CYTOTOXICITY ENHANCEMENT OF LOW-LEVEL LASER IRRADIATION ON GOLD NANOPARTICLES IN HUMAN BREAST CANCER MCF-7 CELL LINE

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by

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LIST OF ABBREVIATIONS

$^1O_2$	Singlet Oxygen
AuNPs	Gold Nanoparticles
Akt	Protein Kinase B
CSC	Cancer Stem Cell
CO_2	Carbon dioxide
DI	Deionized water
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
ECACC	European Collection of Authenticated Cell Cultures
EDTA	Ethylenediaminetetraacetic acid
EPR	Enhanced permeability and retention
FBS	Fetal Bovine Serum
GLOBOCAN	Global Cancer Incidence, Mortality and Prevalence
HIF	Hypoxia-inducible factor
HRTEM	High Resolution Transmission Electron Microscope
IC_{50}	Half maximal inhibitory concentration
LLLT	Low-Level Laser Therapy
MCF-7	Michigan Cancer Foundation-7
miRNA	Micro-Ribonucleic Acid
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
PBS	Phosphate Buffered Saline
PDT	Photodynamic Therapy
PES	Polyethersulfone

PI3K	Phosphatidylinositol 3-kinase
REDOX	Oxidation reduction
ROS	Reactive Oxygen Species
rpm	Rotation per minute
RPMI-1640	Roswell Park Memorial Institute - 1640
SPR	Surface Plasmon Resonance

PENINGKATAN KESITOTOKSIKAN PENYINARAN LASER BERKEAMATAN RENDAH MENGGUNAKAN NANOZARAH AURUM DALAM SEL KANSER PAYUDARA MCF-7

ABSTRAK

Cahaya dalam bentuk laser adalah satu kaedah lain yang digunakan untuk menghasilkan rawatan kanser secara kurang atau tidak invasif. Dalam kajian ini, laser berkeamatan rendah telah digunakan kerana laser ini menghasilkan tenaga yang lebih rendah berbanding laser lain dan digabungkan bersama nanozarah Aurum bagi mempertingkatkan kesan radiasi dalam sel payudara MCF-7. Dalam projek ini, sel-sel MCF-7 dikulturkan di dalam plat 96-perigi dan dirawat dengan nanozarah Aurum bersaiz 50 nm sebelum disinari dengan laser dengan kuasa 0.002, 0.02, 0.06, dan 0.1 W pada masa pendedahan daripada 60 hingga 900 saat. Kemudian, sel-sel yang telah disinari akan dieram selama 24 jam. Selepas itu, kuantiti sel-sel yang telah disinari diukur dengan pengujian Alamar Biru. Keputusan sinaran menunjukkan tanpa nanozarah Aurum, tiada sel yang menunjukkan kematian dengan kadar 50 hingga 70 % daya hidup menandakan laser kurang memberi kesan disebabkan pelbagai faktor rintangan sinar. Walaubagaimanapun, pengurangan dalam kuantiti sel jelas melebihi 90 % apabila sel yang disinari dengan dos 2.40 J/cm^2 dengan kehadiran kepekatan tertinggi iaitu 50 μg/ml nanozarah Aurum berbanding dengan sel tanpa nanozarah Aurum. Ini jelas menunjukkan nanozarah Aurum diperlukan dalam peralihan tenaga haba daripada sinaran laser hijau untuk menggalakkan kematian sel. Dengan pertimbangan terhadap kesan pembiakan sel MCF-7 dan daya hidup sel, rawatan laser yang di bantu oleh nanozarah Aurum telah menghasilkan kesan rawatan yang memuaskan seperti dijangkakan.

CYTOTOXICITY ENHANCEMENT OF LOW-LEVEL LASER IRRADIATION ON GOLD NANOPARTICLES IN HUMAN BREAST CANCER MCF-7 CELL LINE

ABSTRACT

Laser light is another option used to provide non-invasive or less invasive cancer therapy. In this study, low-level laser therapy (LLLT) is used as it is less invasive due to lower energy densities than other types of laser with a combination of gold nanoparticles (AuNPs) to enhance the effects of radiation in the MCF-7 breast cancer cell line. In this work, the cells were seeded in 96-well plate and were treated with 50 nm AuNPs before irradiated with a laser for 0.002 W, 0.02 W, 0.06 W, and 0.1 W at various time from 60 s to 900 s. Then, the irradiated cells with AuNPs were incubated for 24 h. After that, the absorbance of irradiated cells was measured by Alamar Blue to determine the cell viability. The result shows without AuNPs in all irradiation conditions, less cell inhibition occurred with viability percentage in range between 50 to 70 % indicating that the cells were less affected by the laser due to multifactor of radio-resistance. However, a clear reduction in cell density exceeding 90 % was observed for cells irradiated with dose of 2.40 J/cm^2 in the presence of AuNPs with highest concentration 50 μg/ml when compared to AuNPs alone or laser alone. This clearly indicates that the AuNPs are required for photothermal conversion of the green laser irradiation, which induces cell death. Considering the effects on the MCF-7 cells proliferation and the results of viability cells, AuNPs-assisted laser therapy turned out to have a satisfactory treatment effect as expected.

CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter includes the background study of common clinical cancer treatments, laser therapy, and nanoparticles. The major crises in cancer therapy are also discussed in the problem statement. Additionally, the purposes of the study, scope of research and outline of this thesis are also explained in this chapter.

