IRON OXIDE NANOPARTICLES AS RADIOBIOLOGICAL DOSE ENHANCER FOR RADIOTHERAPY

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By

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

List of Symbols

Name	Definition
α	The cell killing per Gy of the initial linear component
β	The cell killing per Gy^2 of the quadratic component
cm^2	Centimeter square
D	Dose
ɣ	Gamma
Gy	Gray
g/mol	Gram per mol
keV	Kilo electron voltage
M	Mega
MeV	Mega electron voltage
mg/ml	Milligram per milliliter
mM	Millimol
mMol/L	Millimol per liter
mol/L	Mol per liter
Mol	Mol
Molar	Molarity
MU/min	Monitor unit per minutes
MV	Megavoltage
n	The extrapolation number
R^2	Goodness of fit of the model
µl	Microliter
µm	Micrometer

List of Abbreviations and Acronyms

Name	Definition
AgNPs	Silver nanoparticles
AuNP	Gold nanoparticles
DEF	Dose Enhancement Factor
DNA	Deoxyribonucleic acid
Gd	Gadolinium
Hf	Hafnium
IONPs	Iron Oxide Nanoparticles
LINAC	Linear Accelerator
LQ	Linear Quadratic
LET	Linear Energy Transfer
MNP	Magnetic Nanoparticle
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NPs	Nanoparticles
PAT	Photon Activated Therapy
PIRT	Particle Induced Radiation Therapy
PIXE	Particle-induced X-ray Emission
RF	Radiofrequency
ROS	Reactive Oxygen Species
Si	Silicon
SPIONs	Superparamagnetic Iron Oxide nanoparticles
T1	The time when 63% of the longitudinal magnetization has recovered

T2	The time when 63% of the transverse magnetization has decayed
Ti	Titanium
TiO_2	Titanium dioxide

List of Equations

NANOPARTIKEL OKSIDA BESI SEBAGAI PENGGALAK DOS RADIOBIOLOGI UNTUK RADIOTERAPI

ABSTRAK

Pengenalan: Nanopartikel oksida besi (IONPs) telah dikaji secara meluas sebagai agen kontras untuk pengimejan resonans magnet (MRI) dan lain-lain aplikasi bioperubatan yang berpotensi seperti penyampaian ubat yang disasarkan. Sifat-sifat IONPs yang menarik bukan sahaja berpotensi untuk multimodaliti aplikasi diagnostik tetapi juga untuk kegunaan terapeutik terutamanya radioterapi. Di dalam tesis ini, potensi aplikasi IONPs dalam meningkatkan kecekapan hasil radioterapi telah disiasat dan diterokai.

Kaedah: Kajian ini dijalankan secara in-vitro menggunakan T_{24} sel kanser pundi kencing, HCT116 sel karsinoma kolon manusia dan F98 sel glioma tikus bersama 15nm IONPs. Pada mulanya penilaian ketoksikan dijalankan untuk memastikan IONPs serasi terhadap sel. Penyiasatan terhadap penggalakan dos telah dijalankan dengan meradiasikan sel yang terkandung dan tidak mengandungi 1mMol/L IONPs menggunakan pancaran foton, elektron, proton dan sinkrotron kilovolt monoenergetik sinar-x yang mempunyai tenaga dan dos yang berbeza. Lekuk kemandirian sel telah diperolehi menggunakan piawaian pengklonian asei dan dianalisis menggunakan LQ model. Faktor penggalakan dos diekstrapolasi sebanyak 90% daripada kemandirian sel dan dikira daripada lekukan yang terbentuk.

Keputusan dan perbincangan: Ujian ketoksikan menunjukkan in vitro serasi terhadap IONPs. Pemerhatian terhadap IONPs mendapati peningkatan ke atas kesan penggalakan dos bagi semua pancaran yang diuji kecuali pancaran elektron. Pancaran foton dengan tenaga 6 MV dan 10 MV menunjukkan peningkatan dos sebanyak 1.71 – 2.50 dengan kehadiran IONPs. Sementara itu, pancaran ion berat proton dengan LET tinggi

menunjukkan faktor penggalakan dos menghampiri 2 kali ganda. Pancaran sinkrotron kilovolt monoenergetik sinar-x menunjukkan peningkatan dos yang paling tinggi dengan nilai DEF sebanyak 9.11. Walaubagaimanapun, iradiasi menggunakan pancaran elektron tidak mengemukakan sebarang kesan penggalakan dos yang mana boleh menjadi kaitan dengan kekurangan interaksi yang mendorong radikal bebas dan spesies oksigen reaktif (ROS) yang menggalakkan kematian sel.

Kesimpulan: IONPs didapati berkesan sebagai penggalak dos untuk rawatan kanser menggunakan sinaran mengion dari segi jenis dan tenaga yang berbeza kecuali terapi pancaran elektron. Kesan yang lebih ketara dapat dilihat dengan menggunakan pancaran sinkrotron kilovolt monoenergetik sinar-x dan pancaran proton dan mencadangkan ia sebagai teknik aplikasi klinikal yang baru untuk IONPs. Keberkesanan penggunaan IONPs di dalam radioterapi kanser dan juga pengimejan diagnostik boleh memberi petunjuk ke arah aplikasi kanser teranostik yang lebih maju pada masa hadapan.

