

THE EFFECT OF LASER IRRADIATION ON SURVIVABILITY OF BREAST
CANCER CELLS SENSITISED WITH GOLD NANOPARTICLES

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Specially dedicated to my beloved mom, Zaiton Hitam, dad,
Badruzzaman Hamzah, and husband, Suhaimi Osman,

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ABSTRACT

Breast cancer is among the major killer diseases for women. Currently chemotherapy is the common method used for such cancer treatment. The drawback with this technique is that it kills both normal and cancerous cells. As a result not many cancer patients can survive after the treatment. Thus, alternative techniques need to be considered. The aim of this project is to introduce laser radiation instead of x-ray for cancer treatment. In order to further enhance the hyperthermia effect, gold nanoparticles (AuNP) are injected to couple with cancerous cell. In this work, laser irradiation with three different wavelengths were used for the treatment namely, 1064 nm, and 532 nm produced by Q-switched Nd:YAG laser and 248 nm produced by Krypton Fluoride Excimer laser. AuNPs were produced using pulse laser ablation in liquid technique. The AuNP diameter was varied in the range of 8-18 nm and concentration was prepared by serial dilution within the range of 0.14-4.50 $\mu\text{g/ml}$. Three different human breast cancer cell lines (MDA-MB-231, MDA-MB-468, and MDA-kb2) and a Chinese Hamster Ovary (CHO) non-cancerous cell were used to study the cell survivability towards hyperthermia effect with and without the presence of AuNPs. The cell survivability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and analyzed using GloMax Multi Microplate Multimode Reader. The results show that survivability rate of MDA-MB-231 cell was 84 percent after treated with 1064 nm laser compared to 74 percent after treated with 532 nm laser. UV radiation from Excimer laser is found to increase the cell survivability rate up to 157 percent. The survivability rate of CHO cell treated with 532 nm laser dropped by 9 percent compared to those of MDA-MB-231, MDA-MB-468 and MDA-kb2 cells which decreased by more than 20 percent after the treatment. The total number of survival cell decreases with the presence of AuNPs. The results indicate that higher concentration and smaller size of AuNPs minimized the MDA-MB-231 breast cancer cell survivability. Therefore, hyperthermia effect can be enhanced by using laser irradiation sensitized with AuNP and has great potential for treating cancer.

ABSTRAK

Kanser payudara adalah antara penyakit pembunuh utama bagi wanita. Pada masa ini kemoterapi ialah kaedah yang biasa digunakan untuk rawatan kanser tersebut. Kelemahan teknik ini adalah ia membunuh kedua-dua sel normal dan kanser. Akibatnya tidak ramai pesakit kanser boleh bermandiri selepas rawatan. Oleh itu, teknik alternatif perlu dipertimbangkan. Tujuan projek ini adalah untuk memperkenalkan sinaran laser sebagai gantian sinaran-x untuk rawatan kanser. Bagi meningkatkan lagi kesan hipertermia, nanopartikel emas (AuNP) disuntik untuk bergabung dengan sel kanser. Dalam projek ini, sinaran laser dengan tiga panjang gelombang yang berbeza telah digunakan untuk rawatan iaitu 1064 nm dan 532 nm yang dihasilkan oleh laser Nd: YAG yang bersuiz-Q dan 248 nm yang dihasilkan oleh laser Eksimer Krypton Fluorida. AuNP telah dihasilkan menggunakan teknik ablasi laser denyut dalam cecair. AuNP telah disediakan dalam pelbagai diameter di dalam lingkungan 8-18 nm dan kepekannya disediakan dengan menggunakan teknik pencairan bersiri dalam julat 0.14-4.50 µg/ml. Tiga turunan sel kanser payudara manusia yang berbeza (MDA-MB-231, MDA-MB-468, dan MDA-kb2) dan satu sel bukan kanser, Chinese Hamster Ovari (CHO) telah digunakan untuk mengkaji kadar kemandirian sel terhadap kesan hipertermia dengan dan tanpa kehadiran AuNP. Kadar kemandirian sel telah ditentukan dengan menggunakan cerakin 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromida (MTT) dan dianalisis menggunakan *GloMax Multi Microplate Multimode Reader*. Keputusan menunjukkan bahawa kadar kemandirian sel MDA-MB-231 ialah 84 peratus selepas dirawat dengan sinaran laser 1064 nm berbanding 74 peratus selepas dirawat dengan sinaran laser 532 nm. Sinaran UV daripada laser Eksimer didapati meningkatkan kadar kemandirian sel sehingga 157 peratus. Kadar kemandirian sel CHO selepas dirawat dengan sinaran laser 532 nm menurun sebanyak 9 peratus berbanding dengan kadar kemandirian sel MDA-MB-231, MDA-MB-468 dan MDA-kb2 yang menurun lebih daripada 20 peratus selepas rawatan. Jumlah sel yang hidup berkurangan dengan kehadiran AuNP. Hasil kajian menunjukkan bahawa AuNP dengan kepekatan yang lebih tinggi dan saiz yang lebih kecil boleh mengurangkan kadar kemandirian sel MDA-MB-231. Oleh itu, kesan hipertermia boleh dipertingkatkan dengan menggunakan sinaran laser dengan AuNP terpeka dan teknik ini mempunyai potensi besar dalam rawatan kanser.

