T.C.

BAHCESEHIR UNIVERSITY

GRADUATE SCHOOL

BIOMEDICAL ENGINEERING HEAD OF THE DEPARTMENT

INVESTIGATION OF THE EFFECTS OF LASER IRRADIATION AT 660nm WAVELENGTH ON CELL VIABILITY IN THE PRESENCE OF GOLD NANOPARTICLES IN A549 LUNG CANCER AND HCT116 COLON CANCER CELL LINES

MASTER'S THESIS

SEDEF ÖZÜNLÜ

ISTANBUL 2023

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ABSTRACT

TITLE OF THE THESIS INVESTIGATION OF THE EFFECTS OF LASER IRRADIATION AT 660nm WAVELENGTH ON CELL VIABILITY IN THE PRESENCE OF GOLD NANOPARTICLES IN A549 LUNG CANCER AND HCT116 COLON CANCER CELL LINES

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The convergence of nanotechnology and laser therapy has ushered in a new era of innovative approaches to the diagnosis and treatment of cancer. With the presence of nanotechnology and laser, advanced therapies can be found as an alternative treatment for cancer.

Nanotechnology has enabled the designing and fabricating of multifunctional nanoparticles with personalized properties for cancer-specific targeting, imaging, and drug delivery. These nanoparticles offer enhanced drug bioavailability, reduced toxicity, and the potential for personalized therapies. Coupled with the precision of laser technology, these nanoparticles can be activated to induce localized effects, including photothermal therapy, photodynamic therapy, and controlled drug release. The utilization of laser energy provides specific targeting, minimizing damage to healthy tissues while maximizing the therapeutic impact on cancerous cells.

The challenges and prospects in this interdisciplinary field are also addressed. These encompass the need for optimized nanoparticle design, comprehensive safety assessments, and effective translation of research findings into clinical applications. Furthermore, the thesis underscores the potential of cancer nanotechnology and laser therapy in primary cancer treatment, adjuvant therapies, targeted drug delivery, and early-stage detection, which is essential.

Herein thesis, lung, and colorectal cancer cell line cell viabilities are investigated with the presence of gold nanoparticles and 660 nm laser with high power.

Keywords: Cancer, Cancer Treatment, Lasers, Nanotechnology

ÖZ

660 nm DALGA BOYUNDA LAZER IŞINLAMANIN A549 AKCİĞER KANSERİ VE HCT116 KOLON KANSERİ HÜCRE HATLARINDA ALTIN NANOPARÇACIKLAR VARLIĞINDA HÜCRE CANLILIĞI ÜZERİNDEKİ ETKİLERİNİN İNCELENMESİ

Sedef, Özünlü

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Nanoteknoloji ve lazer tedavisinin yakınsaması, kanserin teşhis ve tedavisinde yenilikçi yaklaşımlar çağını başlattı. Nanoteknoloji ve lazerin varlığı ile kanser için alternatif bir tedavi olarak gelişmiş tedaviler bulunabilir.

Nanoteknoloji, kansere özgü hedefleme, görüntüleme ve ilaç dağıtımı için kişiselleştirilmiş özelliklere sahip çok işlevli nanoparçacıkların tasarlanmasını ve üretilmesini sağlamıştır. Bu nanoparçacıklar, geliştirilmiş ilaç biyouyumu, azaltılmış toksisite ve kişiselleştirilmiş terapiler için potansiyel sunar. Lazer teknolojisinin kesinliği ile birleştiğinde, bu nanopartiküller fototermal terapi, fotodinamik terapi ve kontrollü ilaç salımı dahil olmak üzere lokalize etkileri tetiklemek için etkinleştirilebilir. Lazer enerjisinin kullanımı, kanserli hücreler üzerindeki terapötik etkiyi en üst düzeye çıkarırken sağlıklı dokulardaki hasarı en aza indirerek spesifik hedefleme sağlar.

Bu disiplinlerarası alandaki zorluklar ve beklentiler de ele alınmaktadır. Bunlar, optimize edilmiş nanoparçacık tasarımı, kapsamlı güvenlik değerlendirmeleri ve araştırma bulgularının klinik uygulamalara etkili bir şekilde dönüştürülmesi ihtiyacını kapsar. Ayrıca, kanser nanoteknolojisinin ve lazer tedavisinin birincil kanser tedavisinde, adjuvan tedavilerde, hedefe yönelik ilaç dağıtımında ve çok önemli olan erken evre tespitindeki potansiyelini vurgulamaktadır.

Bu tezde akciğer ve kolorektal kanser hücre hattı hücre canlılıkları, altın nanopartiküller ve yüksek güçlü 660 nm lazer varlığında araştırılmaktadır.

Anahtar Kelimeler: Kanser, Kanser Tedavisi, Lazerler, Nanoteknoloji

To my beloved family who support me every step of the way

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
CTCs	Circulating Tumor Cells
WHO	World Health Organization
NSCLC	Non-Small Cell Lung Cancer
SCLC	Small Cell Lung Cancer
CT	Computerized Tomography
PET	Positron Emission Tomography
MRI	Magnetic Resonance Imaging
NPs	Nanoparticles
NP	Nanoparticle
PDT	Photodynamic Therapy
PS	Photosensitizer
ROS	Reactive Oxygen Species
PTT	Photothermal Therapy
PEG	Polyethylene Glycol
Au	Gold

SPR	Surface Plasmon Resonance
CdSe	Cadmium Selenide
PdS	Lead Sulfide
Fe3O4	Iron(II,III) oxide
SPIONs	Super Magnetic Iron Oxide NPs
EPR	Enhanced Permeability and Retention
ECM	Extracellular Matrix
AgNO3	Silver Nitrate
XRD	X-ray Diffraction
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
DLS	Dynamic Light Scattering
UV-VIS	Ultraviolet-Visible Spectroscopy
LASER	Light Amplification by Stimulated Emission of Radiation
LLLT	Low-Level Laser Therapy
OCT	Optical Coherence Tomography

CO_2	Carbon Dioxide
HeNe	Helium-Neon
GaAs	Gallium Arsenide
FELs	Free Electron Lasers
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
PBS	Phosphate Buffer Solvent
df	Degree of Freedom

Chapter 1

Introduction

1.1 Literature Review

Cancer is a disease that has affected humanity significantly for many years. Early diagnosis and appropriate treatment increase the survival rate of cancer patients (Ginsburg et al. 2020). The cure for cancer, which differs according to age, gender, cancer type, and similar categories, has been highly studied in laboratory and clinical studies for many years. Chemotherapy has been one of the most common treatments for cancer. However, it has many disadvantages as well. Several methods have been developed to reduce the effect of chemotherapy drugs, which basically is destroying healthy cells while killing cancer cells. One of the different methods is photodynamic therapy, which provides the chemical change of cancer cells by using a laser light source. Many studies have investigated the effects of the therapeutic properties of low-energy laser light on cancer cell viability. The laser causes chemical changes in the materials it interacts with, and it does this thanks to the physical properties of photons, which are its building blocks. The effects of laser radiation duration, energy density, and wavelength on cell viability were investigated. In order to increase the effectiveness of the laser on the tissue, the addition of substances that will increase the light absorption of cancerous tissues has started a change in the literature and the effects of photosensitive substances on cell viability have begun to be investigated. One of these substances is the gold nanoparticle. With the development of nanotechnology, nanoparticles, which are frequently used in biomedicine as well as in every industry, serve different purposes in many fields thanks to their different shapes, sizes, and morphologies. One of them is to investigate the effect on the viability of cancerous cells and to develop an alternative method to chemotherapy. Instead of metal nanoparticles, which can bond with mammalian genes and have toxic effects, gold nanoparticles, which have a more stable structure and do not form metal ions, have been studied in the literature under photodynamic therapy since 2010. It has been studied by many researchers that low-energy density laser beams reduce the effectiveness of cancerous cell lines.

1.2 Purpose of the Study

This thesis research aims to determine the effect of laser irradiation on the viability of lung cancer and colorectal cancer cell lines in the presence of gold nanoparticles. Consequently, it might become feasible to devise an alternative approach to cancer treatment.

Chapter 2
General Information

2.1 Cancer

2.1.1 Cancer definition. In this century, cancer is the most common and severe cell multiplication of all time which is referred to as ‘genomic instability’. It also is such a disease that there is yet no solid applicable treatment that might work for every patient. It is claimed that cancer affects a wide range of human populations with a total number of 19.3 million new cases all around the globe with a death number of 10 million (Sung et al. 2021). Cancer is characterized by a cell's failure to undergo programmed cell death and its uncontrolled multiplication due to genetic alterations (Ferguson et al. 2015). Environmental factors like smoking, stress, and genetic inheritance can cause cancer (Manton, Akushevich, and Kravchenko 2009). Corresponding to the second law of thermodynamics, the cosmic force drives the universe to increased entropy. Life is certainly referred to as order and death is disorder. As Ames's Triage theory in 2010, ‘Nature always favors short-term survival over long-term survival’, the understanding of normal cell transformation to a cancerous cell can be explained accordingly. Free oxygen radicals produced by many essential nutrients can attack and damage nearly any vital molecules and structures in our body. These attacks can cause irreversible and permanent disease and death. For example, attacking the deoxyribonucleic acid (DNA) can cause the transformation of normal cells into cancerous ones. The prevention of carcinogenesis can be ensured via the existence of essential nutrients to produce energy, protein synthesis, DNA repair, etc. The production of antioxidant proteins such as superoxide dismutase, glutathione peroxidase, catalase, etc. can vitiate the free radicals. The uncontrollable cell divisions can be explained as a lack of antioxidant agents. For further specification, normal human cells can divide approximately 50 times and die in the scope of the Hayflick limit. Briefly, the Hayflick limit is the limit of cell replication. After crossing this limit, cell death is observed for normal cells (Groten, Venkatraman, and Mertelsmann 2018). Cell life is limited by telomere shortening with every cell division. Accurate DNA replication needs genetically stable telomeres. Telomeres are end sections of chromosomes that are shortened in every cell division. When telomeres are shortened enough, the cell dies. Cancerous cells activate a reverse transcriptase, telomerase enzyme, to have a longer life than normal cells by having longer telomeres. Hence, the immortality of cells resulting in tumors is the mortality of humans. Every stage of the regulations in our body is interconnected like dots in a circle (Hegde, Mali, and Chandorkar 2013).

Another significant threat posed by cancer is its ability to metastasize and spread to other tissues in the body cancer cells become bulky tumor tissues that cannot fit into their original place and starts to spread through the nearest leaky junctions of blood vessels/tissues. The primary cancerous cells can be found in the bloodstream as circulating tumor cells (CTCs). If the new environment has the proper nutrients, CTCs can be colonized. Every cancerous cell has its own morphology. By investigating the cells under a microscope, specialists can understand the origin of the tissue and diagnose the type (Ju et al. 2022).

There are many types of cancer, more than 100. Bone and muscle, brain and nervous system, breast, skin, etc. In this thesis, lung (A549) and colorectal (HCT116) cancer cells are investigated.

2.1.2 Lung cancer. Lung cancer is a type of cancer that originates in the cells of the lungs. According to World Health Organization (WHO), lung cancer is the most common type of cancer with metastatic properties. Before metastasis, the detection of lung cancer should be diagnosed to prevent the death of the patient. Significant research over the years shows that lung cancer is common among adult men over 60 years old, Afro-Americans, and who come from a family with a cancer history. Air pollution, tobacco smoking (over 80% of cases), e-cigarettes, second-hand smoke inhalation, radon gas (naturally produced mutagenetic uranium decay) inhalation, and

Covid-19 increase the risk of lung cancer (Thandra et al. 2021). As shown in Figure 1, the morphology of normal cells is mutated and becomes unstable.

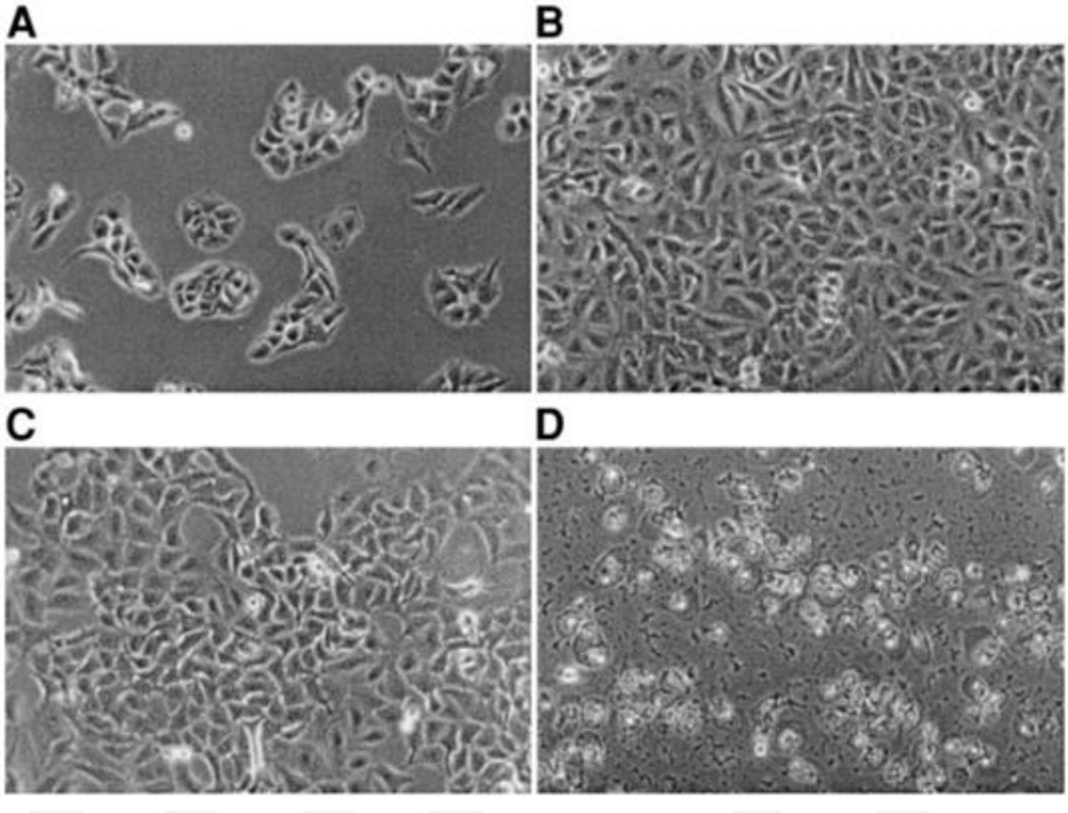

Figure 1. Lung Cancer Cells Under the Light Microscope (Aarbiou et al. 2002).

Lung cancer is generally categorized into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). These types differ in terms of their characteristics, behavior, and treatment options. NSCLC accounts for a great amount of all lung cancer cases. The treatment options are depending on the stage, patients' general health, and tumor characteristics. For the early stage of NSCLC surgical removal can be possible to perform. Radiation therapy can be the primary treatment or combined with surgery and chemotherapy. High-energy rays are used to target and kill cancer cells by radiation. Powerful drugs are used to kill rapidly dividing cancer cells throughout the body but kill healthy cells as well. Targeted therapy includes drugs that target mutations or proteins on the cancer cell surface and lead to cancer cell death specifically. Another treatment is immunotherapy to enhance the body's immune system to attack cancer cells (Mithoowani and Febbraro 2022). For SCLC, it is a less common lung cancer type, and it is more aggressive which is associated with smoking. To shrink tumors and remove them before the surgery, the same procedures as NSCLC treatments are applied for SCLC (Yang, Zhang, and Wang 2019).

2.1.3 Colon cancer. Colorectal cancer is known as a secondary lethal cancer type that occurs in the colorectal or rectum and mortality is tending to be increased (Hossain et al. 2022). Early-stage colorectal cancer might not show any symptoms, but as the disease progresses, common symptoms may include changes in bowel habits (diarrhea or constipation), blood in the stool, abdominal pain or cramping, unexplained weight loss, and fatigue. Several risk factors increase colorectal cancer possibility as a family history of colorectal cancer, inflammatory bowel disease, bad diet habits such as producing high red or processed meats, obesity, smoking, and heavy alcohol consumption.

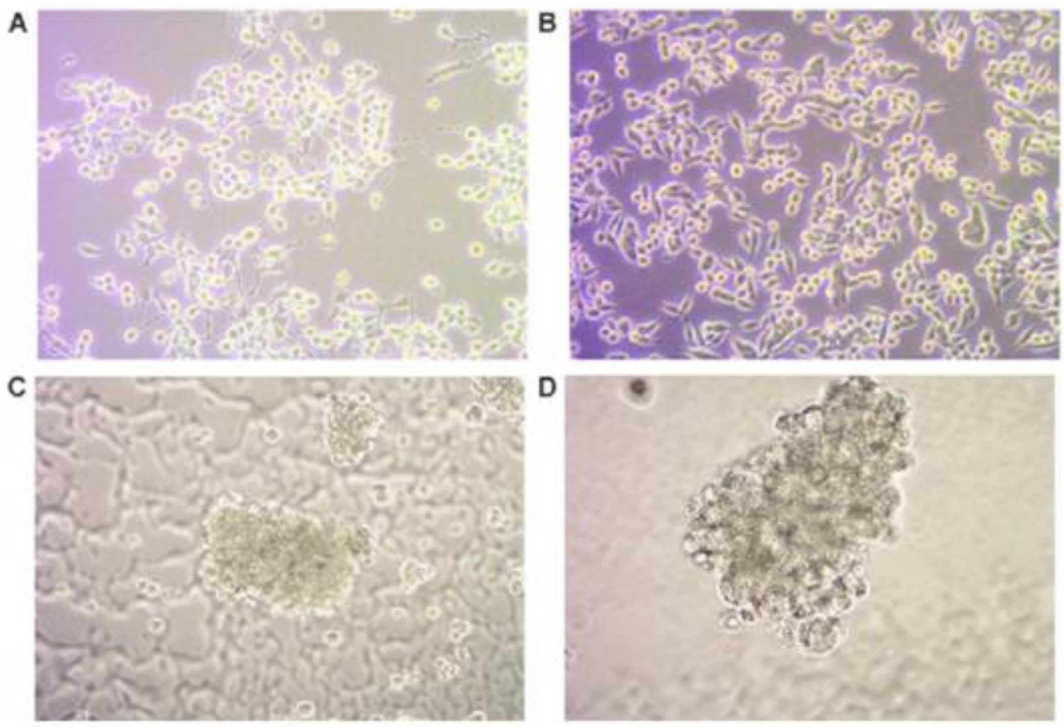

Figure 2. Colorectal Cancer Cells Under the Light Microscope (Guo et al. 2018).

Colorectal cancer is often detected through screenings such as colonoscopy, and blood tests, and the morphology under a light microscope of colorectal cancer cells are shown in Figure 2. The treatment of colorectal cancer is depending on the stage.

As in all cancer treatments, colorectal cancer can be eliminated via chemotherapy to kill and prevent the growth of cancer cells, but the primary treatment can be surgery for early-stage colorectal cancer. Removing cancerous tumors, in some cases a partial/complete removal of the colon might be necessary. Targeted therapy, immunotherapy, and radiation therapy can be applicable if surgery cannot be performed for advanced stages (Granados-Romero et al. 2017).

2.1.4 Cancer detection and diagnosis. The most challenging part of cancer is to detect it in the early stages. Cancer can either have symptoms that can be able to detect in the first stage or don't have any symptoms until the last stage. In order to decide the best pathway to treat cancer, early detection is essential. There are several methods to detect cancer. The main one is to perform a complete physical examination by the doctor, which might include controlling the change in skin color, abnormality tests on specific body parts (organ growth, etc.), and further analysis. If any abnormality is observed, some additional tests should be performed for the diagnosis. For example, white blood cell count and/or specific protein level from blood and urine samples might be the choice of the doctor. Other than these, there are several medical imaging modalities for the diagnosis of cancer. For example, a segmental or whole-body Computerized Tomography (CT) scan might be preferred. Chest, abdomen, and pelvis included CT scan can be a leading answer to a stage investigation but have low sensitivity especially for lung cancer detection since the chest is moving throughout the CT scan and the accuracy of positioning tumor tissue is getting harder. One another imaging modality is Positron Emission Tomography (PET) can also be used for cancer staging as well. The main disadvantage of PET is that it requires the administration of a radioactive drug. The radioactive drug includes fluorodeoxyglucose, which is basically simple sugar that feeds the tumor cells and accumulates inside to provide radioactive radiation and accurately create an image of the tumor tissue. Moreover, Magnetic Resonance Imaging (MRI) is generally used to detect brain cancer and/or control metastasis status, it is not suitable for all cancer types. On the other hand, cancer diagnosis might require biopsy examinations as well. As mentioned before, tumor tissue is examined under the microscope for histological characterization in order to identify the origin of cancerous cells (Crosby et al. 2022).

The cancer cells can be identified via utilizing nanotechnology as well. Without using radioactive drugs, nanoparticles (NPs) can be modified with specific biomarkers as desired to be able to detect cancer cells with improved accuracy. By employing this method, cost-effective and efficient cancer detection for early stages can be ensured (Choksi et al. 2022). Different imaging results are shown in Figure 3.

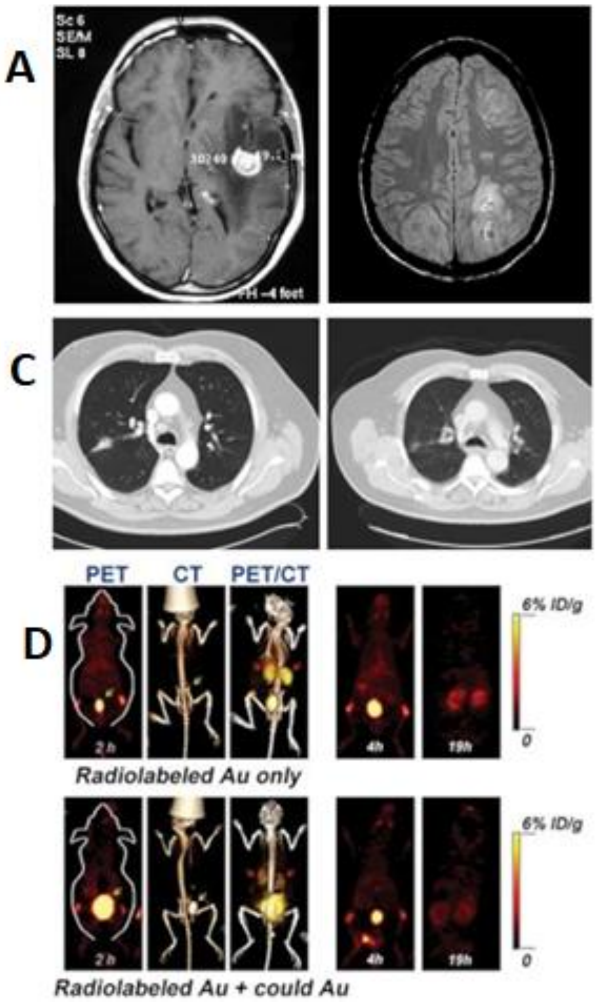

Figure 3. Different Methods to Detect Tumor Tissues (A) CT Scan Results (Idrissi and Ajmi 2014), (B) MRI Scan Results (Idrissi and Ajmi 2014), (C) PET Scan Results (Vansteenkiste and Stroobants 2006), (D) NPs-Assist Analyze Results (Fan et al. 2020).