1.2 Background of study

Conventional therapy of cancer such as surgery, radiotherapy, chemotherapy, hormone therapy and immunotherapy are usually carried out in medical facilities by the qualified and certified medical professional. The most commonly practiced techniques of therapy include surgery, chemotherapy and radiotherapy. Small tumours that could be withdraw entirely by surgery were declared remediable cancer therapy in the early 20th century. Radiation therapy was then introduced as a surgical supplement to control the small tumours that were not surgically removable. Chemotherapy was then created to treat tiny tumours that the surgeon or radiotherapist could not remove. Unfortunately, all of these techniques are often followed by serious side effects as the treatments can also induce damage to normal cells.

To effectively eliminate tumours and minimise injury to surrounding organs, radiation and proton beam therapy are then practised by targeting beams of photons and protons in several directions. Although therapeutic techniques have been improved,

several patients are likely to experience relapse owing to radio-resistance of cancer cells (Wu *et al.*, 2015).

Laser irradiation on cancer cells is a promising alternative in providing a non-invasive treatment of breast cancer. Interestingly, the laser in medical application can be categorised into two which are high intensity and low intensity. Each of these categories has very different therapeutic properties in their designs and applications. High light intensity is quite strong and accurate due to its highly concentrated type of light. Usually it is used to remove small tumours as a laser ablative surgical tool. Another treatment that uses high intensity light is photothermal therapy that applies heat to tumour to shrink them due to the high energy transferred into and absorbed by the tumour. The main benefit of this therapy is that it causes the nearby tissues less bleeding and injury. While high-intensity laser therapy is not a novel approach to cancer treatment, a new strategy that is of concern to this research is the idea of using low-intensity light for cancer treatment.

One promising metal nanoparticle such as gold nanoparticles (AuNPs) show efficacy in cancer therapy owing to its extremely selective toxicity to normal and cancerous cells, which is explained by the formation of reactive oxygen (ROS). Hainfeld and colleagues studied in 2004 as the first group to practice gold nanoparticles to increase the dose of radiation in mice with EMT-6 mammary carcinomas in the thighs and then irradiated the tumour 2 minutes later (Hainfeld *et al*., 2004). Then, the present research also mostly uses gold nanoparticles as the photo-absorbing and anticancer agents due to their strongly enhanced absorption in the visible and Near Infrared Regions (NIR).

This study will be focusing on the both unique features of lasers and the extremely small size of nanoparticles to provide an interesting combined therapeutic effect which creates new physical effects. This combined therapy may offer complete tumour destruction with less psychological morbidity, better cosmetic outcomes and less hospital care compared with conventional method of cancer treatments.

1.3 Problem Statement

The burden of cancer on human life remains to be disastrous despite significant breakthroughs in our medical technology and cancer research attempts. The limitation in cancer treatment is to be able to provide desirable effective therapy, and this needs more studies to provide more efficient, faster, safer and more feasible techniques of diagnosis and therapy. One significant issue in the therapy of breast cancer is representing the treatment of tumours surrounded by vital tissue as there is a probability of uncertain tumour margins. Other limitations of these treatments including poor biodistribution, unwanted side effects, lack of selectivity target toxicities, hazardous radioactive or radiation source and the latent effects of therapy which often persist for a time post-therapy has affected the desired therapeutic advantages of this treatment.

There is also a restriction to treatment such as a therapy that uses light therapy in the presence of photon-absorbing or drug agent in the targeted area. Due to the insufficient selectivity of most agents and their bad solubility, the therapeutic effectiveness of combined therapy in cancer treatment is limited. Most of the agents have bad tissue diffusion, thus restrictive the treatment of internal tumours. To enhance therapeutic protocols, further research is required by adjusting the drug agent, light distribution, power density, and doses of irradiation. Also, by controlling the radiation

delivery can potentially overcome these limitations by limiting radiation in the place of action. Unfortunately, some of the cancer cells often develop radio-resistance, which compromises the efficacy of cancer radiation therapy.

Lastly, the main limitation is patient feel discomfort due to some of the past treatment take a long duration time. Real-time monitoring of temperature and complications is needed to avoid side effects and achieve maximum efficacy (Kerbage *et al*., 2017). Thus, the current method needs improvement for safety and reduce these mentioned limitations. Hence, in this study, the laser and gold nanoparticles combination are expected to provide another alternative in targeting photothermal destruction on MCF-7 cells. If this combination therapy able to gives a better output, the radioactive which usually used can be avoided in medical treatments and at the same time will offer localised therapies that will improve efficacy, less side effects, and recover patient quality of life. This also can help overcome the limitations of traditional therapies like resistance to treatment, late diagnosis and so on. The basis of this therapy is that materials with highly absorb light can be designed and delivered specifically to the tumour cells.

1.4 Objectives

The objectives of the study are:

1. To determine the cytotoxicity effects of AuNPs to the breast cancer MCF-7 cells.
2. To evaluate the effects of different dosage of laser irradiated on MCF-7 cells
3. To investigate the effects of AuNPs in MCF-7 cells by low-level laser of green laser (532 nm) treatment.

1.5 Scope of research

This research focusing on application of Low-Level Laser Therapy (LLLT) by using 532 nm (green) low power visible laser at different doses to stimulate the 50 nm gold nanoparticles (AuNPs) for destruction of breast cancer cells. The MCF-7 cells with and without presence of gold nanoparticles were irradiated at different power of green laser with different exposure times.

1.6 Outline of Thesis

Chapter 1 is an introduction that include the background study of clinical cancer treatments, laser therapy, nanomedicine, and MCF7-cell lines. The later part is the explanation of problem statement including objectives and scope of research of this study.

Chapter 2 contains a detailed statistic of breast cancer and description of the theoretical, mechanism, types of therapy using the laser application. The Gold Nanoparticles (AuNPs) then are described in this chapter. The current and previous review on the therapy using laser and nanoparticle *in vitro* and *in vivo* studies are also provided.