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LIST OF ABBREVIATIONS

°C	-	Degree Celsius
<i>A</i>	-	Absorbance Value
AuNPs	-	Gold Nanoparticles
BSC	-	Biosafety Cabinet
CHO	-	Chinese Hamster Ovary
DMEM	-	Dulbecco's Modified Eagle's Medium
DMSO	-	Dimethyl Sulfoxide
DNA	-	Deoxyribonucleic Acid
ECM	-	Extracellular Matrix
EDTA	-	Ethylenediaminetetraacetic Acid
EFTEM	-	Energy-Filtered Transmission Electron Microscopy
EGFR	-	Epithelial Growth Factor Receptor
EPR	-	Permeability And Retention Effect
EtOH	-	Ethanol
F	-	Fahrenheit
FBS	-	Fetal Bovine Serum
FDA	-	US Food And Drug Administration
HeLa	-	Human Cervical Cancer Cell
HEPA	-	High-Efficiency Particulate Arrestance
Hz	-	Hertz
J	-	Joule
KrF	-	Krypton Fluoride
L	-	Liter
<i>M</i>	-	Cell Density
MDA-Kb2	-	Human Breast Cancer Cell

MDA-MB-231	-	Human Breast Cancer Cell
MDA-MB-468	-	Human Breast Cancer Cell
MIR	-	Mid-Infrared
MRI	-	Magnetic Resonance Imaging
MTT	-	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n	-	Reflective Index
Nd:YAG	-	Neodymium-doped yttrium aluminium garnet
Nd^{+3}	-	Triply Ionized Neodymium
NIR	-	Near-Infrared
θ	-	Angle of Light Ray
Pb	-	Lead
PBS	-	Phosphate-Buffered Saline
PDT	-	Photodynamic Therapy
PLAL	-	Pulse laser ablation in liquid
PTT	-	Photothermal Therapy
RNA	-	Ribonucleic Acid
RPM	-	Rounds Per Minute
s	-	Second
TEM	-	Transmission Electron Microscopy
UV	-	Ultra Violet
V	-	Volt
V	-	Volume
V	-	Volt

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CHAPTER 1

INTRODUCTION

1.1 Overview

The application of laser induced thermal to eliminate, kill or restrain cancer cells is widely acknowledged approach in enhancing and improving cancer therapy (Wust et al. 2002). This non-invasive technique in eliminating cancer cells is generally referred as thermotherapy or hyperthermia. As the temperature raised, it can cause the denature of intracellular protein and the disruption of membrane, leading to death of cell (Liu et al. 1997). The performance of thermotherapy is influenced by power density of the heating source, as well as the energy absorption and thermal conductance of the biological environment (Habash et al. 2007).