Kata kunci: nanopartikel oksida besi, penggalak dos, radioterapi

IRON OXIDE NANOPARTICLES AS RADIOBIOLOGICAL DOSE ENHANCER FOR RADIOTHERAPY

ABSTRACT

Introduction: Iron oxide nanoparticles (IONPs) have extensively been investigated as contrast agents for magnetic resonance imaging (MRI) and other promising biomedical application such as targeted drug delivery. The intriguing properties of IONPs not only promising for multimodality diagnostic application but also for therapeutic purpose especially radiotherapy. In this thesis, potential application of IONPs to increase the efficiency of radiotherapy outcome was investigated and explored.

Methods: The study is conducted *in-vitro* using T_{24} bladder cancer cells, HCT116 human colon carcinoma cell lines and F98 rat glioma cells with 15 nm IONPs. The evaluation on the cytotoxicity was initially conducted to ensure the IONPs biocompatibility to the cells. Investigation on the dose enhancement were done by irradiating the cells with and without 1mMol/L of IONPs using photon, electron, proton and synchrotron's kilovoltage monoenergetic x-rays beams of different energies and doses. The cell survival curves were obtained using standard clonogenic assay and were analyzed using linear quadratic model. Dose enhancement factor (DEF) were extrapolated at 90% cell survival and calculated from the survival curves.

Results and discussions: Cytotoxicity test indicate *in vitro* biocompatibility of IONPs. IONPs were observed to induce dose enhancement effects in all different types of radiotherapy beam tested except the electron beam. Photon beam of energy 6 MV and 10 MV show dose enhancements of 1.71-2.50 folds in the presence IONPs. Meanwhile, the high LET heavy ion of proton beam indicate higher enhancement factor closed to 2 fold. Synchrotron's kilovoltage monoenergetic x-rays beam indicates highest dose

enhancement effects with DEF value 9.11. However, irradiation with electron beams does not produce any significant dose enhancement effects which could be link to the lack of interaction that induce free radical and reactive oxygen species (ROS) that enhance the cell's death.

Conclusions: The IONPs are found to be effective as dose enhancer for cancer treatment using different types of ionizing radiation and energy ranges except for electron beam therapy. The effects are more pronounced for synchrotron kilovoltage monoenergetic x-rays beam and proton beam suggesting the potential new technique for IONPs clinical application. The outcome from this thesis implying the clinical potential of IONPs in increasing the radiotherapy efficiency to treats cancer.

Keywords: iron oxide nanoparticles, dose enhancement, radiotherapy

CHAPTER 1

INTRODUCTION

1.1 Radiotherapy

Radiotherapy is one of the critical techniques in the cancer treatment and management that employ ionizing radiation to produce the lethal effects to the malignant cells. Patients diagnosed with cancer are often referred to radiotherapy as part of their treatment regime (Hoskin, P., 2006). Bombardments of ionizing radiation to the tumour or target are highly localized and conformal which could deliver sufficient dose to kill tumour cell and concurrently prevent dose to the surrounding normal tissue. Uniformly distributed radiation dose to the tumours are also highly aided by the advanced imaging and treatment planning system (Levin, et al., 2005).Therefore radiotherapy remains the most important non-surgical treatment of cancer.

Modern approach of radiotherapy that applied for curative and adjuvant cancer treatment is fundamentally dependent on the concept that radiation induced DNA damage to the cells. Crippling cell growth and mitosis from the damage shrinks the tumours and prevent metastasis. However, it is still a challenge to completely avoid surrounding normal tissue when delivering radiation dose to the tumours. Mechanism of highly conformal treatment are usually delivered through external beam radiotherapy (EBRT) technique or it may come from radioactive material placed in the body near the tumour cells or injected into the bloodstream (internal beam radiotherapy, also called brachytherapy).

EBRT mainly utilizes linear accelerator (LINAC) that equips with beam shaping component such multileaf collimator to increase the specificity towards the

target volume. High energy photon beam of the range from 6 to 25 MV are widely and commonly used to treat many type of cancer especially the deep seated tumours (Hoskin, P., 2006). LINAC also produce electron beam to treat superficial and sub surface cancer. Conformity and targeting capabilities of the EBRT have been going through much improvement with applicability of advance technique such as intensity modulated radiotherapy and image guided radiotherapy.

Augmenting the therapeutic efficiency of radiotherapy is a huge challenge due to diverse nature of cancer. Different treatment techniques are being developed to cater the needs of treating malignancy while preserving the surrounding normal tissue. Conventional LINAC based radiotherapy provides excellent tumour control but sometimes unnecessary exposure to normal tissue cannot be avoided. This is a concern for paediatric patients which has high probability to develop complication and secondary malignancy. Particle beam radiotherapy stimulates interest due to their superior dose distribution compare to photon (Levin, et al., 2005). Distinctive characteristic of particles beam produce another excellent alternative for cancer treatment. Figure 1.1 shows different type of radiation used in radiotherapy.