2.1.5 Cancer treatment. There are some traditional approaches to cancer treatment. To remove the localized tumor clearly, surgery strategy is one of the most common treatment procedures that can only be done for un-risky ones in order not to invade the tumor cells into other tissues. Chemotherapy is another method via certain antineoplastic agent cancer drugs with high side effects such as hair loss, weight loss, and immune system blockers. Lack of targeting of chemo-drugs leads to killing normal cells while killing cancerous cells (Welander, Homesley, and Jobson 1983). Radiation therapy kills tumor cells through high-energy radiation with unnecessary damage to healthy tissues (Baskar et al. 2012). Immunotherapy provides the body fight against infections and other diseases and gives an opportunity for long-term cancer reduction. However, patients may experience a negative impact in the form of severe or potentially fatal allergic reactions (Lahiri et al. 2023).

Besides the traditional approaches, there are some new strategies to treat cancer. One of these strategies is Photodynamic therapy (PDT), so-called photo-radiation therapy that contains a non-invasive cancer-focusing photosensitizer (PS). The PS interacts with a specified wavelength of light and produces reactive oxygen species (ROS) by this interaction with singlet oxygen which leads to cellular death (Bidram et al. 2019).

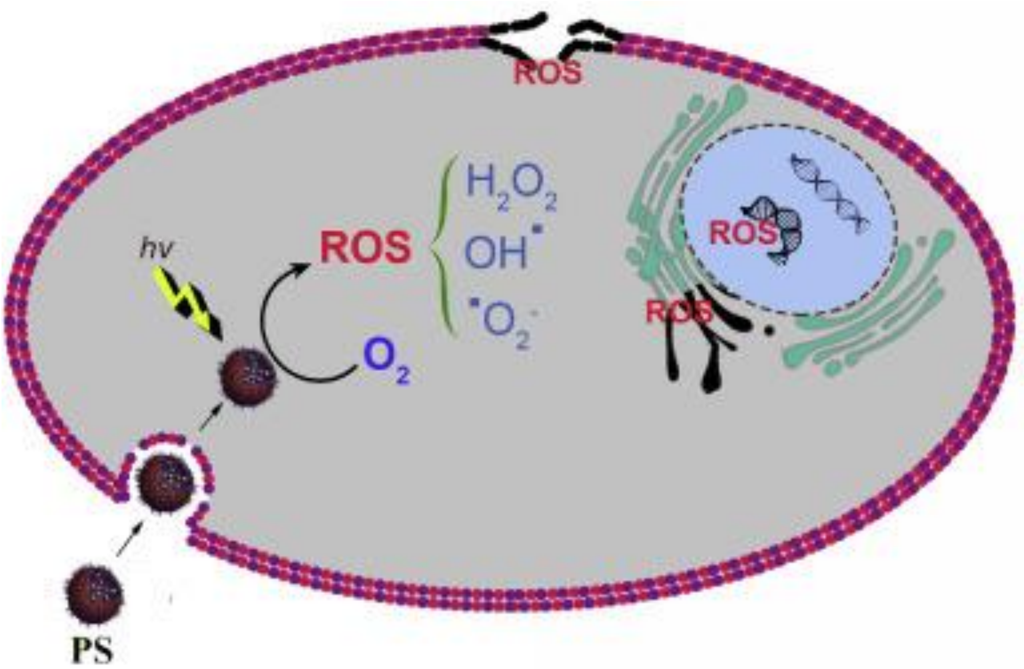

Figure 4. PDT Illustration (Bidram et al. 2019).

In photothermal therapy (PTT), tumor cells are destroyed by heat that NPs gain via radiofrequency, microwaves, magnetic fields, or ultrasound. Generally biocompatible, photo stabilizer agents are utilized to have the potential to absorb light and convert it into heat. Recent studies have reported that metallic NPs such as gold, silver, iron oxide, and carbon-based particles are suggested to have photothermal responses during *in vitro* experiments (Bidram et al. 2019).

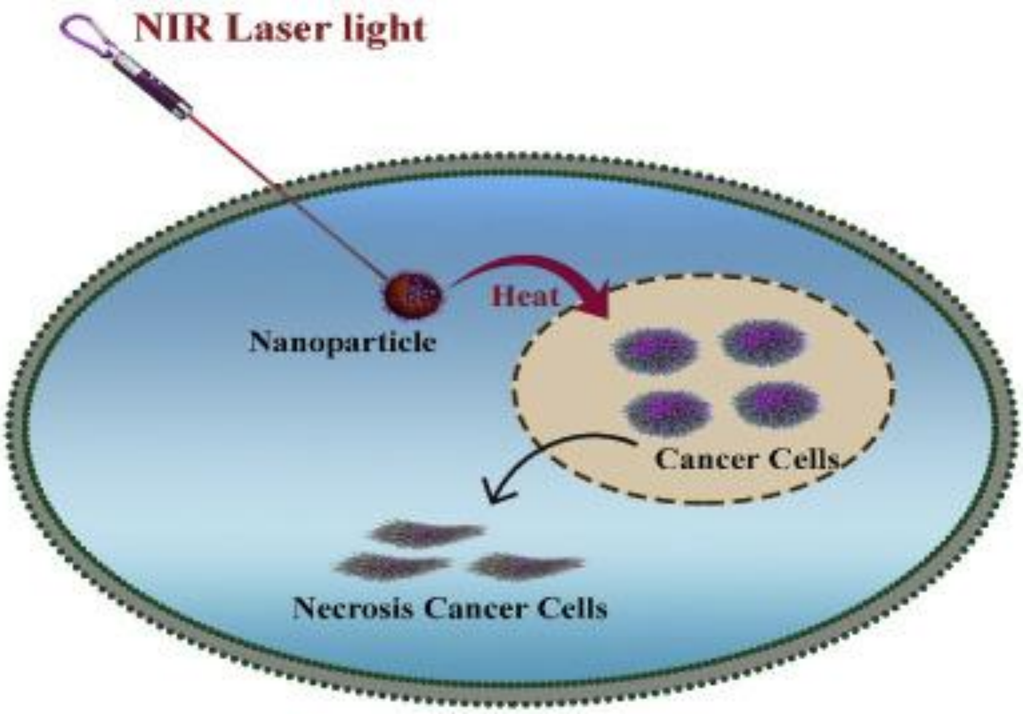

Figure 5. PTT Illustration (Bidram et al. 2019).

2.2 Nanoparticles (NPs)

2.2.1 Definition. NPs are small-scale units with a size range of 1 to 100 nm. After the representation of the term nanotechnology by Nobel laureate physicist Richard P. Feynman in his famous lecture in 1959 ‘There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics’, nanoparticles have gained importance and have been started to be investigated even further (Feynman 1960).

Owing to their extraordinary properties and diverse application paths, nanoparticles have become more popular in many research areas (Mohanraj and Chen 2007).

2.2.2 Classification of NPs. NPs are separated into many categories with respect to their size, shape, color, and surface properties. These features of different nanoparticles vary due to their manufacturing procedures. For instance, in the preparation stage, pH change for a specific procedure can differ in the size and/or shape of the nanoparticle produced. When the purpose of utilization of nanoparticles changes, different ligands can be added to the procedure and make them non-toxic, biocompatible, etc. The surface-to-volume ratio of NPs is essential for the application areas. As the size of the particle decreases, the number of atoms on the surface increases accordingly and this might amplify the activity and efficiency of the particles produces (Saadbin Khan and Hossain 2022).

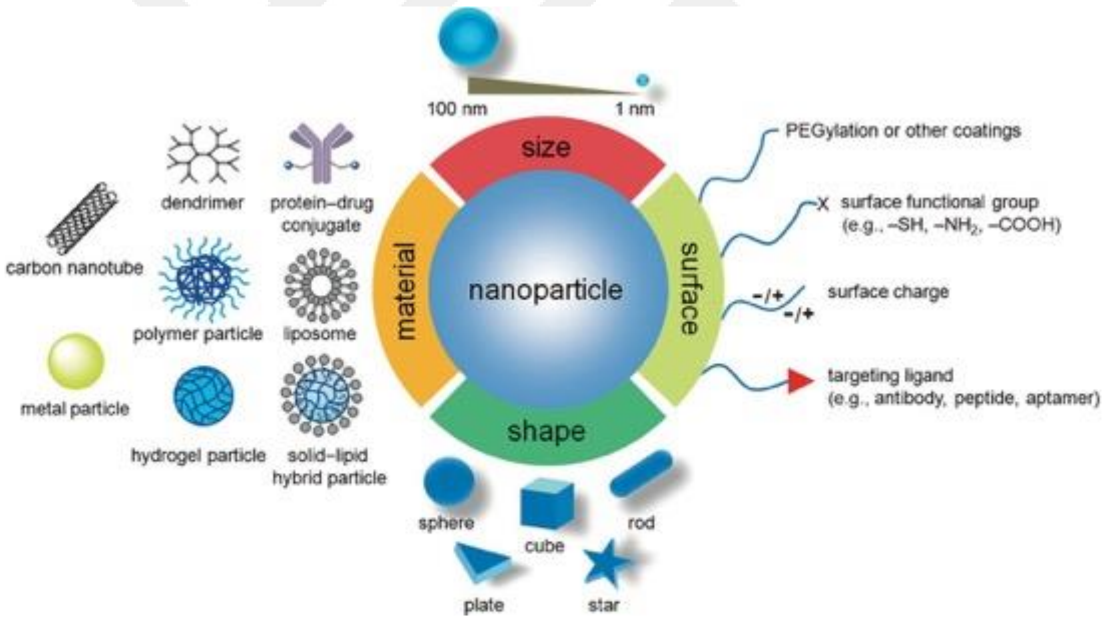

Figure 6. Nanoparticle (NP) Categorization Depending on Their Material, Size, Shape, And Surface (Sun et al. 2014).

NPs can be divided into many groups; herein, organic and inorganic NPs will be explained.

2.2.2.1 Organic NPs. NPs that are made from carbon-based molecules are called organic ones which are aggregated molecules or polymers such as lipids, proteins, and sugars. Biocompatible organic NPs are classified as biocompatible units that can be easily applicable in biomedical fields such as drug delivery systems.

2.2.2.1.1 Micelles. Micelles are nanoparticles formed from amphiphilic molecules (molecules that have both hydrophilic and hydrophobic regions) when they are dispersed in a suitable solvent, usually in aqueous solutions. These molecules arrange themselves in such a way that their hydrophobic portions group together in the center of the particle, shielded from the surrounding solvent by the hydrophilic portions that face externally. The formation of micelles is driven by the hydrophobic effect, which is the tendency of hydrophobic molecules to aggregate in water in order to minimize contact with the aqueous environment (Pawar et al. 2022). This self-assembly results in the formation of spherical or roughly spherical nanoparticles with a core-shell structure. There are various types of micelles such as pH, temperature, enzyme, and magnetism-responsive (Junnuthula et al. 2022). For breast cancer treatment, a pH-responsive core-shell drug-loaded micelle structure was used in *in vitro* studies. The results were promising to enhance antitumor activity in the acidic tumor microenvironment with good biocompatibility (Mehnath et al. 2020).

2.2.2.1.2 Liposome. Liposome nanoparticles are a type of nanoscale drug delivery system that consists of lipid bilayers, mimicking cell membranes, to encapsulate and deliver various therapeutic agents such as drugs, genes, or vaccines to specific target cells or tissues in the body. These nanoparticles have gained significant attention in the field of medicine and pharmaceuticals due to their potential to improve the efficacy and safety of drug delivery. Liposomes are used in drug delivery systems by targeting tumor cells that accumulate on the surface and controlled-release drugs. Breast cancer is known for the high expression of estrogen receptors and epidermal growth factor receptors which can be targeted by modified liposomes (Alavi and Hamidi 2019). Bharti et al. showed significant results with Polyethylene Glycol (PEG) -coated liposomes, a substantial impact on cell division, and angiogenesis of breast tumor cells (Bharti et al. 2017).

2.2.2.1.3 Dendrimer. Dendrimer nanoparticles are a type of nanoscale material that exhibit unique and well-defined structural properties. They are constructed using a branching or tree-like architecture, with a central core and multiple layers of branching molecules radiating outwards. Three parts for dendrimers are core, branch, and end groups. These features allow to use dendrimers in various areas of biomedical. For instance, at the same time, it can include drugs on one side while carrying the target part on the other side (Hou et al. 2016). The multifunctional nature of dendrimers allows researchers to image, target, and treat tumor tissues by drug delivery. The branch structure is allowing dendrimers to bind with other molecules for better CT scans. Zhu et al. modified dendrimers with Gold (Au) NPs and the results are showing better X-ray attenuation properties rather than commercially used agents such as Omnipaque (Zhu et al. 2015).

2.2.2.1.4 Polymeric NPs. Polymeric NPs are small-sized particles made from polymers, which are large molecules composed of repeating subunits. These nanoparticles have gained significant attention in various fields, including medicine, materials science, and engineering, due to their unique properties and potential applications (Zielinska et al. 2020). Wu et al. studies *in vitro* and *in vivo* showed significant anticancer agents' active mechanism on only colorectal cancer cells via targeted delivery with drug-loaded polymeric NPs. The formulation of drug-loaded polymeric NPs allows the reduction of chemoresistance of colorectal cancer cells by increasing oxygen levels (P. Wu et al. 2020).

2.2.2.1.5 Solid lipid and nanostructured based. Solid lipid NPs are colloidal lipid-based nanoparticles that are composed of biocompatible and biodegradable solid lipids. The main feature is the use of solid lipids as the matrix material, which provides structural stability and improved drug loading capacity. Solid lipid NPs have a crystalline structure and can encapsulate both hydrophilic and lipophilic drugs which have gained attention in recent years (Mukherjee, Ray, and Thakur 2009).

Nanostructured-based NPs consist of a mixture of solid lipids and liquid lipids or oil, forming a structured matrix. This mixture of lipids prevents the formation of large lipid crystals, resulting in a more amorphous structure. This structural flexibility offers benefits in terms of drug loading capacity and controlled release. The inclusion of liquid lipids helps in accommodating hydrophobic drugs more effectively and reducing the risk of drug expulsion during storage (Jeevanandam et al. 2018).

2.2.2.2 Inorganic NPs. NPs which configure as non-carbon-based molecules are called inorganic ones which include metals like metal salts, metal oxides, Au NPs, magnetic NPs, quantum dots, and ceramics such as silica particles.

2.2.2.2.1 Carbon-based. Carbon-based nanoparticles are nanoscale structures made primarily from carbon atoms. They have unique properties and a wide range of applications in various fields, including materials science, medicine, electronics, and environmental remediation. These nanoparticles can be classified into several categories based on their structures, properties, and synthesis methods. There are a few notable types of carbon-based nanoparticles such as fullerenes, carbon nanotubes, graphene, etc. (Holmannova et al. 2022) One of the uses of carbon-based nanoparticles is the first photoacoustic measurements. Reported research showed the long-term circulation time of carbon nanoparticles in body imaging of rats. Dubky et al. conclude that elevated capacity to convert absorbed light into heat can open the doors for PTT of cancer (Dubyk et al. 2022).

2.2.2.2.2 Metal NPs. Due to their small size and unique properties, metal nanoparticles exhibit novel behaviors and characteristics that differ from bulk materials of the same composition. The size and shape of metal nanoparticles significantly influence their properties. Small changes in size and shape can result in altered electronic, optical, and catalytic properties. For example, gold nanoparticles can appear red, blue, or green depending on their size and shape due to the localized Surface Plasmon Resonance (SPR) effect (Fathi, Rashidi, and Omidi 2019). One of the most prominent properties of metal nanoparticles is the SPR phenomenon. When exposed to light, the electrons in metal nanoparticles oscillate collectively, leading to enhanced electromagnetic fields near the nanoparticle surface. This property finds applications in sensing, imaging, and PTT. For example, in biomedical applications, Au NPs are used in cancer therapies such as PTT, where they absorb light and convert it into heat to selectively destroy cancer cells where NPs accumulate (Hu et al. 2020).

2.2.2.2.3 Ceramic NPs. Ceramic NPs are solid, inorganic materials that are typically composed of metallic and non-metallic elements, often held together by ionic or covalent bonds. They are known for their hardness, high melting points, and excellent thermal and electrical isolating properties. Ceramic NPs are known as biocompatible and therefore can be used in medical applications such as bone tissues, dental implants, and drug delivery systems. Ceramic NPs can be used as a coating agent as well to resist corrosion and protection from unexpected temperature differences. Specific ceramic nanoparticles can be used in lasers and optical sensors because of their ability to emit or absorb light at specific wavelengths (S. Thomas et al. 2015).

2.2.2.2.4 Semiconductors and quantum dots. Semiconductor nanoparticles and quantum dots are made from semiconductor materials that exhibit unique optical and electronic properties due to their nanoscale size and quantum mechanical effects. These nanoparticles are typically composed of materials like Cadmium Selenide (CdSe), Lead Sulfide (PdS), or other semiconducting compounds. Quantum dots are semiconductor crystals that are generally sized 1-10 nm. Ding et al. synthesized Cadmium-free quantum dots with a Zinc gradient to perform fluorescence MR imaging of mouse tumors as shown in Figure 7 (Ding et al. 2014). The research has shown the high stability of quantum dots for the optical tracing of cells and biological molecules over a long time period (Vu et al. 2015).

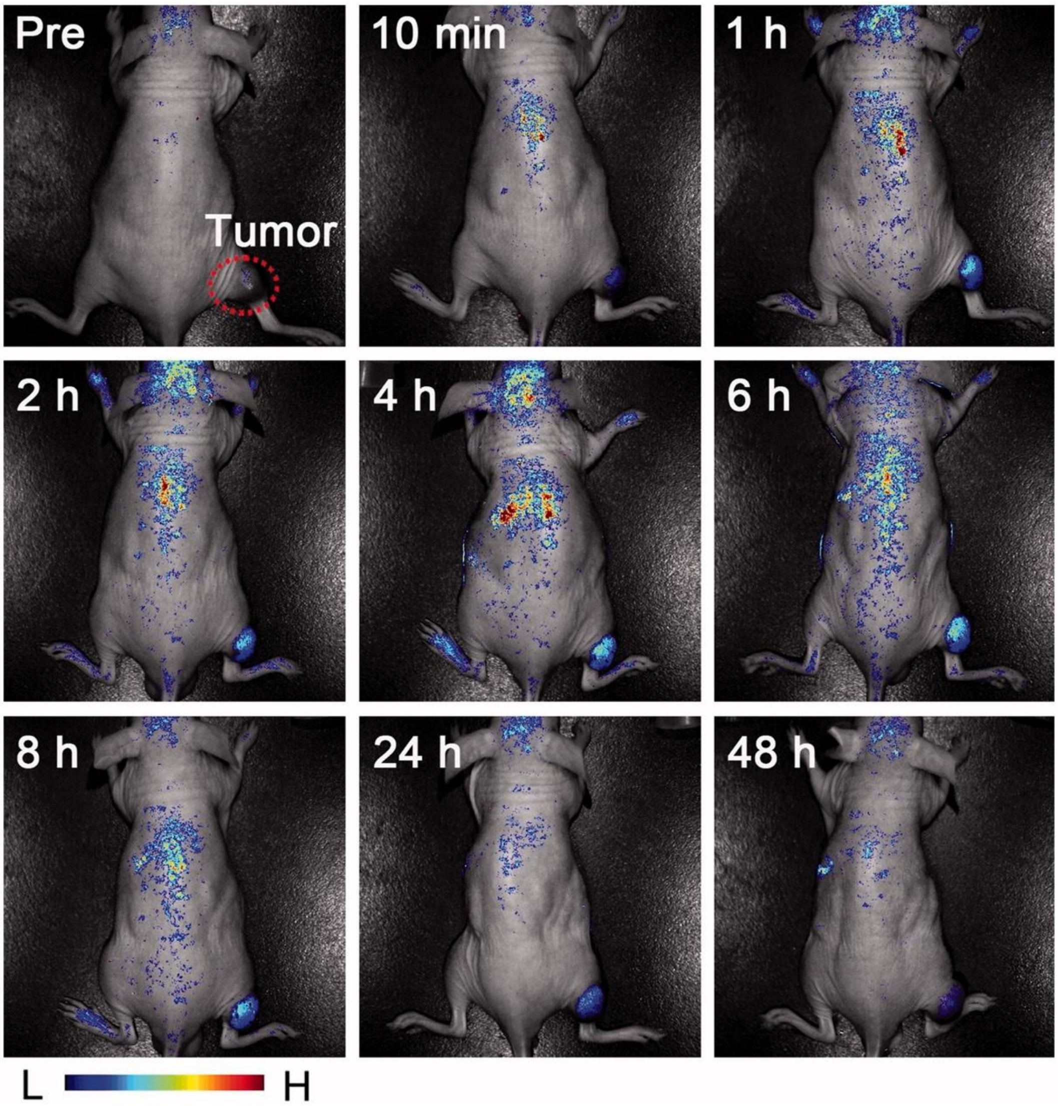

Figure 7. In vivo Fluorescence MR Results of a Mouse With Tumor Tissue Within 48 Hours (Ding et al. 2014).

2.2.2.2.5 Magnetic NPs. Magnetic NPs are typically ranging in size from a few nanometers to several tens of nanometers, that exhibit magnetic properties. These nanoparticles are composed of magnetic materials, often ferromagnetic or superparamagnetic materials, which means they can become magnetized when exposed to an external magnetic field and lose their magnetization when the field is removed. Biomedical application of magnetic NPs is used as contrast agents in MRI; functionalized with drugs to target specific cancer areas using an external magnetic field in drug delivery; generating heat when exposed to alternating magnetic field in hyperthermia therapy. Develop sensitive biosensors to detect specific molecules and substances such as detecting cancer in their early stages by sensing CTCs in microfluidic biosensors (Materón et al. 2021; Mittal, Roy, and Gandhi 2022).

2.2.3 Applications of NPs. NPs are opening various biomedical application areas owing to their adaptable properties via modifications. Below are some examples of the applications of NPs in the medical field.

2.2.3.1 Drug delivery. In recent years, the interest in developing novel drug delivery systems using drug carriers exponentiates. With their high constancy, sustainability, drug loading capacity, controlled drug release, and capability to carry both hydrophilic and hydrophobic drugs. In this matter, NPs are the best fit for the application. The drug release has been developed over time and regulated as slow release by time or triggered by pH change, heat, and stimulated light (De Jong and Borm 2008). Targeted delivery systems can be used in gene therapy, cell sorting, and cancer therapy (Ren et al. 2021; Xia et al. 2021).

The commonly and efficiently used NPs are Iron(II, III) oxide (Fe_3O_4), super magnetic iron oxide NPs (SPIONs), fullerenes, and carbon nanotubes by their surface properties and specific shapes (Rahimi and Doostmohammadi 2020). Being smaller than the blood vessels, and cells, NP application divides into two: passive and active targeting.

2.2.3.1.1 Passive targeting. Passive targeting takes advantage of the natural properties of tumor tissues to selectively accumulate therapeutic agents. Tumor tissues often have abnormal blood vessel structures and poor lymphatic drainage, leading to a phenomenon called the "enhanced permeability and retention" (EPR) effect (J. Wu 2021). This effect allows nanoparticles and drugs to accumulate in the tumor tissue due to increased vascular permeability and reduced clearance from the tumor site. Passive targeting typically involves the use of nanoparticle-based drug delivery systems, such as liposomes, and micelles NPs, which are designed to release their cargo over time at the tumor site. These nanoparticles can be engineered to carry chemotherapy drugs, therapeutic proteins, or nucleic acids that target specific cancer-related genes (Subhan et al. 2021).

