounds [1]. Due to nanoparticle's very small size, which ranges from 1-100nm, they have very large surface area per unit to volume, which gives them unique properties. Nanoparticles exhibit different morphology, such as, sphere, cylinder, platelets, hollow spheres and tubes. Small sized nanoparticles have numerous potential applications in material engineering, health and biomedicine, chemistry, food industry, personal care products, electronics and computers.

Nanoparticles are made from materials with different chemical natures. Most of the nanoparticles are metals, metal oxide, polymers, silicates, carbon, and biomolecules. Many nanomaterials are metal-based nanoparticles, such as gold and silver nanoparticles, nanometallic oxides (zinc oxide, titanium oxide, iron oxide, and copper oxide), and are applied for many uses [2]. Metal-based nanoparticles, in particular silver and copper nanoparticles have a toxic nature that causes concern for their production uses.

Over the past decades, metal-based nanoparticle production has been growing

exponentially due to its nanoparticles' enhanced physicochemical properties and biological activities compared to their bulk parent materials [3].

Iron nanoparticles (FeNPs) are sub micrometer particles of iron metal. Nanosized FeNPs are highly reactive due to their high surface area. FeNPs are unique to other metal-based nanoparticles because of their magnetic properties. When iron particles are below a certain size range, <50nm, their chemical and physical properties react differently under different environments. The positively charged surface of FeNPs interact strongly with the negatively charged cell environment [4]. This allows the FeNPs to be useful in biotechnology, improved MRI imaging, and treating and cleaning up the contamination in ground water.

Copper nanoparticles (CuNPs) are copper based particle size, ranging from 1-100nm. CuNPs are abundant and inexpensive copper metals, which by manufacturing, and synthesis are easily made. Copper nanoparticles can be used in many different areas, such as catalysis, metallic coating, ink and biocide. By nature, CuNPs are a toxic and smaller size. Also, higher concentration of CuNPs has a highly toxic effect. The toxicity of Copper nanoparticles is size and concentration dependent [5]. When copper nanoparticles are exposed to different environments their surface morphology changes. When copper nanoparticles are in a biological environment, they undergo various reactions with different components of the environment; thus, the environmental condition (e.g. solution pH, ionic strength, natural organic matter) that the CuNPs are in plays an important role in their aggregation, degradation and agglomeration [3].

Zinc nanoparticles (ZnNPs) are one of the most commonly used nanoparticles in the biomedical field. The most widespread type of Zinc nanoparticles (ZnNPs) are zinc

oxide (ZnO) nanoparticles. ZnO nanoparticles are believed to be non-toxic, biosafe, and biocompatible, and have been also used as drug delivery carriers, for cosmetics, and filling for medical materials [6]. ZnO nanoparticles are reported by other research studies as nontoxic to human cells; this aspect necessities their usage as antibacterial agent noxious to microorganisms [7]. Antibacterial activities of ZnO nanoparticles are dependent on their size, shape, concentration, and exposure time to the bacterial cells [8].

CuHARS (copper high-aspect ratio structure) is a novel biohybrid material made from copper sulfate and cystine, which has nano- and microscale features [9]. Due to CuHARS containing a copper component, it has a toxic nature. It was discovered and produced at Dr. DeCoster's lab, at Louisiana Tech University, Ruston. Since CuHARS are nanomaterials that have high surface area ratio to volume so that they can be used for targeted drug delivery, bio tracking and tissue engineering. CuHARS are biodegradable material that under physiological conditions (37 ºC and 5% CO_2) in different cell culture media degrade over time [9]. Also, CuHARS in a water environment are extremely mobile for certain amount of time after they stabilize but do not degrade at all [9].

All these nanoparticles have common physical and chemical properties that are essential component for nanotechnology. From size to shape, all nanoparticles have specific structural, optical, electrical and physicochemical properties [7]. Metal based nanoparticles have an important role in determining their mobility, reactivity, toxicity, and potential risk in the cell environment due to their large surface area and size.

1.2 Research Objectives

1. Study metal-containing nanomaterials interacting with biological and physical environments. The research focus is mainly on CuNPs (copper nanoparticles), CuHARS (Copper high-aspect ratio structure) FeNPs (iron nanoparticles), and ZnNPs (zinc microparticles). These nanoparticles and microparticles mostly are characterized by their size, composition, crystallinity, and morphology.

2. Study physical behaviors of nanoparticles in different biological and physical environments.

2a. Test nanoparticles in biological and physical environments and observe their reaction activities, such as whether they can aggregate, degrade or evenly disperse. Biological environments could be sterilized water, deionized water, different types of cell culture media at room temperature or body temperature.

2b. Test nanoparticle's toxicity. Test nanoparticles in real living cells' environments. Nanoparticles interact with cell biomolecules in a unique manner physically transferring into the inner cell structures. Nanoparticles can be tested using primary astrocyte cells and CRL 2303 glioma cells (rat brain tumor cells). Toxicity of nanoparticles can affect the metabolism of the cells. Nanoparticles can damage the cell's normal functionality. After the cells are treated with nanoparticles, they are either dying, stressed or perhaps unaffected.

CHAPTER 2

METHODOLOGY

2.1 Iron Nanoparticles

There are many research papers which have been published in the past few years about applications of iron nanoparticles. Different types of iron nanoparticles are available in the market with suitable prices. Over the last few years, iron nanoparticles have been synthesized by various methods that meet the needs of nanotechnology. Iron nanoparticles are basically iron (III) powder. In this research, Fe_2O_3 nanopowder was purchased from the company Sigma-Aldrich (St. Louis, MO, USA). The particle size is less than 50nm, the surface area is50-245 m^2/mg and molecular weight is 159.69g/mol. Fe_2O_3 nanopowder was diluted in deionized water. Figure 2-1 shows Fe_2O_3 nanopowder and its diluted solution.

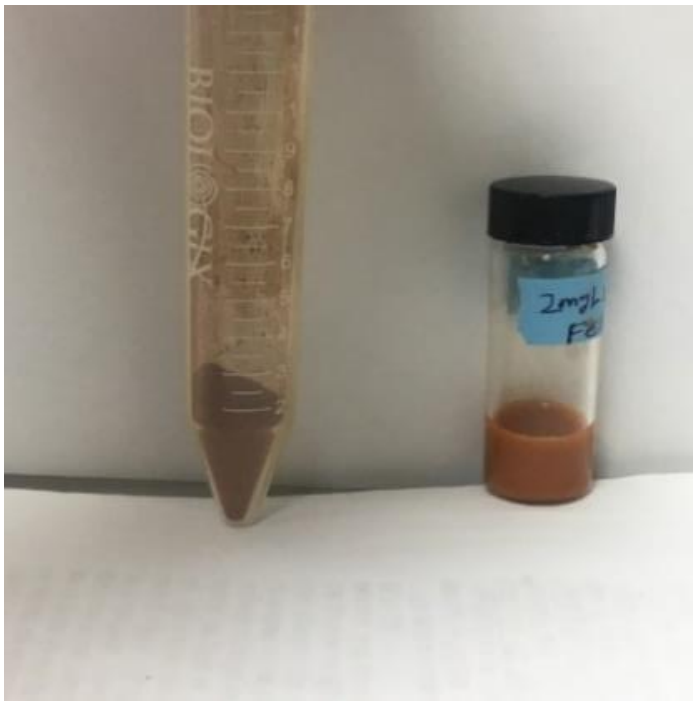

Figure 2-1: Fe_2O_3 nanopowder on the left, diluted Fe_2O_3 nanopowder solution on the right.

After being diluted in the water, the Fe_2O_3 nanopowder is stored in a clear glass vial. The stock solution concentration is 2mg/ml. In order to determine iron nanoparticle's stability, mobility, and other physical dynamics, FeNPs were tested in different environmental and biological condition.

2.1.1 FeNPs in different physical and biological conditions

In this section, iron nanoparticles were tested under various physical conditions. First, iron nanoparticles were tested in sterilized water, deionized water and astrocyte cell culture media in an incubator (37 ºC). The iron nanoparticle's concentrations were diluted from stock solution 2mg/ml to 3ug/ml and 6ug/ml. This was performed easily with the serial dilution method. After dilution process was done, iron nanoparticles were plated in a 48 well suspension culture plate sterilized with a lid. Iron nanoparticles were plated in

12 wells total. Each well volume was 1000ul. In order to get the best results, each concentration of iron nanoparticles were plated into 2 wells, such as 3ug/ml of iron nanoparticles plated into 2 wells in sterilized water, deionized water and astrocyte cell culture media. 6ug/ml of iron nanoparticles were plated using the same method as 3ug/ml. After plating the iron nanoparticles, they were stored in the incubator for 1-4 days to check their stability and mobility in such an environment. This whole experiment was performed by maintaining a sterilized flow hood.

Second, iron nanoparticles under sonicated and non-sonicated conditions were tested with DPBS (Dulbecco's Phosphate-Buffered Saline) solution. DPBS solution was purchased from the Gibco life technology company, Carlsbad, California. DPBS is a balanced salt solution used for a variety of cell culture applications, such as washing the cell before dissociation, transporting the cells, diluting cells for counting and preparing for reagent. The DPBS solution used in this procedure has a pH range of 6.7-7.0 without Calcium Chloride and Magnesium. Iron nanoparticles were diluted from the same stock solution 2mg/ml to 2ug/ml and 5ug/ml with the same method used the in previous section. In this experiment, in order to get consistent pictures for results, iron nanoparticles were tested in sonicated and non-sonicated conditions. Iron nanoparticles were sonicated for 20 mins before plating them into the plate. Sonication was carried out by Branson 1800, and ½ gallon ultra-sonic cleaner, 40kHz. This experiment also used 48 well suspension culture plate sterilized with a lid. Iron nanoparticles were plated into two separated plates: one plate was used for non-sonicated iron nanoparticles with a concentration 2ug/ml and 5ug/ml; another plate used for sonicated iron nanoparticles with concentration of 2ug/ml and 5ug/ml. the volume of each well was 1000ul. The

whole experiment was operated under a sterilized flow hood. After the experiment was done, plates were stored in the incubator for 1-7 days. During this time period, nanoparticles were checked by an electronic digital camera installed with microscope to observe their physical behavior in the DPSB solution.

Finally, iron nanoparticles were tested with living cell environments to check whether they would harm the cells. In this section, iron nanoparticles were tested with astrocyte cells and CRL2303 glioma cells. Primary astrocyte cells were growing from stage 1 to stage 6. Astrocyte cells were regularly fed and split. After stage 6, astrocyte cells were plated into the 48 well cell culture plate sterilized with a lid. When plating the cells, the process was completed, and the cell culture plate was stored in the incubator (37ºC). Astrocyte cells grow slower than CRL2303 glioma tumor cells. Usually, after 3-4 days after the cells can be treated with iron nanoparticles. Each well contains 10,000 astrocyte cells. Iron nanoparticles were diluted from 2mg/ml to 5ug/ml. After treating the cells with iron nanoparticles, the cell culture plate was stored in the incubator. The cell culture plate was checked by the electronic digital camera with scope regularly over time to see if iron nanoparticles would harm the cells or if they wouldn't affect the cells morphology at all.

2.2 Copper Nanoparticles

Copper is one of the most used and important industrial metal. Since copper nanoparticles have different physiochemical properties, they are used for biocide, facial spray, catalysis, sensors, microelectronics, and different electrical applications. Copper nanoparticles can be easily purchased from the market. There are many ways to prepare and synthesize the copper nanoparticles. The preparation process will be challenging

because copper nanoparticles easily oxidized with air. Various methods are used for synthesis of the copper nanoparticles, such as chemical reduction method, the microwave method, the electrochemical method and, biological synthesis. [10]. In this study, copper nanoparticles were purchased from the company Sigma-Aldrich (St. Louis, MO, USA). Copper nanopowder particle size is less than 100nm and 98.8% trace metal basis. In order to test copper nanoparticles with other materials, it needs to be changed to copper nanoparticle solution from nonopowders. Usually it can be made into a stock solution with concentration of 2mg/ml.

2.2.1 Copper nanoparticle and CuHARS in different physical and biological conditions

In this section, copper nanoparticles and copper related CuHARS were tested in different environments to determine their mobility, reactivity, stability and toxicity. First, copper nanoparticles were tested in sterilized water, deionized water, astrocyte cell culture media in the incubator (37ºC). Copper nanoparticles were diluted from stock solution with concentration of 2mg/ml to 3ug/ml and 6ug/ml. This process used serial dilution.

After dilution copper nanoparticles were ready to plate into a 48 well suspension culture plate that was sterilized with a lid. Each well volume is 1000ul. In order to better analyze the data each concentration of copper nanoparticles was plated into two wells. Overall, 3ug/ml and 6ug/ml concentration of copper nanoparticles were plated to 12 wells. After the experiment was completed, plates were stored in the incubator (37ºC). For tracking copper nanoparticle's physical behavior, the digital electronic microscope was used.

Second, CuHARS (Copper high-aspect ratio structure) was tested at room temperature with different concentrations in astrocyte cell culture media. CuHARS were provided by Dr. DeCoster's lab at Louisiana Tech, Ruston. In this section before using the CuHARS, particles in stock solution concentration of 2mg/ml were sonicated for 30mins mins with Branson 1800, and ½ gallon ultra-sonic cleaner, 40kHz. After the sonication process was done, CuHARS were diluted from 2mg/ml to 1ug/ml,2ug/ml and 4ug/ml with a serial dilution method. For this experiment, the 48 well suspension culture plate sterilized with a lid was used. A total of 3 wells were plated for 3 different concentrations, and each well volume was 500ul. After the experiment, the plate was put on one of the shelves at lab room temperature of 22 ºC. At room temperature, molecular dynamics are slower than body temperature, so the whole process for tracking of CuHARS biocompatibility took 1 to 3 weeks, and digital the electronic microscope was used.

Third, copper nanoparticles and CuHARS in astrocyte cell culture have different degrees of reaction rates. CuHARS and copper nanoparticles were plated together in the same well in order to determine reactivity, aggregation and degradation rate. CuHARS were diluted to 10ug/ml from 2mg/ml, and copper nanoparticles were diluted to 5ug/ml. Both CuHARS and copper nanoparticles were sonicated for 20mins with Branson 1800, and ½ gallon ultra-sonic cleaner, 40kHz. Afterwards, sonicated CuHARS and copper nanoparticles were plated into the suspension plate. After plating, the plates were stored in the incubator (37ºC) for 1-5 days. For the tracking of the physical behavior of combined particles, electronic digital microscope was used.

Finally, it must be considered that copper nanoparticles are toxic by nature. In this section in order to determine toxicity of the copper nanoparticles, they were treated with astrocyte cells and CRL2303 glioma rat cancer cells. After growing astrocyte and glioma cells, they were prepared for plating. During the growing section, astrocyte cells were regularly fed with astrocyte cell culture media, and glioma cells were regularly fed with CRL 2303 cell culture media. Usually, glioma cells grow faster than astrocyte cells. In this section, cells were plated to 48 well cell culture plate sterilized with a lid. Astrocyte cells were plated into 6 wells, 2 for control, each well contains 10,000 cells. Glioma cells also plated into 6 wells, 2 for control, each well contains 5000 cells. After plating the cells, plates were stored in the incubator (37ºC) for growing. Usually, it takes 2-4 days to grow the cells. In the last step, cells are treated with copper nanoparticles. Copper nanoparticles are diluted from stock solution of 2mg/ml to 5ug /ml and 10ug/ml. After cells were treated with copper nanoparticles, they were stored in the incubator. For the tracking step, to determine if copper nanoparticles affect the morphology of cells, the electronic microscopic with a digital camera was used. At the end, DAPI staining was used for the analysis of cell morphology. DAPI is a fluorescent stain that strongly binds to cell nuclei.

2.3 Zinc Nanoparticles

Zinc is an essential element in the human system, and with functional, strategic, promising, and versatile inorganic materials with a broad range of applications. Zinc Oxide nanoparticles hold unique optical, chemical sensing, semiconducting, electric conductivity, and piezo electric properties [7]. Nanosized Zinc Oxide particles are non-toxic to the human body; however, they have had antibacterial properties from time

immemorial [8]. Zinc Oxide nanoparticles can be synthesized through various methods by controlling the synthesis parameters [7]. With various synthesis methods, Zinc Oxide nanoparticles can be produced with a different morphology, such as microwave decomposition method making sphere shape, hydrothermal technique making nanorods [7]. In this study, Zinc-cystine microparticles were synthesized at Dr. DeCoster's lab in Louisiana Tech University, Ruston. It used the self-assembly method, which produced different shapes of Zinc-cystine microparticles, such as hexagonal prismatic rods, dumbbell, and rod shaped.

