**Republic of Iraq**

**Ministry of Higher Education and Scientific Research**

**University of Babylon**

**College of Medicine**

**Department of Pharmacology**

# Photodynamic Therapy on Primary Liver Cancer (PLC/PRF/5) and Colon Cancer (SW 480) Cells Using Different Types of Nano-materials.

## A thesis

Submitted to the Council of the College of Medicine, the University of Babylon, as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology/Pharmacology and Toxicology

**By**

**Hawraa Ali Naser**

B.Pharmacy (2012-2013)

**Supervised by**

Prof. Dr.
**Nisreen J. Mohammed**
Ph.D. Pharmacology

Prof. Dr.
**Rana A Ghaleb.**
Ph.D. Biology

**1444 A.H**

**2022 A.D**

بسم الله الرحمن الرحيم

يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ (11)

صدق الله العلي العظيم

سورة المجادلة الآية (11)

## Certification

We certify that this thesis entitled (**Photodynamic Therapy on Primary Liver Cancer (PLC/PRF/5) and Colon Cancer (SW 480) Cells Using Different Types of Nano-materials.**) was prepared by (Hawraa Ali Naser) under our supervision at the Department of Pharmacology, College of Medicine, University of Babylon (Iraq) in partial fulfillment of the requirements for the Master degree of Sciences in Pharmacology and Toxicology.

Signature
Prof. Dr.
**Nisreen Jalal Muhammed**
Supervisor

Signature
Prof. Dr.
**Rana Ayad Ghaleb**
Supervisor

### The Recommendation of the Head of Department

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature
Assist. Prof. Dr.
**Selman Mohammed Selman**
The Head of Department of Pharmacology

## Examining Committee Certificate

We certify that we have read this thesis entitled **"Photodynamic Therapy on Primary Liver Cancer (PLC/PRF/5) and Colon Cancer (SW 480) Cells Using Different Types of Nano-materials"** and as an examining committee examined the student **"Hawraa Ali Naser"** in its contents and that in our opinion it meets the standard of a thesis for the Master degree of Sciences in Pharmacology and Toxicology.

**Prof.**
**Dr. Emad Mohammed Rasheed**
(Chairman)

**Ass. Prof.**
**Dr.Layla Mohammed Al-ameri**
(Member)

**Ass. Prof.**
**Selman Mohammed Selman**
(Member)

**Prof.**
**Dr.Nisreen Jalal Muhammed**
(Member and Supervisor)

**Prof.**
**Dr.Rana Ayad Ghaleb**
(Member and Supervisor)

Approved for the college council

**Prof.**

**Dr.Mohend Abbass Nori Alshalah**

(Dean of College of Medicine)

# Dedication

*Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our heart. My humble effort is dedicated to the helpful and supportive Father, Mother, and lovely Husband. Whose affection, love, encouragement and prays of day and night make me able to get such success and honor. Along with the hardworking and respected supervisors and all who's inspired me especially my sisters and my sons.*

Hawraa Ali

# Acknowledgment

First and foremost, praises and thanks to the God, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I would like to express my deep and sincere gratitude to my research supervisor, Dr. Nisreen J. Muhammed and Dr. Rana A. Ghaleb for giving me the opportunity to do my research and providing invaluable guidance throughout this research.

Their dynamism, vision, sincerity, and motivation have deeply inspired me. They taught me the methodology to carry out the research and to present the research works as clearly as possible.

It was a great privilege and honor to work and study under their guidance. I am extremely grateful to my family for their love, prayers, caring and sacrifices for educating and preparing me for my future.

Finally, special thanks and gratitude to the department of pharmacology and toxicology for the infinite support and cooperation, thanks to all the people who have supported me to complete the research work directly or indirectly.

## Summary:

Cancer arises when certain epithelial cells develop a series of genetic mutations. Treatment may include surgery, radiation therapy, and chemotherapy, which are often associated with serious side effects and toxicity, significantly affecting the quality of life of patients. This led scientists to look for newer alternative treatments and PDT with nanomaterials is one of these treatments.

This study aims to investigate cytotoxic effect of metals-based nanoparticles on cancer cell proliferations of both primary liver cancer (PLC/PRF/5) cells and colon cancer cells by using photodynamic therapy. The cytotoxicity of $Tio_2$ NTs, silver decorated with $Tio_2$ NTs, gold nanoparticles and gold NPs in combination with folic acid was measured on PLCs and SW480 cell line for 24 hours' incubation periods. Nine in vitro experiments were done on these cell lines and the cells of these lines were seeded in 96-well plate. Nano materials were used in six serial dilutions (500,250,125,62.5,31.3,16.2) µg\ml.

The 3-(4,5-dimethylthiazole -2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to determine the number of viable cells, and the intensity of color was measured by ELISA reader.

**Ex (1):** Two plates were seeded with liver cancer cells and colon cancer cells and each plate were exposed to 200 µl of six serial dilutions of $Tio_2$ and Ag decorated $Tio_2$ nanotubes, after 24 hrs. of incubations MTT assay was made to measure viability of cancer cells. The results of these studies showed that $Tio_2$ decrease in cell viability percent($p<0.001$) at higher concentrations on colon cancer cells, while on primary liver cancer cells the results don’t differ from the control. The results of Ag decorated $Tio_2$ nanotubes showed decrease in cell

viability of both cancer cells($p<0.001$) at higher concentrations compared with the control.

**Ex (2):** Two plates were seeded with liver cancer cells and the second with colon cancer cells then the two plates were exposed to diode red laser with 635 nm for different times (1min,3min,5min,7min,9min and 12 min) after 24 hrs. of incubations MTT assay was made to measure viability of cancer cells. The results of this study showed that there is significant decrease in cell viability percent ($p<0.001$) differ from the control on both cell lines at 7,9 and 12 minutes.

**Ex (3):** Two plates were seeded with liver cancer cells and the second with colon cancer cells lines and each plate was exposed to 200µl of six serial dilutions of $Tio_2$ nanotubes and Ag decorated $Tio_2$ nanotubes, then the two plates expose to diode laser with 635 nm for 12 minutes after 24 hrs. of incubations MTT assay was made to measure viability of cancer cells. The results of $Tio_2$ nanotubes on both cancer cell lines showed decrease in cell viability($p<0.001$) at higher concentrations. The results of Ag decorated $Tio_2$ nanotubes on both cancer cell lines showed decrease in cell viability($p<0.001$) at all concentrations.

**Ex (4):** Two plates were seeded with liver cancer cells and the second with colon cancer cells then each plate was exposed to 200 µl (190 µl medium plus 10µl folic acid serial dilutions) ,200 µl of gold nanoparticles six serial dilutions and 200 µl of six serial dilutions of gold nanoparticles combined with folic acid after 24 hrs. of incubation MTT assay was made to measure viability of cancer cells. The results of these studies showed that gold nanoparticles decrease in cell viability of both cancer cell lines at higher concentrations, while results of

Folic acid and gold nanoparticles combined with folic acid caused decrease in cell viability of both cancer cell lines ($p<0.001$) compared with the control.

**Ex (5):** Two plates were seeded with liver cancer cells and the second with colon cancer cells then each plate was exposed to 200 µl (190 µl medium plus 10µl folic acid serial dilutions) ,200 µl of gold nanoparticles six serial dilutions and 200 µl of six serial dilutions of gold nanoparticles combined with folic acid then two plates exposed to diode laser with 635 nm wave length for 12 min at all concentrations after 24 hrs. of incubation MTT assay was made to measure viability of cancer cells. The results of these studied showed decrease in cell viability of both cancer cell lines ($p<0.001$) compared with the control.

## List of Contents


| Item No. | Subjects | Page No. |
|---|---|---|
| | Summary | I |
| | List of Contents | V |
| | List of Tables | XII |
| | List of Figures | XIII |
| | List of Abbreviations | XV |
| **Chapter One: Introduction and Literature Review (1-50)** | | |
| 1.1 | Introduction | 1 |
| 1.2 | cancer | 3 |
| 1.3 | Cancer pathogenesis | 3 |
| 1.4 | Primary liver cancer (PLC/PRF/5) | 5 |
| 1.4.1 | Differentiation between primary and secondary liver cancer | 5 |
| 1.4.2 | Screening and Diagnosing Tools | 6 |
| 1.4.2.1 | Ultrasonography | 6 |
| 1.4.2.2 | Computerized tomography (CT) and magnetic resonance imaging (MRI) | 7 |
| 1.4.2.3 | New serum biomarkers | 7 |
| 1.4.3 | Treatment of primary liver cancer (PLC/PRF/5) | 8 |

| | | |
|---|---|---|
| 1.4.3.1 | Hepatectomy surgical treatment | 8 |
| 1.4.3.2 | Local ablation therapy | 8 |
| 1.4.3.3 | Systemic treatment | 9 |
| 1.4.3.4 | Supportive treatment | 10 |
| 1.5 | Colorectal cancer | 10 |
| 1.5.1 | classification | 11 |
| 1.5.2 | Stages of colorectal cancer | 12 |
| 1.5.3 | Risk factors of colorectal cancer | 13 |
| 1.5.3.1 | Unchangeable risk factor | 13 |
| 1.5.3.2 | Changeable risk factor | 13 |
| 1.5.4 | Test option for screening of colorectal cancer | 13 |
| 1.5.4.1 | Colorectal cancer screening includes several test options | 14 |
| 1.5.6 | Treatment of colorecta cancer | 14 |
| 1.5.6.1 | Treatment for colorectal cancer can include surgery, radiation therapy, and chemotherapy | 15 |
| 1.6 | Phototherapy | 17 |
| 1.6.1 | Photodynamic therapy (PDT) | 19 |
| 1.6.2 | Photodynamic Reaction | 20 |
| 1.6.3 | PDT Essential Elements | 23 |
| 1.6.3.1 | Photosensitizer | 23 |
| 1.6.3.2 | Light | 24 |
| 1.6.3.3 | Oxygen | 26 |

| | | |
|---|---|---|
| 1.6.4 | Applications of PDT | 27 |
| 1.6.5 | Advantages and Limitations of PDT | 28 |
| 1.7 | Nanotechnology | 29 |
| 1.7.1 | Role of nanotechnology in cancer therapy | 30 |
| 1.7.2 | Silver Nanotubes | 31 |
| 1.7.2.1 | Toxicity of silver | 33 |
| 1.7.3 | Titanium dioxide nanotubes ($TiO_2$-NTs) | 34 |
| 1.7.3.1 | Toxicity of $Tio_2$ | 36 |
| 1.7.4 | Gold nanoparticles | 37 |
| 1.7.4.1 | Toxicity of gold Nano -particles | 41 |
| 1.8 | Cell Culture | 42 |
| 1.9 | Cell line | 43 |
| 1.9.1 | Colon cancer cell line (SW480) | 45 |
| 1.9.2 | Primary liver cancer (PLC/PRF/5) cell line | 46 |
| 1.10 | Cell's viability | 46 |
| 2 | Material and methods | 48 |
| 2.1 | Materials | 48 |
| 2.1.1 | chemicals | 48 |
| 2.1.2 | Instruments and Tools | 50 |
| 2.1.3 | Preparation of Reagents and Solutions | 52 |

| | | |
|---|---|---|
| 2.1.3.1 | Phosphate buffer saline | 52 |
| 2.1.3.2 | Trypsin (EDTA)solution | 52 |
| 2.1.3.3 | MTT solution | 52 |
| 2.1.4 | Preparation of Tissue Culture Medium | 53 |
| 2.1.4.1 | Preparation of serum free medium | 53 |
| 2.1.4.2 | Preparation of serum medium | 53 |
| 2.1.4.3 | Preparation of freezing medium | 53 |
| 2.1.5 | Synthesis of gold Nano particles Turkevich method | 54 |
| 2.1.6 | Folic acid stock solution preparation | 54 |
| 2.1.7 | Silver decorated Tio2 nanotubes stock solution preparation | 55 |
| 2.1.8 | $Tio_2$ nanotubes stock solution preparation | 55 |
| 2.1.9 | Laser irradiation | 55 |
| 2.1.10 | Transmission electronic microscope | 56 |
| 2.2 | methods | 56 |
| 2.2.1 | Preparation of PLC/PRF/5 Cell Lines for Cytotoxicity Assays | 56 |
| 2.2.1.1 | Thawing of PLC/PRF/5 and SW480 cell lines | 56 |
| 2.2.1.2 | Harvesting and sub-culturing PLC/PRF/5and SW480 Cell Lines | 57 |
| 2.2.1.3 | Freezing of PLC/PRF/5 and SW480 Cell Lines | 58 |

| | | |
|---|---|---|
| 2.2.2 | The experiments of studying cytotoxic effects of Nano- based metals on (PLC/PRF/5) and (SW480) cell lines | 59 |
| 2.2.2.1 | Study cytotoxic effect of titanium dioxide nanotubes and Ag decorated $Tio_2$ nanotubes | 59 |
| 2.2.2.2 | Study cytotoxicity effect of laser on both primary liver cancer (PLC/PRF/5) (PLCs) and colon cancer (SW480) | 60 |
| 2.2.2.3 | Study cytotoxicity effect of silver decorated $Tio_2$ nanotubes and titanium dioxide nanotubes with laser on both primary liver cancer (PLC/PRF/5) (PLCs) and colon cancer (SW480) | 60 |
| 2.2.2.4 | study cytotoxic effect of gold nanoparticles combined with folic acid | 61 |
| 2.2.2.5 | Study effect of gold NPs combined with folic acid and laser | 62 |
| 2.2.3 | Cytotoxicity assay (MTT) | 64 |
| 2.2.3.1 | principle | 64 |
| 2.2.3.2 | procedure | 65 |
| 2.3 | Statistical analysis | 66 |
| **Chapter Three: Results** | | |
| 3 | Results | 67 |

| | | |
|---|---|---|
| 3.1 | Cytotoxicity evaluation of $Tio_2$ NTs on the viability of PLC and SW480 cancer cell line | 67 |
| 3.2 | Cytotoxicity evaluation of Ag- $Tio_2$ on the viability of PLC and SW480 cancer cell line | 68 |
| 3.3 | Cytotoxicity evaluation of laser on the viability of PLC and SW480 cancer cell line | 69 |
| 3.4 | Cytotoxicity evaluation of $TiO_2$ with laser on the viability of PLC and SW480 cancer cell line | 70 |
| 3.5 | Cytotoxicity evaluation of Ag decorated $Tio_2$ with laser on the viability of PLC and SW480 cancer cell line | 71 |
| 3.6 | Cytotoxicity evaluation of Folic acid on the viability of PLC and SW480 cancer cell line | 72 |
| 3.7 | Cytotoxicity evaluation of Au NPs on the viability of PLC and SW480 cancer cell line | 73 |
| 3.8 | Cytotoxicity evaluation of Folic acid combined Au NPs on the viability of PLC and SW480 cancer cell line | 74 |
| 3.9 | Cytotoxicity evaluation of Au NPs combined folic acid with laser on the viability of PLC and SW480 cancer cell line | 75 |
| 3.10 | Transmission Electron Microscopy results of metals-based Nanotubes | 76 |

| | | |
|---|---|---|
| 3.10.1 | TEM of TiO2 NTs | 76 |
| 3.10.2 | TEM of Ag decorated TiO2 NTs | 76 |
| 3.10.3 | TEM of Gold NPs | 77 |
| **Chapter Four: Discussion** | | |
| **4** | Discussion | 78 |
| **4.1** | cytotoxic effect of $Tio_2$ nanotubes on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 78 |
| **4.2** | Cytotoxic effect of silver modified $Tio_2$ NTs on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 80 |
| **4.3** | Cytotoxicity of laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 82 |
| **4.4** | cytotoxicity $Tio_2$ and laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 84 |
| **4.5** | cytotoxicity of silver modified $Tio_2$ NTs and laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines: | 85 |
| **4.6** | cytotoxic effect of folic acid on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 87 |

| | | |
|---|---|---|
| **4.7** | cytotoxic effect of Au NPs on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 88 |
| **4.8** | cytotoxic effect of folic acid combined Au NPs on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 90 |
| **4.9** | cytotoxic effect of folic acid and Au NPs and laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines | 91 |
| **Conclusions and Recommendations** | | |
| **Conclusions** | 93 | |
| **Recommendations** | 94 | |
| **References** | **95** | |


## List of Tables


| No. | Titles | Pages |
|---|---|---|
| **2.1** | Chemicals used in the study | **48** |
| **2.2** | List of Instruments and Tools Used in the Study | **50** |

## Lists of Figures


| No. | Titles | Pages |
|---|---|---|
| 1.1 | (a) Schematic of PDT macroscopic effect in tissue<br>(b) Schematic of PTT macroscopic effect in tissue over time | 18 |
| 1.2 | Photodynamic reaction Jablonski diagram | 21 |
| 1.3 | Photodynamic mechanism of tumor destruction | 23 |
| 1.4 | Effect of Laser on Tissue | |
| 1.5 | Methods eradicating the cancer cells after nanoparticles accumulation at the tumor site | 39 |
| 2.1 | 96 tissue culture plate seeded with cancer cells and exposed to $Tio_2$ and Ag decorated $Tio_2$ nanotubes. | 59 |
| 2.2 | 96 tissue culture plate seeded with cancer cells and exposed to $Tio_2$ and Ag decorated $Tio_2$ nanotubes with red laser 635 nm exposure. | 60 |
| 2.3 | 96 tissue culture plate seeded with cancer cells and exposed to gold nanoparticles, Folic acid and Gold NPs combined with Folic acid. | 62 |
| 2.4 | 96 tissue culture plate seeded with cancer cells and exposed to gold nanoparticles, Folic acid and Gold NPs combined with Folic acid with red laser 635 nm at different times. | 63 |
| 2.5 | Principle of MTT Assay | 64 |

| | | |
|---|---|---|
| 3.1 | Effect of $Tio_2$ on viability of PLC and SW480 cancer cell line using MTT assay | 67 |
| 3.2 | Effect of Ag- $Tio_2$ on viability of PLC and SW480 cancer cell line using MTT assay | 68 |
| 3.3 | Effect of laser on viability of PLC and SW480 cancer cell line using MTT assay | 69 |
| 3.4 | Effect of $TiO_2$ with laser on viability of PLC and SW480 cancer cell line using MTT assay | 70 |
| 3.5 | Effect of Ag decorated $Tio_2$ with laser on viability of PLC and SW480 cancer cell line using MTT assay. | 71 |
| 3.6 | Effect of Folic acid on viability of PLC and SW480 cancer cell line using MTT assay | 72 |
| 3.7 | Effect of Au NPs on viability of PLC and SW480 cancer cell line using MTT assay | 73 |
| 3.8 | Effect of Folic acid with Au NPs on viability of PLC and SW480 cancer cell line using MTT assay | 74 |
| 3.9 | Effect of Au NPS with Folic acid with laser on viability of PLC and SW480 cancer cell line using MTT assay | 75 |
| 3.10 | Transmission Electron Microscopy results of metals-based Nanotubes | 76 |

## List of Abbreviation

| Abbreviation | Meaning |
|---|---|
| 5-FU | 5-fluorouracil |
| Ag NTs | Silver nanotubes |
| Au NPs | Gold nanoparticles |
| Bcl2 | B cell lymphoma2 |
| CHC | Combined hepatocellular cholangiocarcinoma |
| CT | Computed tomography |
| DNA | Deoxyribonucleic acid |
| EGF | Epidermal growth factor |
| EPR | Enhanced permeability and retention |
| ER | Endoplasmic reticulum |
| FAR | Folic acid receptor |
| FBS | Fetal bovine serum |
| GSH | Glutathione synthetase |
| $H_2O_2$ | Hydrogen peroxide |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| HMGB1 | High mobility group protein -1 |
| HPD | Hematoporphyrin derivatives |
| ICC | Intrahepatic cholangiocarcinoma |
| ICG | Indocyanin green |
| IL1B | Interleukin1B |
| LDH | Lactic acid dehydrogenase |
| LEDs | Light emitting diodes |
| MIP-1α | Macrophage inflammatory protein-1 Alfa |

| | |
|---|---|
| ml | Milliliter |
| MRI | Magnetic resonance imaging |
| MTT | (3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) |
| MWA | Micro wave ablation |
| NAFLD | Non-alcoholic fatty liver disease |
| NP | nanoparticles |
| NT | nanotubes |
| p53 | Tumor suppressor gene |
| PDT | Photodynamic therapy |
| PEI | Percutaneous ethanol injection |
| PLC | Primary liver cancer (PLC/PRF/5) |
| PS | photosensitizer |
| PTT | Photo thermal therapy |
| RES | Reticuloendothelial system |
| RFA | Radiofrequency ablation |
| ROS | Reactive oxygen species |
| SPR | Surface Plasmon resonance |
| TACE | Trans catheter chemoembolization |
| $TIO_2$ NTs | Titanium dioxide nanotubes |
| TNFα | Tumor necrosis factor Alfa |
| US | ultrasonography |

# Chapter One

# Introduction

# &

# Literature Review

## 1.1 Introduction:

Cancer is one of the deadliest diseases reported in developed as well as developing countries, it is mainly characterized by the uncontrolled cell growth and development of normal cells due to genetic alterations or exposure to the carcinogenic substances. The mutation of normal cells leads to abnormal cellular proliferation and develops into tumor, this can be either benign, premalignant (non-cancerous) or malignant (cancerous)(Hester, Kuriakose et al. 2020).

Presently surgery, radiotherapy, and chemotherapy either as monotherapy or as combined treatments are used in the treatment of cancer. These therapies are used routinely based on the pathological stages and clinical signs of the disease. Despite the advances in treatment protocols, patients' long-term survival is low and there is a high incidence of adverse effects of chemotherapy. Often, drugs used in chemotherapy have poor water solubility. Hydrophobic drugs have reduced biocompatibility and therefore need to be administered in higher dosages to achieve therapeutic concentrations(Senapati, Mahanta et al. 2018).

The low hydro solubility translates into reduced drug bioavailability and high systemic toxicity. Besides, chemotherapy drugs generally have little specificity and cause significant damage to normal tissues, inhibit rapid-growing tissues and cells including hair follicles, gastrointestinal tract cells, and bone marrow and consequently, cause adverse reactions. In addition to the problems related to the anticancer drugs addressed, early detection of cancer is also a challenge (Pérez-Herrero and Fernández-Medarde 2015).

To overcome some limitations of conventional chemotherapy photodynamic therapy, nanotechnology might be the solution (Gomes, Martins et al. 2021).

From the point of view of the need for cancer targeting, nanotechnology seems to be potentially the most important way of employing various nanomaterials to overcome the main limitations of current cancer therapies. Through different targeting ligands like vitamins, nanomaterials may be specifically targeted towards cancer tissues, to selectively bind to cancer cells and destroy them (Bazak, Houri et al. 2015)

**Titanium dioxide nanotubes:** is a white, odorless and non-combustible powder. TiO2 are the important nanoscale components which have been widely used in industry as well as medical applications such as antiviral, cancer-cell treatment and antibacterial effect.

**Silver nanotubes**: Silver is a transition metal in one set with Cupper and Gold which is a soft, white, lustrous element possessing high electrical and thermal conductivity. It has been known extensively due to its medical and therapeutic benefits.

**Gold nanoparticles:** gold nanoparticles have been proposed for diverse biomedical applications due to their unique surface, electronic, and optical properties. Because of the strong and size-tunable surface plasmon resonance, fluorescence, and easy-surface functionalization, gold nanoparticles have been widely used in biosensors, cancer cell imaging, cancer therapies photothermal therapy, and drug delivery.

## Aim of study:

The main aim of this study is to find a solution to avoid many limitations related to conventional chemotherapy there by:

1. Study the cytotoxic effects of $Tio_2$ nanotubes and silver decorated Titanium dioxide nanotubes on primary liver cancer (PLC/PRF/5) (PLCs) and colon cancer (SW480) cancer cell lines.

2. Study the cytotoxic effects of gold nanoparticles on primary liver cancer (PLC/PRF/5) (PLCs) and colon cancer (SW480) cancer cell lines.

3- Study the effect of gold nanoparticles combined with folic acid on primary liver cancer (PLC/PRF/5) and colon cancer (SW480) cancer cell lines.

4-study cytotoxic effect of laser alone and in combination with silver, Titanium dioxide nanotubes and with gold NPs combined with folic acid on both cell lines.

## 1.2 Cancer

Cancer is one of the most challenging diseases, characterized by the development of mutated cells that divide uncontrollably. Cancer cells can spread to different organs in a process called metastasis. which may lead eventually to death. Many factors can cause cancer including external factors such as tobacco smoking, radiation, chemicals, and infections) while internal factors include inherited mutations, hormones, immune conditions, and random mutations. (Mathur et al., 2015).

The leading cause of death worldwide is cancer, accounting for nearly 10 million deaths in 2020 (Ferlay, Colombet et al. 2021). The most common types of cancer cause death including liver cancer, breast cancer, colorectal cancer, lung cancer, stomach cancer, prostate cancer and skin cancer (Bray et al., 2018).

According to the Global Cancer Observatory (GCO), approximately 30 million cancer patients will die from cancer each year by 2030(Bray, Ferlay et al. 2018). In addition to the high mortality of cancer, the economic burden on families of cancer patients and society is enormous. Therefore, efforts on cancer prevention, diagnosis and treatment are of great importance

## 1.3 Cancer pathogenesis:

Deoxyribonucleic acid (DNA), alterations that result in dysregulate gene structure and function can cause cancer. the damage to the cellular genetic material alteration in expression of genes is a common feature for virtually all neoplasms, when there is an inherited error in DNA replication, the cells of multicellular organisms face the same certainty of developing neoplasms if they survive long enough.

Many mutations may be inconsequential, but cancer can develop when non-lethal mutations occur in a small subset of the coding and noncoding regions of the genome. The fundamental cause of all cancers is genetic damage, which is usually acquired but is sometimes congenital (Cullen & Breen, 2017).

In general, the genetic dysregulation that gives rise to uncontrolled cell proliferation results from activation of growth-promoting oncogenes and/or deletion/inactivation of growth-inhibiting tumor suppressor genes.

Another contribution to carcinogenesis may result from genes that regulate the programmed cell death called apoptosis process and genes involved in DNA repair (Hill, 2019).

One causes of cancer is the infectious agents, the mechanisms by which each pathogen causes cancer are diverse and include induction of genomic instability as a result of chronic inflammation, impairment of host immunity, and, in some cases, a modulation of the balance between proliferation and antiproliferation signals (Hill, 2019).

The ordinary natural physiological tissue repair mechanisms may become pathophysiological when micro damage occurs due to exogenous factors including chemical, physical and biological carcinogens. as well as the presence of imbalances in the autonomic nervous system stimulation. Sympathetic/hyper sympathetic dominance may cause tissue ischemia and consequently hypoxia this micro damage can occur resulting in chronic inflammation with possible formation of cancer cell that could be permanent (Bukhtoyarov & Samarin, 2015).

## 1.4 Primary liver cancer (PLC/PRF/5):

Primary liver cancer (PLC/PRF/5) is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer mortality worldwide is more common in men than in women(Bray, Ferlay et al. 2018) Primary liver cancer (PLC/PRF/5) , includes hepatocellular carcinoma (HCC) originating from hepatocytes, intrahepatic cholangiocarcinoma (ICC) originating from cholangiocytes, and combined hepatocellular cholangiocarcinoma (CHC) Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) account, respectively, for 85% and 10% of all primary liver cancer (PLC/PRF/5) (Ji, Zhu et al. 2020).

HCC generally occurs in the presence of liver disease or cirrhosis (Ding, Tu et al. 2021), and at least 60% of HCC cases worldwide are caused by viral hepatitis. Chronic hepatitis B virus (HBV), typically acquired at birth or early childhood, remains the leading cause in most high-risk HCC areas (de Martel, Maucort-Boulch et al. 2015).

The predominant etiology in developed countries is more likely to be hepatitis C virus (HCV) infections acquired later in life, other factors include dietary aflatoxin exposure, smoking, and high alcohol consumption, with nonalcoholic fatty liver disease (NAFLD) associated with obesity and diabetes increasingly emerging as a key contributor in the USA and other Western countries (Kulik and El-Serag 2019).

### 1.4.1 Differentiation between primary and secondary liver cancer:

Tumors metastatic to the liver are more common than primary tumors. The most common sites of primary tumor are breast, lung, and colorectal cancer (Ding, Tu et al. 2021). Liver metastases may be rarer in other primary tumors,

with only 10% of distant metastases in head and neck cancers present in the liver (Ferlito, Shaha et al. 2001).

The high incidence of hepatic metastases has been attributed to two mechanisms. (Ferlito, Shaha et al. 2001) First, the dual blood supply of the liver from the portal and systemic circulation increases the likelihood of metastatic deposits in the liver. Second, the hepatic sinusoidal epithelium has fenestrations that enable easier penetration of metastatic cells into the liver parenchyma (Jepsen and Kissmeyer-Nielsen 2008).

### 1.4.3 Treatment of primary liver cancer (PLC/PRF/5):

**1.4.3.1** Hepatectomy surgical treatment is the most important approach for achieving long-term survival for liver cancer patients. These treatments include hepatectomy and liver transplantation(Ward, Sherif et al. 2021)

**1.4.3.2** Local Ablation Therapy only approximately 20–30% of liver cancer patients have the opportunity to undergo surgical resection because a large portion of liver cancer patients have liver cirrhosis or are already at the middle/terminal stage at the time of diagnosis(Peng, Zhang et al. 2013).

However, surgery is the best treatment choice to obtain long-term survival. Local ablation therapy, which has been widely used in recent years, provides early liver cancer patients with a curative outcome. Local ablation therapy is designed to directly destroy tumor tissues using physical or chemical methods under the guidance of imaging techniques, these techniques mainly include RFA, microwave ablation (MWA), cryotherapy, high-power focused ultrasonography ablation, and percutaneous ethanol injection (PEI). Local ablation therapy is most often performed under the guidance of ultrasonography, which has the advantages of real time and high efficiency.

CT and MRI in combination with multimode imaging systems can be used to monitor lesions when ultrasonography is unable to do so, CT and MRI guidance techniques can also be used for the ablation of metastases in the lungs, adrenal glands, and bones (Hasegawa, Aoki et al. 2014)

### 1.4.3.3 Systemic Treatment

Systemic Treatment for patients with advanced liver cancer, systemic treatment may reduce tumor burden, relieve tumor-related symptoms, improve quality of life, and prolong survival**.**

- **Antitumor Treatments and their efficacy molecular target drugs**.

Sorafenib is the only molecular target drug approved at this time for the treatment of advanced liver cancer

- **Systemic Chemotherapy**

Conventional cytotoxic drugs, including Adriamycin, epirubicin, fluorouracil, cisplatin, and mitomycin, have low efficacy, significant side effects, and poor repeatability when they are used as alone or in combination for the treatment of liver cancer. Chemotherapy drugs not only activate HBV replication but also damage liver function, thus leading to a lack of survival benefits from chemotherapy, the EACH study showed that the FOLFOX4 regimen containing oxaliplatin is superior regarding the overall response rate, disease control rate, progression-free survival, and overall survival compared to the conventional chemotherapeutic drug Adriamycin and has good tolerability and safety (Cucchetti, Piscaglia et al. 2017).

- **Antiviral treatment and other Treatments**

Antiviral treatment with nucleoside analogues is essential. Select potent drugs with a low rate of resistance (e.g., entecavir, telbivudine, or tenofovir disoproxil) are recommended, the use of antiviral drugs should be started prior to TACE or chemotherapy because these treatments may lead to active replication of HBV. Antiviral treatment is also associated with lower postoperative recurrence Therefore, antiviral treatment should be performed throughout treatment for liver cancer (Huang, Lau et al. 2015).

### 1.4.3.4 Supportive Treatment

Adequate rehabilitation exercises can increase immunity. In addition, supportive treatments should be administered, including active analgesic treatment, correction of anemia and hypoalbuminemia, enhanced nutritional support, blood sugar control for patients with diabetes, and treatment of concomitant symptoms such as ascites, jaundice, hepatic encephalopathy, and gastrointestinal bleeding in patients with advanced liver cancer.

Doctors should understand the mental status of their patients and their families, take active measures to adjust their mental state by reversing the negative mental state to the active mental state, and reduce depression and anxiety by providing them with a sense of safety and comfort through palliative treatment and care(Xie, Ren et al. 2020).

## 1.5 Colorectal cancer:

Colorectal cancer (CRC) Also known as colorectal adenocarcinoma usually arises from the large intestine's glandular, epithelial cells. When certain epithelium cells acquire a series of genetic mutations, cancer arises. These hyper-proliferative cells give rise to a benign adenoma with an abnormally

increased replication and survival, which may then develop into carcinoma and metastasize over decades (Rawla *etal*., 2019).

The incidence of CRC is increasing because of smoking, lack of physical activity, overweight, and obesity, consuming red and processed meat, and excessive alcohol-consuming (Hamza *et al*., 2017).

### 1.5.1 Classification

Colorectal carcinomas may be classified as sporadic, inherited, and familial depending on the mutation's origin. Mutations that appear in the lifetime are not associated with inherited syndromes and affect only individual cells and their strain. Cancers derived from point mutations are called sporadic cancers and represent 70% of all CRCs. Approximately 70% of CRC cases follow a specific sequence of mutations that are then translated into a specific morphological series, beginning with adenoma formation and ending in the carcinoma state (Mármol, Sánchez-de-Diego et al. 2017). For the inherited cancer, two groups were established to generate a more accurate classification, namely forms of polyposis and non-polyposis.

