T.C.
BIRUNI UNIVERSITY
INSTITUTE OF GRADUATE EDUCATION DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS MOLECULAR AND MEDICAL GENETICS GRADUATE PROGRAM

THE ANTI-PROLIFERATIVE EFFECT OF SERICIN-COATED SILVER NANOPARTICLES ON CANCER CELL LINES

Medine TAŞDEMİR

ADVISOR
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BİRUNİ ÜNİVERSİTESİ
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Biruni Üniversitesi Lisansüstü Eğitim Enstitüsü Moleküler ve Tıbbi Genetik (İngilizce) Anabilim Dalında **Medine TAŞDEMİR** tarafından hazırlanan "The Anti-Proliferative Effect of Sericin-Coated Sılver Nanoparticles on Cancer Cell Lines" adlı tez çalışması jüri tarafından YÜKSEK LİSANS tezi olarak kabul edilmiştir.

Tez Savunma
Tarihi: 02 /08/2021

Jüri Üyesinin Unvanı, Adı, Soyadı	Çalıştığı Kurum	İmza
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Biruni Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliği'nin ilgili maddeleri uyarınca bu tez jüri tarafından onaylanmış ve Lisansüstü Eğitim Enstitüsü Yönetim Kurulu kararıyla kabul edilmiştir.

Prof. Dr. Leman ŞENTURAN
Lisansüstü Eğitim Enstitüsü Müdürü

DECLARATION

I declare that I have designed and performed all experiments in the current study entitled “The Anti-Proliferative Effect of Sericin Coated Sİlver Nanoparticles on Cancer Cell Lines” according to good scientific practices. I obtained all the information contained in this thesis under academic and ethical rules. All the information I have used from secondary literature has been respectively referenced. I also declare that have not violated any patents and copyrights during the preparation and writing of this thesis.

Medine TAŞDEMİR

To my brother,
for his support and encouragement

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ABBREVIATIONS

AgNP:	Silver Nanoparticles
$AgNO_3$:	Silver Nitrate
Tnf-:	Tumour Necrosis Factor Alpha
IL-6:	Interleukin
VEGF:	Vascular Endothelial Growth Factor
PI3k:	Phosphatidylinositol 3-Kinase
Akt:	Protein Kinase B
LDH:	Lactate Dehydrogenase
HeLa:	Human Cervical Cell Line
Saos-2:	Human Osteosarcoma Cell Line
hFob:	Human Osteoblastic Cell Line
DMEM:	Dulbecco's Modified Eagle Medium
PBS :	Phosphate-Buffered Saline
FBS:	Fetal Bovine Serum
EDTA:	Ethylenediaminetetraacetic acid
MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
SDS:	Solubilization Solution

PFA: Paraformaldehyde

DAPI: 4′,6-Diamidino-2-phenylindole

DLS: Dynamic Light Scattering

TEM: Transmission Electron Microscopy

CT: Computed Tomography

MRI: Magnetic Resonance Imaging

PET: Positron Emission Tomography

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ÖZET

Gümüş nanopartiküllerin (AgNP) tanı, tedavi, ilaç salınımı ve tıbbi cihazlar, farmakoloji ve biyoteknoloji dahil pekçok biyomedikal uygulamada geniş kullanım alanına sahip olması diğer nanomateryaller arasında onu en dikkat çekici kılmaktadır. Tıp alanında gümüş nanopartiküllerin temel kullanımı tanı ve terapötik uygulamaları içermektedir. Çoğu terapötik uygulama bu partiküllerin antikanser özelliğini araştırmaya yöneliktir. Bu çalışmada, Bombyx mori ipek kozasından ekstrakte edilen serisin ile kaplanmış gümüş nanopartiküllerin HeLa cervikal karsinoma, Saos-2 osteosarkoma and hFOB osteoblastik hücre hattıüzerindeki sitotoksik etkilerini incelenmiştir. AgNP konsantrasyonları arttıkça hücreler üzerindeki anti-proliferatif etkininde arttığı gözlemlenmişitir. Saos-2ve hFOB hücrelerinin AgNP tedavisinden sonra ise 0.5 µg/mL'den 50 µg/mL'ye kadar doğru orantılı şekilde hücre canlılığında azalma görülmüştür. Saos-2 ve hFOB hücre hatları için IC50 değerleri sırasıyla 1.880 ve 1.522 µg/mL olarak belirlenmiştir. HeLa, Saos-2 ve hFOB hücre hatlarında 0.5- 8 µg/mL konsantrasyon aralığında AgNP etkilerine bakıldığında 2 µg/mL ve artan konsantrasyonlarda üç hücre hattında hücre canlılığında belirgin azalma görülmüştür. HeLa ve Saos-2 kanser hücre hatlarında 0.5-1 µg/mL AgNP konsantrasyonlarında anlamlı bir etki görülmez iken hFOB osteoblast hücrelerinde bu düşük dozlarda hücre canlılığını etkilemiştir. Yapılan incelemelerde HeLa, Saos-2 ve hFOB hücreleri üzerinde uygulanan farklı konsantrasyonlardaki AgNP lerin hücre sitoplazmasında birikime ve hücre hasarına neden olduğu gözlenmiştir. Bu çalışma, üç hücre tipini aktin boyama ile aktin hücre iskeletinin yapısal değişikliklerini gözlemlemiş ve sonuçlar, AgNPlerin HeLA, Saos-2 ve hFOB hücrelerinin aktin hücre iskeletinin yapısna zarar verdiğini göstermiştir.

Anahtar Kelimeler: Gümüş nanoparçacıklar, kanser, hücre canlılığı, aktin sioskeleton

ABSTRACT

The wide use of silver nanoparticles (AgNP) in many biomedical applications including diagnosis, treatment, drug release and medical devices, pharmacology and biotechnology makes it the most remarkable among other nanomaterials. The main use of silver nanoparticles in medicine includes diagnostic and therapeutic applications. Most therapeutic applications are directed towards investigating the anticancer property of these particles. In this study, the cytotoxic effects of sericin-coated silver nanoparticles extracted from Bombyx mori silk cocoon on HeLa cervical carcinoma, Saos-2 osteosarcoma and hFOB bone cell lines were investigated. It was observed that the anti-proliferative effect on cells increased as AgNP concentrations increased. After AgNP treatment of Saos-2 and hFOB cells, a direct proportional decrease in cell viability was observed from 0.5 µg/mL to 50 µg/mL. IC50 values for Saos-2 and hFOB cell lines were determined as 1.880 and 1.522 µg/mL, respectively. Considering the effects of AgNP in the concentration range of 0.5-8 µg/mL in HeLa, Saos-2 and hFOB cell lines, a significant decrease in cell viability was observed at 2 µg/mL and increasing concentrations in three cell lines. While no significant effect was observed at 0.5-1 µg/mL AgNP concentrations in HeLa and Saos-2 cancer cell lines, hFOB affected cell viability at these low doses in osteoblast cells. It has been observed that AgNPs at different concentrations applied on HeLa, Saos-2 and hFOB cells cause accumulation in the cell cytoplasm and cause cell damage. This study observed structural changes of the actin cytoskeleton by actin staining of three cell types, and the results showed that AgNPs damage the structure of the actin cytoskeleton of HeLa, Saos-2 and hFOB cells.

Keywords: Silver nanoparticles, cancer, cell viability, actin cytoskeleton

1. INTRODUCTION and PURPOSE

Nanotechnology is the most striking and developing field of materials science. Nanoparticles (NP) exhibit superior physical and biological properties compared to macro-sized materials thanks to their specific properties such as high surface area / volume ratio. In recent studies, interest in the synthesis of nanoparticles has increased significantly due to its practical applications in many fields such as memory storage devices, sensors, magnetic resonance imaging, drug delivery, catalysis, and the treatment of infectious diseases (Yaqoob, Adnan, Rameez Khan, & Rashid, 2020). Nanoparticles have an important effect in all areas of human life, especially medical science and biotechnology. Nanoparparticles of noble metals such as gold, silver, platinum and zinc oxide are widely used in medical and pharmaceutical applications as well as products that come into direct contact with the human body such as detergents, cosmetics and toothpaste (X. F. Zhang, Liu, Shen, & Gurunathan, 2016b).

Nanoparticles, known as particles with dimensions of 100 nm or less, form the basis of nanometer-sized materials and thus nanotechnology. Different physical and chemical methods are used for the synthesis of nanostructures in nanotechnology. Although the synthesis time and cost of nanoparticles are low with these methods, toxic chemicals are used in the synthesis stage of most of the obtained nanoparticles. Therefore, the nanoparticles obtained have toxic properties (Gao et al., 2016). On the other hand, the toxicity of these nanoparticles, which are used in many living spaces of people today, appears as a major disadvantage. Therefore, there is a need to develop environmentally friendly methods for the synthesis of nanoparticles without using toxic chemicals. Synthesis of nanoparticles using many different methods has been reported in the literature (Dilshad et al., 2020; Lee & Jun, 2019).

Cancer is the leading cause of death in developed countries, with an annual incidence rate of more than twelve million. Available cancer treatment options are restricted to chemotherapy, radiation, and surgery (Y. Zhang, Li, Gao, Chen, & Liu, 2019). Although traditional cancer treatment options have made significant progress in the past few years, cancer treatment is still far from expected because of the nonspecific distribution of anti-cancer drugs; Inadequate concentration of drugs

reaching the tumor site, unacceptable toxicities and increased multidrug resistance are the main problems. Therefore, there is an urgent demand for new innovative technologies that can improve the performance of existing cancer treatments. In recent years, the application of nanotechnology in cancer has attracted considerable attention in this context. Nanotechnology is a new multi-disciplinary approach that covers a wide range of research areas that go beyond engineering, biology, physics and chemistry. Cancer nanotechnology is an evolutionary approach with application in cancer treatment and diagnosis (Cryer & Thorley, 2019). It offers a new tool for early diagnosis and detection of cancer, predicting response to treatment, or developing personalized cancer treatments. At this point, as mentioned earlier, the unique properties of metal nanoparticles provide a better platform for preventing diseased or stressful situations. It shows that AgNPs in particular are used in biomedical applications as antibacterial, antioxidant, antiviral, anticancer, antiangiogenic and antiproliferative. Therefore, AgNPs are considered to be one of the essential key players in the field of science and technology.

In this thesis, we used sericin-coated silver nanoparticles extracted from Bombyx mori silk cocoon. We report our results regarding the effect of these sericin-coated Ag NPs, called protein-corona, on the viability of human cancer cells.

The aim of this study is to examine the concentration of sericin-coated silver nanoparticles and the cytotoxicity on different cell lines and to compare the changes it will cause and to reveal the changes in the cytoskeleton corresponding to these conditions. It is aimed that the results obtained will contribute to the elucidation of the mechanisms underlying the possible toxic effects of AgNP and provide useful information about the approaches that can be applied to prevent these effects.

2. GENERAL INFORMATION

2.1. Nanoparticles

Nanotechnology is a molecular-level multidisciplinary field that includes the of functional systems. It is a branch of applied science concerned with the synthesis, characterization, and application of nanoscale materials and devices. It has become an important area of modern research dealing with the synthesis, characterization, strategy, and manipulation of its structure, with nanoparticles ranging in size from 1 to 100 nm. All properties of individual atoms and molecules vary in this size range as a type of chemical, physical, and biological method (Anselmo & Mitragotri, 2015).

Nanoparticles can be synthesized from a variety of materials, most commonly metals, metal oxides, polymers, silicate, organics, non-oxide ceramics, carbon derivatives, and biomolecules (Figure 2.1). Spherical, cylindrical, cubic, tube, helix, triangle, rod nanoparticle can be found in a variety of morphologies, including. Nanoparticles are typically designed with surface modifications tailored to the needs of the specific applications for which they will be used. (Lee & Jun, 2019; Lopez Ruiz, Bartomeu Garcia, Navarro Gallon, & Webster, 2020; Pillai & Kamat, 2004). The wide chemical nature, shape and morphology of nanoparticles, the environment in which the particles are located, the dispersion state of the particles, and, most importantly, the numerous surface modifications that nanoparticles can make all play a significant role in making nanotechnology an important field of science today. Nanoparticles have a wide range of applications due to their properties, including energy, physics, chemistry, biology, biotechnology, medicine, industry, technology, and industry. (De Matteis, Cascione, Toma, & Leporatti, 2018).

Organic nanoparticles and inorganic nanoparticles containing carbon nanoparticles are the two main types of nanoparticles. Magnetic nanoparticles, noble metal nanoparticles (gold, silver, platinum, palladium, and so on), and semiconductor nanoparticles are examples of inorganic nanoparticles (such as titanium oxide and zinc oxide). Inorganic nanoparticles, particularly noble metal nanoparticles such as gold, platinum, palladium, and silver, are gaining popularity because they combine superior material properties with functional versatility (Benelli, 2016). The most important feature of metal nanoparticles is their high surface area/volume ratio, which allows

them to interact easily with other particles. The sizes of metal nanoparticles are very important for their use in biological systems (Khan, Saeed, & Khan, 2019). Many biological structures, such as genes (2 nm wide and 10-100 nm long), proteins (5-50 nm), and viruses, have nanoscale dimensions (20-450 nm). As a result, nanoparticles can interact at the molecular level or reach complex biosystem regions. Due to ts size and remarkable properties, its potential use in nanomedicine fields such as intra-body drug delivery, disease treatment and diagnosis, biosensors, medical imaging, tissue engineering, bio-electronics, biomolecular interactions, bioassays, biomedical devices, and bone regeneration gains importance (Azharuddin et al., 2019).

