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

AISHAH BADRUZZAMAN

UNIVERSITI TEKNOLOGI MALAYSIA

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

AISHAH BADRUZZAMAN

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Physics)

Faculty of Science
Universiti Teknologi Malaysia

FEBRUARY 2017

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

ACKNOWLEDGEMENT

Alhamdulillah, first praise is to Allah SWT, the Almighty, on whom ultimately we depend for sustenance and guidance. Second, my sincere appreciation goes to my supervisor, Prof. Dr. Noriah Bidin and co-supervisor Dr. Pauliena Mohd Bohari for thier kindness and patience within these years of undergoing this research. Their guidance and supervision, I truly appreciate, with all my heart. May He, Ar-Rahman, bless both of them.

My sincere thanks also go to my Tissue Engineering lab mates, Kavita and Anis who help me a lot in cell culture technique. My appreciation also goes to Dr. Syafiq Affandi, Dr. Munzir, Dr. Ganesan and Mr. Aizat for encouragement, insightful comments and assistant while conducting the experiment. On behalf of it, I thank my fellow labmates under the research group of Laser Center for the stimulating discussion and moral support throughout the years.

Last but not least, deepest gratefulness and appreciation goes to my parents, (Badruzzaman and Zaiton), husband (Suhaimi) and my family members for their support spiritually throughout my life, to whom this thesis is dedicated. Thanks are address to the Malaysian Ministry of Education for the financial support given for me to pursue my Ph.D. Program here in the Universiti Teknologi Malaysia (UTM).

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 µg/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 Kripton Fluorida. AuNP telah dihasilkan menggunakan teknik ablasi laser denyut dalam cecair. AuNP telah disediakan dalam pelbagai diameter di dalam lingkungan 8-18 nm dan kepekatannya 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	-	Degree Celsius
A	-	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
M	-	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

LIST OF APPENDICES

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 Kr_2 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.

REFERENCES

Atkinson, Rachel L. Zhang, Mei Diagaradjane, Parmeswaran Peddibhotla, Sirisha Contreras, Alejandro Hilsenbeck, Susan G. Woodward, Wendy A. Krishnan, Sunil (2010). Thermal Enhancement with Optically Activated Gold Nanoshells Sensitizes Breast Cancer Stem Cells to Radiation Therapy. *Science Translational Medicine*, 2(55), 55-79.

Bass, H. Moore, J.L., (1978). Lethality In Mammalian Cells Due To Hyperthermia Under Oxic And Hypoxic Conditions. *International Journal of Radiation Biology*, 33(1), 57–67.

Bayazitoglu, Y., Kheradmand, S. Tullius, T.K., (2013). An Overview Of Nanoparticle Assisted Laser Therapy. *International Journal of Heat and Mass Transfer*, 67, 469–486.

Bernardi R.J., Lowery A.R., Thompson P.A., Blaney S.M., West J.L. (2008). Immunonanoshells For Targeted Photothermal Ablation In Medulloblastoma And Glioma : An In Vitro Evaluation Using Human Cell Lines. *Journal of Neuro-Oncology*, 86, 165–172.

Bleehen, N.M., (1982). Hyperthermia In The Treatment Of Cancer. *British Journal of Cancer,* 45,96–100.

Cerussi, A., Gratton, E. & Gratton, E., (2011). Breast Cancer Spatial Heterogeneity In Near-Infrared Spectra And The Prediction Of Neoadjuvant Chemotherapy Response Breast Cancer Spatial Heterogeneity In Near-Infrared Spectra And The Prediction Of Neoadjuvant. *Journal of Biomedical Optics*, 16(9), 97007.

Chang, M. et al., 2008. Increased Apoptotic Potential And Dose-Enhancing Effect Of Gold Nanoparticles In Combination With Single-Dose Clinical Electron Beams On Tumor-Bearing Mice. *Cancer Science*, 99(701), 1479–1484.

Cheng, F., Chen, C. Yeh, C., (2009). Comparative Efficiencies Of Photothermal Destruction Of Malignant Cells Using Antibody-Coated Silica @ Au Nanoshells , Hollow Au / Ag Nanospheres And Au Nanorods. *Nanotechnology*, 20(425104).

Cho K., Wang X., Nie S., Chen Z.G., Shin D.M. (2008). Therapeutic Nanoparticles for Drug Delivery in Cancer Therapeutic Nanoparticles for Drug Delivery in Cancer. *Clinical Cancer Research*, 14(5),1310–1316.