2.3.1 Zinc microparticles in different physical and biological environments

Since Zinc-cystine microparticles have different morphologies under different synthesis methods, in this section Zinc-cystine microparticles were tested with sterilized water and astrocyte cell culture media to determine mobility and stability of Zinc-cystine microparticles.

First, Zinc-cystine microparticles were synthesized at Dr. DeCoster's lab in Louisiana Tech University, Ruston. With the self-assembly method, which formed a hexagonal prismatic shape and rod shape. Zinc-cystine microparticles were diluted from solution to different molar concentrations and were tested with sterilized water. Two different shapes of Zinc-cystine microparticles were plated into the 48 well suspension culture plate sterilized with a lid to test their physical morphology. After the experiment was done, plates were stored in the incubator (37ºC). Plates stayed in the incubator for 1-4 days, and Zinc-cystine microparticles' physical activity was tracked by the electronic microscope with the digital camera.

Second, Zinc-cystine microparticles were also tested with astrocyte cell culture media to determine if microparticles are biodegradable in the cell culture media. The whole experiment, just as the Zinc-cystine microparticles was tested with sterilized water.

2.4 Cell Culture

Brain tissues were taken from newborn (less than 3 days old) rat pups. In order to separate brain tissue, trypsin was used in combination with trituration, and work was done in a 15ml tube. After allowing the bulk tissue to settle down, the supernatant containing the desired cells was removed from the tube and put into another 15ml tube. This process was repeated at least three times to maximize the cell yield. Primary cells can grow for many stages: usually astrocyte cells can grow for 7 stages, and glioma cells can grow from15-19 stages. Before plating the cells, cells are taken from the flask and washed with a PBS 1X solution. After cells are washed, trypsin was added to lifting cells from the flask which was put back into the incubator (37ºC) for 5-7 mins. After the cells are taken from the flask, they are moved to a tube that is then ready to be centrifuged. Once 15ml of the tube is completely full of the supernatant, it was centrifugated for 8 mins at a radial centrifugal force (RCF) of 160 and resulted in cell pellets, which were formed at the bottom of the tube. The supernatant was sucked from the tube and fresh new neuronal culture media was added. After adding the fresh media, the pellet was broken up by mild vortexing. A hemocytometer was used for counting the number of living cells under trypan blue added to the cells. Next, cells were plated into 48 well cell culture plate sterilized with a lid. The density of 10,000 cells were plated into the 48 well cell culture plate. The rest of the cells from the tube were stored in the flask with a cell density of over a million cells. Plated cells and the flask stored in the incubator (37ºC) for

several days to grow. For plated cells growing in the incubator (37ºC), astrocyte cells usually take 1-4 days, and glioma cells take 1-3 days. Before treating the cells with nanoparticles, cells are washed by new fresh cell culture media to remove some debris from the plate. Cells in the flask are fed by cell culture media to grow. After cells are grown in the plate, they were treated with copper nanoparticles and iron nanoparticles. Cell culture media ingredients are given in appendix A and B. Cell culture protocol is given by Dr. DeCoster's lab.

2.5 Digital Microscopy

For most of the microscopy work was done by Olympus 1X51 inverted microscope. The IX51 inverted system microscope addresses the observation and imaging needs of high-level laboratory and clinical applications. Fluorescent imaging was taken by the EXFO X-Cite series 120 light source. For DAPI stained nuclei, excitation light in the ultraviolet range (~400nm) was used.

2.6 Image Analysis

All image analysis work was done by open source software Image J 1.52. Image J is a java-based image processing program developed at National Institute of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin). Image J was used to count numbers of nanoparticles, cells and surface areas of total number of cells and nanoparticles in each single image. For each image, Image J program was able to show each nanoparticle's surface area. From the surface area, the nanoparticle's morphology was easily determined, including which nanoparticles were slowly degrading or aggregating. Image J also can determine cell morphology, such as,

whether cell’s nuclei were shrinking or expanding. Example of how the program was used given in the APPENDIX C.

CHAPTER 3

RESULTS

3.1 Iron Nanoparticles in Different Biological and Physical Environments

Nanoparticles' shapes, sizes, and compositions are important to determine their physical morphology in different environments. Sonicated iron nanoparticles were tested in astrocyte cell culture media, sterilized water, and deionized water. Figures 3.1 to 3.6 show iron nanoparticles' activity in different biological environments.

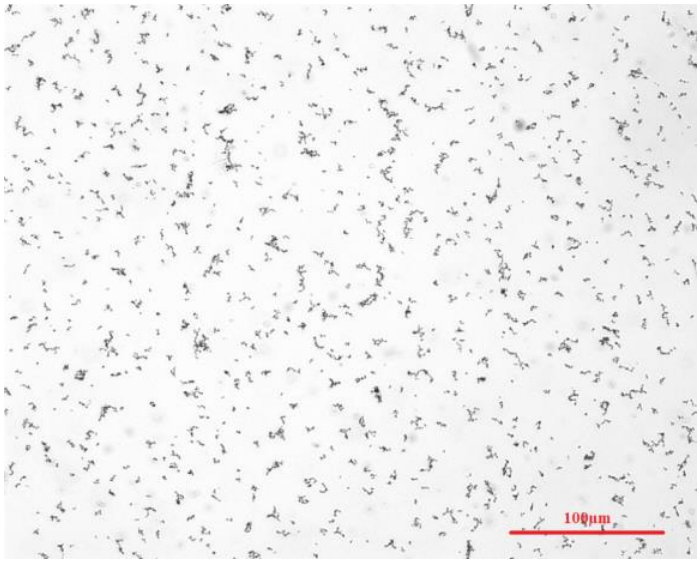

Figure 3.1: 6ug/ml of Iron nanoparticles in the astrocyte cell culture media. Picture was taken 30 mins after the experiment was completed. 10x magnification

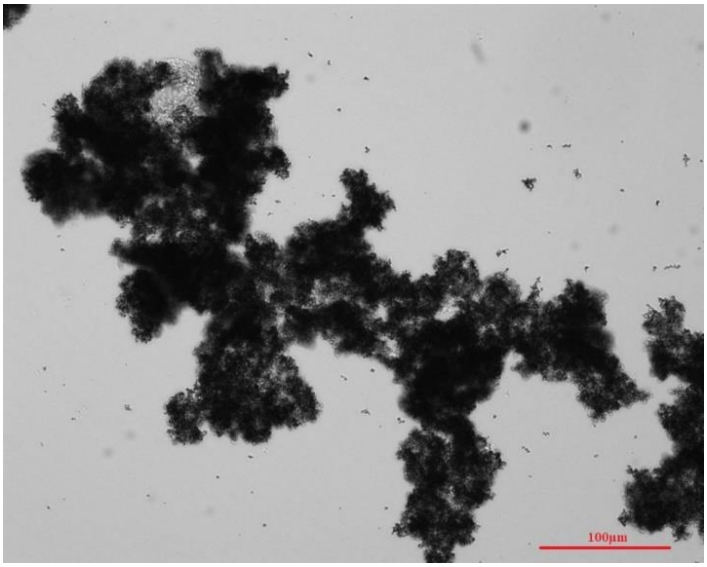

Figure 3.2: 6ug/ml of Iron nanoparticles in the astrocyte cell culture media. Picture was taken 4 days after the experiment was completed. 10x magnification. Over 4 days 6ug/ml of iron nanoparticles were clumped together.

Both Figure 3.1 and Figure3.2 images show that iron nanoparticles in the astrocyte cell culture media from day 0 - day 4 in the incubator(37ºC) changed their morphology. Iron nanoparticles didn't degrade, instead particles were clumped together.

Figure 3.3 and Figure3.4 iron nanoparticles showed unique physical mobility over time in the sterilized water in the incubator (37ºC). Iron nanoparticles changed their morphology from chain shape to aggregate into a net shape.

Figures 3.5 to 3.6 show how iron nanoparticles kept their morphology the same over time. From day 1 to day 4, images show that iron nanoparticles have strong stability in the deionized water environment.

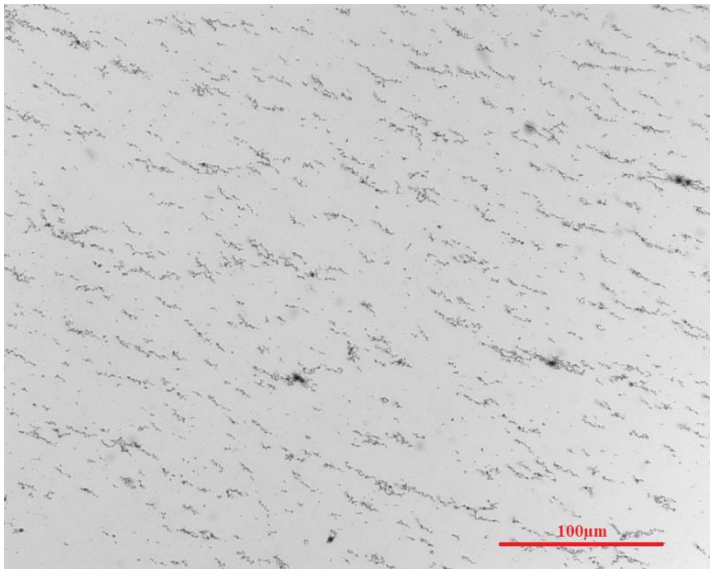

Figure 3.3: 6ug/ml of Iron nanoparticles in sterilized water. Picture was taken 1 hour after when FeNPs plated into the sterilized water in the incubator (37ºC). 10x magnification.

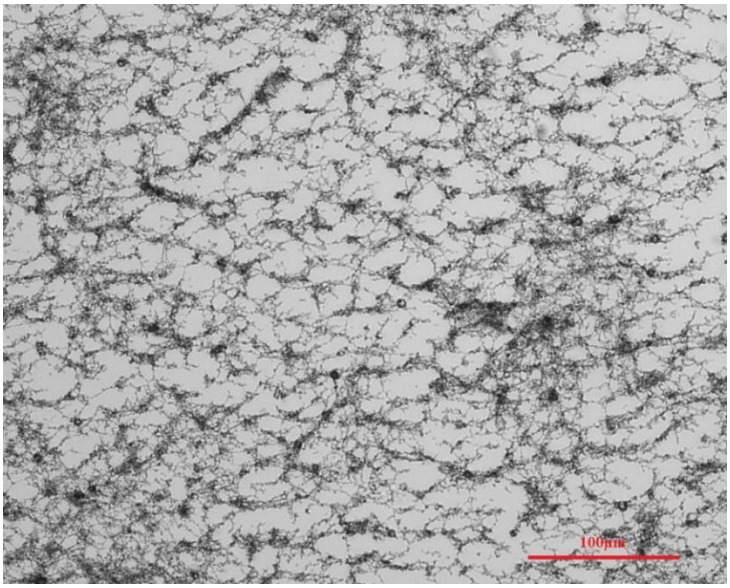

Figure 3.4: 6ug/ml of Iron nanoparticles in the sterilized water. Picture was taken after 4 days when FeNPs are plated onto the sterilized water in the incubator (37ºC). Over 4 days FeNPs are changed their morphology from chain shape to net shape. 10x magnification.

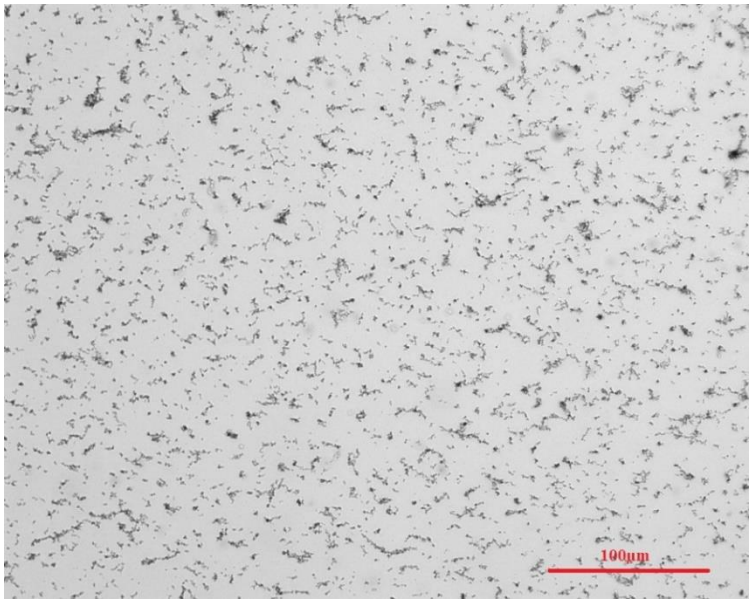

Figure 3.5: 6ug/ml of Iron nanoparticles in the deionized water. Picture was taken 1hour after when FeNPs are plated onto the deionized water in the incubator (37ºC). 10x magnification.

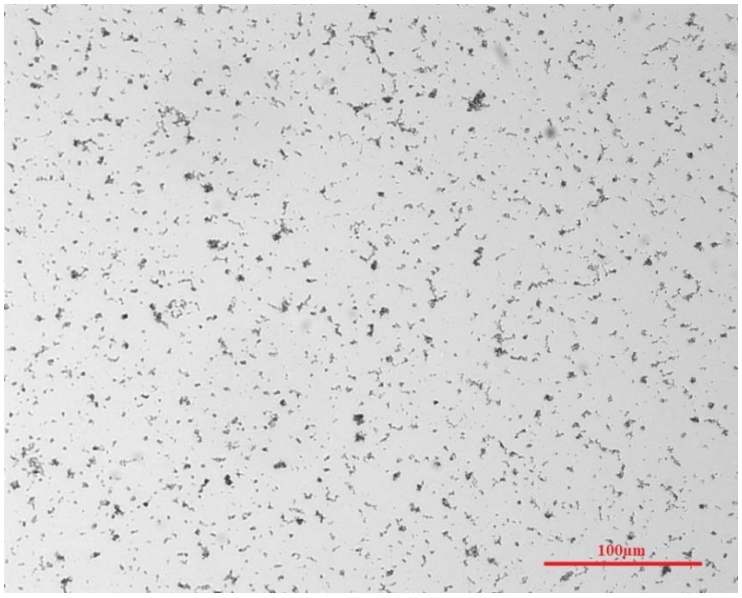

Figure 3.6: 6ug/ml of Iron nanoparticles in the deionized water. Picture was taken 4 days after when FeNPs are plated onto the deionzed water in the incubator (37ºC). Over 4 days nothing has changed. Particles were stable. 10x magnification.

3.2 Iron Nanoparticles in DPBS Solution

Sonicated and non-sonicated iron nanoparticles were tested in the DPBS 1x solution to determine their physical morphology. Figures 3.7 to 3.10 show activity of iron nanoparticles in the DPBS solution in the incubator (37ºC). Figure 3.7 and 3.8 show, morphology of non-sonicated FeNPs in the DPBS1x solution.

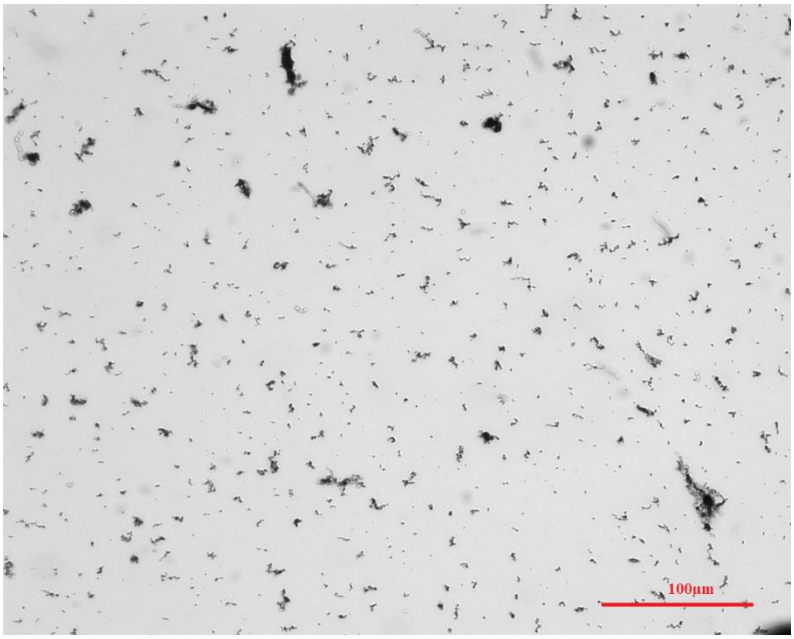

Figure 3.7: Non-sonicated 2ug/ml of iron nanoparticles in the DPBS1x solution. Picture was taken 1hour after iron nanoparticles added. 10x magnification.

Figure 3.9 and 3.10 show sonicated iron nanoparticle's morphology in the DPBS1x solution at body temperature.