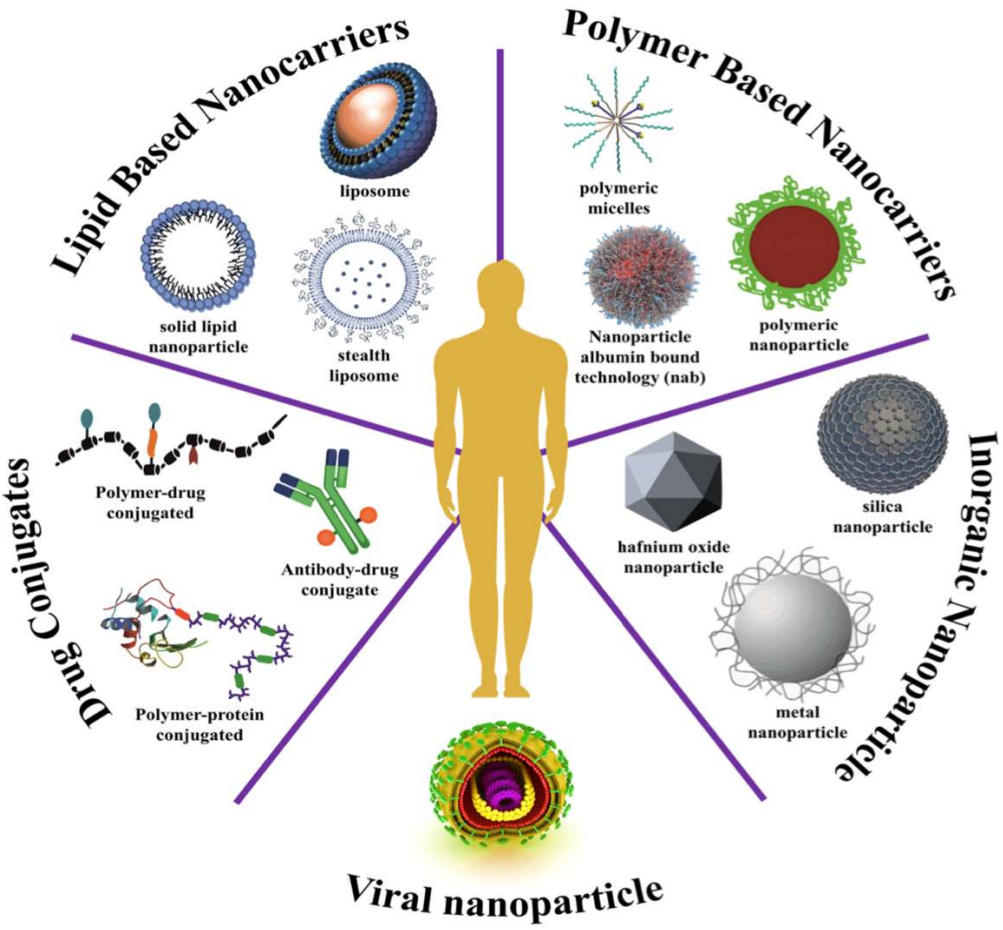

Figure 2.1: **A summary of defined nanomedicines for cancer treatment.** In clinical research, a wide range of platforms, including lipid-based, polymer-based, viral, drug-conjugated, and inorganic nanoparticles, are being investigated as nanocarriers.

2.2. Silver Nanoparticles

Nanoparticles (NPs) can be derived from natural sources, chemically synthesized products, or byproducts. Because of their high surface-to-volume ratio, antibacterial and antimicrobial properties, they find use in the medical field. Among the different nanomaterials available, AgNPs have piqued the interest of researchers due to their distinct physical and biochemical properties, as opposed to their macro and microstructure. (Xu, Piett, Farkas, Qazzaz, & Syed, 2013; Yaqoob et al., 2020). Silver is a safe antimicrobial agent with the ability to kill 650 different disease-causing organisms. AgNPs have been synthesized and shown to have antimicrobial properties. The small particle size of AgNPs causes DNA damage and cell death because their biofilm-producing organisms are resistant to antibacterial agents (Keshari, Srivastava, Singh, Yadav, & Nath, 2020; Valenzuela-Salas et al., 2019).

2.3. Application Areas of Silver Nanoparticles

Silver nanoparticles are one of the inorganic nanoparticles that attract interest in biomedical applications, scientific and technological research due to their easy synthesis, chemical stability, antbacterial and electronic structure (B. Li, Li, Wu, & Zhao, 2014). They are widely used as antibacterial agents in the health sector, food storage, textile coatings, and a variety of environmental applications due to these properties. As a result, AgNP has been used in a wide range of applications, from medical device and white goods disinfection to water purification. In addition to these, AgNPs are used in fabrics in the textile industry (Yetisen et al., 2016). Cotton fibers containing AgNP have been reported to have high antibacterial activity on Escherichia coli (Lopez Ruiz et al., 2020). In addition, it is seen as a candidate for use in biomedical applications for imaging (computed tomography (CT), photoacoustic imaging), transport (drug, gene, RNA), treatment (photothermal therapy) and diagnosis (biological and chemical detection). Cancer nanotechnology has evolved into an interdisciplinary field with numerous potential applications in cancer treatment, such as molecular imaging, molecular diagnosis, targeted therapy, and bioinformatics. (De Matteis et al., 2018). Cancer nanotechnology shows promise for one-to-one effect and

personalized oncological treatment based on the molecular profile of patients. AgNPs can be used together in diagnosis and treatment in cancer nanotechnology, with both targeted imaging and drug delivery (Saravanan et al., 2021).

2.4. Interactions of Nanoparticles with Biological Systems and Protein Corona

Nanoparticles and other nanomaterials are being researched for their potential use in biomedical applications such as imaging, drug transport, and hyperthermia. As a result, understanding how nanomaterials interact with biological systems is critical for safe and efficient applications (Benelli, 2016).

The physicochemical properties of nanoparticles have a greatly affected on nanoparticle-cell interactions. Features such as size, shape, surface chemistry, surface charge have a great effect on the binding of NP to the cell membrane and its absorption into the cell. The NP surface provides the driving forces (electrosatic, hydrophobic, hydrophilic) for cellular uptake and determines the uptake pathway (Borel & Sabliov, 2014). Positively charged NPs interact with the cell membrane more than neutral or negatively charged NPs. This situation occurs due to the negative charge of the cell membranes and positive electrostatic interactions.

In addition to these, NPs are covered by proteins that rapidly adsorb to their surface in a biological environment. This situation, which creates a new interface by changing the physicochemical properties of NP, is called "protein corona". At the same time, it is said that electrostatic interactions between NP and cell membranes occur with the effect of "protein corona". NP is encapsulated by protein macromolecules and can cause nonspecific interactions with cellular membranes (Park, 2020) .

The structure and composition of the protein corona are determined by the NP's physicochemical properties (size, shape, structure, functional groups on the surface, surface load), physiological environment properties (blood, interstitial fluid, cell cytoplasm), and exposure time (Nguyen & Lee, 2017). Protein corona consists of a "hard" corona, which consists of tightly bound proteins, adsorbed with high affinity, and a "soft" corona that is adsorbed with low affinity and can be easily separated from the surface (Figure 2.2).

While the corona adsorbed on the NP surface does not completely mask the surface, it can change the physiological response such as aggregation, cellular uptake, circulatory life, accumulation, and toxicity (Duran, Silveira, Duran, & Martinez, 2015).

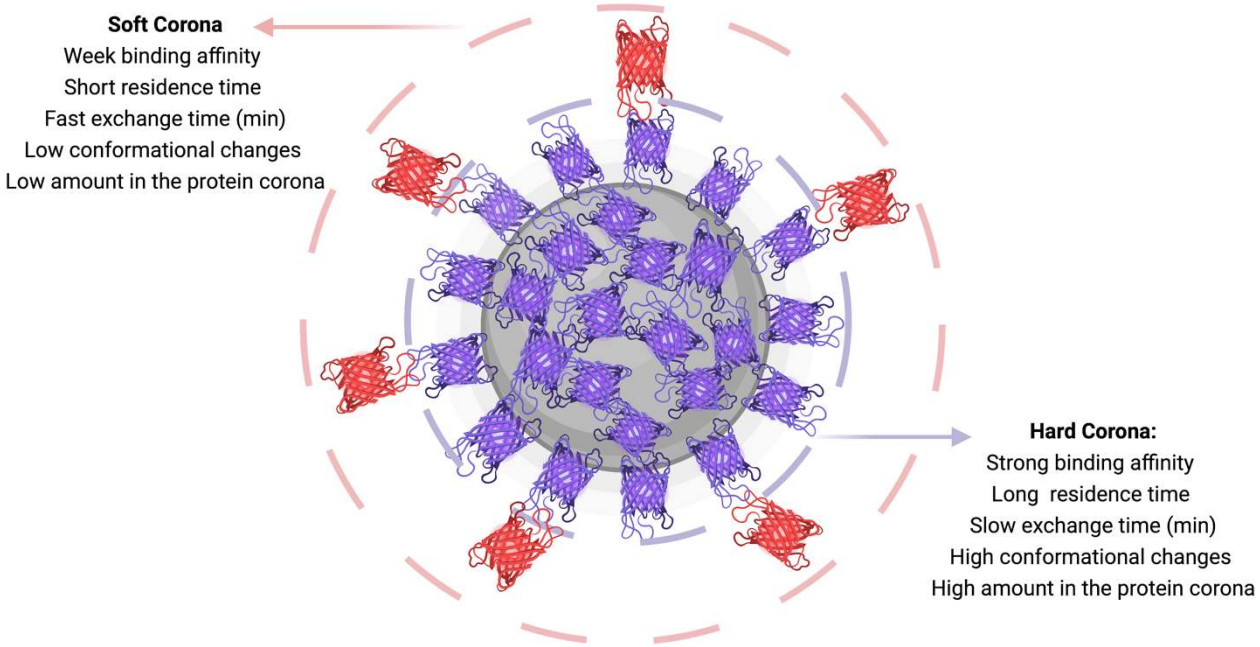

Figure 2.2: The properties of hard and soft coronas in nanoparticle–protein corona complexes.

2.5. Nanotechnology in Cancer Diagnosis and Therapy

Cancer is one of the maximum essential sicknesses of our age. Millions of people around the world get this disease every year and the treatment process is very likely to result in death (Baetke, Lammers, & Kiessling, 2015; Perez-Herrero & Fernandez-Medarde, 2015). Therefore, the most important criterion for the success of the cancer treatment process is early diagnosis. The next step after the early diagnosis of cancer is the implementation of a correct and effective treatment process (Wicki, Witzigmann, Balasubramanian, & Huwyler, 2015). Normal human cells divide and develop to form new cells as the body requires them. Cells die when they become old or damaged, and new cells form in their place. In cancer cells, the opposite is true. (Riaz Ahmed et al., 2017). In its most general definition, cancer is the uncontrolled and continuous proliferation of cells. Errors and mutations occurring at the gene level are the basis of uncontrolled reproduction here.

One of the most important reasons for these errors to occur is that genes such as p53, which are involved in cancer control, are blocked and errors in gene scanning cannot be corrected (Bieging & Attardi, 2012). The genetic changes that cause cancer can be inherited from our parents. However, the percentage (5-10%) of having cancer genetically in the family is quite low. The occurrence of gene errors during cell division is usually caused by damage to DNA as a result of exposure to environmental factors (carcinogenic and mutagenic agents, environmental pollution, dietary habits, alcohol, smoking, viruses, bacteria, UV rays, etc.)

Tumors are divided into 2 classes according to their presence in the body. Benign tumors (Bogart et al., 2014) are limited by their location and do not have any spreading hazards. However, they can impair their functions by putting pressure on the place or organ where they originate. Usually they do not cause death. They remain local and can sometimes shrink spontaneously. Malignant tumors (Chaffer & Weinberg, 2011) proliferate faster than benign tumors. They display an anarchic structure that does not recognize any rules in division and reproduction and they metastasize. Both tumor types are classified into three main groups: carcinomas, lymphomas, and sarcomas (Geller & Gorlick, 2010). Carcinomas are the type of tumor that consists of epithelial cells, and 80-90% of human cancers are carcinomas. Lymphomas are stimulated from immune system and blood-forming cells, which comprise 5-10% of total human malignancies. Finally, sarcomas are extremely rare in humans and are of solid tumor origins from connective tissue (Ferguson & Turner, 2018).

2.5.1. Cancer Diagnosis Methods

Early detection of cancerous cells is an important factor for the success of treatment (Aung, Campbell, & Mitchell, 2019). In addition, determining whether the tumor is malignant or benign is important in determining the treatment. However, identifying the cancerous area is still one of the biggest problems today. Detection of the mass formed by the accumulation of cancerous cells can be detected with the help of the physician's physical examination and Magnetic Resonance (MRI) techniques as a result of the tumor reaching a certain size (Huang et al., 2020).The methods used to diagnose cancer cells can be summarized as follows:

a) In detecting cancer by imaging method; The various imaging techniques used today are designed to find the best treatment option for each patient. Imaging techniques are frequently used in tandem to obtain sufficient data. Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET), which provide cross-sectional imaging by computer, are the most commonly used imaging methods for detecting cancer and monitoring its spread (Baetke et al., 2015).

b) Laboratory tests and cancer markers; It is performed at the point where the patient is suspected of having cancer. A blood sample is taken from the person to monitor their blood counts. The use of cancer markers in some cancers is useful for detection (Knowles & Wu, 2012).

c) Biopsy; It is the most widely used cancer diagnosis method today. In this method, tissue or cells are examined by a pathologist under a microscope (Aung et al., 2019).

d) Genetic testing; genetic research has attracted considerable attention in medicine in recent years. Genetic testing can be useful by linking some cancer cases at the gene level. However, genetic testing cannot detect cancer, but can identify some of the genetic defects that predispose to developing cancer so that a genetic susceptibility to cancer is detected. Recent studies suggest that cancer treatment may be more effective with individual treatment (Cryer & Thorley, 2019). Targeted treatments are available for many types of cancer that involve targeted drug delivery that affect certain genes or gene errors. For this reason, genetic tests may be beneficial before cancer treatment.

2.5.2. Cancer treatment methods

Treatment varies greatly depending on the type and stage of the cancer, as well as the patient's overall health. Surgery, radiotherapy, and chemotherapy are the most commonly used treatments. Targeted / biological therapies, hematopoietic stem cell transplantation, angiogenesis inhibitors, cryosurgery, and photodynamic therapy are some of the other treatments available.

a) Surgical Method: It is one of the most popular cancer treatment methods and is used to remove solid tumors. It is especially useful for early-stage benign tumors. Cancer

cannot always be completely removed surgically due to the risk of damaging vital organs or tissues. In this case, mass removal surgery is used to remove the tumor as safely as possible. However, organ loss, the risk of cancer recurrence and not being applied to all types of cancer are among the disadvantages of these methods (de Wall, Bauersachs, & Berliner, 2021).

b) Radiotherapy; It is a widely used cancer treatment. Approximately 50% of cancer patients receive radiation therapy before, during or after surgery and / or chemotherapy. In the radioelectric method, ionizing radiation is used to break cancer cells with X-rays, gamma rays or other high-energy particles. Cancer cells are killed by directly releasing reactive oxygen species that inhibit DNA damage or cell division. This method has disadvantages such as damage to healthy cells as well as cancerous cells, the radiation distribution not being in equal intensity to all cancer cells, and loss of function in the tissue exposed to radiation (Song, Cheng, Chao, Yang, & Liu, 2017).

c) Chemotherapy; Refers to more than 100 different drugs used to treat cancer. Chemotherapy aims to kill cancer cells with toxic drugs or to eliminate the mechanisms that allow cancer cells to divide. Classical chemotherapy drugs do not act towards the target cancer cell in the body. The drugs used affect healthy cells as well as cancerous cells. In addition, the doses of medication required for treatment cannot reach cancer cells. .Chemotherapy weakens the affected person's immune immune system and the patient becomes more susceptible to other diseases. Another trouble encountered is the MDR (Multi Drug Resistance) state of affairs that develops in opposition to anticancer compounds. The motive for those essential aspect results is that chemotherapy pills do now no longer display unique results on cancerous tissue. These situations are among the disadvantages of the chemotherapy method (Hanna & Mayden, 2021; Javidnia et al., 2014; Y. Zhang et al., 2019).

d) Targeted or biological treatments; It aims to cure cancer and strengthen the body's immune system while causing the least amount of harm to normal, healthy cells. Targeted or biological therapies include monoclonal antibodies, immunomodulating drugs, vaccines, and cytokines (Harada, Baba, & Ajani, 2018).