- The Familial Adenomatosis Polyposis (FAP)

It is a mutation in the gene of adenomatous polyposis coli (APC), a tumor suppressor gene that triggers the formation of non-malignant adenomas, also known as polyps. About 15% of these adenomas are expected to be developed into carcinoma within 10 years; (<1% of colorectal cancer cases) (Wong *et al.*, 2019).

- The Hereditary Non-Polyposis Colorectal Cancer (HNPCC)

(Lynch syndrome) with several mutations in one of the DNA repair genes; (approximately 5% of cases of colorectal cancer) (Mármol *et al.*, 2017). The

mean age of onset of colorectal cancer in both of these syndromes is much lower (less than 50 years old) than in sporadic colorectal cancers, and extra-colonic malignancies are also noticed (Angelis, 2019). Familial colorectal cancer responsible for about 25% of all cases and is also caused by hereditary mutations, even though they're not classified as hereditary cancers since they cannot be included in any hereditary cancer version (Mármol *et al.*, 2017).

The proximal colon is the most common tumor location in CRC followed by the rectum and distal colon. Different CRC tumor sites have different clinical and biological presentations, prognoses, and treatment-resistant (Wong *et al.*, 2019).

### 1.5.2 Stages of Colorectal Cancer

Once a cancer diagnosis is made, one stage will describe cancer. CRC staging describes the tumor's size, how far it has grown into the colon or rectum wall and whether cancer has spread to lymph nodes or other parts in the body past the place where it started to grow

- Stage 0: This is the first stage, when cancer remains within the colon or rectum's mucosa, or inner layer. It is also termed in situ carcinoma.
- Stage1: Cancer cells have spread through the middle layers of the colon or rectum's muscular wall from the inner lining.
- Stage 2: Cancer has spread through or into the colon or rectum wall, but still has not reached neighboring lymph nodes.
- Stage 3: Cancer has invaded the adjacent lymph nodes, but other parts of the body have not yet been affected.

- Stage 4: Cancer spreads to other body parts, including other organs such as the liver, the membrane that lines the abdominal cavity, the lung, or the ovaries (Hamza et al.,2017).

### 1.5.3 Risk Factors

#### 1.5.3.1 Unchangeable risk factors:

- Elderly.
- Has breast, ovary, or cervical cancer.
- Family history of colorectal cancer or adenomatous polyps.
- Personal history of (IBD): Ulcerative colitis and Crohn's disease are the main causes behind IBD.
- Personal history of adenomatous polyps, the presence of polyps in the colon or rectum, which can sooner or later become cancerous.
- Genetically inherited risk: The most common inheritance conditions are FAP and HNPCC, also called Lynch syndrome (Hamza et al.,2017).

#### 1.5.3.2 Changeable risk factors:

- Overweight and obesity, and a lack of exercise.
- High consumption of alcohol.
- Smoking.
- A diet that is rich in animal protein, saturated fats, calories, and a diet that has low fiber (Rawla et al., 2019).

### 1.5.6 Treatment

Endoscopic diagnosis plays an important role in determining therapeutic strategy. Endoscopic diagnosis is intended to predict the oncological

characteristics of a lesion based on endoscopic characteristics, such as size, and also to distinguish between malignant and benign tumors (Ishida and Koda, 2019). Since not all CRCs share similar driving mutations, it has been difficult to design a “catch-all” molecular therapy.

Colorectal cancer treatment options depend on the stage of the tumor that is, how far it has spread, or how deeply it affects the intestinal wall and other tissues, and whether it is located in the colon or rectum.

Patients with colon cancer generally receive post-operational chemotherapy if the lymph nodes are positive. For rectal cancer, most patients with positive nodes or tumors that extend into the fat surrounding the rectum are treated with chemotherapy plus radiation before surgery. Treatment is also adjusted to the age, medical history of the patient, overall health, and tolerance of specific medications and therapies(Lech, Słotwiński et al. 2016).

**1.5.6.1Treatment for colorectal cancer can include surgery, radiation therapy, and chemotherapy.**

- Surgery

Among all therapeutic options, surgery remains the primary course of treatment in early diagnosis cases where surgery with a curative purpose is still possible but is no longer effective in advanced cases where cancer has metastasized, as is the case in about 25% of diagnoses (Rawla et al., 2019). In the early stages, surgery is used to cure cancer by completely removing the tumor and tissues it affects. The type of colectomy is based on the location of the tumor and the lymphatic vascular drainage. Recent evidence supports the principle of complete mesocolic excision (CME) as the optimal approach to be applied to all colon cancer (Angelis, 2019).

❖ Radiation Therapy

Radiation may be used as ultimate therapy in combination with surgery or may be used to decrease or alleviate the symptoms of colorectal cancer such as pain, bleeding, or blockage when curative therapy is not possible.

In selected patients with rectal cancer, radiation therapy is often given before surgery to improve outcomes. It is used before surgery to shrink tumors, for the destruction of cancer cells that may remain after the surgery ( Mehmet, 2015).

❖ Chemotherapy

For the treatment of CRC, drugs such as fluorouracil (5-FU), irinotecan, oxaliplatin, and humanized monoclonal antibodies (bevacizumab, cetuximab, and panitumumab) were approved. Also, the introduction of an orally active drug, capecitabine in the chemotherapeutic protocol for the CRC (Angelis, 2019). Standard cytotoxic chemotherapy remains the mainstay of treatment for metastatic CRC; however, a growing appreciation for the molecular subtypes of CRC has led to recent advancements in the manner in which this disease is treated. FOLFOX (leucovorin, 5-FU, and oxaliplatin) (Greally & Ilson, 2019), or FOLFIRI (leucovorin, 5-FU, and irinotecan) chemotherapy regimens are largely utilized in the first-line setting, commonly in conjunction with bevacizumab, bevacizumab is a humanized monoclonal antibody that targets the vascular endothelial growth factor (Destefanis et al., 2019).

❖ Target Therapy

These drugs work in different ways from standard chemotherapy drugs. They affect particular parts of cancer cells that differentiate them from normal cells. However, cytotoxic chemotherapy remains the primary backbone of treatment, this subtype like agents against the receptor of the epidermal growth factor

(EGFR) is targeted. EGFR is a transmembrane glycoprotein with an extracellular ligand-binding domain and an intracellular tyrosine kinase domain that mediates downstream signals. EGFR functions resulting in cell growth, proliferation, and regulation of other critical cellular functions upon ligand binding. EGFR inhibitors including cetuximab and panitumumab have been extensively investigated and showed the first-line benefit combined with chemotherapy (Destefanis et al., 2019).

## 1.6 Phototherapy:

There are a variety of phototherapies, that is, treatments that exploit the photochemical, photo-thermal or photomechanical interactions of light with cells and tissues (Agostinis, Berg et al. 2011)

Photodynamic therapy (PDT) is based on photochemistry through the use of light-activatable molecules (or, more recently, nanoparticles), with the activation leading to generation of free radicals or reactive oxygen species (most commonly, singlet-state oxygen, $^{1}O_2$ that are cytotoxic as shown in figure (1.1a), the photosensitizer (or a precursor that leads to endogenous synthesis of photosensitizer) is administered, either systemically (orally or by intravenous injection), or in some cases topically to the surface of the target tissue (Wilson and Weersink 2020). Photo thermal therapy (PTT) uses only light as shown in figure (1.1b), usually in the near-infrared in order to achieve maximum tissue penetration, to kill cells/tissues directly by heat. This requires achieving coagulation-threshold temperatures of around 55–60°C. Photo thermal therapy should be distinguished from lower-temperature light-induced hyperthermia that, unless applied for an extended time, is not directly cytotoxic by itself. Traditionally, PTT has been applied without the use of any administered (exogenous) compounds, although the use of molecular absorbers such as

indocyanine green (ICG) was proposed in the early 1990s for enhanced tumor control and is also used in ophthalmology for non-oncologic condition (Wilson and Weersink 2020).

More recently, nanoparticles of various forms have been incorporated to increase the efficiency or target specificity of the treatment, as recently will use the term "exogenous photo-absorbers" to indicate either molecular or nanoparticle PDT photosensitizers or PTT thermal-enhancement agents (Chitgupi, Qin et al. 2017)

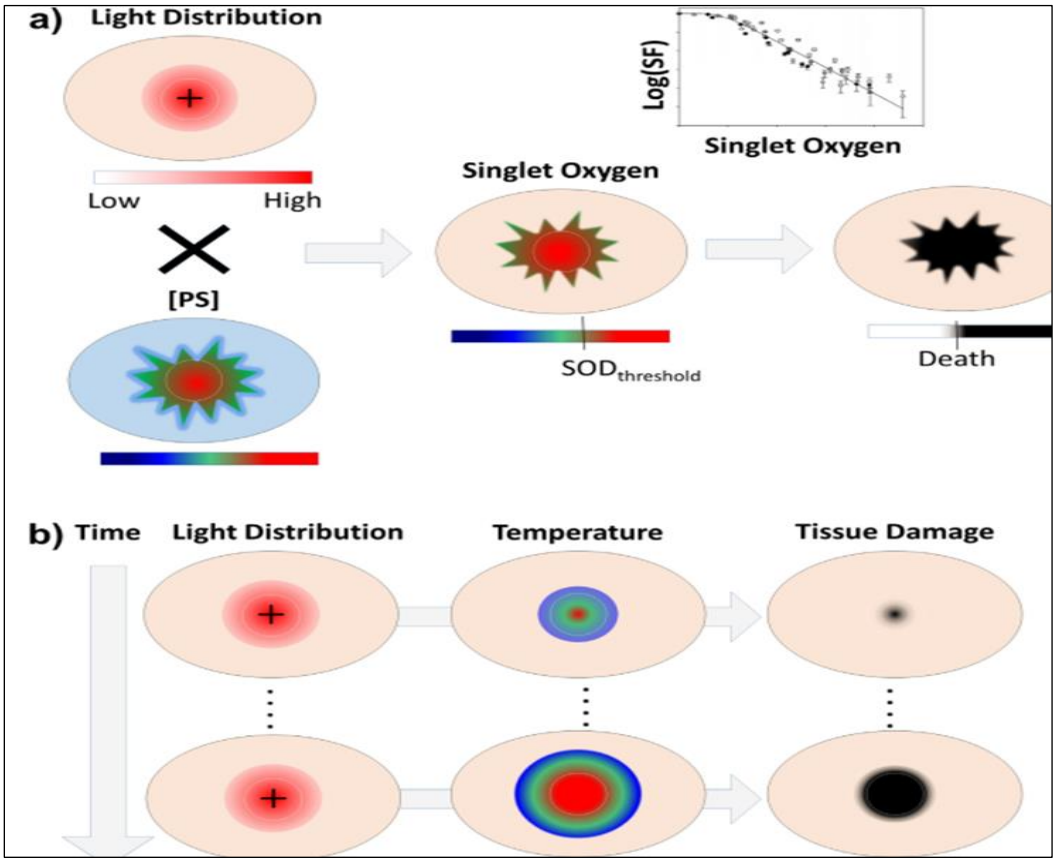



**Figure (1.1): (a) Schematic of PDT macroscopic effect in tissue**

**(b) Schematic of PTT macroscopic effect in tissue over time (Lukač, Lozar et al. 2019)**

### 1.6.1 Photodynamic therapy (PDT):

Photodynamic therapy is a modern and non-invasive form of therapy, used in the treatment of non-oncological diseases as well as cancers of various types and locations.

Good therapeutic results and the possibility of the parallel application of PDT with other therapeutic protocols make it more commonly used in many fields of medicine(Correia, Rodrigues et al. 2021).

In 1903, Von Tappeiner and Jesionek demonstrated the first clinical application of PDT using basal cell carcinomas and now it has been approved to treat various cancers such as melanoma, esophagus, and bladder cancer (Celli, Spring et al. 2010, Saeed, Ren et al. 2018).

Photodynamic therapy is based on the local or systemic application of a photosensitive compound - the photosensitizer, which is intensely accumulated in pathological tissues, the photosensitizer molecules absorb light of the appropriate wavelength, initiating the activation processes leading to the selective destruction of the inappropriate cells(Fitzgerald 2017). Photodynamic therapy is well tolerated by patients because of its selective action in addition PD protocols are painless, and the simplicity of their application allows for outpatient use (Kwiatkowski, Knap et al. 2018). Currently PDT has been on the forefront of cancer research. As an anticancer therapy, PDT kills cancer cells through oxidative stress produced by the highly cytotoxic Reactive Oxygen Species (ROS), generated by the PS in its activated state. The main form of ROS produced in PDT is singlet oxygen ($^1O_2$), which initiates reactions and leads to activation of apoptosis, necrosis, and macro-autophagy (MA) in cells as well as activation of the immune system and the destruction of tumor vasculature in vivo, the establishment of PDT as an alternative treatment modality for most

localized cancers has given more hope for the possibility of maximum cancer eradication with a good prognosis of cancer (Chizenga and Abrahamse 2020).

### 1.6.2 Photodynamic Reaction:

A photosensitizer absorbs a photon of light and becomes activated from ground state to a short-lived excited singlet state (1 PS*). The excited PS may either decay back to the ground state by emitting fluorescence, a property that can be clinically exploited for imaging and photo detection; or it can undergo intersystem crossing whereby the spin of its excited electron inverts to form a relatively long-lived triplet state (3 PS*)(Lee, Hsu et al. 2020).

The triplet excited PS can then directly interact with a substrate, such as the cell membrane or a molecule, and transfer a proton or an electron to form a radical anion or cation, respectively, which then reacts with oxygen to produce oxygenated products such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxides (type I reaction). Alternatively, the energy of the excited PS can be directly transferred to molecular oxygen (itself a triplet in the ground state), to form $^1O_2$ (type II reaction) as shown in figure (1.2). The energy required for the transition of oxygen from triplet ground state to excited singlet state is 22 kcal $mol^{-1}$, which corresponds to a wavelength of 1274 nm. Thus, a relatively small energy is only needed to produce $^1O_2$(Fitzgerald 2017). The byproducts formed as a result of the type I and type II reactions are responsible for the cell-killing and therapeutic effect in PDT. It is to be noted that both type I and type II reactions can occur simultaneously, and the ratio between these processes depends on the type of PS, as well as the concentrations of molecular oxygen and substrate present (Lucky, Soo et al. 2015).

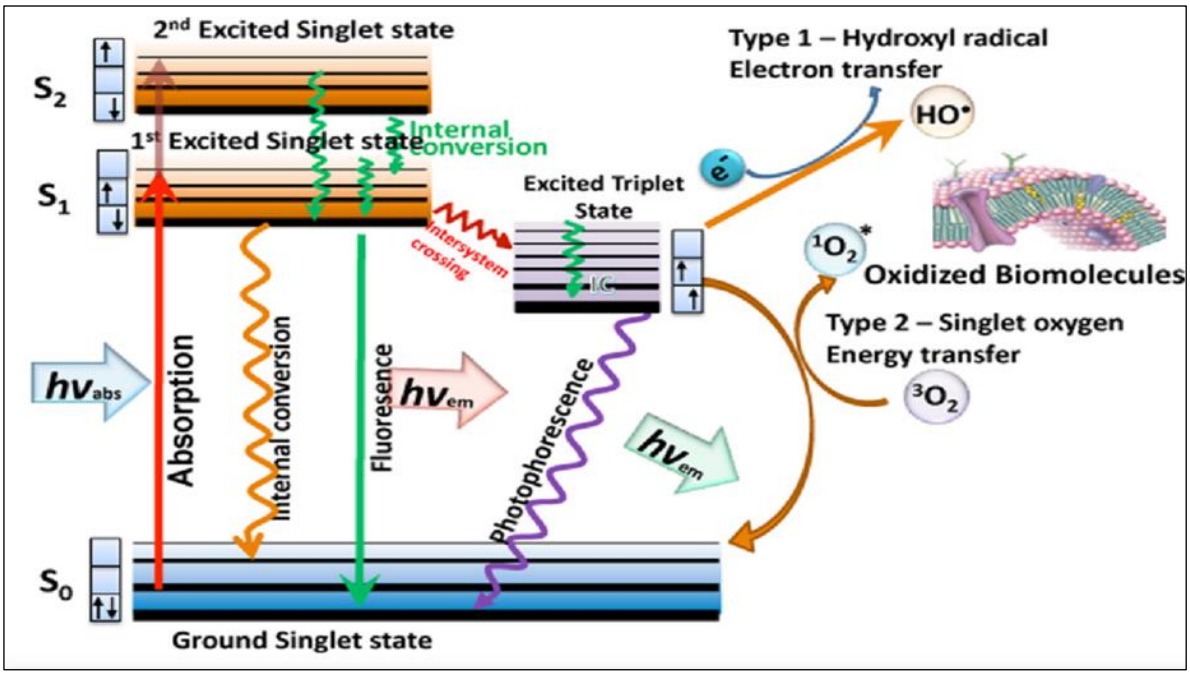



**Figure (1.2): photodynamic reaction Jablonski diagram (Silva, Bussadori et al. 2015)**

When light (hv) is absorbed by the PS the electron moves from a non-excited, low-energy singlet state into a high-energy singlet state. This excited state can lose energy by emitting a photon (fluorescence) or by internal conversion (non-radiative decay). The process known as inter-system crossing, involves flipping of the spin of the high-energy electron, leading to a long-lived excited triplet state. In the presence of molecular oxygen, superoxide and hydroxyl radicals are formed in type I reactions and singlet oxygen in type II reactions(Jin, Fatima et al. 2022). These ROS can damage most types of biomolecules (proteins, lipids, nucleic acids). The mechanism of killing cancer cells firstly occurs by direct tumor cell kill. Generally, the site of photo damage is considered to coincide with the location of accumulation of the PS in the tumor cells Oxidation of the lipids, amino-acids, and proteins by $^{1}O_2$ induces irreversible photo damage to vital subcellular targets such as plasma membrane and organelles like mitochondria, lysosomes, Golgi apparatus, and endoplasmic reticulum (ER)

Photosensitizers that are less hydrophobic tend to be too polar to diffuse across the plasma membrane and are therefore taken up by endocytosis (Castano, Demidova et al. 2004). PDT induced cell death can occur by apoptosis, necrosis, or autophagy and does not depend on the phase of the cell cycle (Mroz, Yaroslavsky et al. 2011). Apoptotic cell death (programmed cell death) remains the preferred mode of cell death following PDT, which is characterized by cell shrinkage, membrane blebbing, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and formation of apoptotic bodies that are scavenged by phagocytes in vivo thus preventing an inflammatory response (Li, Lee et al. 2018).

Secondly, the PS that adsorbed to the dense blood vessel network of tumor tissues causes potent anti-vascular effects that destroy the tumor vasculature leading to thrombosis and hemorrhaging that subsequently, lead to tumor death via deprivation of oxygen and nutrients. Lastly, an ancillary mechanism that follows is the direct activation of the immune system (Mroz, Hashmi et al. 2011), as shown in figure (1.3).

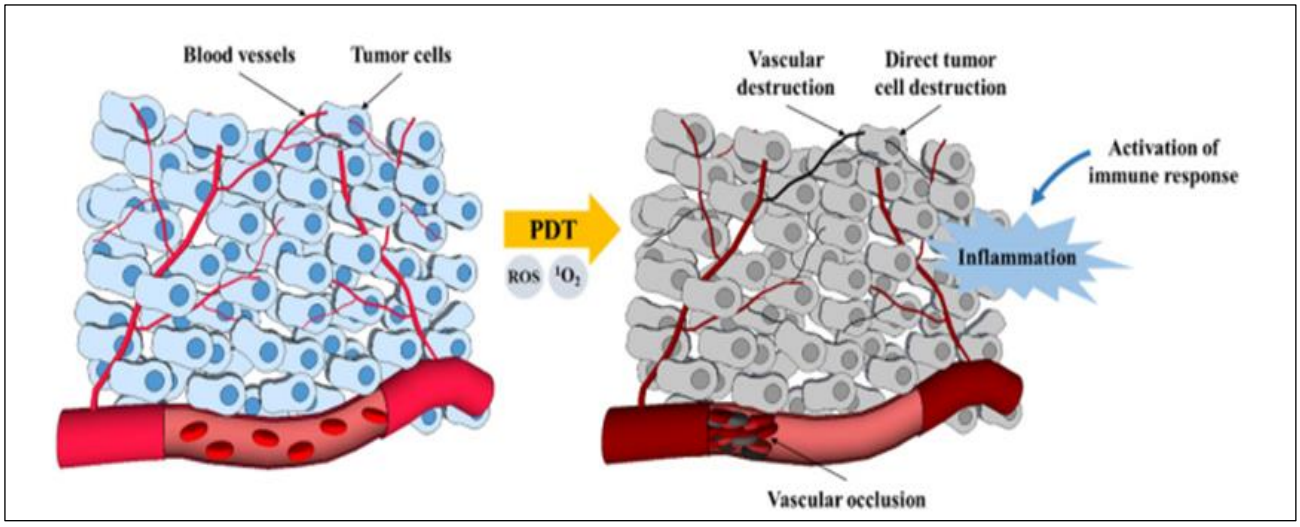



**Figure (1.3): Photodynamic mechanism of tumor destruction (Agostinis, Berg et al. 2011)**

### 1.6.3 PDT essential elements:

#### 1.6.3.1 Photosensitizers:

Photosensitizers are key elements for PDT. Ideally, these molecules should accumulate preferentially in the tumors, have a high singlet oxygen quantum yield, have low activity in the absence of light, be quickly eliminated from the patient body, have amphiphilicity, and have a light absorption peak between approximately 600 nm and 800 nm (Simões, Sarpaki et al. 2020)

It is possible to divide photosensitizers into three generations. The porfimer sodium and the hematoporphyrin derivatives (HpD) are first-generation photosensitizers. These PSs have relatively weak absorption of light in the red portion of the spectrum where tissue penetration of light is optimal, thus significantly reducing the penetration abilities and thus the effectiveness of the treatment, in addition due to their low extinction coefficients, administration of a large amount of the drug is often required to obtain a satisfactory phototherapeutic response, in addition they readily accumulate and are retained in the normal tissue and skin for prolonged period, leading to severe photosensitivity for approximately 4−6 weeks after treatment. But these side effects could be prevented by avoiding sunlight and high-energy light, or by wearing protective clothing and sunglasses for approximately 6 weeks after treatment. However, lack of tumor selectivity, poor bioavailability, and unfavorable bio distribution, as well as prolonged photosensitivity was a major limitation during the initial clinical trials with first generation PSs(Allison, Bagnato et al. 2006).

The second-generation photosensitizers arise to overcome some drawbacks of the first-generation ones, related to light absorption at a specific spectral region.

Some examples of second-generation photosensitizers are the derivate of chlorines, bacteriochlorin, and phthalocyanines, which can have a stronger action on the tumor regions due to their strong absorbance in the deep red region, and consequently, increased light penetration. Hydrophobicity could hamper its solubility in physiological solvents and body fluids, limiting clinical applications of these PSs. Thus, clinically successful PS must have balance between the degree of hydrophilicity and lipophilicity(Simões, Sarpaki et al. 2020).

Finally, the third-generation photosensitizers are molecules with improved selectivity for tumor regions, due to the conjunction of the PS with targeting molecules or its encapsulation into carriers. Today, the functionalization of photosensitizers seems to be the best strategy to achieve a high selectivity to the tumor regions, combining photosensitizers with biomolecules or carriers (Abrahamse and Hamblin 2016).

Photosensitizers can be covalently bonded to several biomolecules, which have affinity to tumors. These biomolecules include antibodies, proteins, carbohydrates, and others. Photosensitizers can also be encapsulated into carriers, such as gold nanoparticles, silica nanoparticles, quantum dots, carbon nanotubes, or others carriers, to guide the photosensitizers to tumors (Li and Yan 2018, Montaseri, Kruger et al. 2021).

### 1.6.3.2 Light:

Photodynamic therapy has been performed with various light sources, including lasers, incandescent light, and laser-emitting diodes (Yanovsky, Bartenstein et al. 2019).

Laser light sources are usually expensive and require the use of an optical system to expand the light beam for irradiation of a larger tissue area. Non-laser light sources (e.g., conventional lamps) can be used with optical fibers to specify the light wavelength for tissue irradiation. However, conventional lamps may have thermal effects, which must be avoided in PDT.

Light-emitting diodes (LEDs) have also been used in PDT as light sources. LEDs are less expensive, less hazardous thermally non-destructive, and easily available in flexible arrays (Chen, Zheng et al. 2012). Light penetration into tumor tissue is very complex, as it can be reflected, scattered, or absorbed. The extent of these processes depends on the type of tissue and the wavelength of light as shown in figure(1.4) (Yoon, Li et al. 2013). Light absorption is mainly due to endogenous chromophores existing in tissues, such as hemoglobin, myoglobin, and cytochromes, which can reduce the photodynamic process by competing with PS in the absorption process (Allison and Sibata 2010).

Light absorption by tissues decreases with increasing wavelength, so longer wavelengths of light (red light) penetrate more efficiently through tissue.

The region between 600 and 1200 nm is often called the “tissue optical window”. Shorter wavelengths (<600 nm) have less tissue penetration and are more absorbed, resulting in increased skin photosensitivity (Agostinis, Berg et al. 2011).

On the other hand, longer wavelengths (>850 nm) do not have enough energy to excite oxygen in its state of singlet and to produce enough reactive oxygen species. Therefore, the highest tissue permeability occurs between 600 and 850 nm. This range, called the “phototherapeutic window,” is predominantly used in PDT (Kwiatkowski, Knap et al. 2018, Correia, Rodrigues et al. 2021).

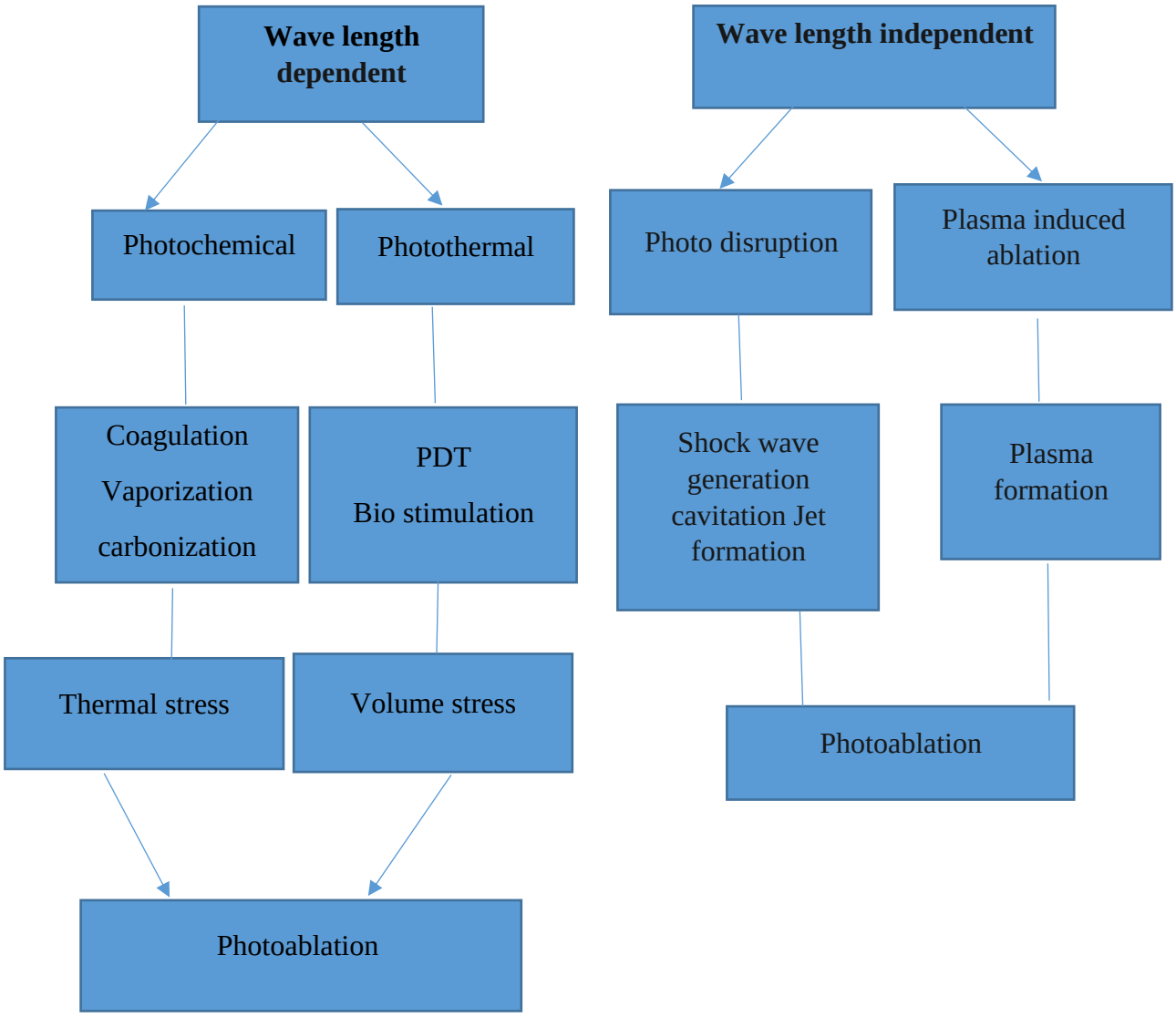



**Figure (1.4) Effect of Laser on Tissue (Aboud, 2005)**

### 1.6.3.3 Oxygen:

The third key component in the PDT mechanism is molecular oxygen. Oxygen is crucial for the production of ROS during PDT. Oxygen concentration in the tissues truly affects the effectiveness of the PDT treatment. In fact, oxygen concentration can vary significantly between different tumors and even between different regions of the same tumor, depending on the density of the vasculature(Rocha 2016).

Especially in deeper solid tumors, often characterized by their anoxic microenvironment, lack of oxygen can be a limiting factor. the irradiation of the tumor with a high light fluence rate can lead to a temporary local oxygen depletion. This leads to interruption of ROS production and reduced treatment effectiveness. Oxygen depletion occurs when the rate of oxygen consumption by the photodynamic reaction is greater than the rate of oxygen diffusion in the irradiated area. In addition, PDT can cause occlusion of the tumor vasculature, reducing blood flow to the tumor tissue, further increasing hypoxia (Correia, Rodrigues et al. 2021).

Two available methods to increase oxygen availability in the tumor have been tested: indirect introduction of oxygen and direct introduction of oxygen. One indirect way to increase oxygen concentration in tumor cells is using catalase enzyme to decompose the intracellular hydrogen peroxide into oxygen. The direct delivery of oxygen into tumors is achieved by using oxygen carriers, such as perfluorocarbons and hemoglobin, commonly used to overcome tumor hypoxia in the PDT procedure (Ortiz-Rodríguez, Fang et al. 2021).

### 1.6.4 Applications of PDT:

Photodynamic therapy is a minimally invasive procedure that is clinically used in the treatment of several oncologic human diseases, such as skin, esophageal, head and neck, lung, and bladder cancers (Plaetzer, Berneburg et al. 2013).

However, PDT also has several non-oncologic applications (Yoo, Oh et al. 2021), including the treatment of non-cancerous human diseases, such as dermatologic acne (Monfrecola, Megna et al. 2021), warts (Bastuji-Garin, Laurent et al. 2001), photoaging (Shin, Kim et al. 2015), psoriasis (Choi, Adelzadeh et al. 2015), hirsutism (Comacchi, Bencini et al. 2012), and alopecia

areata (Linares-González, Ródenas-Herranz et al. 2020), ophthalmologic central serous chorioretinopathy (van Dijk, Fauser et al. 2018)and cardiovascular (atherosclerosis (Houthoofd, Vuylsteke et al. 2020) and dental oral lichen planus (Cosgarea, Pollmann et al. 2020), neurologic (Alzheimer's disease (Lee, Suh et al. 2017)) skeletal (rheumatoid arthritis (Gallardo-Villagrán, Leger et al. 2019), and gastrointestinal (Crohn's disease) (Favre, Borle et al. 2011)).

Photodynamic therapy utilized for inactivation of viruses and microorganisms, including bacteria, yeasts, and fungi, named as photodynamic inactivation of microorganisms (PDI). The viruses or microorganisms are inactivated by combining non-toxic dyes (photosensitizers) with harmless visible light applied intravenously can be a solution during the clinical treatment of infections. Very recently, the use of the PDT procedure to treat patients with COVID-19 performed a study to evaluate if the PDT procedure with Riboflavin (also known as vitamin B2) and blue light can be used effectively as a therapy for patients infected with acute COVID-19.

The study used COVID-19-positive patients who received PDT therapy and COVID-19-positive patients who received conventional care. The patients that received PDT treatment showed significant improvement in clinical symptoms and viral load within 5 days, while the control patients had no significant improvement in clinical symptoms or viral load within 5 days. The results prove the potential of PDT procedure to treat patients infected with COVID-19 virus at an early infection stage and with mild to moderate clinical symptoms. This new application of PDT procedure can prevent hospitalization and intensive care treatments (Fekrazad 2020, Weber, Mehran et al. 2020).