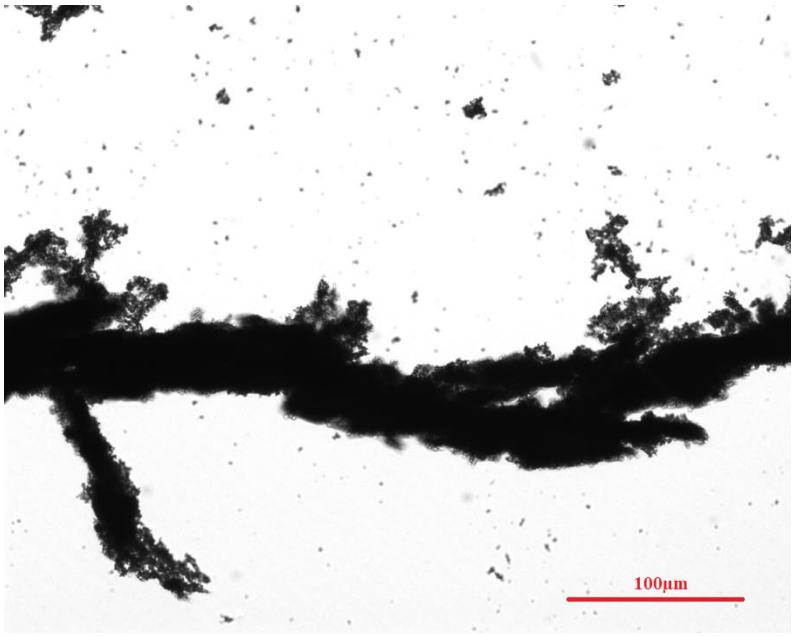

Figure 3.8: Non-sonicated 2ug/ml of iron nanoparticles in the DPBS1x solution. Picture was taken 3days after when iron nanoparticles were added. 10x magnification.

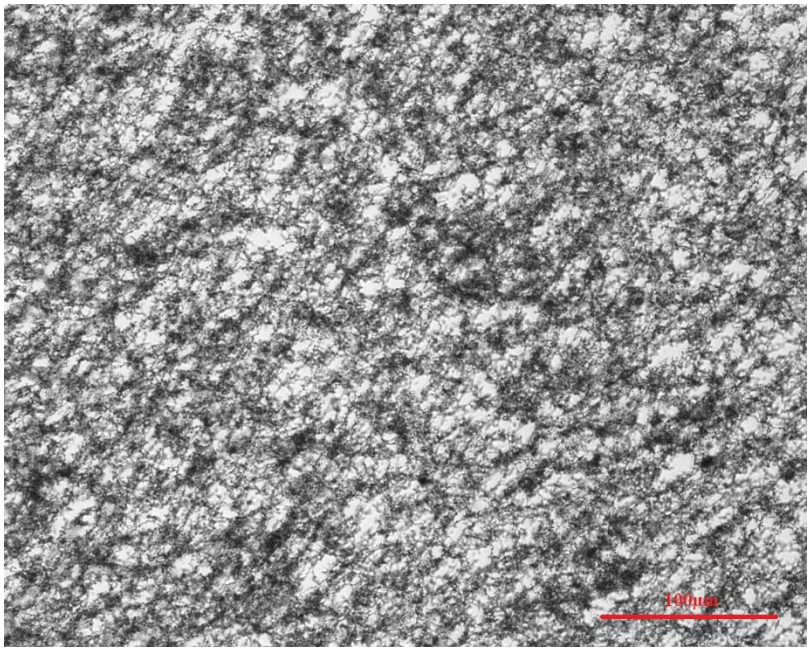

Figure 3.9: Sonicated 2ug/ml of iron nanoparticles in the DPBS1x solution. Picture was taken 5hours after when iron nanoparticles were added. 10x magnification.

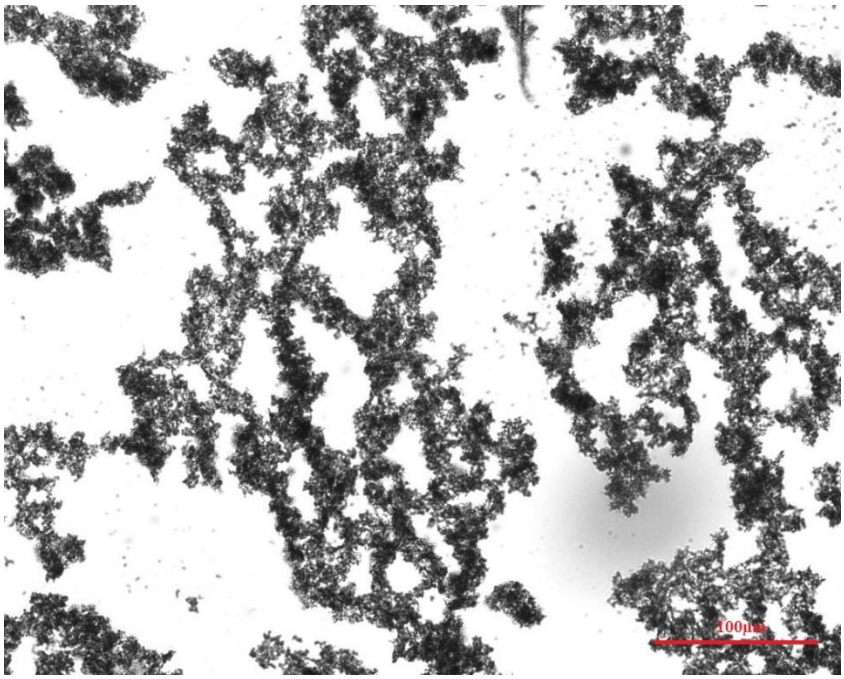

Figure 3.10: Non-sonicated 2ug/ml of iron nanoparticles in the DPBS1x solution. Picture are taken day3 after iron nanoparticles were added. 10x magnification.

Iron nanoparticles have different activities in the DPBS 1x solution in the incubator (37ºC). Non-sonicated iron nanoparticles were slowly aggregated to long chain shapes over time. Sonicated iron nanoparticles changed their morphology from high density disperse into clumped chain shape.

3.3 Iron Nanoparticles in The Living Cell Environment

In order to determine whether iron nanoparticles are affecting the growth rate of the living cells, particles were tested with astrocyte cells and CRL2303 glioma rat cancer cells. Figures 3.11 to 3.16 show how iron nanoparticles affect the cells' morphologies. Figure 3.11 shows 10,000 control astrocyte cells without iron nanoparticles treated. Figure 3.12 shows 10,000 astrocyte cells were treated with 10ug/ml of iron nanoparticles.

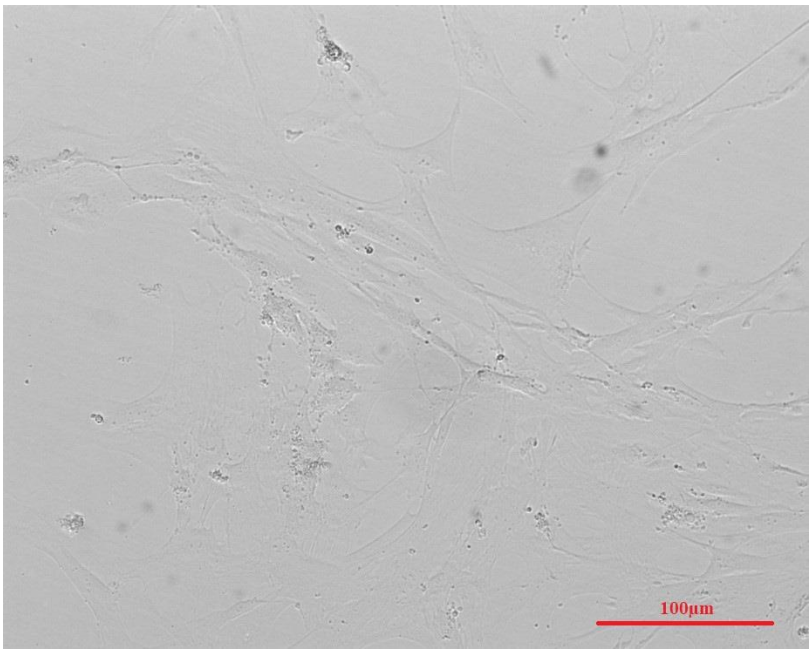

Figure 3.11: 10,000 astrocyte cells in the cell culture plate. The Picture is shown as a control cells to compare with other cells are treated with iron nanoparticles. Picture was taken 17 hours after when cells are treated with iron nanoparticles. Picture represents small portion of 10,000 astrocyte cells in single cell culture plate well. 10x magnification.

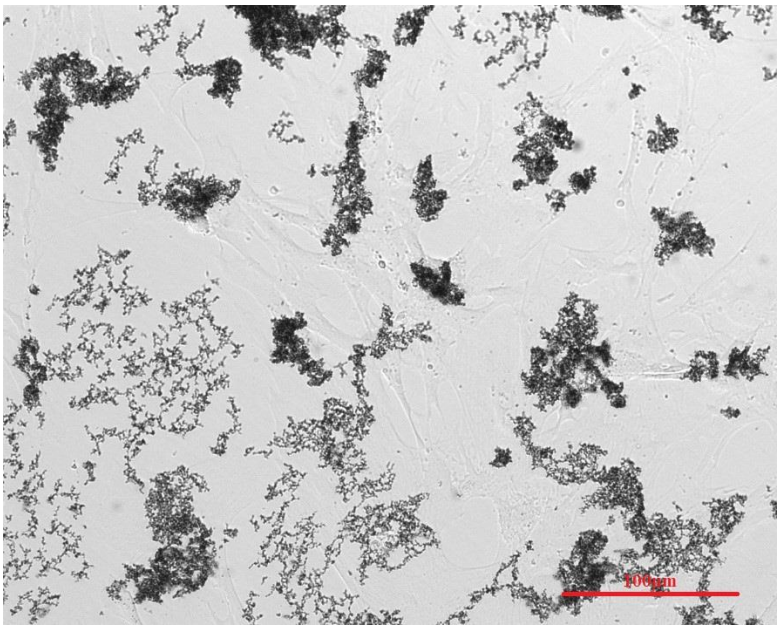

Figure 3.12: 10,000 astrocyte cells treated with 10ug/ml of iron nanoparticles. Picture was taken 17 hours after cells were treated with iron nanoparticles. Picture represents small portion of 10,000 astrocyte cells in single cell culture plate well. within 17 hours iron nanoparticles already aggregated and clumped. 10x magnification.

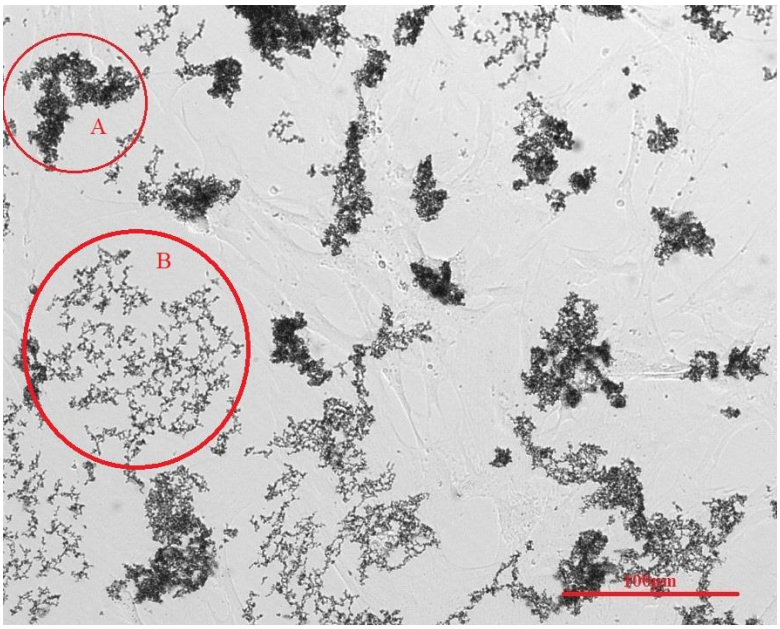

Figure 3.12a: Aggregation and clumping of 10ug/ml of iron nanoparticles with10,000 astrocyte cells. Picture represents small portion of 10,000 astrocyte cells in single cell culture plate well. 10x magnification.

In the Figure 3.12a shows 10,000 astrocyte cells are treated with concentration of 10ug/ml iron nanoparticles. Circle A shows some iron nanoparticles are started clump when they attached to the cell membrane within 17 hours. Circle B shows other iron nanoparticles in the space between cells are did not clump but they are aggregated in different degrees. Although iron nanoparticles didn't break down, but they are not toxic.

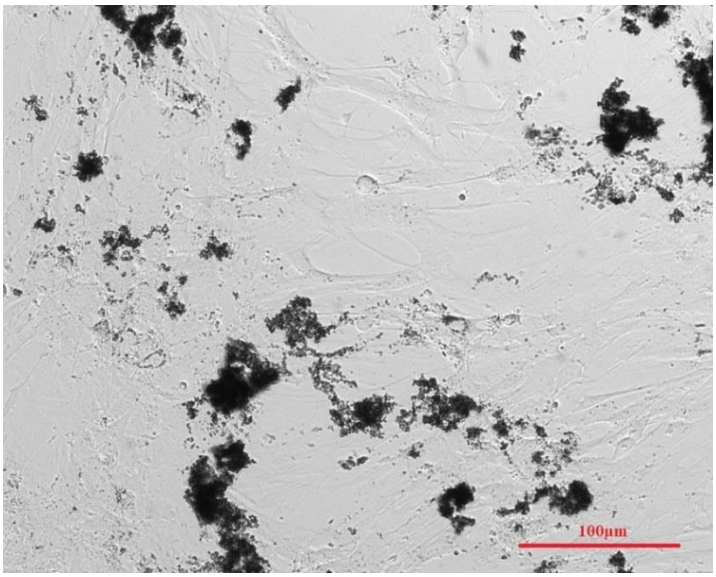

Figure 3.13: 10,000 astrocyte cells were treated with 10ug/ml of iron nanoparticles. The picture was taken 8 days after cells were treated with iron nanoparticles. Picture represents small portion of 10,000 astrocyte cells in single cell culture plate well.10x magnification.

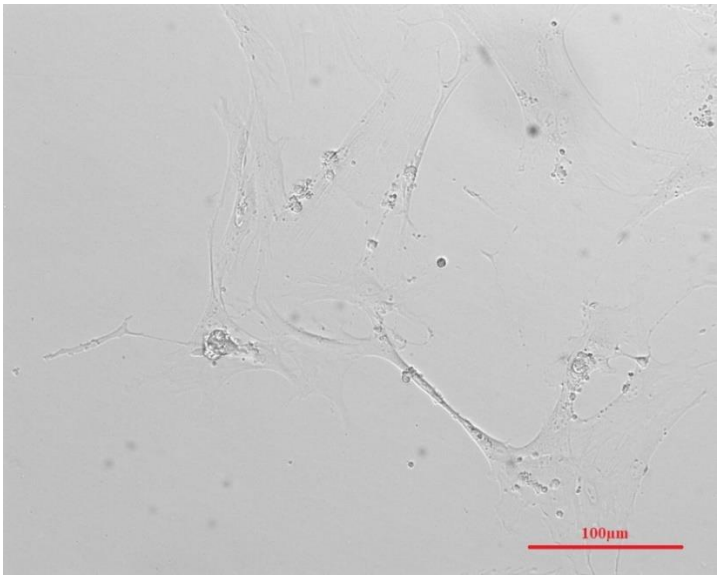

Figure 3.14: 10,000 astrocyte cells control plate without treated with iron nanoparticles. The picture was taken 8 days after cells were treated with iron nanoparticles. Picture represents small portion of 10,000 astrocyte cells in single cell culture plate well. 10x magnification.

From Figures 3.11 to 3.14, 10,000 astrocyte cells are shown treated with 10ug/ml of iron nanoparticles to test whether nanoparticles are harmful to a cells' morphology or the normal functionality. Images show that after treating astrocyte cells with iron nanoparticles, the cells are growing normally and didn't change their morphology over 8 days. Control cells are growing normally for 8 days. During these 8 days, fresh astrocyte cell culture media was added to the well to feed the cells. During the changing cell culture media process iron nanoparticles in the space between cells were sucked out but other iron nanoparticles were attached to the cells are stayed and clumped.

Iron nanoparticles were also used with 5000 CRL 2303 glioma rat cancer cells. Glioma cells by nature grow faster than astrocyte cells. Figures 3.15 to 3.17 show how iron nanoparticles interact with CRL 2303 glioma cells and affect cell morphology in the incubator (37ºC).