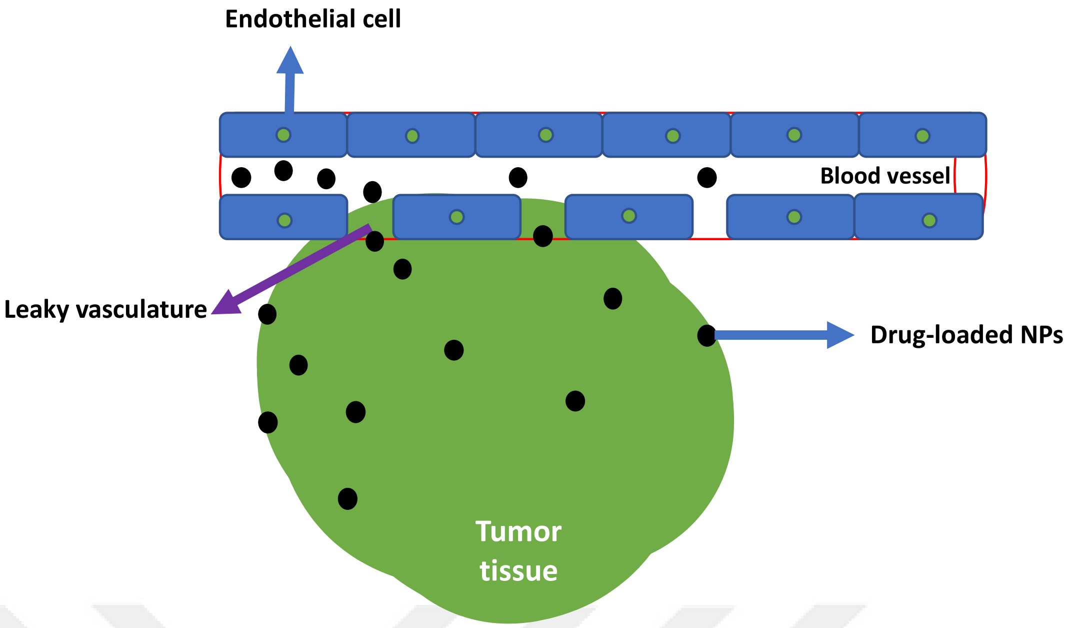

Figure 8. Illustration of Passive Targeting.

2.2.3.1.2 Active targeting. Active targeting is more specific to cancerous cells. The specifically modified nanoparticles with ligands can bind to the receptors and/or antigens of cancer cells. These types of ligands can be recognized by the surface of cancer cells and lead the way for cell death via releasing drugs and/or laser treatment to increase the temperature (Feng et al. 2022). The ligands used for active targeting can be antibodies, peptides, aptamers, or small molecules that have a high affinity for the cancer cells' unique receptors. By using active targeting, drug delivery systems can improve the selectivity and efficiency of drug delivery to cancer cells, reducing the damage to healthy tissues (Gumala and Sutriyo 2022).

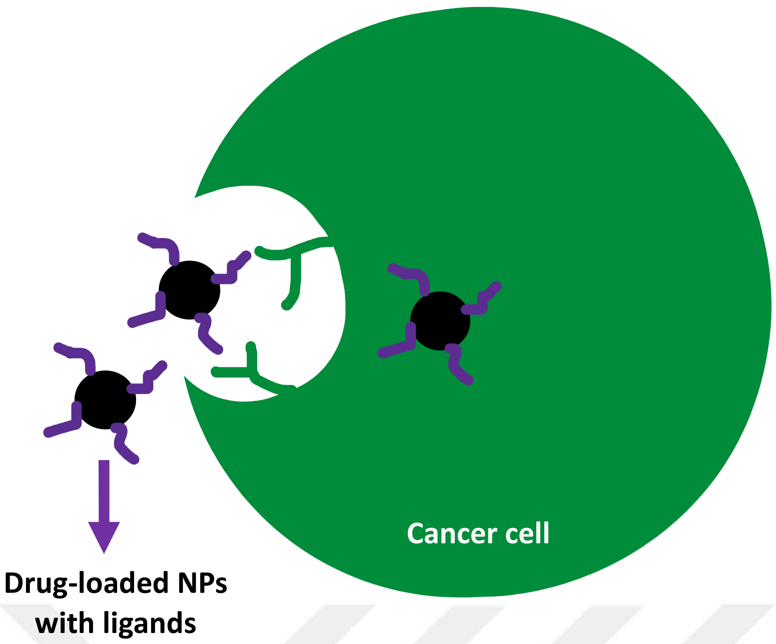

Figure 9. Illustration of Active Targeting.

Passive and active targeting strategies can significantly enhance the accumulation of therapeutic agents at the tumor site while sparing normal tissues. This can lead to improved treatment outcomes and reduced side effects in cancer patients (Alavi and Hamidi 2019).

2.2.3.2 Anti-bacterial agent. The emerging need for novel antibacterial agent development is becoming a serious public health problem since bacterial strain resistance is increasing. Metal, organic, and modified inorganic NPs can be used for antibacterial purposes. For instance, by covering inorganic NPs with organic compounds such as hydrogels, PEG, etc., biocompatibility will be ensured. The principle of antimicrobial activity is to induce ROS generation in cells which affects DNA and proteins and leads to cell death by excess radical production. Especially silver NPs are used as an antibacterial agent (Selem et al. 2022).

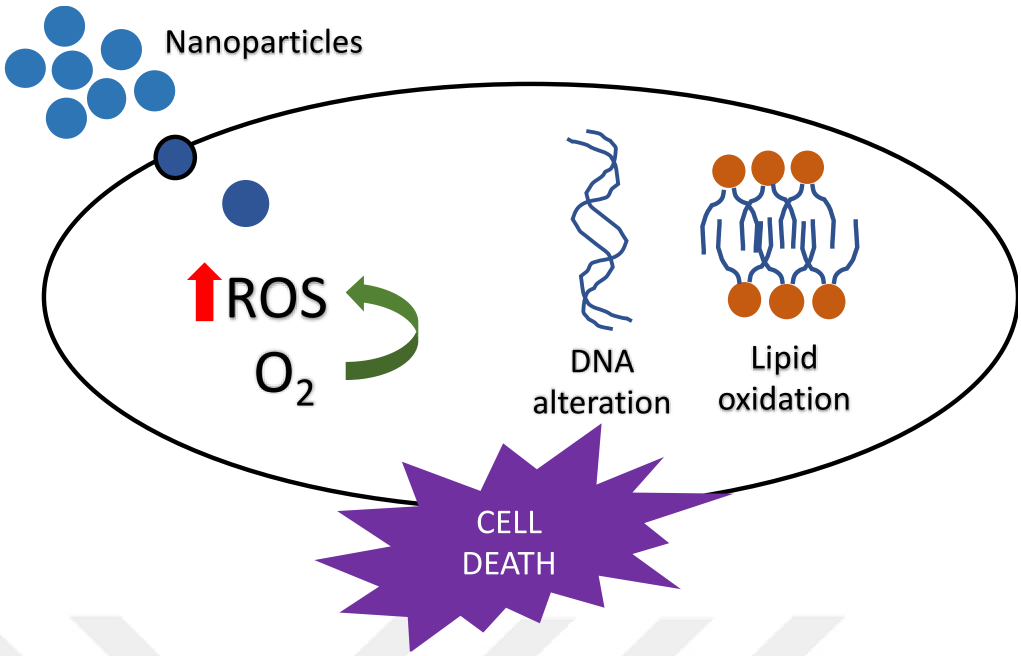

Figure 10. The Illustration of Cell Death by the Antibacterial Effect.

2.2.3.3 Wound Healing Agent. Polymeric NPs, which are categorized as biocompatible, are used as wound healing agents due to their stimulation of cell proliferation via re-epithelization. Therapeutic agents can be placed inside polymeric NPs because hydrophilic and hydrophobic parts can be able to trap the agents through the accurate release position. Polymers such as collagen, lipids, polyesters, and self-assembled peptides are suitable for wound dressing owing to their extracellular matrix (ECM) mimicking ability (Augustine et al. 2020). Metallic and ion-release NPs show antimicrobial features as well as promote wound healing. The important part is to cover the NPs before utilizing them to ensure non-toxicity (Loo et al. 2022).

It has been reported in the literature that cellulose nanocrystal matrix with Silver Nitrate ($AgNO_3$) NPs has shown promising results in healing chronic wounds of diabetic patients. The wound healing process had similar effects as that of the antibacterial agents with an increase in collagen deposition, angiogenesis, and re-epithelization observation (Blanco-Fernandez et al. 2021; W. Wang et al. 2019).

2.2.3.4 Catalytical Agent. Catalytical agents' main roles are to support the reaction routes such as decreasing activation energy without changing chemical configuration. As per the same principle, nanoparticles offer several advantages over traditional catalysts, including increased surface area, and enhanced reactivity. Nanoparticles have a high surface-to-volume ratio which allows for activating more site areas and promoting efficient interactions between the catalyst and reactants. Exponentiates the rate of reaction via their small size with a higher concentration of atoms/molecules at the surface. Absorption and activation of reactant molecules accelerate which leads to an improved catalytic performance (Fouladi-Fard et al. 2022).

2.2.3.5 Diagnostic Agent. NPs have gained significant properties in recent years and elevated their unicity and capabilities. As diagnostic agents, NPs offer substantial advantages like enhanced sensitivity, targeted delivery, and imaging contrast agents. NP-based tumor targeting contrast agent with enhanced sensitivity and specificity for tumor imaging enabling early detection of metastases (Naseri et al. 2018; X. Wang et al. 2008). The most essential part for NP use as targeting agents is the coating process to prevent aggregation and not contact with proteins by creating neutral and hydrophilic surfaces. For *in vivo* applications polymeric materials such as PEG, chitosan, and starch are used. After coating, selected NPs can be used with less toxic effects. Liposomes, micelles, polymeric NPs, and Au NPs can be used as contrast agents preferred over Gadolinium ion complexes in novel approaches. Au NPs are gaining attention as an X-ray contrast agent for their biocompatibility and targeting tumor cells by EPR effect and have high X-ray absorption which is proper for CT imaging (Giljohann et al. 2010; Lee et al. 2013). For Au NPs both passive and active targeting are applicable. As explained in drug delivery, passive targeting is the accumulation of Au NPs in tumor tissue by crossing through leaky vasculature (Albanese, Tang, and Chan 2012). X-ray attenuation increases on tumor tissue with Au NP presence on the surface (Hayashi et al. 2013; Reuveni et al. 2011).

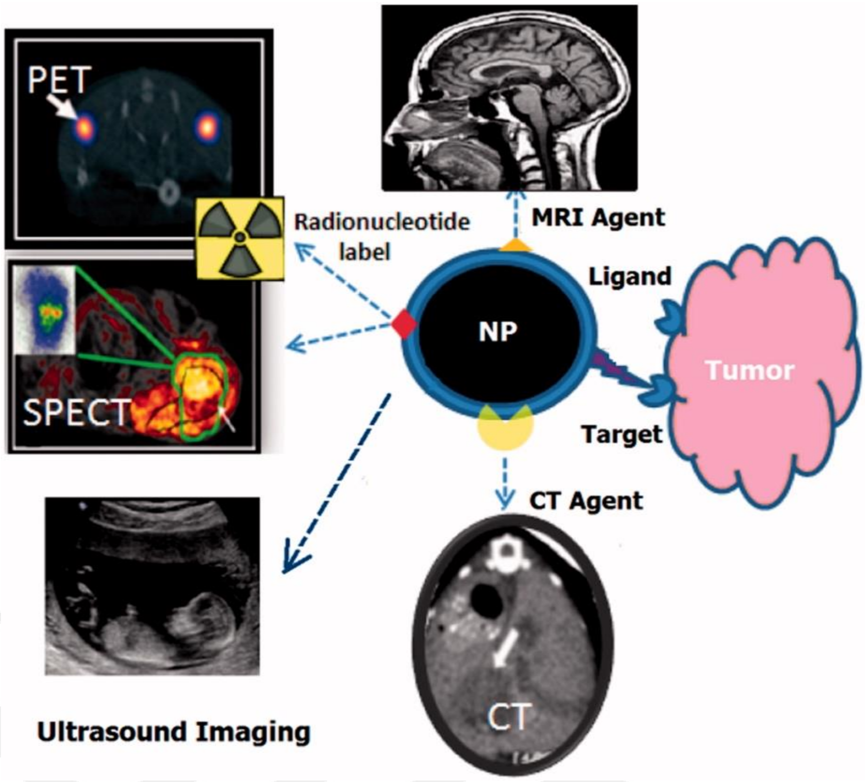

Figure 11. The Main Usages of NPs in Different Modalities of Imaging (R. Thomas, Park, and Jeong 2013).

2.2.3.6 Biosensor. The sensitivity, accuracy, and precision of a biosensor depend on its size, shape, and surface charge. The main principle of biosensors is to have functionalized thin film with bonded bioreceptors. The signal intensity increases when the analyte bonds with the bioreceptor. Most extensively used gold NP-based biosensors are analyte-sensitive due to their biocompatibility, unique electronic properties, and comparatively simple manufacture and adaptation steps. As mentioned before, high surface-to-volume is essential for application areas of NPs at which Au NPs have significantly higher surface energy than other NPs. Au NPs can promote rapid and direct electron transfer between a broad range of electrode resources. Au NPs utilized as signal amplification tags owing to their light-scattering properties and large augmentation ability of local electromagnetic fields (Banerjee, Maity, and Mastrangelo 2021).

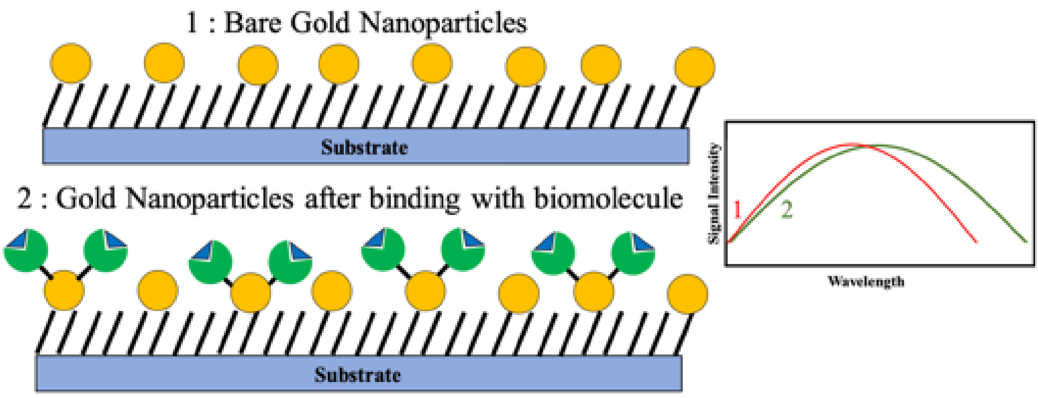

Figure 12. An Illustration of an Au-NP-Based Biosensor that Captured Analyte Molecules (Banerjee, Maity, and Mastrangelo 2021).

2.2.4 Preparation. Synthesizing nanoparticles involves creating nanoscale materials with specific properties and characteristics for various applications, ranging from medicine to electronics. The process typically involves controlling the size, shape, composition, and surface properties of the nanoparticles. Selecting materials, methods, wanted size-shape and surface functionalization are essential for specific areas of interest. The preparation of nanoparticles can be separated into two main groups. Top-down and bottom-up synthesizing methods are represented in Figure 13.

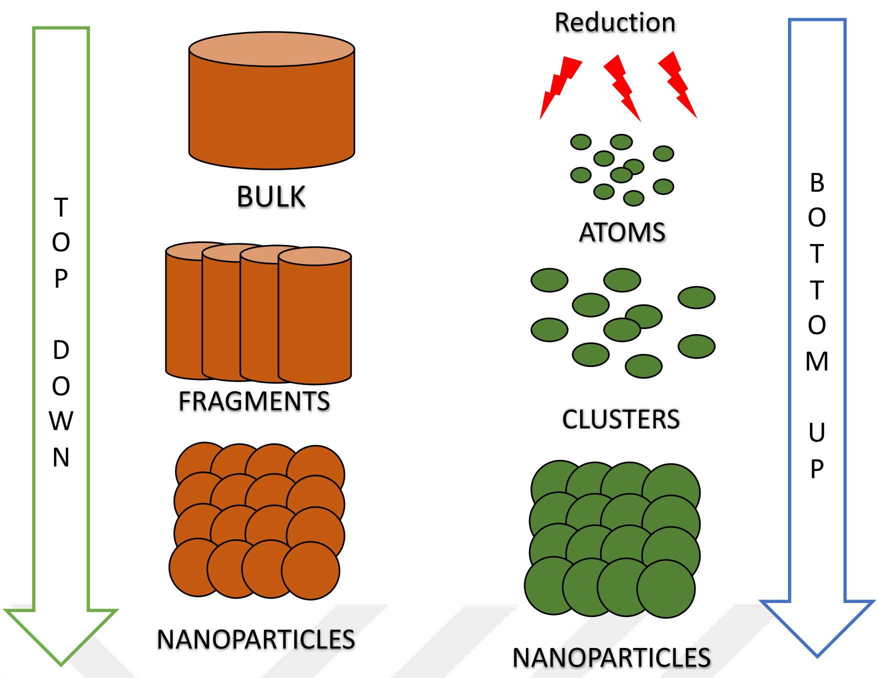

Figure 13. Nanoparticle Preparation Approaches' Illustration.

2.2.4.1 Top-Down Synthesis. Top-down is described as shredding bulky materials into small-sized fragments which leads the nanoparticle formation. The most efficient and simple approach is the ball milling process. Top-down synthesis of nanoparticles refers to a set of techniques used to produce nanoparticles by breaking down larger materials into smaller particles. This approach is particularly useful when working with materials that are difficult to synthesize using bottom-up methods, where nanoparticles are built up from smaller components (Baig et al. 2021). Some of the common methods are laser ablation ball milling, sputtering, and electron beam lithography. Laser ablation is based on a high-energy laser-focused on a target material, causing it to vaporize and form nanoparticles. The vaporized material condenses to form nanoparticles as it cools down (Kim et al. 2017). Ball milling is a technique to grind and mill bulk materials into smaller ones as nano-micro particles (Ullah, Ali, and Hamid 2014). Sputtering involves bombarding a target material with high-energy ions which causes atoms to be emitted from the surface of the target material. These emitted atoms then deposit onto a substrate as nanoparticles (Okoye et al. 2023). Electron beam lithography uses a focused electron beam to selectively remove materials from a substrate and leave nanoparticles behind (Fu et al. 2018).

2.2.4.2 Bottom-Up Synthesis. Bottom-up synthesis is a strategy used in nanotechnology to create nanoparticles and other nanoscale materials by building them from smaller components or molecules. This approach is the opposite of top-down synthesis, where larger materials are broken down into smaller pieces. In the context of nanoparticle synthesis, bottom-up methods involve the controlled assembly of atoms, molecules, or ions to form nanoparticles. These methods offer precise control over the size, shape, composition, and properties of the nanoparticles being created (Indiarto et al. 2022). The growth and self-assembly of atoms and molecules into clusters lead the nanoparticle formation. The most common bottom-up syntheses are chemical precipitation, Sol-Gel process, self-assembly, and electrochemical synthesis. Chemical precipitation is the controlled reaction of precursor chemicals in a solution to form nanoparticles as the products precipitate out of the solution. Different temperatures, concentrations, and pH levels differ in the nanoparticle size and morphology hence, with different conditions (even different scientists with the same formulations) the error of accurate nanoparticle production is high (Li et al. 2018). Sol-Gel is a technique in which a solution or sol containing precursor molecules is polymerized to form a gel, which is then dried or heat-treated to obtain nanoparticles. The sol-gel process is widely used to create nanoparticles with controlled compositions (Al-luhaibi and Sendi 2022). Self-assembly involves the spontaneous organization of molecules or particles into ordered structures due to specific interactions between the components. This method is often used to create nanoparticles with well-defined structures and properties (Mishra and Ekielski 2019). Electrochemical methods involve using electrical currents to drive chemical reactions that lead to the formation of nanoparticles (Lebedeva, Kultin, and Kustov 2021).

2.2.5 Characterization. Characterization of nanoparticles involves the comprehensive analysis and understanding of their physical, chemical, structural, and morphological properties. Nanoparticles are incredibly small particles with dimensions typically in the range of 1 to 100 nanometers. Due to their unique size-dependent properties, characterizing nanoparticles is crucial for a wide range of applications in fields such as materials science, medicine, electronics, and environmental science. Several techniques are commonly used for nanoparticle characterization (Mittu 2016).

2.2.5.1 X-ray Diffraction (XRD). It is a technique used to analyze the structure of crystalline materials by measuring the scattering pattern of X-rays as they interact with the atomic arrangement of the material. XRD is widely used in various scientific disciplines, including solid-state physics, chemistry, materials science, geology, and biology. In XRD analysis, a sample is bombarded with a beam of X-rays, and the X-rays are diffracted by the atoms in the crystal lattice. The diffracted X-rays produce a unique pattern of constructive and destructive interference, which can be captured on a detector and converted into a diffraction pattern. From this diffraction pattern, valuable information about the atomic arrangement, crystal structure, lattice parameters, and other characteristics of the material can be obtained. XRD can determine the crystal structure of a wide range of materials, including metals, minerals, ceramics, polymers, and organic compounds. By comparing the experimental diffraction pattern with known patterns from a database, scientists can identify the crystalline phases present in a sample and determine the orientation, size, and shape of the crystals. This information is essential for understanding the physical, chemical, and mechanical properties of materials, as well as for quality control and the development of new materials with specific properties (Ameh 2019; Jain 2022).

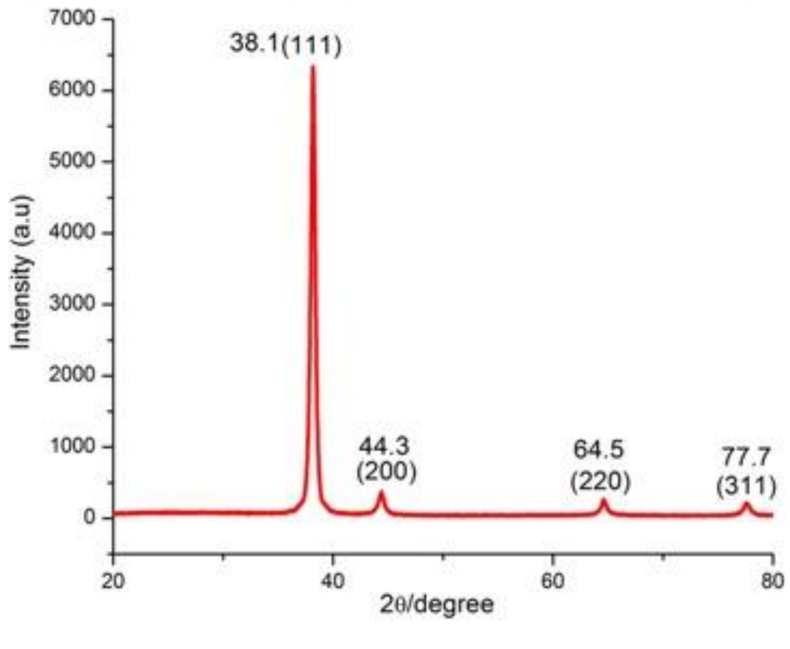

Figure 14. XRD Analysis for Au NPs (Krishnamurthy et al. 2014).