Despite the variety of cancer treatment methods, prominent problems are common. It is aimed to eliminate the limitations of traditional treatments with multi-functional, new generation nanostructures.

2.6. Potential Therapeutic Applications of AgNPs in Cancer

AgNPs frequently enter mammalian cells by clustering endocytosis and, due to their small size, they can also cross the blood-brain barrier. When they enter the cell within an endocytic vesicle, it is distributed via intracellular traffic to the cytoplasm and nucleus (De Matteis et al., 2018; Riaz Ahmed et al., 2017). Because of differences in their physicochemical properties, they can affect different cells through different cellular processes. In an attempt to assess their toxicity to human health, it was discovered that they were toxic to both normal and cancer cells. Many in vitro studies have been conducted to investigate the microenvironmental mechanisms by which AgNPs induce genotoxicity or cytotoxicity. In vitro anti-cancer properties have been studied against a variety of cancer cells, including human hepatoma cells, lung cancer, breast cancer, and cervical carcinoma. (Kovacs et al., 2016; Pillai & Kamat, 2004; Raja et al., 2020; Zhu et al., 2016).

They cause toxicity in cancer cells by decreasing mitochondrial function, producing reactive oxygen species (ROS) (Moloney & Cotter, 2018), secreting lactate dehydrogenase (LDH), deregulating the cell cycle, inducing apoptotic genes such as Bax, micronuclei formation, chromosome aberration, and DNA damage (Kovacs et al., 2020).

Some studies have revealed that AgNPs cause inflammation in the treated cell, shedding light on the interaction between AgNPs and the immune system. The inflammatory response begins with macrophages ingesting AgNP. These activated macrophages produce reactive oxygen species (ROS), TNF-, inflammatory cytokines, and interleukins (IL-6) (Parnsamut & Brimson, 2015). Aside from these cellular mechanisms, AgNPs were found to have anti-angiogenic and anti-proliferative properties. VEGF binds to its receptor on endothelial cells, causing angiogenesis and activation of the PI3K / Akt signaling pathway in normal tissue cells. Ag-NPs are anti-angiogenic because they inhibit the phosphorylation of Akt by PI3K (Rodriguez-

Razon et al., 2018; X. F. Zhang, Shen, & Gurunathan, 2016). Since the signal pathway cannot be completed, angiogenesis is eventually terminated, the cell is starved, deprived of oxygen, and the tumor cell is killed (Figure 2.3) (Chugh et al., 2018).

During metastasis, tumor cells undergo morphological and structural changes, and the actin cytoskeleton plays an important role in cancer progression by allowing tumor cell invasion and migration. A characteristic polarized morphology is observed first in migrating cells in response to various extracellular factors and signals (Zhao, Jiao, & Zhang, 2015). Filopodia (finger-like protrusions) and lamellipodia (smooth membrane protrusions) extend forward and activate actin assembly. Cell adhesion at the anterior end of the lamellipod then attaches the actin cytoskeleton to the extracellular matrix, resulting in cell migration (Al Absi et al., 2018). Importantly, actin activated by external stimuli induces the formation and reorganization of the actin cytoskeleton. Actin induced by AgNPs damages and instabilizes the cytoskeleton. (Tang & Gerlach, 2017; P. Zhang, Teng, & Wang, 2020). Cytoskeletal damage inhibits cell turnover and division by promoting the anti-proliferative activity of cancer cells.

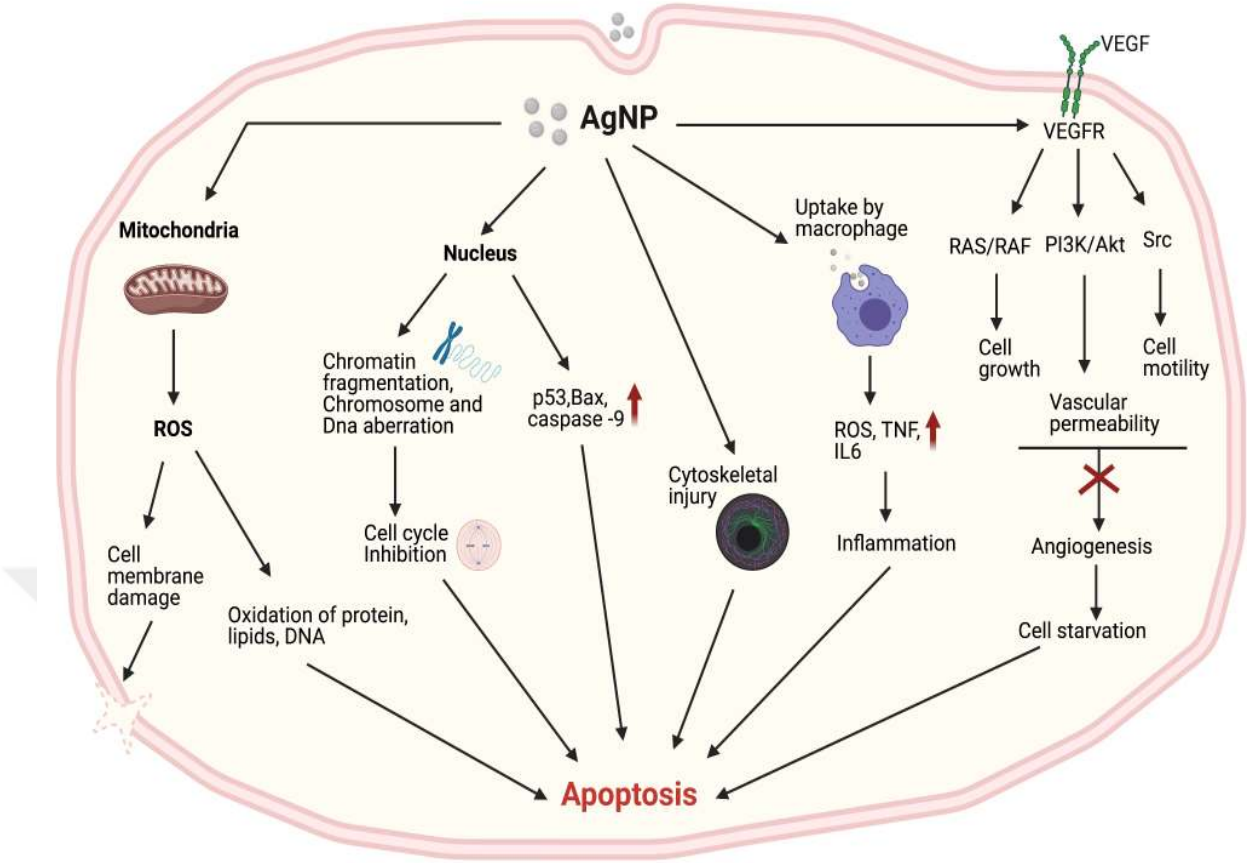

Figure 2.3: Schematic map of AgNP toxicity showing cellular processes affected by AgNPs**.** (ROS – reactive oxygen species, VEGF – Vascular endothelial growth factor, VEGFR – Vascular endothelial growth factor receptor, Src- Proto-oncogene tyrosine-protein kinase, TNF – Tumour necrosis factor, IL-6 – interleukin-6)

Creating a large amount of toxicological data on nanoparticles can sometimes lead to a negative perception of their use. Toxicity, on the other hand, can be beneficial for cancer treatments on its own. When AgNPs were included in cancer treatments, positive results were obtained. AgNPs not only interact with cells passively, but also actively mediate molecular processes that regulate cell functions.

3. MATERIALS AND METHODS

3.1. Cells Culture Conditions

Cervical carcinoma in humans ATTC provided HeLa, human osteosarcoma SaOS-2 cells, and fetal human osteoblastic hFOB cell lines (Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Waltham, USA) will be used to culture the cells, which will be supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, USA) and 1% penicillin and streptomycin. The cells were cultured in an incubator with 5% CO2 at 37 degrees Celsius, and the medium will be changed every three days, followed by passage when 80 percent of them will fuse. When 80 percent of the culture cells have fused, the cell culture medium will be removed and rinsed with Phosphate-buffered saline PBS, followed by 3 minutes of digestion with percent 0,125 Trypsin-EDTA and gentle pipetting to digest the adherent cells. To stop digestion, DMEM medium containing 10% FBS was added, followed by 5 minutes of centrifugation at 15,000 x rpm. The supernatant will then be removed, medium will be added for resuspension, and the cells will continue to be cultured (Figure 3.1). Finally, the cells will be gathered for research purposes.

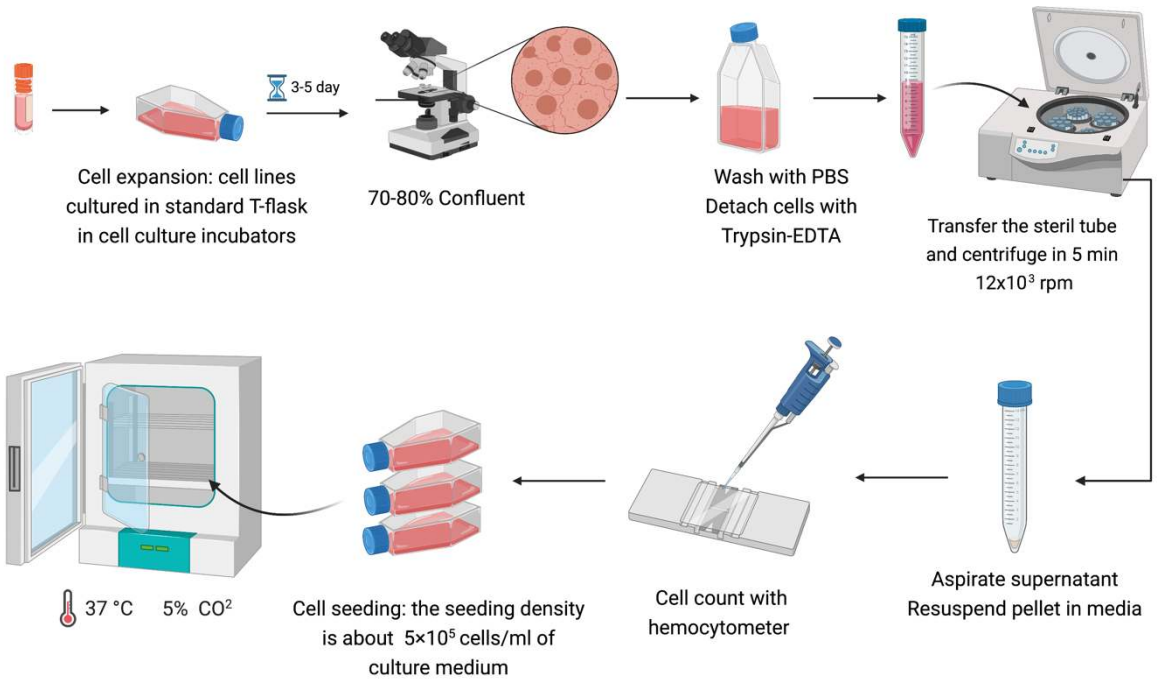

Figure 3.1: Schematic illustration of the cell culture method.

3.2. Synthesis and properties of Ag-NPs

A biofunctional nanoparticle synthesis pathway for biomedical applications has been designed and implemented. Sericin extracted from Bombyx mori silk cocoon was used to coated silver nanoparticles. In the presence of sericin, the silver ion is reduced by the particle formation of the sugar, thus forming stable silver nanoparticles and maintaining long-term functionality for at least 4 months. UV visible spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM), and gel electrophoresis were used to characterize the newly synthesized protein corona-based nanoparticles. Conjoined nanoparticles, spherical in nature, have a very narrow ($\sigma = \pm$ 5) size in dispersion and peak at 20 nm.

3.3. Detection of cell proliferation by MTT Assay

The cell viability was evaluated by [3–(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay. Cells were seeded in 96-well plates at a density of 2 × 104 cells/well. Unused wells in each plate were used as blanks or medium controls. Then, the cells will expose in the different concentrations of AgNP. The cells will culture in an incubator with 5% CO2 at 37℃ for 24 h. After 24h, 10 µL of MTT solution will add to each well and incubated for 4 h at 37 °C. Then, the medium will replace with 100 µL of the Solubilization solution (SDS) into each well to dissolve the precipitate formazan and incubate for another 24h. All the results will performe in triplicate, and all data will express as the mean ± SD. IC 50 values obtained with dose-response curves for cell lines (n =3, two independent experiments) were obtained by nonlinear regression analysis using GraphPad Prism 7.0 (Figure 3.2).

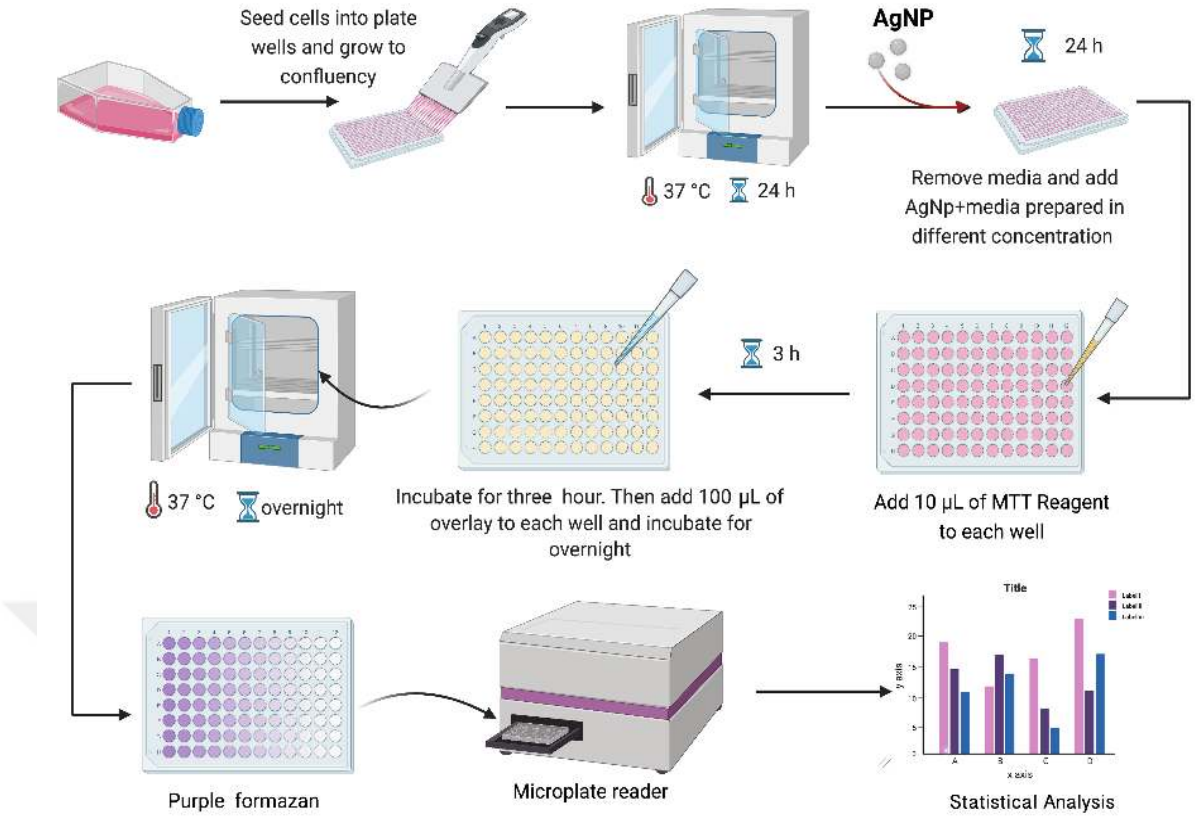

Figure 3.2: Schematic illustration of the MTT assay.