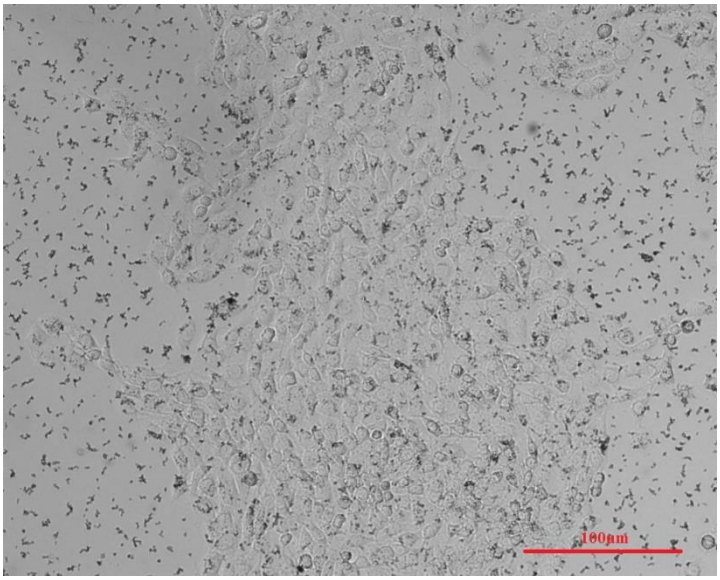

Figure 3.15: 5000 CRL 2303 glioma cells were treated with 5ug/ml of sonicated iron nanoparticles. The picture was taken 2 hours after cells were treated with iron nanoparticles in the incubator (37ºC). 10x magnification. Picture represents small portion of 5000 CRL 2303 glioma cells in single cell culture plate well.

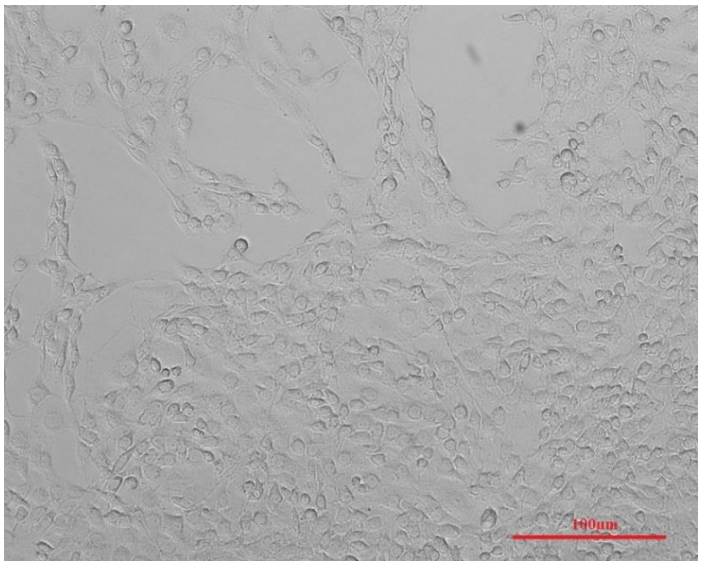

Figure 3.16: 5000 CRL 2303 glioma cells, control cells without iron nanoparticles treated. The picture was taken 2 hours after other cells treated with iron nanoparticles. Picture represents small portion of 5000 CRL 2303 glioma cells in single cell culture plate well. 10x magnification.

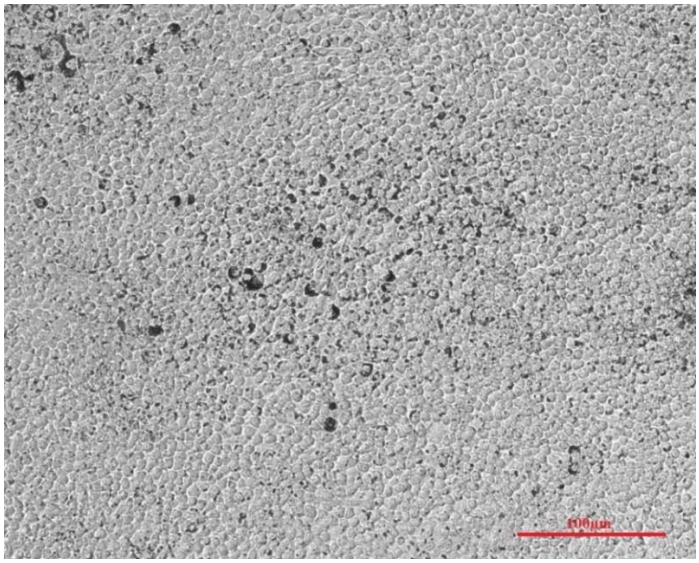

Figure 3.17: 5000 CRL 2303 glioma cells were treated with 5ug/ml of iron nanoparticles. The picture was taken 3 days after cells were treated with iron nanoparticles in the incubator (37ºC). Picture represents small portion of 5000 CRL 2303 glioma cells in single cell culture plate well. 10x magnification.

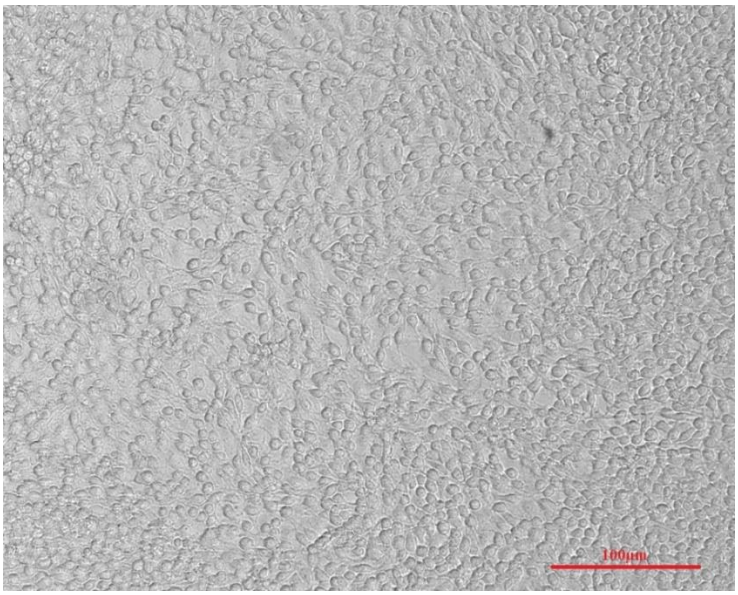

Figure 3.18: 5000 CRL 2303 glioma control cells without iron nanoparticles shown. The picture was taken 3 days after cells growing without iron nanoparticles were treated in the incubator (37ºC). Picture represents small portion of 5000 CRL 2303 glioma cells in single cell culture plate well. 10x magnification.

From Figure 3.15 to 3.18 5000 CRL 2303 glioma rat cancer cells are shown treated with a concentration of 5ug/ml of sonicated iron nanoparticles. CRL 2303 glioma rat cancer cells grow very fast, so within 3 days the cells have spread to every edge of the well. Figure 3.15 and Figure 3.17 show cells growing normally, and iron nanoparticles not impacting the cell's morphology. In the Figure 3.17 iron nanoparticles are clumped and attached to the cell membrane. Figure 3.16 and Figure 3.18 show how fast cells can grow.

3.4 Copper Nanoparticles in Biological Environments

Copper nanoparticles are characterized by small size between 10-100nm, which accounts for their good interactions with different biological environment, such as sterilized water, deionized water, different type of cell culture media, astrocyte cells, and CRL 2303 glioma rat cancer cells. High surface area and particle size determined copper nanoparticle's activity, mobility, and stability. Figures 3.19 to 3.24 show copper nanoparticles have different morphologies in different biological environments. Figure 3.19shows, 6ug/ml of CuNPs are added into the astrocyte cell culture media. Figure 3.20 shows degradation of the CuNPs in the astrocyte cell culture media at body temperature. The Figure 3.2o shows CuNPs in the well are completely disappeared.

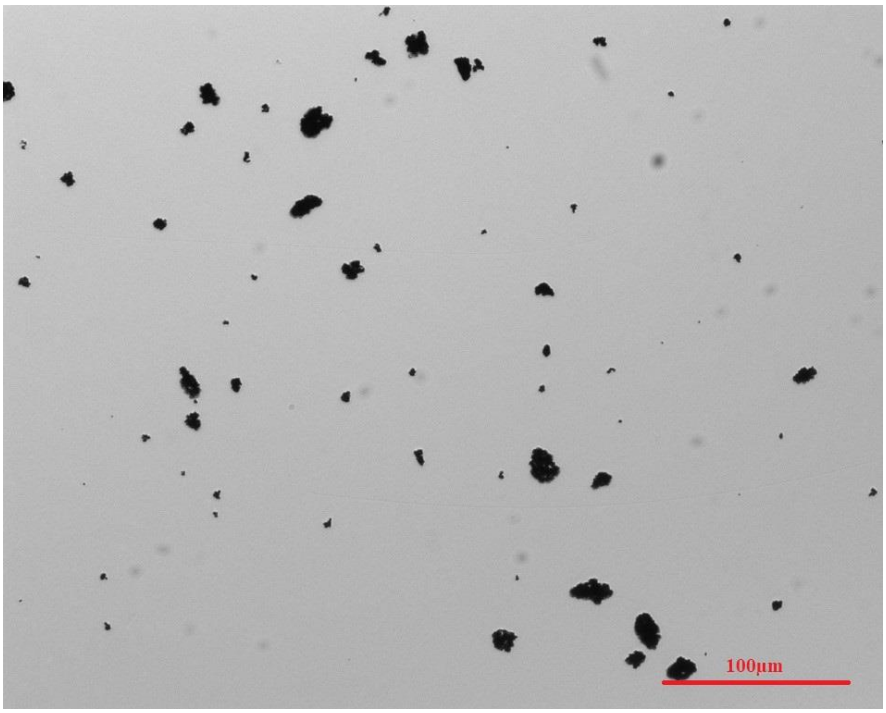

Figure 3.19: 6ug/ml of non-sonicated copper nanoparticles in the astrocyte cell culture media. The picture was taken 15mins after copper nanoparticles added to astrocyte cell culture media in the incubator (37ºC). 10x magnification.

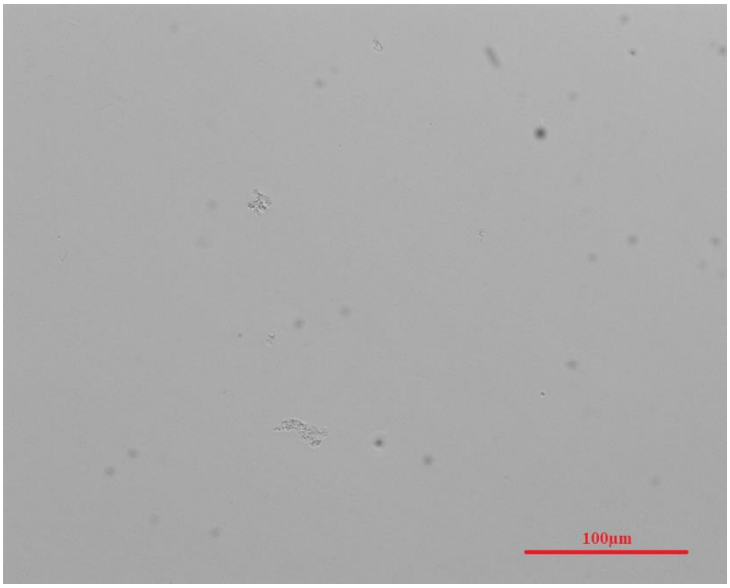

Figure 3.20: 6ug/ml of non-sonicated copper nanoparticles in the astrocyte cell culture media. The picture was taken 12hours after copper nanoparticles added to the astrocyte cell culture media in the incubator (37ºC). 10x magnification.

Figure 3.21 shows, 6ug/ml of CuNPs are added into the sterilized water in the incubator (37ºC). Figure 3.22 shows, aggregation of CuNPs in the sterilized water at body temperature.

Copper nanoparticles were tested in the deionized water to observe whether CuNPs are able to degrade or aggregate. Figure 3.23 shows 6ug/ml of CuNPs were tested in the deionized water. Figure 3.24 shows, morphology of CuNPs in the deionized water.

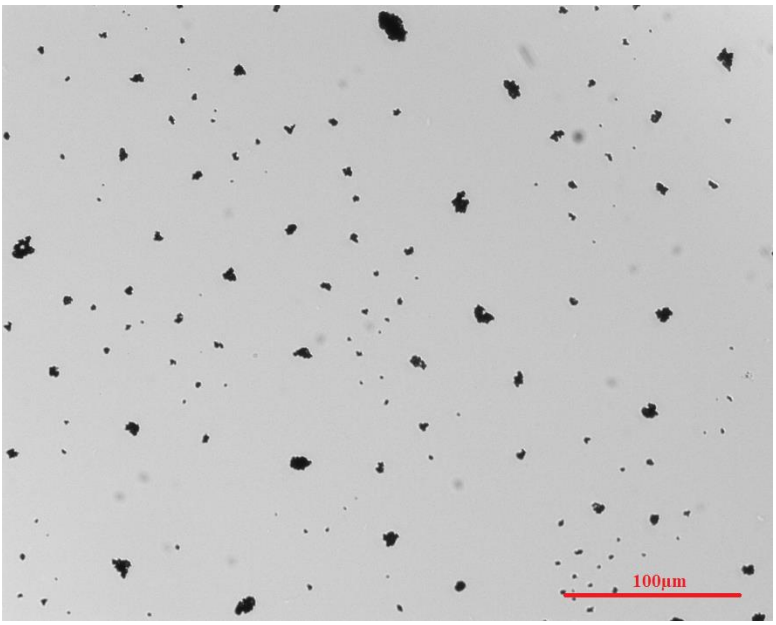

Figure 3.21: 6ug/ml of non-sonicated copper nanoparticles in the sterilized water. The picture was taken 15mins after copper nanoparticles added to the sterilized water in the incubator (37ºC). 10x magnification.

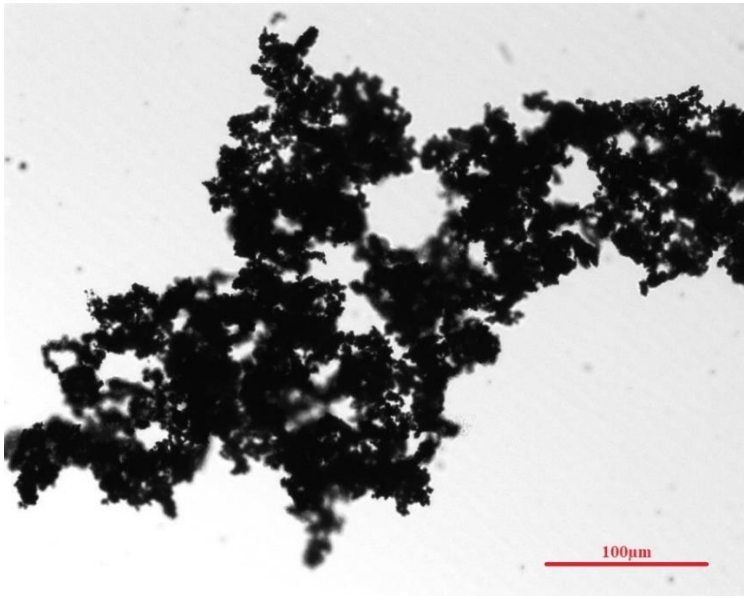

Figure 3.22: 6ug/ml of non-sonicated copper nanoparticles in the sterilized water. The picture was taken 4 days after copper nanoparticles added to the sterilized water in the incubator (37ºC). 10x magnification.

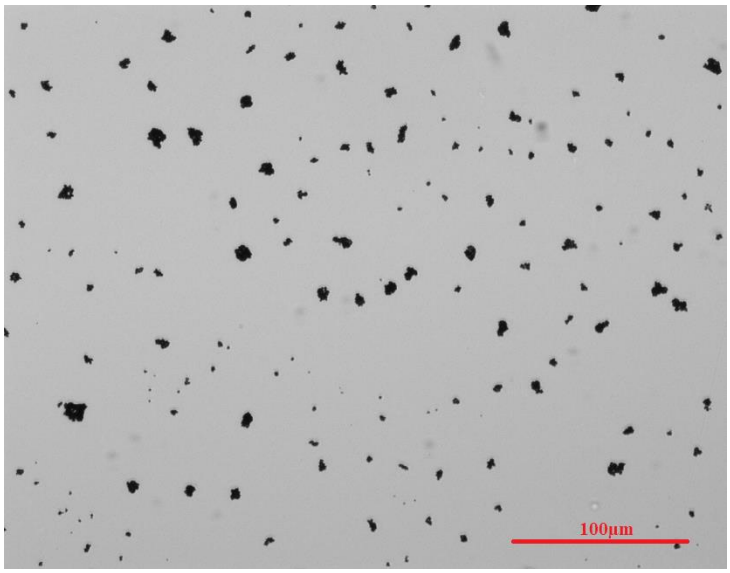

Figure 3.23: 6ug/ml of non-sonicated copper nanoparticles in the deionized water. The picture was taken 15 mins after copper nanoparticles added to the deionized water in the incubator (37ºC). 10x magnification.