2.2.5.2 Scanning Electron Microscopy (SEM). SEM is a powerful magnification technique that is used to image the surface of samples at very high magnifications. It is an advanced type of electron microscope that uses a focused beam of electrons to generate detailed images of the sample. In an SEM, a beam of electrons is produced by an electron gun and accelerated to high energy. This beam is then focused onto the sample using electromagnetic lenses. When the electron beam interacts with the sample's surface, various signals are generated. These signals include secondary electrons, backscattered electrons, and characteristic X-rays. The secondary electrons are emitted from the surface of the sample and provide information about the topography and composition of the material. Backscattered electrons are electrons that are deflected from the sample's atoms at high angles and are sensitive to the atomic number of the elements in the sample. Characteristic X-rays are emitted when electrons from the beam interact with the inner shells of atoms in the sample. The signals produced by these interactions are detected by specialized detectors in the SEM. The detected signals are then used to create an image of the sample's surface. The SEM can produce highly detailed and three-dimensional images of the sample at magnifications ranging from a few times to several hundred thousand times (Mohammed and Abdullah 2018).

In addition to imaging, scanning electron microscopes can also be equipped with various accessories and detectors for additional analysis. For example, energy-dispersive X-ray spectroscopy (EDS) detectors can be used to analyze the elemental composition of the sample. Electron backscatter diffraction (EBSD) detectors can provide information about the crystal structure and orientation of the sample.

SEM is widely used in various scientific fields, including materials science, biology, geology, and nanotechnology. It allows researchers to examine the surface morphology, composition, and structural characteristics of a wide range of samples at a high level of detail, contributing to advancements in numerous fields of study.

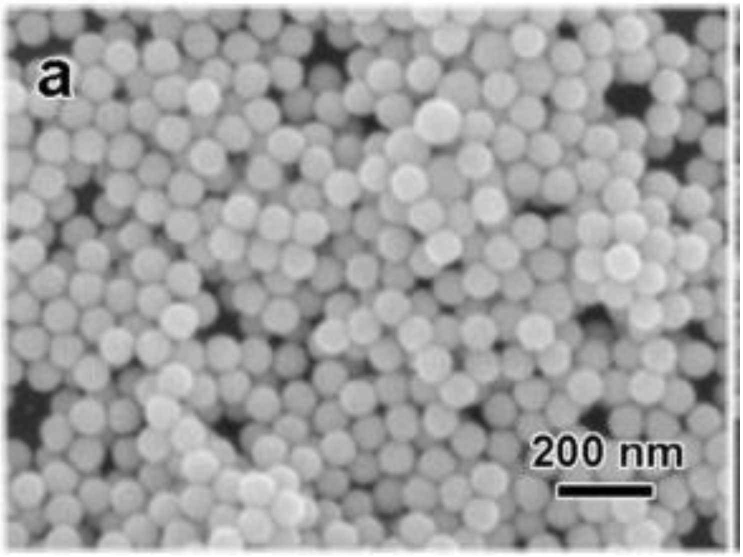

Figure 15. SEM Analysis for the Au NPs (I. Khan, Saeed, and Khan 2019).

2.2.5.3 Transmission Electron Microscopy (TEM). TEM is a powerful imaging technique that allows for the high-resolution visualization of the internal structure of materials at the nanoscale. It is a type of microscopy that uses a beam of electrons instead of light to probe the specimen, enabling much higher magnification and resolution compared to traditional light microscopy. In a transmission electron microscope, a beam of electrons is generated by an electron source, typically a heated filament or a field emission gun. The electron beam is accelerated to high energies using electromagnetic fields and focused onto the specimen using magnetic lenses. The electrons pass through the thin specimen, and their interaction with the specimen's atoms and their electrons generates signals that can be used to form an image. As the name suggests, in TEM, the electrons transmitted through the specimen are used to form the image. These transmitted electrons carry valuable information about the specimen's structure and composition. They interact with the specimen through various processes, including scattering, diffraction, and absorption, which can be detected and recorded. A detector placed on the opposite side of the specimen collects the transmitted electrons, and their intensity is used to form a two-dimensional image. By manipulating the electron beam and using different types of detectors, TEM can provide information about the sample's morphology, crystal structure, chemical composition, and even elemental mapping at the atomic scale (Jian et al. 2021).

TEM has revolutionized our understanding of materials science, nanotechnology, biology, and many other fields. It has been instrumental in studying the structure and properties of materials such as metals, ceramics, polymers, nanoparticles, biological samples, and even individual atoms. The technique has widespread applications in research, development, and quality control across various disciplines, contributing to advancements in materials science, nanotechnology, medicine, and other areas of scientific exploration (Rattanawongwiboon et al. 2022).

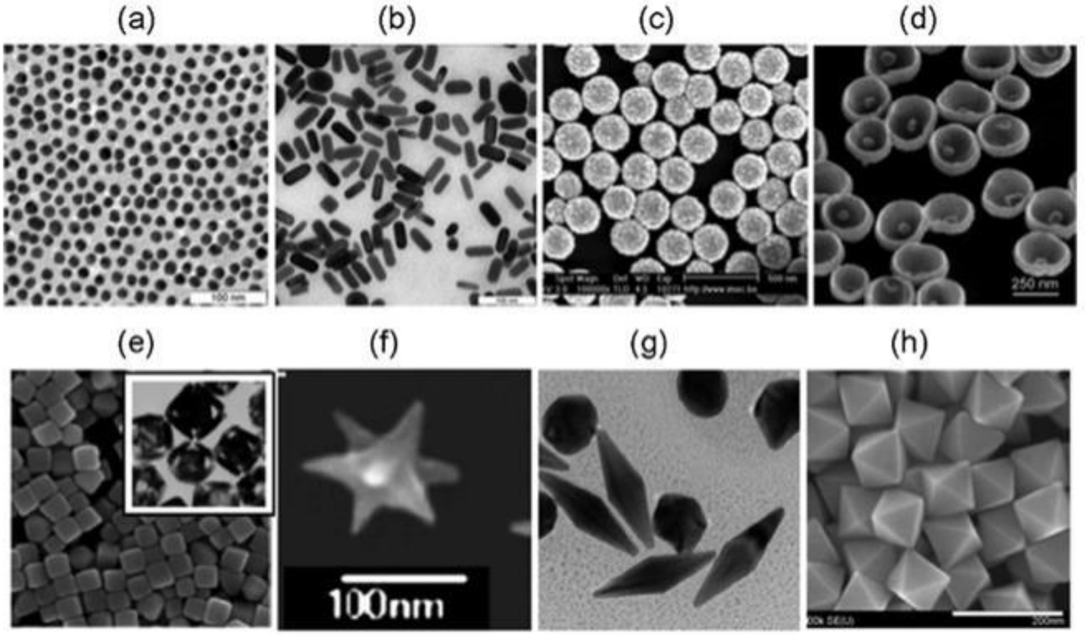

Figure 16. TEM Analysis for Various Forms of Au NPs (N. Khlebtsov and Dykman 2010; N. Khlebtsov and Dykmana 2011; N. G. Khlebtsov and Dykman 2010).

2.2.5.4 Fourier Transform Infrared Spectroscopy (FTIR). It is a technique used to analyze a material's infrared (IR) absorption or emission. IR radiation is passed through a sample, and the resulting spectrum is used to identify the material's chemical bonds and functional groups. In FTIR spectroscopy, the sample is exposed to a wide range of infrared wavelengths. The instrument measures the intensity of the transmitted or absorbed light as a function of wavelength. This data is then mathematically processed using Fourier transformation to obtain a high-resolution infrared spectrum. The IR spectrum obtained from FTIR spectroscopy provides information about the molecular composition of the sample. Different chemical bonds absorb specific wavelengths of infrared radiation, resulting in characteristic peaks in the spectrum. By comparing these peaks with known spectra of various compounds, scientists can identify the presence of specific functional groups or compounds in the sample (Baudot, Tan, and Kong 2010).

FTIR spectroscopy has applications in various fields, including chemistry, materials science, pharmaceuticals, forensics, environmental analysis, and more. It is a powerful analytical tool for identifying unknown substances, characterizing polymers, studying chemical reactions, and monitoring industrial processes (Eid 2022).

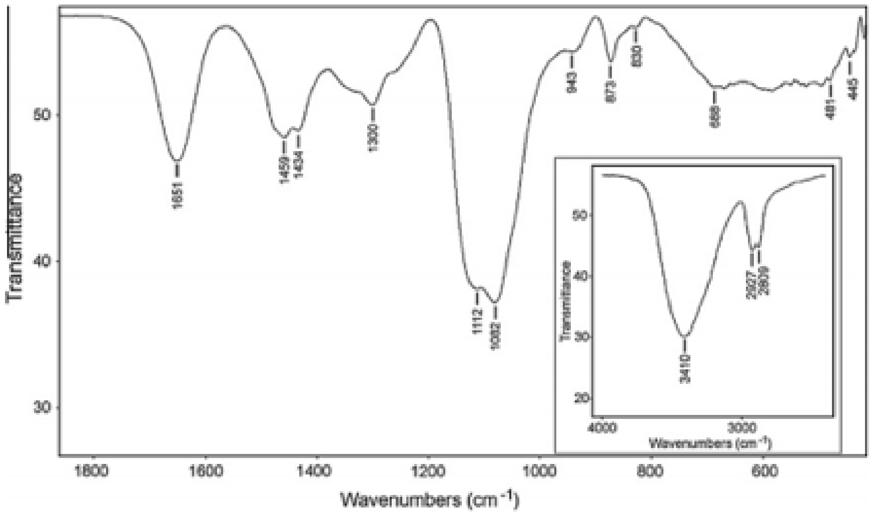

Figure 17. FTIR Analysis of Carbon Nanotubes (Baudot, Tan, and Kong 2010).

2.2.5.5 Dynamic Light Scattering (DLS). It is a technique used to study the size and motion of particles or molecules in a solution. It is a non-invasive and non-destructive method that relies on the principles of light scattering. In DLS, a laser beam is directed at a sample containing particles or molecules in a liquid medium. These particles can be anything from nanoparticles, proteins, and polymers to even larger aggregates. As the laser beam interacts with the particles, it scatters light in different directions. The scattered light is collected at various angles and detected by a photodetector. The fluctuations in the intensity of the scattered light are analyzed to obtain information about the size distribution and dynamics of the particles. The analysis is based on the phenomenon of Brownian motion, where particles undergo random motion due to collisions with solvent molecules. DLS provides information about the hydrodynamic radius, which is related to the size of the particles and their diffusion coefficient, a measure of their mobility. The autocorrelation function is calculated by analyzing the intensity fluctuations over time, which provides information about the distribution of particle sizes and their dynamics (Rahdar et al. 2019).

DLS is a versatile technique widely used in various fields, including colloid science, biophysics, nanotechnology, and pharmaceutical research. It can provide valuable insights into the behavior and interactions of particles in solution, such as aggregation, stability, and the effect of environmental factors like temperature or pH (Farkas and Kramar 2021).

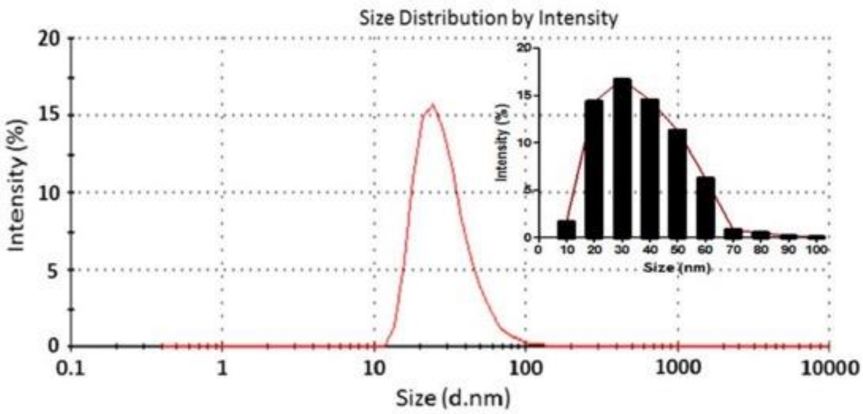

Figure 18. DLS Size Spreading Histogram of Au NPs (Singh et al. 2014).

2.2.5.6 Ultraviolet-Visible Spectroscopy (UV-VIS). It is a widely used analytical technique that measures ultraviolet (UV) and visible (VIS) light absorption by molecules in a sample. It provides information about the electronic transitions within molecules and is commonly employed in various scientific disciplines, including chemistry, biochemistry, physics, and materials science. The principle behind UV-VIS spectroscopy is based on the fact that molecules absorb light in the UV-VIS range when the energy of the light matches the energy difference between two electronic states within the molecule. The absorbed light causes the electrons in the molecule to transition from the ground state (lower energy) to an excited state (higher energy). In a typical UV-VIS spectrophotometer, a light source emits a broad spectrum of light, which is then passed through a sample cell containing the substance of interest. The sample absorbs specific wavelengths of light depending on its molecular structure and composition. A detector measures the intensity of light transmitted through the sample, and the instrument generates a UV-VIS absorption spectrum that shows how much light was absorbed at each wavelength. UV-VIS spectroscopy is particularly useful for determining the concentration of a substance in a sample, as the absorption of light is directly proportional to the concentration of the absorbing species. This property makes UV-VIS spectroscopy a valuable tool for quantitative analysis in fields such as pharmaceuticals, environmental monitoring, and chemical research (Haiss et al. 2007). Additionally, UV-VIS spectroscopy can provide qualitative information about the structure and bonding of molecules. Different functional groups and chromophores exhibit characteristic absorption patterns, allowing scientists to identify compounds or analyze chemical reactions based on their UV-VIS spectra.

Overall, UV-VIS spectroscopy is a versatile technique that enables researchers to study the absorption properties of molecules, determine their concentrations, and gain insights into their electronic structure and reactivity (Abbas 2019).

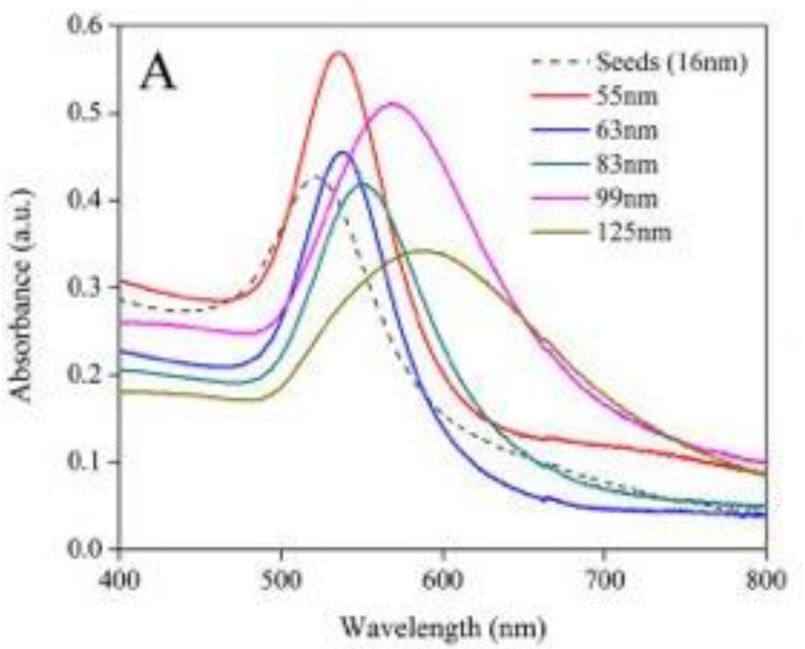

Figure 19. UV–VIS Analysis of the Au NPs (López-Muñoz et al. 2012).

2.3 LASER (Light amplification by stimulated emission of radiation).

Lasers are devices that can trigger the emission of photons from excited atoms or molecules to produce coherent beams of monochromatic light. A laser, which stands for "Light Amplification by Stimulated Emission of Radiation," is a device that emits a concentrated beam of light through a process called stimulated emission. The laser beam is characterized by its coherence, monochromaticity (single color), and high intensity. The basic principle of a laser involves the stimulation of atoms or molecules to release photons (particles of light) in a specific manner. This process begins by exciting the atoms or molecules in a medium, such as a crystal, gas, or semiconductor, to a higher energy state. When these excited particles return to their lower energy state, they release photons (Tay et al. 2022).

In a laser, this release of photons is amplified and directed into a narrow, focused beam by a series of mirrors or other optical components. The amplification is achieved by placing the medium between two mirrors, creating an optical cavity. One of the mirrors is partially transparent, allowing a portion of the photons to escape as the laser beam. The other mirror reflects the photons back into the medium, stimulating further emission. The properties of laser light make it useful in various applications. The coherence of laser light means that the waves are in phase with each other, allowing the beam to stay focused over long distances. The monochromatic nature of laser light means it consists of a single wavelength, which can be precisely controlled.

Lasers are used in diverse applications, including cutting and welding metals (Changdar and Chakraborty 2021), medical surgeries (Khalkhal et al. 2019), barcode scanners (Mor Cases et al. 2018), laser printers to fabricate microfluidics (Ng and Hashimoto 2020), weapons (Lyu and Zhan 2022). The specific design and type of laser depend on the intended application and the properties of the desired laser beam.

2.3.1 Lasers in medicine. In biomedical engineering, lasers find extensive use in different areas, including diagnostics, therapeutics, surgery, imaging, and research. Here are a few examples of their applications:

2.3.1.1 Diagnostics. Lasers are employed in diagnostic techniques such as laser-induced fluorescence, where specific molecules are labeled with fluorescent dyes and then excited by laser light to detect diseases or biomarkers. They can also be used in flow cytometry, a method that analyzes cells or particles suspended in a fluid stream by passing them through laser beams (Musgrave et al. 2015).

2.3.1.2 Therapeutics. Laser therapy, also known as phototherapy, utilizes lasers to treat various medical conditions. Low-level laser therapy (LLLT) or cold laser therapy involves using low-power lasers to stimulate tissue repair and reduce pain in conditions like musculoskeletal disorders, wounds, and inflammation (Muniz et al. 2021).

2.3.1.3 Surgery. Lasers have revolutionized surgical procedures in several ways. They offer high precision and minimize damage to surrounding tissues, making them suitable for delicate procedures. Lasers are used in various surgical disciplines such as ophthalmology (e.g., laser eye surgery) (Sakimoto, Rosenblatt, and Azar 2006), dermatology (e.g., laser ablation of skin lesions) (Boord 2006), and dentistry (e.g., laser gum surgery) (Khalkhal et al. 2019).

2.3.1.4 Imaging. Lasers are integral to imaging technologies like laser scanning microscopy (Xue Wang, Lu, and Huang 2021), optical coherence tomography (OCT**)** (Hiratsuka, Morisaki, and Yoshimura 2000), and laser speckle imaging (Postnov et al. 2019). These techniques utilize lasers to obtain high-resolution images of tissues, cells, and organs, aiding in diagnostics, research, and monitoring of diseases.

2.3.1.5 Research. Lasers serve as versatile tools in biomedical research. They are used in spectroscopy to study molecular structures and identify biomarkers. Laser microdissection enables the precise isolation of specific cells or tissues for further analysis. Additionally, lasers are employed in optogenetics, a technique that uses light-sensitive proteins to control and study the function of cells in living organisms (Asavei et al. 2019).

It's important to note that laser applications in biomedical engineering can vary significantly depending on the specific field, research focus, and advancements in technology.

2.3.2 Types of lasers.

2.3.2.1 Gas lasers. These lasers use a gas mixture as the gain medium. Examples include Helium-Neon (HeNe) lasers, Carbon Dioxide (CO_2) lasers, argon-ion lasers, and excimer lasers. Gas lasers are often used in scientific research, medical procedures, and industrial applications (Vasilyevich et al. 2021).

2.3.2.2 Semiconductor lasers. Also known as diode lasers, semiconductor lasers are based on semiconductor materials such as Gallium Arsenide (GaAs). They are compact, efficient, and commonly used in telecommunications, laser pointers, barcode readers, and optical storage devices like DVD and Blu-ray players (Peng et al. 2021).

2.3.2.3 Dye lasers. Dye lasers use an organic dye dissolved in a solvent as the gain medium. These lasers can produce a wide range of wavelengths and are used in spectroscopy, medical research, and laser-induced fluorescence (Said, Ghoneimy, and Abdelshafy 2022).

2.3.2.4 Fiber lasers. Fiber lasers use optical fiber as the gain medium. They offer high power, and efficiency and are commonly used in materials processing, laser marking, telecommunications, and sensing applications (Zervas and Codemard 2014).

2.3.2.5 Free electron lasers. Free electron lasers (FELs) produce coherent radiation by accelerating electrons to relativistic speeds and passing them through an undulating magnetic field. FELs can generate laser light across a wide range of wavelengths and are used in scientific research, synchrotron radiation facilities, and material science studies (S. Khan 2006).

2.4 Materials and Methods

2.4.1 Materials. For cell culture Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solvent, trypsin-EDTA solvent, sterile phosphate buffer solvent (PBS), flasks, 96 vail plate, proliferation kit, Au nanoparticles (purchased from NanoSilver Inc.).

2.4.2 Methods.

Cell Culture Preparation

Cells are seeded into T25 Flasks in DMEM medium supplemented with 10% FBS and 1% Penicillin Streptomycin. Cells are kept in the incubator (37ºC, 5% CO_2) until the cells are confluent for the experiments.

Cell counting was done by using Trypan Blue. (500 µL of cell suspension from the cell suspension with a cell count of $1x10^5$ cells/mL is put into an Eppendorf tube. 100 µL of 0.4% Trypan blue solution is added to the cell suspension. It waits for 5 minutes. The sample was placed on a Thoma slide. All cells were counted. The following formula quantifies the percentage of viable cells:
Proportion of viable cells (%) = (Number of unstained cells/ Total number of cells) x 100

Determination of suitable laser parameters (energy/power density, application time)

660 nm wavelength laser was used to perform the experiments. For 6 well plate experiments, the selected parameters are 300 mW (P1) and 500 mW (P2) power with 962 seconds of irritation. For 96 well plate experiments, 300 mW (P1) and 500 mW (P2) power with 32 seconds irritation time. The calculations of time spent are comparable to the surface area of wells. 6 well plates' surface area (9.6 cm^2) is greater than 96 well plates (0.32 cm^2). Therefore, the irradiation time is getting smaller for a smaller surface area.

The application of nanoparticle and laser treatment to cancer cell lines

Two doses of nanoparticle concentration were selected: 5% (D1) and 10% (D2) Au NPs/DMEM concentration for the beginning of the research. After investigation of the toxicity of Au NPs for cancer cells, the percentages can be changed accordingly. After the treatments of NPs and laser, cell proliferation and viability tests have been done.

Evaluation of cell proliferation with scratch assay tests:

Scratch Assay

Scratch assay is an *in vitro analysis* technique to identify the molecular migration of cells. Before the treatments, cells were seeded on the Petri dish and let them reach to highest confluency. When cells completely cover the surface, the cells are at their maximum confluency level. After incubation at 37ºC, 5% CO_2, the A549, and HCT116 cancer cells reach their confluency in DMEM. The experiment proceeds by making a scratch (gap in a cell monolayer) in the middle of the petri dishes. Each cancer cell line, 8 experiment groups, and one control group were analyzed quantitively. The expected results for each experiment set were a cell-free gap after the treatments with no closure and a conclusion of ‘no migration’ in cancer cell lines. The main purpose is to study cell migration, proliferation, and cell-cell interaction, and observe and analyze how quickly and efficiently cells move to fill that gap. The scratch assay principle is shown in Figure 20.