### 1.6.5 Advantages and Limitations of PDT:

Photodynamic therapy has several advantages over conventional approaches to cancer treatment. PDT can be applied directly and accurately in the target tissue, due to its dual selectivity. The two main factors that contribute to the selectivity of PDT are the intrinsic capacity of some photosensitizers to preferentially accumulate in tumor tissue and light irradiation exclusively in the target tissue.

The selective accumulation of the PS in the tumor is facilitated in the case of topical application, since PS is applied directly and only to the lesions to be treated. When PS is given intravenously, it needs to remain in circulation long enough to reach and accumulate in the tumor (Calixto, Bernegossi et al. 2016, Correia, Rodrigues et al. 2021).

First generation photosensitizers cause increased skin photosensitivity. However, PDT has no long-term side effects when correctly used. It is less invasive than surgical procedures and can be performed on an outpatient basis.

In addition to the tumor itself, PDT can also destroy the vasculature associated with it, greatly contributing to tumor death (Calixto, Bernegossi et al. 2016).

Furthermore, PDT can be repeated several times in the same location, unlike radiation. There is little or no scarring after healing. Finally, it usually costs less than other therapeutic modalities in cancer treatment (dos Santos, de Almeida et al. 2019). Like every therapeutic modality. Photodynamic therapy also has some limitations, Tissue oxygenation is crucial for the photodynamic effect to occur, so tumors surrounded by necrotic tissue or dense tumor masses can lead to ineffective PDT.

Finally, the accuracy of target tissue irradiation is the most important point when considering PDT as a treatment option. Therefore, deep tumors (not easily accessible without surgical intervention) are difficult to treat due to the low penetration of visible light into the tissue (dos Santos, de Almeida et al. 2019).

## 1.7 Nanotechnology:

Nanotechnology is a multidisciplinary field and has emerged from the junction of chemistry, biology, applied physics, optics, digital analysis, and materials science(Soares, Sousa et al. 2018).

This evolving and interdisciplinary field mainly involves the design, characterization, manufacture, manipulation, and application of structures at the nanometer scale such structures are known as nanoparticles and are of particular interest as seen by the growing popularity and publications on nanoparticles or nanomaterials (Werengowska-Ciećwierz, Wiśniewski et al. 2015).

These can be attributed to their physicochemical features, among which are their rigidity, hydrophobicity, size, and charge, which characterized this technology.

When used to monitor, repair, and regulate molecular activities of human biological systems, it is known as Nano-medicine; the specialized application of nanotechnology to achieve a reliable diagnosis as well as effective therapy (Jain, Hirst et al. 2012).

It includes aspects such as nanoparticle drug delivery, which currently constitutes an extensively studied area and shows potentialities for both molecular nanotechnology and nano-vaccinology (Gopukumar, Sana-Fathima et al. 2016).

### 1.7.1 Role of nanotechnology in cancer therapy:

As with any cancer therapy, the key issue is to achieve the desired concentration of therapeutic agent in tumor sites, thereby destroying cancerous cells while minimizing damage to normal cells. With this vision, it is imperative to create single agents with tremendous potential to make an important contribution in cancer prevention, detection and treatment. Cancer nanotechnology is emerging as a new field of interdisciplinary research cutting across the disciplines of biology, chemistry, engineering and medicine ,and is expected to lead to major advances in cancer detection, diagnosis and treatment (Misra, Acharya et al. 2010).

The idea of crafting more effective cancer treatments by engineering matter at the nanoscale provides a compelling panacea for preferential elimination of cancer cells without serious damage to normal cells. These Nano systems have four unique properties that distinguish them from other cancer therapeutics:

**(i)** the Nano systems can themselves have therapeutic or diagnostic properties and can be designed to carry a large therapeutic ‘payload’;(Zhou, Peng et al. 2018, Pedziwiatr-Werbicka, Horodecka et al. 2021).

**(ii)** Nano systems can be attached to multivalent targeting ligands, which yield high affinity and specificity for target cells.

**(iii)** Nano systems can be made to accommodate multiple drug molecules that simultaneously enable combinatorial cancer therapy.

**(iv)** Nano systems can bypass traditional drug resistance mechanisms.

**(v)** NTS are also involved in the mitotic index, cell apoptosis, oxidative stress, metabolic processes, cell cycle, signal transduction and cell proliferation, and most of these effects might be caused by a genotoxic mechanism (Xia, He et al. 2018).

### 1.7.2 Silver Nanotubes:

Is a transition metal in one set with Cupper and Gold which is soft, white, lustrous element possessing high electrical and thermal conductivity. It has been known extensively due to its medical and therapeutic benefits before the recognition that microbes are agents for infections. It is practiced in many forms as coins, vessels, solutions, foil, sutures, and colloids as lotions, unguents, and thus onwards. Since the 19th century, silver-based compounds have been engaged in the antimicrobial application (Husen and Siddiqi 2014). Silver nanotubes play a great role in the study of biology and medicine due to their attractive physicochemical properties.

Silver products have ex-tended been familiar to have strong inhibitory and bactericidal effects as considerably as a wide spectrum of antimicrobial activities (Nam, Purushothaman et al. 2016), which has been practiced for centuries to prevent and care for various diseases, most notably infections (Shankar, Rai et al. 2005, Ahmed, Ahmad et al. 2016).

Silver nanoparticles are accounted to own antifungal, anti-inflammatory, antiviral, and anti-platelet activity (Gawish, Shaban et al. 2018).

Other important medical applications of Ag-NTs are drug delivery and anticancer agents (Schluesener and Schluesener 2013) , the mechanisms of cytotoxicity of silver nanoparticles (NTs) including cell viability, oxidative stress and genes expression involvement in processes that contribute to silver NTs induced apoptosis .The effect created on ROS was surveyed, as direct document of oxidative stress as mitochondria are more susceptible to nanosilver particles (Govender, Phulukdaree et al. 2013).

Nano-silver particles, because of their capability to form lump in outward the mitochondria (Bressan, Ferroni et al. 2013) or localize inside mitochondria, have indirect and direct collaboration in the mitochondrial toxicity. The mitochondria are the main source of ROS production within the cell (Eftekhari, Ahmadian et al. 2018).

Increase in ROS production in mitochondria could be the result of impression of toxic nanoparticle on respiratory chain (Ahmadian, Babaei et al. 2016). Breaking the electron chain in the mitochondria via nanoparticles increase the production of ROS and decreases the synthesis of ATP (Ahmadian, Eftekhari et al. 2017, Ahmadian, Khosroushahi et al. 2018).

This indicates that Nano-silver mediated apoptosis is in a mitochondrial-dependent mechanism that occurs with an intrinsic pathway (Gengan, Anand et al. 2013).

### 1.7.2.1 Toxicity of silver:

Although nanotechnology has become increasingly important today, there is still reduced and confirmed information about the risks of exposure of humans, animals, and environment to NTs, particularly those of silver, in terms of toxicity at short and long term (Vazquez-Muñoz, Borrego et al. 2017, Ivanova, Gugleva et al. 2018).

The synthesis of NTs may presuppose the use of substances that are naturally toxic to living organisms or the environment. Most studies address these effects only when they are administered by inhalation, damaging the respiratory tract.

There are in vitro studies that concluded that Ag NTs are toxic to several organs, such as the lungs, liver, brain, vascular system, and reproductive organs. Possible mechanisms of toxicity include inducing the formation of reactive

oxygen species (ROS) and oxidative stress, thus leading to DNA damage and apoptosis (He, Li et al. 2017). (De Matteis, Malvindi et al. 2015) concluded that the toxicity in cells treated with Ag NTs is mainly caused by the release of Ag+ ions in the cytosol, after internalization of Ag NTs through endocytosis and their dissolution in acidic environment.

Hence, the oxidative stress, DNA damage, and cell death verified in the presence of AgNTs are mainly due to the impairment of physiological metabolic and cell cycle mechanism by silver ions present in the cytosol. The activation of metallothioneins and the prevention of cytotoxicity using Ag+ chelating agents strengthen this hypothesis. The authors found that there was a rapid entry of soluble silver ions into the nucleus, where it accumulates and impairs nuclear receptor activity, which is detrimental to liver metabolism. There are also some in vivo studies focused on the cytotoxicity and geno-toxicity of AgNTs.

Due to their small size, they have high mobility and humans are easily exposed through the skin, inhalation, ingestion, etc. AgNTs can travel from the site of exposure to other organs easily, penetrating cells (Ivanova, Gugleva et al. 2018).

In the work conducted by (Ahamed, AlSalhi et al. 2010), it was revealed that exposure to Ag NTs modulates the expression of several genes associated with motor neuron disorders, neurodegenerative diseases, and immune function, indicating potential neurotoxicity and immunotoxicity resulting from exposure, also Ag NTs cause reproductive failures, malformations during development, and morphological deformations in some animal models. The geno-toxicity and cytotoxicity of Ag NTs are influenced by several physicochemical characteristics, such as concentration, charge, and surface functionalization, size, and shape (de Lima, Seabra et al. 2012).

The experimental results reported until recently are not enough to conclude with precision which are the effects and mechanisms of toxicity of Ag NTs. However, toxicity is a limiting factor for its in vivo use (Rosenblum, Joshi et al. 2018, Karuppaiah, Siram et al. 2020).

### 1.7.3 Titanium dioxide nanotubes ($TiO_2$-NTs):

Titanium dioxide ($TiO_2$) is categorized as a chemically inert mineral that usually occurs naturally in anatase, brookite and rutile forms.(Nasr, Hasanzadeh et al. 2018) . $TiO_2$ is an important abundantly available inorganic compound in nature and possesses specific properties, such as excellent biocompatibility, thermal stability, low ion release, high refractive index, high corrosion resistance and resistance to photodegradation (Raja, Cao et al. 2020). Therefore, $TiO_2$ is widely used in several applications such as sunscreens, paints, skin care products, food products and the pharmaceutical industry.

Furthermore, titanium-mediated nickel alloys are extensively used in various surgical implants (Ziental, Czarczynska-Goslinska et al. 2020)In addition, the titanium dioxide nanoparticles ($TiO_2$ NTs) have significant medicinal potential, including inertia, magnificent biocompatibility with body tissues and thus $TiO_2$ NTS have been employed in various biomedical applications such as artificial hips, bone plates, scaffolds, dental implants and coating, as well as drug and gene delivery systems (Kim, Im et al. 2020).

Moreover, $TiO_2$ NTs possess antimicrobial, wound healing, larvicidal, antioxidant and anticancer application potentials showing their promising role in the biomedical field $TiO_2$ anti-tumor effects revealed that it occurs through oxidative stress when reactive oxygen species (ROS) disrupt the balance between oxidative pressure and antioxidant defense. ROS (such as hydroxyl radical, superoxide, etc.) could be produced by photo-activated, some chemicals

on the particle surface, or a consequence of the interaction between particles and cellular components(Akinola, Lateef et al. 2020). The mitochondria are the target of $TiO_2$ NTs that have been phagocytosed by cells as well as a source for ROS production, and the disruption of mitochondria would also lead to the increase in ROS production, then the decrease of mitochondrial membrane potential and apoptosis. Furthermore, ROS can also cause damage to protein, lipids, and DNA in cells(El-Said, Ali et al. 2014).

### 1.7.3.1 Toxicity of $Tio_2$:

The potential toxicity of $TiO_2$ in vivo, it can be assumed that there will be no light exposure and activation of $TiO_2$ photo-catalysis of cellular components; however, there is still a need to consider the surface reactivity of $TiO_2$ nanomaterials, their ability to bind to proteins and nucleic acids, and a general propensity of nanomaterials to interact with molecules within a similar size range (Dobrovolskaia, Patri et al. 2009).

In addition to the fact that biological responses caused by nanoscale materials may be very different from those caused by bulk materials, the increased surface reactivity of $Tio_2$ at the nanoscale may, potentially, lead to an aggregation of nanoparticles, and to the triggering of immune responses, the clogging of vessels and ducts, and an accumulation in organs associated with the filtration of blood and lymph (Patri, Umbreit et al. 2009).

At present, the conditions that either increase or decrease the rate of aggregation of $Tio_2$ nanomaterials have not yet been widely studied in biological systems, in part because there are so many factors to consider, such as crystal structure and size, pH, and the ionic strength of the colloid.

Moreover, in a study of microscale $Tio_2$ particles, anatase $Tio_2$ was found to produce more ROS than its rutile counterpart, adding crystal poly-morphology as yet another potential factor to be controlled when evaluating the toxicity of $Tio_2$ nanoparticles in vivo. It has been argued that the total surface area - per - unit - mass is the best predictor of $Tio_2$ toxicity in vivo, whereas others have maintained that this characteristic is not indicative of toxicity at all (Olmedo, Tasat et al. 2008).

Whilst photocatalytic reactivity of $Tio_2$ in vivo is unlikely, concern has been expressed that ROS may be generated at the particle surface when $Tio_2$ nanoparticles interact with biological processes, potentially causing oxidative damage to adjacent tissues. The expected clearance of $Tio_2$ nanomaterials is via the reticuloendothelial system (RES) and, because of their small size, they would be expected to locate to the liver, spleen, lymph nodes, and kidneys.

In the fi eld of cancer diagnostics and therapeutics, nanoscale molecules would potentially accumulate in tumors because of the poorly developed and largely fenestrated blood vessels of the neo-vasculature. Poor lymphatic drainage would reduce the clearance of these nanoscale structures from the tumor. This phenomenon, which is referred to as the enhanced permeability and retention (EPR) effect, is a mainstay of most so - called “passive targeting” approaches to concentrate nanoparticles within tumors.

### 1.7.4 Gold nanoparticles:

Chemistry of gold colloids began from the nineteenth century, when Michael Faraday performed his well-known experiments for gold colloids generation. His experiments yield deep red gold sols by reduction of tetrachloroaurate with the help of white phosphorus. At the start of the 20th century, Wilhelm Ostwald contributed positively to the supplementary progress of colloid science.

He pointed out that in the nm range, the properties of metal particles are mainly defined by surface atoms and he reasoned that these nanoparticles, called colloids, should show novel properties with respect to bulk particles. The trimness of gold to the nanometer range has dramatic consequences for its physical and chemical properties (Khan, Saeed et al. 2019).

The promising candidate is gold because of its outstanding surface properties. These surface properties can be used in biotechnological, optical and electrochemical applications. There are various advantages of the gold nanoparticles like non-toxicity, strong scattering length, bio-conjugation and long-term stability. These features are fundamental for a secure and responsive bio-sensing policy (Yadav 2018). Gold nanoparticles (GNPs) are the most well-suited nanomaterial for groundwork of engineered Nano-platforms in smart sensing devices. Surface Plasmon resonance property of GNP makes them useful nanomaterial in various fields like bio-imaging, biomedical therapeutics and bio-diagnostic tool (Lata, Arvind et al. 2014).

Gold nanoparticles are also called as gold colloids. There are huge global demands of gold colloids because of their significant requirement in many industrial and commercial applications. Biomolecule GNPs are used in the medicine and in cosmetic products as these have anti-aging components for skin protection.

Gold nanoparticles are used for doing permanent coloration of wool or cotton fibers. These are also used for novel coatings and catalysis purposes over the years (Rao, Gowda et al. 2017).

Gold NPs consider the most important laser-responsive nanomaterial, into the tissue changes optical properties of the medium and increases local conversion of laser energy into heat as shown in figure (1.5).

Gold NPS possess surface Plasmon resonance (SPR), a well-known property that implies the resonant oscillation of free electrons on the particle surface induced by incident light, following this phenomenon, the electrons in Au atoms absorb the laser photons and become excited to higher energy levels. Through electron-phonon relaxation, the absorbed photon energy is converted to heat and is transferred into the particle lattice (Raoof, Cisneros et al. 2013)

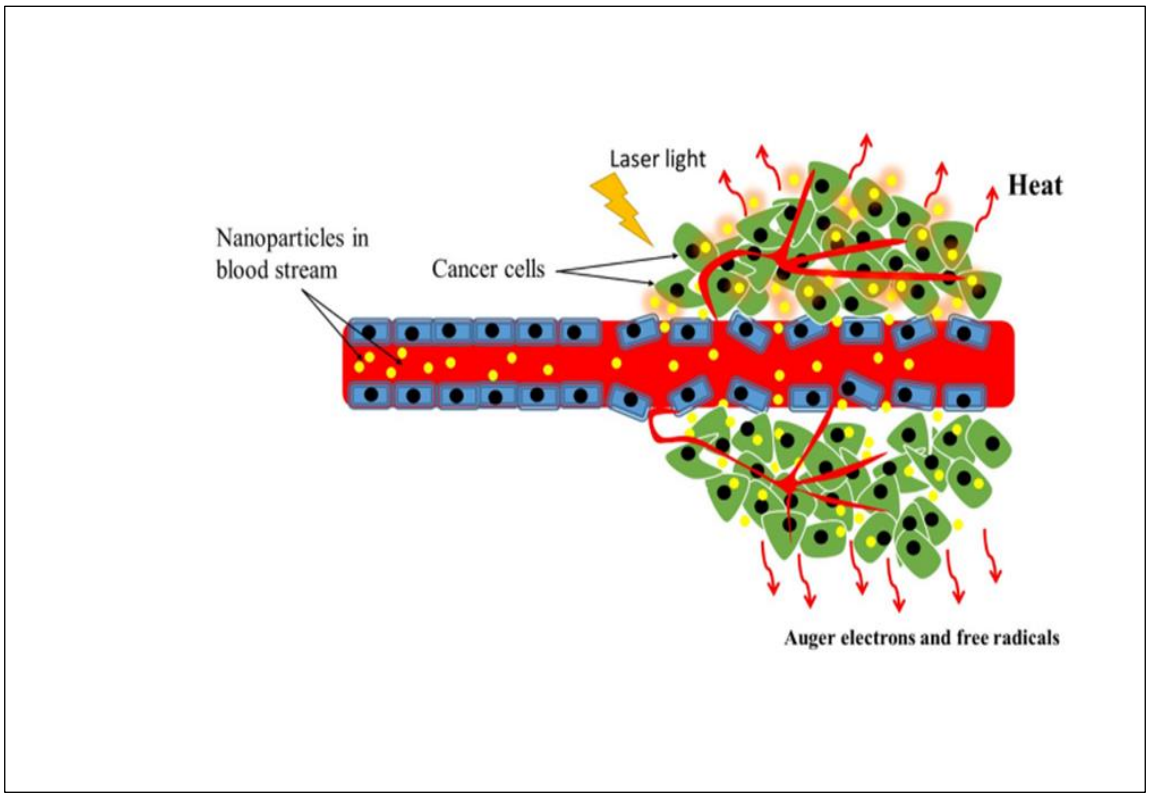



**Figure (1.5): Methods eradicating the cancer cells after nanoparticles accumulation at the tumor site (Samadian, Hosseini-Nami et al. 2016)**

To limit such released heat and its resulted thermal damages to the tumor, and also to increase targeting of nanoparticles toward tumor cells it is necessary to target Au NPs towards cancer cells using various targeting ligands as folate.

The combination of F-Au NPS with laser exposure leads to selectively heat and destroy the cancer cells (Mehdizadeh, Pandesh et al. 2014).

Folic acid or folate (pteroyl glutamate) is a water-soluble vitamin, an ingredient needed for DNA synthesis and cell division. The closed chemical formula of folic acid is $C_{19}$ $H_{19}$ $N_7$ $O_6$ (Mw = 441.4 Da) (the salt of folic acid) is an ingredient needed for biosynthesis of purines and pyrimidines in DNA synthesis pathway (Ghaznavi, Soheili et al. 2015).

Rapidly dividing cancer cells overexpress folate receptors in order to compensate the increased requirement of folate for DNA synthesis and rapid growth. Folate has several properties that make it suitable as a targeting agent such as its stability, simple conjugation chemistry, non-immunogenicity, very high affinity to its receptors and rapid internalization into tumor cells (Talekar, Kendall et al. 2011). Folate is brought into both healthy and cancerous cells by the folate-receptor, this receptor is used to transport folate into the cytosol for the synthesis of thymine by dihydrofolate reductase. The expression level of the folate receptors on a cell's surface is regulated by the cell's function.

Owing to their rapid division rate and a higher requirement for folate, cancer cells generally require a much greater amount of folate and, consequently, tend to overexpress a high level of folate receptors on their surface in comparison with the normal cells (Birn, Spiegelstein et al. 2005). Therefore, it would be expected that the folate-conjugated nanoparticles are preferentially directed towards the cancer cells due to the abundant presence of folate receptors on the cancer cell (Beik, Khademi et al. 2017). Tumor targeting of folic acid occur with either active targeting or passive targeting. Active technique based on specific identification of a ligand by its target substrate and an active targeting approach is the alternative for passive methods to increase specificity of targeting procedure and reduced side effects on normal tissues (Golla, Cherukuvada Bhaskar et al. 2013).

Folate conjugated nanoparticles are either taken up into endocytotic vesicles or released into the cytoplasm. From this point of view, folate conjugated nanoparticles have an advantage over antibodies or other targeting ligands in which the targeted nanocomplex is transported into the lysosome to get destroyed (Samadian, Hosseini-Nami et al. 2016).

Some ligands such as monoclonal antibodies, vitamins, and hormones act based on molecular recognition processes to deliver therapeutic agents to pathological sites or to pass through biological barriers (Li et al. 2015).

### 1.7.4.1 Toxicity of gold Nano -particles:

Colloidal gold nanoparticles have been proposed for diverse biomedical applications due to their unique surface, electronic, and optical properties (Castano, Demidova et al. 2004). Because of the strong and size-tunable surface Plasmon resonance, fluorescence, and easy-surface functionalization, gold nanoparticles have been widely used in biosensors, cancer cell imaging, photo thermal therapy, and drug delivery (Anker, Hall et al. 2010).

Today, gold nanoparticles have been suggested to be potentially useful as a novel radio sensitizer in radiotherapy, because the strong photoelectric absorption and secondary electron caused by gamma or X-ray irradiation can accelerate DNA strand breaks. In general, the toxicity of gold nanoparticles depends on their physical dimensions (such as size and shape) and surface chemistry (such as coating) gold nanoparticles in human cells has been studied in detail, and the results have shown that gold nanoparticles are nontoxic up to 250 mM, while ionic gold shows obvious cytotoxicity at 25 mM (Liu, Wang et al. 2008).

Actually, in vitro cultures cannot replicate the complexity of an in vivo system or provide meaningful data about the response of a physiologic system to an agent. A case in point is carbon nanotubes (Manna, Sarkar et al. 2005). The toxicity in vivo is determined by many parameters including dose, route of exposure, metabolism, excretion, and immune response. The toxicological profiles of nanomaterials might also be determined by nanomaterial chemical composition, size, shape, aggregation, and surface coating.

Very recently, the size-dependent organ distributions of gold nanoparticles have been investigated, and the results showed that small gold nanoparticles of 5–15 nm had wider organ distribution than that of large gold nanoparticles of 50–100 nm, and liver and spleen were the dominant targeted organs (Semmler-Behnke, Kreyling et al. 2008)

Meanwhile, it has been found that gold nanoparticles with a long blood circulation time can accumulate in the liver and spleen, and have obvious effects on gene expression Increasing concentrations of gold nanoparticles induce decreases in weight but no significant statistical difference is observed. Obvious effects on organ index have been observed at high concentration (Balasubramanian, Jittiwat et al. 2010).

Moreover, of the three different administration routes, the oral and intraperitoneal injection show the highest toxicity, and tail vein injection shows the least toxicity. Considering the results of all of these studies, targeting gold nanoparticles by tail vein injection is promising for possible biomedical application (Zhang, Wu et al. 2010).

## 1.8 Cell Culture:

Cell culture is a process in which the cells are removed from an organism and placed in fluid medium. Under proper conditions, the cells can stay live and even grow and replicate. The growth can be identified and characterized by cell division (mitosis) or by other processes, such as differentiation, during which the cells can convert into specific types that are capable of functions analogous to tissues or organs in the whole organism (Lynn 2009).

It is a method that are done to enable the growth of cells in physiological desired conditions, it was first introduced to study tissue growth and maturation, virus biology, and vaccine evolution, role of genes in the disease and health, and for use of hybrid cell to produce biopharmaceutics. In a clinical context, cell culture is most commonly linked to evolve model systems in order to study basic cell biology, replicate disease mechanisms, or investigate the toxicity of novel drug compounds. The main advantages of using this cell culture system are to manipulate genes and molecular pathways. (Segeritz and Vallier 2017).

The goal and advantage of cell culture is to train the researcher and become familiar with the technique of undergoing cell setup and successfully propagate cells in vitro, and to ensure safety of the researcher and carrying out aseptic conditions that is free of contamination (Segeritz and Vallier 2017).

Primary culture is derived from tissue removed directly from a living animal rather than immortalized cells that divide indefinitely, Primary tissue culture allows scientists to directly investigate cells of interest in a carefully controlled in vitro environment (Ventura, Toullec et al. 2018). The preparation of primary cultures is intensive labor, and they can be maintained in vitro only for a limited period. Primary cultures usually retain most of the characteristics of the cells in vivo.

Primary cultures mean that the cells have not been passaged and when they passaged, they become a cell line and no longer called primary cultures, the primary cell culture, when sub-cultured, it becomes a cell line or cell strain that can be finite or continuous, depending on its life span in culture (Philippeos, Hughes et al. 2012)

**1.9 Cell line:**

The term cell line refers to the propagation of cell culture after the first subculture. In other words, a cell line is a permanently established cell culture that will proliferate through given appropriate fresh medium and space. A given cell line contains several cell lineages of either similar or distinct phenotypes. Cloning or physical cell separation or any other technique could be used to select particular cell linage. This cell line that obtained by cloning or selection is referred to as cell strain. Cell strains do not have infinite life, as they die after some divisions (Chaudhary & Singh, 2019) The choice of a cell line for cell culture depends heavily on the functional properties and specific readouts required of the cell model.

Cells cultured in the lab can be classified into three different types: primary cells, transformed cells, and self-renewing cells, However, there are strict regulations and rules that restrict handling of these cell types to ensure researchers safety and aseptic condition. Furthermore, primary cells are generally characterized as “finite” and therefore rely on a continuous supply of stocks since their proliferation ceases after a limited amount of cell divisions and cell expansion is oftentimes impossible. Transformed cells can be generated either naturally or by genetic manipulation (Segeritz and Vallier 2017).

Cell lines can be obtained commercially, where certain quality control measures are in place that guarantee genomic stability and absence of

contaminants. Other places to source cell lines from can be cell banks or other cell culture laboratories. The introduction of new cell lines in a lab should always be accompanied by a Mycoplasma PCR test to ensure clean cultures (Segeritz and Vallier 2017).

Contamination with bacteria, yeast and other fungi can result in the complete loss of cultures. Undetected contamination with slow growing micro-organisms, or with micro-organisms resistant to antibiotics, can have a significant impact on the quality and/or validity of data obtained from in vitro systems. The most common example of such an infection is mycoplasma follow up of the tissue culture is a mandatory process via macroscope or microscope (Coecke, Balls et al. 2005).

The two major factors that determine the proliferation of the cell culture, the first is the free space in the culture flask and the second is the decline in nutritional support provided by the medium, therefore, cell passage is required in these conditions, the passage is required according to the cell conditions such as cell concentration, pH of the environment, time of the last passaged and the conditions that the specific study brings. Sometimes in cell cultures, there is a need for cells to be frozen and stored, also cell viability testing is carried out most often to determine viability after exposing cells to traumatic conditions such as freezing, thawing and sub culturing, the cells will continue its proliferation but at the end of a certain time scale, the cells lose its ability to proliferate and survive due to many changes that are physiological or pathological, this process is somehow similar to cellular aging and apoptosis, therefore, in cell culture it is always recommended to measure cell viability and apoptosis periodically (Philippeos, Hughes et al. 2012)

### 1.9.1 Colon cancer cell line (SW480):

One of the most fatal and prevalent cancerous world-wide is the colorectal cancer, despite the great advance in the medical field and chemotherapeutic agents, the drug resistance remains the unresolved problem in cancer. Therefore, establishing an effective compound with lowest side effects like Nanomaterials in PDTs (Khan, Albalawi et al. 2022) to fight cancer is of central priority The colonic cancer SW480 cell line originates from primary tumor of an adenocarcinoma of the colon in a 50-year-old male (Siekmann, Tina et al. 2019).

### 1.9.2 Primary liver cancer (PLC/PRF/5) cell line (PLCs):

A continuously growing cell line has been established from autopsy material taken from a primary liver carcinoma, a cancer which has a high incidence in Southern Africa. The cell line was initiated from multifocal areas of outgrowth in the primary culture, and adaptation to in vitro conditions was completed after 18 months. The cells resemble hepatocytes in culture, have a doubling time of 35 - 40 hours and a plating efficiency of 40 - 50%. No virus particles have been found in the cells by ultrastructural examination.

The isozyme pattern and the karyology of the cells are human. The chromosome pattern is heteroploid (mean number 56) and there are marker chromosomes (Ishii, Tamura et al. 2020).

## 1.10 Cell's viability:

Viability levels or proliferation rates of cells are used as indicators of cell health. Physical and chemical agents can affect cell health and metabolism. Different mechanisms can cause damage or even death to the cultured cells such as cell membrane destruction, inhibiting protein synthesis, enzymatic reactions,

irreversible binding to receptors, and inhibition of polydeoxynucleotide elongation. In order to determine the cell death caused by these mechanisms, there is a need for inexpensive, reliable and reproducible short-term cytotoxicity and cell viability assays (Riss, Moravec et al. 2016).

# Chapter two

# Materials and Methods

# 2.Materials and methods:

This study was done during the period from September 2021- April 2022 in the Tissue Culture Laboratory at the College of Medicine/ Babylon University.

## 2.1 Materials:

### 2.1.1 Chemicals:

The chemicals used in this study are listed in (Table2-1) with their suppliers

**Table 2-1 List of Chemicals Used in the Study**

| Chemical | Company | Country |
|---|---|---|
| Alcohol spray (ethanol 70%) | AMEYA FZE | UAE |
| Chloroauric acid | Sigma | USA |
| De-ionized water | PR omega | USA |
| Dimethyl sulfoxide (DMSO) | Roth | Germany |
| Fetal bovine serum (FBS) | Gibco | UK |
| Folic acid powder | | india |
| Gentamycin (80 mg vial) | The Arab pharm. Salat | Jordan |
| Liquid nitrogen | Clever | USA |
| MTT(3-(4,5-Dimethylthiazole-2-yl)- 2,5-diphenyl-2H-tetrazolium bromide) dye powder | Roth | Germany |

| | | |
|---|---|---|
| Phosphate buffer saline tablet | Gibco | UK |
| Roswell Park Memorial Institute1640 (RPMI-1640) powder medium | Gibco | UK |
| Sodium bicarbonate powder | Ludeco | Belgium |
| Trisodiumcitrate dehydrate(TCD) | Sigma | USA |
| Trypsin-Ethylenediaminetetraacetic acid (EDTA) powder | US biological | USA |

### 2.1.2 Instruments and Tools:

The instruments that used in the study listed in table (2-2)

Table 2-2 List of instruments used in this study

| **Equipment** | **Manufacturers** | **Origin** |
|---|---|---|
| (Sterile freezing vial (1.5 ml) | Biofil | Australia |
| Autoclave | Jeiotech | Korea |
| Automatic micropipettes (different sizes) | Human | Germany |
| Cell culture flask (25ml) | SPL | Korea |
| Cell culture plate (96- wells) | SPL | Korea |
| Deep freezer -80°C | Labtech | Korea |
| Digital camera | Sony | Japan |
| Disposable face masks | KY | China |
| Disposable gloves | Fe | Malaysia |
| Distiller | ROWA | Germany |
| Double distillation water stills | GFL | Germany |
| Electric oven | Memmert | Germany |
| ELISA Reader | Human | Germany |

| | | |
|---|---|---|
| Eppendorf tubes 2.0ml | Chemo Lab | China |
| Freezer -20 °C | Mettler | Switzerland |
| Incubator | Memmert | Germany |
| Inverted microscope | T.C Meiji techno | Japan |
| Laminar air flow cabinet | Labtech | Korea |
| Laser MDL-III-635nm-300Mw | Dragon Laser | China |
| Liquid nitrogen container GT38 | Air Liquide | France |
| Magnetic stirrer | Labinco | Netherland |
| Microcentrifuge | Hettich | Germany |
| Micropipette | Jiangsu xinkang medical | China |
| Millipore filter (0.45, 0.22µm) | BiofilJET | Australia |
| Paraffin film | Analab | Ireland |
| Sensitive Balance | Labtech | Korea |
| Syringe | Chemo Lab | China |
| Ultrasonic | Binder | Germany |
| Refrigerator | Arcelik | Turkey |

### 2.1.3 Preparation of Reagents and Solutions:

#### 2.1.3.1 Phosphate Buffer Saline (PBS):

The PBS was prepared according to Gibco manufacturer manual by dissolving one tablet of PBS in 500 ml deionized distilled water (DDW) with stirring constantly on a magnetic stirrer at room temperature, the pH will be 7.45 and requires no adjustment. Sterilization was done by autoclaving and kept sterile in a closed bottle until use.