3.4. Immunofluorescence Analysis

Human cervical cell line HeLa, osteosarcoma cell line Saos-2, and human osteoblastic cell line hFOB were used in this study. Cells were grown at 37°C in a 5% CO 2 environment in culture media recommended by the provider, with 10% FBS added. For immunofluorescent experiments, 12-well glass bottom plates with 100,000 cells seeded per well were used. For 15 minutes, the cells were treated with freshly prepared ice-cold 4 percent paraformaldehyde (PFA). For 10 minutes, fixed cells were permeabilized in PBS with 0.1 percent Triton-X100. PBS was added three times for a total of five minutes per round. After staining the cells with ActinGreen (Invitrogen, R37110), they were incubated overnight at 4°C. After washing, stain with 4,6-diamidino-2-phenylindole (DAPI) and store at 4°C in the dark. Micrographs were taken with a Zeiss microscope and an Axiocam color charge-coupled device camera. Images were captured and processed on a computer using the Axiovisiontm software. For image processing, Adobe Photoshop (Adobe Systems) was used..

3.5. Statistical analysis

Measurements in each experiment will be repeated three times in triplicate independently. In the case of quantitative data, the results will be reported as the mean S.D. GrapPad software will be used to perform one-way analysis of variance (ANOVA) to determine the differences between groups (version 17.0). A P value of 0.05 or less is considered statistically significant.

4. RESULTS

4.1. Evaluation of Cell Lines Morphology

To test synthesized sericin coated silver nanoparticles we have selected three types of cell lines. Human cervical carcinoma HeLa are the oldest and most widely used cell line. Human osteosarcoma SaOS-2 cells and fetal human osteoblastic hFOB cell lines are widely used in bone research.These cell lines well charaterizated and excellent model organism to study nanoparticles in vitro. For the chracterization we first optimized the cell growth conditions. Cells cultivated in a flask were observed three days after the initiation of the cell culture. (Figure 4.1) We have shown that there are easily and rapidly growing cells that can reproduce themselves.

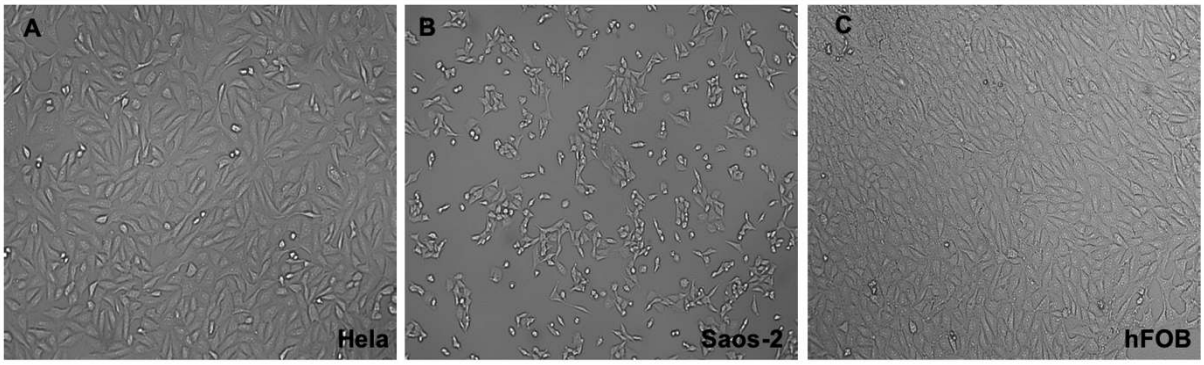

Figure 4.1: **Bright field images of model organism.** HeLa cervical carcinoma(A), Saos-2 osteosarcoma (B) and hFOB osteoblast (C) cell lines. Magnification = 20x

4.2. Targeting of Saos-2 and hFOB cells by sericin coated silver nanoparticles

Cytotoxic effects of silver nanoparticles against Saos-2 osteosarcoma and hFOB osteoblast cell lines were evaluated by the dose-dependent cytotoxicity-cell viability test. The well-known MTT assay, which evaluates the activity of mitochondrial dehydrogenases, was used to assess cell viability. MTT tests were done 24 hours after the application of the solutions. According to the MTT results, sericin-coated AgNPs obtained by chemical method against Saos-2 osteosarcoma and hFOB bone cell lines

were higher with increasing concentration in the concentration range of 0.5 µg / mL - 50 µg / mL compared to control cell lines. It was observed to have a cytotoxic effect. AgNPs were found to have a high anti-proliferative effect in Saos-2 and hFOB cell lines at a concentration of 50 µg / mL. (Figure 4.2 A and B).

After treatment with Ag NPs for 24 hours, the minimum inhibitory concentration (IC50) values shown in the Figure were calculated as 1.880 µg / mL and 1.522 µg / mL for Saos-2 and hFOB cell lines, respectively (Figure 4.2 C and D).

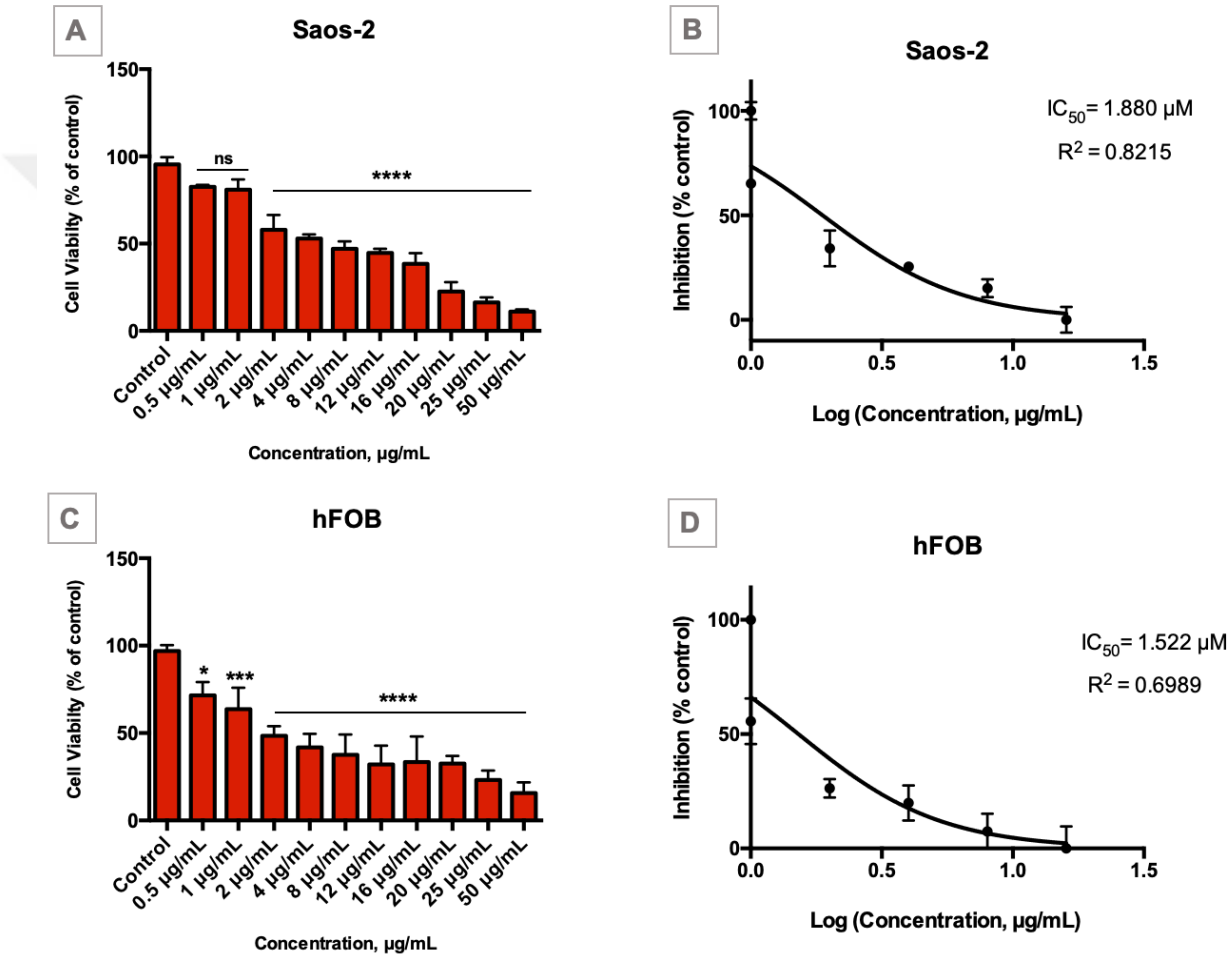

Figure 4.2: Viability of Saos-2 (A) and hFOB (B) cells after 24 h treatment with different concentrations of AgNP (0.5 to 50 µg/ml), according to MTT assay. IC50 graph of Saos-2 (B) and hFOB cells (D) exposed to AgNP. The IC50 value of AgNP was calculated as 1.880 for Saos-2 cells and 1.522 for hFOB cells. (ns:non significant, *P < 0.05, ***P < 0.001, ****P < 0.0001)

4.3. Dose Dependent Cytotoxic Effect of Sericin, AgNO3 and AgNP on Cell Lines

To test the similar effect of sericin-coated AgNP, we applied different concentrations to the three cell lines. According to the obtained MTT results, low dose values were determined and AgNP, sericin and silver ion solutions were applied to three different cell lines. To control the effect of sericin and to shed light on the role of silver ions in the toxic effect of Ag NPs, cells were incubated with Sericin or silver nitrate (AgNO3) solutions in parallel with the silver nanoparticle solution. Here, Sericin and silver ion concentrations were prepared equal to AgNP concentrations (as if all nanoparticles would dissociate into ions). According to our results, Sericin doses were found to have no noticeable effect on cell viability in all three cell lines (Figure 4.3.1, 4.3.2 and 4.3.3).

Percentage effects of silver ion concentration in HeLa, Saos-2 and hFOB cells are presented in Figure. For HeLa, Saos-2 and hFOB cells (Figures A, B and C), viability decreased significantly with the concentration of 0.5 µg / mL and 2 µg / mL. Interestingly, an increase in viability was observed at concentrations of 4 µg / mL and 8 µg / mL (Figure 4.3.4, 4.3.5 and 4.3.6).

HeLa, Saos-2 and hFOB cell lines were cultured at doses ranging from 0.5 µg / ml to 8 µg / ml in the absence or presence of AgNPs to evaluate the effect of silver nanoparticles consisting of Sericin and silver nitrate combination on cell viability. It was observed that the cytotoxic effect of AgNPs on HeLa cervical and Saos-2 osteosarcoma cancer cells was significantly more effective than the control group with increasing dose values starting at 2 µg / ml. However, in the HeLa cancer cell line at concentrations of 0.5 µg / ml and 1 µg / ml, there was no significant effect on cell viability in Saos-2 osteosarcoma cells at a concentration of 0.5 µg / ml. Nevertheless, in hFOB bone cells, the cytotoxicity of silver nanoparticles increased as the drug concentration increased from 0.5 µg / ml to 8 µg / ml compared to control cells (Figure 4.3.7, 4.3.8 and 4.3.9). A plot graph was created to compare the effect of all solutions (Sericin, AgNO3 and AgNP) on cells with each cell lines (HeLa, Saos-2 and hFOB) (Figure 4.3.10, 4.3.11 and 4.3.12).

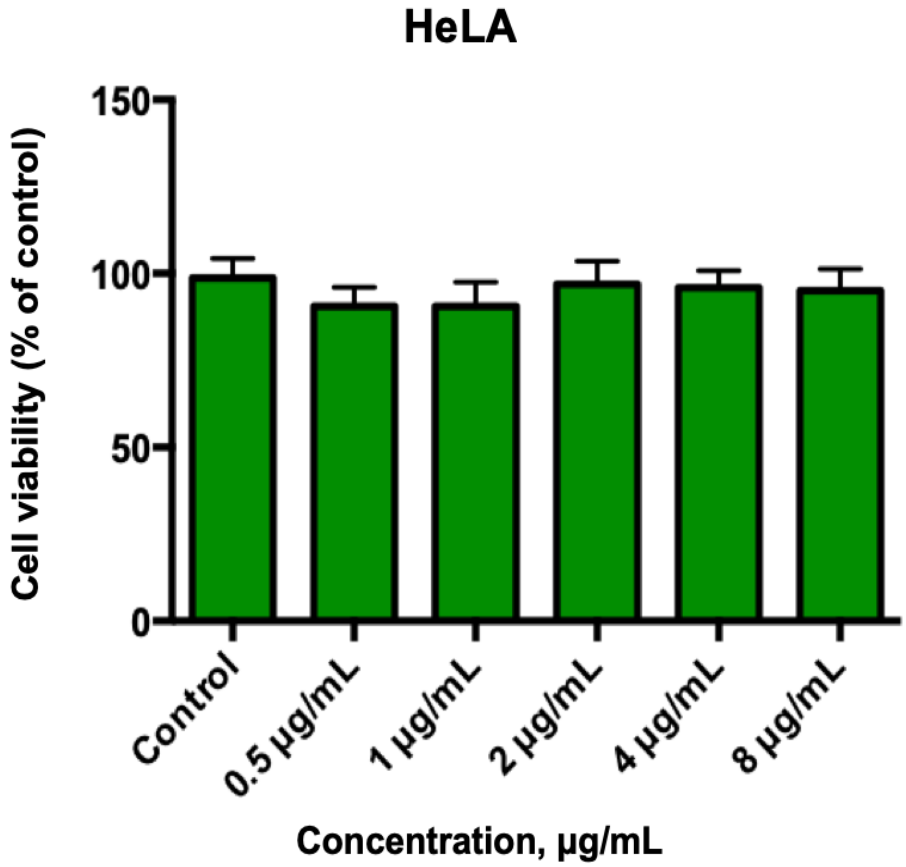

Figure 4.3.1: **Dose-dependent effect of sericin on cell viability of HeLa cells.** Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ±standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test (ns: nonsignificant).