Chu M., Shao Y., Peng J., Dai X., Li H., Wu Q., Shi D. (2013). Near-Infrared Laser Light Mediated Cancer Therapy By Photothermal Effect Of Fe_3O_4 Magnetic Nanoparticles. *Biomaterials*, 34(16),4078-88.

Curry, A.C. Crow, M.,(2015). Molecular Imaging Of Epidermal Growth Factor Receptor In Live Cells With Refractive Index Sensitivity Using Dark-Field Microspectroscopy And Immunotargeted Nanoparticles. *Journal of Biomedical Optics*, (13),1–7.

Dennis, D., Fukumura, D. Jain, R. k., (2003). Photodynamic Therapy For Cancer Skin Of Mice. *Nature Review Cancer*, 3(May), 380–387.

Diagaradjane P., Shetty A., Wang J.C., Elliott A.M., Schwartz J., Shentu S., Park H.C., Deorukhkar A., Stafford R.J., Cho S.H., Tunnell J.W., Hazle J.D., Krishnan S. (2008). Modulation of in Vivo Tumor Radiation Response via Gold Nanoshell-Mediated Vascular-Focused Hyperthermia : Characterizing an Integrated Antihypoxic and Localized Vascular Disrupting Targeting Strategy. *Nano Letters*, 8(5),1492–1500.

Dickerson E.B., Dreaden E.C., Huang X., El-Sayed I.H., Chu H., Pushpanketh S., McDonald J.F., El-Sayed M.A. (2008). Gold Nanorod Assisted Near-Infrared Plasmonic Photothermal Therapy (PPTT) Of Squamous Cell Carcinoma In Mice. *Cancer Letters*, 269(1), 57–66.

Farma J.M., Puhlmann M., Soriano P.A., Cox D., Paciotti G.F., Tamarkin L., Alexander H.R. (2007). Direct Evidence For Rapid And Selective Induction Of Tumor Neovascular Permeability By Tumor Necrosis Factor And A Novel Derivative , Colloidal Gold Bound Tumor Necrosis Factor. *International Journal of Cancer*, 120, 2474–2480.

Fuller K.J., Issels R.D., Slosman D.O., Guillet J.G., Soussi T., Polla B.S. (1993). Cancer and the Heat Shock Response. *European Journal of Cancer*, 30A(12), 1884–1891.

Ganesh Gollavelli, Ling, Y.C., (2014). Magnetic and Fluorescent Graphene for Dual Modal Imaging and Single Light Induced Photothermal and Photodynamic Therapy of Cancer Cells. *Biomaterials*, 35(15), 4499–4507.

Gannon C.J., Patra C.R., Bhattacharya R., Mukherjee P., Curley S.A. (2008). Intracellular Gold Nanoparticles Enhance Non-Invasive Radiofrequency Thermal Destruction Of Human Gastrointestinal Cancer Cells. *Journal of Nanobiotechnology*, 6(2),1–9.

Habash, R.W.Y., Bansal, R. Krewski, D., (2007). Thermal Therapy , Part IV : Electromagnetic and Thermal Dosimetry. *Critical Reviews™ in Biomedical Engineering*, 35(1–2), 123–182.

Hainfeld, J.F., Dilmanian, F.A. Zhong, Z., (2010). Gold Nanoparticles Enhance The Radiation Therapy Of A Murine Squamous Cell Carcinoma. *Physics in Medicine and Biology*, 55,3045–3059.

Hakil, A.B.S., Sborn, J.A.L.O. Ong, C.H.W.S., (1999). Changes In Oxygenation Status And Blood Flow In A Rat Tumor Model By Mild Temperature Hyperthermia. *International Journal of Radiation Oncology * Biology * Physics*, 43(4), 859–865.

Harmont, B. V, Takano, Y.S. Winterford, C.M., (1991). The Role Of Apoptosis In The Response Of Cells And Tumours To Mild Hyperthermia. *International Journal of Radiation Biology*, 59(2), 489–501.

Hartman, K.B., Wilson, L.J.,Rosenblum, M.G., (2008). Detecting and Treating Cancer with Nanotechnology. *Molecular Diagnosis & Therapy*, 12(1), 1–14.