#### 2.1.3.2 Trypsin-(EDTA) Solution:

According to US Biological directions, a weight of 10.1 gm of trypsin-EDTA (ethylene diaminetetra-acetic acid) powder was dissolved in 900ml of DDW and constantly mixed by stirring at room temperature. The pH of the solution was adjusted to 7.2, and the volume completed to one liter. The solution was sterilized by filtration using 0.45 and 0.22 µm millipore filters subsequently. The content was stored at (- 20C˚).

#### 2.1.3.3 MTT Solution:

A weight of 0.5 g of MTT powder was dissolved in 100 ml PBS to obtain a concentration of 5 mg/ml. Then the MTT solution was sterilized by filtration through a 0.2 µm Millipore filter into a sterile and light protected container and stored at 4°C for frequent use or at (-20)°C for long term storage (Van Meerloo, Kaspers et al. 2011).

### 2.1.4 Preparation of Tissue Culture Medium:

#### 2.1.4.1 Preparation of Serum-Free Medium:

Liquid RPMI-1640 medium was prepared from powdered RPMI-1640 medium according to the Gibco product manual as the following: From the RPMI-1640 powdered medium, 10.43 g was dissolved in approximately 900 ml of DDW in a volumetric flask. The other components include: 2 g sodium bicarbonate powder or according to need and 1.25 ml from gentamycin stock solution had been added with continuous stirring. The volume was completed by DDW to one liter and the pH of the medium adjusted to 7.4. Sterilization was done by 0.4 and 0.2 μm Millipore filters subsequently.

After the end of the procedure, 5 ml of the medium was incubated at 37 ºC in a sterile flask for 4 days with daily examination for signs of bacterial and fungal contamination. It was considered sterile only in case of no signs of contamination during the four days of incubation. Then the medium was stored at 4ºC until use.

#### 2.1.4.2. Preparation of Serum-Medium:

Serum-medium was prepared as described in (2.1.4.1) with the addition of 10% FBS.

#### 2.1.4.3 Preparation of Freezing Medium:

Ten ml of freezing medium was prepared from the following constituents: six ml serum-free medium, three ml FBS, and one ml DMSO.The solution was stored in between uses around (- 20˚C) (Barrette, 2016).

### 2.1.5 Synthesis of gold Nano particles (Turkevich method):

It's a chemical procedure were gold Nano particles (Au NPs) prepared as a solution, it's simple and result in aspherical gold nanoparticles which is testing by TEM (Transmission Electron Microscopy) (Wang and Dellago 2003).

Materials for synthesizing gold Nano particles include:

**A-**Chloroauric acid

**B-**Trisodium citrate dihydrate (TCD) as reducing agent.

For preparing the gold Nano particles a stock solution was made from Chloroauric acid and trisodium citrate dihydrate, then to prepare this stock solution of 2%Chloroauric acid we dissolved 1g of Chloroa and for preparing 1%stock solution of Trisodium citrate dihydrate dissolve 1g in 100ml deionized water. After preparing the stock solutions, 150µl of Chloroauric acid solution plus 50ml of deionized water must heat up to 100°C then 500µl of Trisodium citrate solution was added (stirrer with heating at 100°C) until the clear color change into red color indicating the gold Nano particles.

### 2.1.6 Folic acid stock solution preparation:

Folic acid (pure drug powder) was solubilized with DMSO ,20 milligrams of folic acid powder was dissolved in 1ml of DMSO to obtain a stock solution of the drug 20,000 µg\ml) and from this stock a serial dilution was made(Zu, Wang et al. 2011).

### 2.1.7 Silver decorated $Tio_2$ nanotubes stock solution preparation:

Silver decorated titanium dioxide Nano tubes was solubilized with deionized water then 10 milligrams were dissolved in 10 ml of deionized water to obtain a stock solution of the 1000 μg\ml and from this stock a serial dilution was made.

### 2.1.8 $Tio_2$ nanotubes stock solution preparation:

$Tio_2$ Nano tubes was solubilized with deionized water then 20 milligrams were dissolved in 10 ml of deionized water to obtain a stock solution of the 2000 μg\ml and from this stock a serial dilution was made.

### 2.1.9 Laser Irradiation

The irradiation was done in a 96-well plate after 24 hours of cell seeding. The cells were uniformly irradiated and there was a distance of 10 centimeters between the laser tip and the bottom of the plate, which corresponded to a single well size from the 96-well plate (Dompe, Moncrieff et al. 2020).

In this study used red Diode laser with wave length 635 nm, laser power 300 Mw with continuous wave.

For all treated groups, the energy density (fluence) ($J/cm^2$) was calculated by multiplying the exposure time (s) by the laser power density $W/cm^2$. The exposed surface area selected for the study was identical to the surface area of each culture well on a 96-well plate which is (0.2826 $cm^2$). Surface area ($cm^2$) = $\pi \times r^2$. When the power is divided by the area on which is it distributed, then we got the power density (irradiance): The power density (irradiance) ($W/cm^2$) =Power/Area.

The energy density (fluence) ($J/cm^2$) = Time (s) x [Power (W)/ Area ($cm^2$)] (Gagnon, 2015).

Dose (energy density) of red diode laser that was used in this study is 765 $J/cm^2$ for 12 minutes.

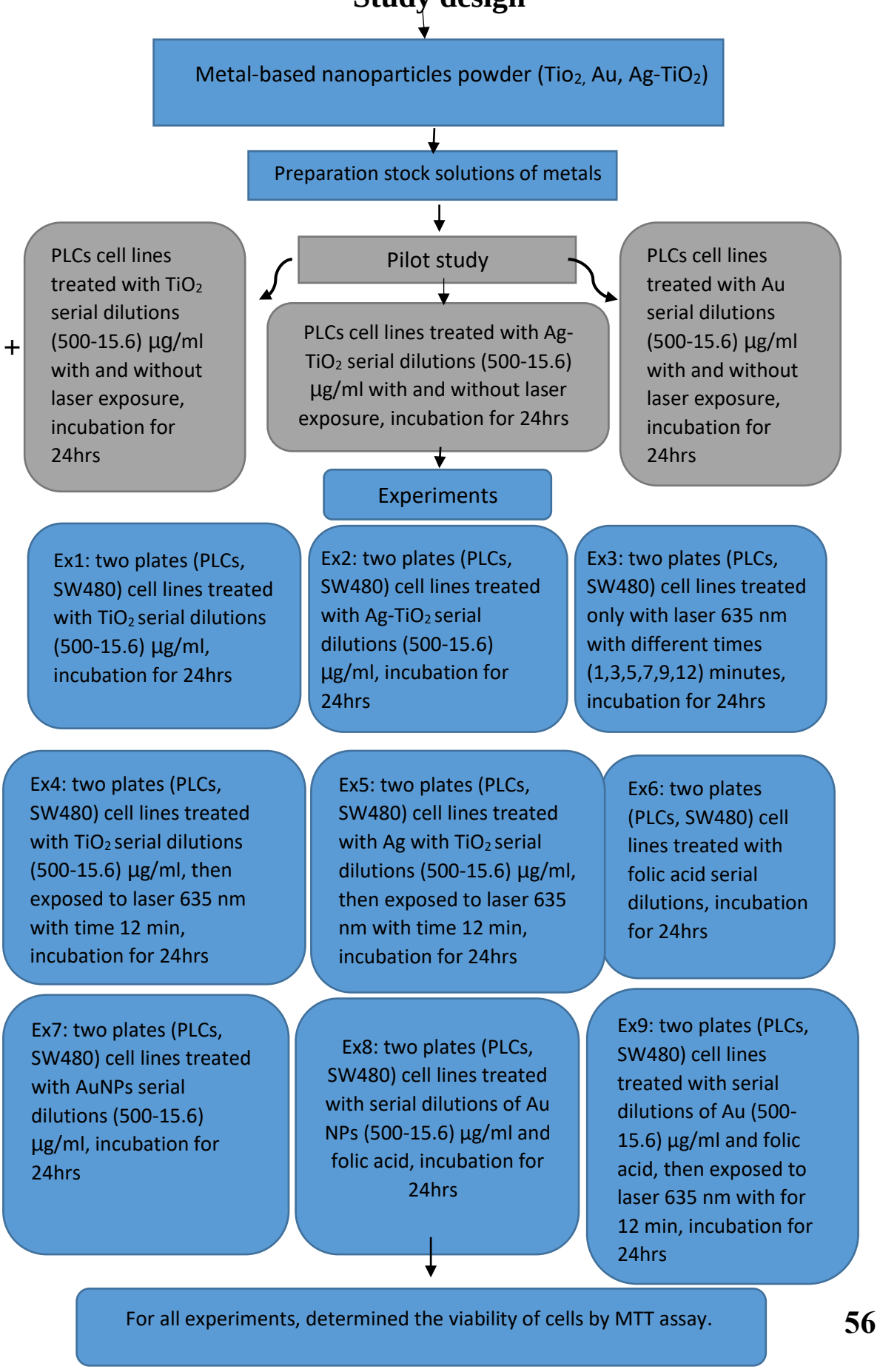
### 2.1.10 Transmission Electron Microscopy (TEM):

Transmission electron microscopy is used for material imaging and elemental composition analysis. The Transmission Electron Microscope (TEM) is a versatile analytical microscope for material characterization.

In a TEM, a very high-energy electron beam is placed on a sample that is thin enough to be partially electron transparent, and the electron "shadow" of the sample is viewed and digitally recorded.

High resolution imaging shows morphology, aggregate form and internal structure of particles and materials. In addition, the images of the particles can be used to measure the particle, size distribution of the material(Velez, Allen et al. 2017).

## Study design



## 2.2 method:

### 2.2.1 Preparation of PLC/PRF/5 Cell Lines for Cytotoxicity Assays:

#### 2.2.1.1 Thawing of PLC/PRF/5 and SW480 cell lines:

The frozen cell line vial was removed from liquid nitrogen container with caution and directly placed into a beaker containing pre-warmed (37°C) sterile DDW. The vial was removed from the water before the ice floccule dissolved completely, then it was wiped with 70% ethanol.

Without delay, the cell suspension content of the vial was pipetted under laminar flow cabinet into a 15 ml sterile plastic centrifuge tube containing 10 ml of pre-warmed serum free medium.

Centrifugation was done at 1000 rpm for 5 minutes and the supernatant was aspirated and decanted.

The cells pellet was re-suspended into 5ml warm (37C˚) serum-medium and transferred into 25 ml size cell culture flask, incubated at 37C˚ and the serum medium replaced on the next day.

#### 2.2.1.2 Harvesting and sub-culturing PLC/PRF/5and SW480 Cell Lines:

Harvesting is a technique that uses the proteolytic enzyme trypsin to detach and disaggregate the adherent monolayer cells from the bottom of the culture flask. It was performed whenever the cells need to be harvested for cell counting and sub-culturing of the cell line.

This procedure was done according to the following:

**1-** Cells were examined using the inverted microscope to ensure that the cells are healthy and sub-confluent (in the exponential phase of growth) and are free from contamination.

**2-** The spent medium was removed using a pipette and wash the monolayer with a sufficient volume of pre-warmed trypsin- (EDTA) solution to ensure the removal of all medium from the flask. This washing step was repeated if the cells were known to adhere strongly.

**3-** Appropriate volume of trypsin- (EDTA) solution was added into the washed cell monolayer using (1-2 ml) per 25-cm 2 flask. Flask was rotated to cover the monolayer with trypsin.

**4-** The flask was returned to the incubator at 37°C to allow the cells to detach from the inside surface of the flask (the length of time depends on the cell line, but usually this will occur within 2–10 minutes).

**5-** The cells were examined using an inverted microscope to ensure that all the cells are detached and in suspension. The side of the flasks may be gently tapped to release any remaining attached cells.

**6-** Inactivate the trypsin by adding an equal volume of serum-containing medium to the flask.

**7-** According to the required density, an aliquot of cells was transferred to a new labeled flask containing a pre-warmed serum-containing medium (5–7ml for a 25-cm 2 flask).

**8-** The flask was incubated at 37C°.

**9-** This process was repeated as demanded by the growth characteristics of the cell line

**2.2.1.3 Freezing of PLC/PRF/5 and SW480 Cell Lines:**

Cell lines source was kept frozen at (-196) °C 0 in nitrogen tank according to the following protocol:

**1-** Cultures were checked using an inverted microscope to assess the degree of cellular growth and to ensure that the cells are free of contamination. Adherent cells are harvested (as mentioned above) for cryopreservation in the exponential phase of growth.

**2-** A volume of 1 ml of the freezing medium was added and then the content of the flask was transferred into 1.5 ml sterile freezing vial. Each freezing vial was clearly marked with the cell line name, passage number, and date of freezing.

**3-** These vials were placed in the vapor phase of a liquid nitrogen container, which is equivalent to a temperature of -80°C for a minimum of three hours (or overnight).

**4-** The vials were removed from the vapor phase of the liquid nitrogen container and transfer them to the liquid phase for storage (-196°C).

### 2.2.2 The experiments of studying cytotoxic effects of Nano- based metals on (PLC/PRF/5) and (SW480) cell lines.

#### 2.2.2.1 Study cytotoxic effect of titanium dioxide nanotubes and Ag decorated $Tio_2$ nanotubes as following:

Cell line including primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) were seeded and labeled in 96 tissue culture plate in concentration $5*10^5$.

One replicate well considered as control group and three replicates well were exposed to 200µl of serial dilution with different concentrations (500,250,125,62.5,31.3,15.6) µg/ml of silver decorated $Tio_2$ nanotubes.

The remaining well were exposed to 200 µl of $Tio_2$ nanotubes with the same concentrations. After incubation for 24hrs, MTT assay was done to assess

cytotoxic effect of metals-based nanoparticles for each cell lines.as shown in figure (2.1).

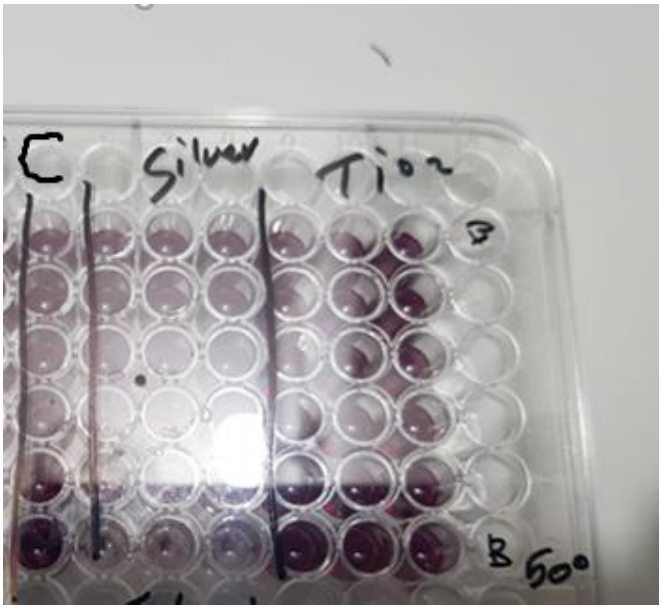



**Figure (2.1) 96 tissue culture plate seeded with cancer cells and exposed to $Tio_2$ and Ag decorated $Tio_2$ nanotubes**

### 2.2.2.2 Study cytotoxicity effect of Diode laser on both primary liver cancer (PLC/PRF/5) (PLCs) and colon cancer (SW480) as following:

Cell line including PLCs and SW480 were seeded and labeled in 96 tissue culture plate in concentration $5* 10^5$. Each plate was exposed to diode laser with 635nm for (1,3,5,7,9,12) minutes after that the two plates incubated for 24hrs then MTT assay was made to assess cytotoxicity effect of laser.

### 2.2.2.3 Study cytotoxicity effect of silver decorated $Tio_2$ nanotubes and titanium dioxide nanotubes with laser on both primary liver cancer (PLC/PRF/5) (PLCs) and colon cancer (SW480) as following:

Cell line including PLCs and SW480 were seeded and labeled in 96 tissue culture plate in concentration $5* 10^5$. Three replicates well of columns no.2,3

and 4were exposed to 200 µl of silver decorated Tio2 nanotubes of serial dilution with different concentrations (500,250,125,62.5,31.3,15.6) µg/ml.

Two replicates considered as a control, three replicates well of columns no.8,9 and 10 were exposed to 200 µL of different concentrations (500,250,125,62.5,31.3,15.6) µg/ml of Tio2 nanotubes then the plate exposed to diode laser for 12 minutes with wave length 635nm at all concentrations, after that the plate was incubated for 24 hrs. and at the end of incubation cell lines growth was assessed by cytotoxicity assay MTT assay. As shown in figure (2.2).

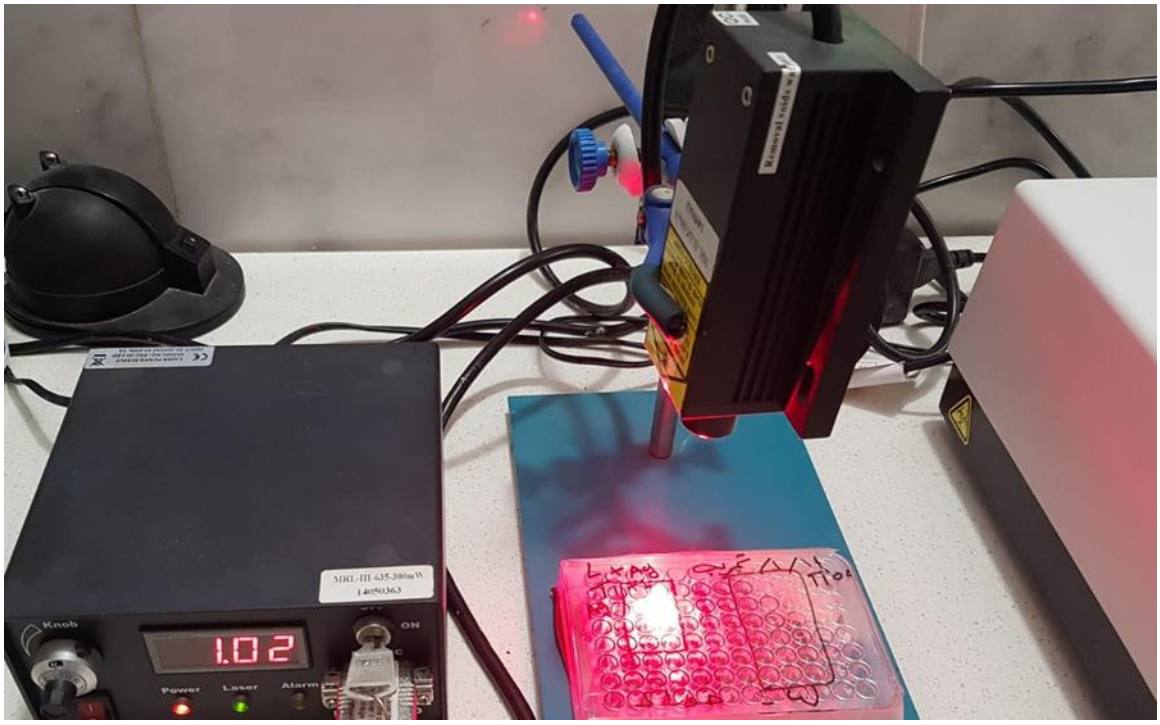



**Figure (2.2) 96 tissue culture plate seeded with cancer cells and exposed to $Tio_2$ and Ag decorated $Tio_2$ nanotubes with red laser 635 nm exposure.**

**2.2.2.4 study cytotoxic effect of gold nanoparticles, Folic acid and Gold NPs combined with Folic acid as following:**

Primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines were seeded and labeled in 96 tissue culture plate in concentration $5* 10^5$.

Three replicates well of column no.2,3 and 4 were exposed to200 µL of gold nanoparticles serial dilutions (500,250,125,62.5,31.3,15.6) µg/ml.

Three replicates well in columns 5,6 and 7 were exposed to200 µl of folic acid serial dilutions (0.05 - 0.00156) µg/ml. One replicate well in column 8 consider as control, the remaining column exposed to 200 µl of gold nanoparticles serial dilutions combined with folic acid serial dilutions were high concentration of gold nanoparticles with high concentration of folic acid and low concentration of gold nanoparticles with low concentration of folic acid.

After that plate was incubated for 24 hrs. and MTT assay was done to assess cytotoxic effect of folic acid and gold nanoparticles on each cell lines, as shown in figure (2.3).

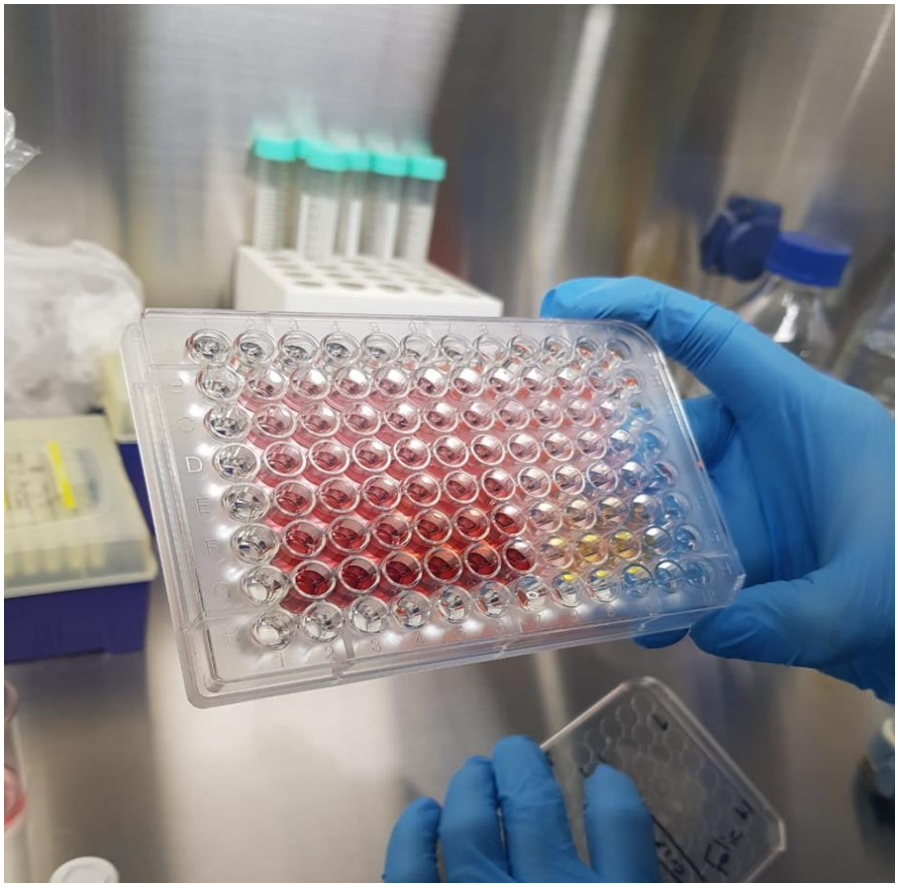


**Figure (2.3) 96 tissue culture plate seeded with cancer cells and exposed to gold nanoparticles, Folic acid and Gold NPs combined with Folic acid.**

### 2.2.2.5 Study effect of Gold NPS combined with Folic acid and laser as following:

Cell line including primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) were seeded and labeled in 96 tissue culture plate in concentration 5* $10^5$. Three replicates well in columns no.2,3 and4 exposed to 200 µL of serial dilutions of different concentrations of gold NPs with folic acid serial dilutions, were high concentrations of gold NPs with high concentration of folic acid and low concentration of gold NPs with low concentration of folic acid. Two replicates of columns no.5 and 6 considered as control after that the plate exposed to diode laser with 635 nm wavelength for 12 minutes on each well of

all concentrations and at the end of exposure the plate was incubated for 24 hrs. then the cell line growth was assessed by cytotoxicity assay MTT assay, as shown in figure (2.4).

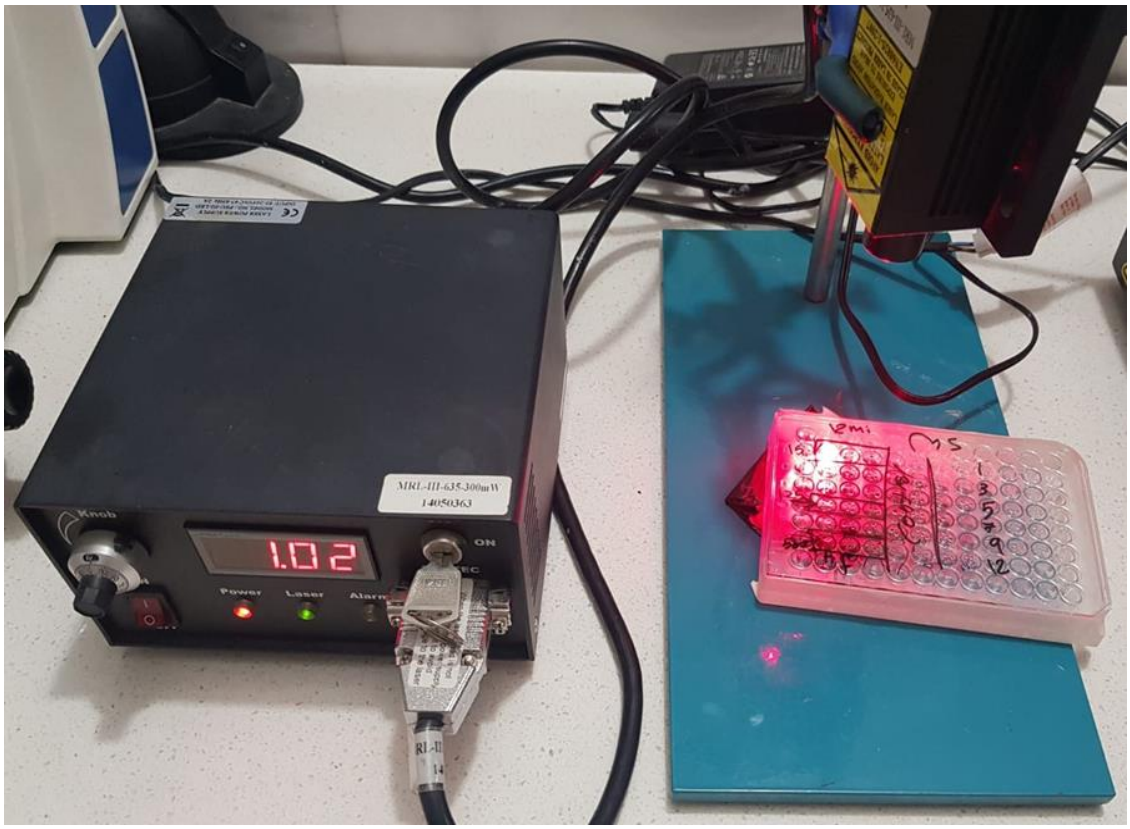



**Figure (2.4)96 tissue culture plate seeded with cancer cells and exposed to gold nanoparticles, combined with Folic acid with red laser 635 nm at different times.**

### 2.2.3 Cytotoxicity assays (MTT Assay):

#### 2.2.3.1 Principle

The general purpose of the MTT assay is to measure viable cells in relatively high throughput (96-well plates) without the need for elaborate cell counting. Therefore, the most common use is to determine the cytotoxicity of several drugs at different concentrations. The principle of the MTT assay is that for most viable cells mitochondrial activity is constant and thereby an increase or decrease in the number of viable cells is linearly related to the mitochondrial activity. The mitochondrial activity of the cells is reflected by the conversion of the pale-yellow tetrazolium salt (MTT dye) into dark purple formazan crystals by NADH (Figure below) which can be solubilized for homogenous measurement.

Thus, any increase or decrease in viable cell number can be detected by measuring formazan concentration reflected in optical density (absorbance) using a plate reader at 570 nm. The darker the solution, the greater the number of viable and metabolically active cells (Van Meerloo, Kaspers et al. 2011), as shown in figure (2.5).

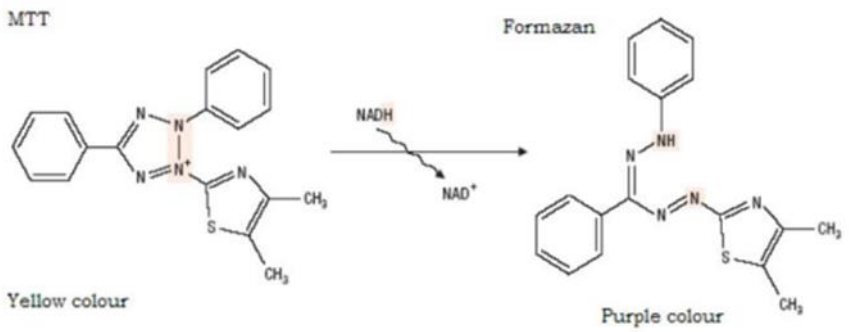



**Figure (2.5) Principle of MTT Assay (Sukhramani, Sukhramani et al. 2011)**

**2.2.3.2 Procedure (Meerloo et al; 2011):**

**1-** At the end of the drug exposure period, the medium was removed from the wells and then the cells were washed with PBS. A blank control was carried to assess unspecific formazan conversion.

**2-** A volume of 1.2 ml of MTT solution (5 mg/ ml) was added to 10.8 ml medium to obtain final concentration of 60 mg/mL. Then, 200 µl of the resulting solution was added in each well.

**3-** The plate was incubated for 3 hours at 37°C until intracellular purple formazan crystals were visible under the inverted microscope.

**4-** The supernatant was removed and 100 µl DMSO was added in each well to dissolve the resultant formazan crystals.

**5-** The plate was incubated at room temperature for 30 minutes until the cells have lysed and purple crystals have dissolved.

**6-** Absorbance was measured by a microplate reader at 570 nm. The absorbance reading of the blank must be subtracted from all samples. Absorbance readings from test samples must then be divided by those of the control and multiplied by 100 to give percentage cell viability or proliferation. Absorbance values greater than the control indicate cell proliferation, while lower values suggest cell death or inhibition of proliferation. Percent of cell viability or percent of inhibition was calculated by the following formula:

% viability = (AT – AB) / (AC – AB) × 100% Where, AT = Absorbance of treated cells (drug). AB = Absorbance of blank (only medium).

AC = Absorbance of control (untreated).

% Inhibition = 100 – % viability.

## 2.3 Statistical Analysis:

All data were collected and analyzed by Microsoft Office Excel 2016 and Sigma plot version 12.5 software. ANOVA one-way test was used to assess significant differences among the means of data. the p-value ($p<0.001$, $p<0.05$) were considered statistically significant(Hamida, Albasher et al. 2020).

# Chapter Three

# Results

## 3. Results:

### 3.1 Cytotoxicity evaluation of $Tio_2$ nanotubes on the viability of PLC and SW480 cancer cell line

The results of Titanium dioxide NTs on SW480 colon cancer cell line showed that Concentrations of (62.5, 125,250, and 500) µg/ml caused significant decrease in cell viability (P<0.001) while a concentration of 31.25µg/ml statistically (P<0.050) differ from the control. The results of $Tio_2$ NTs showed that there are no statistical differences from the control liver cancer cell line. (As shown in figure 3.1).

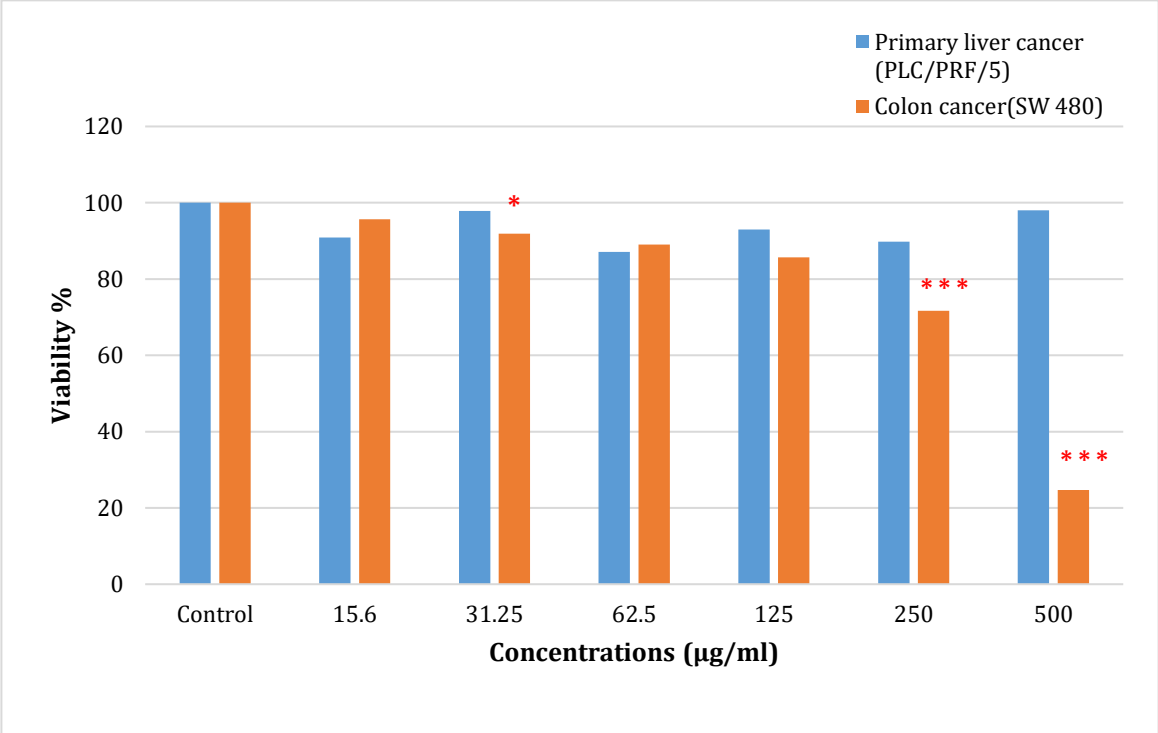



**Figure 3.1. effect of $Tio_2$ NTs on viability of PLC and SW480 cancer cell line using MTT assay**

***= Significantly decrease (p<0.05)**

****= significantly decrease (p<0.001)**

### 3.2 Cytotoxicity evaluation of Ag decorated $Tio_2$ on the viability of PLC and SW480 cancer cell lines.

The results of Ag decorated $Tio_2$ NTs on liver cancer cell line showed that the concentrations (250 and 500) µg/ml were significantly decrease in cell viability($P<0.001$) compared with the control. While the concentration 125µg/ml was significantly decrease cell viability ($P<0.050$) in comparison with the control. The results on colon cancer cell line showed that (125, 250µg/ml and 500) µg/ml were significantly decrease cell viability ($P<0.001$) compared with the control (As shown in figure 3.2).