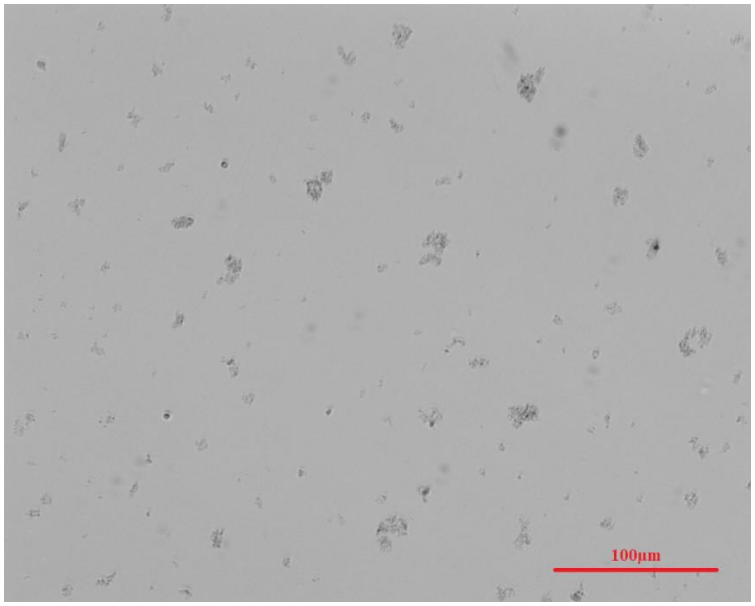

Figure 3.24: 6ug/ml of non-sonicated copper nanoparticles in the deionized water. The picture was taken 24hours after copper nanoparticles added to the deionized water in the incubator (37ºC). 10x magnification.

Figure 3.25 showed 6ug/ml copper nanoparticles in astrocyte media degrades over time. Comparing to the starting area coverage of copper nanoparticles at 3 hours, the copper nanoparticles are decreased by 99.5% by 12 hours. Figure 3.26 shows the numbers of copper nanoparticles in the astrocyte cell culture media decrease over time.

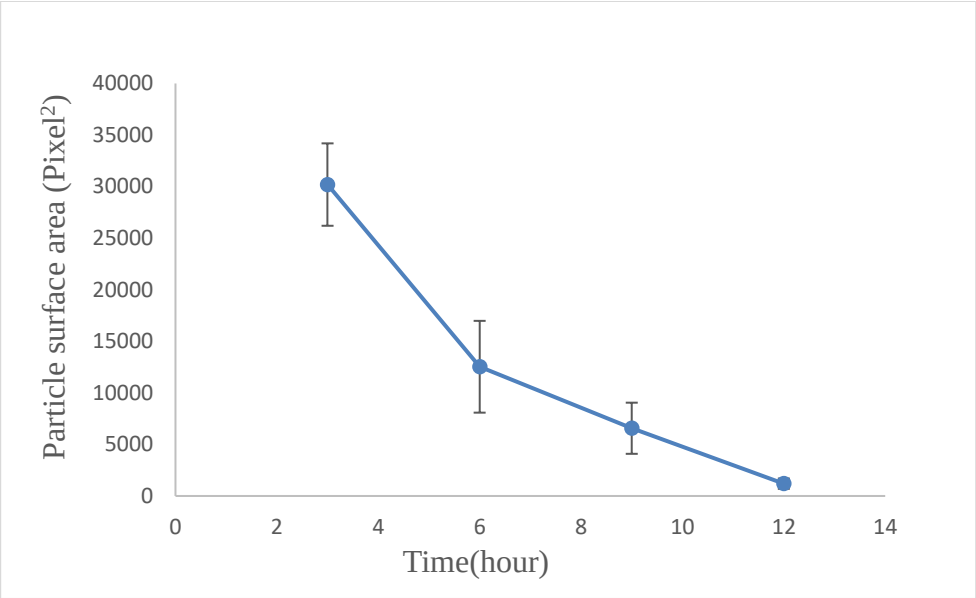

Figure 3.25: 6ug/ml of non-sonicated copper nanoparticles in the astrocyte media degrading over 12 hours. Error bar represents standard deviations.

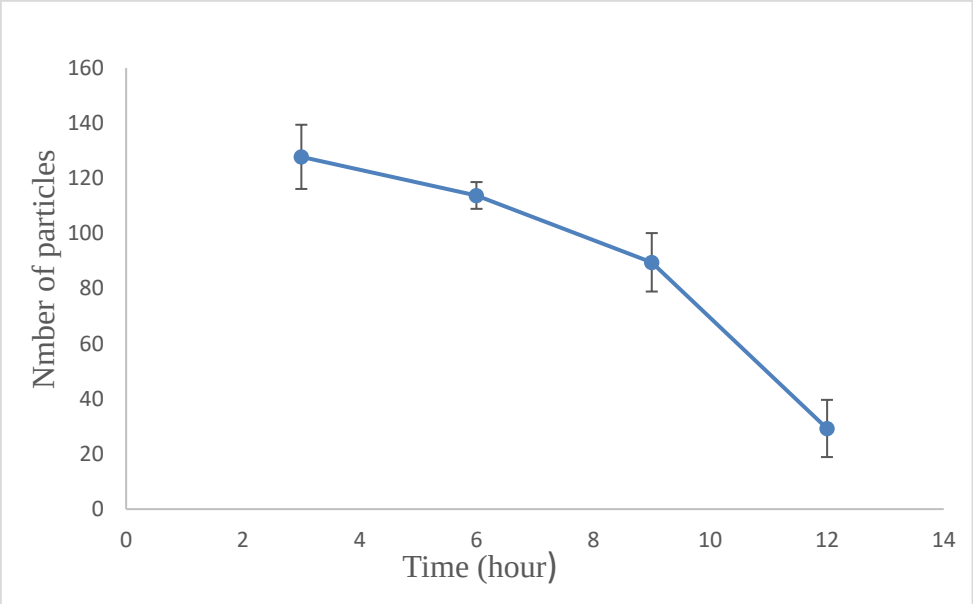

Figure 3.26: 6ug/ml of non-sonicated copper nanoparticles in astrocyte media degrading over 12 hours. The number of copper nanoparticles are decreasing over time. Error bar represents standard deviations.

From Figure 3.21 to 3.22, 6ug/ml of copper nanoparticles were plated into the sterilized water. Figure 3.22 shows all nanoparticles in the plate were agglomerated into a big clump. Results shows nanoparticles in the sterilized water wouldn't degrade or evenly disperse. Figures 3.23 and 3.24 show 6ug/ml of copper nanoparticles were plated into the deionized water, which didn't change their morphology over 24 hours. Copper nanoparticles have high stability in the deionized water and do not move in the deionized water. In the deionized water, the zeta potential of copper nanoparticles does not change, and the particles stay in the same location over time.

3.5 CuHARS (Copper high-aspect ratio structure) in Astrocyte Cell Culture Media

Figures 3.27 to 3.28 show 4ug/ml CuHARS (Copper high-aspect ratio structure) tested in the astrocyte cell culture media for 12 days at a room temperature of 22ºC.

Figure 3.27 to 3.28 shows that there are some CuHARS are degraded partially over time. Figure 3.28 shows there are still some of the CuHARS that stayed in the well and didn't degrade. Figures 3.29 and 3.30 show the degradation rate of CuHARS in the astrocyte cell culture media.

Figure 3.27: 4ug/ml of CuHARS (Copper high-aspect ratio structure) tested in the astrocyte cell culture media at room temperature 22ºC. The picture was taken on day 1. 10x magnification.

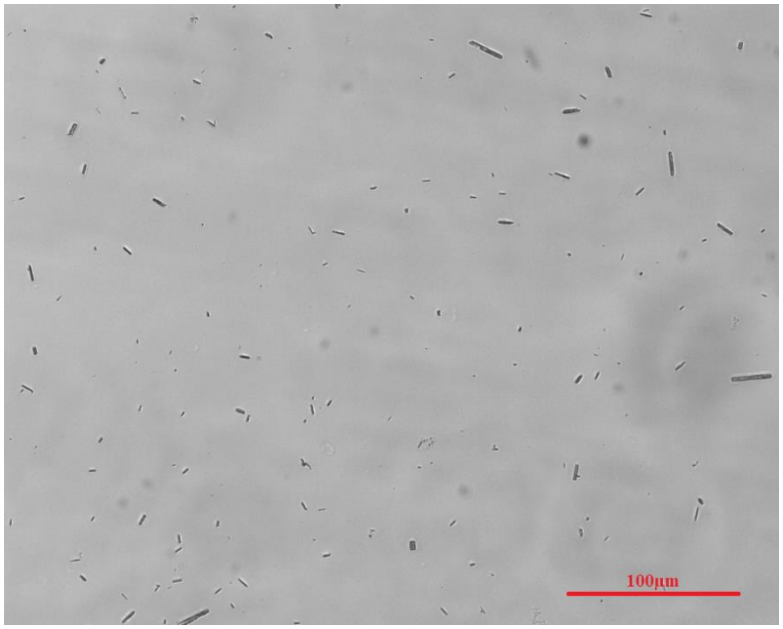

Figure 3.28: 4ug/ml of CuHARS (Copper high-aspect ratio structure) tested in the astrocyte cell culture media at room temperature of 22ºC. The picture was taken on day 12. 10x magnification.

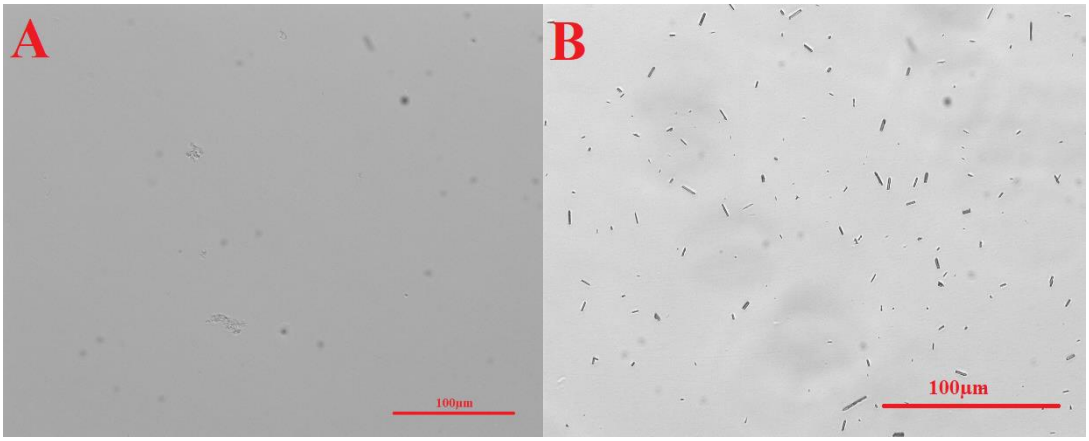

Figure 3.29: Figure A shown 6ug/ml of Copper nanoparticles completely degraded within 12 hours in the astrocyte media. Figure B shown 4ug/ml of CuHARS (Copper high-aspect ratio structure) hasn't completely degraded in 5 days in the astrocyte media. 10x magnification.

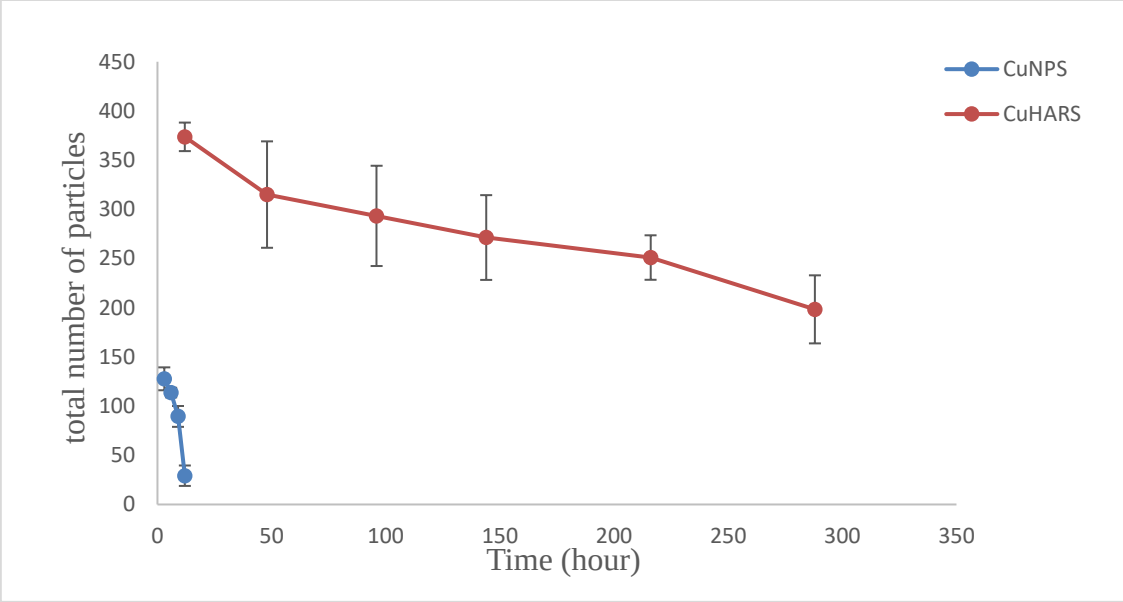

Figure 3.30: Degradation of 4ug/ml of CuHARS in the astrocyte cell culture media over 12days, showing decreasing number of CuHARS particles over 12 days. Error bar represents standard deviations.

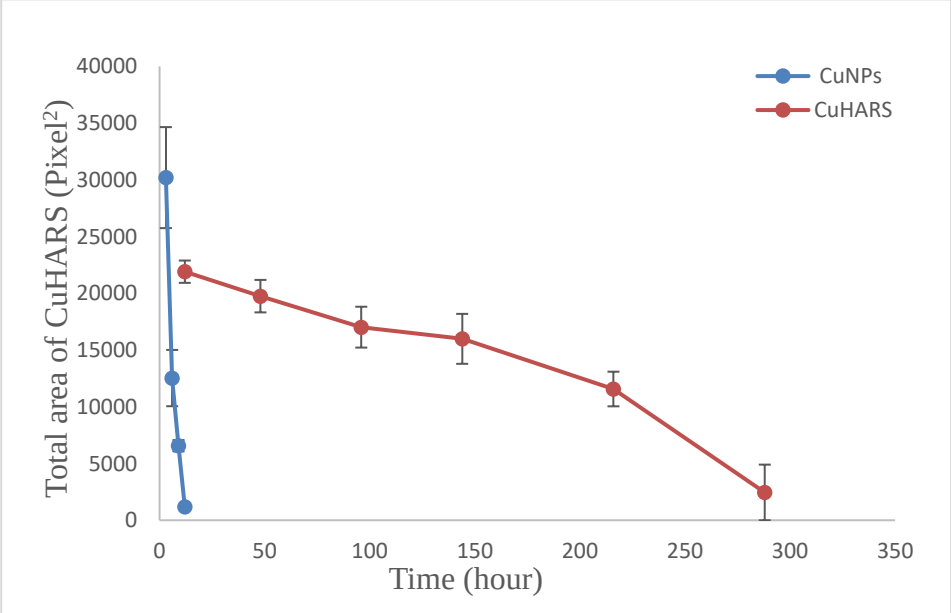

Figure 3.31: Degradation of 4ug/ml of CuHARS in the astrocyte cell culture media over 12days, showing decreasing the CuHARS' surface area over 12 days. Error bar represents standard deviations.

3.6 Combination of CuHARS and CuNPs in The CRL 2303 Cell Culture Media

Figures 3.31 to 3.34 show combination of sonicated CuHARS and copper nanoparticles in the CRL 2303 cell culture media at the same well. 5ug/ml of copper nanoparticles were sonicated for 15mins, and 10ug/ml of CuHARS were sonicated for 25 mins. Figures 3.33 and 3.34 show degradation of combination of CuHARS and copper nanoparticles.