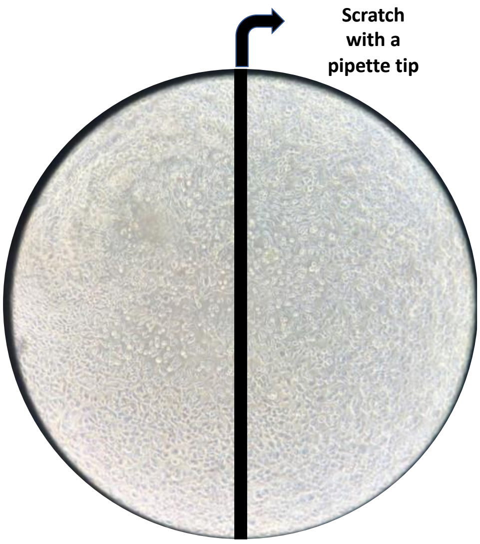

Figure 20. Scratch Assay Principle.

Cells were seeded in 6-well plates at 105 cells per well. A scratch was made in the middle of the well with a 1-10ul tip. Images were taken with an inverted microscope at 0 and 24 hours for A549, and for HCT116 at 48, and 72 hours were taken as well. Evaluations were made over the closing distance (Gulei et al. 2018). For the scratch assay migration test, the experimental group set showed as follows.

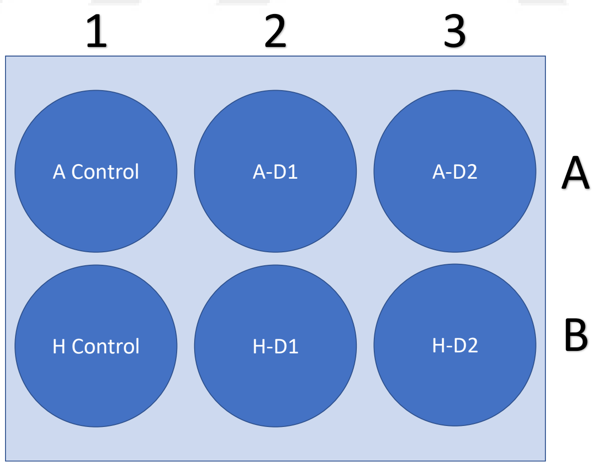

Figure 21. 6-well Plate Orientation for Control Group of A549 (A1), Control Group of HCT116 (B1), A549 with 5% Concentration Au NPs/DMEM (A2), HCT116 with 5% Concentration Au NPs/DMEM (B2), A549 with 10% Concentration Au NPs/DMEM (A3), HCT116 with 10% Concentration Au NPs/DMEM (B3) without Laser Treatments.

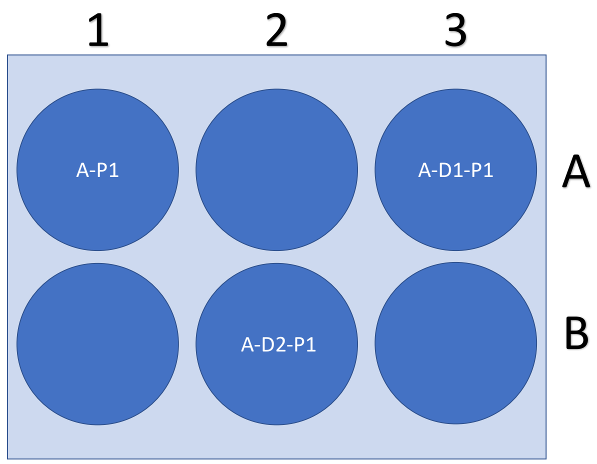

Figure 22. 6-well Plate Orientation for A549 Cell Lines without Au NPs/DMEM (A1), with 5% Concentration Au NPs/DMEM (A3), and 10% Concentration Au NPs/DMEM (B2) Treated by Laser at 660 nm 300 mW.

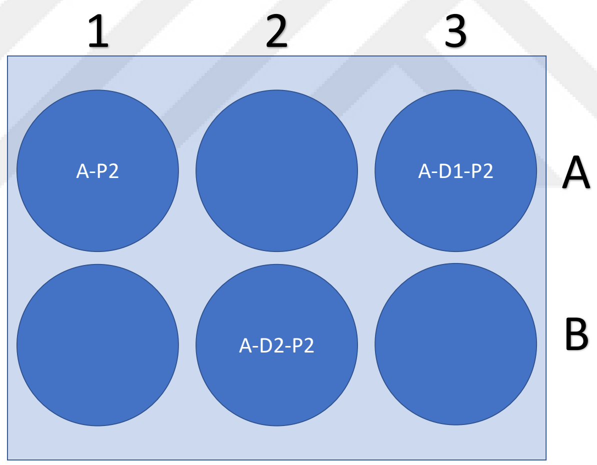

Figure 23. 6-well Plate Orientation for A549 Cell Lines without Au NPs/DMEM (A1), with 5% Concentration Au NPs/DMEM (A3), and 10% Concentration Au NPs/DMEM (B2) Treated by Laser at 660 nm 500 mW.

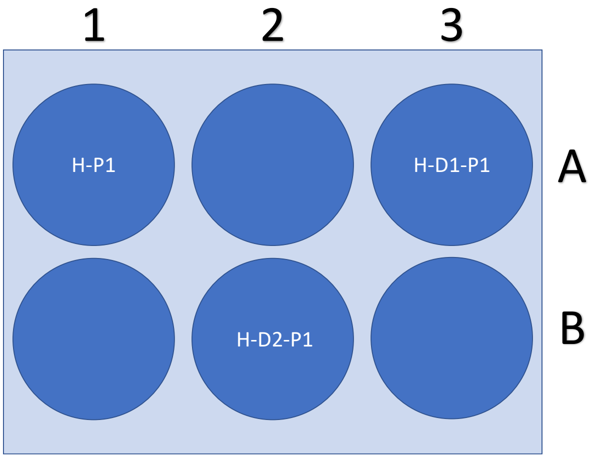

Figure 24. 6-well Plate Orientation for HCT116 Cell Lines without Au NPs/DMEM (A1), with 5% Concentration Au NPs/DMEM (A3), and 10% Concentration Au NPs/DMEM (B2) Treated by Laser at 660 nm 300 mW.

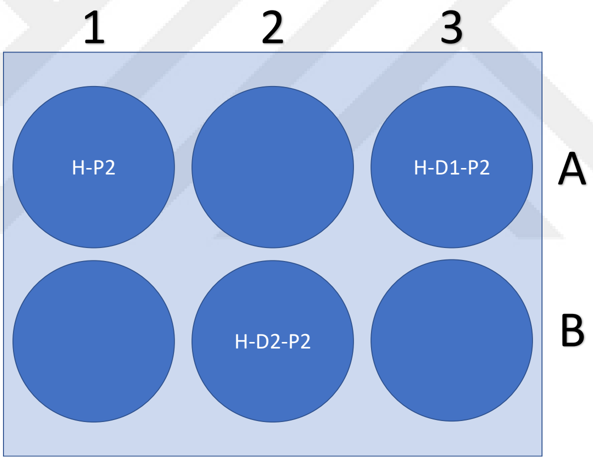

Figure 25. 6-well Plate Orientation for HCT116 Cell Lines without Au NPs/DMEM (A1), with 5% Concentration Au NPs/DMEM (A3), and 10% Concentration Au NPs/DMEM (B2) Treated by Laser at 660 nm 500 mW.

Performing cell viability test:

For the MTT cell viability test, the main purpose is to analyze the samples at a colorimetric level. The MTT test involves the color transformation of yellow MTT dye to a purple formazan product by living cells' mitochondria. Only viable cells have an absorbance in the MTT test. Therefore, the expected absorbance levels are low values for the treated cancer cell lines compared with the control groups' viabilities. The principle of MTT is shown in Figure 26.

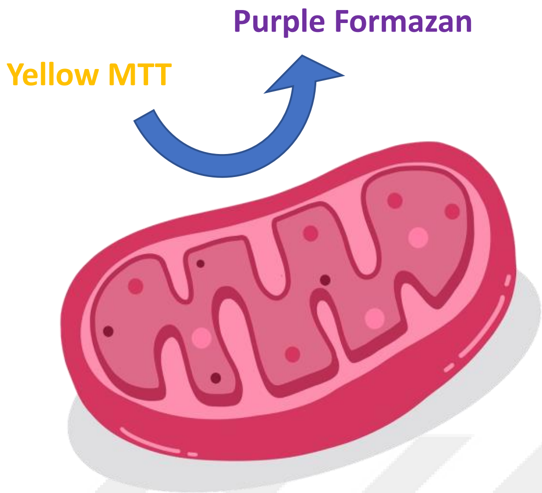

Figure 26. MTT test principle.

MTT Protocol

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) protocol was done for cell proliferation and viability determination. 104 cells per well were seeded in 96-well cell plates for this test and the measurements were done for the 24th hour (Liang, Park, and Guan 2007; Sahin et al. 2022). The protocol proceeds as, adding 1 mL of sterile 1x PBS (phosphate-buffered saline) to a vial of 5 mg MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and 12 mM MTT (3-(4,5-) dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) a stock solution was prepared. It was vortexed until dissolved. Afterward, 10 mL of 0.01 M HCl was added to a tube containing 1 mg of SDS (sodium dodecyl sulfate). The solution was gently mixed by inversion or by sonication until SDS (sodium dodecyl sulfate) dissolved. The solvent solution should be used immediately after preparation. The steps of the test were adding 50ul of MTT solution to each well and left to incubate for 2-3 hours at room temperature. Then, 150ul solvent solution was added to all wells and incubated for 15 minutes at room temperature. After the procedures, the measurements were taken in the cell plate reading device at a wavelength of 570 nm.

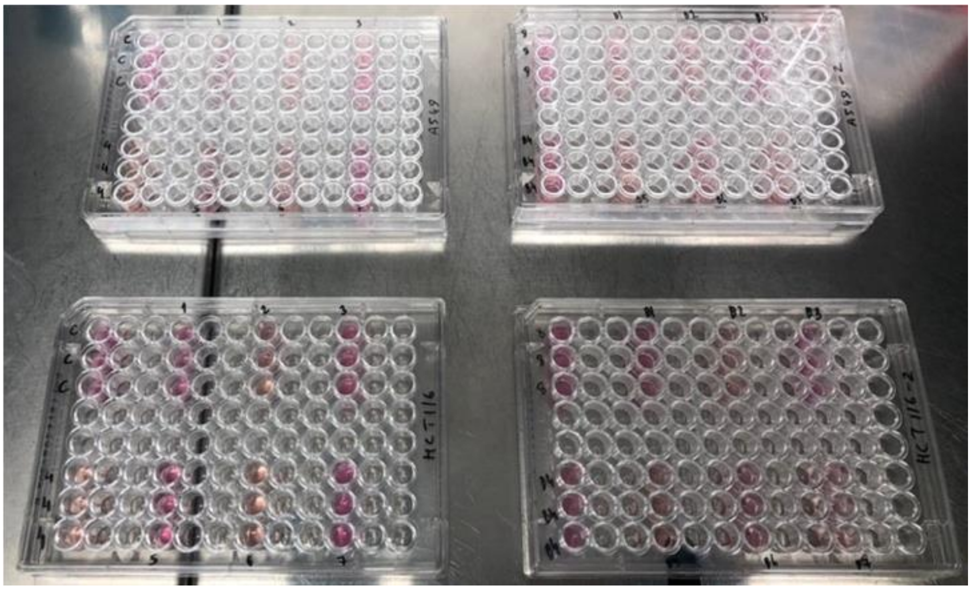

Figure 27. 96-well Plate Configuration for MTT Test

Examining the findings and making statistical evaluations:

Statistical evaluation of the MTT results was done by applying the one-way ANOVA test in SPSS Statistics statistical software. First, the results for both A549 and HCT116 cancer cell lines were explored. ANOVA tests were performed on data that were considered normal. The p-value (significance) was accepted as 0,05.

2.5 Ethical issues.

Nanoparticles in medicine hold great promise for advanced diagnostics, targeted drug delivery, imaging, and other therapeutic applications. However, like any emerging technology, the use of nanoparticles in medicine also raises several ethical issues that need to be carefully considered and addressed. Some of these ethical concerns include:

Safety and Risk Assessment: Nanoparticles are extremely small and can potentially interact with biological systems in unexpected ways. There's a concern about nanoparticles' long-term safety and potential adverse effects on human health. Proper risk assessment and rigorous testing are essential to ensure patient safety (Tsuji et al. 2006).

Environmental Impact: Nanoparticles used in medical applications can find their way into the environment through various routes, such as improper disposal of medical waste or excretion by patients. It's important to assess the potential environmental impact of nanoparticles and their potential to accumulate in ecosystems (Martínez et al. 2021).

Informed Consent: Patients should be fully informed about the use of nanoparticles in their medical treatment. However, explaining nanoparticles' complex nature and potential effects on patients may be challenging. Ensuring that patients provide informed consent while understanding the potential risks and benefits is crucial (Tang et al. 2019).

Long-Term Effects: The long-term effects of nanoparticles on human health are not yet fully understood. Ethical concerns arise when using nanoparticles for treatments that could have impacts on patients' health many years down the line (Dreaden et al. 2012).

Chapter 3
Results and Discussion

In this study, the effects of Au NPs and laser treatments were investigated. NPs are purchased from NanoSilver Inc. 100 ppm NPs have an absorbance of 513 nm. As shown in Figure 19, the particle size of Au NPs varies with respect to the wavelength of incident light which gives maximum relative absorbance. In this study, it has been measured that maximum absorbance was obtained at a wavelength of approximately 520-530 nm which corresponds to a size of an NPs smaller than 20 nm via UV-VIS analysis. The results show mono-peak consequently, there is no agglomeration.

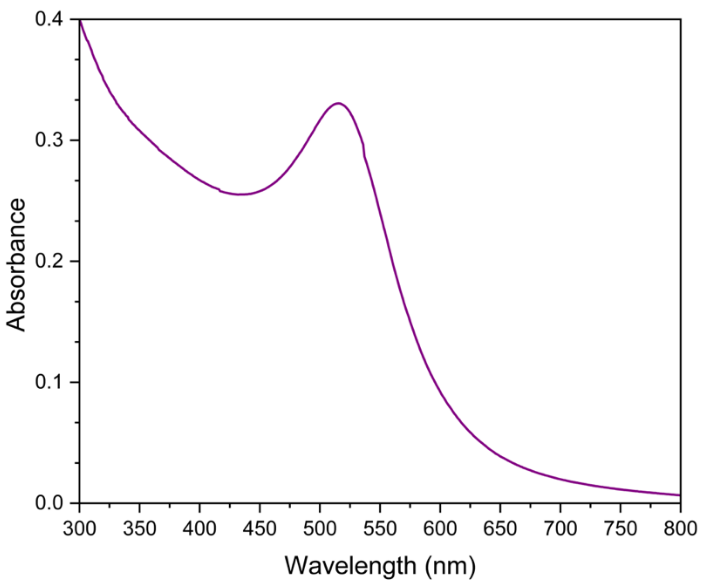

Figure 28. Absorbance Graph of Purchased Au NPs.

Before the experiments, the proliferation of cell lines differed from each other. As an observation, the A549 lung cancer cell lines' proliferation was lower than the HCT116 colon cancer cell lines. The expected result for the scratch assay was, the proliferation of HCT116 cell lines to be greater than A549 cell lines.

In order to analyze the cell migration and viability, scratch assay and MTT test, were done accordingly.

Scratch Assay Results:

The expected result of the scratch assay is that the gap will not close and there will be no migration. Compared to the control groups' closure distance, the experiment groups' closure percentage was expected to be less after the treatments. On the other hand, the expected results were in control groups after the treatments for the A549 cell line to proliferate slower than HCT116 cells.

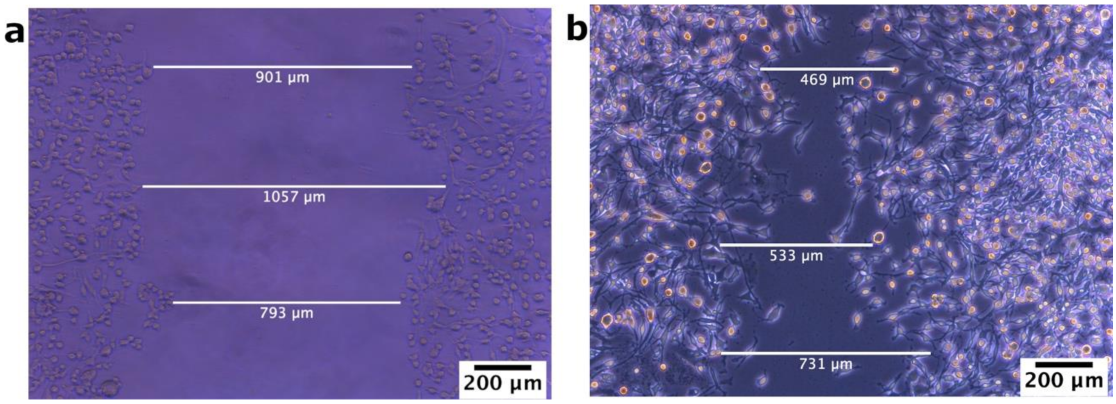

Figure 29. Control Groups of A549 Cell Lines (a) 0 Hours, (b) 24 Hours.

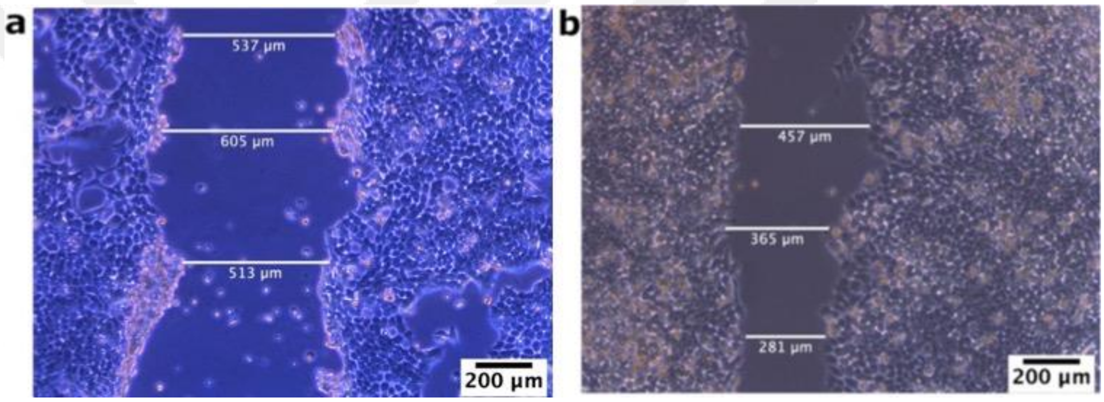

Figure 30. Control Groups of HCT116 Cell Lines (a) 0 Hours, (b) 24 Hours.

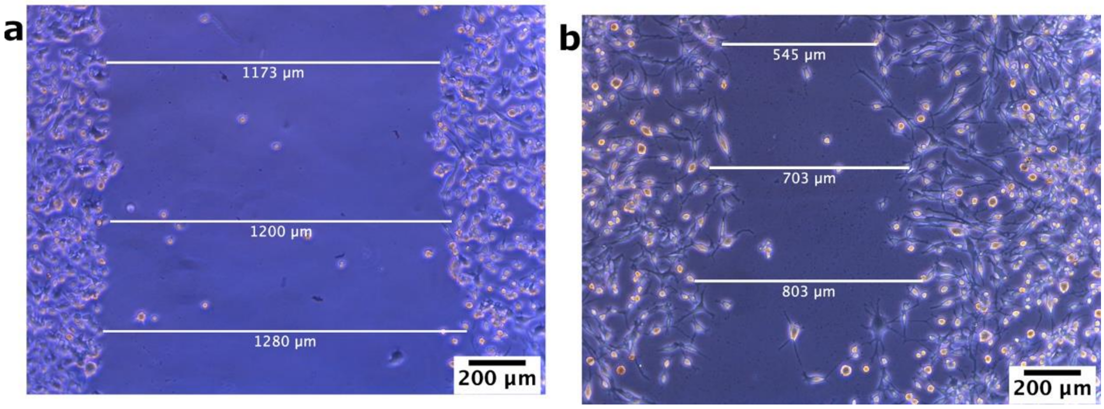

Figure 31. 5% Au NPs/DMEM Concentration Treatment of A549 (a) 0 Hours, (b) 24 Hours.

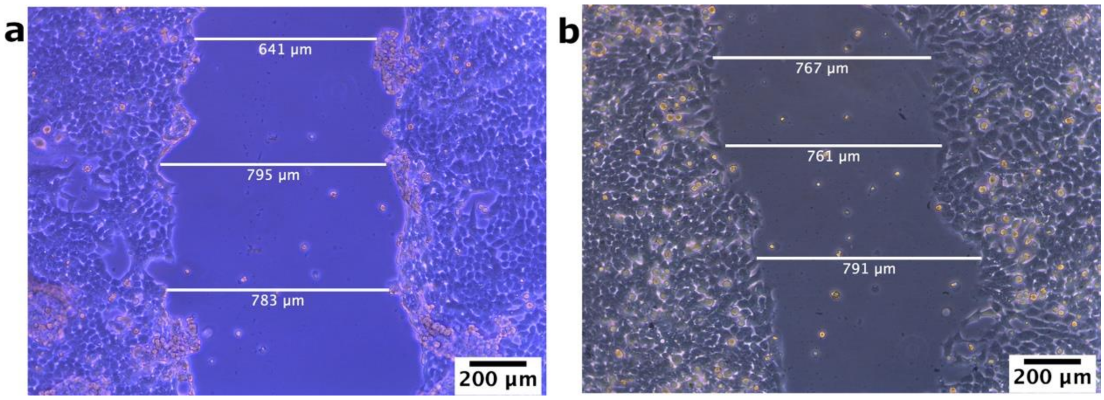

Figure 32. 5% Au NPs/DMEM Concentration Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

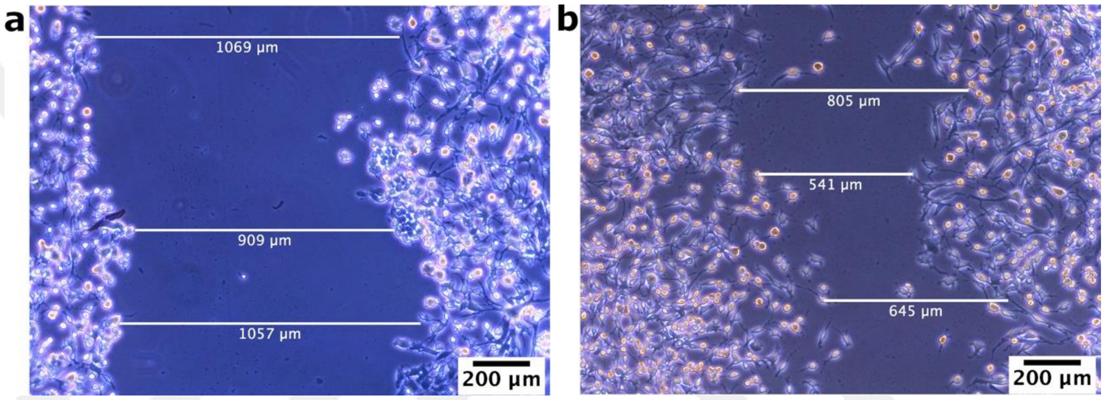

Figure 33. 10% Au NPs/DMEM Concentration Treatment of A549 (a) 0 Hours, (b) 24 Hours.