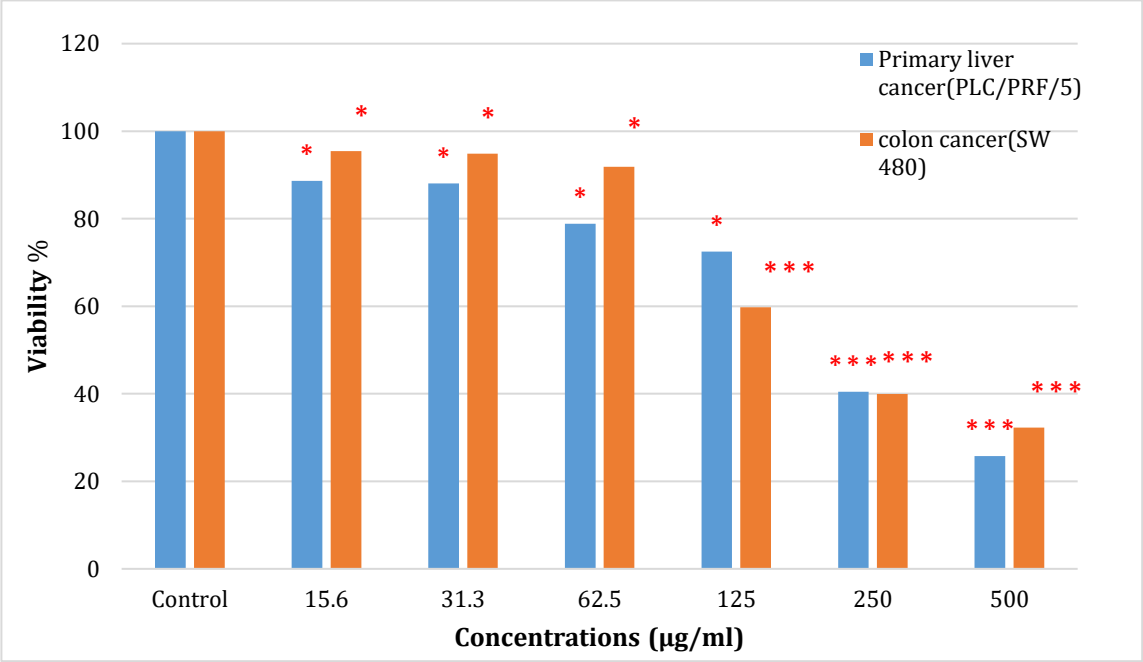



**Figure 3.2 effect of Ag decorated $Tio_2$ on viability of PLC and SW480 cancer cell line using MTT assay**

***= Significantly decrease ($p<0.05$) ***= significantly decrease ($p<0.001$)**

### 3.3 Cytotoxicity evaluation of laser on the viability of PLC and SW480 cancer cell lines.

The results of laser on liver cancer cell line showed that Only (9 &12) minutes. significantly decrease cell viability ($p<0.001$) differ from the control.

The results of laser on colon cancer cell line showed that time from (7 to 12) minutes statistically decrease cell viability ($p<0.001$) while time of 5 minutes' decrease cell viability ($p<0.05$) compared with the control (As shown in figure 3.3).

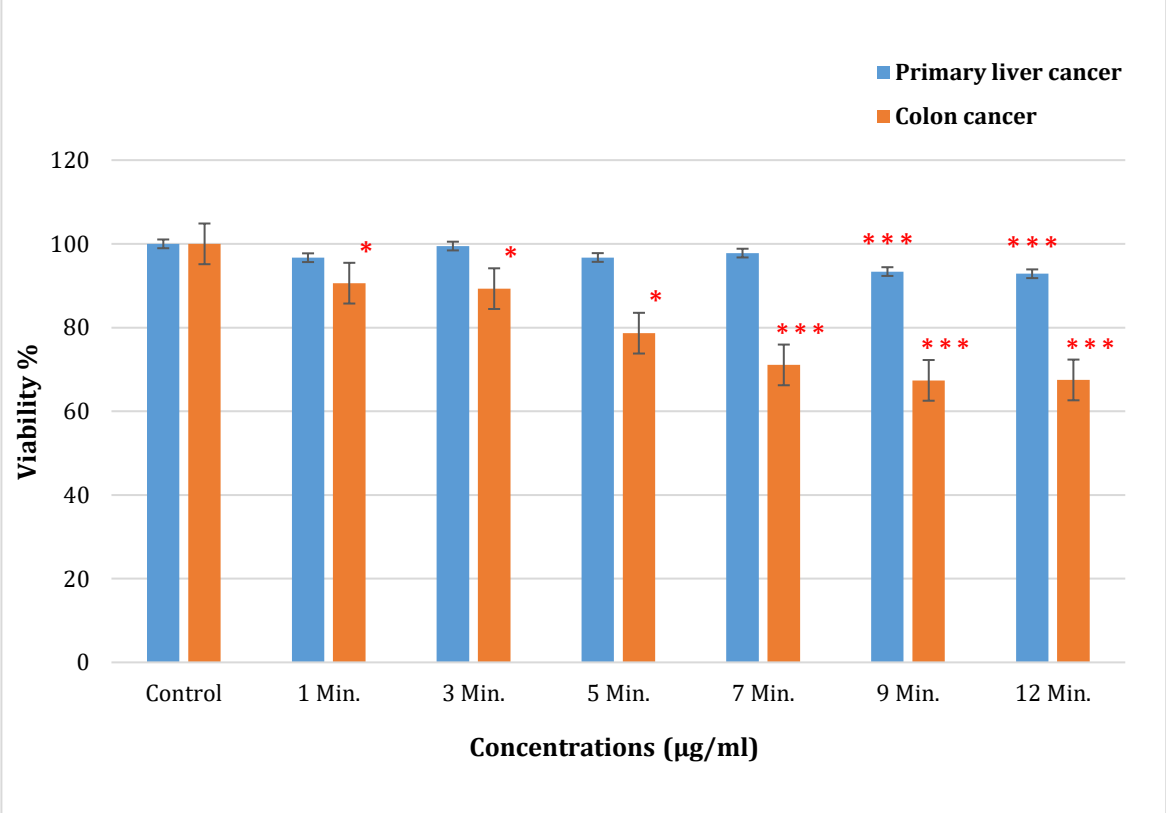



**Figure 3.3 effect of laser on viability of PLC and SW480 cancer cell line using MTT assay**

***= Significantly decrease ($p<0.05$) ***= significantly decrease ($p<0.001$)**

### 3.4 Cytotoxicity evaluation of $TiO_2$ NTs with laser on the viability of PLC and SW480 cancer cell lines.

The results of Titanium dioxide NTs with laser on liver cancer cell line showed that the concentrations of (250 and 500) µg/ml significantly decrease in cell viability ($p<0.001$), while all the remaining concentrations statistically decrease in cell viability ($p<0.050$) compared with the control.

The results of Titanium dioxide with laser on colon cancer cell line showed that concentrations of 500 µg/ml significantly decrease in cell viability ($p<0.001$) differ from the control while the concentrations (of 125 and 250) µg/ml statistically ($p<0.050$) differ from the control (As shown in figure 3.4).

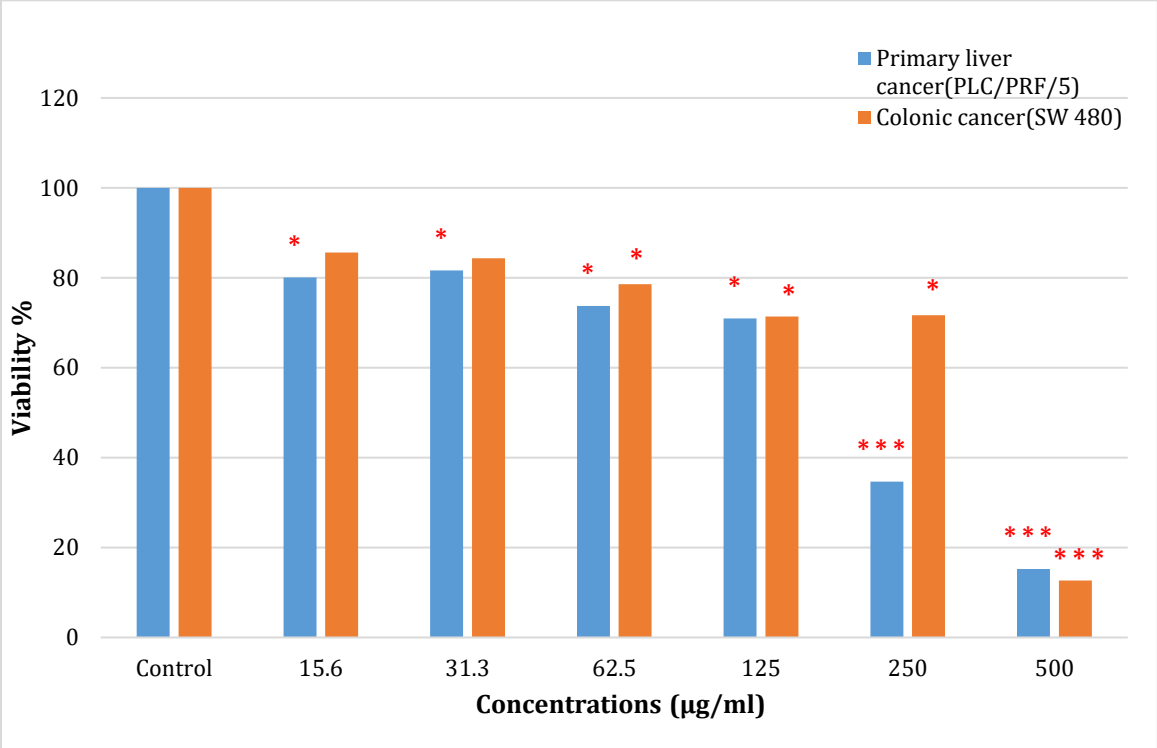



**Figure 3.4 effect of $TiO_2$ with laser on viability of PLC and SW480 cancer cell line using MTT assay**

***= Significantly decrease ($p<0.05$) , ***= significantly decrease ($p<0.001$)**

### 3.5 Cytotoxicity evaluation of Ag decorated $Tio_2$ with laser on the viability of PLC and SW480 cancer cell lines.

The results of Ag decorated $Tio_2$ with laser on liver cancer and colon cancer cell lines showed that all the concentrations decrease in cell viability significantly ($p<0.001$) when compared with the control (As shown in figure 3.5).

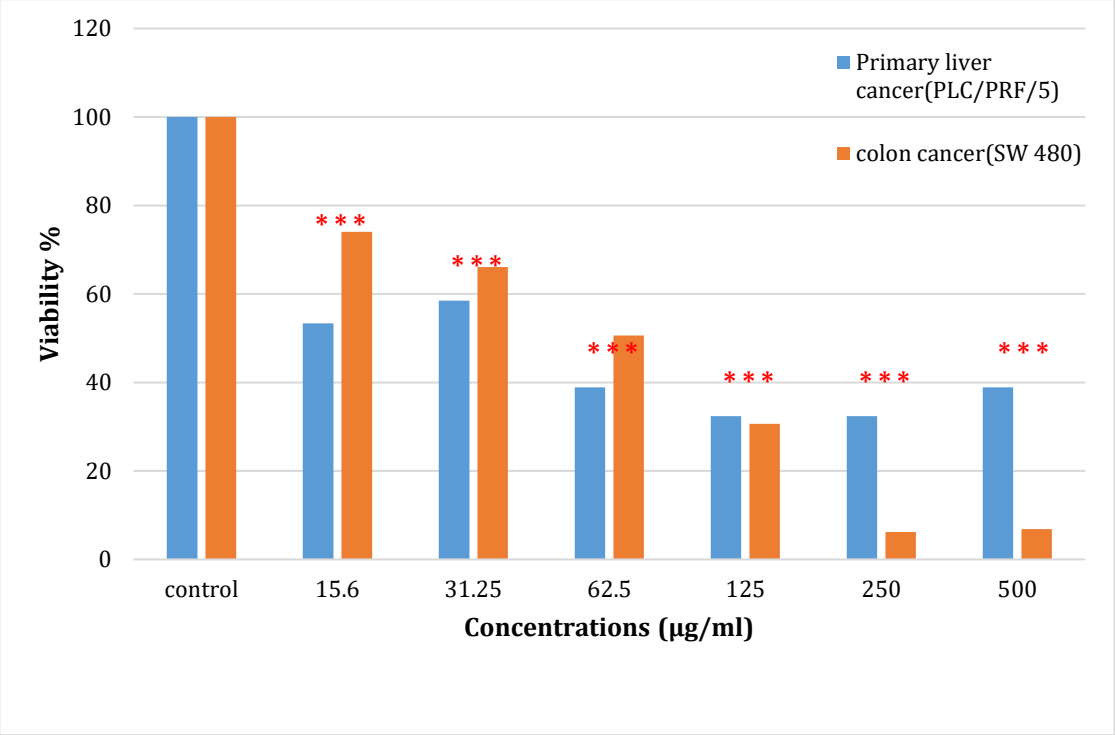



**Figure 3.5 effect of Ag decorated $Tio_2$ with laser on viability of PLC and SW480 cancer cell line using MTT assay**

***= Significantly decrease ($p<0.05$)**

****= significantly decrease ($p<0.001$)**

### 3.6 Cytotoxicity evaluation of Folic acid on the viability of PLC and SW480 cancer cell lines.

The results of folic acid on liver cancer and colon cancer cell lines showed that all the concentrations significantly decrease in cell viability ($p<0.001$) compared with the control (As shown in figure 3.6).

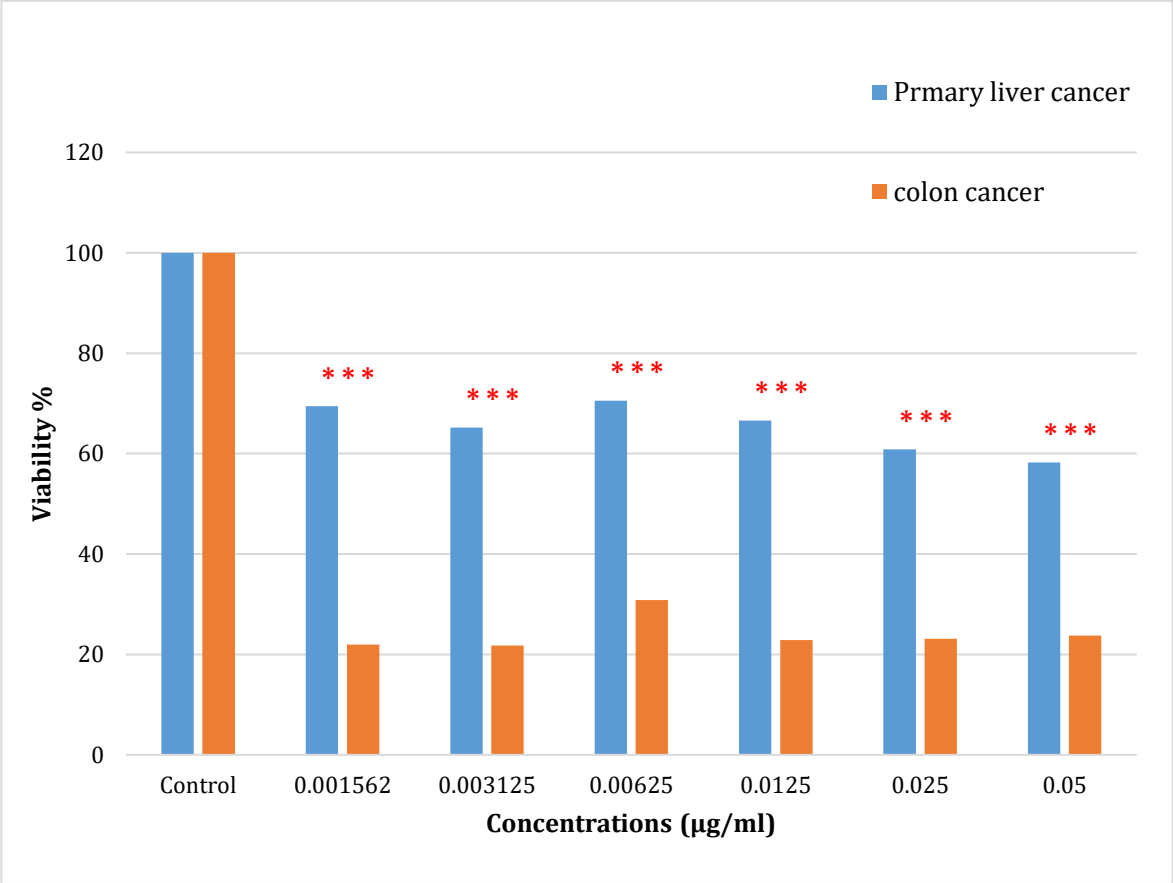



**Figure 3.6 effect of Folic acid on viability of PLC and SW480 cancer cell line using MTT assay**

***= Significantly decrease ($p<0.05$)**

****= significantly decrease ($p<0.001$)**

### 3.7 Cytotoxicity evaluation of Au NPs on the viability of PLC and SW480 cancer cell lines.

The results of Gold NPs on liver cancer cell line showed that the concentrations (250 and 500) µg/ml significantly decrease in cell viability (P<0.050) differ from the control. While The results of Gold NPs on colon cancer cell line showed that the Concentrations from (62-500) µg/ml significantly decrease cell viability (P<0.001) compared with the control (As shown in figure 3.7).

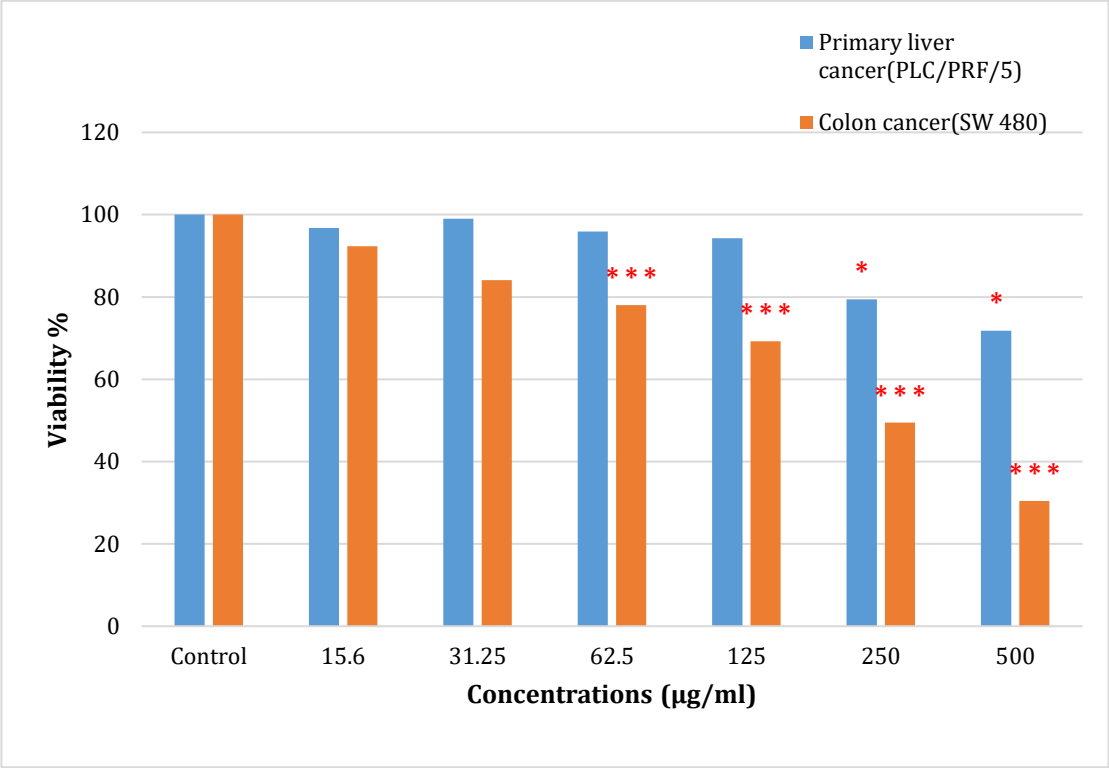



**Figure 3.7 effect of Au NPs on viability of PLC and SW480 cancer cell line using MTT assay*= Significantly decrease (p<0.05)**

*****= significantly decrease (p<0.001)**

### 3.8 Cytotoxicity evaluation of Folic acid combined Gold NPs on the viability of PLC and SW480 cancer cell lines.

The results of Gold NPs with folic acid on liver cancer and colon cancer cell lines showed that all the concentrations significantly decrease in cell viability ($p<0.001$) when compared with the control (As shown in figure 3.8).

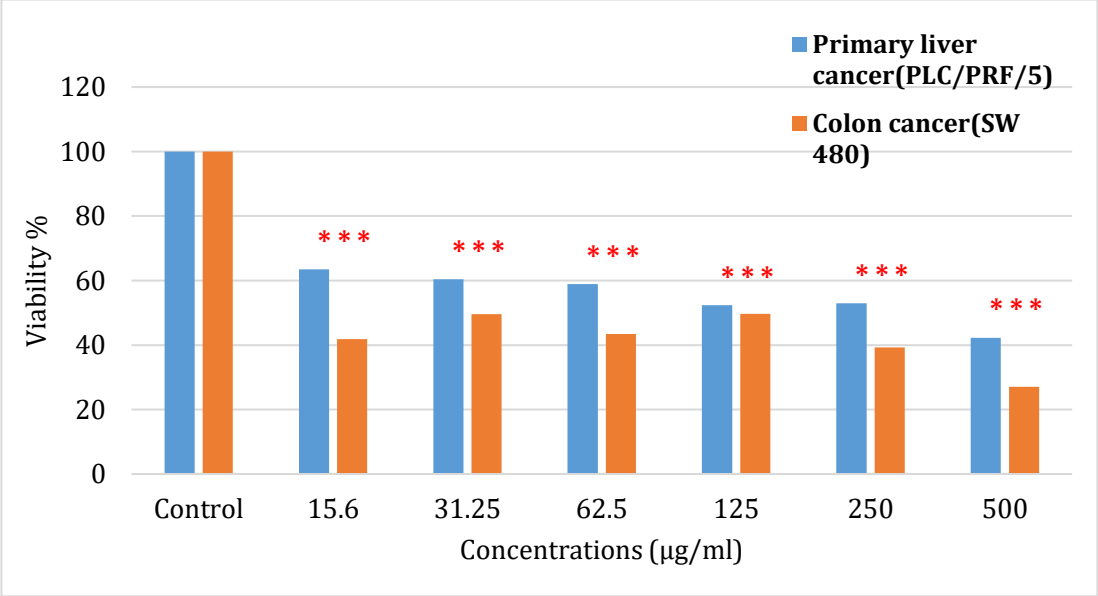



**Figure 3.8 effect of Folic acid combined Au NPs on viability of PLC and SW480 cancer cell line using MTT assay**

***= Significantly decrease ($p<0.05$)**

****= significantly decrease ($p<0.001$)**

### 3.9 Cytotoxicity evaluation of Gold NPS combined folic acid with laser on the viability of PLC and SW480 cancer cell lines.

The results of Gold NPs with folic acid and laser on liver cancer and colon cancer cell lines showed that all the concentrations significantly decrease in cell viability ($p<0.001$) when compared with the control (as shown in figure 3.9).

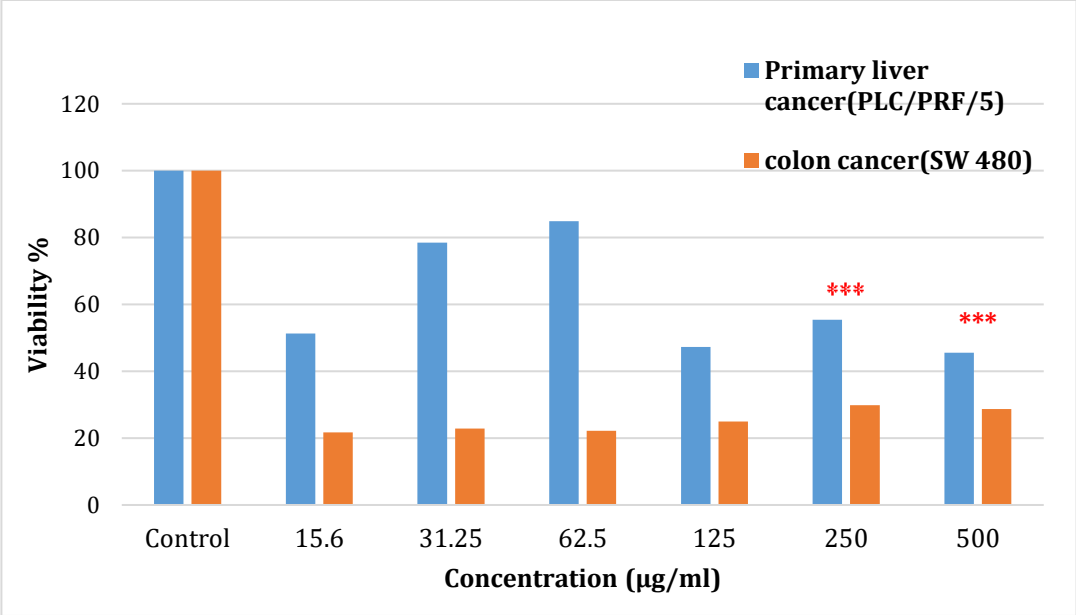


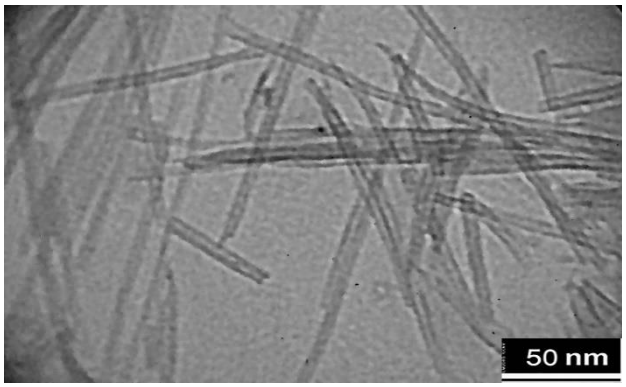

**Figure 3.9 effect of Au NPS combined folic acid with laser on viability of PLC and SW480 cancer cell line using MTT assay**

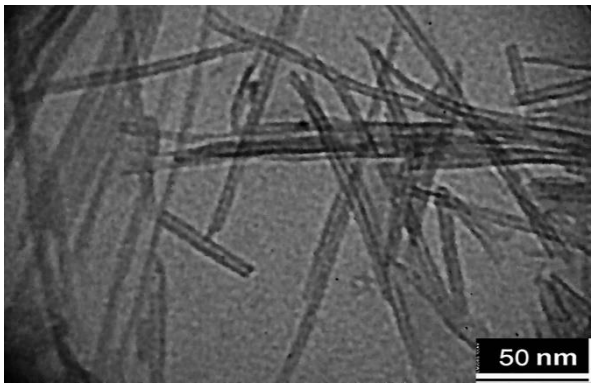
***= Significantly decrease ($p<0.05$)**

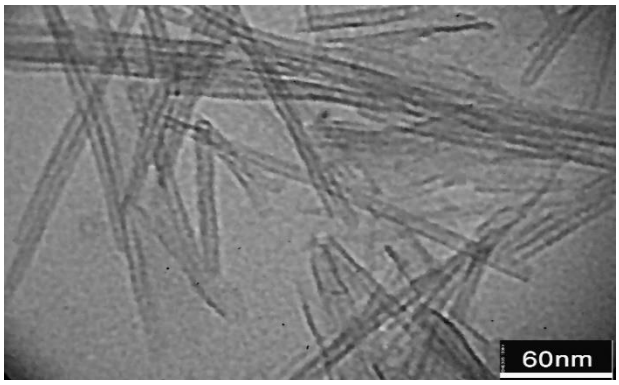
****= significantly decrease ($p<0.001$)**

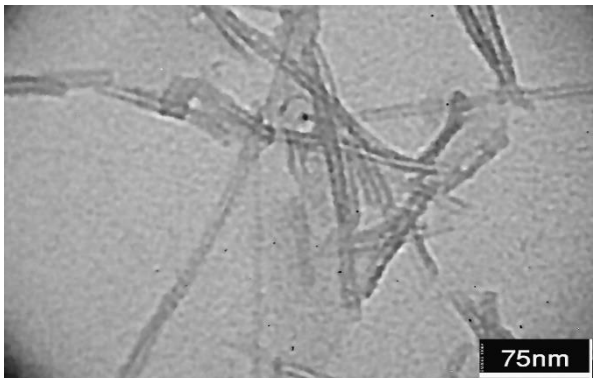
## 3.10 Transmission Electron Microscopy results of metals-based Nanotubes:

### 3.10.1TEM of $TiO_2$ NTs:

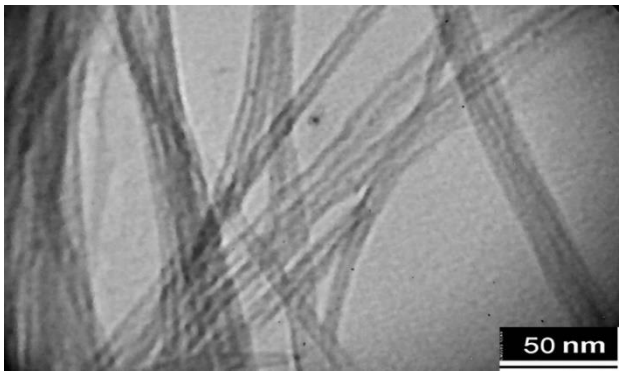


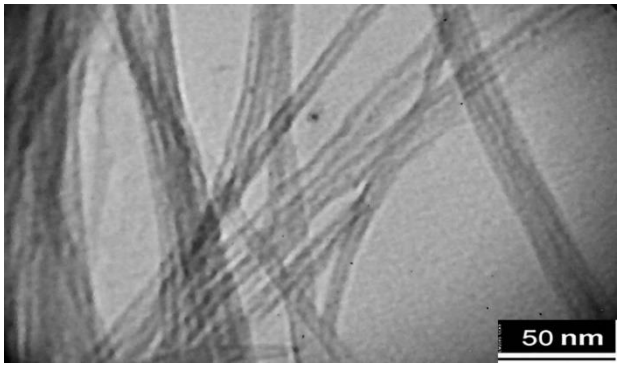



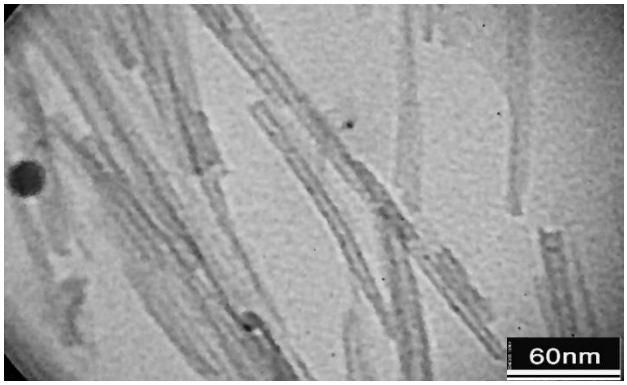



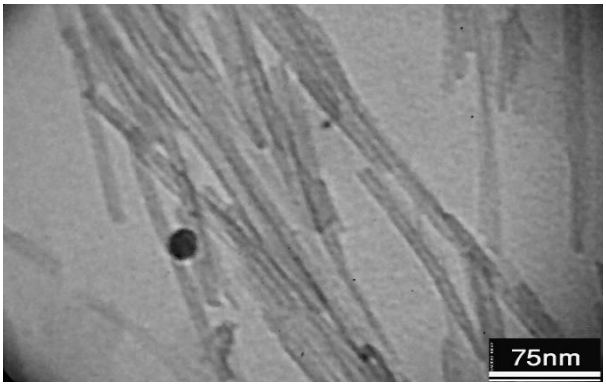




### 3.10.2 TEM of Ag decorated $TiO_2$ NTs:

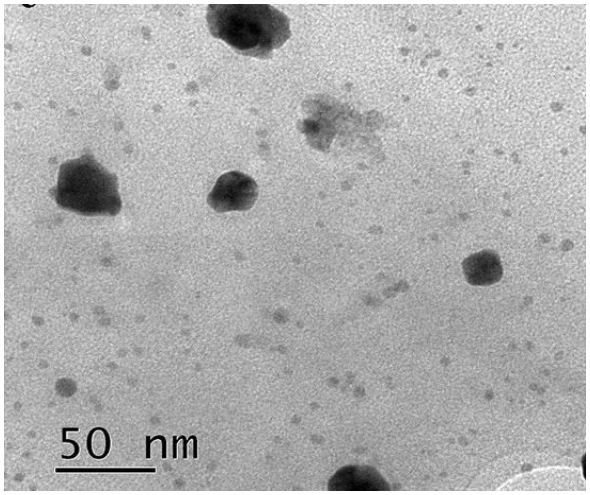


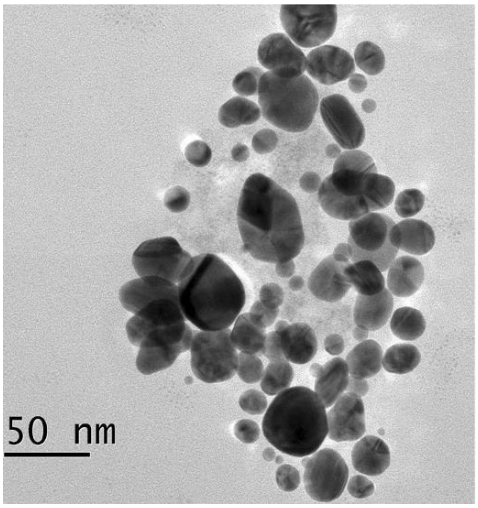






### 3.10.3 TEM of Gold NPs:



# Chapter Four

# Discussion

# 4. Discussion

## 4.1 Cytotoxic effect of $Tio_2$ nanotubes on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines:

The results showed that there is significant decrease in cell viability percent ($p<0.001$) for colon cancer cell line. While the results showed that there is no statistically significant decrease in the cell viability ($p< 0.794$) for primary liver cancer (PLC/PRF/5) cell line.