Thermotherapy causes far fewer restrictive side effects than conventional chemotherapy and radiotherapy. It also has greater potential in evading any development of intracellular resistance mechanism. It is beneficial in dealing with some types of malignant tumor cells (van der Zee 2002). Thermotherapy is still under investigation.

The main challenge is to achieve highly localized thermal effect on tumor cells with defined lesion boundaries within a short period of time (Bayazitoglu et al. 2013). Recently, thermotherapy based on near infrared (NIR) irradiation-activated nanomaterials has received significant attention due to their efficacy in killing cancer cells. This is because of the unique optical and thermal properties of nanomaterials that gained significant interest (Terentyuk et al. 2013). This is a minimal invasive and localized treatment for cancer which lead towards the development of gold nanomaterials as novel light absorbing agents (Jain et al. 2012). The application of nanoparticles as exogenous agents in laser therapy or photothermal therapy is rapidly expand to include nanostructures of various geometries and composition (Zhang 2010). The particles will enhance the magnitude of light absorption resulting in more precise delivery of energy at low laser powers and prevent healthy tissue nearby from damage. However, the approach is by no means perfect, and the investigation into exploring superior materials and methodology is still crucial (Nguyen, 2012).

Gold nanoparticles as photothermal therapy agents absorb light and cause electron transition from ground state to excited state. This transition causes the increases of kinetic energy that lead to overheat of the surrounding from the light absorption species. The production of heat can lead to destruction of cells called hyperthermia effect (Huang et al. 2008). Recently, many researchers have done investigations about the application of gold nanoparticles in photothermal therapy on cancer treatment. Hainfeld et al. 2010 had studied by using x-rays source (ionization radiation) and enhanced the radiation therapy by gold nanoparticles to treat the mouse head and neck squamous cell carcinoma model, SCCVII. The harmful effects from X-rays and its related scan usually become significant after a person had substantial number of high radiation-dose treatment.

Gold nanoparticles (AuNPs), echogenic nanoparticles and iron oxide nanoparticles have been proposed as injectable agents in order to improve the localized delivery of heat in thermal therapies. In particular, AuNPs shown to be an ideal agent in enhancing laser-based thermal therapies mostly because of their

tunable optical properties and surface plasmon resonance effect (Paulo et al. 2013; Hirsch et al. 2006). Van der Zee (2002), had used two oral squamous carcinoma cell lines (HSC 313 and HOC 3 Clone 8) and one benign epithelial cell line (HaCaT) which incubated with anti-epithelial growth factor receptor (EGFR) antibody conjugated gold nanoparticles. Then, it exposes with continuous visible argon ion laser at 514 nm. They found that the malignant cells required less than half of the laser energy to kill than the benign cells after incubation with anti-EGFR antibody conjugated Au nanoparticles. However, it is reported that there are harmful effects of EGFR inhibitors that found in more than 90% of patients, which is a papulopustular rash that spreads across the face and chest. The presence of rash is correlated to antitumor effect of EGFR. In about 10% to 15% of affected patients were critical and required treatments (Bayazitoglu et al. 2013).

In this work, the effects of the gold nanoparticles (AuNPs) in photothermal therapy was studied by using Kr₂ Excimer laser of 248 nm and Q-switched Nd:YAG laser of 1064, and 532 nm. The survivability of human breast cells MDA-MB-231, MDA-MB-468, and MDA-Kb2, were investigated and the results were compared with normal cell from Chinese Hamster Ovary (CHO).

1.2 Problem Statement

Breast cancer is the most common cancer in the world with one out of eight women having cancer in Malaysia and 80% of the them are over forty years old, but still younger women and men, also can get breast cancer. Latest, in Feb 2014 in Kelantan, six years old girl was detected with breast cancer (Pride Foundation). From the researches, eight out of ten will not survive due to late detection cancer.