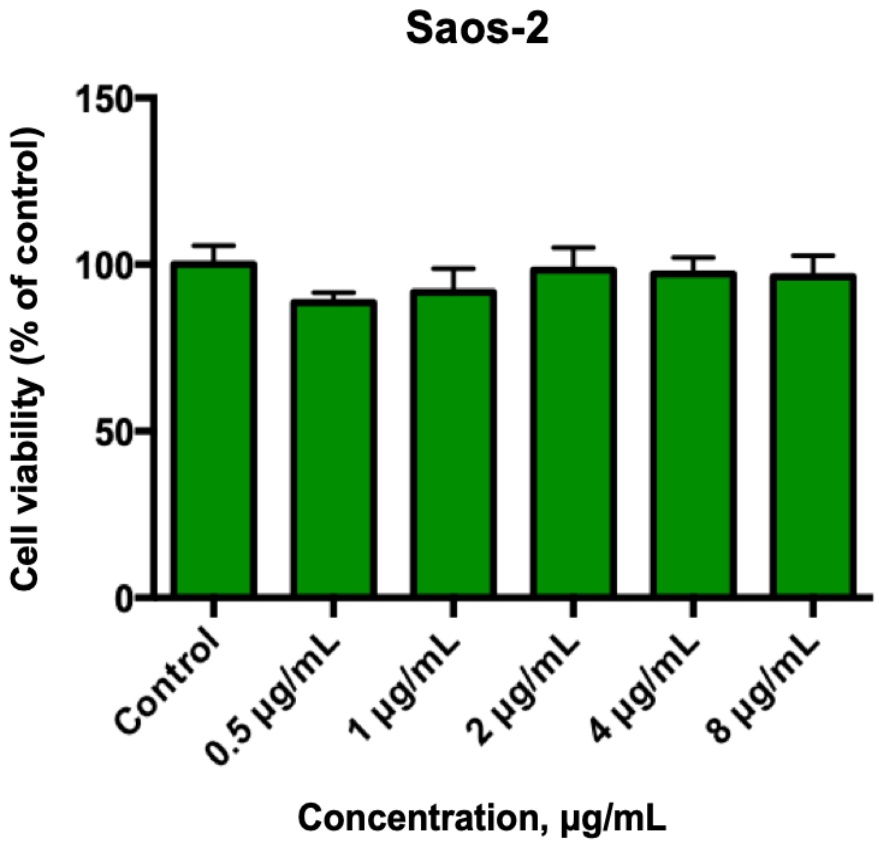

Figure 4.3.2: **Dose-dependent effect of sericin on cell viability of Saos-2 cells.** Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ±standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test (ns: nonsignificant).

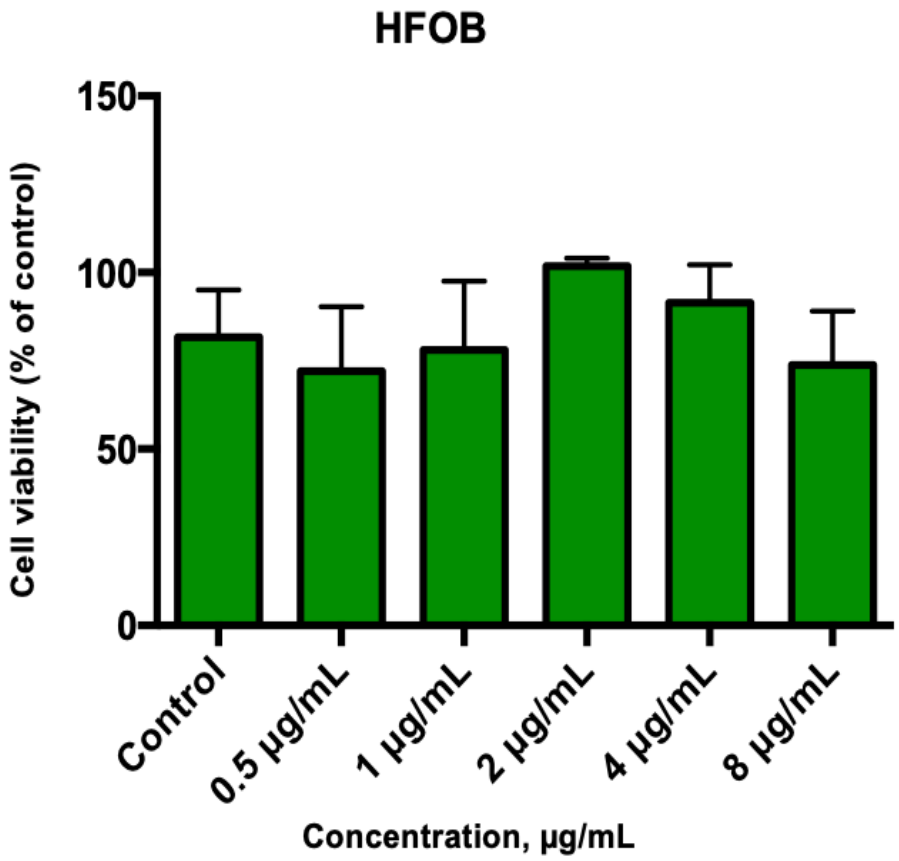

Figure 4.3.3: **Dose-dependent effect of sericin on cell viability of hFOB cells.** Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ±standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test (ns: nonsignificant).

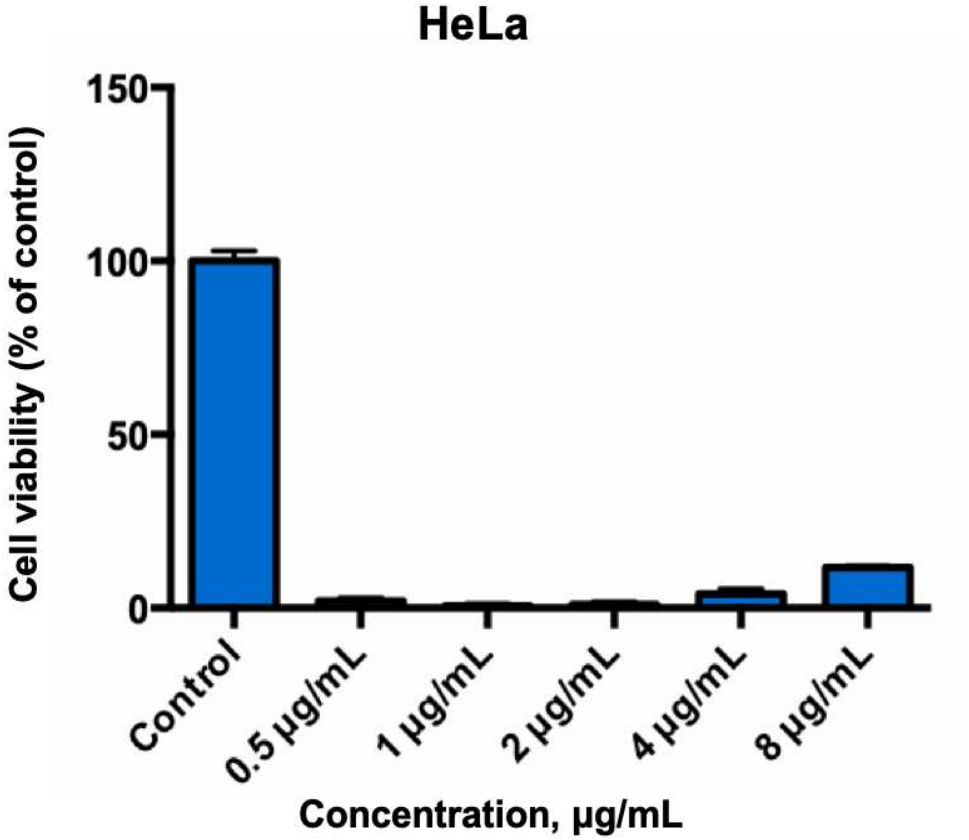

Figure 4.3.4: **Dose-dependent effect of silver nitrate (AgNO3) on cell viability of HeLa cells.** Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ±standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test(****p <0.001).

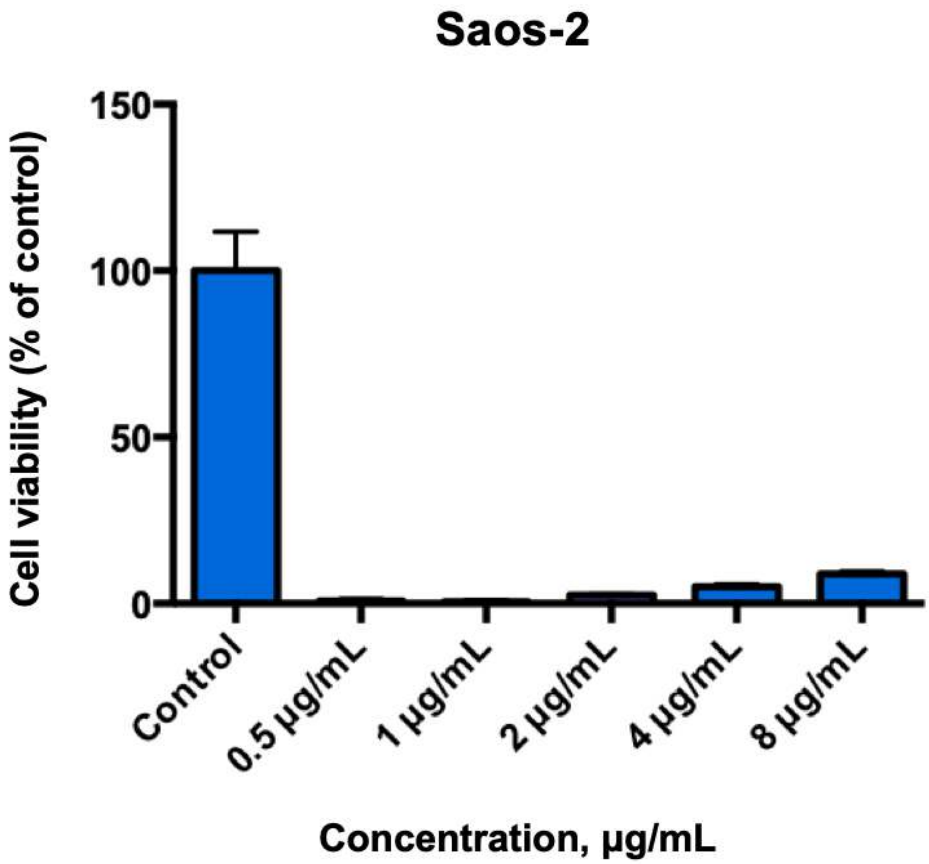

Figure 4.3.5: **Dose-dependent effect of silver nitrate (AgNO3) on cell viability of Saos-2 cells.** Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ±standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test (****p <0.001).

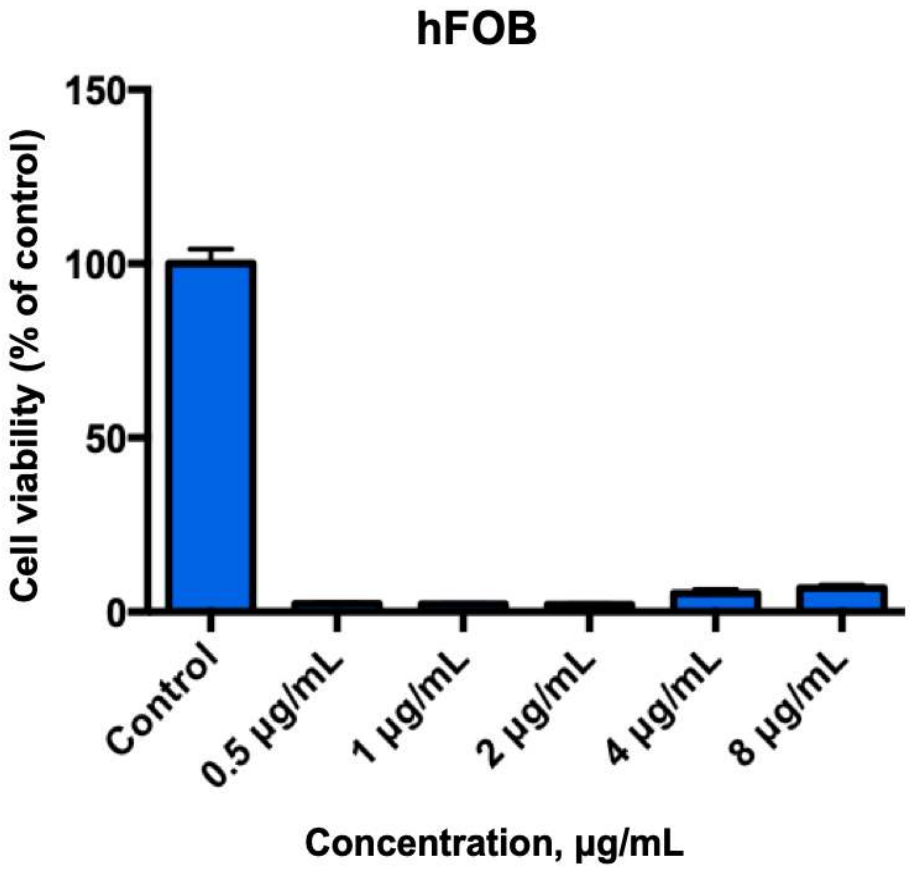

Figure 4.3.6: **Dose-dependent effect of silver nitrate (AgNO3) on cell viability of hFOB cells.** Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ±standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test
(****p <0.001).

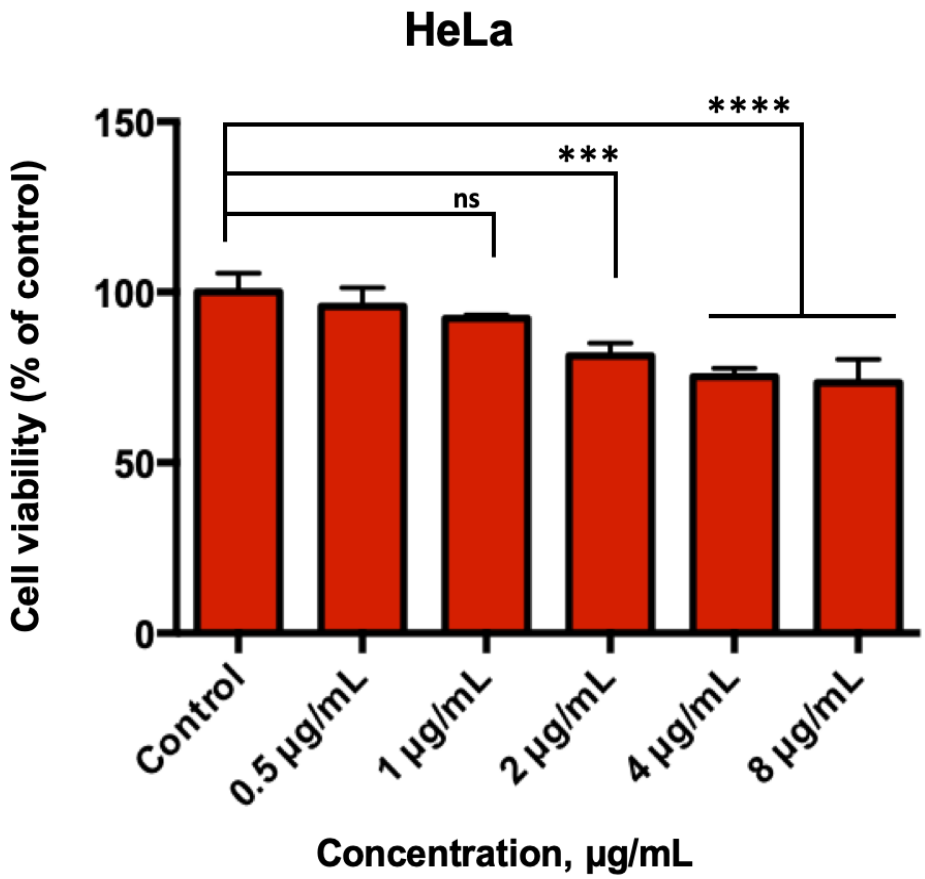

Figure 4.3.7: Dose-dependent effect of silver nanoparticles (AgNP) on cell viability of HeLa cells. Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ± standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test. (****p <0.001, ns: nonsignificant).