Antitumor effects of $TiO_2$-NTs affected by NP size, aggregation tendency and agglomeration are key factors that determine cell viability and genetic alteration in tumor cells ( Behzadi, 2017 ) and even the type of disease may substantially change the biological identity of NPs and their biological fates, including their cellular uptake and toxicity (Colapicchioni, Tilio et al. 2016).

Nanoscale $TiO_2$ particles have unique medicinal properties, including inertia and biocompatibility with body tissues and, thus, have been invested in many biomedical applications, such as bone plates, dental implants, artificial hips, scaffolds, and coatings, as well as in gene and drug delivery systems. Ultrafine $TiO_2$-NPs (<100 nm) possess anti-tumor and anti-bacterial properties, showing promise in biomedical fields(Raja, Cao et al. 2020).

$TiO_2$-NPs enter the cell via pinocytosis, phagocytosis, or micropinocytosis and, then, get accumulated in the cells at specific locations, such as the vesicles, cytoplasm, or mitochondria (Rauch, Kolch et al. 2013). The interactions of NPs with cells resulted in the generation of ROS, and the resultant oxidative stress may cause DNA fragmentation, in addition the increased levels of hydrogen peroxide ($H_2O_2$), hydroxyl radical ($^{\bullet}OH$), superoxide ($O2^{\bullet-}$), hydroperoxyl

($HO2^{\bullet}$) induced oxidative stress in cells, which can cause oxidative DNA damage and lead to the activation of p53 tumor suppressors and bcl-2 apoptotic factors. Oxidative stress can affect the mitochondria, the richest source of ROS, in which oxygen is metabolized and converted to $O^{-2}$ by several components of the mitochondrial respiratory chain (Armand, 2016; El-Said, 2014).

Cell cycle arrest is closely associated with cell growth retardation and apoptosis. The cell cycle can be divided into G1, S, and G2/M phases. In the current study, $TiO_2$ treatment increased the percentage of G1 phase and decreased the percentages of S and G2/M phase cells, suggesting that the synthesis of RNA and ribosomes were blocked, thus preventing the cells from entering the next stage of the cell cycle and leading to increased apoptosis(Lagunes, Martın-Batista et al. 2018).

$TiO_2$ Nano filaments exhibited enhanced cytotoxic action and a strong dose-dependent effect on cell proliferation and cell death (Magrez, Horváth et al. 2009).

$TiO_2$ particles can induce malignant cell apoptotic death via the production of ROS and ROS on the surface of $TiO_2$ nanoparticles caused oxidative stress in adjacent cells or tissues, and preferentially damaged cancer cells(Imani, Dillert et al. 2017) .ROS-induced accumulation of misfolded and unfolded polypeptides in the ER activates the adaptive intracellular stress response (unfolded protein response) (Kritsiligkou, Rand et al. 2018).

The ROS-induced ER stress response can trigger cell apoptosis by inducing the expression of ER sensor proteins, including protein kinase RNA-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), eukaryotic initiation factor (eIF) 1, and X-box binding protein 1, and their

downstream signaling pathways, such as the PERK/eIF2a/ATF4/CCAAT enhancer-binding protein homologous protein (CHOP) pathway(Chalmers, van Lith et al. 2017) Nanoparticle-induced ER stress is an early biomarker for nontoxicity evaluation(Chen, Huo et al. 2014)**.**

## 4.2 Cytotoxic effect of silver modified $Tio_2$ NTs on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines:

Cancer is a multifaceted disease, extremely variable in its presentation, development and outcome. It is well established that cancer is a multifactorial disease caused by a complex mixture of genetic and environmental factors. However, the knowledge of the genetic, molecular, and cellular basis of cancer can provide new targets and strategies for therapy. Many anticancer drugs are unable to reach their target site in sufficient concentrations and efficiently exert the pharmacological effect without causing irreversible unwanted injury to healthy tissues and cells, nanotechnology offers a wealth of tools to treat cancer by passing biological barriers to exert their effect .The unique physicochemical characteristics of metal NPs, such as high surface-to-volume ratio, broad optical properties, ease of synthesis and surface functionalization offer new opportunities for cancer therapeutics(Buttacavoli, Albanese et al. 2018).

The results of this study showed significant decrease in cell viability percent ($p<0.001$) differ from the control at higher concentrations for primary liver cancer (PLC/PRF/5) cell line, while on colon cancer cell line the results showed there is significant decrease in cell viability percent ($p<0.001$) at concentrations (500,250 and 125) µg/ml compared with the control.

Ag NTs and $Tio_2$ NTs each of them exerts its cytotoxic effect by specific mechanisms were silver NTs caused morphological changes; including cell shrinkage, detachment, clustering, and rounding (Bin-Meferij and Hamida

2019). These cytomorphological changes may be attributed to the small size of N-SNPs, which enables them to interact with cellular structures, such as membranes, mitochondria, endoplasmic reticulum, and the nucleus, and cellular components, such as nucleic acids, proteins, and enzymes, leading to cellular dysfunction and cell death (Firdhouse and Lalitha 2015).Imbalances in ATPase, LDH, GPx, and GSH activities. LDH is a soluble cytoplasmic enzymes that exists in nearly all cells and it is liberated into the extracellular space through damaged plasma membranes the significant increase in LDH levels observed in the assay revealed that N-SNPs caused membrane disruption that influenced membrane integrity and permeability, The decrease in ATPase activity after exposure of cells could be attributed to the induction of oxidative stress, which inhibits the activity and expression of ATPase, resulting in imbalances in cellular homeostasis and eventual damage to cells(Chan, Moriwaki et al. 2013).

Enhanced cell death pathways by suppressing the expression of anti-apoptotic proteins (Akt, p-Akt, mTOR, Bcl-2) and promoting the activity of apoptotic proteins (p53 and caspase 3), possibly via inducing oxidative stress and/or through interactions with cellular components and organelles, such as DNA and mitochondria. The tumor suppressor p53 has a crucial role in the response to cellular stress; it regulates many genes that are involved in numerous cellular process, including DNA damage repair, cell cycle arrest, apoptosis, and cell survival (Hafner, Bulyk et al. 2019) The observed upregulation of p53 protein may indicate that N-SNPs directly and/or indirectly interact (via oxidative stress) with DNA, causing DNA damage that stimulates cell death pathways (Satapathy, Mohapatra et al. 2013), Caspase 3 is a well-known major executor of the apoptosis pathway that orchestrates cellular destruction with proteolytic cascades (Nakajima and Kuranaga 2017).

Its acted as potent antiproliferative agents in a dose dependent manner were the response of cancer cells toward NPs differs according to the cell type. (Hasegawa, Aoki et al. 2014, Lagopati, Kotsinas et al. 2021).

$Tio_2$ NTs can cause cytotoxicity (Galata, Georgakopoulou et al. 2019) and induce apoptosis (Lagopati, Tsilibary et al. 2014) as mention previously . In this study combined Ag with $Tio_2$ in order to improved $Tio_2$ NTs cytotoxic effects that compatible with these studies (Chao, Yun et al. 2003, Nbelayim, Kawamura et al. 2017) were silver increases the Specific Surface Area (SSA) of the $TiO_2$. In fact, silver modification promotes the ROS generation prolonging the bioactivity duration of the $TiO_2$ NTs.

### 4.3 Cytotoxicity of Diode laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines:

Lasers are widely used for superficial and interstitial PDT. A unique property of lasers is that the generated monochromatic light, with a very narrow bandwidth, is coherent.(Kim and Darafsheh 2020). Main components of a laser are a gain medium, a resonant cavity, and an energy source (Saleh and Teich 2019)

Fundamental interactions of light with tissue include reflection, refraction, scattering, and absorption were Scattering leads to dispersion of light and eventual reduction in the light intensity major highly absorbing molecules in tissue include water, oxyhemoglobin, deoxyhemoglobin, melanin, and cytochromes (Wong and Ilgner 2016) .

Hence, even if the surface of the target area is illuminated evenly, the energy of the incident light will fall dramatically at increasing depths below the surface (or at increasing distances within tissue from an interstitial fiber). This is

because most tissue chromophores (hemoglobin, melanin, fat etc.) absorb light strongly in the visible spectrum, Thus, effectiveness of PDT greatly decrease with tissue thickness due to strong attenuation of visible light with increasing tissue depth, leading to incomplete treatment and tumor relapse(Lucky, Soo et al. 2015) .

Treatment light is affected by the tissue optics and must be taken into consideration when determining photodynamic effect. The optimal therapeutic window for PDT, however, is with light between 600–800 nm in wavelength is often called the “optical window” of tissue(Kirillin, Shakhova et al. 2016).

Use of longer wavelengths (>800 nm) is not practical due to their inefficiency in exciting an oxygen molecule from triplet ground state to the excited singlet state and the depth to which light penetrates tissue is dependent upon the optical properties of the tissue and the wavelength of the light employed. (Hester, Kuriakose et al. 2020) .

The results of this study showed that there is significant decrease in cell viability percent ($p<0.001$) differ from the control on both cell lines at 7,9 and 12 minutes.

Cell injury occur as laser therapy mainly works on cytochrome c oxidase (COX) in the mitochondrial, Apoptotic responses to PDT are carried out primarily through the intrinsic pathway in which the mitochondria play a crucial role. This pathway is induced by diverse factors including developmental and environmental cues, cellular stress, DNA damage, heat shock, nutrient deprivation, and cytotoxic(Parsons and Green 2010). One of key events in the intrinsic pathway is the release of apoptogenic proteins from the mitochondrial intermembrane space into the cytosol; these proteins include cytochrome c, apoptosis-inducing factor (AIF), and endonuclease G (EndoG) (Jendrossek

2012)Upon release into the cytosol, cytochrome c associates with apoptotic protease activating factor 1 (Apaf-1), forming a heptameric complex (i.e., apoptosome) with caspase-9 in the presence of ATP. This leads to the conformational change and activation of caspase-9, in turn, the activated caspase-9 cleaves and activates downstream caspases (e.g., caspase-3 and caspase-7), which carry out apoptosis)Tait and Green 2010)

### 4.4 Cytotoxicity $TiO_2$ and laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines:

The results of this study showed there is a statistically significant decrease in cell viability percent ($p<0.001$) at concentrations (500 and 250) µg/ml while the concentrations (125,62.31 and 15) µg/ml showed decrease in cell viability percent ($p<0.050$) for PLCs cell line.

There is significant decrease in cell viability of colon cancer percent($p<0.001$) at concentration 500 µg/ml but with concentrations (250 and 125) µg/ml ($p<0.050$) compared with the control.

These results closed to the study (Zhang, 2014) that indicated the viability of the cancer cells treated with $TiO_2$ nanoparticles in the presence of UV irradiation decreased remarkably compared to those under UV irradiation or $TiO_2$ nanoparticles alone, which indicated that the photocatalytic activity of $TiO_2$ nanoparticles could have increased the mortality of cells in dose dependent manner.

Semiconductor nanoparticles made of $TiO_2$ used in new applications in biology and medicine, e.g., such as photosensitizers in photodynamic therapy (PDT) for cancer therapy(Li, He et al. 2020).

$TiO_2$ NPs behave as photo catalysts, when they are excited by photon energy derived from light , pairs of holes and electrons are generated, reacting with the available water and oxygen, yielding reactive oxygen species (ROS) (Liao, Li et al. 2020). This phenomenon can take place when $TiO_2$ NPs enter the cell and since the produced free radicals might be potentially very harmful (Barbouti, Lagopati et al. 2021) $TiO_2$ NPs may prove efficient damaging agents against crucial biomolecules of cancer cells were Reactive oxygen species (ROS) have high oxidative potential and they damage DNA and cell membrane in cancer cells and as the membrane of a cancer cell damages, this causes necrosis. Nucleus and mitochondria damage lead to apoptosis, whereas damage in endoplasmic reticulum induce autophagy(Çeşmeli and Biray Avci 2019). If this event is triggered controllably, it can specifically target the cancer cells, sparing the healthy ones **(Li, 2020).**

## 4.5 Cytotoxicity of silver modified $TiO_2$ NTs and Diode laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines:

The results of this study showed there is a significant decrease in cell viability percent($p<0.001$) at all concentrations compared with the control for primary liver cancer (PLC/PRF/5) and colon cancer cell lines.

This study aim to improve photo catalytic activity of $TiO_2$ by addition of silver NTs according to this study(Kim, Lin et al. 2017, Liao, Li et al. 2020). Silver is used as a dopant, since it has the ability to trap the photo-excited electrons from $TiO_2$, allowing to the holes remain active as collective oscillation of conduction electrons can be induced in metal NPs by irradiating with light. This is because the collective oscillation of surface electrons resonates with the electromagnetic field of the incident light.

This behavior is generally termed as the localized surface Plasmon resonance (LSPR). LSPR covers a wide range of solar spectrum, particularly in the visible and near-infrared (NIR) regions After excitation, LSPR decays non-radiatively into hot electrons and holes through Landau damping, generating highly energetic charge carriers that are typically termed 'hot carriers. This ultrafast relaxation renders the hot carriers capable of rapidly separating and transferring into semiconductors to drive chemical reactions and create ROS on adsorbed molecules.

The LSPR effect is more pronounced Ag nanoparticles compared with other metal. Enhanced photocatalytic activity also was observed, since the time needed for the efficient photo-activation was approximately 10 min, Previous studies had shown that $TiO_2$ induced apoptosis only after a 20-min irradiation with light. Thus, silver modification allowed an optimization of the photocatalytic cytotoxicity that was controllably triggered in order to lead cancer cells to apoptosis according to this study (Lagopati, Kotsinas et al. 2021).

Possibly, the composition of the cell membrane proteins is different in each cell line and consequently the interactions between those proteins and $Ag/TiO_2$ NPs are different in each case**.**

**4.6 Cytotoxic effect of folic acid on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines**:

Folic acid is a small water-soluble molecule and vitamin in the B group. The FA molecule consists of three components: a pterin residue, p-aminobenzoate and a glutamic acid residue. Folic acid can be coupled via either α- or γ-carboxyl groups of its glutamate residue, but it is described that the γ-conjugate is mostly capable of binding to FR (Stallivieri, Baros et al. 2015) . Folate plays an important role in regulating DNA replication and various metabolic

pathways such as the biosynthesis of thymidylates and purines so a sufficient intake of folate is needed in any rapidly proliferating cells like cancer cells. Many cancer cells try to overexpress the folate receptors (e.g., breast, colon, ovary, lung, kidney, and other cancers) on their surface to uptake much more folic acid in competition with their neighboring cells (Cheung, Bax et al. 2016).

Generally, Folic acid can protect against the development of cancer by helping in methionine regeneration and reducing the error rate during DNA replication In addition, FA has been shown to exert the scavenging potential for free radical and antioxidant properties, which might also contribute to its anti-tumor properties (Li, Rong et al. 2006) ..

The results of this study showed that there is a statistically significant decrease in cell viability percent($p<0.001$) at all concentrations for both cell lines (PLCs and SW480) this results closed to this study (Majumdar, Kodali et al. 2004)that indicated FA inhibit cell proliferation by inhibits tyrosine kinase activity particularly epidermal growth factor receptor (EGFR) suggests a role for these enzymes in regulating the folic acid-induced inhibition of proliferation, tyrosine kinases play a crucial role in regulating proliferation, differentiation, and transformation of cells.

The present study has demonstrated that folic acid was able to induce the apoptosis this apoptosis may be mediated by down-regulating the expression of apoptosis-associated gene *bcl-2* and up-regulating the expression of tumor suppressor gene *p53* as the members of the Bcl-2 family play a central role to inhibit or promote apoptosis(Ting, Lee et al. 2019). Levels of Bcl-2 within cells are critical to antiapoptotic activity, decreasing Bcl-2 could be a mechanism to sensitize cells to apoptosis(Merlin, Rupasinghe et al. 2021)

## 4.7 **Cytotoxic effect of gold NPs on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell line**s:

Cell proliferation was measured after incubation period for 24 h at 37 ° the result show there was a significant decrease in the viability of cells ($p<0.05$) at higher concentration (500,250) µg/ml for primary liver cancer (PLC/PRF/5) cell line, while for colon cancer cell line all concentrations showed statistically significant decrease in cell viability percent($p<0.001$) compared with control.

The cytotoxic effect Au NPs on cells may be due to the higher-level concentration of Au NPs which can interact with other component in the growth media. Cellular media contains vitamins, serum proteins and antibiotics and other chemicals.

The high concentration of gold nanoparticles will change their properties such as size, surface chemistry, shape, aggregation state, and surface charge (Alkilany and Murphy 2010) .

AuNPs at high concentration induce damage to cell membrane, plasma membrane, cellular metabolic activity, mitochondrial activity and a LDH leakage that led to the damage of cells (Ávalos, Haza et al. 2018).Another reason may be due to the oxidative stress as a results of increasing reactive oxygen species (ROS) in mitochondria which is consider toxic to the cells and cause damage to cellular molecules such as DNA, lipids and proteins(Khanna, Ong et al. 2015)

The cytotoxic mechanism of AuNPs include damage of normal morphology resulting in rounded cells, cell cycle arrest at G1 phase, increased expression levels of proapoptotic genes caspase-9 and caspase-3 and generation of ROS(Grijalva, Vallejo-López et al. 2018).AuNPs induce change in the structure

of cellular cytoskeleton network due to high concentration which effect on cells morphology and cause change in energy metabolism , cell adhesion and signal transduction to the cells(Pavlovich, Volkova et al. 2017).

It was shown that AuNPs have many cytotoxic interactions with Cell membrane, mitochondria, proliferation related gene and nucleus. Previous study has shown toxicity of AuNPs to be dose dependent, area and surface charge. The surface charge is one of the major reasons that lead to increase Au NPs toxicity. The authors found that interaction of a positive charge on the ammonium species with a negative charge on the lipid bilayer of cell membranes can cause cytotoxic effect to cells (Pivodová, Franková et al. 2015).

### 4.8 Cytotoxic effect of folic acid and gold NPs on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines:

Tumor targeting therapy is a promising therapeutic strategy (active targeting and passive targeting) It can be achieved via the vectorization of PSs meaning either the introduction of the latter into liposomes or their encapsulation in nanoparticles (NPs) (Talekar, Kendall et al. 2011). It can also be carried out using the addressing strategy by conjugation of the PS with molecules known to have specific interactions with receptors, which are overexpressed on tumor cells as this enhances their selectivity for cancerous tissue as opposed to healthy tissue.

In this study uses folic acid for improving nanoparticles targeting and also for utilization cytotoxic effects of folic acid against cancer cell proliferation that agreed with these studies(Samadian, Hosseini-Nami et al. 2016, Liu, Turyanska et al. 2019).

The results of this study showed that all concentrations of both cell lines (PLCs and SW480) decrease cell viability significantly ($p<0.001$) differ from control the results were closed to these studies (Liu, 2019, Li, 2015). Gold nanoparticles have been identified as promising candidates for new cancer therapy modalities because of biocompatibility, easy synthesis and functionalization, chemo-physical stability, and optical tunable characteristics(Mehdizadeh, Pandesh et al. 2014) .

Gold NPs were directly attached to folic acid can be conjugated to Au nanoparticles by 4-aminothiophenol producing folate-targeted Au nanoparticles (Ramzy, Nasr et al. 2017) in order to avoid significant increases in the overall size and hence properties of the Au NP(Galchenko, Schuster et al. 2019) (Stallivieri, Baros et al. 2015) cytotoxicity of folic acid conjugated gold NPs occur more on colon cancer cell than primary liver cell as colon cancer cells express FARs more than PLCs according to the study (Stallivieri, Baros et al. 2015) so colon cancer cell more sensitive to cytotoxic effect of this conjugation.

**4.9 Cytotoxic effect of folic acid and Au NPs and laser on primary liver cancer (PLC/PRF/5) (PLC) and colon cancer (SW480) cell lines**:

The results of this study showed that all concentrations of both cell lines (PLCs and SW480) decrease cell viability significantly ($p<0.001$) comparing with the control, the results were closed to these studies (Zeinizade, 2018, Beik, 2017).

Gold nanoparticle (AuNP) is of great interest for developing a unique system with high potential for biological applications such as Nano-photo thermolysis.

When short laser pulses irradiate AuNPs, a process named Nano-photo thermolysis occurs. In this process, AuNPs heat up quickly.

It has been determined that the absorbed light by AuNP converts to heat on the picosecond time scale. AuNPs are the most promising candidates for nano photo thermolysis since they are strong absorbers, photo stable, nontoxic, and have adjustable optical properties.

The absorption maximum of AuNP is tunable from the mid visible region into the infrared region based on the size, shape and material. AuNPs strongly absorb laser irradiation, this absorbed energy transforms quickly into heat, which could cause fatal damage to cancer cells through local overheating effects. Accordingly, AuNPs are potentially very practical and efficient photo thermal agents in therapeutic applications, especially in cancer treatment(Akhter, Ahmad et al. 2012).

The introduction of AuNPs, the most important laser-responsive nanomaterial, into the tissue changes optical properties of the medium and increases local conversion of laser energy into heat. As stated earlier, AuNPs possess surface Plasmon resonance (SPR), a well-known property that implies the resonant oscillation of free electrons on the particle surface induced by incident light. Following this phenomenon, the electrons in Au atoms absorb the laser photons and become excited to higher energy levels. Through electron-phonon relaxation, the absorbed photon energy is converted to heat and is transferred into the particle lattice (Manna, Sarkar et al. 2005, Beik, Khademi et al. 2017) To limit such released heat and its resulted thermal damages to the tumor, it is necessary to target AuNPs towards cancer cells using various targeting ligands such as folate. Therefore, it is expected the combination of F-AuNPs with laser exposure leads to selectively heat and destroy the cancer cells(Shakeri-Zadeh, Mansoori et al. 2010).

# Conclusions

# and

# Recommendations

## Conclusions:

1. $TiO_2$ showed cytotoxic effects on liver cancer and colon cancer cells in a different sensitivity.
2. Ag and $TiO_2$ each of them exerted cytotoxic effects on liver cancer and colon cancer cells with specific mechanisms and $TiO_2$ modification by addition Ag NTs enhanced cytotoxic effects of $TiO_2$ NTs.
3. Laser showed cytotoxic effects on liver cancer and colon cancer cells in a time dependent manner and laser caused improvement in cytotoxic effects of NTs on tumor cells.
4. Folic acid (pure drug powder) showed cytotoxic effects on both cancer cells even at low concentrations while gold NPs showed cytotoxic effects at higher concentrations.
5. Combination of folic acid with gold NPs lead to increase targeting of nanoparticles in tumor cells and so enhancement cytotoxic effects of NPs.

**Recommendations:**

We recommended that:

1. Utilization of nanomaterials for drug delivery system to increase targeting of the drugs towards cancer cells.
2. Use PDT to treat the same or other type of cancerous disease on animal groups (in vivo).
3. Studying cytotoxic effects of other types of photosensitizers on cancer cells.
4. Use other types of ligands like monoclonal antibodies, hormones, other types of vitamins combined with nanomaterials to increase cancer cells targeting.

# References

## References:


1. Abrahamse, H. and M. R. Hamblin (2016). "New photosensitizers for photodynamic therapy." Biochemical Journal 473(4): 347-364.
2. Agostinis, P., K. Berg, K. A. Cengel, T. H. Foster, A. W. Girotti, S. O. Gollnick, S. M. Hahn, M. R. Hamblin, A. Juzeniene and D. Kessel (2011). "Photodynamic therapy of cancer: an update." CA: a cancer journal for clinicians 61(4): 250-281.
3. Ahamed, M., M. J. Akhtar, Z. M. Alaizeri and H. A. Alhadlaq (2020). "TiO 2 nanoparticles potentiated the cytotoxicity, oxidative stress and apoptosis response of cadmium in two different human cells." Environmental Science and Pollution Research 27(10): 10425-10435.
4. Ahamed, M., M. M. Khan, M. J. Akhtar, H. A. Alhadlaq and A. Alshamsan (2017). "Ag-doping regulates the cytotoxicity of $TiO_2$ nanoparticles via oxidative stress in human cancer cells." Scientific reports 7(1): 1-14.
5. Ahamed, M., M. S. AlSalhi and M. Siddiqui (2010). "Silver nanoparticle applications and human health." Clinica chimica acta 411(23-24): 1841-1848.
6. Ahamed, M., M. S. AlSalhi and M. Siddiqui (2010). "Silver nanoparticle applications and human health." Clinica chimica acta 411(23-24): 1841-1848.
7. Ahmadian, E., A. Eftekhari, J. K. Fard, H. Babaei, A. M. Nayebi, D. Mohammadnejad and M. A. Eghbal (2017). "In vitro and in vivo evaluation of the mechanisms of citalopram-induced hepatotoxicity." Archives of pharmacal research 40(11): 1296-1313.
8. Ahmadian, E., A. Y. Khosroushahi, M. A. Eghbal and A. Eftekhari (2018). "Betanin reduces organophosphate induced cytotoxicity in primary hepatocyte via an anti-oxidative and mitochondrial dependent pathway." Pesticide biochemistry and physiology 144: 71-78.

9. Ahmadian, E., H. Babaei, A. M. Nayebi, A. Eftekhari and M. A. Eghbal (2016). "Venlafaxine-induced cytotoxicity towards isolated rat hepatocytes involves oxidative stress and mitochondrial/lysosomal dysfunction." Advanced pharmaceutical bulletin 6(4): 521.
10. Ahmed, S., M. Ahmad, B. L. Swami and S. Ikram (2016). "A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise." Journal of advanced research 7(1): 17-28.
11. Akhter, S., M. Z. Ahmad, F. J. Ahmad, G. Storm and R. J. Kok (2012). "Gold nanoparticles in theranostic oncology: current state-of-the-art." Expert opinion on drug delivery 9(10): 1225-1243.
12. Akinola, P., A. Lateef, T. Asafa, L. Beukes, A. Hakeem and H. Irshad (2020). "Multifunctional titanium dioxide nanoparticles biofabricated via phytosynthetic route using extracts of Cola nitida: antimicrobial, dye degradation, antioxidant and anticoagulant activities." Heliyon 6(8): e04610.
13. Alkilany, A. M. and C. J. Murphy (2010). "Toxicity and cellular uptake of gold nanoparticles: what we have learned so far?" Journal of nanoparticle research 12(7): 2313-2333.
14. Allison, R. R. and C. H. Sibata (2010). "Oncologic photodynamic therapy photosensitizers: a clinical review." Photodiagnosis and photodynamic therapy 7(2): 61-75.
15. Allison, R. R., V. S. Bagnato, R. Cuenca, G. H. Downie and C. H. Sibata (2006). "The future of photodynamic therapy in oncology".
16. Anker, J. N., W. P. Hall, O. Lyandres, N. C. Shah, J. Zhao and R. P. Van Duyne (2010). "Biosensing with plasmonic nanosensors." Nanoscience and Technology: A Collection of Reviews from Nature Journals: 308-319.
17. Armand, L., A. Tarantini, D. Beal, M. Biola-Clier, L. Bobyk, S. Sorieul, K. Pernet-Gallay, C. Marie-Desvergne, I. Lynch and N. Herlin-Boime (2016).

"Long-term exposure of A549 cells to titanium dioxide nanoparticles induces DNA damage and sensitizes cells towards genotoxic agents." Nanotoxicology 10(7): 913-923.

18. Ávalos, A., A. Haza, D. Mateo and P. Morales (2018). "In vitro and in vivo genotoxicity assessment of gold nanoparticles of different sizes by comet and SMART assays." Food and chemical toxicology 120: 81-88.

19. Balasubramanian, S. K., J. Jittiwat, J. Manikandan, C.-N. Ong, E. Y. Liya and W.-Y. Ong (2010). "Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats." Biomaterials 31(8): 2034-2042.

20. Barbouti, A., N. Lagopati, D. Veroutis, V. Goulas, K. Evangelou, P. Kanavaros, V. G. Gorgoulis and D. Galaris (2021). "Implication of dietary iron-chelating bioactive compounds in molecular mechanisms of oxidative stress-induced cell ageing." Antioxidants 10(3): 491.

21. Bastuji-Garin, S., R. Laurent, N. Basset-Seguin, C. Bédane, P. Combemale and L. Dubertret (2001). Photodynamic therapy with 5-aminolevulinic acid or placebo for recalcitrant foot and hand warts: randomised double-blind trial. Annales de Dermatologie et de Vénéréologie.

22. Bazak, R., M. Houri, S. El Achy, S. Kamel and T. Refaat (2015). "Cancer active targeting by nanoparticles: a comprehensive review of literature." Journal of cancer research and clinical oncology 141(5): 769-784.

23. Behzadnia, A., M. Montazer, A. Rashidi and M. M. Rad (2014). "Sonosynthesis of nano $TiO_2$ on wool using titanium isopropoxide or butoxide in acidic media producing multifunctional fabric." Ultrasonics sonochemistry 21(5): 1815-1826.

24. Beik, J., S. Khademi, N. Attaran, S. Sarkar, A. Shakeri-Zadeh, H. Ghaznavi and H. Ghadiri (2017). "A nanotechnology-based strategy to

increase the efficiency of cancer diagnosis and therapy: folate-conjugated gold nanoparticles." Current medicinal chemistry 24(39): 4399-4416.

25. Belade, E., L. Armand, L. Martinon, L. Kheuang, J. Fleury-Feith, A. Baeza-Squiban, S. Lanone, M.-A. Billon-Galland, J.-C. Pairon and J. Boczkowski (2012). "A comparative transmission electron microscopy study of titanium dioxide and carbon black nanoparticles uptake in human lung epithelial and fibroblast cell lines." Toxicology in Vitro 26(1): 57-66.
26. Bin-Meferij, M. M. and R. S. Hamida (2019). "Biofabrication and antitumor activity of silver nanoparticles utilizing novel nostoc sp. Bahar M." International journal of nanomedicine 14: 9019.
27. Birn, H., O. Spiegelstein, E. I. Christensen and R. H. Finnell (2005). "Renal tubular reabsorption of folate mediated by folate binding protein 1." Journal of the American Society of Nephrology 16(3): 608-615.
28. Bray, F., J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre and A. Jemal (2018). "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." CA: a cancer journal for clinicians 68(6): 394-424.
29. Bressan, E., L. Ferroni, C. Gardin, C. Rigo, M. Stocchero, V. Vindigni, W. Cairns and B. Zavan (2013). "Silver nanoparticles and mitochondrial interaction." International journal of dentistry 2013.
30. Bupathi, M., A. Kaseb, F. Meric-Bernstam and A. Naing (2015). "Hepatocellular carcinoma: where there is unmet need." Molecular oncology 9(8): 1501-1509.
31. Buttacavoli, M., N. N. Albanese, G. Di Cara, R. Alduina, C. Faleri, M. Gallo, G. Pizzolanti, G. Gallo, S. Feo and F. Baldi (2018). "Anticancer activity of biogenerated silver nanoparticles: an integrated proteomic investigation." Oncotarget 9(11): 9685.

32. Calixto, G. M. F., J. Bernegossi, L. M. De Freitas, C. R. Fontana and M. Chorilli (2016). "Nanotechnology-based drug delivery systems for photodynamic therapy of cancer: a review." Molecules 21(3): 342.