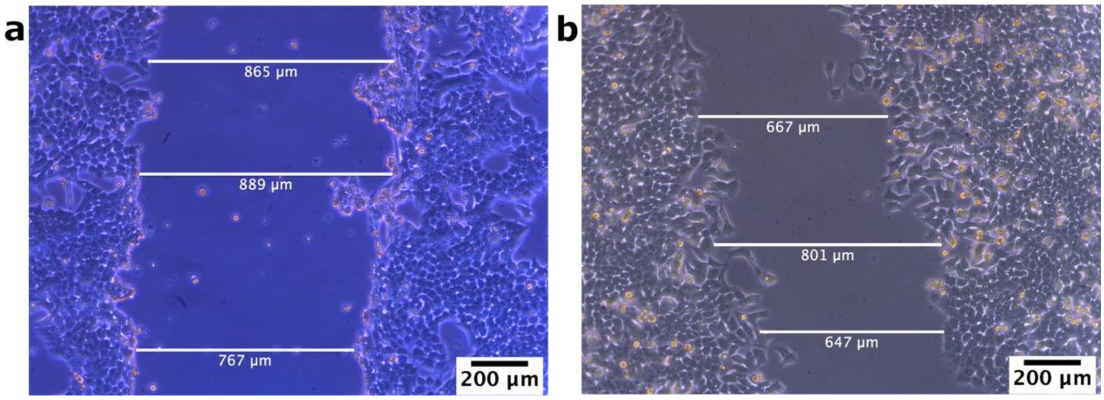

Figure 34. 10% Au NPs/DMEM Concentration Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

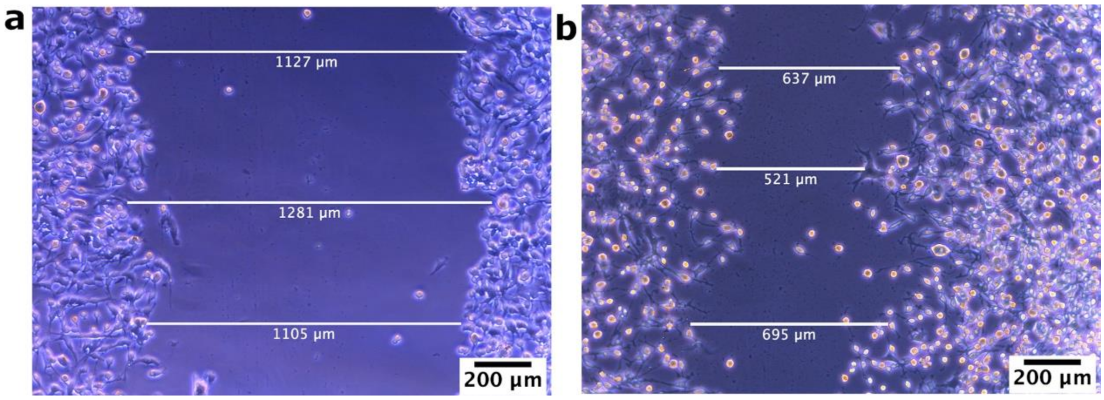

Figure 35. 5% Au NPs/DMEM Concentration and 660 nm 300 mW Treatment of A549 (a) 0 Hours, (b) 24 Hours.

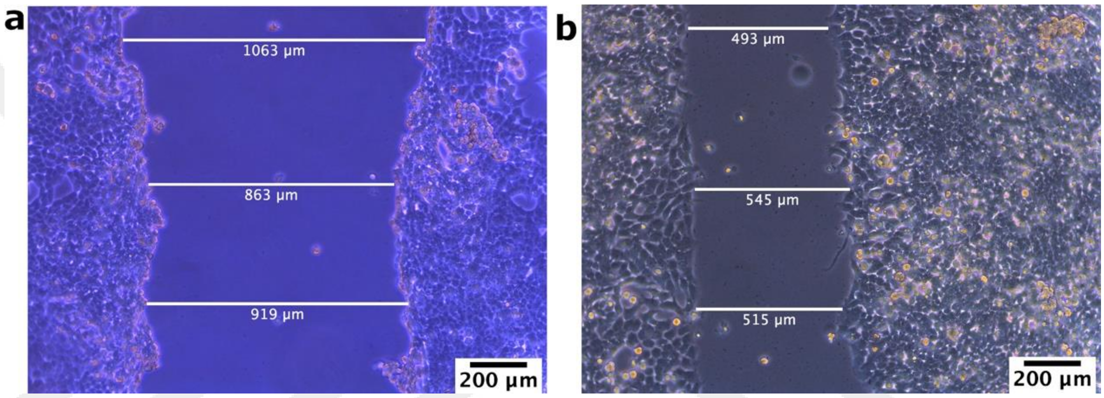

Figure 36. 5% Au NPs/DMEM Concentration and 660 nm 300 mW Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

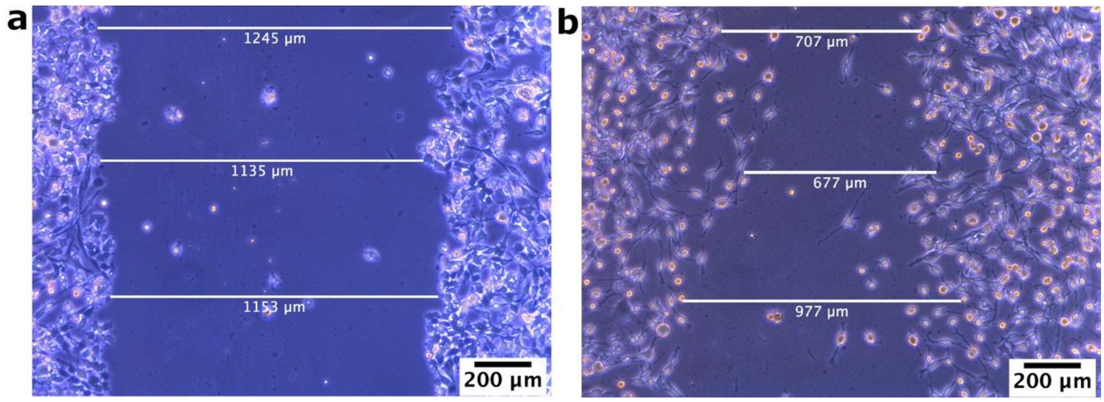

Figure 37. 5% Au NPs/DMEM Concentration and 660 nm 500 mW Treatment of A549 (a) 0 Hours, (b) 24 Hours.

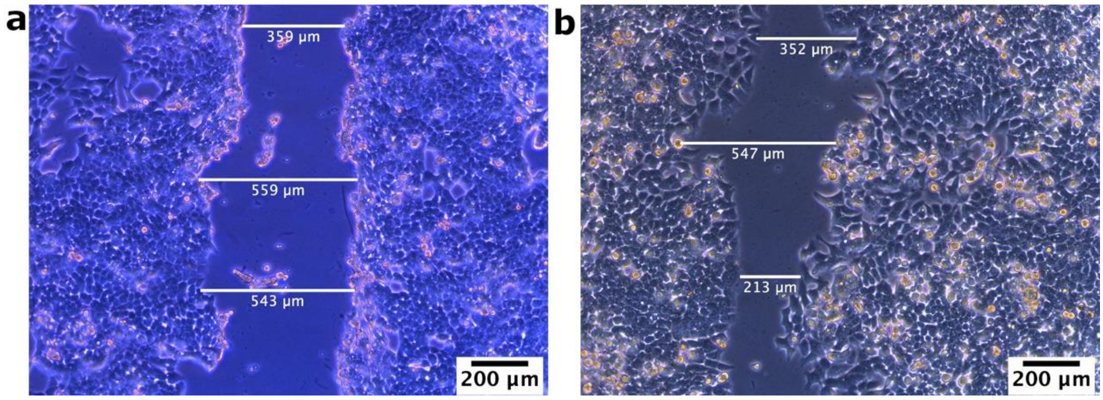

Figure 38. 5% Au NPs/DMEM Concentration and 660 nm 500 mW Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

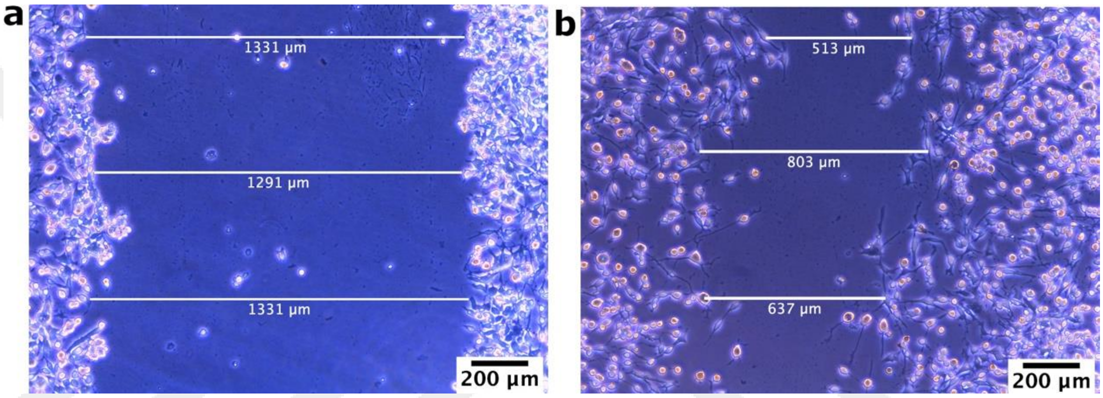

Figure 39. 10% Au NPs/DMEM Concentration and 660 nm 300 mW Treatment of A549 (a) 0 Hours, (b) 24 Hours.

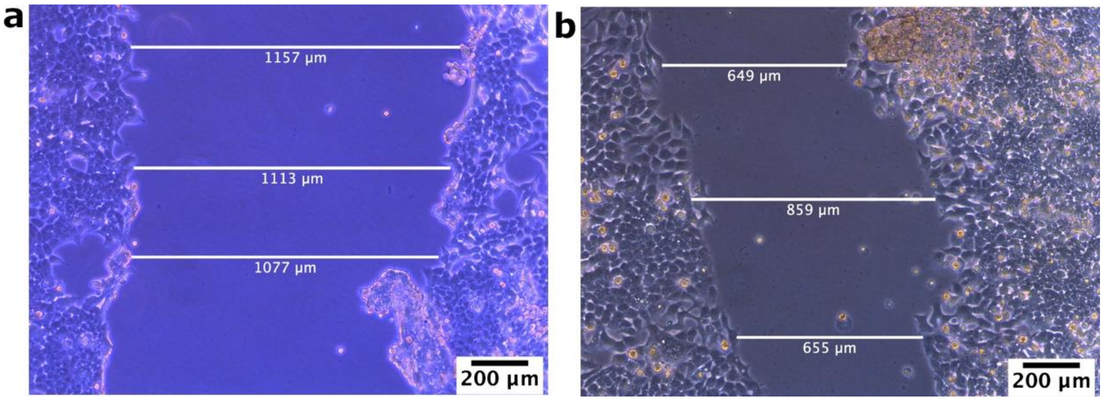

Figure 40. 10% Au NPs/DMEM Concentration and 660 nm 300 mW Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

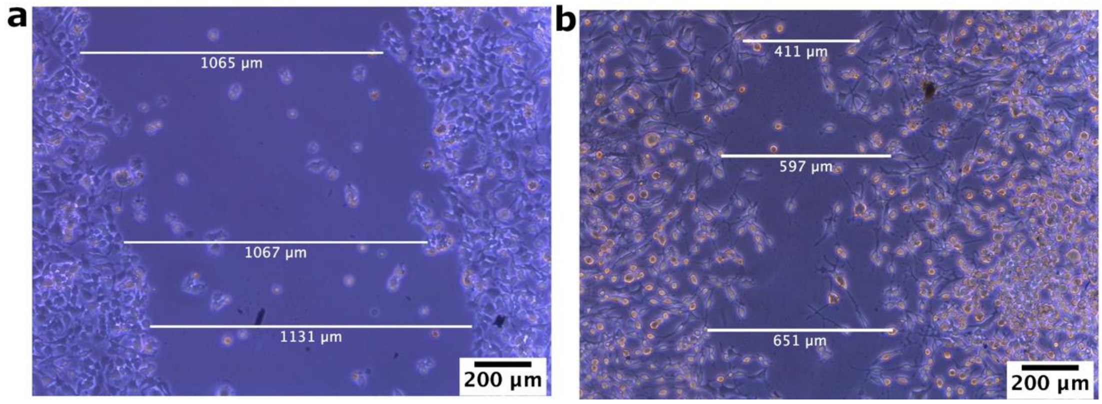

Figure 41. 10% Au NPs/DMEM Concentration and 660 nm 500 mW Treatment of A549 (a) 0 Hours, (b) 24 Hours.

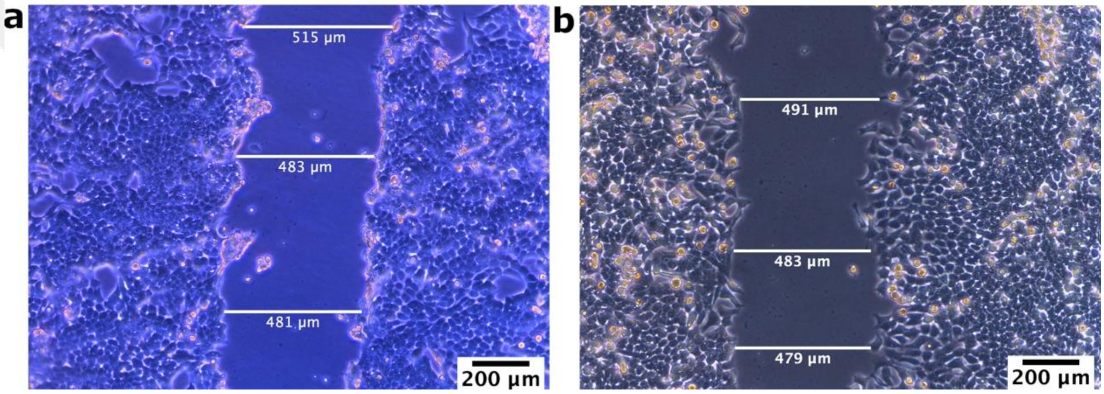

Figure 42. 10% Au NPs/DMEM Concentration and 660 nm 500 mW Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

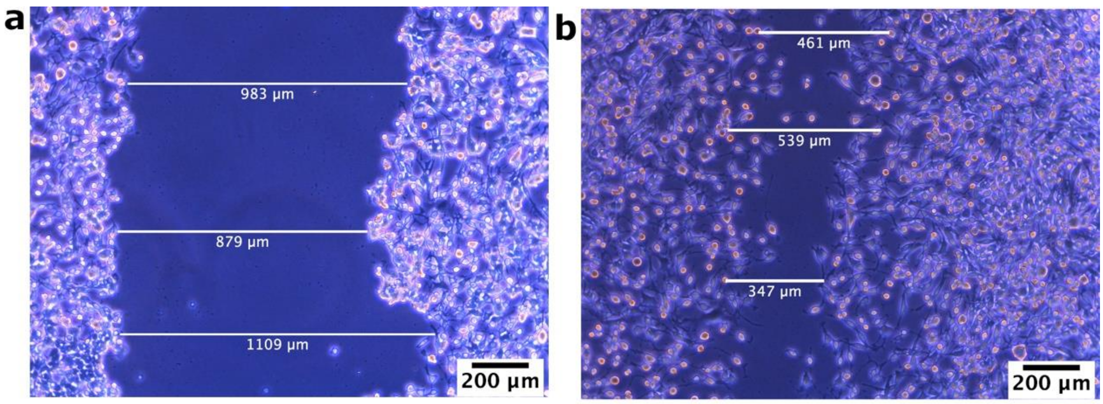

Figure 43. 660 nm 300 mW Treatment of A549 (a) 0 Hours, (b) 24 Hours.

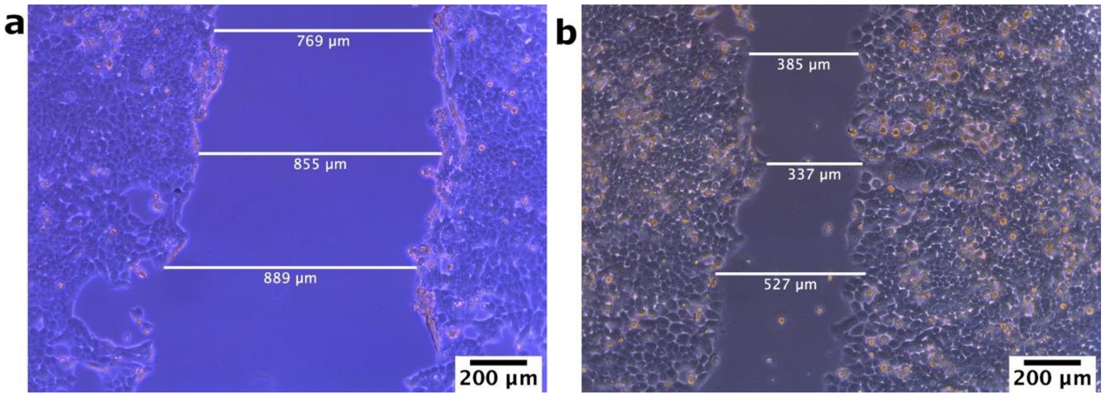

Figure 44. 660 nm 300 mW Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

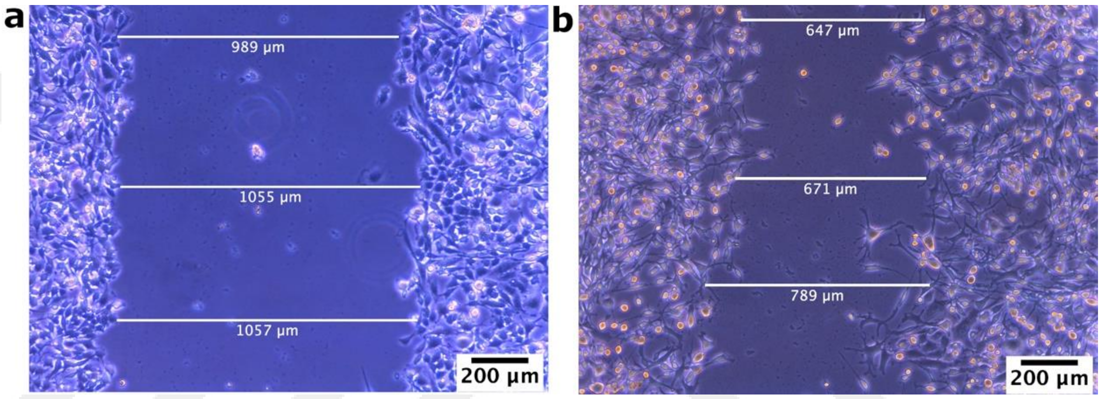

Figure 45. 660 nm 500 mW Treatment of A549 (a) 0 Hours, (b) 24 Hours.

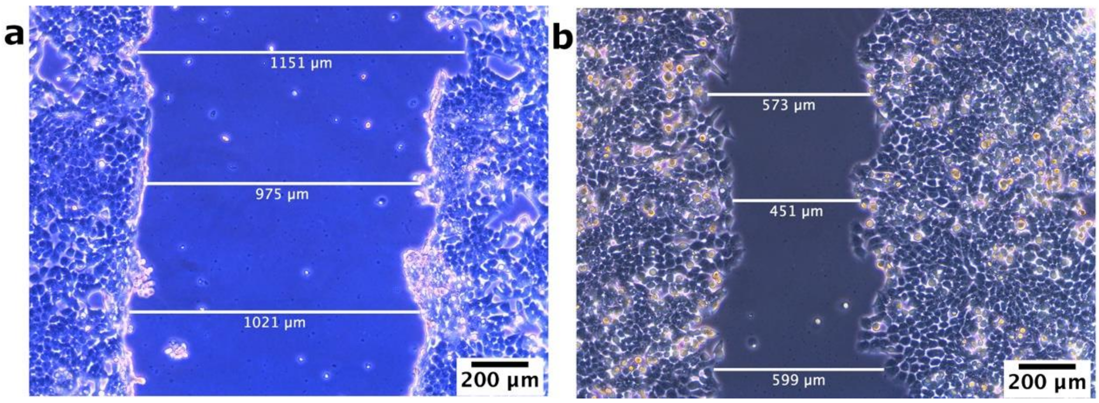

Figure 46. 660 nm 500 mW Treatment of HCT116 (a) 0 Hours, (b) 24 Hours.

However, the results appear to be the opposite way; HCT116 cells migrate slower than A549 in 24 hours As a result, the treatments were efficient for HCT116 colon cancer cells whereas inefficient for A549 lung cancer cells (Figure 29-46). Since the migration of HCT116 colon cancer cells was slower than A549, microscopic images have been taken for a significant conclusion. After the results were shown by HCT116 cell lines, further microscopic images were taken in 48 hours to support the valid success of treatments (Figure 47 and Figure 48). The distances between the cell walls are written under the experimental groups. After the microscopic images, 48 hours of closure for experimental groups for A549 reached 100%. However, for HCT116, at 48 and 72 hours, the results showed a significantly low migration level (Figure 47-49). Hence, it is a conclusion of success in the scratch assay experiment for the treated HCT116 colon cancer cell line.

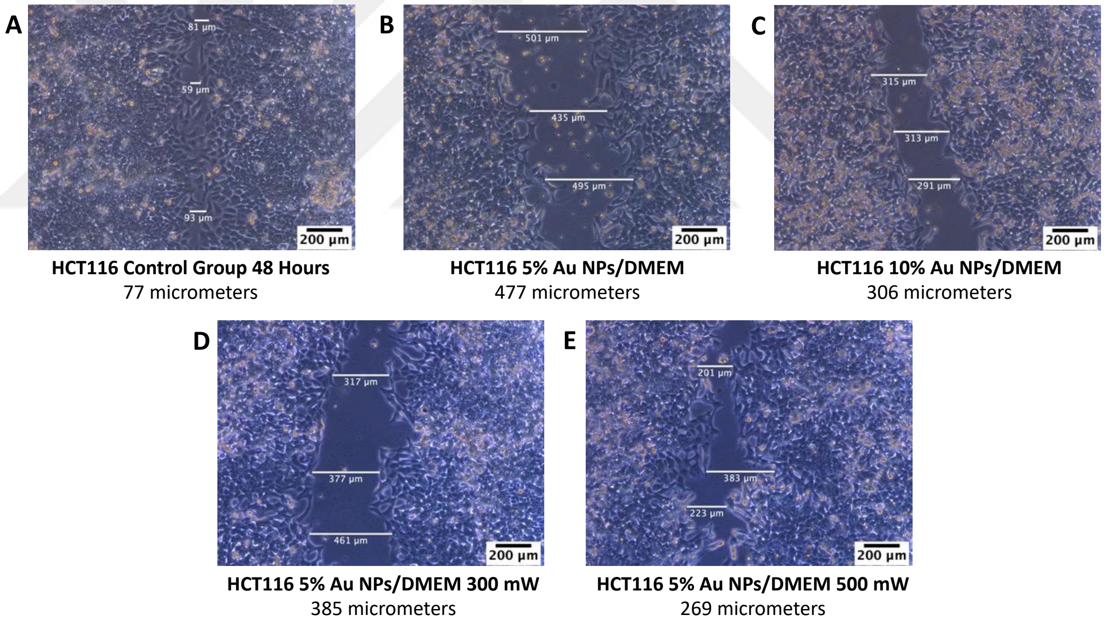

Figure 47. HCT116 Cell Lines After Treatments in 48 Hours.