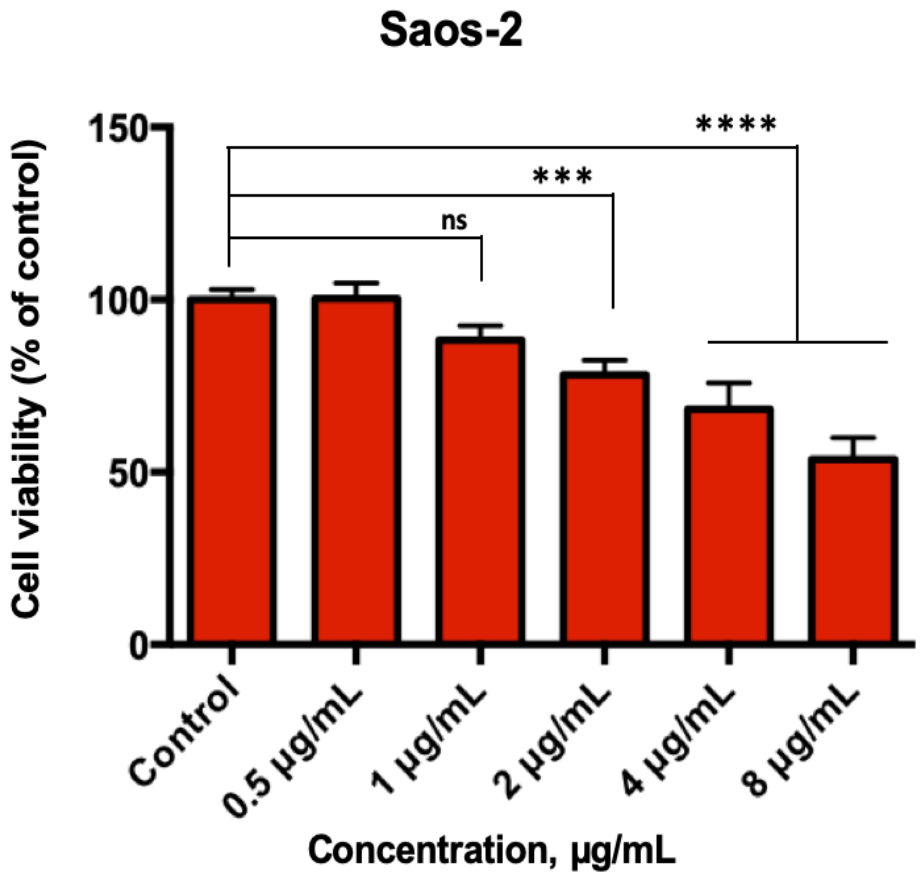

Figure 4.3.8: Dose-dependent effect of silver nanoparticles (AgNP) on cell viability of Saos-2 cells. Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ± standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test. (****p <0.001, ns: nonsignificant).

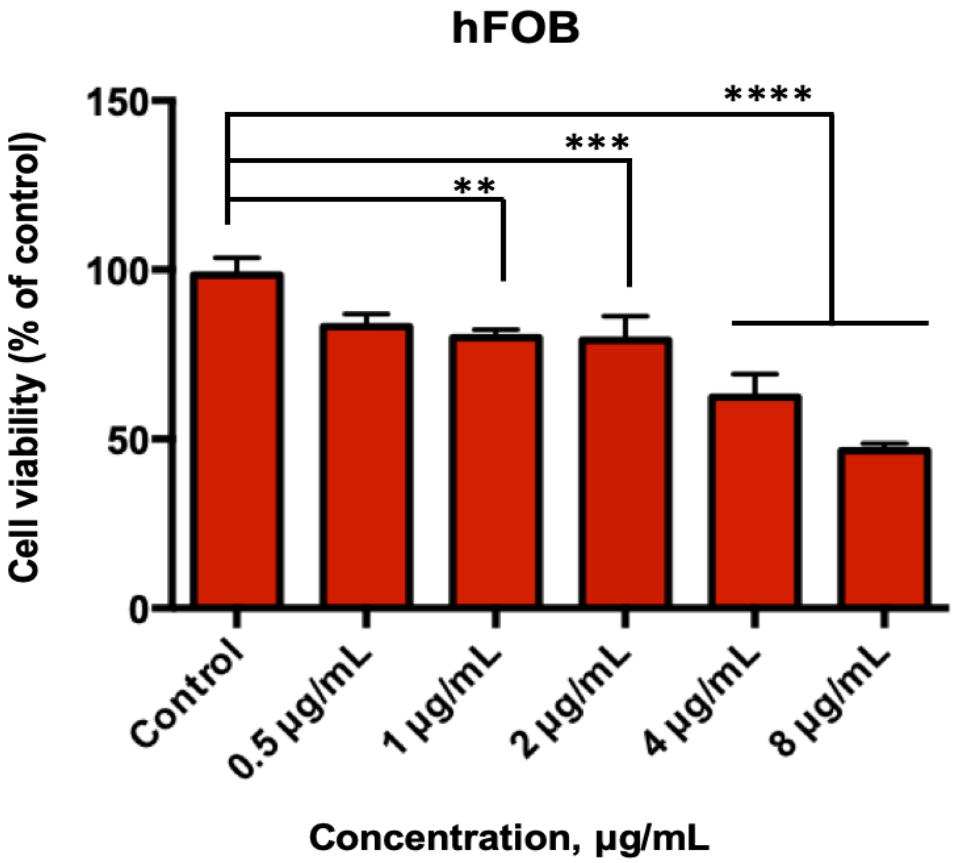

Figure 4.3.9: Dose-dependent effect of silver nanoparticles (AgNP) on cell viability of hFOB cells. Cells were treated for 24 hours at various AgNP concentrations and cytotoxicity was determined by the MTT method. The mean ± standard deviation of three independent experiments is used to calculate the results. Statistical significance of the viability rates of treated cells compared to control cells was determined using the One way Anova test. (****p <0.001, ns: nonsignificant).

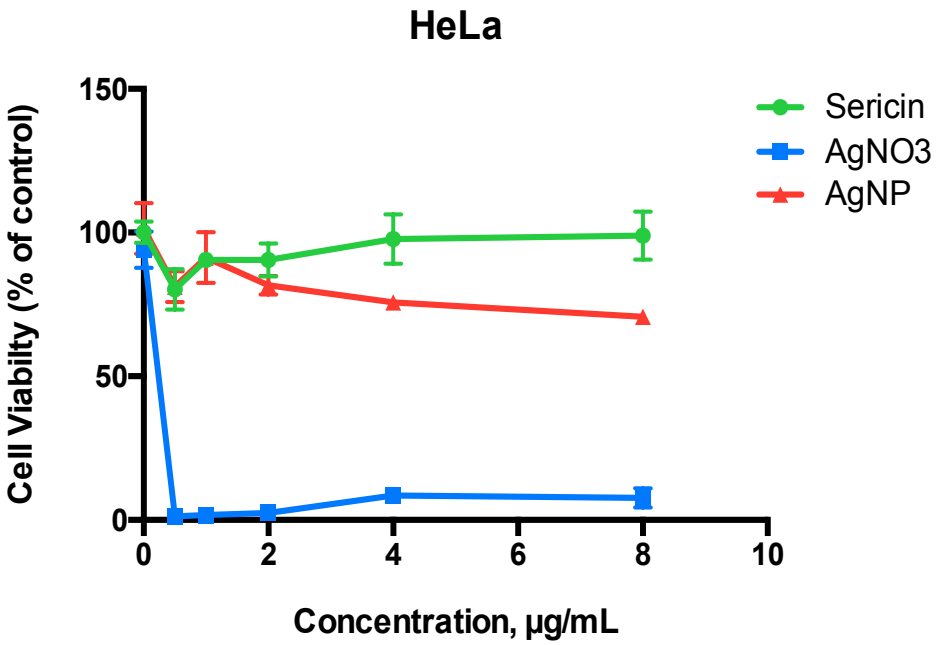

Figure 4.3.10: Viability of HeLa cells with Sericin AgNO3 and AgNPs, at their various concentrations and after 24 hours incubation time.

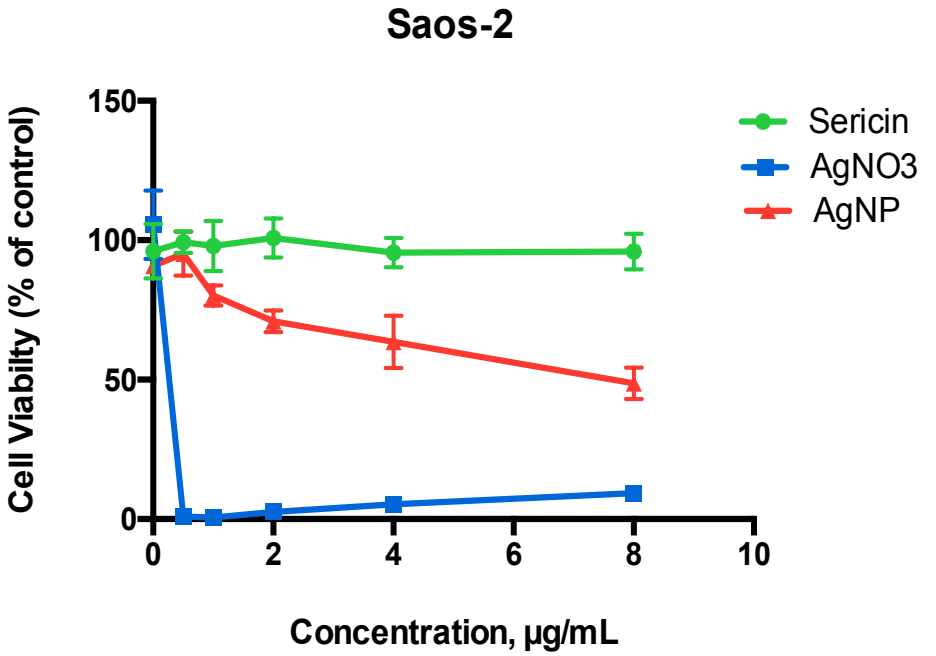

Figure 4.3.11: Viability of Saos-2 cells with Sericin AgNO3 and AgNPs, at their various concentrations and after 24 hours incubation time.

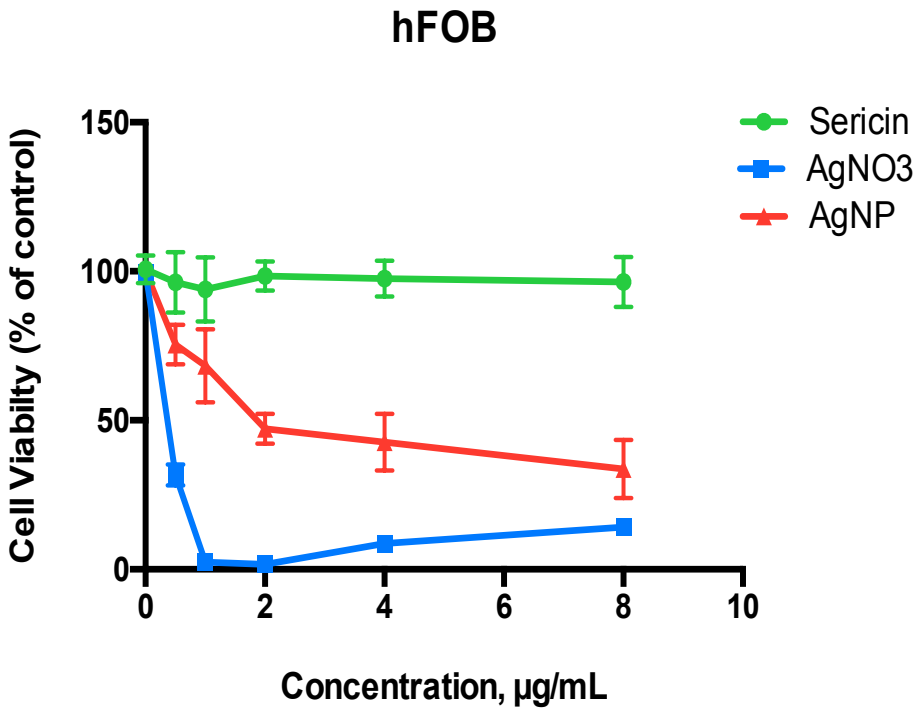

Figure 4.3.12: Viability of hFOB cells with Sericin AgNO3 and AgNPs, at their various concentrations and after 24 hours incubation time.