33. Castano, A. P., T. N. Demidova and M. R. Hamblin (2004). "Mechanisms in photodynamic therapy: part one—photosensitizers, photochemistry and cellular localization." Photodiagnosis and photodynamic therapy 1(4): 279-293.

34. Celli, J. P., B. Q. Spring, I. Rizvi, C. L. Evans, K. S. Samkoe, S. Verma, B. W. Pogue and T. Hasan (2010). "Imaging and photodynamic therapy: mechanisms, monitoring, and optimization." Chemical reviews 110(5): 2795-2838.

35. Çeşmeli, S. and C. Biray Avci (2019). "Application of titanium dioxide ($TiO_2$) nanoparticles in cancer therapies." Journal of drug targeting 27(7): 762-766.

36. Chalmers, F., M. van Lith, B. Sweeney, K. Cain and N. J. Bulleid (2017). "Inhibition of IRE1α-mediated XBP1 mRNA cleavage by XBP1 reveals a novel regulatory process during the unfolded protein response." Wellcome Open Research 2.

37. Chan, F. K.-M., K. Moriwaki and M. J. De Rosa (2013). Detection of necrosis by release of lactate dehydrogenase activity. Immune Homeostasis, Springer: 65-70.

38. Chao, H., Y. Yun, H. Xingfang and A. Larbot (2003). "Effect of silver doping on the phase transformation and grain growth of sol-gel titania powder." Journal of the European Ceramic Society 23(9): 1457-1464.

39. Chen, D., H. Zheng, Z. Huang, H. Lin, Z. Ke, S. Xie and B. Li (2012). "Light-emitting diode-based illumination system for in vitro photodynamic therapy." International Journal of Photoenergy 2012.

40. Chen, R., L. Huo, X. Shi, R. Bai, Z. Zhang, Y. Zhao, Y. Chang and C. Chen (2014). "Endoplasmic reticulum stress induced by zinc oxide nanoparticles is an earlier biomarker for nanotoxicological evaluation." ACS nano 8(3): 2562-2574.
41. Cheung, A., H. J. Bax, D. H. Josephs, K. M. Ilieva, G. Pellizzari, J. Opzoomer, J. Bloomfield, M. Fittall, A. Grigoriadis and M. Figini (2016). "Targeting folate receptor alpha for cancer treatment." Oncotarget 7(32): 52553.
42. Chitgupi, U., Y. Qin and J. F. Lovell (2017). "Targeted nanomaterials for phototherapy." Nanotheranostics 1(1): 38.
43. Chizenga, E. P. and H. Abrahamse (2020). "Nanotechnology in modern photodynamic therapy of cancer: A review of cellular resistance patterns affecting the therapeutic response." Pharmaceutics 12(7): 632.
44. Choi, Y. M., L. Adelzadeh and J. J. Wu (2015). "Photodynamic therapy for psoriasis." Journal of Dermatological Treatment 26(3): 202-207.
45. Choudhary, G. S., S. Al-Harbi and A. Almasan (2015). Caspase-3 activation is a critical determinant of genotoxic stress-induced apoptosis. Apoptosis and Cancer, Springer: 1-9.
46. Chuang, S.-C., M. Rota, M. J. Gunter, A. Zeleniuch-Jacquotte, S. J. Eussen, S. E. Vollset, P. M. Ueland, T. Norat, R. G. Ziegler and P. Vineis (2013). "Quantifying the dose-response relationship between circulating folate concentrations and colorectal cancer in cohort studies: a meta-analysis based on a flexible meta-regression model." American journal of epidemiology 178(7): 1028-1037.
47. Coecke, S., M. Balls, G. Bowe, J. Davis, G. Gstraunthaler, T. Hartung, R. Hay, O.-W. Merten, A. Price and L. Schechtman (2005). "Guidance on good

cell culture practice: a report of the second ECVAM task force on good cell culture practice." Alternatives to Laboratory Animals 33(3): 261-287.

48. Colapicchioni, V., M. Tilio, L. Digiacomo, V. Gambini, S. Palchetti, C. Marchini, D. Pozzi, S. Occhipinti, A. Amici and G. Caracciolo (2016). "Personalized liposome–protein corona in the blood of breast, gastric and pancreatic cancer patients." The international journal of biochemistry & cell biology 75: 180-187.

49. Comacchi, C., P. L. Bencini, M. G. Galimberti, P. Cappugi and D. Torchia (2012). "Topical photodynamic therapy for idiopathic hirsutism and hypertrichosis." Plastic and reconstructive surgery 129(6): 1012e-1014e.

50. Correia, J. H., J. A. Rodrigues, S. Pimenta, T. Dong and Z. Yang (2021). "Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions." Pharmaceutics 13(9): 1332.

51. Cosgarea, R., R. Pollmann, J. Sharif, T. Schmidt, R. Stein, A. Bodea, T. Auschill, A. Sculean, R. Eming and B. Greene (2020). "Photodynamic therapy in oral lichen planus: A prospective case-controlled pilot study." Scientific reports 10(1): 1-11.

52. Cucchetti, A., F. Piscaglia, A. D. Pinna, B. Djulbegovic, F. Mazzotti and L. Bolondi (2017). "Efficacy and safety of systemic therapies for advanced hepatocellular carcinoma: a network meta-analysis of phase III trials." Liver Cancer 6(4): 337-348.

53. de Lima, R., A. B. Seabra and N. Durán (2012). "Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles." Journal of Applied Toxicology 32(11): 867-879.

54. de Martel, C., D. Maucort-Boulch, M. Plummer and S. Franceschi (2015). "World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma." Hepatology 62(4): 1190-1200.

55. De Matteis, V., M. A. Malvindi, A. Galeone, V. Brunetti, E. De Luca, S. Kote, P. Kshirsagar, S. Sabella, G. Bardi and P. P. Pompa (2015). "Negligible particle-specific toxicity mechanism of silver nanoparticles: the role of Ag+ ion release in the cytosol." Nanomedicine: Nanotechnology, Biology and Medicine 11(3): 731-739.

56. Ding, H., H. Tu, C. Qu, G. Cao, H. Zhuang, P. Zhao, X. Xu, Y. Yang, S. Lu and C. o. H. o. C. R. H. Association (2021). "Guideline for stratified screening and surveillance in patients with high risk of primary liver cancer (PLC/PRF/5) (2020)." Hepatoma Research 7.

57. Dobrovolskaia, M. A., A. K. Patri, J. Zheng, J. D. Clogston, N. Ayub, P. Aggarwal, B. W. Neun, J. B. Hall and S. E. McNeil (2009). "Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles." Nanomedicine: Nanotechnology, Biology and Medicine 5(2): 106-117.

58. dos Santos, A. l. F., D. R. Q. de Almeida, L. F. Terra, M. c. S. Baptista and L. Labriola (2019). "Photodynamic therapy in cancer treatment-an update review." Journal of cancer metastasis and treatment 5.

59. Eftekhari, A., E. Ahmadian, V. Panahi-Azar, H. Hosseini, M. Tabibiazar and S. Maleki Dizaj (2018). "Hepatoprotective and free radical scavenging actions of quercetin nanoparticles on aflatoxin B1-induced liver damage: in vitro/in vivo studies." Artificial cells, nanomedicine, and biotechnology 46(2): 411-420.

60. El-Said, K. S., E. M. Ali, K. Kanehira and A. Taniguchi (2014). "Molecular mechanism of DNA damage induced by titanium dioxide nanoparticles in toll-like receptor 3 or 4 expressing human hepatocarcinoma cell lines." Journal of Nanobiotechnology 12(1): 1-10.

61. Engstrom, P. F., J. P. Arnoletti, A. B. Benson, Y.-J. Chen, M. A. Choti, H. S. Cooper, A. Covey, R. A. Dilawari, D. S. Early and P. C. Enzinger (2009). "Colon cancer." Journal of the National Comprehensive Cancer Network 7(8): 778-831.

62. epidermal carcinoma cells." Lasers in medical science 29(3): 939-948.

63. Erkan, B., J. Meier, T. J. Clark, J. Kaplan, J. R. Lambert and S. Chang (2019). "Non-invasive diagnostic criteria of hepatocellular carcinoma: comparison of diagnostic accuracy of updated LI-RADS with clinical practice guidelines of OPTN-UNOS, AASLD, NCCN, EASL-EORTC, and KLSCG-NCC." Plos one 14(12): e0226291.

64. Favre, L., F. Borle, D. Velin, D. Bachmann, H. Bouzourene, G. Wagnieres, H. van den Bergh, P. Ballabeni, T. Gabrecht and P. Michetti (2011). "Low dose endoluminal photodynamic therapy improves murine T cell-mediated colitis." Endoscopy 43(07): 604-616.

65. Fekrazad, R. (2020). Photobiomodulation and antiviral photodynamic therapy as a possible novel approach in COVID-19 management, Mary Ann Liebert, Inc., publishers 140 Huguenot Street, 3rd Floor New …. 38: 255-257.

66. Ferlay, J., M. Colombet, I. Soerjomataram, D. M. Parkin, M. Piñeros, A. Znaor and F. Bray (2021). "Cancer statistics for the year 2020: An overview." International journal of cancer 149(4): 778-789.

67. Ferlito, A., A. R. Shaha, C. E. Silver, A. Rinaldo and V. Mondin (2001). "Incidence and sites of distant metastases from head and neck cancer." ORL 63(4): 202-207.

68. Firdhouse, J. and P. Lalitha (2015). "Apoptotic efficacy of biogenic silver nanoparticles on human breast cancer MCF-7 cell lines." Progress in biomaterials 4(2): 113-121.

69. Fitzgerald, F. (2017). Photodynamic Therapy (PDT), Nova Science Publishers, Incorporated.

70. Galata, E., E. A. Georgakopoulou, M.-E. Kassalia, N. Papadopoulou-Fermeli and E. A. Pavlatou (2019). "Development of smart composites based on doped-$TiO_2$ nanoparticles with visible light anticancer properties." Materials 12(16): 2589.

71. Galchenko, M., R. Schuster, A. Black, M. Riedner and C. Klinke (2019). "Preparation of high-yield and ultra-pure Au 25 nanoclusters: towards their implementation in real-world applications." Nanoscale 11(4): 1988-1994.

72. Gallardo-Villagrán, M., D. Y. Leger, B. Liagre and B. Therrien (2019). "Photosensitizers used in the photodynamic therapy of rheumatoid arthritis." International journal of molecular sciences 20(13): 3339.

73. Gawish, M., S. Shaban, S. Abdel Aal and S. Shalabi (2018). "CEffect of Silver Nanoparticles Versus Titanium Dioxide Nanoparticles on the Lung of Adult Male Albino Rats: A histological and immunohistochemical study." Journal of Medical Histology 2(2): 181-200.

74. Gengan, R., K. Anand, A. Phulukdaree and A. Chuturgoon (2013). "A549 lung cell line activity of biosynthesized silver nanoparticles using Albizia adianthifolia leaf." Colloids and Surfaces B: Biointerfaces 105: 87-91.

75. Ghaznavi, H., Z. Soheili, S. Samiei and M. S. Soltanpour (2015). "Association study of methylenetetrahydrofolate reductase C677T mutation with cerebral venous thrombosis in an Iranian population." Blood Coagulation & Fibrinolysis 26(8): 869-873.

76. Golla, K., F. A. Cherukuvada Bhaskar and A. K. Kondapi (2013). "A target-specific oral formulation of doxorubicin-protein nanoparticles: efficacy and safety in hepatocellular cancer." Journal of Cancer 4(8): 644.

77. Gomes, H. I., C. S. Martins and J. A. Prior (2021). "Silver Nanoparticles as Carriers of Anticancer Drugs for Efficient Target Treatment of Cancer Cells." Nanomaterials 11(4): 964.
78. Goo, S., Y. J. Choi, Y. Lee, S. Lee and H. W. Chung (2013). "Selective effects of curcumin on CdSe/ZnS quantum-dot-induced phototoxicity using UVA irradiation in normal human lymphocytes and leukemia cells." Toxicological research 29(1): 35-42.
79. Gopukumar, S., T. Sana-Fathima, P. Alexander, V. Alex and P. Praseetha (2016). "Evaluation of antioxidant properties of silver nanoparticle embedded medicinal patch." Nanomed. Nanotech. Op. Acc 1.
80. Govender, R., A. Phulukdaree, R. M. Gengan, K. Anand and A. A. Chuturgoon (2013). "Silver nanoparticles of Albizia adianthifolia: the induction of apoptosis in human lung carcinoma cell line." Journal of nanobiotechnology 11(1): 1-9.
81. Grijalva, M., M. J. Vallejo-López, L. Salazar, J. Camacho and B. Kumar (2018). "Cytotoxic and antiproliferative effects of nanomaterials on cancer cell lines: a review." Unraveling the Safety Profile of Nanoscale Particles and Materials—From Biomedical to Environmental Applications: 63-85.
82. Hafner, A., M. L. Bulyk, A. Jambhekar and G. Lahav (2019). "The multiple mechanisms that regulate p53 activity and cell fate." Nature reviews Molecular cell biology 20(4): 199-210.
83. Harris, P. S., R. M. Hansen, M. E. Gray, O. I. Massoud, B. M. McGuire and M. G. Shoreibah (2019). "Hepatocellular carcinoma surveillance: an evidence-based approach." World journal of gastroenterology 25(13): 1550.
84. Hasegawa, K., T. Aoki, T. Ishizawa, J. Kaneko, Y. Sakamoto, Y. Sugawara and N. Kokudo (2014). "Comparison of the therapeutic outcomes

between surgical resection and percutaneous ablation for small hepatocellular carcinoma." Annals of surgical oncology 21(3): 348-355.

85. Hasegawa, K., T. Aoki, T. Ishizawa, J. Kaneko, Y. Sakamoto, Y. Sugawara and N. Kokudo (2014). "Comparison of the therapeutic outcomes between surgical resection and percutaneous ablation for small hepatocellular carcinoma." Annals of surgical oncology 3): 348-355.

86. He, Y., X. Li, J. Wang, Q. Yang, B. Yao, Y. Zhao, A. Zhao, W. Sun and Q. Zhang (2017). "Synthesis, characterization and evaluation cytotoxic activity of silver nanoparticles synthesized by Chinese herbal Cornus officinalis via environment friendly approach." Environmental toxicology and pharmacology 56: 56-60.

87. Hester, S. C., M. Kuriakose, C. D. Nguyen and S. Mallidi (2020). "Role of ultrasound and photoacoustic imaging in photodynamic therapy for cancer." Photochemistry and photobiology 96(2): 260-279.

88. Houthoofd, S., M. Vuylsteke, S. Mordon and I. Fourneau (2020). "Photodynamic therapy for atherosclerosis. The potential of indocyanine green." Photodiagnosis and photodynamic therapy 29: 101568.

89. Huang, G., W. Y. Lau, Z.-G. Wang, Z.-Y. Pan, S.-X. Yuan, F. Shen, W.-P. Zhou and M.-C. Wu (2015). "Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial." Annals of surgery 261(1): 56-66.

90. Husen, A. and K. S. Siddiqi (2014). "Phytosynthesis of nanoparticles: concept, controversy and application." Nanoscale research letters 9(1): 1-24.

91. Imani, R., R. Dillert, D. W. Bahnemann, M. Pazoki, T. Apih, V. Kononenko, N. Repar, V. Kralj-Iglič, G. Boschloo and D. Drobne (2017). "Multifunctional gadolinium-doped mesoporous $TiO_2$ nanobeads: photoluminescence, enhanced spin relaxation, and reactive oxygen species

photogeneration, beneficial for cancer diagnosis and treatment." small 13(20): 1700349.

92. Ishii, T., A. Tamura, T. Shibata, K. Kuroda, T. Kanda, M. Sugiyama, M. Mizokami and M. Moriyama (2020). "Analysis of HBV genomes integrated into the genomes of human hepatoma PLC/PRF/5 cells by HBV sequence capture-based next-generation sequencing." Genes 11(6): 661.

93. Ivanova, N., V. Gugleva, M. Dobreva, I. Pehlivanov, S. Stefanov and V. Andonova (2018). Silver nanoparticles as multi-functional drug delivery systems. Nanomedicines, IntechOpen.

94. Jain, S., D. Hirst and J. O'Sullivan (2012). "Gold nanoparticles as novel agents for cancer therapy." The British journal of radiology 85(1010): 101-113.

95. Jaszewski, R., S. Misra, M. Tobi, N. Ullah, J. A. Naumoff, O. Kucuk, E. Levi, B. N. Axelrod, B. B. Patel and A. P. Majumdar (2008). "Folic acid supplementation inhibits recurrence of colorectal adenomas: a randomized chemoprevention trial." World journal of gastroenterology: WJG 14(28): 4492.

96. Jendrossek, V. (2012). "The intrinsic apoptosis pathways as a target in anticancer therapy." Current pharmaceutical biotechnology 13(8): 1426-1438.

97. Jepsen, P. and P. Kissmeyer-Nielsen (2008). "Epidemiology of primary and secondary liver cancers." Ugeskrift for laeger 170(16): 1323-1325.

98. Ji, Y., J. Zhu, L. Zhu, Y. Zhu and H. Zhao (2020). "High-intensity focused ultrasound ablation for unresectable primary and metastatic liver cancer: real-world research in a Chinese tertiary center with 275 cases." Frontiers in oncology 10: 2307.

99. Jin, Z. Y., H. Fatima, Y. Zhang, Z. Shao and X. J. Chen (2022). "Recent Advances in Bio-Compatible Oxygen Singlet Generation and Its Tumor Treatment." Advanced Therapeutics 5(1): 2100176.
100. Kalluru, P., R. Vankayala, C. S. Chiang and K. C. Hwang (2013). "Photosensitization of singlet oxygen and in vivo photodynamic therapeutic effects mediated by PEGylated W18O49 nanowires." Angewandte Chemie International Edition 52(47): 12332-12336.
101. Karuppaiah, A., K. Siram, D. Selvaraj, M. Ramasamy, D. Babu and V. Sankar (2020). "Synergistic and enhanced anticancer effect of a facile surface modified non-cytotoxic silver nanoparticle conjugated with gemcitabine in metastatic breast cancer cells." Materials Today Communications 23: 100884.
102. Khan, F. A., R. Albalawi and F. H. Pottoo (2022). "Trends in targeted delivery of nanomaterials in colon cancer diagnosis and treatment." Medicinal Research Reviews 42(1): 227-258.
103. Khan, I., K. Saeed and I. Khan (2019). "Nanoparticles: Properties, applications and toxicities." Arabian journal of chemistry 12(7): 908-931.
104. Khanna, P., C. Ong, B. H. Bay and G. H. Baeg (2015). "Nanotoxicity: an interplay of oxidative stress, inflammation and cell death." Nanomaterials 5(3): 1163-1180.
105. Kim, H., D. Jeon, S. Oh, K. Nam, S. Son, M. C. Gye and I. Shin (2019). "Titanium dioxide nanoparticles induce apoptosis by interfering with EGFR signaling in human breast cancer cells." Environmental research 175: 117-123.
106. Kim, M. M. and A. Darafsheh (2020). "Light sources and dosimetry techniques for photodynamic therapy." Photochemistry and photobiology 96(2): 280-294.

107.Kim, M., M. Lin, J. Son, H. Xu and J. M. Nam (2017). "Hot-Electron-Mediated Photochemical Reactions: Principles, Recent Advances, and Challenges." Advanced Optical Materials 5(15): 1700004.

108.Kim, S., S. Im, E.-Y. Park, J. Lee, C. Kim, T.-i. Kim and W. J. Kim (2020). "Drug-loaded titanium dioxide nanoparticle coated with tumor targeting polymer as a sonodynamic chemotherapeutic agent for anti-cancer therapy." Nanomedicine: Nanotechnology, Biology and Medicine 24: 102110.

109.Kim, T.-H., J. H. Yoon and J. M. Lee (2019). "Emerging role of hepatobiliary magnetic resonance contrast media and contrast-enhanced ultrasound for noninvasive diagnosis of hepatocellular carcinoma: emphasis on recent updates in major guidelines." Korean journal of radiology 20(6): 863-879.

110.Kirillin, M. Y., N. M. Shakhova and I. V. Turchin (2016). "From optical bioimaging to clinical biophotonics." Photonics & Lasers in Medicine 5(2): 81-83.

111.Kritsiligkou, P., J. D. Rand, A. J. Weids, X. Wang, C. J. Kershaw and C. M. Grant (2018). "Endoplasmic reticulum (ER) stress–induced reactive oxygen species (ROS) are detrimental for the fitness of a thioredoxin reductase mutant." Journal of Biological Chemistry 293(31): 11984-11995.

112.Kukia, N. R., Y. Rasmi, A. Abbasi, N. Koshoridze, A. Shirpoor, G. Burjanadze and E. Saboory (2018). "Bio-effects of $TiO_2$ nanoparticles on human colorectal cancer and umbilical vein endothelial cell lines." Asian Pacific journal of cancer prevention: APJCP 19(10): 2821.

113.Kulik, L. and H. B. El-Serag (2019). "Epidemiology and management of hepatocellular carcinoma." Gastroenterology 156(2): 477-491. e471.

114.Kuo, C.-T., C. Chang and W.-S. Lee (2015). "Folic acid inhibits COLO-205 colon cancer cell proliferation through activating the FRα/c-

SRC/ERK1/2/NFκB/TP53 pathway: in vitro and in vivo studies." Scientific reports 5(1): 1-13.

115.Kwiatkowski, S., B. Knap, D. Przystupski, J. Saczko, E. Kędzierska, K. Knap-Czop, J. Kotlińska, O. Michel, K. Kotowski and J. Kulbacka (2018). "Photodynamic therapy–mechanisms, photosensitizers and combinations." Biomedicine & pharmacotherapy 106: 1098-1107.

116.Lagopati, N., A. Kotsinas, D. Veroutis, K. Evangelou, A. Papaspyropoulos, M. Arfanis, P. Falaras, P. V. Kitsiou, I. Pateras and A. Bergonzini (2021). "Biological Effect of Silver-modified Nanostructured Titanium Dioxide in Cancer." Cancer Genomics & Proteomics 18(3 Suppl): 425-439.

117.Lagopati, N., E.-P. Tsilibary, P. Falaras, P. Papazafiri, E. A. Pavlatou, E. Kotsopoulou and P. Kitsiou (2014). "Effect of nanostructured $TiO_2$ crystal phase on photoinduced apoptosis of breast cancer epithelial cells." International journal of nanomedicine 9: 3219.

118.Lagunes, I., E. Martın-Batista, G. Silveira-Dorta, M. X. Fernandes and J. M. Padrón (2018). "Differential mechanism of action of the CK1ε inhibitor GSD0054." J Mol Clin Med| Vol 1.(2)

119.Lata, K., K. Arvind, N. Laxmana and S. Rajan (2014). "Gold nanoparticles: preparation, characterization and its stability in buffer." A Journal of Nanotechnology and Its Applications 17(1): 1-10.

120.Lech, G., R. Słotwiński, M. Słodkowski and I. W. Krasnodębski (2016). "Colorectal cancer tumour markers and biomarkers: Recent therapeutic advances." World journal of gastroenterology 22(5): 1745.

121.Lee, B. I., Y. S. Suh, Y. J. Chung, K. Yu and C. B. Park (2017). "Shedding light on Alzheimer's β-amyloidosis: photosensitized methylene blue inhibits

self-assembly of β-amyloid peptides and disintegrates their aggregates." Scientific reports 7(1): 1-10.

122.Lee, C.-N., R. Hsu, H. Chen and T.-W. Wong (2020). "Daylight photodynamic therapy: An update." Molecules 25(21): 5195.

123.Li, S. Y., M. Rong and B. Iacopetta (2006). "Germ-line variants in methyl-group metabolism genes and susceptibility to DNA methylation in human breast cancer." Oncology reports 15(1): 221-225.

124.Li, T. and L. Yan (2018). "Functional polymer nanocarriers for photodynamic therapy." Pharmaceuticals 11(4): 133.

125.Li, X., S. Lee and J. Yoon (2018). "Supramolecular photosensitizers rejuvenate photodynamic therapy." Chemical Society Reviews 47(4): 1174-1188.

126.Li, Z., J. He, B. Li, J. Zhang, K. He, X. Duan, R. Huang, Z. Wu and G. Xiang (2020). "Titanium dioxide nanoparticles induce endoplasmic reticulum stress-mediated apoptotic cell death in liver cancer cells." Journal of International Medical Research 48(4): 0300060520903652.

127.Liao, C., Y. Li and S. C. Tjong (2020). "Visible-light active titanium dioxide nanomaterials with bactericidal properties." Nanomaterials 10(1): 124.

128.Lin, Y., G. Huang, Y. Huang, T. R. J. Tzeng and D. Chrisey (2010). "Effect of laser fluence in laser-assisted direct writing of human colon cancer cell." Rapid Prototyping Journal.

129.Linares-González, L., T. Ródenas-Herranz, S. Sáenz-Guirado and R. Ruiz-Villaverde (2020). "Successful response to photodynamic therapy with 5-aminolevulinic acid nanoemulsified gel in a patient with universal alopecia areata refractory to conventional treatment." Dermatologic therapy 33(3): e13416.

130.Liu, C.-J., C.-H. Wang, C.-C. Chien, T.-Y. Yang, S.-T. Chen, W.-H. Leng, C.-F. Lee, K.-H. Lee, Y. Hwu and Y.-C. Lee (2008). "Enhanced x-ray irradiation-induced cancer cell damage by gold nanoparticles treated by a new synthesis method of polyethylene glycol modification." Nanotechnology 19(29): 295104.

131.Liu, X., A. Baecker, M. Wu, J. Y. Zhou, J. Yang, R. Q. Han, P. H. Wang, Z. Y. Jin, A. M. Liu and X. Gu (2019). "Family history of liver cancer may modify the association between HBV infection and liver cancer in a Chinese population." Liver International 39(8): 1490-1503.

132.Liu, Z., L. Turyanska, F. Zamberlan, S. Pacifico, T. D. Bradshaw, F. Moro, M. W. Fay, H. E. Williams and N. R. Thomas (2019). "Synthesis of folic acid functionalized gold nanoclusters for targeting folate receptor-positive cells." Nanotechnology 30(50): 505102.

133.Lucky, S. S., K. C. Soo and Y. Zhang (2015). "Nanoparticles in photodynamic therapy." Chemical reviews 115(4): 1990-2042.

134.Lucky, S. S., K. C. Soo and Y. Zhang (2015). "Nanoparticles in photodynamic therapy." Chemical reviews 115(4): 1990-2042.

135.Lukač, M., A. Lozar, T. Perhavec and F. Bajd (2019). "Variable heat shock response model for medical laser procedures." Lasers in medical science 34(6): 1147-1158.

136.Lynn, J. A. (2009). Battle: a history of combat and culture, Hachette UK.

137.Magrez, A., L. Horváth, R. Smajda, V. Salicio, N. Pasquier, L. Forro and B. Schwaller (2009). "Cellular toxicity of $TiO_2$-based nanofilaments." Acs Nano 3(8): 2274-2280.

138.Majumdar, A. P., U. Kodali and R. Jaszewski (2004). "Chemopreventive role of folic acid in colorectal cancer." Frontiers in Bioscience-Landmark 9(4): 2725-2732.

139.Manna, S. K., S. Sarkar, J. Barr, K. Wise, E. V. Barrera, O. Jejelowo, A. C. Rice-Ficht and G. T. Ramesh (2005). "Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-κB in human keratinocytes." Nano letters 5(9): 1676-1684.

140.Manna, S. K., S. Sarkar, J. Barr, K. Wise, E. V. Barrera, O. Jejelowo, A. C. Rice-Ficht and G. T. Ramesh (2005). "Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-κB in human keratinocytes." Nano letters 5(9): 1676-1684.

141.Mármol, I., C. Sánchez-de-Diego, A. Pradilla Dieste, E. Cerrada and M. J. Rodriguez Yoldi (2017). "Colorectal carcinoma: a general overview and future perspectives in colorectal cancer." International journal of molecular sciences 18(1): 197.

142.Masuda, Y. and K. Kato (2014). "Halogen-and Acid-Free Syntheses of $TiO_2$ Nanocrystal Coatings and High Surface Area $TiO_2$ Nanocrystal-Assembled Particles." Journal of nanoscience and nanotechnology 14(3): 2231-2237.

143.Mehdizadeh, A., S. Pandesh, A. Shakeri-Zadeh, S. K. Kamrava, M. Habib-Agahi, M. Farhadi, M. Pishghadam, A. Ahmadi, S. Arami and Y. Fedutik (2014). "The effects of folate-conjugated gold nanorods in combination with plasmonic photothermal therapy on mouth epidermal carcinoma cells." Lasers in medical science 29(3): 939-948.

144.Mehdizadeh, A., S. Pandesh, A. Shakeri-Zadeh, S. K. Kamrava, M. Habib-Agahi, M. Farhadi, M. Pishghadam, A. Ahmadi, S. Arami and Y. Fedutik (2014). "The effects of folate-conjugated gold nanorods in combination with plasmonic photothermal therapy on mouth

145.Meleady, P. and R. O'Connor (2006). General procedures for cell culture. Cell Biology, Elsevier: 13-20.

146.Merlin, J., H. Rupasinghe, G. Dellaire and K. Murphy (2021). "Role of dietary antioxidants in p53-mediated cancer chemoprevention and tumor suppression." Oxidative Medicine and Cellular Longevity 2021.

147.Misra, R., S. Acharya and S. K. Sahoo (2010). "Cancer nanotechnology: application of nanotechnology in cancer therapy." Drug discovery today 15(19-20): 842-850.

148.Monfrecola, G., M. Megna, C. Rovati, M. Arisi, M. Rossi, I. Calzavara-Pinton, G. Fabbrocini and P. Calzavara-Pinton (2021). "A critical reappraisal of off-label use of photodynamic therapy for the treatment of non-neoplastic skin conditions." Dermatology 237(2): 262-276.

149.Montaseri, H., C. A. Kruger and H. Abrahamse (2021). "Inorganic Nanoparticles Applied for Active Targeted Photodynamic Therapy of Breast Cancer." Pharmaceutics 13(3): 296.

150.Mroz, P., A. Yaroslavsky, G. B. Kharkwal and M. R. Hamblin (2011). "Cell death pathways in photodynamic therapy of cancer." Cancers 3(2): 2516-2539.

151.Mroz, P., J. T. Hashmi, Y.-Y. Huang, N. Lange and M. R. Hamblin (2011). "Stimulation of anti-tumor immunity by photodynamic therapy." Expert review of clinical immunology 7(1): 75-91.

152.Hamida, R. S., G. Albasher and M. M. Bin-Meferij (2020). "Oxidative stress and apoptotic responses elicited by nostoc-synthesized silver nanoparticles against different cancer cell lines." Cancers **12**(8): 2099.

153.Nakajima, Y.-i. and E. Kuranaga (2017). "Caspase-dependent non-apoptotic processes in development." Cell Death & Differentiation 24(8): 1422-1430.

154.Nam, G., B. Purushothaman, S. Rangasamy and J. M. Song (2016). "Investigating the versatility of multifunctional silver nanoparticles:

preparation and inspection of their potential as wound treatment agents." International Nano Letters 6(1): 51-63.

155.Nasr, R., H. Hasanzadeh, A. Khaleghian, A. Moshtaghian, A. Emadi and S. Moshfegh (2018). "Induction of apoptosis and inhibition of invasion in gastric cancer cells by titanium dioxide nanoparticles." Oman medical journal 33(2): 111.

156.Nbelayim, P., G. Kawamura, W. K. Tan, H. Muto and A. Matsuda (2017). "Systematic characterization of the effect of Ag@ TiO 2 nanoparticles on the performance of plasmonic dye-sensitized solar cells." Scientific reports 7(1): 1-12.

157.Olmedo, D. G., D. R. Tasat, P. Evelson, M. B. Guglielmotti and R. L. Cabrini (2008). "Biological response of tissues with macrophagic activity to titanium dioxide." Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials 84(4): 1087-1093.

158.omics 18(3 Suppl): 425-439.

159.Ortiz-Rodríguez, L. A., Y.-G. Fang, G. Niogret, K. Hadidi, Y. Tor, G. Cui and C. Crespo-Hernández (2021). "Thionation of a Fluorescent Thieno [3, 4-d] pyrimidine Derivative for the Development of a Heavy-Atom-Free Photosensitizer".

160.Parsons, M. J. and D. R. Green (2010). "Mitochondria in cell death." Essays in biochemistry 47(1): 99.

161.Patri, A., T. Umbreit, J. Zheng, K. Nagashima, P. Goering, S. Francke-Carroll, E. Gordon, J. Weaver, T. Miller and N. Sadrieh (2009). "Energy dispersive X-ray analysis of titanium dioxide nanoparticle distribution after

intravenous and subcutaneous injection in mice." Journal of Applied Toxicology: An International Journal 29(8): 662-672.

162.Pavlovich, E., N. Volkova, E. Yakymchuk, O. Perepelitsyna, M. Sydorenko and A. Goltsev (2017). "In vitro study of influence of Au nanoparticles on HT29 and SPEV cell lines." Nanoscale research letters 12(1): 1-9.