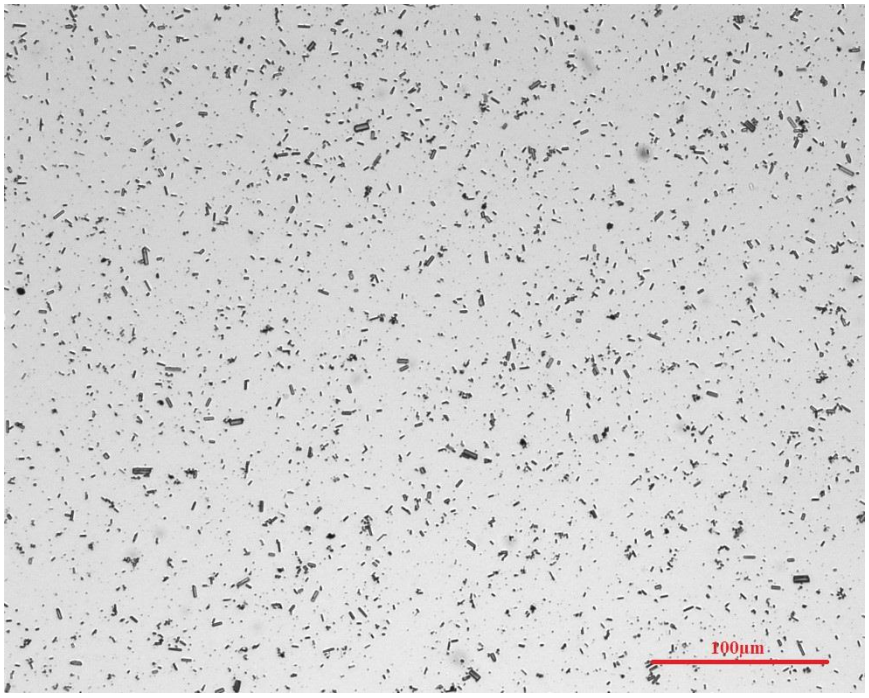

Figure 3.32: Combination of sonicated 10ug/ml of CuHARS and 5ug/ml of copper nanoparticles in CRL 2303 cell culture media at same well in the incubator (37ºC). The picture was taken 3hours after particles plated into the well. 10x magnification.

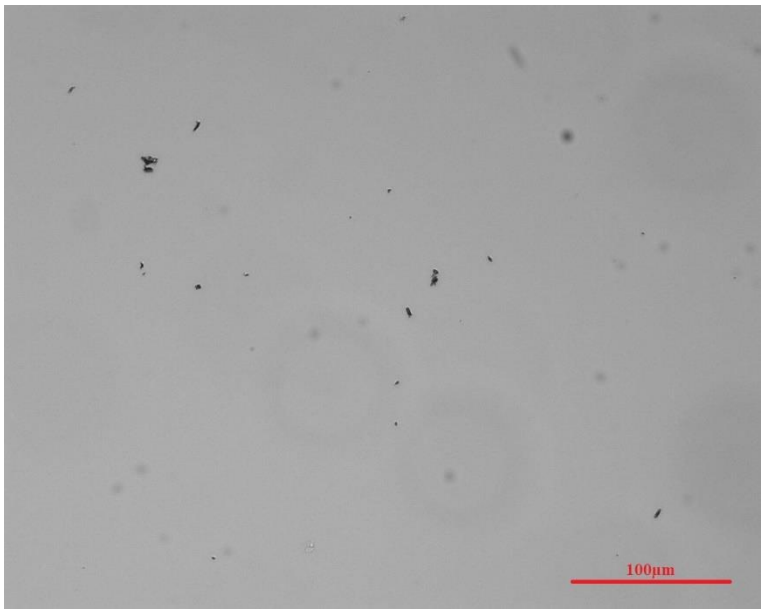

Figure 3.33: Combination of sonicated 10ug/ml of CuHARS and 5ug/ml of copper nanoparticles in CRL 2303 cell culture at same well in the incubator (37ºC). The picture was taken 7 days after particles were plated into the well. 10x magnification.

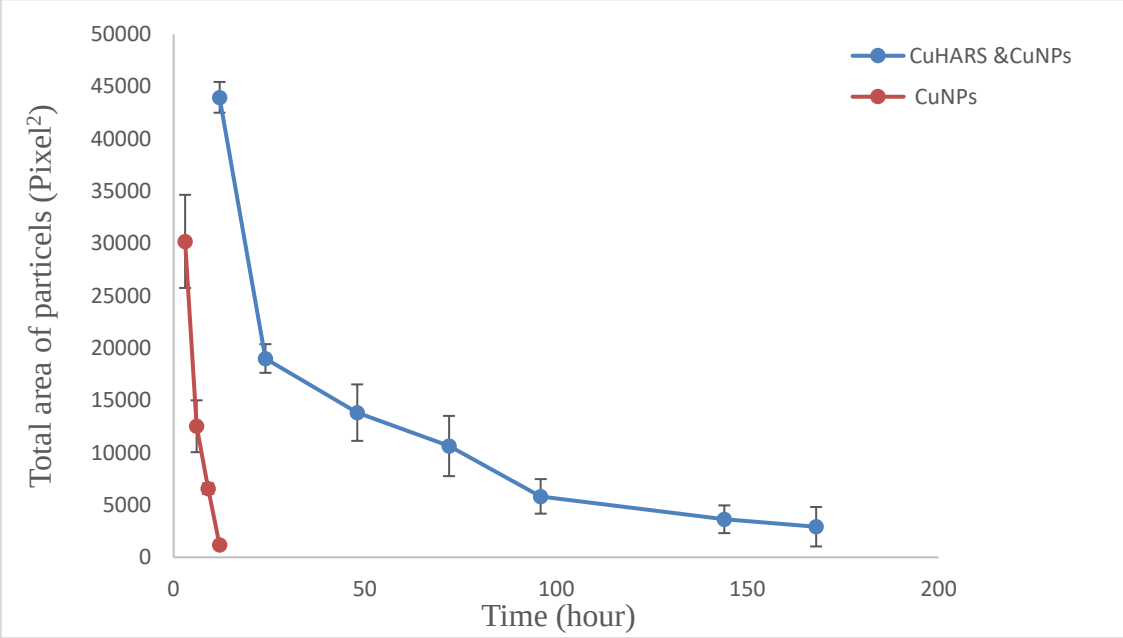

Figure 3.34: Degradation for combination of CuHARS and copper nanoparticles. Decreasing surface area of total 10ug/ml CuHARS and 5ug/ml CuNPs in same well. Error bar represents standard deviations.

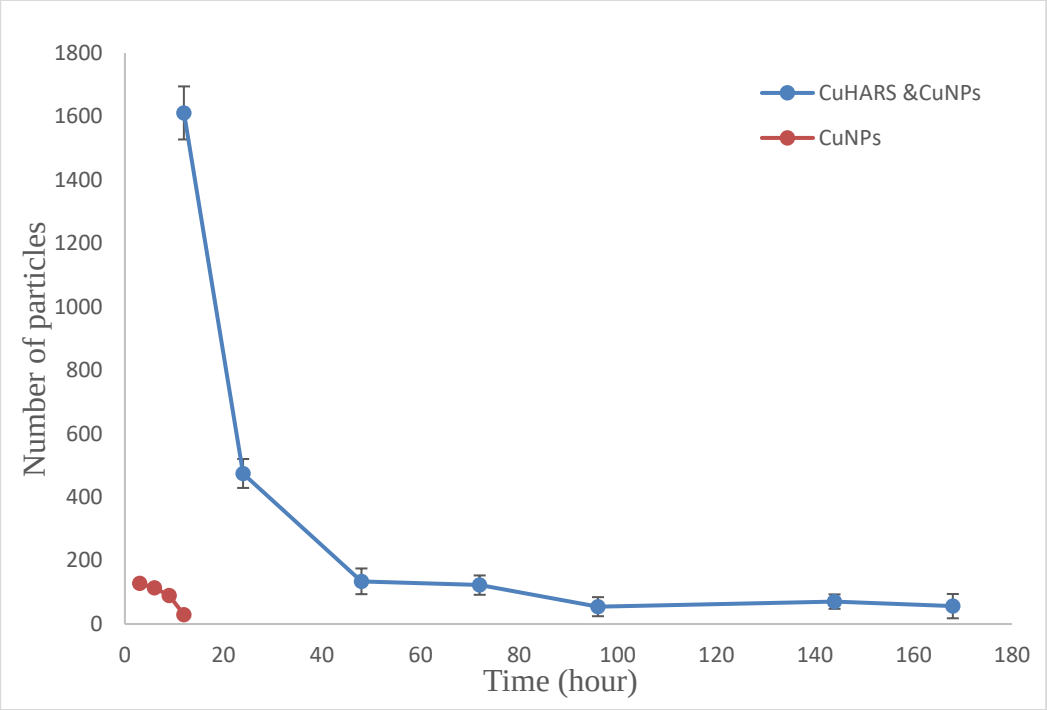

Figure 3.35: Degradation for combination of CuHARS and copper nanoparticles. Decreasing total number of 10ug/ml CuHARS and 5ug/ml CuNPs particles in the same well. Error bar represents standard deviations.

Figures 3.33 and 3.34 show sonicated 10ug/ml CuHARS and 5ug/ml CuNPs were degraded over 7 days in the CRL 2303 cell culture media in the incubator (37ºC). The number of particles decreased by 98% over 7 days. The total surface area of particles decreased by 94% over 7 days.

3.7 Copper Nanoparticles in The Living cell environment

Copper nanoparticles were treated with astrocyte cells and glioma rat cancer cell to testify how toxicity of Copper nanoparticles affect the cells' normal growth rate. Figures 3.36 to 3.37 show how copper nanoparticles affect the cells' morphologies.

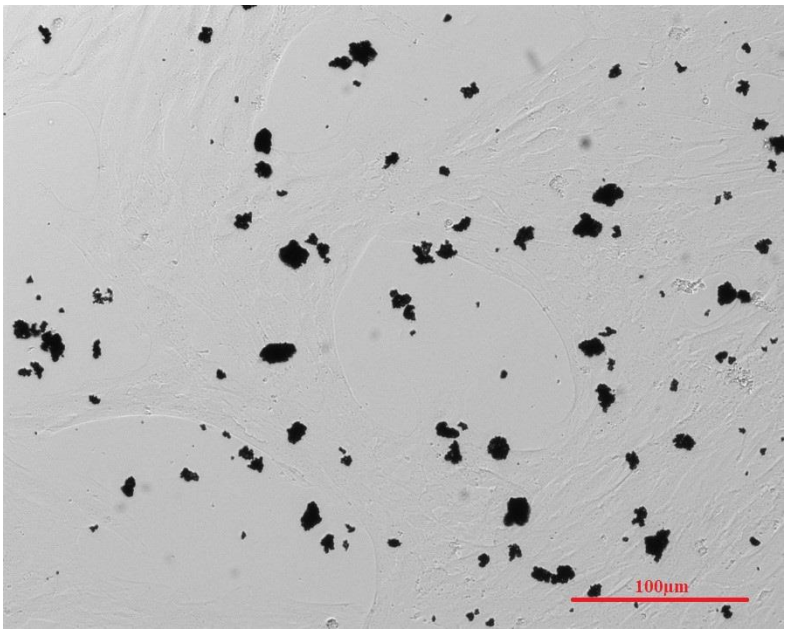

Figure 3.36: 5ug/ml of CuNPs are treated with 10,000 astrocyte cells in the incubator (37ºC). The picture was taken after 20 minutes cells were treated with CuNPs. 10x magnification.

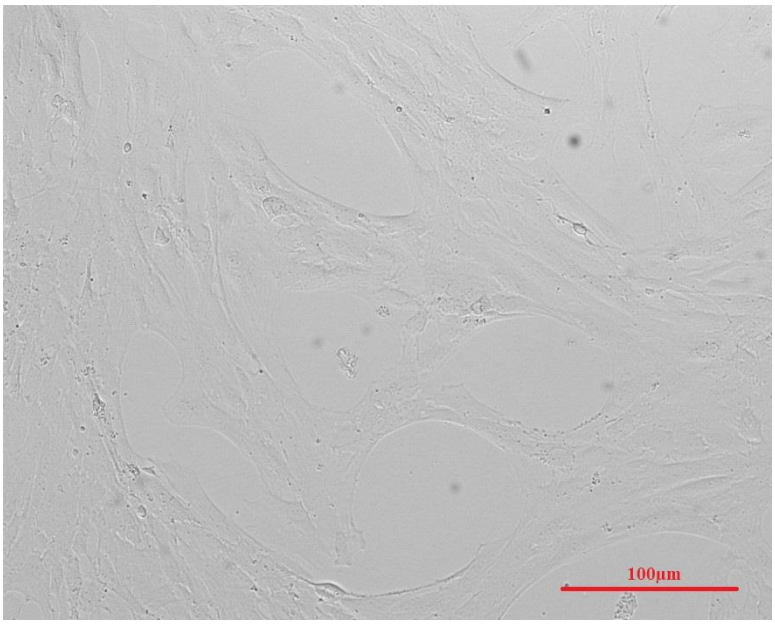

Figure 3.37: 10,000 astrocyte cells without CuNPs in the incubator (37ºC). Picture is a control to compare with CuNPs treated with the cells. The picture is taken 20 mins after other wells were treated with CuNPs.

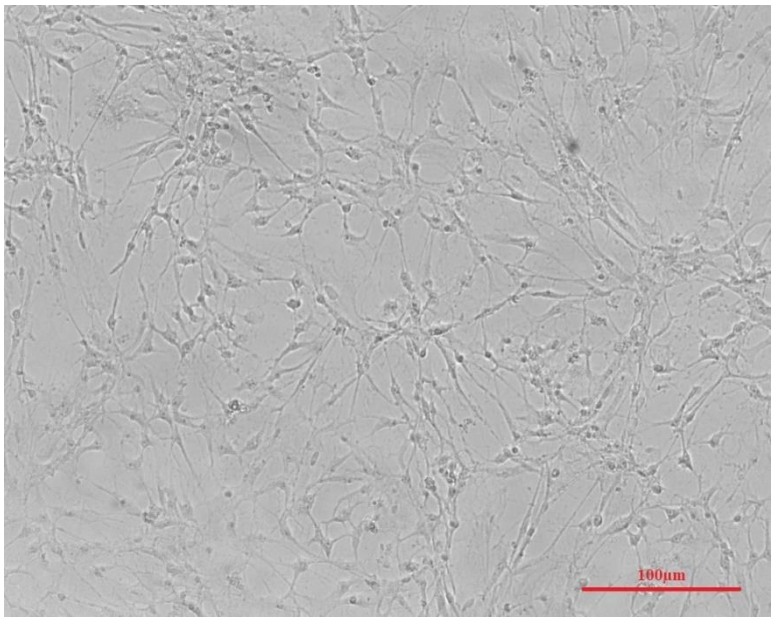

Figure 3.38: 5ug/ml of CuNPs are treated with 10,000 astrocyte cells in the incubator (37ºC). The picture was taken 2 days after cells were treated with CuNPs.

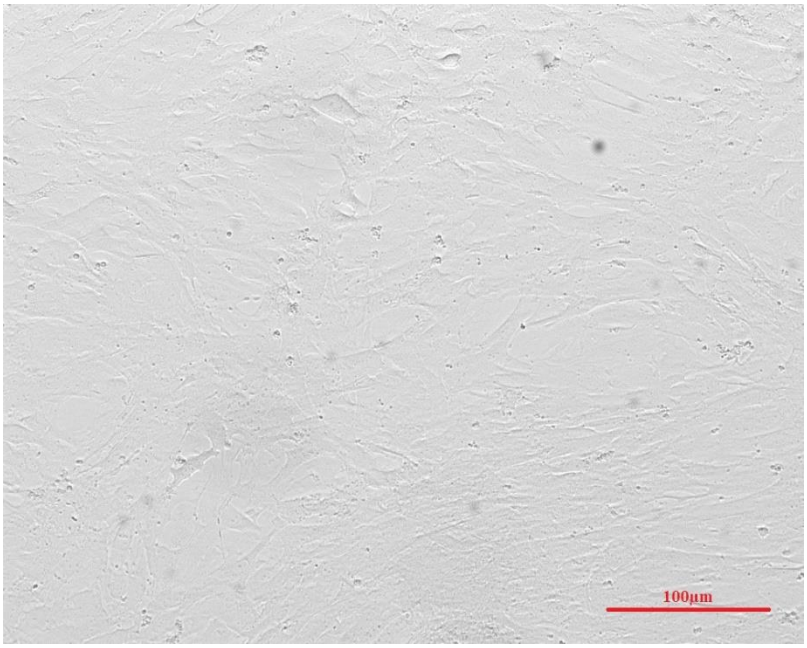

Figure 3.39: Control cells without CuNPs. The picture was taken 2days after other cells were treated with CuNPs. 10x magnification.