Hildebrandt B., Wust P., Ahlers O., Dieing A., Sreenivasa G., Kerner T., Felix R., Riess H. (2002). The Cellular And Molecular Basis Of Hyperthermia. *Critical Reviews in Oncology/Hematology*, 43, 33–56.

Hirsch L.R., Gobin A.M., Lowery A.R., Tam F., Drezek R.A., Halas N.J., West J.L. (2006). Metal Nanoshells. *Annals of Biomedical Engineering*, 34(1), 15–22.

Hirsch L.R., Stafford R.J., Bankson J.A., Sershen S.R., Rivera B., Price R.E., Hazle J.D., Halas N.J., West J.L. (2003). Nanoshell-Mediated Near-Infrared Thermal Therapy Of Tumors Under Magnetic Resonance Guidance. *Proceedings of the National Academy of Sciences of the United States of America*, 100(23), 13549–54.

Holliday, D.L. Speirs, V., (2011). Choosing The Right Cell Line For Breast Cancer Research. *Breast cancer research* , 13(4), p.215.

Huang, X., Jain, P.K. El-sayed, I.H., (2008). Plasmonic Photothermal Therapy (PPTT) Using Gold Nanoparticles. *Lasers in Medical Science*, 23, 217–228.

Hulander, M. Hong, J. Andersson, M Gerve, F. Ohrlander, M. Tengvall, P. Elwing, Hm, (2009). Blood Interactions with Noble Metals : Coagulation and Immune Complement Activation. *Applied Materials & Interfaces*, 1(5), 1053–1062.

Hwang H.Y., Kim I.S., Kwon I.C., Kim Y.H. (2008). Tumor Targetability And Antitumor Effect Of Docetaxel-Loaded Hydrophobically Modified Glycol Chitosan Nanoparticles. *Journal of Controlled Release*, 128, 23–31.

Iancu, C., (2013). Photothermal Therapy of Human Cancers (PTT) Using Gold Nanoparticles. *Biotechnology, Molecular Biology, and Nanomedicine*, 1(1).

Jain P.K., Lee K.S., El-Sayed I.H., El-Sayed M.A. (2006). Calculated Absorption and Scattering Properties of Gold Nanoparticles of Different Size , Shape , and Composition : Applications in Biological Imaging and Biomedicine. *The Journal of Physical Chemistry B*, 110, 7238–7248.

Jain S., Hirst D.G., O'Sullivan J.M. (2012). Gold Nanoparticles As Novel Agents For Cancer Therapy. *The British Journal of Radiology*, 85(February), 101–113.

Kam, N.W.S., Liu, Z. Da, H., (2006). Carbon Nanotubes as Intracellular Transporters for Proteins and DNA: An Investigation of the Uptake Mechanism and Pathway. *Angewandte Chemie International Edition*, 45(4), 577–581.

Kimberly A. Kelly, Sunita R. Setlur, Robert Ross, Rajesh Anbazhagan, Peter Waterman, Mark A. Rubin, and Ralph Weissleder (2008). Detection of Early Prostate Cancer Using a Hepsin-Targeted Imaging Agent. *Cancer Research*, 68(7), 2286–2292.

Knight, M.W. Halas, N.J., (2008). Nanoshells To Nanoeggs To Nanocups: Optical Properties Of Reduced Symmetry Core–Shell Nanoparticles Beyond The Quasistatic Limit. *New Journal of Physics*, 10, 105006.

Kou G. Wang S. Cheng C. Gao J. Li B. Wang H. Qian W. Hou S. Zhang D. Dai J. Gu H. Guo Y. (2008). Biochemical And Biophysical Research Communications Development Of SM5-1-Conjugated Ultrasmall Superparamagnetic Iron Oxide Nanoparticles For Hepatoma Detection. *Biochemical and Biophysical Research Communications*, 374, 192–197.

Jing-Liang Li, Min Gu (2010). Gold-Nanoparticle-Enhanced Cancer Photothermal Therapy. *IEEE Journal of Selected Topics in Quantum Electronic*, 16(4), 989–996.

Li S., Wang A., Jiang W., Guan Z. (2008). Pharmacokinetic Characteristics And Anticancer Effects Of 5-Fluorouracil Loaded Nanoparticles. *BMC Cancer*, 8(103).