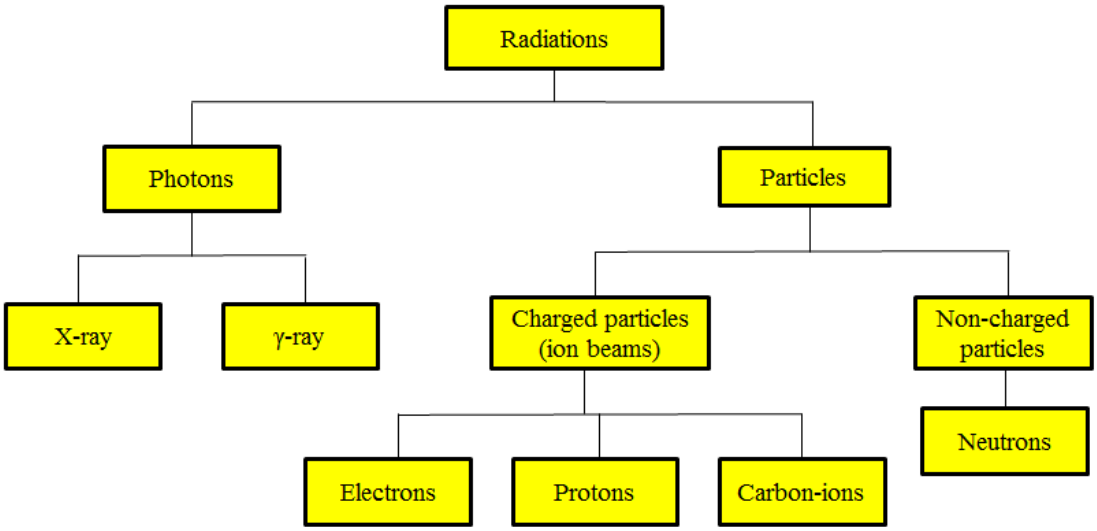

Figure 1.1: Type of radiation used in radiotherapy

1.1.1 Photon Beam Radiotherapy

Conventional radiotherapy mostly utilize photon beam to treat various type of cancer. Photon beam in the form of x-ray or gamma ray is used to achieve tumour control by inducing DNA damage to the cancerous cells. The cellular damage increase proportionally with radiation dose that represent the amount of energy that the ionizing radiation deposits (Trikalinos, et al., 2009; Peter Hoskin, 2006).The sources of photon beam usually originate from LINAC which is the most commonly used beam delivery device for radiotherapy. Meanwhile treatments with gamma ray are normally done using the gamma emitting radioisotope such as Co-60.

Figure 1.2 shows the basic components of LINAC in production of high-energy photon beams.

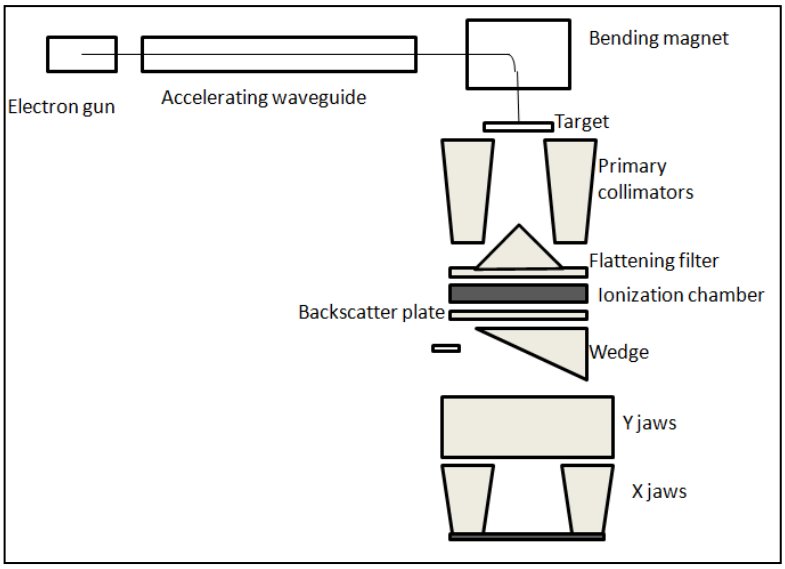

Figure 1.2: Basic components of linear accelerator

High energy photon beam produced by medical LINAC are yielded when electron from accelerator guide smashed into heavy metal target. The electron originated from the heated filament will travel through accelerator waveguide to acquire the required kinetic energy from the radiofrequency power source. The electron beam will then go through the electron beam transport system in which the beam is focused through the angle between 90° and 270° before hitting the target. The high energy photon or x-ray produced from the target need to be tuned to ensure uniform intensity and homogenous dose distribution. Uniform dose distribution and homogenous dose variation will ease the treatment planning and dose calculation to the patient. This is usually being achieved by having flattening filter in LINAC which is normally located between the primary collimator and the monitor chamber. The flattening filter is made off high atomic number (Z) material and conical in shape to flatten the forward peaked bremsstrahlung spectrum of megavoltage photon beams (Sharma, 2011). The flat dose profile of photon beam will then be collimated using jaws collimator and multileaf collimator (MLC) to produce clinically useful

beam to treat various type of cancer. In the advance technique, the intensity of the photon beam will be modulated so that much more conform dose to the tumour is produce without hitting critical adjacent organs.