4.4. Uptake and retention of AgNPs in HeLa Cells

After optimization of Hela cells, certain concentrations of AgNP were applied on the cells to study of cell viability and impairs cell morphology. The control group growing in the absence of AgNP characterizes healthy cell bodies and good growth process (Figure 4.4). It has been observed that AgNPs at a concentration of 2 µg / mL cause intracellular accumulation and cell damage. Cells cultured in AgNPs were found to have an increased number of degraded cells by 4 µg / mL and significant intracellular accumulation. Cell death and abnormal / limited growth were seen in most cells in the presence of AgNP at the final dose of 8 µg / mL. (Figure 4.4)

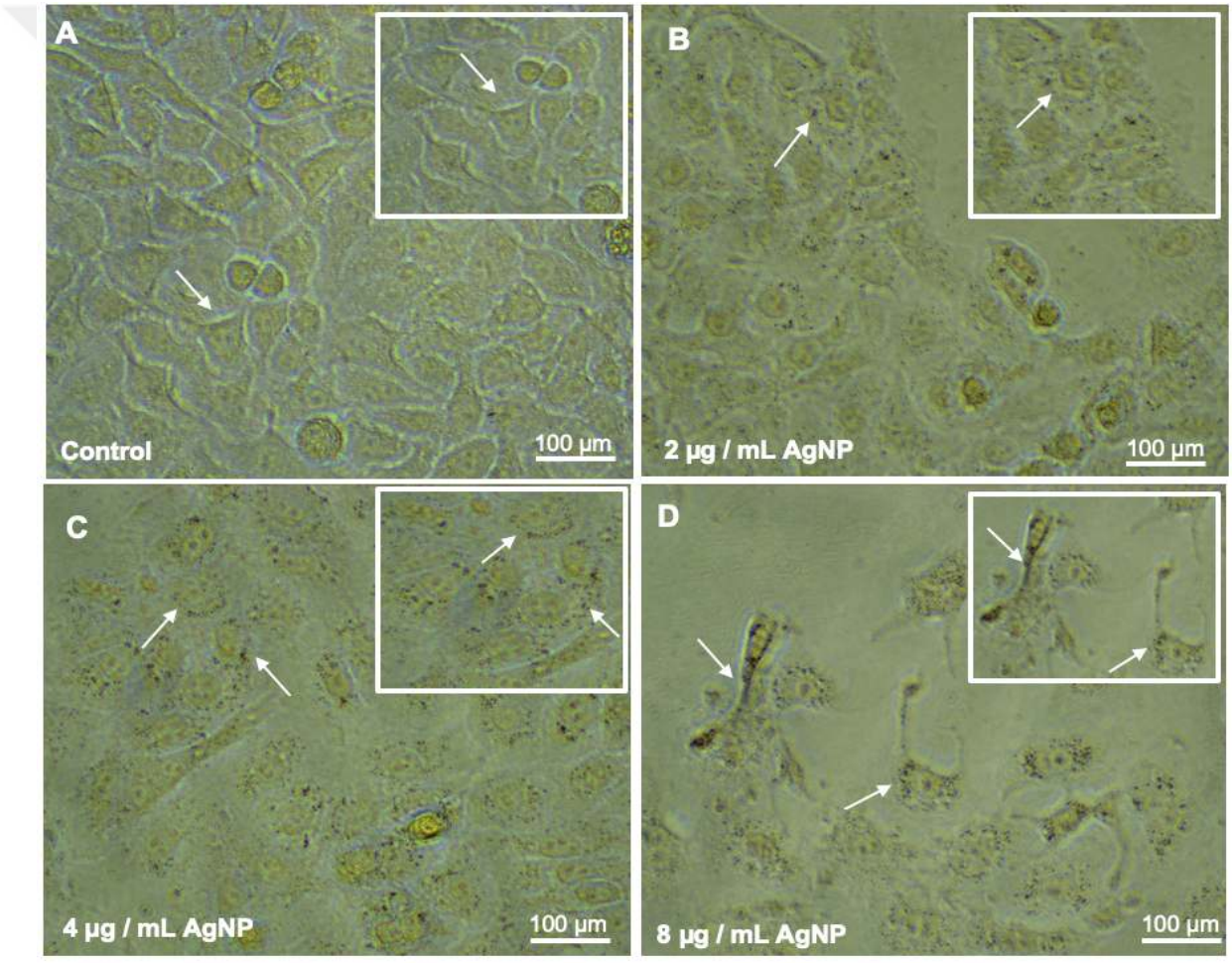

Figure 4.4: Exposure to AgNPs compromises cell viability and impairs cell morphology. Images of Hela cancer cells cultured either in the absence (A, control) or in the presence of various AgNP concentrations at 2 (B), 4 (C) and 8 (D) µg / ml for 24 hours. The arrows indicate the localization of nanoparticles inside cell cytoplasm. Scale bar, 100 µm.

4.5. Effect of AgNPs on actin cytoskeleton of HeLa, Saos-2 and hFOB cell lines

The actin cytoskeleton, which has a dynamic nature (polymerase / depolymerization), is necessary for cell migration and invasion. Therefore, the actin cytoskeleton of HeLA, Saos-2 and hFOB cells was examined after treatment with AgNPs.

In the cellular cytoplasm of the control cell, clearly visible thick actin filaments were observed. In contrast, after AgNP treatment, HeLa, Saos-2 and hFOB cells were found to damage the structure of the actin cytoskeleton in a dose-dependent manner.

When examined morphologically, finger-like protrusions (filopodia) and smooth membrane protrusions (lamellipodia) in the cells were seen as non-elongation, shrinkage into the cell itself (Figure 4.8, 4.9 and 4.10). The alteration of the actin cytoskeleton after treatment with AgNP was observed to be similar in all three cell types. Thus, this study observed structural changes in the actin cytoskeleton by actin staining, and the results showed that AgNPs damage the structure of the actin cytoskeleton of HeLa, Saos-2 and hFOB cells. (Figure 4.5.1, 4.5.2 and 4.5.3)

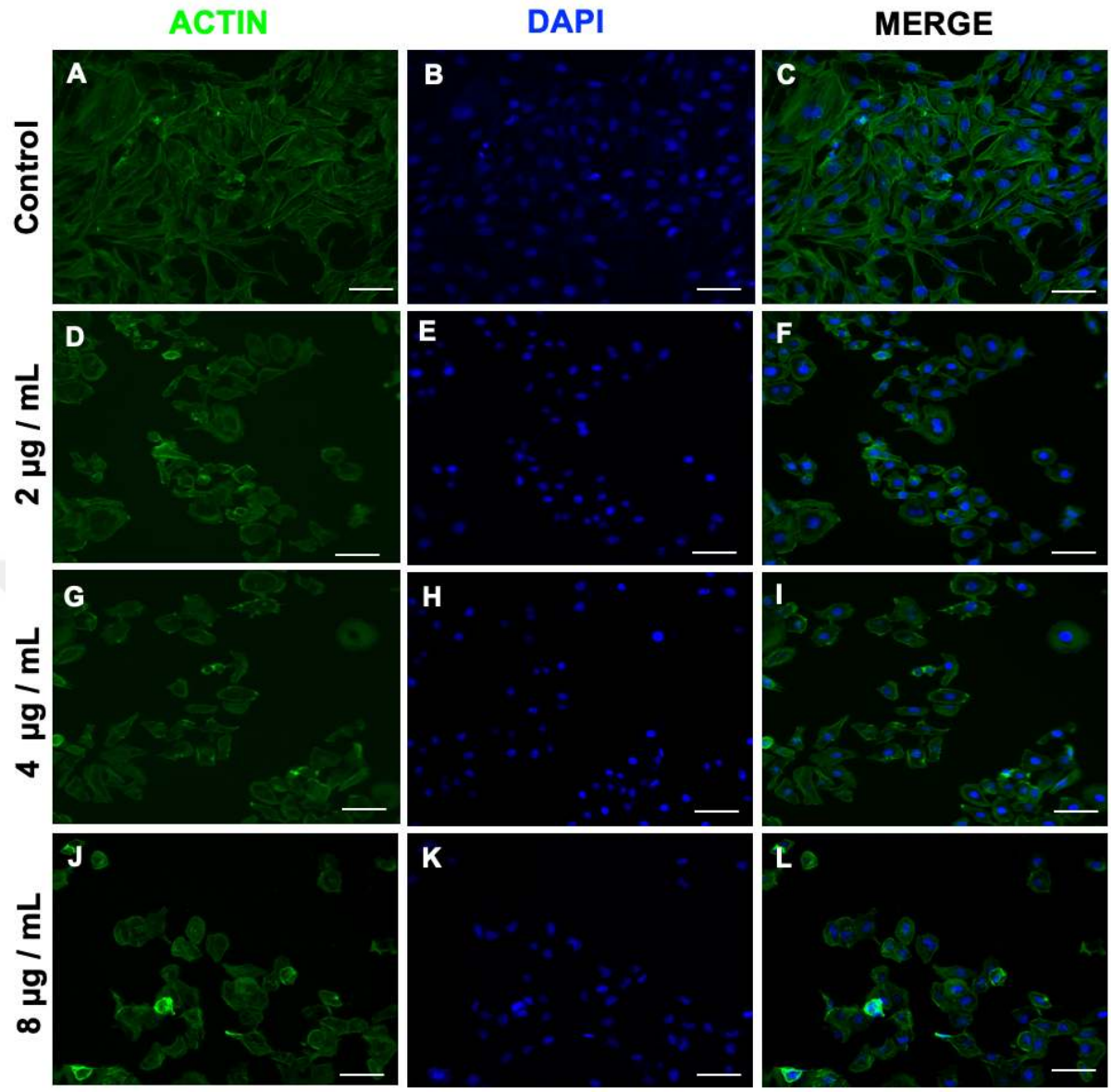

Figure 4.5.1: Actin immunofluorescence images in Hela cervical cancer cells exposed to different AgNP concentrations. (A-C) Control group showing actin positive stained cells. (D-F) Shows cells treated with 2 µl of AgNP. (G-I) Shows cells treated with 4 µl of AgNP. (J-L) Shows cells treated with 8 µl of AgNP. Green indicates actin staining and blue for the nuclear marker DAPI. The merged images of A, B, D, E, G, H or J, K are shown in the C, F, I, L panels respectively, and an overlap of blue and green appears. Data were collected from an independent immunofluorescent staining experiment performed three times each. All images have the same magnification. Scale bar:100 µm..

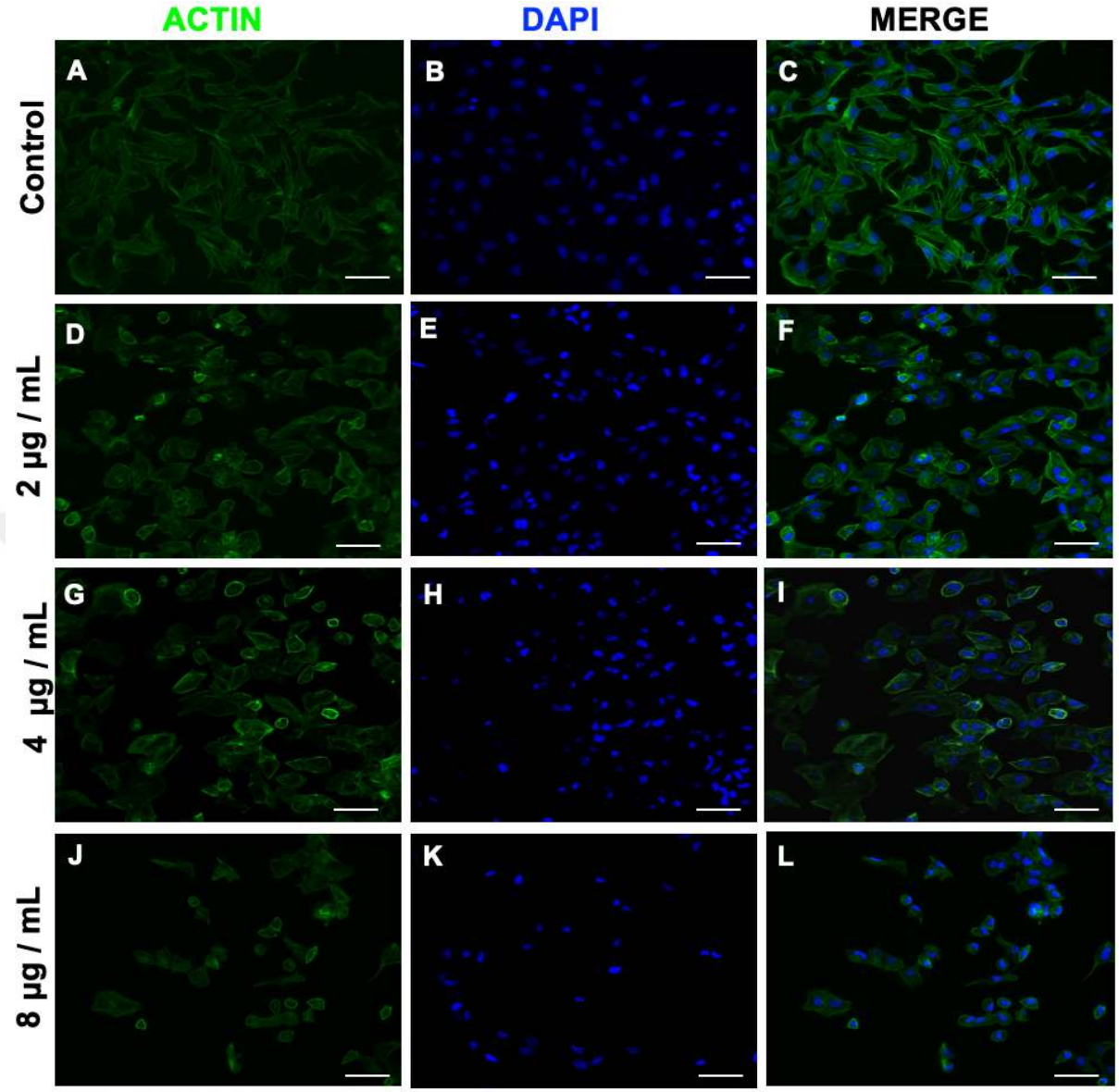

Figure 4.5.2: Actin immunofluorescence images in Saos-2 osteosarcoma cells exposure to different AgNP concentrations. (A-C) Control group showing actin positive stained cells. (D-F) Shows cells treated with 2 µl of AgNP. (G-I) Shows cells treated with 4 µl of AgNP. (J-L) Shows cells treated with 8 µl of AgNP. Green indicates actin staining and blue for the nuclear marker DAPI. The merged images of A, B, D, E, G, H or J, K are shown in the C, F, I, L panels respectively, and an overlap of blue and green appears. Data were collected from an independent immunofluorescent staining experiment performed three times each. All images have the same magnification. Scale bar:100 µm.