Link, S. El-Sayed, M.A., (2000). Shape And Size Dependence Of Radiative, Non-Radiative And Photothermal Properties Of Gold Nanocrystals, *International Reviews in Physical Chemistry*, 19 (3), 409-453

Liu D.L., Andersson-Engels S., Sturesson C., Svanberg K., Håkansson C.H., Svanberg S. (1997). Tumour Vessel Damage Resulting From Laser-Induced Hyperthermia Alone And In Combination With Photodynamic Therapy. *Cancer letters*, 111(1–2), pp.157–65.

Liu Z., Cai W., He L., Nakayama N., Chen K., Sun X., Chen X., Dai H. (2006). In Vivo Biodistribution And Highly Efficient Tumour Targeting Of Carbon Nanotubes In Mice. *Nature Nanotechnology*, 2, 47–52.

Lu S., Xia D., Huang G., Jing H., Wang Y., Gu H. (2010). Colloids And Surfaces B : Biointerfaces Concentration Effect Of Gold Nanoparticles On Proliferation Of Keratinocytes. *Colloids and Surfaces B: Biointerfaces*, 81(2), .406–411.

Matson, J.B.. Grubbs, R.H., (2008). Synthesis of Fluorine-18 Functionalized Nanoparticles for use as in vivo Molecular Imaging Agents. *Journal of the American Chemical Society*, 130, 6731–6733.

Milleron, R.S. Bratton, S.B., (2007). Heated Debates in Apoptosis. *Cellular and Molecular Life Sciences*, 64, 2329–2333.

M. R. Arnfield, R. P. Mathew, J. Tulip and M. S. McPhee (1992). Analysis Of Tissue Optical Coefficients Using An Approximate Equation Valid For Comparable Absorption And Scattering. *Physics in Medicine and Biology*, 37(6), 1219–1230.

Mukherjee Priyabrata, Resham Bhattacharya, Ping Wang, Ling Wang, Sujit Basu, Janice A. Nagy, Anthony Atala, Debabrata Mukhopadhyay, Shay Soker (2005). Preclinical Antiangiogenic Properties of Gold Nanoparticles. *Clinical Cancer Research* , 11(9), 3530–3535.

Mukherjee P., Bhattacharya R., Bone N., Lee Y.K., Patra C.R., Wang S., Lu L., Secreto C., Banerjee P.C., Yaszemski M.J., Kay N.E., Mukhopadhyay D. 2(007). Potential Therapeutic Application Of Gold Nanoparticles In B-Chronic Lymphocytic Leukemia (BCLL): Enhancing Apoptosis. *Journal of Nanobiotechnology*, 13, 1–13.

Neumaier C.E., Baio G., Ferrini S., Corte G., Daga A. (2008). MR And Iron Magnetic Nanoparticles . Imaging Opportunities In Preclinical And Translational Research. *Tumori*, pp.226–233.

Nguyen, K.T., (2012). Bioengineering & Biomedical Science Photothermal Therapy and Nanomaterials. *Theranostics* , 3(3): 152–166..

No, M.U.R.A., Kuroda, M. ishimur, Y.A., (1999). For the clinical application of thermochemotherapy given at mild temperatures. *International Journal of Hyperthermia* , 15(2):79-107

O'Neal, D.P. Leon R. Hirschb, Naomi J. Halasc, J. Donald P., Jennifer L. West, (2004). Photo-Thermal Tumor Ablation In Mice Using Near Infrared-Absorbing Nanoparticles. *Cancer Letters*, 209(2), 171–176.

Opanasopit P., Apirakaramwong A., Ngawhirunpat T., Rojanarata T., Ruktanonchai U. (2008). Development and Characterization of Pectinate Micro / Nanoparticles for Gene Delivery. *AAPS PharmSciTech*, 9(1):67-74.

Patra CR1, Bhattacharya R, Wang E, Katarya A, Lau JS, Dutta S, Muders M, Wang S, Buhrow SA, Safgren SL, Yaszemski MJ, Reid JM, Ames MM, Mukherjee P, Mukhopadhyay D. 2008. Targeted Delivery of Gemcitabine to Pancreatic Adenocarcinoma Using Cetuximab as a Targeting Agent.*Cancer Research* , 15;68(6):1970-8

Powell K., Low P., McDonnell P.A., Laakso E.L., Ralph S.J. (2010). The Effect of Laser Irradiation on Proliferation of Human Breast Carcinoma, Melanoma, and Immortalized Mammary Epithelial Cells. *Photomedicine and Laser Surgery*, 28(1), 115–123.