Chapter 3 explains the general protocols for biological study using cell culture and also the characterisation of AuNPs. Procedure for measuring the cytotoxicity of AuNPs is also described. In addition, the comparison of dose laser energy of 532 nm with different power and exposure times is explained. Lastly, the combination therapy by using both AuNPs and laser treatment is designated.

Chapter 4 reports the results and discussion of each method experiments conducted in this study. The results are divided into six parts which are introduction,

characterisation of AuNPs, cell growth curve, cytotoxicity of AuNPs, laser dose optimisation, for photodynamic therapy and finally the effects of AuNPs enhance the laser irradiation are also explained.

Lastly, the final chapter of this study is a conclusion that summarised the overall results of this experiment and the recommendation for further works.

CHAPTER 2

THEORY AND LITERATURE REVIEW

2.1 Introduction

Cancer is an extraordinary complex disease. Applications that are commonly used in physics can develop an effective strategy for cancer treatment by minimising the complexity of cancer to a manageable set of underlying principles and phenomena. Hence, in this chapter the statistics of breast cancer and theory of laser application, nanoparticles, cancer therapy are described including the previous and current research related to these applications. Moreover, details on MCF-7 cancer cells line are also discussed in this chapter.

2.2 Statistic of Breast Cancer

A press release by World Health Organisation (WHO) stated that cancer is one of the major health issues and the second leading cause of death globally with 9.6 million of deaths (World Health Organization, 2018). According to a report from Malaysian Study on Cancer Survival (MySCan), cancer has been identified as the fourth most common cause of death in Malaysia with 12.6 % of all deaths in government hospitals but the rate in private hospitals has more than doubled to 26.7 %. (National Cancer Registry Department, 2018). The breast cancer is the commonest cancer among women in this nation, regardless of ethnic group and from the age of 15 years onwards (Akhtari *et al*., 2015). With 99 % of cases occurring in women and only 1% in men, breast cancer is more of a concern for women and is estimated to develop in one out of 19 women (Rudlowski, 2008). In 2018, as reported by Global Cancer

Incidence, Mortality and Prevalence, GLOBOCAN as many as 11.6 % of cases of cancer (2.1 million cases) were caused by of breast cancer followed by lung, colorectum, prostate and stomach cancer (Globocan, 2018) (refer in Figure 2.1).

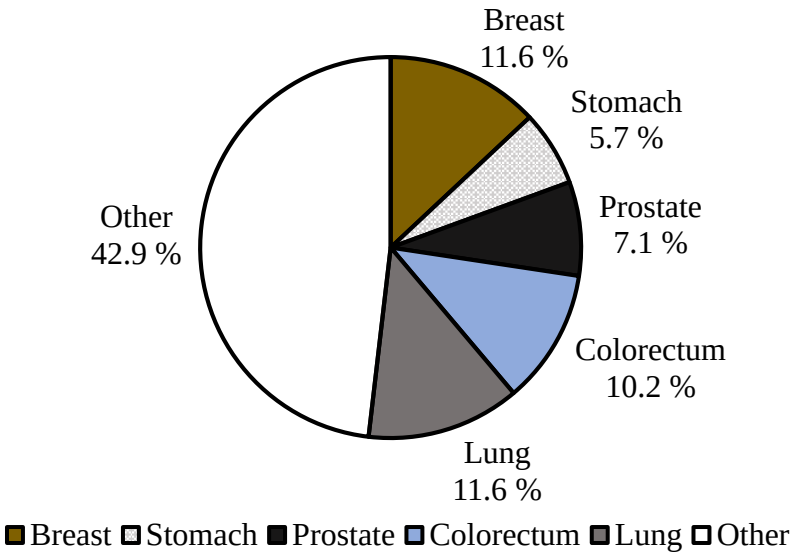


Figure 2.1 The percentage number of cancer cases in 2018 for both sexes and all ages in worldwide (Globocan, 2018)

Women in Malaysia have poor survival from breast cancer and it is estimated that half of the deaths caused by this cancer could be prevented (Yip *et al*., 2005). There has been an increasing trend of breast cancer patients from 2007-2016 from 11.3 % in 2007 to 12.6 % in 2016 (Azizah *et al*., 2016). However, the rate differs between the three main races; the Malays, Chinese and Indians. The rate of incidence in Chinese is the highest, with 59.7 per 100,000, followed by the Indians at 55.8 per 100,000 and Malays have the lowest incidence of 33.9 per 100,000 population. This means that 1 in 16 Chinese, 1 in 16 Indian and 1 in 28 Malay women would develop breast cancer at some stage in their lives (Yu *et al*., 2016). Even though, Malay patients present at later stages and with larger tumours their survival is worse than with other races women (Yip *et al*., 2005). Furthermore, report from MyScan also revealed that

roughly 37,000 newly diagnosed cancer cases every year, and the number is expected to increase to over 55,000 by 2030. The report shows that therapeutic methods for cancer treatment especially breast cancer and patient quality of life need to be reconsidered. Application and theory in physics can be implemented by reducing the complexity of cancer to create efficient therapy.

2.3 Laser

The word of “LASER” is an acronym for light amplification by stimulated emission of radiation. In 1960, Theodore H. Maiman developed the first LASER or "Microwave Amplification by Stimulated Radiation Emission (MASER)" (Kohale *et al*., 2016). After lasers were discovered, it was proved that laser could enhance wound healing and relieve pain, swelling and inflammation (Chung *et al*., 2012).