Ability to penetrate deeper into human tissues granting the popularity of megavoltage photon beam in treating majority of deep seated tumours in patients. The usefulness of the photon beam depends on their energy and volume to be treated. Versatility in term of beam choice and excellent skin sparing effects give major advantages for high energy LINAC based radiotherapy compare to earlier version of radiotherapy using kilovoltage x-rays and Co-60 teletherapy unit. Megavoltage photon beams exhibit low surface dose, maximum dose at depth, gradual loss of dose with depth and sharp physical penumbra at the beam edge. Photon beams with higher energy show reduced attenuation compare to lower energy photon beams. Meanwhile, the maximum dose of kilovoltage beams occurs at the surface giving unnecessary skin dose. Therefore, kilovoltage beams are typically only useful for treatment superficial lesions. Photon energies < 150 keV are limited to lesions with thickness < 0.5 cm due to rapid dose fall off. Orthovoltage treatments usually limited to < 2 cm lesions thickness. Beam energy also determined the rate of dose fall. Dose falloff is sharper for high energy beams. Compare to kilovoltage beams, megavoltage beams have very sharp penumbra at the surface.

In order to achieve uniform dose distribution, photon beam radiotherapy was carried out by cross firing multiple beams towards the target volume. Beam configurations are designed and planned to deliver homogenous dose to the tumours while ensuring normal tissue receive minimal dose as possible. Current modern radiotherapy planning system provide visualisation of dose distribution that assist in more effective cancer treatment.

1.1.2 Electron Beam Radiotherapy

Electron beam used in radiotherapy is commonly produced by similar medical LINAC that generates photon beams. It is widely available at radiotherapy center with energy ranging from 6 to 25 MeV and the application are popular for superficial types of malignant and diseases. The method used in the production clinically useful electron beam is different from photons production. Electron beams used for treatment can be produced by rapidly scanning the narrow beam of electrons that originate directly from the electron gun and accelerator waveguide. The pencil beam electron was then broadened using the scattering foils. The thin metal sheets scattered the electron beam, thereby expanding the useful size of the therapy beam. The broadened electron beam is collimated to appropriate size for treatment using lower and upper jaws and additional electron collimators known as electron cones. A series of openings in an electron applicator are used to collimate the beam at or close to patient's skin (Stanton, et al., 1996). The field size of the electron beams were determined by the applicator's size (Ajjitchandran, 2008).

In comparison to photon beam, electron are subatomic particle that loss energy continuously when they interact with matter. The electrons interact with atoms by variety of processes when its travel through medium. The processes occurs are inelastic collision with atomic electrons (ionization and excitation), inelastic collisions with nuclei (bremsstrahlung), elastic collisions with atomic electrons and elastic collisions with nuclei. The electron will lose their energy when they travel through the medium as results of collisional and radiative processes. In bremsstrahlung process, the increasing number of kinetic energy and high atomic number (Z) material will increase the probability of radiation loss (Ajjitchandran, 2008). The electron interaction with the medium resulting in decrease in energy and

determine the characteristic of depth dose curve that give clinical benefit of electron beam.

Electron beams are used to treat the tumour disease within approximately 6 cm from the surface depending on the beam energy. Treatment of cancer such as skin and lips, upper-respiratory and tumour bed boost for breast cancer take advantages of electron therapeutic ranges. Dose delivery from the surface to a specific depth where the dose will fall off rapidly until the value is near to zero are particularly useful for variety of clinical situation (Ajjitchandran, 2008).

1.1.3 Proton Beam Radiotherapy

Proton was discovered in 1919 by Ernest Rutherford in an experiment conducted on the hydrogen nucleus that was extracted from nuclei of nitrogen by collision. The hydrogen nucleus was named as Proton, after the neuter singular of the Greek word for "first" (William, 2013). Proton is a subatomic particle with positive electric charge and mass slightly less than neutron but much heavier than electron (the proton is 1,836 times more massive than an electron). Proton interaction depends on their energy which represents the velocity. The interaction could be inelastic or elastic collisions with the nucleus, with the bound atomic electrons or with the whole atom. This interaction of proton with matter creates the basis for therapeutic potential of proton beam in cancer treatment and become an alternative treatment modality instead of photons (Mazal, 2007; Thomas, et al., 2009).