Figures 3.35 and 3.37 show 10,000 astrocyte cells treated with concentration of 5ug/ml of CuNPs to determine whether CuNPs are harmful to astrocyte cells and change their morphologies. These two figures show CuNPs completely disappeared within 2 days. All CuNPs are degraded, but astrocyte cells have changed their morphologies. Cells in Figure 3.37 are very stressed and shrunk. Figure 3.36 and 3.38 show cells are normally growing, and nothing has changed. The result shows CuNPs are impacted astrocyte cells.

Figures 3.39 to Figure 3.42 show 5000 glioma rat cancer cells treated with concentration of 5ug/ml CuNPs.

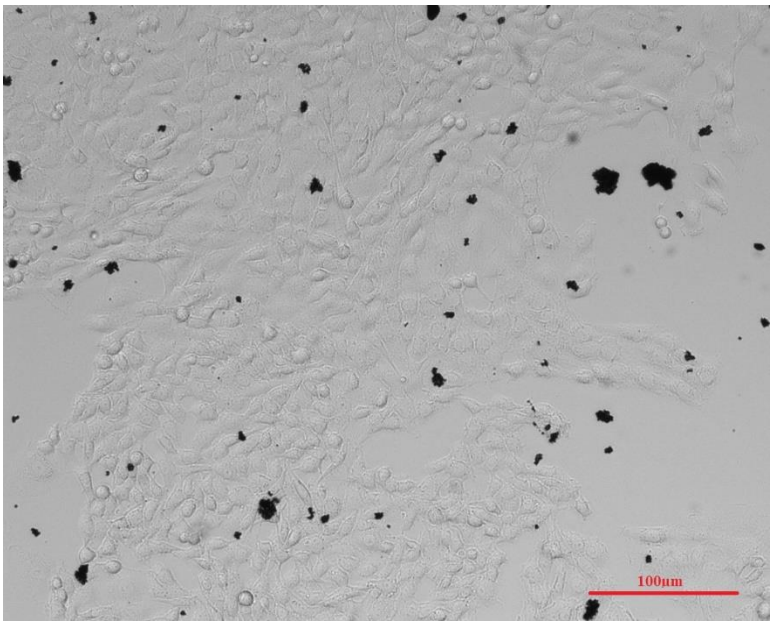

Figure 3.40: 5000 glioma rat cancer cells treated with 5ug/ml of CuNPs in the incubator (37ºC). The picture was taken 2 hours after glioma cells were treated with CuNPs. 10x magnification.

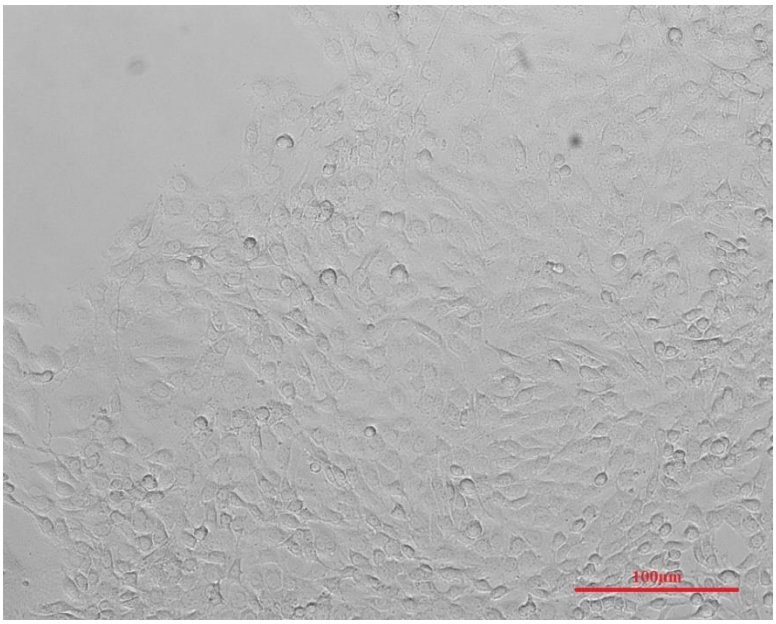

Figure 3.41: Control cells without CuNPs. The picture was taken 2 hours after other cells treated with CuNPs. 10x magnification.

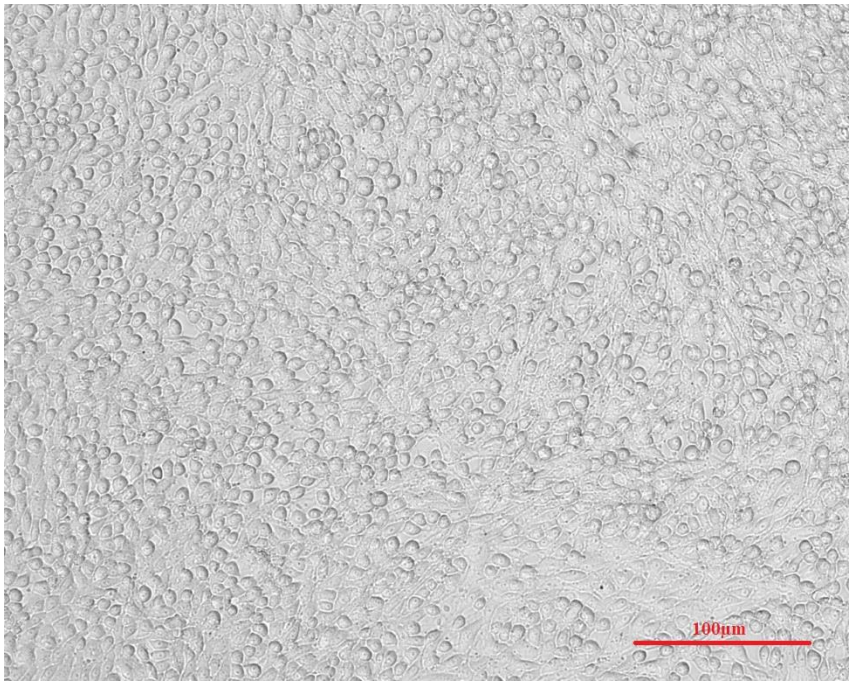

Figure 3.42: 5000 glioma rat cancer cells treated with 5ug/ml of CuNPs. The picture was taken 3days after treated with CuNPs. 10x magnification.

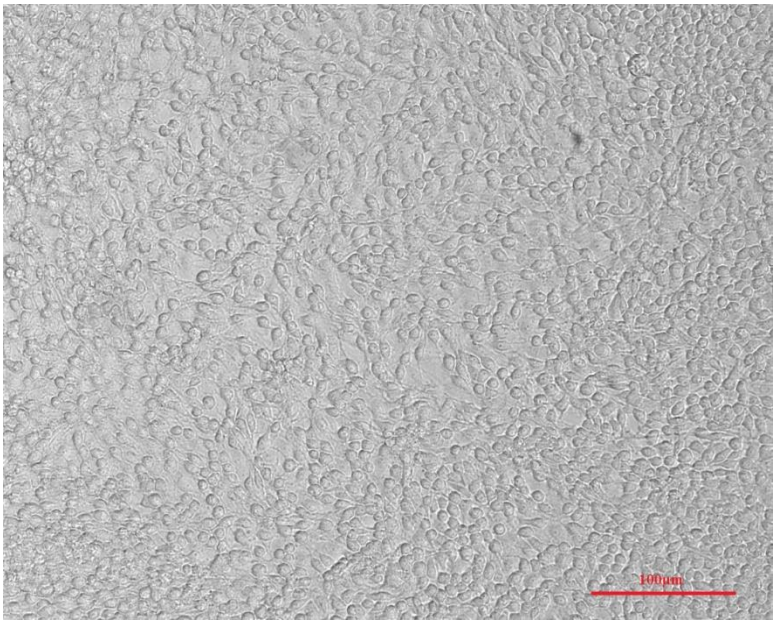

Figure 3.43: Control cells (glioma cells) without CuNPs. The picture was taken 3 days after other cells were treated with CuNPs. 10x magnification.

Figures 3.39 and 3.41 show 5000 CRL 2303 glioma rat cancer cells were treated with 5ug/ml non-sonicated CuNPs in the incubator (37ºC) for 3 days. The result show in Figure 3.41 that CuNPs in the cells were completely degraded. Also, cells have changed their morphologies, they have grown fast, they are stressed, and cells' volume is enlarged. The control cells kept growing and nothing major changed.

3.8 Zinc Nanoparticles in Different Biological and Physical Environments

Micro sized Zinc-cystine microparticles were tested in the sterilized water and astrocyte cell culture media in the incubator (37ºC) to determine their mobility and activity. Figures 3.43 to 3.46 show activities of Zinc-cystine microparticles in the sterilized water and astrocyte cell media in the incubator (37 ºC).

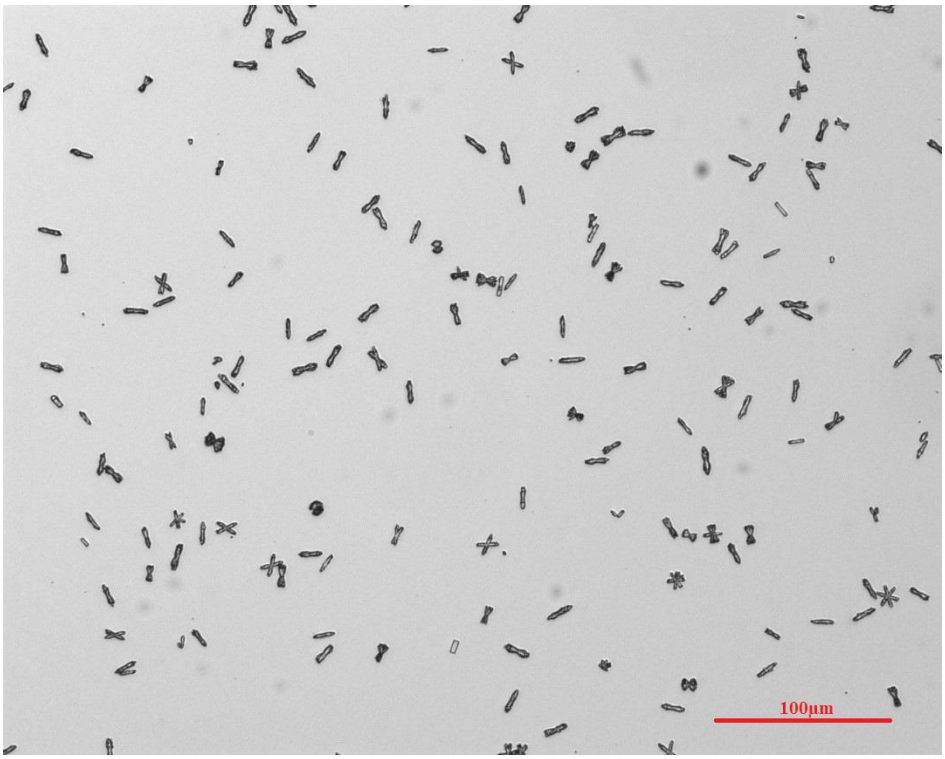

Figure 3.44: 0.2 mM of Zinc-cystine microparticles in sterilized water in the incubator (37 ºC). The picture was taken 3 hours after microparticles were added into the plate. 10x magnification.

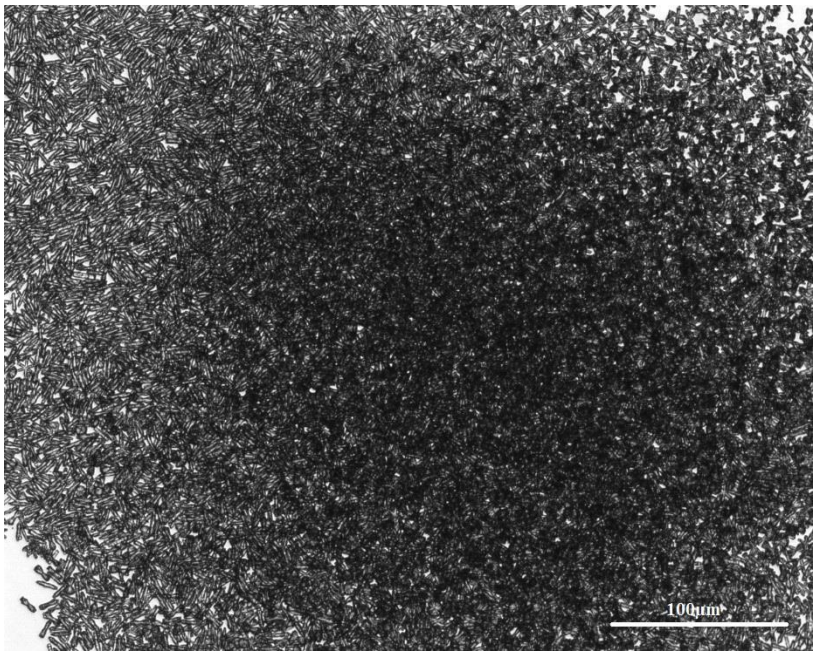

Figure 3.45: 0.2 mM of Zinc-cystine microparticles in the sterilized water at incubator (37 ºC). The picture was taken 5 days after microparticles were added into the plate. 10x magnification.

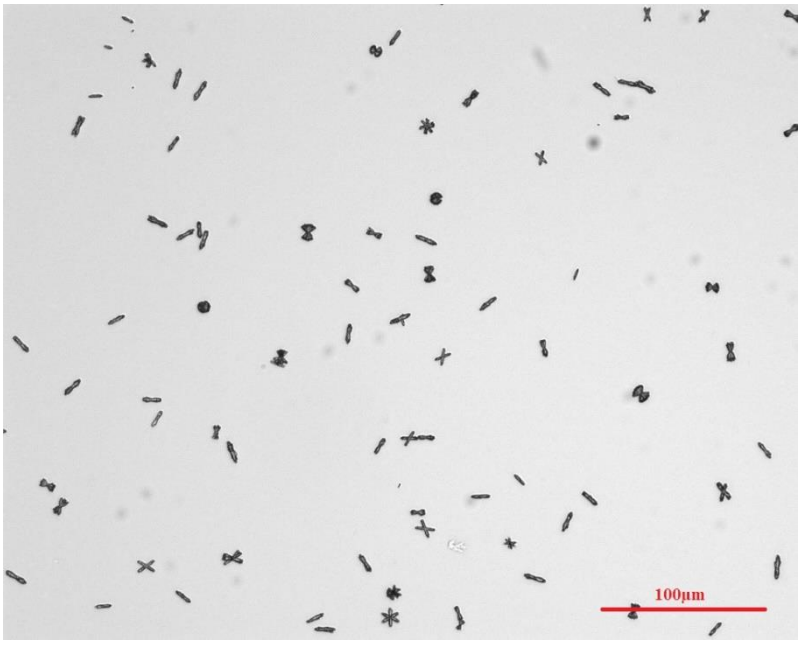

Figure 3.46: 0.2 mM of Zinc-cystine microparticles in the astrocyte cell culture media in the incubator (37 ºC). The picture was taken 3 hours after microparticles were added into the plate. 10x magnification.

Figure 3.47: 0.2 mM of Zinc-cystine microparticles in the astrocyte cell culture media in the incubator (37 ºC). The picture was taken 5 days after microparticles were added into the plate.

Figures 3.43 to 3.46 show a concentration of 2mM Zinc-cystine microparticles tested in the sterilized water in the incubator (37ºC). Although Zinc-cystine microparticles were tested in different solutions, in both solutions Zinc-cystine microparticles aggregated slowly within 5 days. Due to different surface properties, particle distribution of Zinc-cystine microparticles directly affected the agglomeration behavior under different biological environments. When Zinc-cystine microparticles were added to the sterilized water and astrocyte cell culture media, microparticles were clumped very big that there are only two data points which can't provide aggregation plot graph. Table 3.1 shows all nanoparticles' activities in different biological environments. Table will be explained in discussion section.

Table 3.1: All nanoparticles' physical behaviors in different physiological environments

Particles	**Sterilized water**	**Deionized water**	**Astrocyte media**	**CRL 2303 media**	**Astrocyte cells**	**CRL 2303 Cells**
FeNPs	Aggregate	Stable	aggregate	____	Aggregate/not harmful to the cells	Aggregate/not harmful to the cells
CuNPs	Aggregate	Break down	Degrade	Degrade	Degrades/ harmful to the cells	Degrades/ not harmful to the cells
CuHARS	Stable	Stable	Degrade	Degrade	Degrades/ harmful to the cells	Degrades/ not harmful to the cells
ZnNPs	Aggregate	____	Aggregate	____	____	____

CHAPTER 4

DISCUSSION

In this study, different types of metal related nanoparticles were tested in different biological environments to investigate their morphology change, stability, mobility and activity. Due to nanomaterials having huge surface area per unit volume, they are biocompatible, stable, aggregating and degrading under different physiological conditions.