The properties of laser are different with other sources of light. The laser light is monochromatic, which means a wavelength or colour. By contrast, ordinary light is a combination of many different colours or wavelengths. Furthermore, lasers emit highly directional light. Thus, the light coming from the laser is very powerful and concentrated in comparison with normal light such as coming from the sun, bulb, or flashlight, as the light is weak and diffuse as it is emitted from the source in many directions. The wavelengths of the laser light are in phase in space and time (coherent) while wavelengths of other light sources have a phase that varies randomly with time and space (incoherent).

Lasers are classified as hard and soft tissue lasers based on the type of laser–tissue interaction and not on the type of tissue exposed. Various wavelengths used are in the range of visible and near infrared spectrum (ranging from 400 to 900 nm) and it has a unique interaction with the respective target tissues. As stated by Kohale and

colleagues, high-power lasers cut tissue while low-power lasers stimulate various tissues and help the cells to function (Kohale *et al*., 2016). However, high doses of laser have certain disadvantages and to overcome these matters, extensive research is going on with low-level laser therapy as this therapy has a stimulatory effect on cells at low dosage and a suppressive effect at high dosage.

There are two ways of treating cancer using laser application either by shrinking tumour with heat coming from laser photon energy or destroying tumour by using photon energy to activate a chemical called drug agent which has been injected onto tumour site. Previous research has shown that laser heating at a temperature of 43 ° C for 30 min may cause apoptosis and temperatures of 45 ° C or higher may trigger necrosis in certain sensitive tissues (Heike *et al*., 1996). This claim has also been supported by Suriyanto *et al*., (2017) study, stated that hyperthermia occurs when heat is used to increase the body temperature from a normal body temperature of 37 °C to the temperature ranging of 41 to 45 °C. This heating has a possibility to selectively kill cancerous cells without damaging healthy tissues. Therefore, the critical temperature at which cancer cells can be killed while normal tissues remain alive is 45 ° C (Tang *et al*., 2014). However, study by Habash *et al*., (2007) the application of a high temperature or known as thermal ablative, above 45 °C, with their aim to produce significant tissue destruction heating also lead to cell death of normal tissues even it has positive effects on killing tumour cells, hence this therapy has to be applied with caution.

The finding that the tumour is more sensitive to heat than healthy tissues is one of the reasons for using hyperthermia theory as a cancer therapy technique. This is due to high cancer cell acidity as a result of increased glycolytic activity within cancer cells

(Gerweck *et al*., 1980). Furthermore, the heat reduced by blood flow protects the healthy tissue from overheating (Suriyanto *et al*., 2017).

2.3.1 Components of laser

The three main components of the laser system are the lasing medium, pumping source system and optical resonator. Lasing medium is an active medium within laser which contains atoms that can emit light by stimulated emission. As seen in Figure 2.2, the image shows schematic diagram of laser diagram.

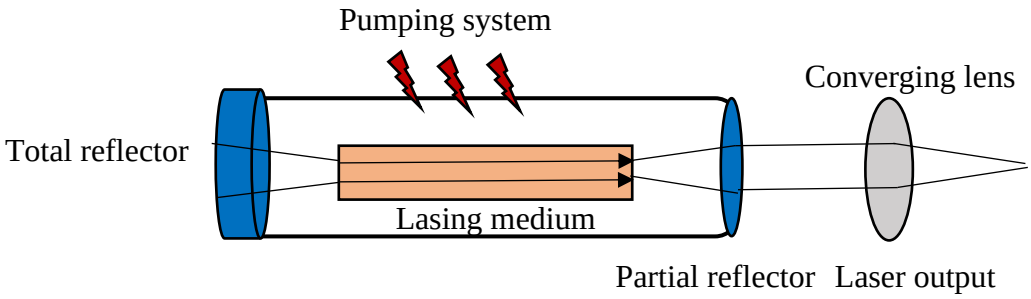


Figure 2.2 Solid state laser diagram

The active medium may be a crystal, gas, semiconductor, and dye. The pumping system is a source of energy to excite the atoms to the proper energy state. The pumping by energy source can be optical, electrical or chemical depending on the active medium. Optical resonator consists of active medium enclosed between two mirrors which are one of it is totally reflective mirror (100% reflective) and the other is partially transmissive mirror (99% reflective). It is used to reflect the laser beam through the active medium for amplification (Wenlong & Xichun, 2010).

The process begins when the light energy from the pumping system is exposed to the atoms of the lasing medium. Normally, the electron in lasing medium reside in

a steady state lower energy level. Then, majority of these electrons are excited to a higher energy level after energy is added to lasing medium.

The state of these electrons is unstable, known as population inversion phenomena. These unstable electrons will remain in this state for a short period of time, and then return to their original energy state. This decay occurs in two ways including spontaneous decay and stimulated decay (Singh *et al.*, 2012).

This stimulated transition will harvest energy in the form of photons travelling in phase as the photon incident. If the direction is parallel to the optical axis, the photons emitted travel back and forth through the lasing material between the fully reflective mirror and the partially reflective mirror within the optical cavity. In this technique, the light energy is increased until sufficient energy is generated to transmit the laser light through a partially reflective mirror.

2.3.2 Working principle of laser

The principle of a photons interacted with atoms is based on three processes which are absorption of radiation, spontaneous and stimulated emission. Figure 2.3 shows an absorption of radiation process which occurs when the lower-energy electron absorbs photon energy to jump into a higher-energy state. In general, the electron at ground state need enough energy in order to jump into the higher energy state (Cherif *et al.,* 1999). When photons (light energy) equal to the energy difference of the two energy levels (E2 – E1) is incident on the atom, the ground state electrons gain enough energy and jump from ground state (E1) to the excited state (E2) (Herd *et al.*, 1997).