Production of proton beams for medical used involve highly sophisticated accelerator facilities, beam transport system, shielding, beam shaping device , patient positioning system and the control system. Proton originally coming from the ion

sources where hydrogen atoms are separated into the electrons and protons. The protons are ejected into the cyclotron where they were accelerated. High power of protons was sent through the energy selection system and degrader to determine and adjust the proton energy. The beam transport system will conduct the protons with right energy and their trajectory. Once the desired amount of energy is achieved, a magnetic field steers the protons into the treatment room through gantry. Gantry can rotates up to 360° and deliver beams at any angles. The beams move through the custom made devices called aperture and the compensator shapes the exact size and depth of the tumor. The proton beams deliver powerful therapeutic dose to the tumor with no exit dose which provides great advantages to the deep seated tumor. Figure 1.3 shows the proton beam therapy machine (Hyogo Ion Beam Medical Center, Japan).

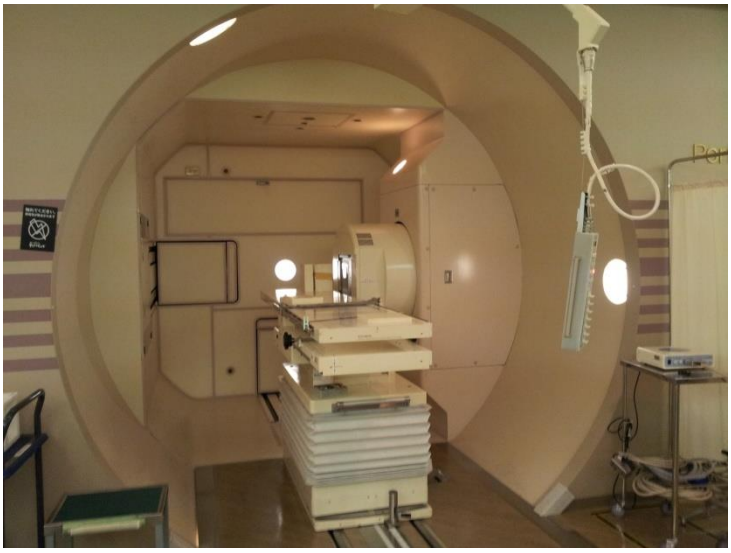

Figure 1.3: Proton Beam Radiotherapy Machine

The proton interaction creates a peak of the energy directly targeted into the tumor without unnecessary dose to the surrounding tissue (Mazal, 2007). Energy

ranging between 65 to 250 MeV is normally employeddue to their depth dose properties and characteristic that is favourable for clinical application. Charged particles proton beams have a very rapid energy loss after a few millimetres of penetration which results in Bragg peak. Bragg peak is a sharp and localized peak of dose. The penetration depth of the Bragg peak is determined by the initial energy (speed) of the charged particles. Desired dose can be delivered to anywhere in the patient's body with high precision by adjusting the energy of the charged particles and intensity of the beam (Levin, et al., 2005; Thomas, et al., 2009). The Figure 1.4 shows the graph of the Bragg Peak of proton beam therapy.

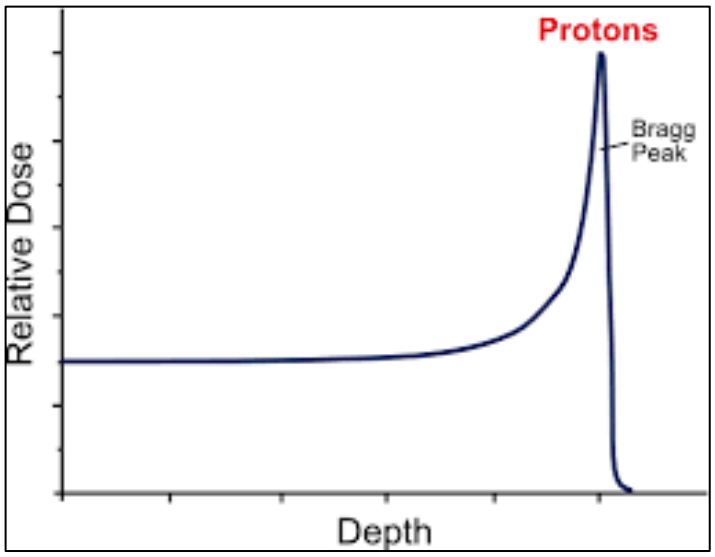

Figure 1.4: The Graph of the Bragg Peak of Proton Therapy

The interesting characteristics of proton beams depth dose provide superior spatial dose distribution to the tumour while reducing the total dose to critical organ. The dose delivered to tumour volume is more conformal and uniform which increase the therapeutic ratio of radiotherapy. The term of therapeutic ratio can be defined as the ratio of the probabilities for tumour eradication and normal tissue complication

and is often used to consider the balancing between administering the prescribed target dose and dose to healthy tissue.