163.Pedziwiatr-Werbicka, E., K. Horodecka, D. Shcharbin and M. Bryszewska (2021). "Nanoparticles in Combating Cancer: Opportunities and Limitations: A Brief Review." Current medicinal chemistry 28(2): 346-359.

164.Peng, C., Y. Ye, Z. Wang, L. Guan, S. Bao, B. Li and W. Li (2019). "Circulating microRNAs for the diagnosis of hepatocellular carcinoma." Digestive and Liver Disease 51(5): 621-631.

165.Peng, Z.-W., Y.-J. Zhang, M.-S. Chen, L. Xu, H.-H. Liang, X.-J. Lin, R.-P. Guo, Y.-Q. Zhang and W. Y. Lau (2013). "Radiofrequency ablation with or without transcatheter arterial chemoembolization in the treatment of hepatocellular carcinoma: a prospective randomized trial." Journal of clinical oncology 31(4): 426-432.

166.Pérez-Herrero, E. and A. Fernández-Medarde (2015). "Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy." European journal of pharmaceutics and biopharmaceutics 93: 52-79.

167.Philippeos, C., R. D. Hughes, A. Dhawan and R. R. Mitry (2012). Introduction to cell culture. Human Cell Culture Protocols, Springer: 1-13.

168.Pivodová, V., J. Franková, A. Galandáková and J. Ulrichová (2015). "In vitro AuNPs' cytotoxicity and their effect on wound healing." Nanobiomedicine 2: 7.

169.Plaetzer, K., M. Berneburg, T. Kiesslich and T. Maisch (2013). New applications of photodynamic therapy in biomedicine and biotechnology, Hindawi. 2013.

170.Qudayr, A., A. M. Kabel, M. A. AbdElmaaboud, W. Y. Alghamdi and A. Alghorabi (2018). "Colorectal Cancer: New Perspectives." J. Cancer Res. Treat 6(3): 80-83.

171.Raja, G., S. Cao, D.-H. Kim and T.-J. Kim (2020). "Mechanoregulation of titanium dioxide nanoparticles in cancer therapy." Materials Science and Engineering: C 107: 110303.

172.Ramzy, L., M. Nasr, A. A. Metwally and G. A. Awad (2017). "Cancer nanotheranostics: a review of the role of conjugated ligands for overexpressed receptors." European Journal of Pharmaceutical Sciences 104: 273-292.

173.Rao, B. L., M. Gowda, S. Asha, K. Byrappa, B. Narayana, R. Somashekar, Y. Wang, L. Madhu and Y. Sangappa (2017). "Rapid synthesis of gold nanoparticles using silk fibroin: characterization, antibacterial activity, and anticancer properties." Gold Bulletin 50(4): 289-297.

174.Raoof, M., B. T. Cisneros, S. J. Corr, F. Palalon, S. A. Curley and N. V. Koshkina (2013). "Tumor selective hyperthermia induced by short-wave capacitively-coupled RF electric-fields." PloS one 8(7): e68506.

175.Rauch, J., W. Kolch, S. Laurent and M. Mahmoudi (2013). "Big signals from small particles: regulation of cell signaling pathways by nanoparticles." Chemical reviews 113(5): 3391-3406.

176.Riss, T. L., R. A. Moravec, A. L. Niles, S. Duellman, H. A. Benink, T. J. Worzella and L. Minor (2016). "Cell viability assays." Assay Guidance Manual [Internet].

177.Rocha, L. G. B. (2016). Development of a novel photosensitizer for Photodynamic Therapy of cancer, Universidade de Coimbra (Portugal).

178.Rosenblum, D., N. Joshi, W. Tao, J. M. Karp and D. Peer (2018). "Progress and challenges towards targeted delivery of cancer therapeutics." Nature communications 9(1): 1-12.

179.Saeed, M., W. Ren and A. Wu (2018). "Therapeutic applications of iron oxide based nanoparticles in cancer: basic concepts and recent advances." Biomaterials science 6(4): 708-725.

180.Saleh, B. E. and M. C. Teich (2019). Fundamentals of photonics, john Wiley & sons.

181.Samadian, H., S. Hosseini-Nami, S. K. Kamrava, H. Ghaznavi and A. Shakeri-Zadeh (2016). "Folate-conjugated gold nanoparticle as a new nanoplatform for targeted cancer therapy." Journal of cancer research and clinical oncology 142(11): 2217-2229.

182.Satapathy, S. R., P. Mohapatra, R. Preet, D. Das, B. Sarkar, T. Choudhuri, M. D. Wyatt and C. N. Kundu (2013). "Silver-based nanoparticles induce apoptosis in human colon cancer cells mediated through p53." Nanomedicine 8(8): 1307-1322.

183.Schluesener, J. K. and H. J. Schluesener (2013). "Nanosilver: application and novel aspects of toxicology." Archives of toxicology 87(4): 569-576.

184.Segeritz, C.-P. and L. Vallier (2017). Cell culture: growing cells as model systems in vitro. Basic Science Methods for Clinical Researchers, Elsevier: 151-172.

185.Semmler-Behnke, M., W. G. Kreyling, J. Lipka, S. Fertsch, A. Wenk, S. Takenaka, G. Schmid and W. Brandau (2008). "Biodistribution of 1.4-and 18-nm gold particles in rats." Small 4(12): 2108-2111.

186.Senapati, S., A. K. Mahanta, S. Kumar and P. Maiti (2018). "Controlled drug delivery vehicles for cancer treatment and their performance." Signal transduction and targeted therapy 3(1): 1-19.

187.Shakeri-Zadeh, A., G. A. Mansoori, A. R. Hashemian, H. Eshghi, A. Sazgarnia and A. R. Montazerabadi (2010). "Cancerous cells targeting and destruction using folate conjugated gold nanoparticles." Dyn Biochem Process Biotechnol Mol Biol 4(1): 6-12.

188.Shankar, S. S., A. Rai, A. Ahmad and M. Sastry (2005). "Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings." Chemistry of Materials 17(3): 566-572.

189.Shin, H. T., J. H. Kim, J. Shim, J. H. Lee, D. Y. Lee, J. H. Lee and J. M. Yang (2015). "Photodynamic therapy using a new formulation of 5-aminolevulinic acid for wrinkles in Asian skin: A randomized controlled split face study." Journal of Dermatological Treatment 26(3): 246-251.

190.Siekmann, W., E. Tina, A. Koskela von Sydow and A. Gupta (2019). "Effect of lidocaine and ropivacaine on primary (SW480) and metastatic (SW620) colon cancer cell lines." Oncology letters 18(1): 395-401.

191.Silva, Z. S., S. K. Bussadori, K. P. S. Fernandes, Y.-Y. Huang and M. R. Hamblin (2015). "Animal models for photodynamic therapy (PDT)." Bioscience reports 35.(6)

192.Simões, J. C., S. Sarpaki, P. Papadimitroulas, B. Therrien and G. Loudos (2020). "Conjugated photosensitizers for imaging and PDT in cancer research." Journal of Medicinal Chemistry 63(23): 14119-14150.

193.Soares, S., J. Sousa, A. Pais and C. Vitorino (2018). "Nanomedicine: principles, properties, and regulatory issues." Frontiers in chemistry 6: 360.

194.Stallivieri, A., F. Baros, G. Jetpisbayeva, B. Myrzakhmetov and C. Frochot (2015). "The interest of folic acid in targeted photodynamic therapy." Current medicinal chemistry 22(27): 3185-3207.

195.Stintzing, S. (2014). "Management of colorectal cancer." F1000prime reports 6.

196.Sukhramani, P. S., P. S. Sukhramani, S. R. Tirthani, S. A. Desai and M. P. Suthar (2011). "Biological cytotoxicity evaluation of spiro [azetidine-2, 3'-indole]-2', 4 (1'H)-dione derivatives for anti-lung and anti-breast cancer activity." Der Pharmacia Lettre 3(5): 236-243.

197.Tait, S. W. and D. R. Green (2010). "Mitochondria and cell death: outer membrane permeabilization and beyond." Nature reviews Molecular cell biology 11(9): 621-632

198.Tait, S. W. and D. R. Green (2010). "Mitochondria and cell death: outer membrane permeabilization and beyond." Nature reviews Molecular cell biology 11(9): 621-632.

199.Talekar, M., J. Kendall, W. Denny and S. Garg (2011). "Targeting of nanoparticles in cancer: drug delivery and diagnostics." Anti-Cancer Drugs 22(10): 949-962.

200.Talekar, M., J. Kendall, W. Denny and S. Garg (2011). "Targeting of nanoparticles in cancer: drug delivery and diagnostics." Anti-Cancer Drugs 22(10): 949-962.

201.Ting, P.-C., W.-R. Lee, Y.-N. Huo, S.-P. Hsu and W.-S. Lee (2019). "Folic acid inhibits colorectal cancer cell migration." The Journal of nutritional biochemistry 63: 157-164.

202.Ulanja, M. B., M. Rishi, B. D. Beutler, M. Sharma, D. R. Patterson, N. Gullapalli and S. Ambika (2019). "Colon cancer sidedness, presentation, and survival at different stages." Journal of oncology 2019.

203.van Dijk, E. H., S. Fauser, M. B. Breukink, R. Blanco-Garavito, J. M. Groenewoud, J. E. Keunen, P. J. Peters, G. Dijkman, E. H. Souied and R. E. MacLaren (2018). "Half-dose photodynamic therapy versus high-density

subthreshold micropulse laser treatment in patients with chronic central serous chorioretinopathy: the PLACE trial." Ophthalmology 125(10): 1547-1555.

204. Van Meerloo, J., G. J. Kaspers and J. Cloos (2011). Cell sensitivity assays: the MTT assay. Cancer cell culture, Springer: 237-245.

205. Vazquez-Muñoz, R., B. Borrego, K. Juarez-Moreno, M. Garcia-Garcia, J. D. M. Morales, N. Bogdanchikova and A. Huerta-Saquero (2017). "Toxicity of silver nanoparticles in biological systems: does the complexity of biological systems matter?" Toxicology letters 276: 11-20.

206. Velez, N., F. Allen, M. A. Jones, G. Meyers and A. M. Minor (2017). "Development of Quantitative In Situ TEM Nanomechanical Testing for Polymers." Microscopy and Microanalysis 23(S1): 742-743.

207. Ventura, P., G. Toullec, C. Fricano, L. Chapron, V. Meunier, E. Röttinger, P. Furla and S. Barnay-Verdier (2018). "Cnidarian primary cell culture as a tool to investigate the effect of thermal stress at cellular level." Marine Biotechnology 20(2): 144-154.

208. Wan, J. C., C. Massie, J. Garcia-Corbacho, F. Mouliere, J. D. Brenton, C. Caldas, S. Pacey, R. Baird and N. Rosenfeld (2017). "Liquid biopsies come of age: towards implementation of circulating tumour DNA." Nature Reviews Cancer 17(4): 223-238.

209. Wang, Y. and C. Dellago (2003). "Structural and morphological transitions in gold nanorods: A computer simulation study." The Journal of Physical Chemistry B 107(35): 9214-9219.

210. Ward, E. M., A. E. Sherif, S. O'Neill, M. Hughes, H. Ireland, S. J. Wigmore and A. Adair (2021). "Clinical Outcomes of Ablation Compared with Resection for Single Hepatocellular Carcinoma Lesions, as a Primary Treatment or Bridging to Liver Transplantation: A Retrospective Comparative Study." Annals of Transplantation 26: e931980-931981.

211.Weber, H. M., Y. Z. Mehran, A. Orthaber, H. H. Saadat, R. Weber and M. Wojcik (2020). "Successful reduction of SARS-CoV-2 viral load by photodynamic therapy (PDT) verified by QPCR—a novel approach in treating patients in early infection stages." Med Clin Res 5: 311-325.

212.Werengowska-Ciećwierz, K., M. Wiśniewski, A. P. Terzyk and S. Furmaniak (2015). "The chemistry of bioconjugation in nanoparticles-based drug delivery system." Advances in Condensed Matter Physics 2015.

213.Wilson, B. C. and R. A. Weersink (2020). "The Yin and Yang of PDT and PTT." Photochemistry and photobiology 96(2): 219-231.

214.Wong, B. J.-F. and J. Ilgner (2016). Biomedical optics in otorhinolaryngology: Head and neck surgery, Springer.

215.Xia, Z., J. He, B. Li, K. He, W. Yang, X. Chen, J. Zhang and G. Xiang (2018). "Titanium dioxide nanoparticles induce mitochondria-associated apoptosis in HepG2 cells." RSC advances 8(55): 31764-31776.

216.Xia, Z., J. He, B. Li, K. He, W. Yang, X. Chen, J. Zhang and G. Xiang (2018). "Titanium dioxide nanoparticles induce mitochondria-associated apoptosis in HepG2 cells." RSC advances 8(55): 31764-31776.

217.Xie, D.-Y., Z.-G. Ren, J. Zhou, J. Fan and Q. Gao (2020). "2019 Chinese clinical guidelines for the management of hepatocellular carcinoma: updates and insights." Hepatobiliary surgery and nutrition 9(4): 452.

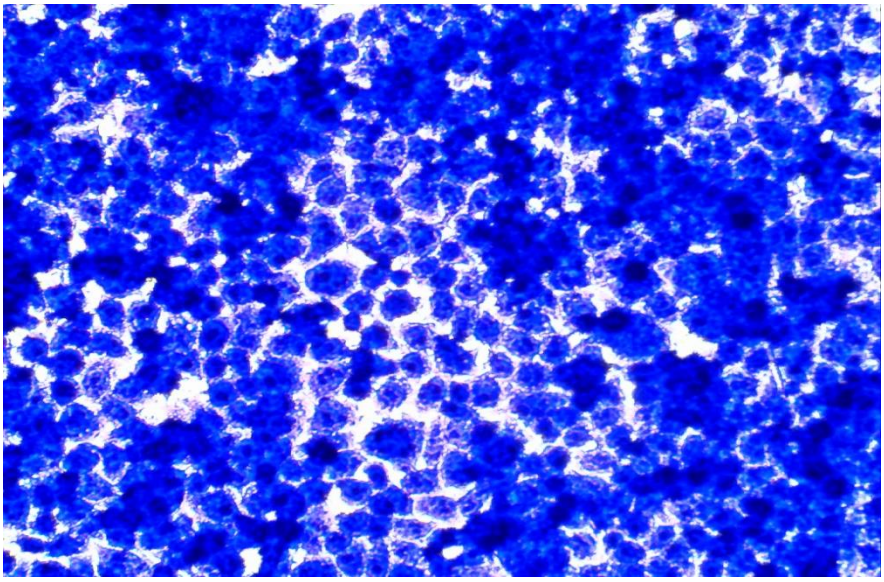
218.Yadav, J. (2018). "Synthesis and characterization of gold nanoparticles." International Journal on Future Revolution in Computer Science & Communication Engineering 4(4): 220-230.

219.Yanovsky, R. L., D. W. Bartenstein, G. S. Rogers, S. J. Isakoff and S. T. Chen (2019). "Photodynamic therapy for solid tumors: A review of the literature." Photodermatology, photoimmunology & photomedicine 35(5): 295-303.

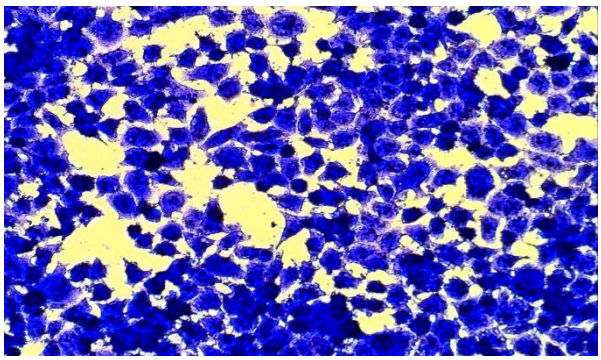
220.Yoo, S. W., G. Oh, J. C. Ahn and E. Chung (2021). "Non-Oncologic Applications of Nanomedicine-Based Phototherapy." Biomedicines 9(2): 113.
221.Yoon, I., J. Z. Li and Y. K. Shim (2013). "Advance in photosensitizers and light delivery for photodynamic therapy." Clinical endoscopy 46(1): 7.
222.Yu, K.-N., S.-H. Chang, S. J. Park, J. Lim, J. Lee, T.-J. Yoon, J.-S. Kim and M.-H. Cho (2015). "Titanium dioxide nanoparticles induce endoplasmic reticulum stress-mediated autophagic cell death via mitochondria-associated endoplasmic reticulum membrane disruption in normal lung cells." PloS one 10(6): e0131208.
223.Zhang, X.-D., H.-Y. Wu, Y.-Y. W. Di Wu, J.-H. Chang, Z.-B. Zhai, A.-M. Meng, P.-X. Liu, L.-A. Zhang and F.-Y. Fan (2010). "Toxicologic effects of gold nanoparticles in vivo by different administration routes." International journal of nanomedicine 5: 771.
224.Zhang, X.-D., H.-Y. Wu, Y.-Y. W. Di Wu, J.-H. Chang, Z.-B. Zhai, A.-M. Meng, P.-X. Liu, L.-A. Zhang and F.-Y. Fan (2010). "Toxicologic effects of gold nanoparticles in vivo by different administration routes." International journal of nanomedicine 5: 771.
225.Zhou, Y., Z. Peng, E. S. Seven and R. M. Leblanc (2018). "Crossing the blood-brain barrier with nanoparticles." Journal of controlled release 270: 290-303.
226.Zhu, Y., J. W. Eaton and C. Li (2012). "Titanium dioxide ($TiO_2$) nanoparticles preferentially induce cell death in transformed cells in a Bak/Bax-independent fashion." PloS one 7(11): e50607.
227.Ziental, D., B. Czarczynska-Goslinska, D. T. Mlynarczyk, A. Glowacka-Sobotta, B. Stanisz, T. Goslinski and L. Sobotta (2020). "Titanium dioxide nanoparticles: prospects and applications in medicine." Nanomaterials 10(2): 387.

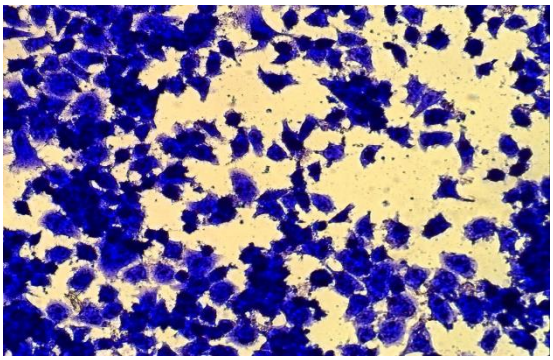
228.Aboud, G.T., 2005. Treatment of oral ulcerations. M.Sc. Thesis, University of Baghdad.

# Appendix



**Effect Of Titanium Dioxide NTs 500g/ml On Primary Liver Cancer Cells**

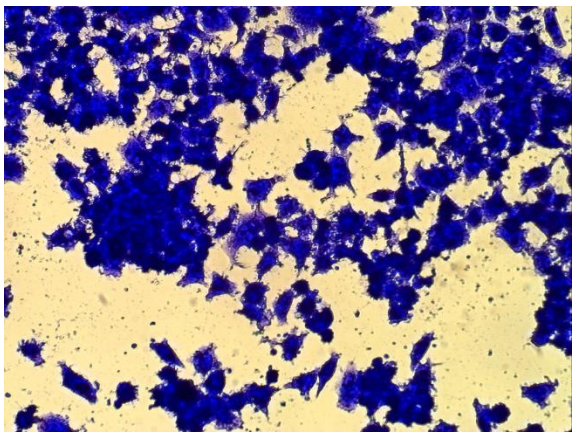




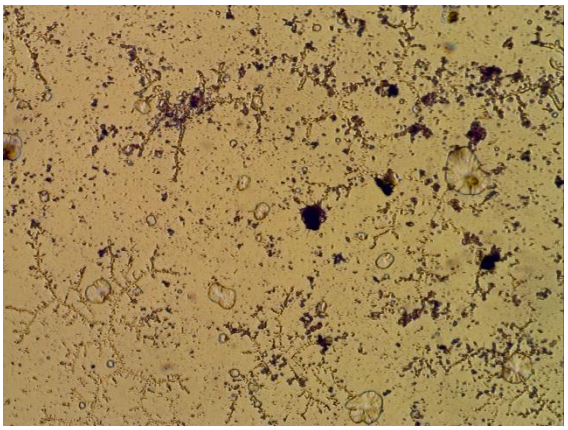
$Tio_2$ 250µg/ml with laser 635 nm

$Tio_2$ 500µg/ml with laser 635 nm

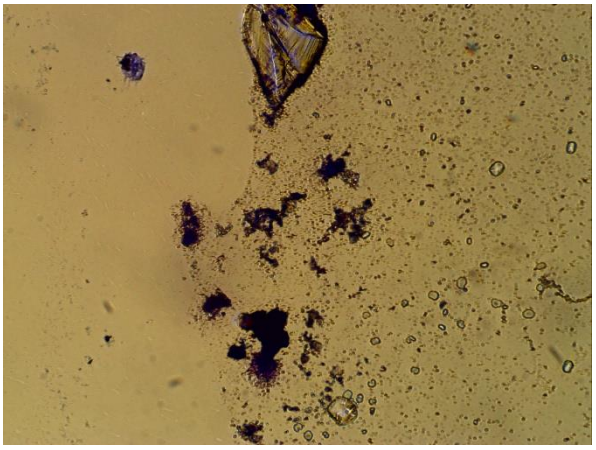
**Effect Of Titanium Dioxide NTs With Laser on Primary Liver Cancer Cells**



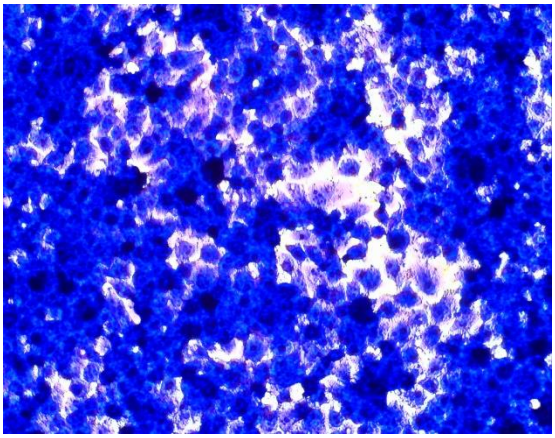
Ag@Tio2 125µg/ml with laser



Ag@Tio2 250µg/ml with laser

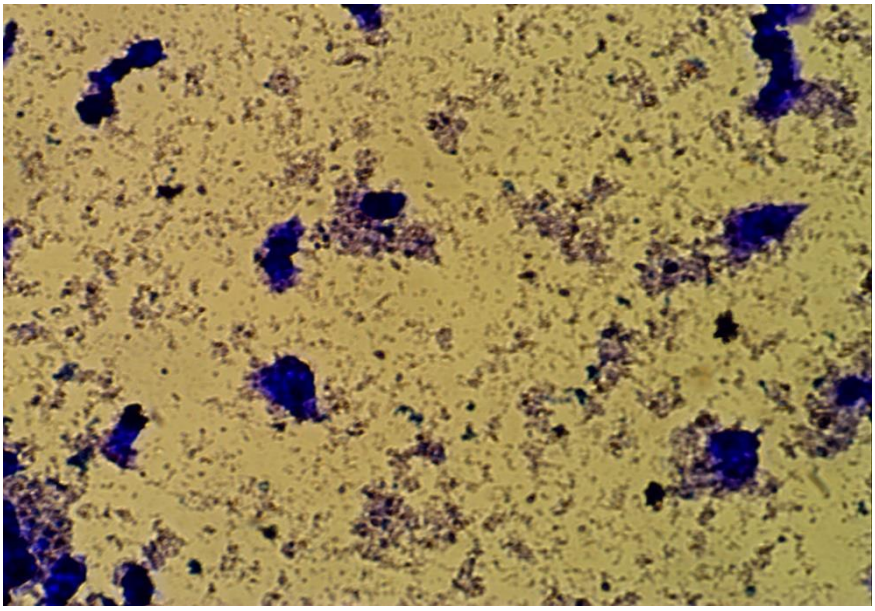


Ag@Tio2 500µg/ml with laser

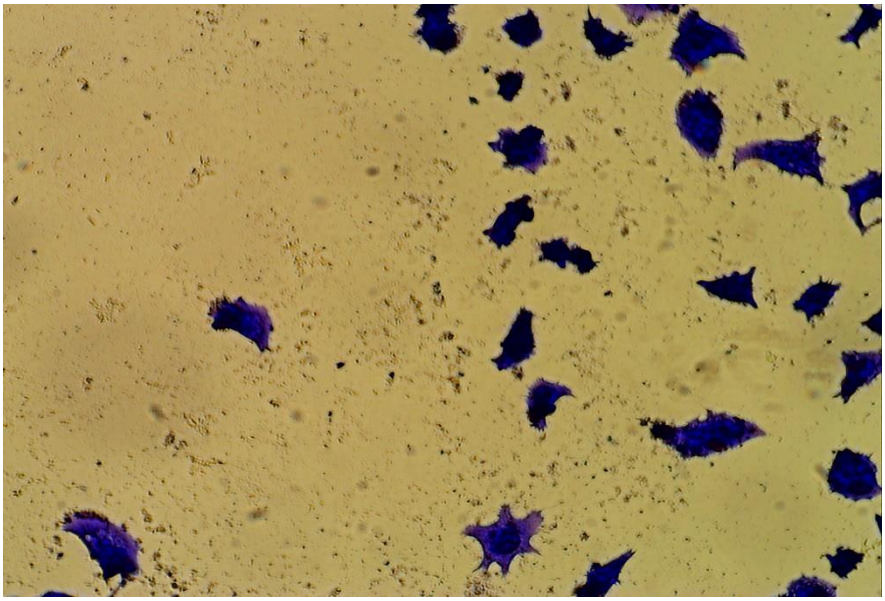


Ag@Tio2 62.5µg/ml with laser

**Effect Of Silver Decorated Titanium Dioxide NTs With Laser On PLC**



**Au with FA and lase 250µg/ ml plus 0.025 µg/ ml**



**Au with FA and Laser 500µg/ ml Plus 0.05µg/ml**

الخلاصة:

ينشأ السرطان عندما تطور خلايا طلائية معينة سلسلة من الطفرات الجينية. قد يشمل العلاج الجراحة والعلاج الإشعاعي والعلاج الكيميائي، والتي غالبًا ما ترتبط بآثار جانبية خطيرة وسمية، مما يؤثر بشكل كبير على نوعية حياة المرضى. أدى هذا بالعلماء إلى البحث عن علاجات بديلة أحدث، ويعد العلاج الضوئي باستخدام المواد النانوية أحد هذه العلاجات.

تهدف هذه الدراسة إلى التحقيق في التأثير السام للخلايا للجسيمات النانوية القائمة على المعادن على تكاثر الخلايا السرطانية لكل من خلايا سرطان الكبد الأولية وخلايا سرطان القولون باستخدام العلاج الضوئي. تم قياس السمية الخلوية لـ الأنابيب النانوية لثاني أكسيد التيتانيوم والفضة المزينة بـ الأنابيب النانوية لثاني أكسيد التيتانيوم وجسيمات الذهب النانوية والذهب NPs بالاشتراك مع حمض الفوليك على خط الخلايا PLCs وSW480 لمدة 24 ساعة من فترات الحضانة. أجريت تسع تجارب في المختبر على خطوط الخلايا هذه وزُرعت خلايا هذه الخطوط في صفيحة 96 مكان زرعي، تم استخدام مواد النانو في ست تخفيفات متسلسل (16.2،31.3،62.5،125،250،500) ميكروغرام / مل . تم استخدام مقايسة 3-(4،5-ثنائي ميثيلثيازول -2-يل) -2،5-ثنائي فينيل -2H- تترازوليوم بروميد (MTT) لتحديد عدد الخلايا القابلة للحياة، وتم قياس شدة اللون بواسطة قارئ ELISA.

التجربة (1): تم زرع صفيحتين بخلايا سرطان الكبد وخلايا سرطان القولون وتم تعريض كل لوحة إلى 200 ميكرولتر من ست تخفيفات متسلسلة من الأنابيب النانوية Tio2 المحملة بـ Tio2 وAg، بعد 24 ساعة من الحضن، تم إجراء اختبار MTT لقياس حيوية الخلايا السرطانية. أظهرت نتائج هذه الدراسات أن Tio2 ينخفض في نسبة بقاء الخلية (P <0.001) عند التراكيز الأعلى على خلايا سرطان القولون، بينما في خلايا سرطان الكبد الأولية لا تختلف النتائج عن المجموعة الضابطة. أظهرت نتائج الأنابيب النانوية Tio2 المزخرفة بـ Ag انخفاضًا في قابلية بقاء الخلية للخلايا السرطانية (P <0.001) بتركيزات أعلى مقارنةً بمجموعة السيطرة السلبية.

التجربة (2): تم زرع صفيحتين بخلايا سرطان الكبد والثاني بخلايا سرطان القولون ثم تعرض اللوحان لليزر الأحمر الثنائي بقدرة 635 نانومتر لأوقات مختلفة (دقيقة واحدة، 3 دقائق، 5 دقائق، 7 دقائق، 9 دقائق، 12 دقيقة) بعد 24 ساعة من الحضن تم إجراء اختبار MTT لقياس حيوية الخلايا السرطانية. أظهرت نتائج هذه الدراسة أن هناك انخفاضًا ملحوظًا في نسبة بقاء الخلية ($p<0.001$) تختلف عن مجموعة السيطرة السلبية في كلا خطي الخلايا عند 7،9 و12 دقيقة.

التجربة (3): تم زرع صفيحتين بخلايا سرطان الكبد والثانية بخلايا سرطان القولون وتم تعريض كل لوحة إلى 200 ميكرولتر من ست تخفيفات متسلسلة للأنابيب النانوية Tio2 والأنابيب النانوية Tio2 المحملة بـ Ag، ثم تعرض الصفيحتان لليزر الصمام الثنائي مع 635 نانومتر لمدة 12 دقيقة بعد 24 ساعة من الحضن تم إجراء اختبار MTT لقياس حيوية الخلايا السرطانية. أظهرت نتائج الأنابيب النانوية Tio2 على خطي الخلايا السرطانية انخفاضًا في حيوية الخلية ($p<0.001$) بتراكيز أعلى. أظهرت نتائج الأنابيب النانوية Tio2 المحملة بـ Ag على كلا خطي الخلايا السرطانية انخفاضًا في حيوية الخلية ($p<0.001$) في جميع التراكيز.

التجربة (4): تم زرع صفيحتين بخلايا سرطان الكبد والثاني بخلايا سرطان القولون ثم تعرض كل طبق إلى 200 ميكرولتر (190 ميكرولتر وسط زائد 10 ميكرولتر من تخفيفات حمض الفوليك التسلسلي) و200 ميكرولتر من جزيئات الذهب النانوية ست تخفيفات متسلسلة و200 ميكرولتر من ست تخفيفات متسلسلة من جزيئات الذهب النانوية مع حمض الفوليك بعد 24 ساعة من الحضن تم إجراء اختبار MTT لقياس حيوية الخلايا السرطانية. أظهرت نتائج هذه الدراسات أن جزيئات الذهب النانوية تقلل في حيوية الخلايا لكل من خطي الخلايا السرطانية بتراكيز أعلى، في حين أن نتائج حمض الفوليك وجزيئات الذهب النانوية مع حمض الفوليك تسببت في انخفاض قابلية بقاء الخلايا لكلا خطي الخلايا السرطانية ($p<0.001$) مقارنة بمجموعة السيطرة السلبية.

التجربة (5): تم زرع صفيحتين بخلايا سرطان الكبد والثاني بخلايا سرطان القولون ثم تعرض كل طبق إلى 200 ميكرولتر (190 ميكرولتر وسط زائد 10 ميكرولتر من تخفيفات حمض الفوليك التسلسلي) و200 ميكرولتر من جزيئات الذهب النانوية ست تخفيفات متسلسلة و200 ميكرولتر من ست تخفيفات متسلسلة من جزيئات الذهب النانوية ممزوجة بحمض الفوليك ثم لوحين معرضين لليزر الصمام الثنائي بطول موجة 635 نانومتر لمدة 12 دقيقة عند جميع التراكيز، بعد 24 ساعة من الحضن تم إجراء اختبار MTT لقياس حيوية الخلايا السرطانية. أظهرت نتائج هذه الدراسة انخفاضًا في حيوية الخلايا لكلا خطي الخلايا السرطانية ($p<0.001$) مقارنةً بمجموعة السيطرة السلبية.

**جمهورية العراق**

**وزارة التعليم العالي والبحث العلمي**

**جامـــعة بابـل**

**كليـة الطب**

# العلاج الضوئي على خلايا سرطان الكبد الأولي ( PLC / PRF 5 /) وسرطان القولون (SW 480) باستخدام أنواع مختلفة من المواد النانوية.

رسالة

مقدمة الى مجلس كلية الطب/جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في الادوية/الادوية والسموم

من قبل

حوراء علي ناصر

(بكالوريوس صيدلة , 2012-2013)

إشراف

أ.د. نسرين جلال محمد

دكتوراه علم الادوية

أ.د. رنا اياد غالب

دكتوراه علم الاحياء

1444 هـ

2022 م