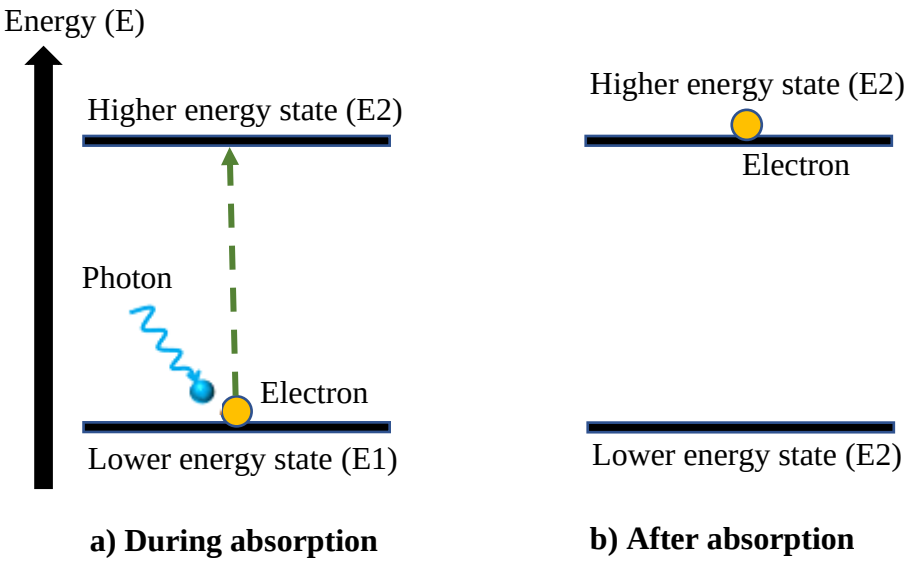
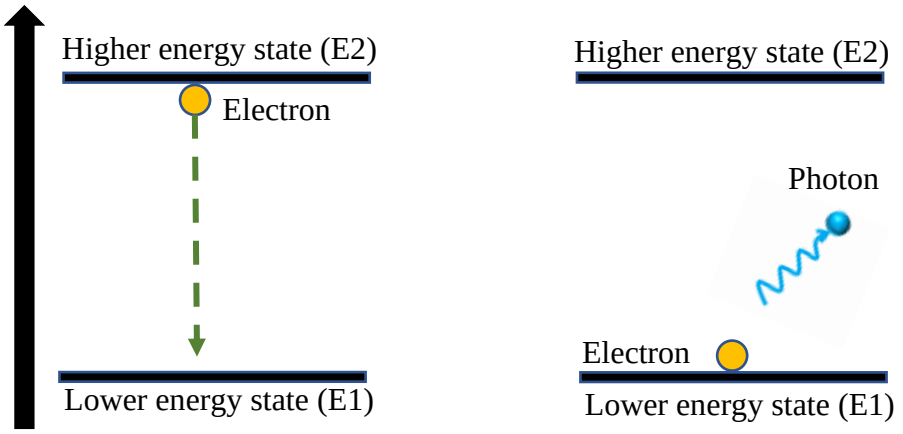


Figure 2.3 Schematic diagram of absorption radiation

As shown in Figure 2.4, spontaneous emission is the process where the electron at higher energy state returns spontaneously to the ground state by emitting photons as the electrons in the excited state (high energy level) can stay only for a short period until its lifetime is over. This emission of photons occurs naturally unlike stimulated emission. The photons emitted in spontaneous emission process constitute ordinary incoherent light. Incoherent light is a beam of photons with frequent and random changes of phase between them. In other words, the photons emitted during the spontaneous emission process do not flow exactly in the similar way as the incident photons.

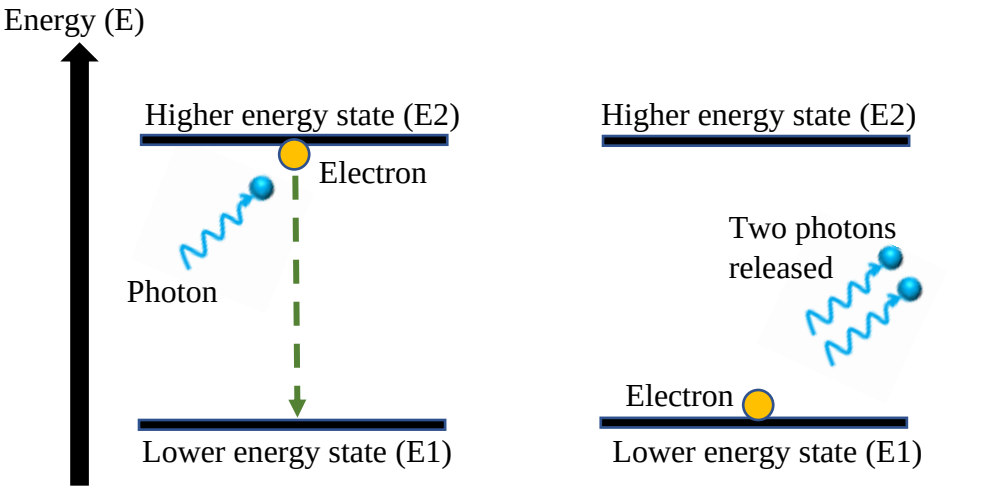
In Figure 2.5, stimulated emission is the process by which incident photon collides with the excited electron and forces it to return to the ground state before completion of their lifetime by receiving energy supplied directly to the excited electron instead of supplying energy to the electron in ground state (Singh *et al.*, 2012). This excited electron releases two energies in the form of light while falling to the ground state. The two photons emitted is due to the incident photon and another one is

due to energy release of excited electron. The stimulated emission process is quicker than the spontaneous emission process. All the emitted photons in stimulated emission have the equal energy, similar frequency and are in phase (Thomas & Isaacs, 2011). Therefore, all photons in the stimulated emission travel in the same direction.