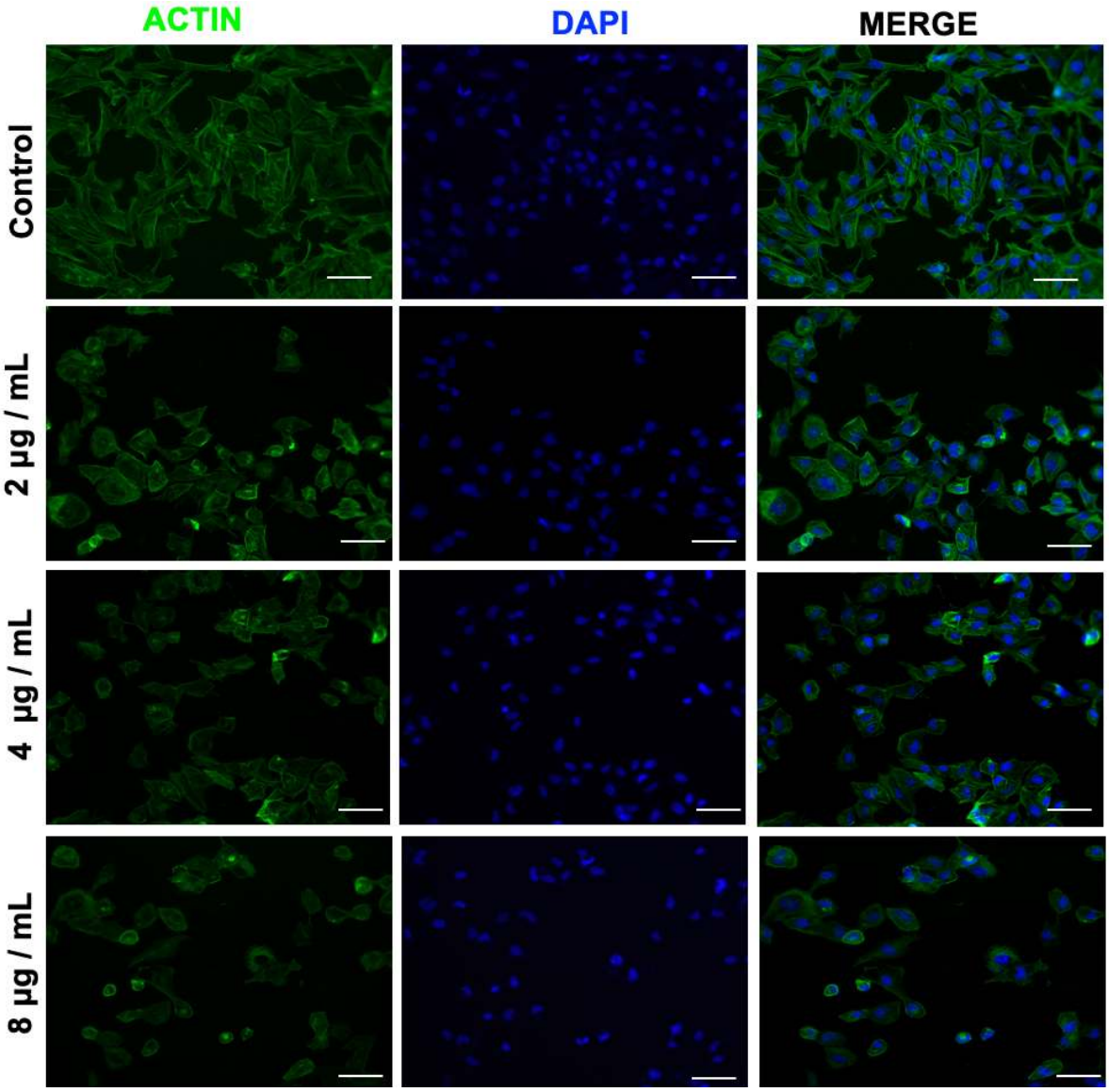

Figure 4.5.3: Actin immunofluorescence images in hFOB osteoblast cells exposed to different AgNP concentrations. (A-C) Control group showing actin positive stained cells. (D-F) Shows cells treated with 2 µl of AgNP. (G-I) Shows cells treated with 4 µl of AgNP. (J-L) Shows cells treated with 8 µl of AgNP. Green indicates actin staining and blue for the nuclear marker DAPI. The merged images of A, B, D, E, G, H or J, K are shown in the C, F, I, L panels respectively, and an overlap of blue and green appears. Data were collected from an independent immunofluorescent staining experiment performed three times each. All images have the same magnification. Scale bar:100 µm..

5. DISCUSSION, CONCLUSION, and RECOMMENDATIONS

5.1. Discussion

Nanotechnology has enabled the emergence of new and improved materials for biomedical applications in the diagnosis and treatment of diseases. NPs, which are approximately 1-100 thousand times smaller than human cells, can easily interact with biomolecules at the cell surface and within the cell, providing improved signals and target specificity for diagnostics and therapeutics (X. F. Zhang, Liu, Shen, & Gurunathan, 2016a).

Among the metal nanoparticles, AgNPs have attracted great interest for many years due to their high surface-to-volume ratio, antibacterial properties, electrical and physicochemical properties. In addition, the surfaces of Ag NPs can be easily modified to increase their biocompatibility, thus drugs and genetic material can be attached to the surfaces and drugs can be transported to target cells (Y. Li, Ma, Ren, & Li, 2013). To design a biocompatible NP, proteins that will surround AgNP and prevent non-specific interactions with biomolecules can be used. These structures, called protein corona, increase the stability of Ag NPs and the hydrophilicity of the outer surface, and make the solubility and AgNP size adjustable (Pei, Fu, Jiang, & Sun, 2019). Considering the advantages of AgNPs, which are one of the metal NPs, in drug distribution, diagnosis and treatment applications, these particles should be investigated extensively. For this reason, besides benefiting from its unique properties in biomedical applications, experimental studies have also focused on the evaluation of the toxicity of AgNPs, and studies on in vivo biodistribution and in vitro toxicity have started to gain importance (Anselmo & Mitragotri, 2015; Pillai & Kamat, 2004; X. F. Zhang, Shen, et al., 2016). Besides, despite technological advances, there is not enough information about the molecular processes that direct the interactions of AgNPs with cells. This lack of information hinders the design of nanomaterials that have low cytotoxicity and can be used in effective diagnosis and treatment. AgNPs have been defined as toxic and non-toxic particles in some studies. Studies have shown that size, surface load, degree of aggregation and shape are the basic parameters related to the potential toxicity and stability of AgNPs (El-Naggar, Hussein, & El-Sawah,

2017; Liao et al., 2019). In addition to the toxicity caused by size, surface load, degree of aggregation and shape, DNA damage (genotoxicity) is thought to be an important issue that needs to be investigated in the use of AgNPs.

When the literature on the cytotoxicity of silver nanoparticles was evaluated, many studies under cancer nanotechnology were seen. Many in vitro studies have emphasized the cytotoxic effects of Ag NPs in different cell lines (Abel, Poonga, & Panicker, 2016; Bin-Meferij & Hamida, 2019; Pillai & Kamat, 2004; Valenzuela-Salas et al., 2019). It has been stated that many factors such as the concentration and size of nanoparticles, surface charge, coating or the cell line used may play a role in cytotoxicity (Khan et al., 2019). In this thesis, which was planned and carried out in the light of this information, the cytotoxic effects of sericin coated silver nanoparticles synthesized by biochemical method in different cell lines and changes in the cytoskeleton were examined. In a study evaluating the cytotoxicity of silver nanoparticles, the cytotoxicity of Ag NPs with a diameter of 13.2 +4.72 nm in human cervical HeLa (adherent) and human lymphoma U937 (suspended) cell lines was investigated using the MTT method AgNP concentrations of 0.5 – 8 µg/mL were applied between 4 and 24 hours. In this study, Ag NPs obtained by an original biochemical synthesis method were used and according to the results, strong toxicity was observed against tumor cells in the investigated concentration range. In Hela and U937 cells, viability was significantly reduced at 2 µg/mL AgNP concentration at both incubation times. Cell viability was found to be higher at 8.0 µg/mL Ag NPs in both cell lines than at 4.0 µg/mL at both incubation times (Kaba & Egorova, 2015). The researchers suggested that this result may be related to nanoparticle aggregation and sedimentation in the cell environment, which leads to a reduction in the concentration of biologically active particles. In different study, Ranjitham et al. examined the in vitro cytotoxicity of AgNPs against the MCF-7 breast cancer cell line at different concentrations. AgNPs exhibited potent cytotoxic activity against MCF-7 and the inhibitory concentration (IC50) was recorded as 27.79 ± 2.3 µg/mL. The samples showed significant cytotoxicity against the MCF-7 cell line (Unal et al., 2020).

Within the scope of this thesis, the cytotoxicity test performed against sericin-coated AgNPs Saos-2 osteosarcoma and hFOB bone cell lines at a concentration range of 0.5-

50 μg / mL in 24 hours of incubation was evaluated with the MTT assay. In Saos-2 line, it was discovered that the anti-proliferative impact at the cells improved as the AgNP dose value multiplied in any respect concentrations besides 0.5 and 1 μg / mL. After AgNP remedy of hFOB cells, a proportional lower in cellular viability changed into determined from 0.5 μg/mL to 50 μg/mL. It is able to be anticipated that the purpose why 0.5 and 1 μg/mL concentrations showed nonsignificant outcomes in Saos-2 cellular line as compared to hFOB cellular line is because of the extra resistant and invasive nature of cancer cells. A nonlinear regression analysis was used to determine the IC50 price of each cell line. The IC50 values for the Saos-2 and hFOB lines were determined to be 1.880 and 1.522 g/mL, respectively. Those findings imply that AgNPs have sizable cytotoxicity against one-of-a-kind cellular types ($P<0.0001$ by way of evaluation of variance). The cytotoxicity of AgNPs is relatively dependent on intracellular physiological situations consisting of pH, redox reaction, and the like, which can range from type to every other, resulting in a exceptional cell response to AgNPs. To control the effect of sericin on the synthesized sericin coated AgNPs and to shed light on the role of silver ions in the toxic effect of AgNP, sericin, silver nitrate and AgNP solutions were applied on three different cell lines, with reference to the above research dose values.

According to our findings, sericin concentrations had no effect on cell viability in all three cell lines, whereas silver ion concentration had a cytotoxic effect on cells. Interestingly, an increase in viability was observed for HeLa, Saos-2 and hFOB cells at concentrations of 4 μg/mL and 8 μg/mL, while viability decreased significantly with the concentration of 0.5 μg/mL and 2 μg/mL (Figure 4.3). It is possible that Ag ions agents can be considered to cause hypoxia, leading to an activation of Hypoxia-inducible factor-1 (HIF-1) (Yang et al., 2016). Since it is a transcription factor in hypoxic conditions, it thus may increase the survival of these cells in higher concentrations of AgNPs (J. Li et al., 2013). The mechanisms by which this protein acts include gene expression regulation, cell differentiation, vascularization, autocrine growth factor production, proliferation, invasion, and metastatic spread, metabolic modulating, and enhanced tumor growth (J. H. Li et al., 2020). Mainly controlled by hypoxia, HIF-1 plays a role in the regulation of many genes.

Exposure of cells to several metal ions stabilizes the HIF-1α protein. However, the molecular mechanisms are not fully understood.

As a result, the anti-prolative effect of the silver nitrate solution on cell lines is obvious. Considering the effects of AgNP in the concentration range of 0.5-8 µg/mL in HeLa, Saos-2 and hFOB cell lines, a significant decrease in cell viability was observed at 2 µg/mL and increasing concentrations in three cell lines. While there was no significant effect at 0.5-1 µg / mL AgNP concentrations in HeLa and Saos-2 cancer cell lines, hFOB affected cell viability at low doses in osteoblast cells. The reason for this result can be interpreted as cancer cells are more resistant and invasive than normal cells.

The organization and dynamic polymerization/depolymerization of the cytoskeleton are important in cell migration and motility. There is ample evidence that cells exposed to nanoparticles can alter the morphology of the cytoskeleton. Studies in Perni et al., have shown that Ag NPs cause significant cellular structure degeneration by changing F-actin and β-tubulin proteins (Perni, Yang, Preedy, & Prokopovich, 2018). In addition, C6 and U373 glial tumor cells exposed to nanoparticles have been shown to undergo significant morphology changes and reduced F-actin organization (Marquez-Ramirez, Delgado-Buenrostro, Chirino, Iglesias, & Lopez-Marure, 2012). However, cell morphology changes are observed after treatment with nanoparticles due to conformational changes in the cytoskeleton and this deserves further investigation.

In our study, bright visuals of HeLa cells exposed to AgNPs were presented regarding the uptake and retention of nanoparticles. It has been observed that AgNPs at different concentrations applied on HeLa cells cause accumulation in the cell cytoplasm and cause cell damage. AgNPs can affect the assembly of the actin cytoskeleton by disrupting cell morphology, thereby inhibiting the migration and invasion of cells. This study observed structural changes of actin cytoskeleton of HeLA, Saos-2 and hFOB cells by actin staining, and the results showed that AgNPs damage the structure of the actin cytoskeleton of HeLA, Saos-2 and hFOB cells. In conclusion, the findings from this study revealed that AgNPs exert inhibitory effects on cell migration and invasion by destroying the actin cytoskeleton.

All these findings indicate that sericin-coated silver nanoparticles cause cytotoxicity, cell morphological damage and cell actin skeleton degradation. Further studies are

needed to elucidate the mechanisms responsible for AgNP-induced changes in cell viability and cytoskeleton.

5.2. Conclusion

Nanotechnology has shown heaps of promise in cancer treatment over the years. Nanomaterials improved pharmacokinetic and pharmacodynamic properties have aided in the advancement of cancer identification and treatment. Because of their specificity, the subject field enables targeted drug delivery in affected organs with the lowest overall toxicity. However, engineering science, like alternative therapeutic options, is not completely free of toxicities and poses many challenges in its use, as well as general and specific organ toxicities, causing disruptions in its clinical applications. Given the constraints of engineering science, more progress should be made to improve drug delivery, maximizing effectiveness while minimizing disadvantages. By increasing the interactions between the chemistry properties of the nanomaterials used, safer and more practical derivatives for cancer identification and treatment will be provided.

Despite the fact that numerous studies have addressed nanotoxicity or cellular behavior, data gaps persist due to treatment, design, concentration, model type, and a variety of other factors. Understanding how AgNPs interact with specific cells and stimulate signal cascades, as well as the reasons for a wide range of responses in different cell lines, is a complex task when all of these parameters are taken into account. Any pharmacology study of a new nanomaterial necessitates in vitro biocompatibility testing with various cell types in various cell culture media formulations, as well as testing with nanomaterial suspensions with identical physical and chemical properties. To determine the toxicity and biocompatibility of AgNPs, many cell lines are used as in vitro model systems. Throughout this thesis, we have a tendency to choose the required cell varieties involved in numerous diseases to summarize the various responses of various cell varieties to AgNPs. Numerous studies have concluded that the cellular response of each cell type is determined by the following factors: physical and chemical properties; concentration; incubation period;

the presence of macromolecule coronas that affect body fluid and thus cell uptake; particle release; bio-distribution; biological activity; toxicity; and biocompatibility. Secondary aspects of the cellular response embrace agglomeration within the cellular setting, animate thing localization, and conductor particle unleash. AgNP toxicity, cytoskeletal injury and necrobiosis were ascertained in most of the cell lines.

5.3. Recommendations

In the future, we will test response levels of caspase-3, reactive oxygen species (ROS), mitochondrial membrane potential in addition to held in this study testing to identify subcellular responses and mechanisms against AgNP. To solve the mystery of the increase in cell viability of Ag ion at high concentration, qRT-PCR analysis of HIF-1 a mRNA will be revealed by western blot analysis. We believe that these further studies will elucidate the direct role of AgNP in each cell types, and direct effects of AgNP on the cell methabolism.

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THE ANTI-PROLIFERATİVE EFFECT OF SERICIN-COATED SILVER NANOPARTICLES ON CANCER CELL LINES

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THE ANTI-PROLIFERATİVE EFFECT OF SERICIN-COATED SILVER NANOPARTICLES ON CANCER CELL LINES

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