R. D.K. Misra, (2008). Quantum Dots For Tumor-Targeted Drug Delivery And Cell Imaging. *Nanomedicine*, 3(3), 271–274.

Richard, C., Chermont, M. De, Scherman, D., (2008). Nanoparticles For Imaging And Tumor Gene Delivery. *Tumori* , 94(2), 264-70.

Samantaa, B. Haoheng Y., Nicholas O. Fischer, Jing Shi, D. Joseph J., Vincent M. Rotello, (2009). Protein-Passivated Fe3O4 Nanoparticles: Low Toxicity And Rapid Heating For Thermal Therapy. *Journal of Materials Chemistry*, 18(11), 1204–1208.

Skrabalak S.E., Au L., Lu X., Li X., Xia Y., (2007). Gold Nanocages For Cancer Detection And Treatment. *Nanomedicine*, 2(5), 657–668.

Sra Amandeep K., Carlos Bárcena, Girija S. Chaubey, Chalermchai Khemtong, J. Ping Liub Jinming Gao, (2008). Zinc Ferrite Nanoparticles As MRI Contrast Agents. *Chemical Communications*, 2224–2226.

Steiner, R., (2011). Laser-Tissue Interactions. *Laser and IPL Technology in*

Dermatology and Aesthetic Medicine, 23-36

Steven A. Curley, (2001). Radiofrequency Ablation of Malignant Liver Tumors. *The Oncologist*, 6(1), 14–23.

Sumer, B., Jinming Gao, (2008). Theranostic Nanomedicine For Cancer. *Nanomedicine*, 137–140.

Terentyuk G.S., Maslyakova G.N., Suleymanova L.V., Khlebtsov N.G., Khlebtsov B.N., Akchurin G.G., Maksimova I.L., Tuchin V.V., (2013). Laser-Induced Tissue Hyperthermia Mediated By Gold Nanoparticles : Toward Cancer Phototherapy. *Journal of Biomedical Optics* , 14(2), 021016.

Visaria R.K., Griffin R.J., Williams B.W., Ebbini E.S., Paciotti G.F., Song C.W., Bischof J.C., (2006). Enhancement Of Tumor Thermal Therapy Using Gold Nanoparticle-Assisted Tumor Necrosis Factor-Alpha Delivery. *Molecular Cancer Therapeutics*, 5(4), 1014-20.

Wang, A.,Li, S., (2008). Hydroxycamptothecin-Loaded Nanoparticles Enhance Target Drug Delivery And Anticancer Effect. *BMC Biotechnology*, 4;8:46

Wu J., Yan F., Zhang X., Yan Y., Tang J., Ju H., (2008). Disposable Reagentless Electrochemical Immunosensor Array Based On A Biopolymer/Sol-Gel Membrane For Simultaneous Measurement Of Several Tumor Markers. *Clinical Chemistry*, 1488, pp.1481–1488.

Wust P., Hildebrandt B., Sreenivasa G., Rau B., Gellermann J., Riess H., Felix R., Schlag P.M., (2002). Hyperthermia In Combined Treatment Of Cancer. *The lancet Oncology*, 3(8), 487–497.

Xiao J. W., Shi-Xuan Fan, Feng Wang, Ling-Dong Sun, Xiao-Yu Zhenga, Chun-Hua Yan, (2014). Porous Pd Nanoparticles With High Photothermal Conversion E Ffi Ciency For E Ffi Cient Ablation Of Cancer Cells. *Nanoscale* , 6, 4345–4351.

Yuan A., Wu J., Tang X., Zhao L., Xu F., Hu Y., (2013). Application of Near-Infrared Dyes for Tumor Imaging , Photothermal , and Photodynamic Therapies. *Journal of Pharmaceutical Sciences*, 102(1), 6–28.

Van Der Zee, J., (2002). Heating The Patient: A Promising Approach?. *Annals of Oncology*, 13(8), 1173–1184.

Zhang, H., Yee, D. Wang, C., (2008). Quantum Dots For Cancer Diagnosis And Therapy : Biological And Clinical Perspectives. N*anomedicine (Lond)*, 3, 83–91.

Zhang, J.Z., (2010). Biomedical Applications of Shape-Controlled Plasmonic Nanostructures: A Case Study of Hollow Gold Nanospheres for Photothermal

Ablation Therapy of Cancer. *The Journal of Physical Chemistry Letters*, 1(4), 686–695.