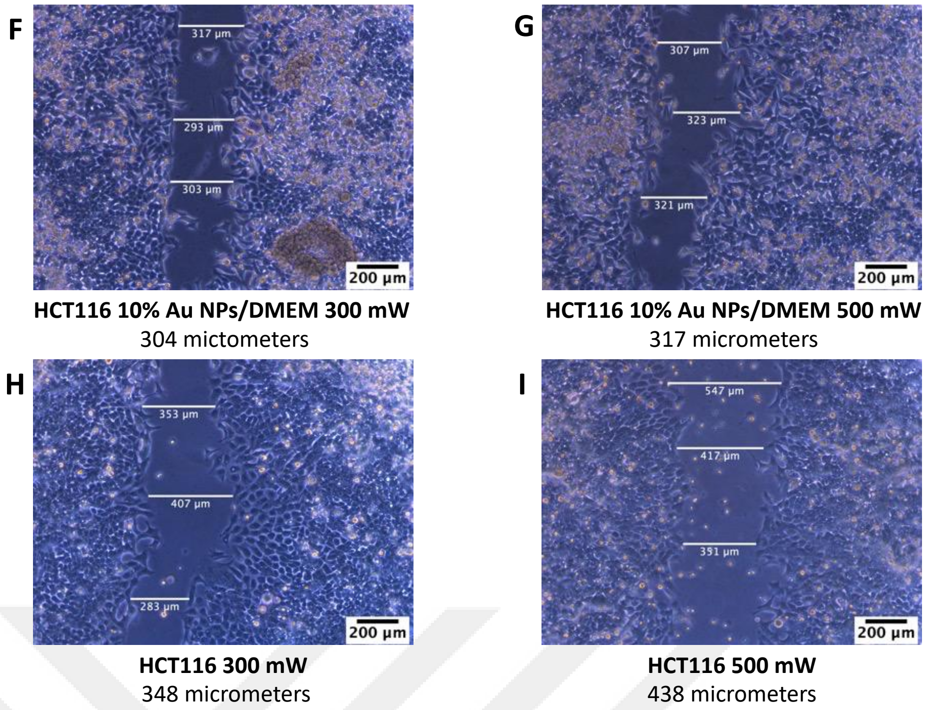

Figure 48. HCT116 Cell Lines After Treatments in 48 Hours.

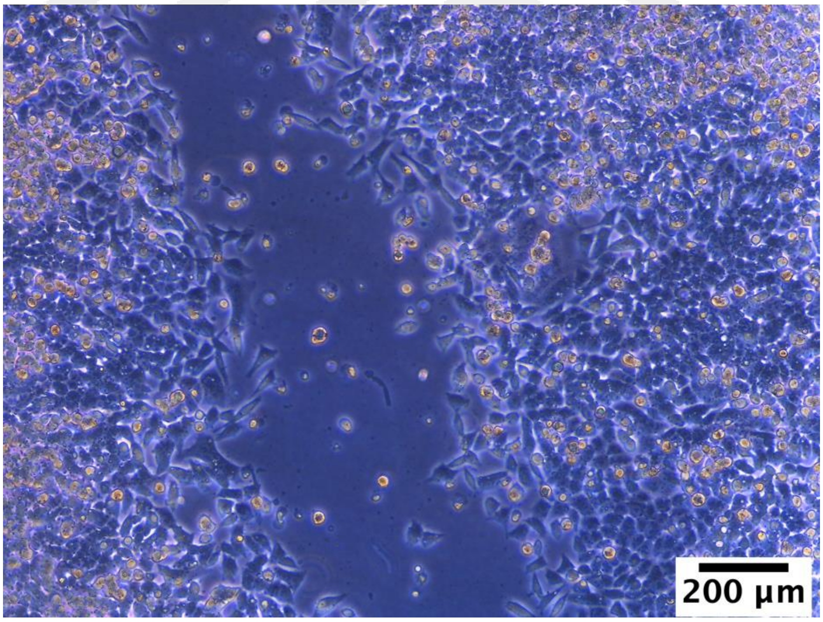

Figure 49. HCT116 Treatment with 10% Concentrated Au NPs/DMEM and 660 nm with 300 mV Powered Laser in 72 Hours.

As a result, the treatments were efficient for HCT116 colon cancer cells whereas inefficient for A549 lung cancer cells.

Scratch Assay Results for A549 Lung Cancer Cell Line:

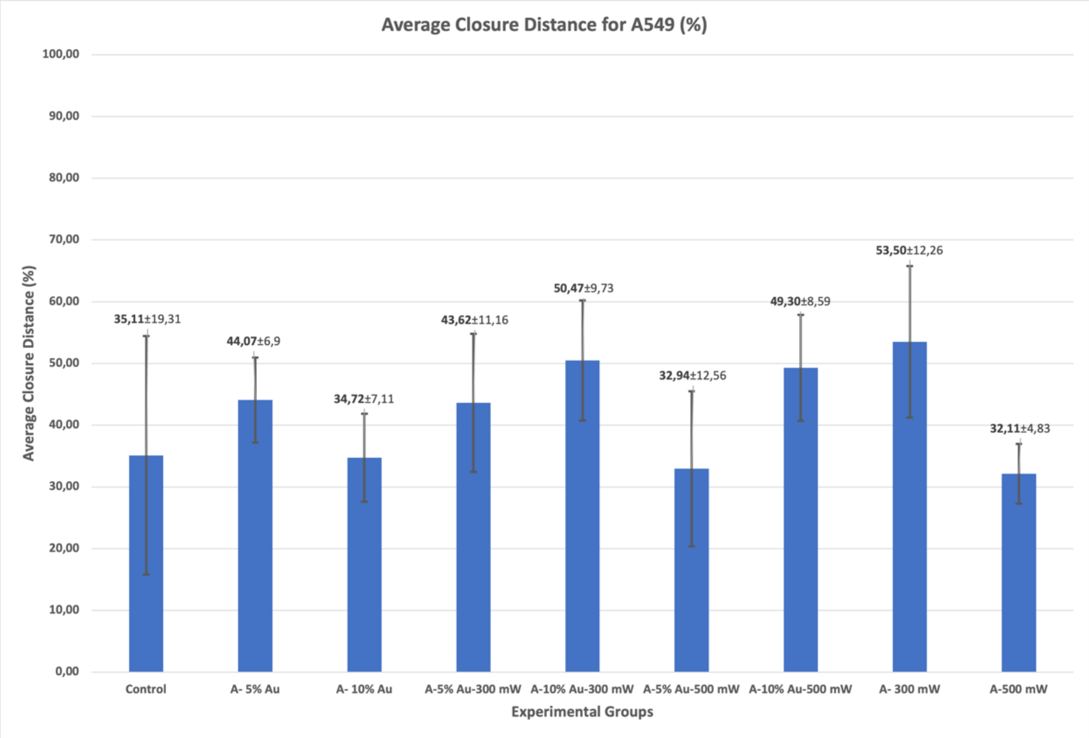

Figure 50. Average Closure of A549 Lung Cancer Cell Line in 24 Hours.

With the presence of Au NPs and laser irradiation, the aggression of the A549 lung cancer cell line was found to increase and led to more migration of cells especially for the experiment groups of 5% Au NPs, 5% Au NPs & 300 mW laser, 10% Au NPs & 300 mW laser, 10% Au NPs & 500 mW and 300 mW laser irradiations.

Cell migration was less for the experimental groups of 10% Au NPs, 5% Au NPs & 500 mW laser, and 500 mW laser treated ones. For the A549 lung cancer cell line, the combined therapy was not effective. In order to examine the cell viability, an MTT assay was done.

__Scratch Assay Results for HCT116 Colon Cancer Cell Line:__

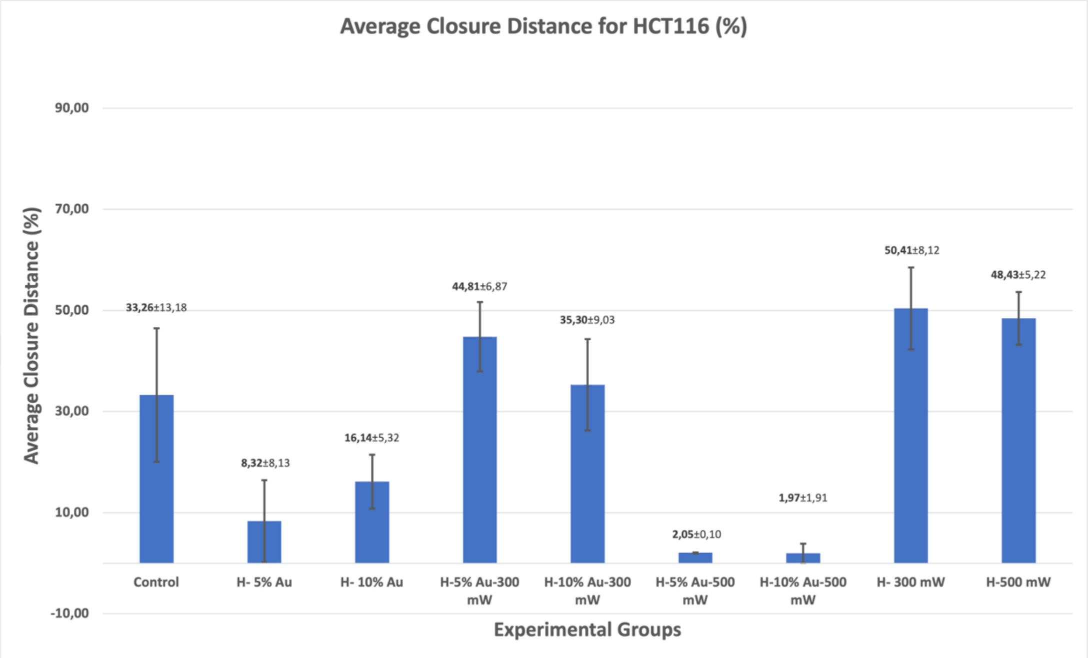

Figure 51. Average Closure of HCT116 Colon Cancer Cell Line in 24 Hours.

With the presence of 5% Au, 10% Au, 5% Au & 500 mW laser, 10% Au & 500 mW laser irradiation, cell migration was decreased for HCT116 in 24 hours. The results show that the combined therapy for some parameters can apply to HCT116 colon cancer cells in *in vitro* experiments. As well as the A549 cell line, just the laser irradiation of 660 nm stimulates cancer cells and activates proliferation.

After the scratch assay, MTT was performed to investigate the cell viability of cancer cell lines.

MTT Cell Viability Test Results:

After performing the cell migration assay, the cell viability tests were done to investigate the treatment effects on cells in 24 hours (n=3 for each cancer cell line, 3 replicates). The complementary results for A549 and HCT116 cancer cell lines was explained below:

A549 MTT Test Results:

For the A549 lung cancer cell line, scratch assay showed significant cell migration after the treatments in 24 hours which led to a conclusion of unwanted treatment effects. In this section, the relative absorbance and cell viability in percentages after the treatments are shown in Graphs 3 and 4, respectively, for the A549 lung cancer cell line in 24 hours.

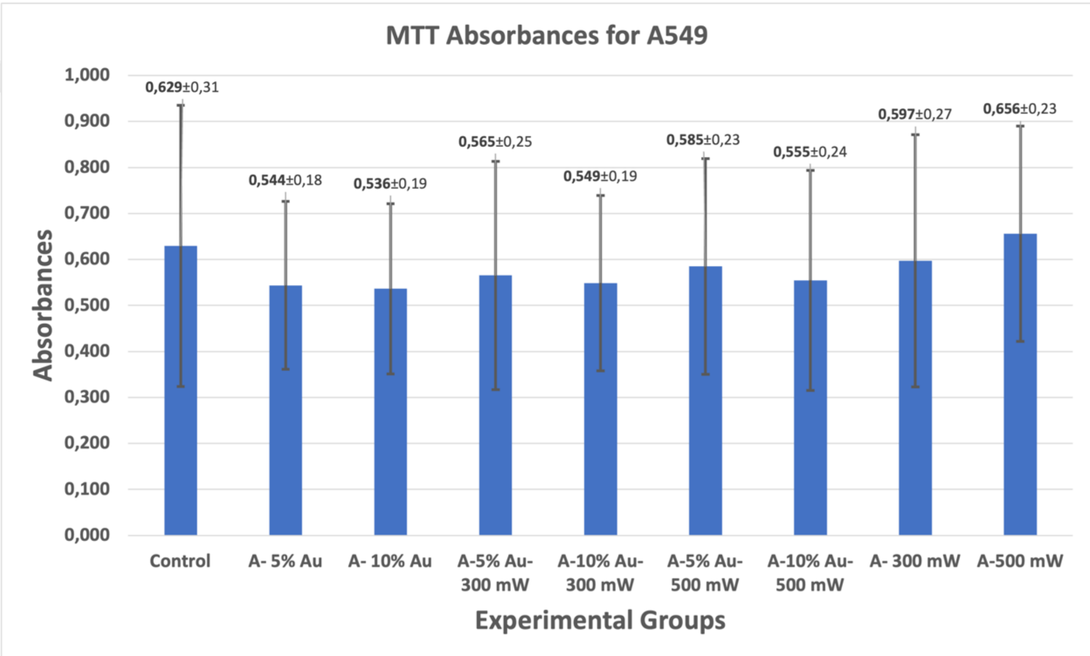

Figure 52. Absorbances of A549 Lung Cancer Cell Lines After Treatments in 24 Hours.

After evaluating the MTT results for the A549 lung cancer cell line, the normal distribution of data was checked, and a one-way ANOVA test was done. The degree of freedom (df) was calculated as 8 and significance as 0,972 (p-value=0,05). According to post hoc multiple comparisons by Bonferroni, Tukey, and Duncan's methods, there was no difference between the data. For the Tukey HSD post hoc test, the graphs are shown below.

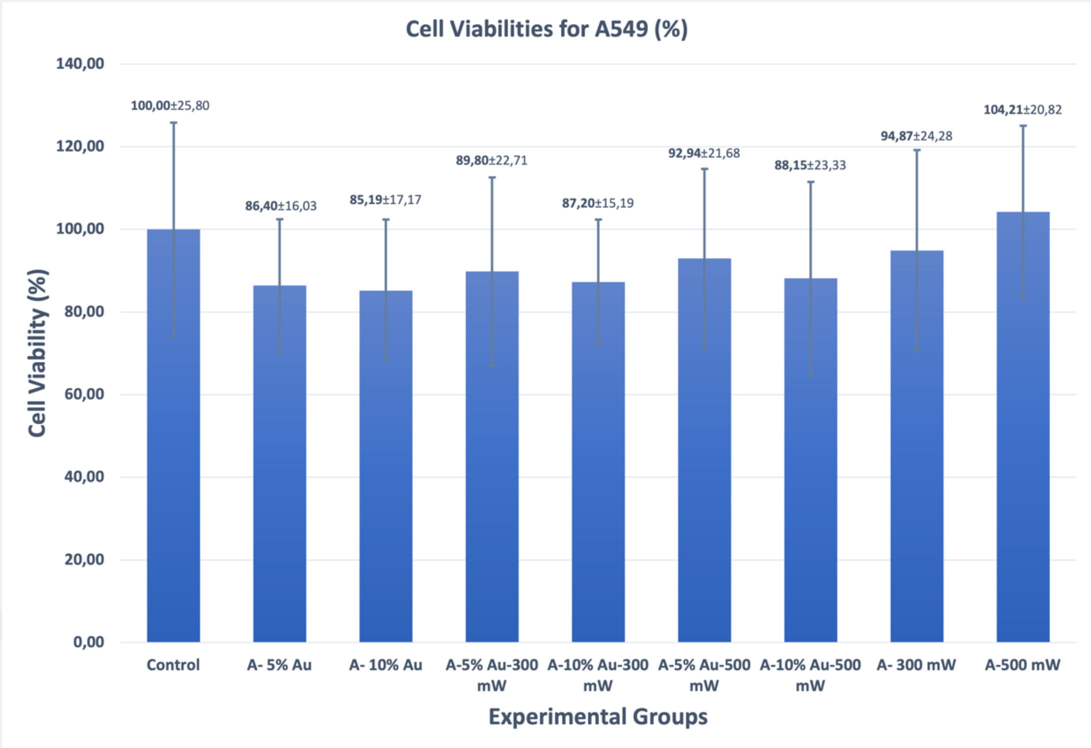

Figure 53. Cell Viabilities of A549 Lung Cancer Cell Line (%) After Treatments in 24 Hours.

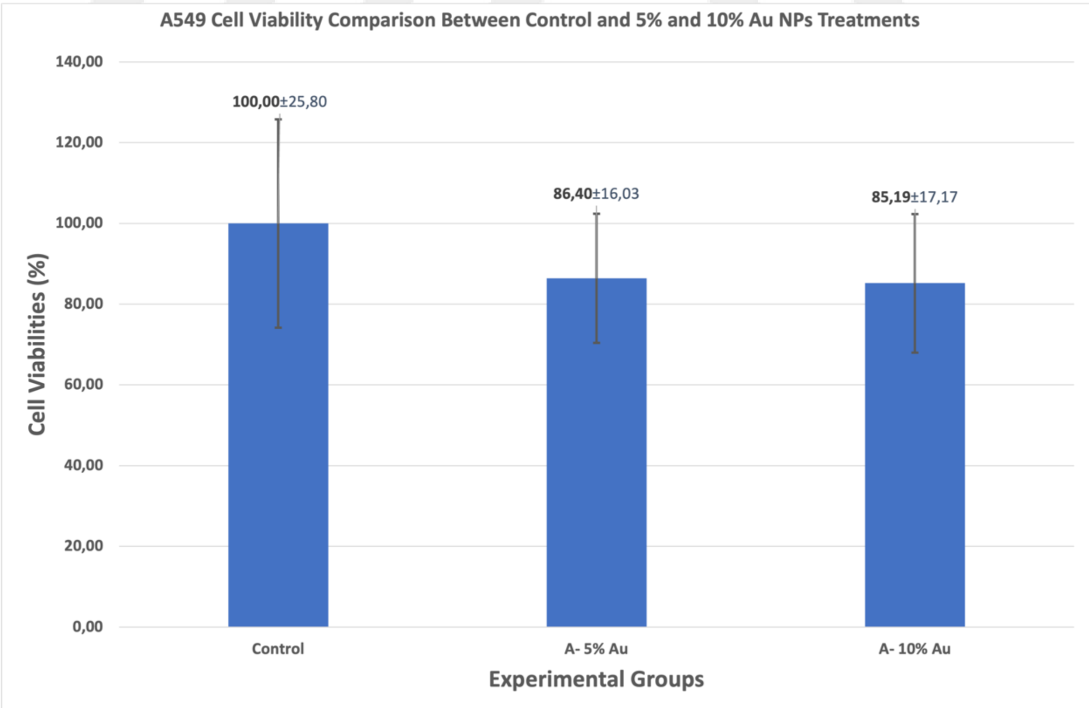

Figure 54. A549 Cell Viability Comparison Between Control Group and Au NPs Treatment Groups with a p-value>0,05, significance of 0,997, and 0,995, respectively.

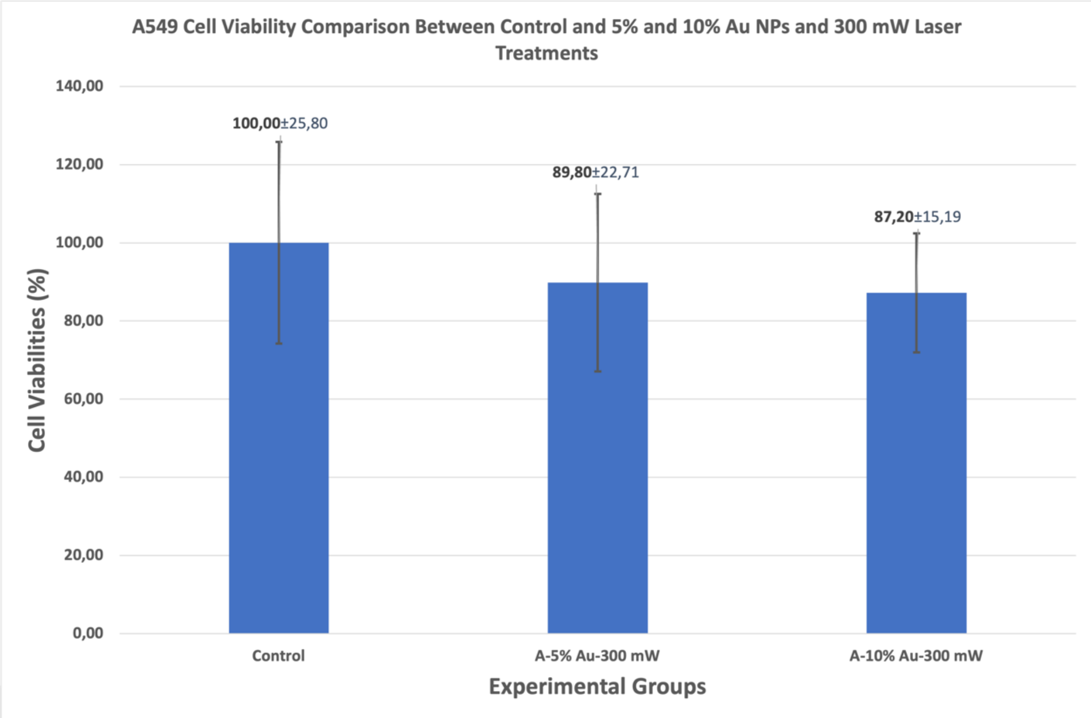

Figure 55. A549 Cell Viability Comparison Between Control Group and 5% and 10% Au NPs with 660 nm 300 mW Laser Treatments, p-value>0,05, significance of 1,000, and 0,998, respectively.