Currently breast cancer treatment is through chemotherapy which involves chemical and x-ray radiation. The problem with such chemical treatment is depending on the stages and immunity of the body. Plus the x-ray is conventional radiation which penetrates into the body and killing not only the cancer cells but also the normal ones. As a result such treatment prone to increase the number of death instead of saving life, therefore alternative route need to be considered. Nowadays many researches are conducted to find the solution. To throw some light on this matter, a fundamental study is carried out to look at the potential of laser to replace the x-ray. Furthermore to enhance the absorption of laser radiation on the cancer cell, inert metal such as gold nanoparticles are employed. Through the best of our knowledge during this study, this is the first intension of using laser in cancer cell treatment. Hopefully the effort to introduce laser in the cancer treatment will open the window for optical technology to be involved in the medical industry.

1.3 Research Objectives

The main objective of this project is to determine the effects of laser heating on cancer cell associated with gold nanoparticles. In attempt to achieve this goal various studies will be carried out including:

- i. To characterize the fabricated gold nanoparticles (AuNPs) by pulse laser ablation in liquid (PLAL) process
- ii. To determine the effects of irradiated cancer cell to laser with various parameters including different wavelength, energy density, and exposed duration.
- iii. To determine the effects of irradiated cancer cells associates with gold AuNPs at different concentration and size with laser

1.4 Research Scope

In this study, breast cancer cells MDA-MB-231, MDA-MB-468, MDA-Kb2 and normal cell from Chinese human ovary, CHO. All cells are cultured in Faculty of Bioscience and Medical Engineering, UTM and used as a sample to interact with non-ionized radiation. Gold nanoparticle (AuNPs) was fabricated by pulse laser ablation in liquid (PLAL) process and will be sterilized by several treatment techniques including ethanol, autoclaving, and ultraviolet exposure. The fabricated AuNPs will be characterized using Energy-filtered transmission electron microscopy (EFTEM) and the interested concentration and size will be verified. Kr2 Excimer, and Nd:YAG laser with tuneable wavelength including fundamental, and second harmonic generation were utilized as non-ionization sources. Energy density, and exposed duration for each of the source radiation will verified. The cell survivability rates after exposure were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay to observe the effect of cancer cell before and after radiation. Cell changes in morphology will be observed by inverted microscope.

1.5 Research Significance

The Effect of Laser Irradiation to Nanoparticles on Cancer Cells study will guide to the potential advantages such as

- i. Localized cancer treatment without harmful effects on surrounding healthy tissues
- ii. Reduce or eliminate undesired side effects in comparison to chemotherapy
- iii. Potentially fast and economy treatment that involve just a few laser shots
- iv. Patients will have less pain, bleeding, swelling, and scarring as laser are more precise and less likely to get infections compared to traditional surgeries

1.6 Thesis Outline

Chapter 1 describes the overview of the thesis, problem statement, list of objectives and the scope of the research. The complete theory of hyperthermia effect is explained in Chapter 2. Several major problems in laser on cancer treatment process are described briefly. It is followed by the literature review of different methods of implementation and techniques used as compared to this research.

The subculture techniques, AuNPs fabrication process, AuNPs sterilization techniques, laser experimental setups, and MTT cell survivability assay are covered in Chapter 3. The media, reagent, and material used in the process are specified. Sample preparation for several characterization techniques used is also included.

All the results are presented and discussed in Chapter 4. The initial work comprised of laser system calibration, cell optimization, and cell morphology observation. This is followed by the explanation of laser heating effect on human breast cancer cells, and normal cells combined with different concentration and size of AuNPs. Finally the cell changes in morphology is discussed. Chapter 5 is the conclusion and the summary of the results achieved from the experimental works together with some recommendations for future study.

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