Iron nanoparticles were tested with sterilized water, deionized water, astrocyte cell culture media and DPBS1x solution. Only iron nanoparticles in deionized water did not change their morphology, they were stable. However, iron nanoparticles in sterilized water, astrocyte cell culture media, and DPBS1x solution formed chain-like aggregates. The surface charge or the zeta potential of iron nanoparticles has important implications on their suspension stability and mobility in water environment. Aquifer materials generally have universal negative surface charge in the neutral pH range. Hence, the iron nanoparticles with positive charge at pH lower than 8.3 are attractive to aquifer materials [11]. Sterilized water, astrocyte cell culture media, and DPBS1x solution have lower pH than iron nanoparticles, which can explain the apparent aggregation of iron nanoparticles and low mobility. When astrocyte cells and CRL 2303 cells were treated with sonicated iron nanoparticles, they didn’t change their morphologies, and the cells were growing

normally. Iron nanoparticles aggregated and attached to the cell membrane, which didn't harm the cells.

Copper nanoparticles were tested with sterilized water, deionized water, and astrocyte cell culture media. Copper nanoparticles in sterilized water exhibited highly aggregated morphology. Aggregated size is influenced by ionic strength (IS) and pH via charge regulation, whereby the effective repulsive surface charge of the copper nanoparticles is decreased through ionic shielding and surface de/protonation [12]. Surface characterization of copper nanoparticles control their surface charge and potential interactions with environmental components, which play critical roles in determining nanoparticles aggregation [3]. Copper nanoparticles in deionized water didn't aggregate due to deionized water having neutral pH, zeta potential of copper nanoparticles didn't change. However, copper nanoparticles in deionized water changed the color due to changing of their dielectric properties and size.

Morphology of copper nanoparticles is one of the important properties affecting their toxicity. Copper nanoparticles added to astrocyte cells and glioma rat cancer cells which affected the cells' growth, metabolism, and replication. Copper nanoparticles attached to the cell environment produce high amounts of reactive oxygen species (ROS). Numerous studies have considered ROS generation as the major cause of nanotoxicity [7]. The copper nanoparticles generated ROS, leading to lipid peroxidation, DNA damage, protein oxidation, and interaction with cell membrane via electrostatic interaction resulting in disruption of cell functions [4].

CuHASRS and CuNPs in astrocyte cell culture media degrade in different degrees. CuNPs degrade faster, so within 12 hours CuNPs completely disappeared.

However, CuHARS materials are largely non-aggregating in many aqueous conditions and are less toxic to cells than CuNPs. This could be benefit for CuHARS and CuNPs are biodegradable in certain cell culture media that contains fetal bovine or horse sera. likely copper binding proteins to be considered could include ceruloplasmin which us a serum ferroxidase that contains 95% of the copper found in plasma, and albumin, which also has copper binding properties [9].

Zinc-cystine microparticles were tested in sterilized water and astrocyte cell culture media, and different shape and size of Zinc-cystine microparticles aggregated over time. The charge released from Zinc-cystine microparticles and their electrophoretic mobility determined the aggregation of the nanoparticles.

CHAPTER 5

CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

Nano sized metal related nanoparticles have unique physical and chemical properties; fully understanding and predicting their environmental behaviors remains challenging [3]. The critical factors determining the mobility of CuNPs, CuHARS, FeNPs, and ZnNPs include their speciation, size, surface charge and physical and chemical condition of the environmental medium. CuNPs, FeNPs, and ZnNPs are aggregated in sterilized water. CuHARS and CuNPs are degraded in astrocyte and CRL 2303 cell culture media in different degrees. Due to deionized water having neutral pH, which nanoparticles didn't aggregate.

For toxicity evaluation, identifying whether the toxicity is due to charge release or interaction with cells, or both combinations is really challenging. CuNPs and CuHARS have negative impact on astrocyte cells and CRL 2303 glioma rat cancer cells. When astrocyte cells and CRL 2303 glioma rat cancer cells were treated with CuNPs and CuHARS, particles attached to the membrane. In the cell environment CuNPs and CuHARS produce ROS, which disturbs normal function of the cells, so eventually the cells will die. Also, astrocyte cells and CRL 2303 glioma rat cancer cells were treated with FeNPs. After FeNPs attached to the cells, the cells were normally growing. FeNPs

did not harm the cells.

5.2 Future Work

Nanotechnology has been considered as a key component of sustainable development. However, the promise of nanotechnology can only be achieved if the exposure and toxicity can be fully evaluated and properly managed [3]. Metal-based nanoparticles exhibit good cellular interactions with biomolecules within the cell and on cell surfaces. They can also be engineered by introducing selected biological moieties with specific binding activity to selected targets [4]. Due to most of the metal- related nanoparticles having toxicity by nature, they can be used for anti-bacterial, anti-infection, anti-viral drugs, and other therapy applications.

Metal- related nanoparticles have important roles in targeted drug delivery fields. In this technique, nanoparticles maybe attached to a selective drug delivery system that will help therapeutic efficacy, bio availability and biodistribution. However, in order for metal-related nanoparticles to continue to be used in the targeted drug delivery field, a multidisciplinary approach is required from both basic and clinical research backgrounds to achieve sustained innovation [13].

APPENDIX A

ASTROCYTE CULTURE MEDIA

For 250mL total media, use the following amounts:

- 12.5mL Horse serum (5.0%)
- 12.5mL Fetal Bovine Serum [FBS] (0.5%)
- 12.5mL Penicillin/Streptomycin [P/S] (0.5%)
- 223.75Ml Ham's F-12K media with L-Glutamine (89.5%)

In a sterile environment, add the component together in the following manner:

1. Add 100mL of Ham's F-12K media to sterile vacuum filtration unit.
2. Add horse serum, FBS, and P/S to vacuum filtration unit.
3. Add 123.5 mL of Ham's F-12K media to unit.
4. Place cap on unit, carefully turn on vacuum
5. Allow the liquid to pass through the filter, turn off vacuum before bubbles from
6. Once vacuum is off, remove cap and place screw on cap on the container
7. Label media including name and date made, store in refrigerator

APPENDIX B

CRL 2303 CULTURE MEDIA

For 250 mL total media, use the following amounts:

- 25 mL of Fetal Bovine Serum [FBS]
- 2.5 mL Amino acid Solution (1%)
- 221.5 mL Dulbecco's Modified Eagle's Medium [DMEM]
- 1.25 mL Penicillin/Streptomycin [P/S] solution (0.5%)

In sterile environment, add the component together in the following manner:

1. Add 110.25 mL DMEM to sterile filtration unit
2. Add Fetal Bovine Serum, Amino acid solution, and P/S to sterile filtration unit
3. Add 111 mL DMEM to sterile filtration
4. Place the lit over sterile filtration unit and connect it to vacuum nozzle
5. Carefully turn on the vacuum and allow the liquid to pass through the filter. Make sure to hold sterile filtration unit during this step so that unit dose not turn over
6. Twist top of the filtration unit off carefully. Screw sterile cap onto container of media
7. Label media including name and date made, store in refrigerator

APPENDIX C

IMAGE -J

1. Open image J

2. Go to file → Open→ Select the image

3. Convert the image to 8bit

4. Go to analyze and the set the scale distance for 1 pixel

5. Adjust the thresh hold of the image and set to white and black apply it

6. Go to analyze →analyze particles

7. Display results

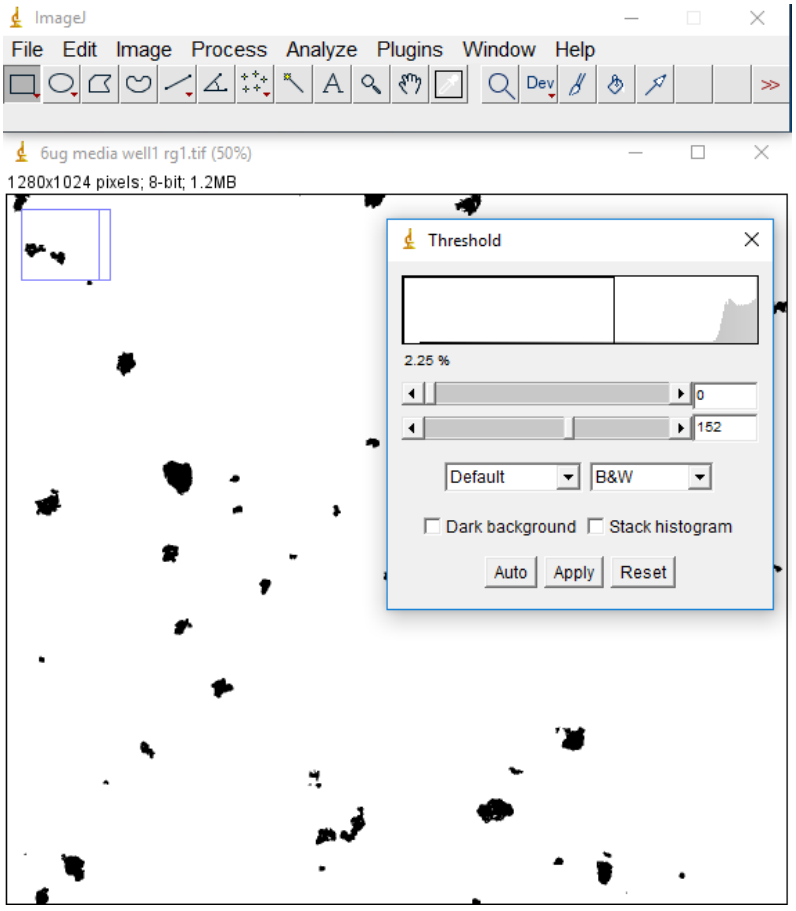

Figure C.1: An example of image analysis to justify thresh hold of the image.

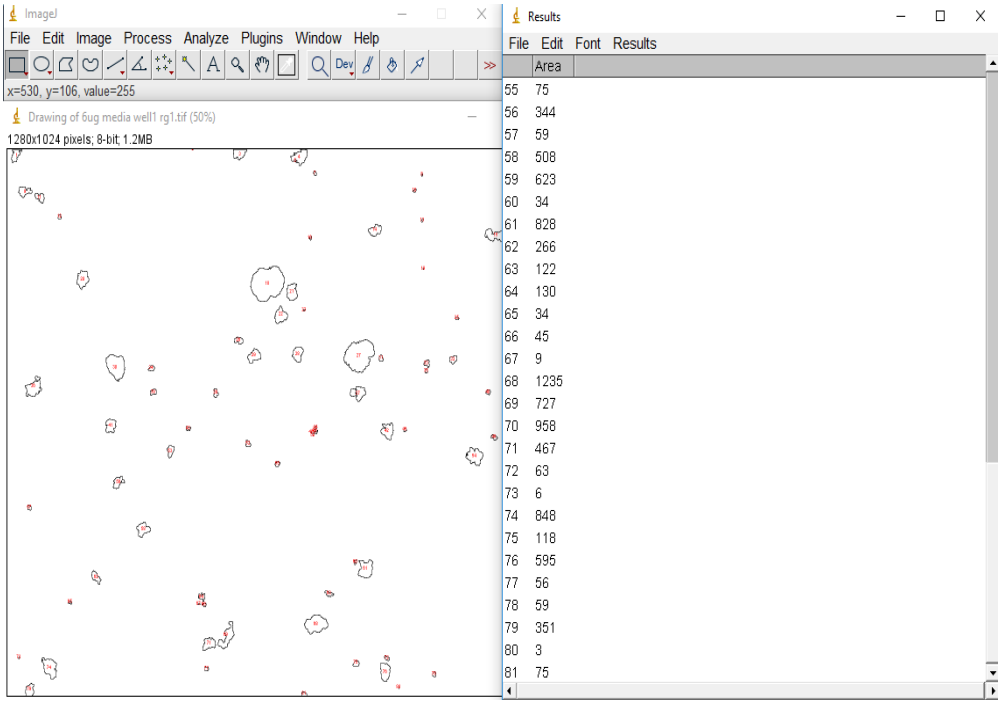

Figure C.2: An example of image analysis to calculate CuNPs' total surface area and the number of particles.

BIBLIOGRAPHY

[1] Nagranjan R. Nanoparticles: Syntheis, Stablization, Passivation, and Functionalization ACS Symposium Series. American Chemical Society: Washington, DC, 2008

[2] Luo YH, Chang LW, Lin P. review article Metal-Based Nanoparticles and the Immune System: Activation, Inflammation, and potenial applications. Hindawi Publishing Corporation Volume 2015 Article ID 1443720, 2015

[3] Chen H. Metal based nanoparticles in agriculture system: behaviour, transport, and interation with plants. Chemical Speciation & Bioavailability, 30:1 123-134, 2018

[4] Aderibigbe BA. review Metal-Based Nanoparticles for the Treatment of Infectious Disease. Molecules 22,1370, 2017

[5] PRABHU BM, ALI SF, MURDOCK RC, HUSSAIN SM, SRIVATSAN M. Copper nanoparticles exert size and concentraion dependent toxicity on somatosensory of rat. Nanotoxicology 4 (2): 150-160, 2010

[6] Raghupathi KR, Koodali RT, Manna AC. Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles. Langmuir 27, 4020-4028, 2011

[7] Sirelkhatim A, Mahmud S, Seeni A, Kaus NH, Mohamad HH. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Lett 7 (23):219-242, 2015

[8] Siddiqi KS, Rahman AU, Husen A. Properties of Zinc Oxide Nanoparticles and Their Acticity Against Microbes. Nanoscale Research Letters 13: 141, 2018

[9] Karan A, Darder M, Kansakar U, Norcross Z, DeCoster MA. Integration of a Copper-Containing Biohybrid (CuHARS) with Cellulose for Subsequent Degradation and Biomedical Control. International Journal of Environmental Research and Public Health 15 (5):844, 2018

[10] Kumar SS, Venkateswarlu P, Rao VR, Rao GN. Synthesis, characterization and optical properties of zinc oxide nanoparticles. International Nano Letters 3:30, 2013

[11] Conway JR, Adeleye AS, Torresdey JG, Keller AA. Aggregation, Dissolution, and Transformation of Copper Nanoparticles in Natural Waters. Environmental SciencE & Technology 49, 2749-2756, 2015

[12] Sun YP, Li XQ, Cao J, Zhang WX, Wang HP. Characterization of zero- valent iron nanoparticles. Advanced in Colloid and Interface Science 120: 47-56, 2006

[13] Kingsley JD, Gendelman HE, Destache CJ. Nanotechnology: A Focus on Nanoparticles as a Drug Delivery System. Neuroimmune Pharmacology 1: 340-350, 2006

[14] Salik G, Tenailleau C, Dufour P, Fritisch SG. Room temperature inorganic polycondensation of oxide (Cu_2O and ZnO) nanoparticles and thin films preparation by the dip-coating technique. Thin Solid Films 589: 872-876, 2015

[15] James T, Paul G, Bare DR, Pecher K. Characterization and Properties of Metalic Iron nanoparticles: Spectroscopy, Electrochemistry, and Kinetics. Environmental Science & Technology 39: 1221-1230, 2005

[16] Mott D, Galkowski J, Wang LY, Zhong CJ. Synthesis of size -Controlled and Shaped Copper Nanoparticles. Langnuir 23: 5740-5745, 2007

[17] Bodker F, Morup S, Linderoth S. Surface Effects in Metallic Iron Nanoparticles. Physical Review Letters 72 (2), 1993

[18] Morones JR, Elechiguerra JL, Camacho A, Kouro JB. The bacterial effect of silver nanoparticles. Nanotechnology 16: 2346-2353, 2005

[19] Yang C, Yu P, Wang J, Ge W, Wong L. Synthesis and Characterization of Poly(vinylpyrrolidone)-Modified Zinc Oxide Nanoparticles. Chemistry of Material 12: 2268-2274, 2000

[20] Connolly M, Song L, Cruz MF, Vijer MG, Fernandez M, Navas JM. Species-specific toxicity of copper nanoparticles among mammalian and piscine cell lines. Nanotoxicology 8 (4): 383-393, 2014

[21] Roco MC. Nanoparticles and nanotechnology research. Nanoparticle Research1: 1-6, 1999

[22] Kenzaoui BH, Ceridono M, Urban P, Bogni A, Ponti J, Gioria S. The agglomeration state of nanoparticles can influence the mechanism of their cellular internalization. Nanobiotechnology 15: 48, 2017

[23] Bantz C, Koshkina O, Lang T, Galla HJ, Kirkpatrick CJ, Stauber RH, Maskos M. The surface properties of nanoparticles determine the agglomeration state and the size of particles under physiological conditions. Nanotechnology 5: 1774-1786, 2014