a) Before photon emission **b) Photon emission during transition**

Figure 2.4 Schematic diagram of spontaneous Emission of photon



a) Before photon emission **b) Photon emission during transition**

Figure 2.5 Schematic diagram of stimulated emission

2.3.3 Low-Level Laser Therapy

Low-level laser therapy (LLLT) is an application of light using low power energy. It is also known as “cold laser” due to less thermal effect generated from the low power energy used. In general, the power densities used for LLLT are lesser than those required to generate heating in tissue depending on wavelength and tissue type (M. Hamblin & Demidova, 2006) . Due to its wavelength and biphasic dose at a cellular level, LLLT has shown significant outcomes for a wide range of medical techniques. (Hamblin *et al*., 2011). LLLT clinical practice has been discovered and used for about 20 years. It is reported that LLLT could improve the process of wound healing and also has stimulating effects on bone cells and can hasten the repair process of the bone (Kohale *et al*., 2016). However, researchers and therapists doubted the clinical advantages of laser treatment due to gaps in the methodological standardisation and clinical applicability (Aparecida *et al*., 2011). The biphasic dose-response curve or Arndt – Schulz curve is a crucial part of LLLT. This principle specifies that optimum parameters provide an advantage to the specific disease, and if these parameters are significantly surpassed, the advantages will disappear and may even result in harmful outcomes when the dose is extraordinarily high (Hamblin *et al*., 2018).

Previous studies always came out with different conclusions which difficult for clinical team to select the optimum parameters. The first study used the LLLT in cancer was done by Mester and his team. Their aim of research was to cure cancer on the shaved dorsal skin of mice. Despite LLLT did not cure tumours they observed a higher rate of hair development and better wound healing. This was the first sign that low-level laser light could have its own useful medical applications (Mester *et al.*,

1971). In spite of that, study by Pinheiro *et al.* (2002) found that after 635 and 670 nm irradiation on H.Ep.2 cells could significantly increase proliferation of laryngeal cancer cells. Work by Kara *et al.* (2018) then concluded that LLLT promotes cancer cell proliferation and could activate precancerous cells, depending on the power of the laser and the number of treatments. . In their study, they observed percentage of lung cancer cell proliferation were higher in the treated group by using Nd:YAG laser compared to the control groups . Their study proved that the low-level laser therapy using Nd: YAG did not inhibit lung cancer cell proliferation.

Their results can also be supported by previous research in 2015, the human leukemic cells that were irradiated (810 nm) with different doses, 20 J/cm^2 showed significant increase in cell proliferation after two exposures but there were no changes in the growth rate of cells treated with 5 J/cm^2 and 10 J/cm^2 (Dastanpour *et al*., 2015). In the same year, breast cancer line of MDA-MB-231 cell viability increased after being treated by laser with 248 nm but slightly decreased after irradiated with both 1064 and 532 nm lasers were found in study by Badruzzaman *et al.* (2016).

Additionally, study by Cerchiaro *et al*. (2012) using He-Ne (632nm) laser found that MCF-7 which exposed to 5 mJ/cm^2, the maximum number of dead cells was observed at all times in the treated group except at 48 h. Meanwhile, for the group irradiated with 28.8 mJ/cm^2, the percentage of dead cells was significantly higher at 24 to 72 h.

Because LLLT has been shown to stimulate the development of cancer cells (Sroka, Schaffer, Fuchs, Pongratz, & Schrader-Reichard, 1999) and may also enhance the aggressiveness of some cancer cells (Sperandio *et al.*, 2013), some researchers have claimed that LLLT may be contraindicated in clinical use in cancer patients

(Navratil & Kymplova, 2002). However, not all of the researchers found the same results. In contrast, it was found that LLLT was very effective at minimising many distressing side effects that occur as a result of a range of different cancer treatments (Zecha, Raber-durlacher, Nair, & Epstein, 2017).

2.3.4 Action mechanism of low-level laser therapy

Laser light can interact with tissue through transmission, reflection, scattering and absorption. Transmission relates to the light passing through tissue without affecting it while reflection refers to the light that repels from the surface of the tissue without passing through the tissue. Light scattering takes place after light has entered the tissue due to heterogenous structure of tissue and different index of refraction between parts of tissue. Interaction of light scattering can change both direction and power (inelastic) or only the direction (elastic scattering) of photons. The light interactions in visible and NIR range with biological tissue generally generate elastic scattering (Jacques, 2013). Absorption occurs when light photon is absorbed and interacted with an atom, bond, or molecule and the photon energy is transmitted to the atom or molecule.

The exact action mechanism of LLLT is not well understood, and several theories exist. As stated by Houreld (2014), most of the studies and the best understood in mechanism of this therapy being that of cytochrome-c oxidase (Complex IV) in the cellular mitochondrial respiratory chain. Complex IV appears to be a chromophore or photoreceptor that absorbs energy from photons moving on wavelengths in the near infrared spectrum which accelerates electron transfer rate (Kohale *et al*., 2016). After the photon absorption by photoreceptor, the photon tends to have delocalised electrons in molecular orbital which can be excited from lowest state to higher state and result

in activate or deactivate enzymes which can alter macromolecules such as DNA and RNA. This alteration then stimulates cellular metabolism (Matić *et al.,* 2003). The photon absorption by photoreceptor can also transfer the energy to other molecules that leads to photochemical reactions in surrounding tissue and give rise to observable biological impacts (Pinheiro *et al*., 2002).