The proton beams have been clinically adopted to treat various types of cancer such as ophthalmic and intracranial malignancy, glioblastoma multiforme, artero-venous malformations and prostate carcinoma. Besides that, it is also able to treat tumors in the vertebral and paravertebral regions, central nervous system, lung and pelvic tumors (Mazal, 2007; Slater, 2006). Proton radiation therapy also could be used in combination with photon irradiation such as radiosurgery and stereotactic radiotherapy, surgery and chemotherapy.

1.1.4 Synchrotron Radiation

Synchrotrons radiations are electromagnetic radiations consist of the entire electromagnetic spectrum from radiowaves, infrared light, visible light, ultraviolet light, x-rays and gamma rays. Synchrotron radiations are generated when electrons moving at almost the speed of light in a curved trajectory under the influence of a circular magnetic field. The electromagnetic waves emitted have ranges of wavelengths from very low such as x-rays and some of much longer such as the visible light range. Synchrotron radiation has special characterization such as high intensity, brightness, directionality and variable polarisation, which is distinctive from clinical conventional x-rays.

The synchrotron radiation produce artificially by synchrotron is the brightest man made x-rays source. Figure 1.5 shows the schematic diagram of the production of synchrotron radiation. The process begins when electrons are generated by electron gun through heating a barium compound cathode. The electrons are accelerated in a linear accelerator by electric field and it is accelerated even more by

means of a strong magnetic field until they reach the 99.999% of the speed of light. After that, electrons are transferred from the linear accelerator to the booster ring where their energy is augmented using radiofrequency (RF) energy. Dipole of electromagnets force is used to direct the electron path into circular trajectory. The electrons were then passed into the storage ring when they approximately complete 1 million laps in half a second.

The electrons accelerated at constant speed will remain in a circular orbit when they are injected into the storage ring. They will circulate for approximately 30-40 hours and continuously emits intense synchrotron radiation. The synchrotron radiation from the storage ring is then transferred to the beamlines for the utilization in research and experimental work. Each beamline include different types of filters, mirrors and other optical components that prepare the radiation for application in different fields.

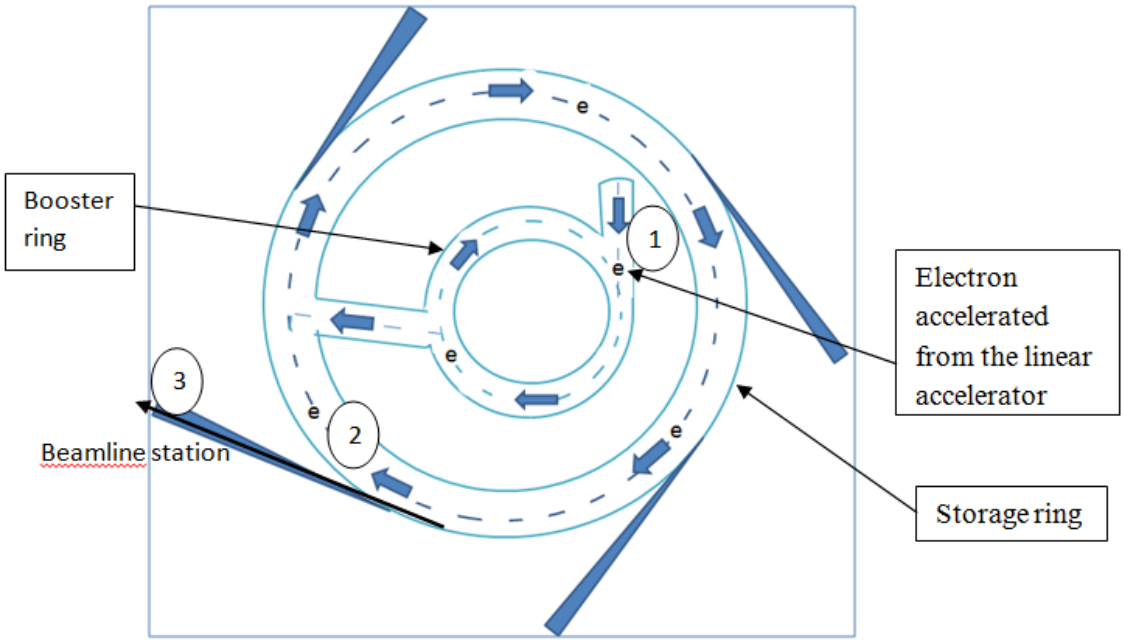

Figure 1.5: The schematic diagram of the production of synchrotron radiation

Synchrotron radiation has several advantages compare to conventional clinical x-rays. Synchrotron radiation is special because of the intensity is extremely high which is brighter than the sun. The high level of intensity that synchrotron produce can reveal the details that cannot be seen using any other method. This allows the researcher to observe highly detailed and accurate image or information. Moreover, the radiation emitted covers a continuous, wide range of wavelengths simultaneously. The wavelength or range of wavelengths can be specifically selected according to the required specification of a research. The radiation light emitted is outside the visible spectrum, mostly from infrared to x-rays and it's depends on the energy of the electrons. The visible light might be produced when the electrons travel the curve with lower energy; with higher energy they might produce x-rays. The synchrotron light beam is precisely focused, result in a very small divergence which allows extremely small areas of a sample to be investigated. The results are superior in terms of accuracy, quality, robustness, the level of detail that can be seen and the amount time required to analyse a sample is extremely short.