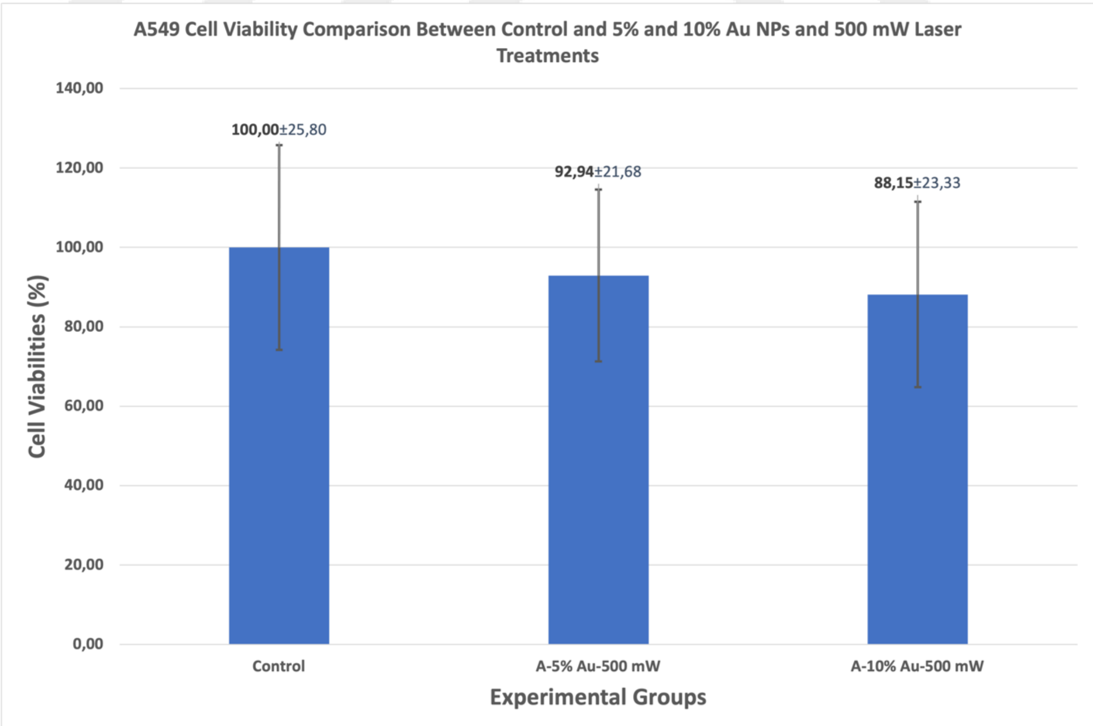

Figure 56. A549 Cell Viability Comparison Between Control Group to 5% and 10% Au NPs with 660 nm 500 mW Laser Treatments, p-value>0,05, significance of 1,000 and, 0,999, respectively.

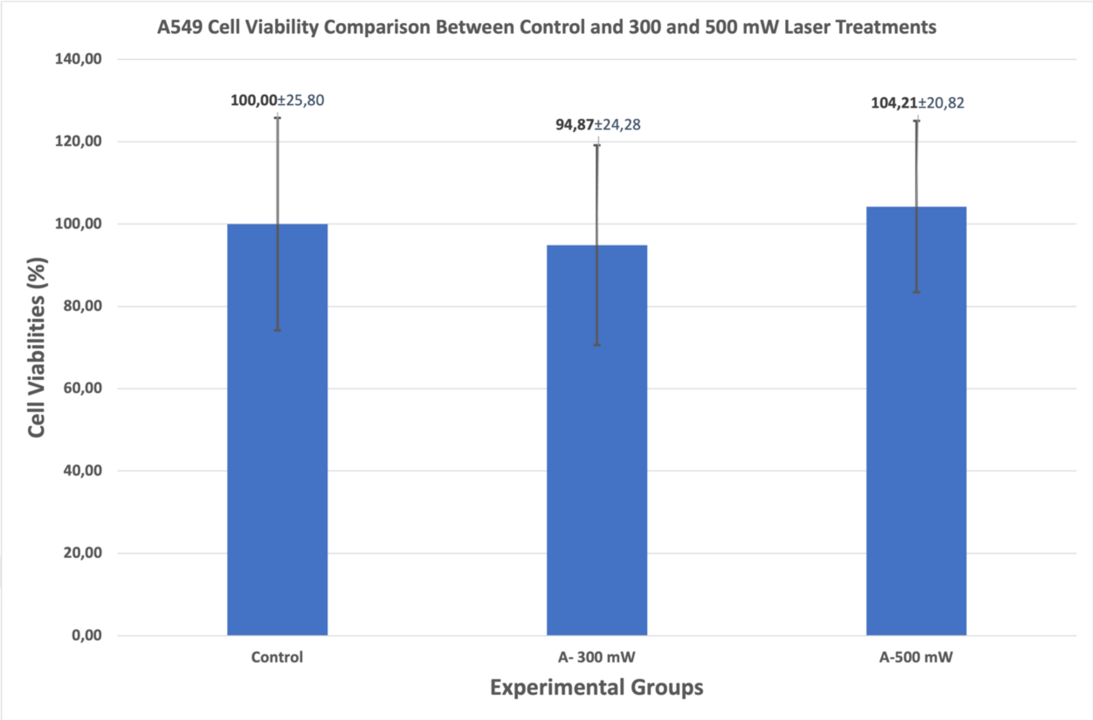

Figure 57. A549 Cell Viability Comparison Between Control Group to 300 and 500 mW Laser Treatments, p-value>0,05, significance of 1,000.

In 24 hours, for the A549 cancer cell line, the expected cell viability of experimental groups was higher compared with the control group because of the migration rate. Hence, except for 500 mW laser irradiation cell viability of the experimental groups was lower than the control group. These results can be explained by multilayered cancer cell line formation. Stimulation of A549 lung cancer cell lines can cause a collapse of cancer cells and might lead to cell death. For the cell viability of 500 mW, the migration was lower than the control group, so the cells were monolayered after the irradiation with high viability *in vitro*. But for the other experimental groups, because of high migration, the cells are dividing on top of each other as their nature of proliferation. Since the *in vitro* tumor microenvironment is monolayered, confluency will reach maximum and lead to cell death because of the multilayered configuration. For cancer cells in a Petri dish, there is no suitable extracellular matrix like human tissue, and the cancer cells will not adapt to their new environment conditions. For this reason, the results of MTT and scratch assay overlap each other.

HCT116 MTT Test Results:

For the HCT116 colon cancer cell lines, the expected results for cell viability tests were significant cell death compared with control groups. HCT116 colon cancer cell lines' viabilities after the treatments were shown in Graph 9. As well as the A549 cell line results, HCT116 results have no significant difference between each other.

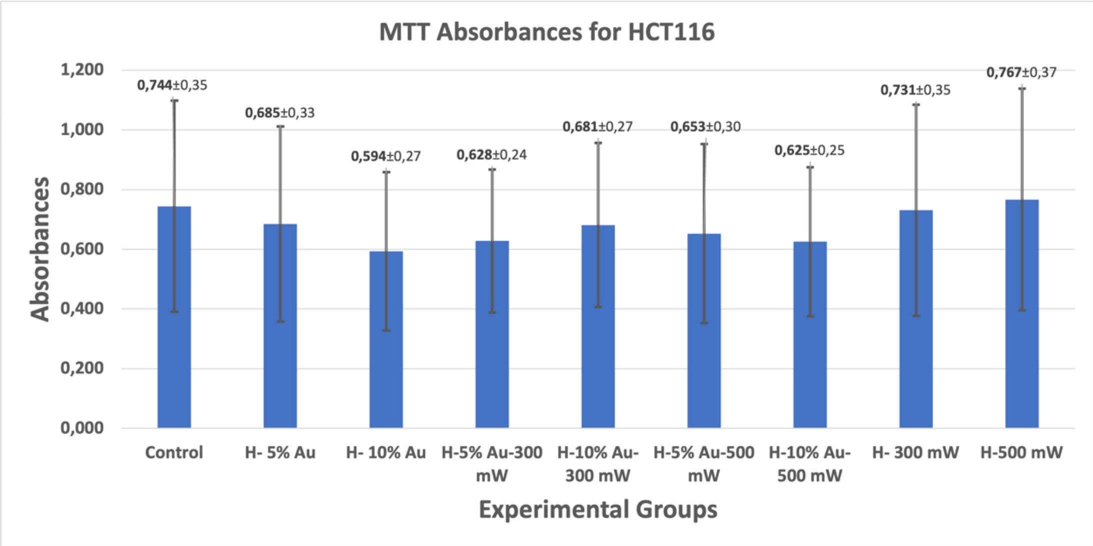

Figure 58. Absorbances of HCT116 Colon Cancer Cell Lines After Treatments.

After evaluating the MTT results for the HCT116 colon cancer cell line, the normal distribution of data was checked, and a one-way ANOVA test was done. The df was calculated as 8 and the significance as 0,950 (p-value=0,05). According to post hoc multiple comparisons by Bonferroni, Tukey, and Duncan's methods, there was no difference between the data. For the Tukey HSD post hoc test, the graphs are shown below.

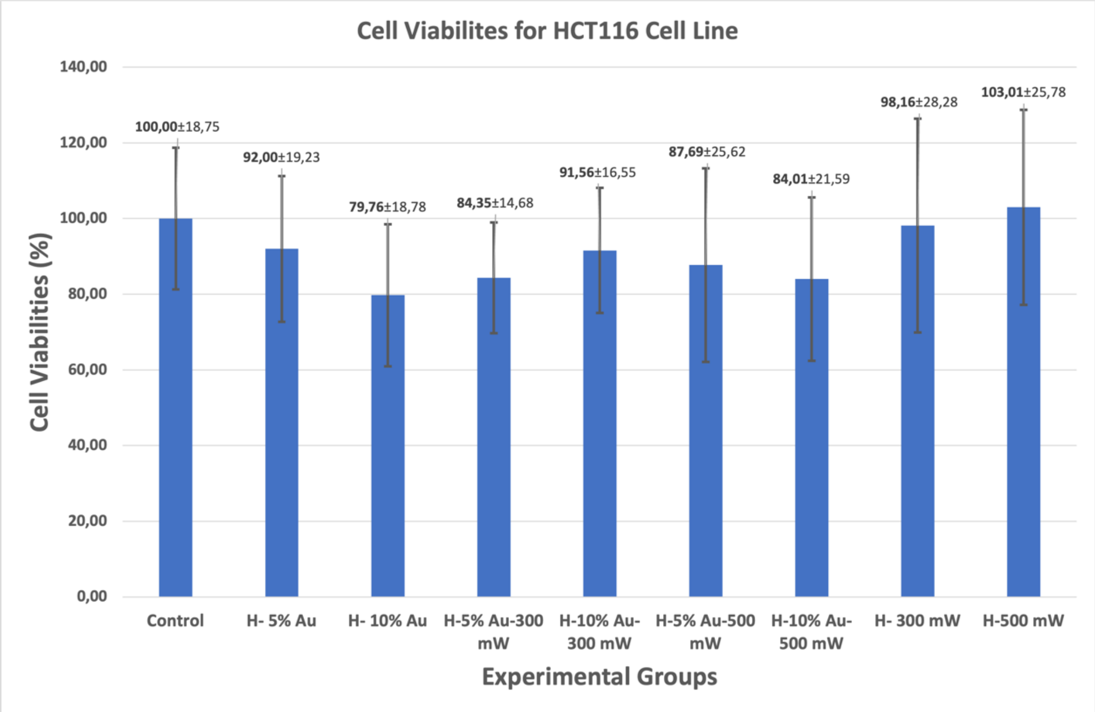

Figure 59. Cell Viabilities of HCT116 Colon Cancer Cell Line (%) After Treatments.

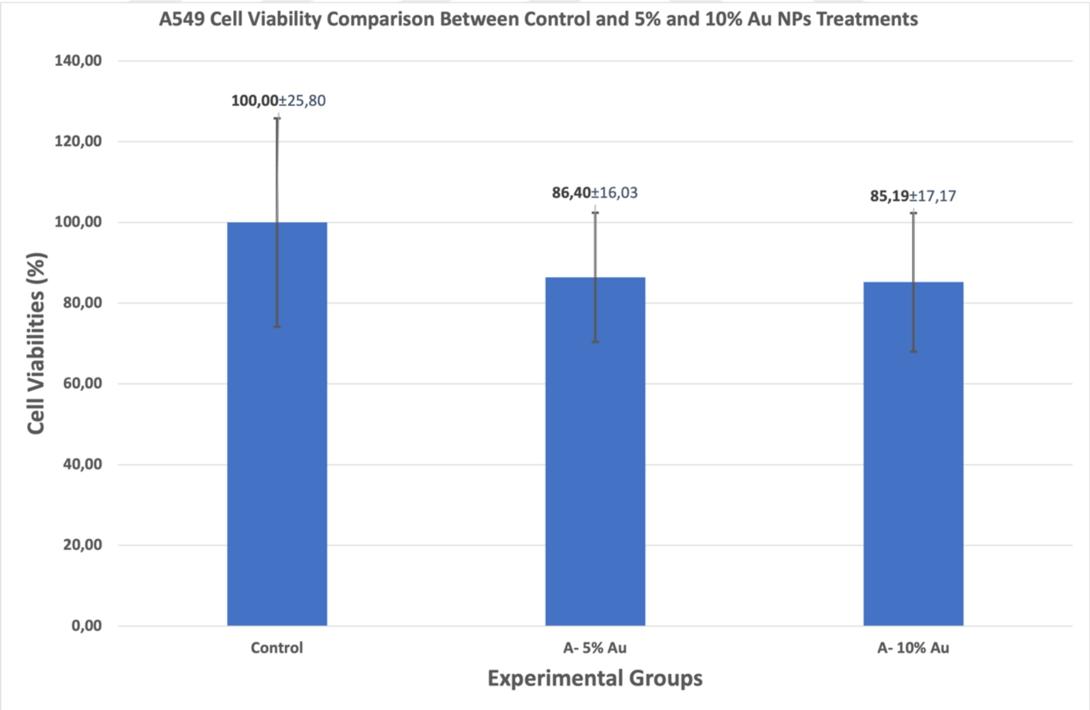

Figure 60. HCT116 Cell Viability Comparison Between Control Group and, 5% and 10% Au NPs Treatments, p-value>0,05, significance of 1,000, and 0,981, respectively.

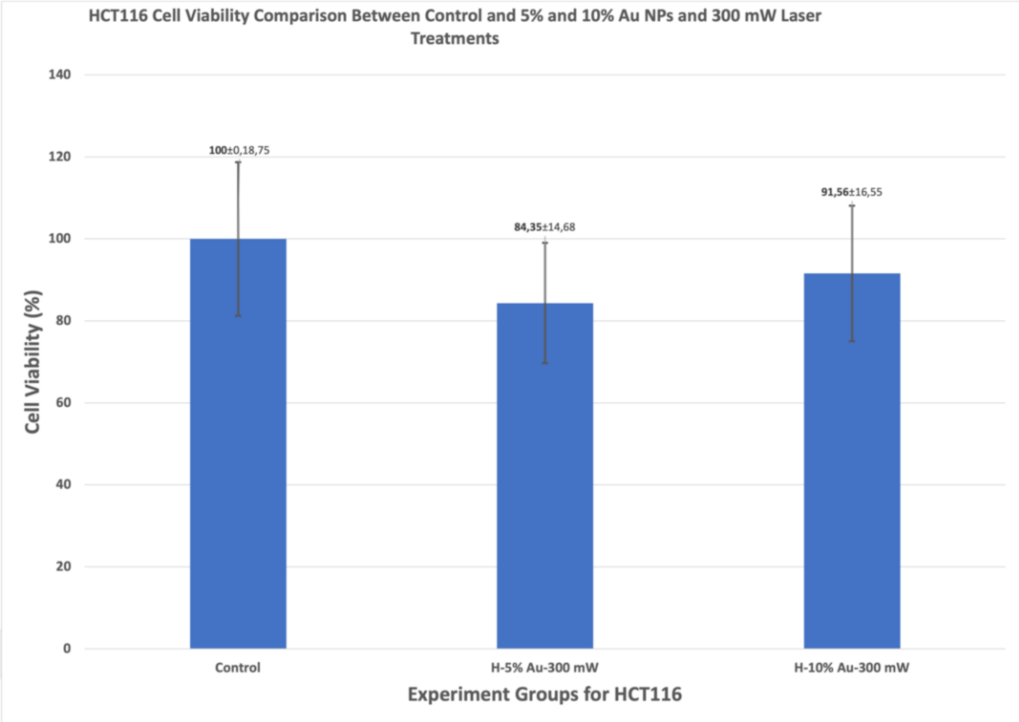

Figure 61. HCT116 Cell Viability Comparison Between Control Group to 5% and 10% Au NPs with 660 nm 300 mW Laser Treatments, p-value>0,05, significance of 0,996, and 1,000, respectively.

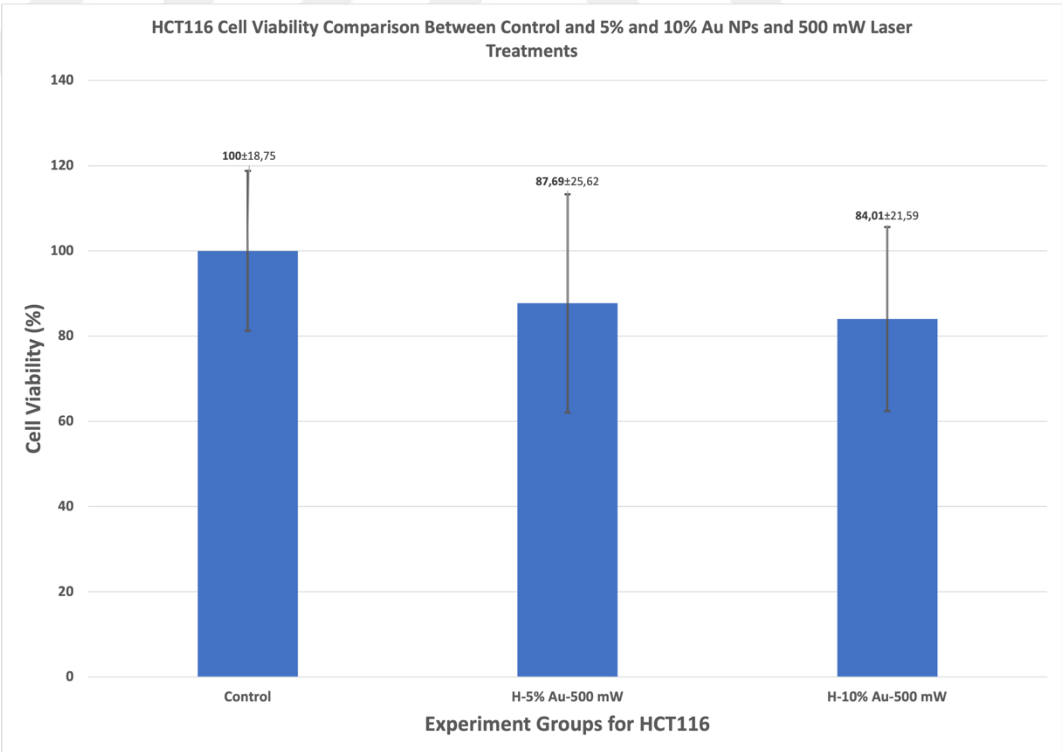

Figure 62. HCT116 Cell Viability Comparison Between Control Group to 5% and 10% Au NPs with 660 nm 500 mW Laser Treatments, p-value>0,05, significance of 0,999, and 0,996, respectively.

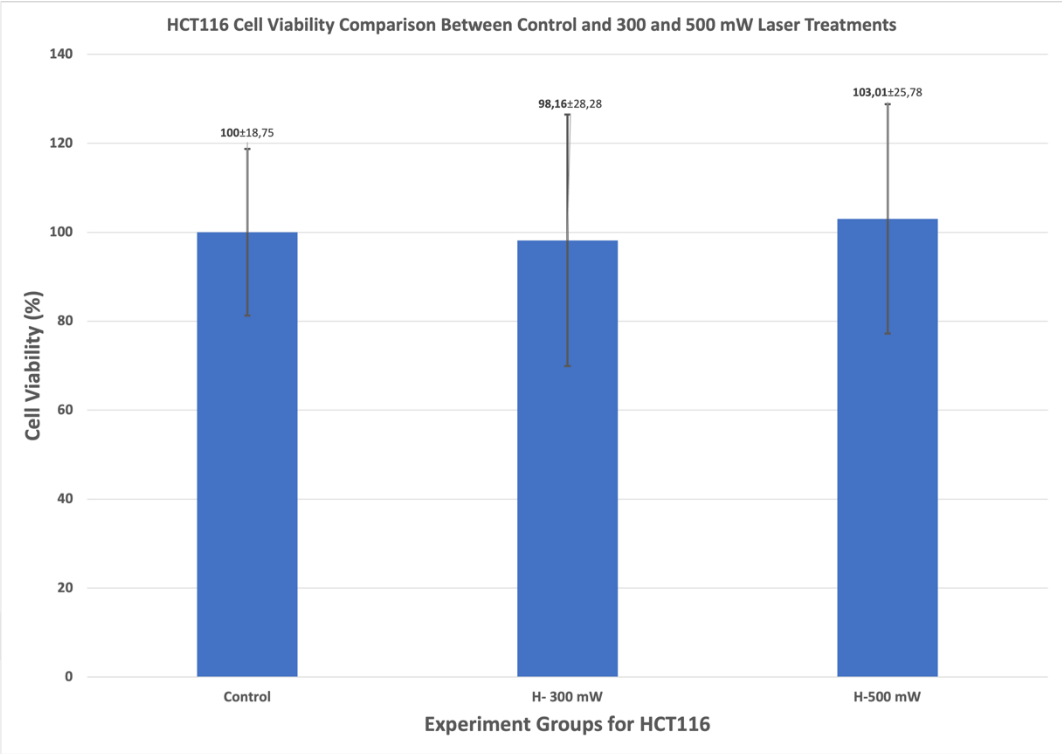

Figure 63. HCT116 Cell Viability Comparison Between Control Group to 300 and 500 mW Laser Treatments, p-value>0,05, significance of 1,000.

For the HCT116 results, the prospect was cell death in several experimental groups such as 5% Au, 10% Au, 5% Au & 500 mW, and 10% Au & 500 mW laser irradiation. Except for 500 mW laser irradiation, cell death of the HCT116 colon cancer cell line was observed. Therein, the HCT116 cancer cell behavior can differ from the A549 cell line. The migration of A549 is higher than HCT116 cells, but the cell viabilities were lower because the A549 cell line proliferates on top of each other which causes an unwelcoming multilayered environment and leads to cell death. HCT116 cells can migrate and overlap each other while proliferating, and reach maximum confluency levels, but stay alive at the same time. In conclusion, specific treatment groups as mentioned before can prevent metastasis, but cell viability may still exist.

In this thesis, the effects of Au NPs and laser treatments were investigated. After the treatments of Au NPs and laser irradiation, there is a change in migration and cell viability in both of cancer cell lines. For migration evaluation, the scratch assay was used. Before the treatments, a gap between cancer cells was opened in the middle of the 6-well plate. The predictable results after the treatments were, the weakness of cancer cells for not to migrate and not to attach to the surface to close the vacancy. For A549 lung cancer cell lines, the migration occurred, and cells closed the gap in 48 hours. As an observation at the beginning of the experiments, the growth of the A549 cell line was slower than the HCT116 cell line. Consequently, the expected closure of the gap for HCT116 was greater than A549. However, the results showed a significantly opposite direction. The gap in the middle of the HCT116 cell line remained for 72 hours, as a conclusion of treatment success. After the migration tests, the cell viability test was performed with the MTT test. Via the MTT principle, the absorbances of experiment groups must be lower than the control group. On the other hand, the predictable results for A549 lung cancer cell line viability were higher than the HCT116 colon cancer cell line. Cell viabilities of A549 for each experimental group, except the 500 mW laser irradiated one, were decreased compared to the control group. The explanation for the increment of viability for the group was the aggression of the A549 lung cancer cell line response to 660 nm 500 mW laser. Cell viabilities for HCT116 except for 500 mW were lower than the control group as well. The laser parameter of 660 nm and 500 mW by itself stimulates viabilities for both of the cancer cell lines. The conflict between migration and viability tests for A549 can caused by the overlapping cancer cells and become multilayered by proliferation aggressively. Although HCT116 colon cancer cells have a multilayered structure after proliferating on top of each other, they do not lose their cell viability.

Further experiments will be done to support these findings to investigate more *in vivo* applications such as toxicity effects of Au NPs with 5% and 10% on cancer cell lines and adjust the laser parameters correspondingly.

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