2.4 Nanoparticles

In recent years, nanoparticle has become interesting in forefront fields in Physics, Chemistry, Biology, and Engineering. Nanoparticle in medicine can be explained as the design of therapeutic agents in the nanoscale range with diameters ranging from 1 to 1000 nm. With their unique characteristics and long circulation time in blood compared with small molecules, nanoparticles have developed as an excellent candidates for optimised therapy through personalised medicine (Sanna *et al*., 2014).

2.4.1 Gold nanoparticles

Gold nanoparticles; AuNPs, are the most potential candidate among various metallic nanoparticles as therapeutic agent in LLLT as they are powerfully absorbed laser light, nontoxic, and have tunable optical properties (Priyanka *et al*., 2018). AuNPs display distinctive physicochemical characteristics, including their strong surface plasmon resonance (SPR) bands in the visible region and not suffering photobleaching (Mendes *et al.*, 2017). This phenomenon significantly improves both the scattering and the absorption of light by the AuNP which make it suitable for various biomedical applications (Xiaohua Huang & El-Sayed, 2010).

AuNPs have been found to have cytotoxicity effect against breast cancer cells (Park *et al*., 2016). Among the various shape of nanoparticles, spherical (colloidal)

AuNPs have been used for photothermal cancer therapies during the last 20 years (Mohseni *et al.*, 2016). As mentioned by Rosli *et a*l. (2015), colloidal AuNPs is the most preferred among the nanoparticles because they can easily produce to a desirable size from 0.8 to 200 nm, easy preparation, can be easily altered for various functionalities. The triangular AuNPs showed more effective cellular uptake than spherical with similar surface area but rod shape like is less efficient compared to the spherical shape (Xie *et al*., 2017) as shown in Figure 2.6. Usually, colloidal AuNPs show a single absorption peak in the visible range between 510 to 550 nm (Cai *et al*., 2008) which have enough energy to absorb photon emitted from 532 nm green laser. Unfortunately, the visible laser treatment using this AuNPs has less application compared to near-infrared region laser. Majority of reports have focused on the near-infrared region laser to treat cancer cells by increasing temperature as these cancer cells sensitive to heat (Mendes *et al*., 2017).

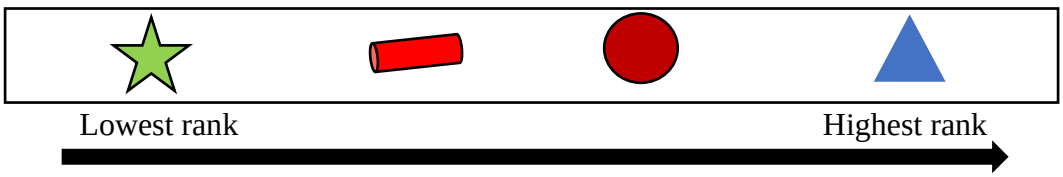


Figure 2.6 The rank of cellular uptake of shape- dependent. The image demonstrated that triangular shape of AuNPs is more effective cellular uptake and the lowest rank is star- shaped AuNPs

Size and concentration of nanoparticles are another important parameter for their applications in oncology treatment. In 2016, study by Suganya *et al*. (2016) concluded that 12 nm spherical AuNPs against MDA-MB-231 and MCF-7 have toxicity varies with concentration and time. They also determined that the spherical AuNPs did not affect the viability of normal Human Mammary Epithelial Cells, HMEC

However, in previous study in 2013 by Song and colleagues, they claimed that 50 nm is the excellent choice for size of AuNPs for cancer therapy which have the ability to penetrate and accumulate around cancer cells due to enhanced permeability and retention effect (EPR) (Song, Xu, & Meng, 2013). However, for small particles size is too small to take advantage of the EPR effect in both in-vivo and in-vitro study (Saad *et al*., 2013). EPR is a phenomenon where the certain size of particles tends to accumulate in cancer cells better than in normal tissue (Pillai, 2014). Song and colleagues (2013) also concluded that the best time to apply irradiation following the AuNP application was 12 h for MCF-7 cells. This study can be contended by Sztandera *et al*. (2019) which indicates the maximum uptake of AuNPs occurs with diameter of 50 nm spheres, which may be attributed to the fact that their size is within the range of viruses and lipid-carrying proteins, efficiently endocytosed by the cell. Another study also stated that AuNPs can be used as targeted cancer treatment due to AuNPs only increase the radiation sensitivity in cancer cells but not in normal cells (Jain, Coulter, Hounsell, & Karl, 2011).

2.4.2 Nanoparticle interaction with light and tissue

These nanoparticles show unique properties in their extremely small size allowing it to penetrate the cancer cells easily as well as capability to absorb light energy from laser source at specific wavelength and manipulate the cellular function of the cell, leading to cytotoxicity which provides huge therapeutic benefits (Alkilany & Murphy, 2010). AuNPs can easily enter tumour vasculature and remain in tumours site due to the EPR effects (Lee *et al.*, 2014).

When AuNPs was injected into the cancer cells, it would differentiate tumour cells from normal cells easily and able to absorb the laser beam and thus tumour cells treated with this nanoparticle can be killed quickly (Mohseni *et al*., 2016). As studied

by Servatkhah and Goodarzi (2017), AuNPs are very good at scattering and absorbing light for cancer cells than noncancerous cells. The excretion of nanoparticles is through renal clearance (Fekrazad *et al*., 2016).

2.5 Combined Therapy

In this therapy, laser with combination of drug agent can generate reactive oxygen species (ROS) and singlet oxygen ($^1O_2$) to kill cancer cells (Wang *et al*., 2017). ROS is well known to stimulate cellular proliferation of LLLT but inhibits proliferation and kills cells at higher-levels laser (Kumari *et al.*, 2018). According to Li *et al*. (2010) the hematoporphyrin derivative and photofrin (porfimer sodium) are officially one of the first-generation drug agents used in the treatment of cervical cancer. However, these synthetic organic dyes that are used as photosensitisers or drug agents are likely to suffer photobleaching which results in loss of anticancer activity (Abrahamse & Hamblin, 2017). In order to solve this issue, new generation of photothermal nanoscale agents, such as AuNPs, with greater absorption effectiveness and without suffering photobleaching have been introduced as new anti-cancer treatment (Abadeer & Murphy, 2016).