Synchrotron radiation is applied many application in physics, chemistry, biology and medicine. In medical for instance, absorption contrast imaging is performed using synchrotron radiation. It is made possible to select a narrow wavelength band by crystal monochromator because of the high intensity and good natural collimation of synchrotron radiation. Besides that, synchrotron radiation is also used in coronary angiography and micro-angiography as diagnostic method which provides detailed high-resolution images. Another potential clinical applications using synchrotron radiation are mammography screening, micro-tomography with spatial resolution of a few micrometres and computed tomography to eliminate the effects of beam hardening (Suortti, et al., 2003). Figure 1.6 shows

the Australian Synchrotron situated in Clayton, Victoria. Some part of the experimental work in this thesis was performed using the Imaging and Medical Beamline, Australian Synchrotron.

Figure 1.6: The Australian Synchrotron

1.1.5 Comparison of Photon, Electron, Proton and Synchrotron Radiotherapy

Radiotherapy employs different type of radiation beam such as photons, electron, proton and recently synchrotron radiation to treat cancer. Each type of beam has advantages and disadvantages that require great optimization in order to produce high conformal dose to the tumours and reduced dose to normal tissue.

Conventional external beams radiotherapy that use the high energy photons and electrons beams are the most commonly and widely used to treat the tumours cells. The megavoltage photon beams provide greater penetration or depth dose due to the lower mass attenuation coefficient of high energy x-ray (Stanton & Donna, 1996). In clinical applications, 4-8 MV photon beams are the most useful for providing a balance between penetrations through tissues. Megavoltage photon

beams also provide skin sparing effects which can reduced the skin reactions towards radiation (Stanton & Donna, 1996; Peter Hoskin, 2006). Meanwhile, megavoltage electrons beam is widely used for superficial tumours due to the depth dose characteristic that to deposit the energy immediately on entering the patient.

Proton beam radiotherapy provide different alternative in cancer treatment. Proton have dense ionization region which is called Bragg Peaks. Heavy charged particles penetrate the tissue and steadily increase the linear energy transfer (LET) to the maximum. The increase of the LET produce more dense ionization near the end of their path compare to the beginning which produced the Bragg peaks. Compare to the photon beam, proton beams have no exit dose which could reduce the normal tissue complication. Highly conformal dose distributions can be achieved and less normal tissues dose can be achieved. This allows the proton therapy for better acute tolerance of combined chemotherapy and radiation therapy (Levin, et al., 2005).

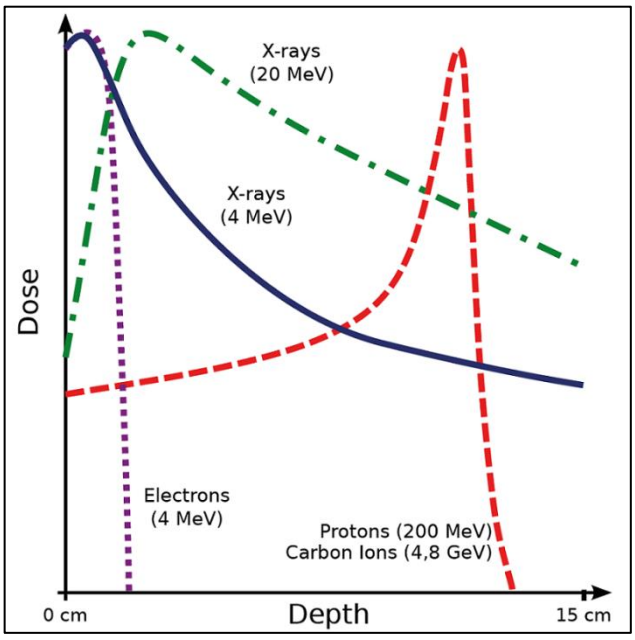

Figure 1.7: The graph of dose versus depth for megavoltage photon, electron and proton beams

The Figure 1.7 shows the graph of depth dose for megavoltage photon, electron and proton beams. The depth dose curves of electron beams contrast sharply with the photon beams when the electron beams' depth dose fall rapidly compared to the photon beams. The depth dose characteristics of protons are enticing because of their high dose at depth and very tiny doses at the beginning and the end of the peak. In real clinical cases, majority tumours are larger than the Bragg peaks and more useful Bragg peak width can be produce by spreading the particle energy (Stanton & Donna, 1996).