Usually this treatment relies on the supply of oxygen in tumours, but as the commonly used drug agent can be replaced with nanoparticles and laser application, there is no such limitation and can be used as alternative methods. Different type of laser can be used depending on the maximum peak absorption of nanoparticles. The combination of these applications of nanoparticles and laser eliminates cancer cells via two mechanisms (Fekrazad *et al*., 2016). First, the nanoparticles conjugated to the cell surface molecules of cancer cells. The laser light then exposed to targeted area releasing heat onto the nanoparticles and causing the selective death of cancer cells.

For the second mechanism is via murine macrophage. Some studies used macrophage as nanocarriers, through phagocytosis. The function of macrophages is to digest alien material in the body system, so they easily move due to their migration capability and can surround cancer cells (Fekrazad *et al*., 2016).

As stated by Farivar *et al*. (2014), gold nanospheres of 30 nm diameter were chosen as potential drug agent based on their pros and cons and 530 nm green light emitting diode (LED) of 30 mW used as irradiation source to study the death effect of Vero, Hela and Hep 2 cell lines. They observed localised apoptotic and necrosis in irradiated spot, leading to irreversible cell. Pasparakis (2013) found that the 40 nm AuNPs could generate $^1O_2$ upon either 532 nm pulse or continuous wave laser irradiation. Most previous studies had been done in this field using combination between near-infrared range (NIR) laser with AuNPs. In 2012, Ma *et al*. reported that AuNPs capped with magnetic core exposed to 808 nm at power density of 2.0 W/cm^2 for 5 minutes showed a cytotoxic effect on MCF-7 cells.

2.5.1 Physical Mechanism of Nanoparticles and Laser Irradiation

When the AuNP localised within cancer cell, the electron in nanoparticles will absorb photon from the laser as shown in Figure 2.7. The electron then will boost up into higher energy level state and emit photoelectrons by lost it energy. The emitted photoelectrons then transfer a photon or electron to form a free radical anion or radical cation. These radicals may further interact with oxygen to produce Reactive Oxygen Species (ROS) and cause significant cytotoxicity effect leading to cell death via apoptosis or necrosis (Dayem, Hossain, & Lee, 2017). Since the lifetime of these radicals is very short, only the targeted area is directly affected by the therapy.

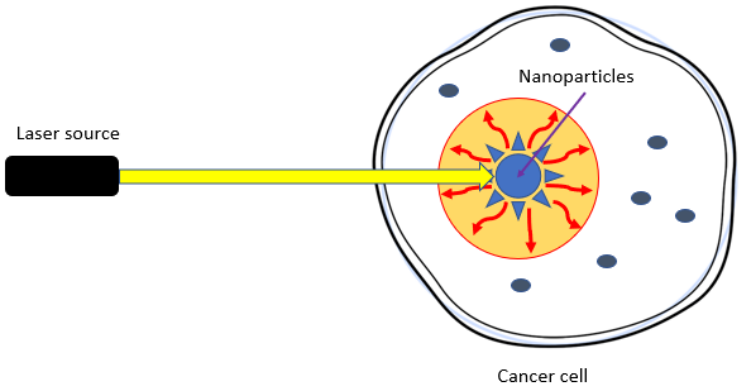


Figure 2.7 Schematic diagram of Nano-laser irradiation on cancer cell tissue

2.6 MCF-7 Cancer cells line

Human breast tumour cell lines, MCF-7 cells are commonly used in research that has been propagated for many years by multiple groups. MCF-7 is the acronym of Michigan Cancer Foundation-7 which has been isolated from the pleural effusion of a 69-year-old woman with metastatic disease (Levenson & Jordan, 1997). The cell line demonstrates to be an appropriate candidate cell line for studies of breast cancer around the world, including those related to anticancer drugs (Sweeney *et al*., 2012). As in Figure 2.8, the image of cell line from The European Collection of Authenticated Cell Cultures (ECACC) shows an epithelial-like shape and monolayers form dome structures of the cells.

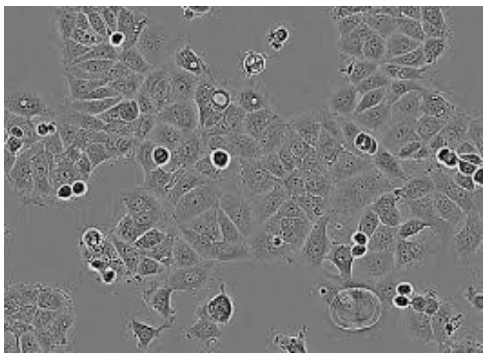


Figure 2.8 Morphology of MCF-7 Cells 48 h post seeding (Cooper, 2012).

CHAPTER 3

MATERIALS & METHODS

3.1 Introduction

This chapter consists of materials and experimental procedure used for studying the effects of AuNPs on MCF-7 Cell treatment by laser irradiation in cell culture. The experimental methods are divided into several subtopics. The first part of subtopics describes the preparation and characterisation of AuNPs followed by the cell culture protocols for biological sample preparation. This chapter also includes the procedure for treatment and cytotoxicity test. Then, the crucial part of this research is the laser irradiation procedure using 532 nm green laser with AuNPs as a drug agent. Figure 3.1 shows the flow chart summaries the procedure for this experiment.