1.2 Radiobiological Aspect in Radiotherapy

Radiobiological effect occurs with the combination actions of both ionizing radiation (photon and electron) and biological human cell. The charged particles absorbed in the human cell interact directly with the important component of the DNA strands. The interaction causes the biological effects which is cell death. The clinical biology focused on the relationship of absorbed dose and biological response. Radiobiological modelling is used for quantitative radiation biology that will explain both dose-response and time-dose relationships. Radiobiological modelling are used to predict the outcome of radiation therapy based on dose distribution characteristics. The radiobiological model used in this study is Linear Quadratic model (LQ model).

1.2.1 Linear Quadratic Model

Linear quadratic model is the models that commonly used to analyse radiation response of both *in-vitro* and *in-vivo* data. LQ model is commonly and widely used to describes the cell killing in terms of DNA double strand breaks

(DSBs) produced by the radiation with the proportionate to the dose. The DSBs may be repaired with different rate constants and LQ is one of them (Brenner, 2008). The LQ model describes the relationship between cell survival and irradiation dose, based on this formula:

$$S = \exp^{-(\alpha D + \beta D2)} \quad (1)$$

Where S is the survival, α and β are dependents and D is the absorbed dose. In the other word, the LQ model assumes that S is made of two terms; a linear term, αD and a quadratic term, $\beta D^{2.}$

The LQ model is accurate in the fractionation region from 1.5 to 4 Gy. However, the accuracy becomes less as the range of the radiotherapy application widen with the classical LQ model (Brahme, 2011). The LQ model is not suitable at the high dose region where it underestimates the surviving fraction in the high dose range. The parameter of the model is essential. However, realistic model is more desirable to describe more complex treatment (Andisheh, et al., 2013).

1.3 Problem Statement and Rationale of the Study

Radiotherapy in conjunction with chemotherapy and surgery is the primary option for cancer treatment. High energy ionizing radiation is delivered to the tumours site with high accuracy and precision to induce cellular damage and DNA breakage of cancer cells. Despite the technological advancement in radiotherapy technique, the curative potential of radiotherapy is always restricted by normal tissue tolerance and the tumour cell resistance. This constraint impales the therapeutic efficacy of radiotherapy to remain dismal.

Gold nanoparticles have been extensively investigated to increase the cancer cell radiosensitivity but to date no clinical application of gold nanoparticles have been approved. IONPs have been clinically applied as contrast agent for MRI and widely studied for other promising biomedical application. Application of IONPs as dose enhancer in radiotherapy is not widely explored and their therapeutic potential are still unknown. The advantages of IONPs over AuNPs such as magnetic properties, biocompatibility and cost effectiveness could have benefits radiotherapy especially for hypoxic and radioresistant tumours.

In this study, the potential of IONPs as novel radiation dose enhancer for radiotherapy was investigated. The applicability of IONPs as radiation dose enhancer was evaluated with wide range of radiotherapy beam from conventional megavoltage x-ray beam to monoenergetic kilovoltage x-ray beam, electron beam and proton beam. This thesis present novel results of the radiobiological effects induced by ionizing radiation with augmented impact by IONPs that could be promising for radiotherapy cancer patient.

1.4 Aims of the Study

To investigate the dose enhancement effects by the Iron Oxides Nanoparticles (IONPs) using different types of radiotherapy beams.

1.5 Objectives of the Study

i. To assess the cytotoxicity of IONPs.

ii. To quantify the dose enhancement effects produced by IONPs for photon, electron, proton and monoenergetic kilovoltage x-ray synchrotron beam.

iii. To evaluate the radiobiologic characteristic of *in vitro* cell survival curves in the presence of IONPs using LQ model.

iv. To analyze the effect of IONPs elemental molar concentration to the radiation dose enhancement effects.

CHAPTER 2

LITERATURE REVIEW

2.1 Nanomedicine in Radiotherapy

Nanometer size materials had a big impact on many scientific fields and area including medicine. This has fascinate scientist into exploring the possible application of nanomaterials in various field especially medicine. Nanoparticles are defined as nanostructures consisting of a number of atoms or molecules in the 1-1000 nm diameter range in at least one dimension. They are believed to have specific advantages over conventional drugs in the treatment of diseases and are used for various biomedical applications where they facilitate laboratory diagnostics and treatment of diseases, especially cancer (Mahmoudi, et al., 2010).

In radiotherapy, nanoparticles have potential application as radiosensitizer in enhancing the efficacy of the treatment. There are many types of the radiation sensitizers that are grouped in metal-based nanoparticles, quantum dots, superparamagnetic iron oxides and non-metal based nanoparticles. Examples of metal-based nanoparticles are the gold nanoparticles (AuNP), Gadolinium based nanoparticles (Gd-NP), Titanium based (Ti-NP), Silver based nanoparticles (Ag-NP) and Hafnium-based nanoparticles (Hf-NP). The non-metal based nanoparticles can be classed as Silicon-based nanoparticles (Si-), Fullerene-based nanoparticles and chemotherapeutic entrapped nanoparticles (Kwatra, et al., 2013).

Currently, a large number of nanoparticles have been synthesized especially those made from noble metals such as gold (Thakor, et al., 2011). In previous and recent studies, gold are mostly used as laboratory based clinical diagnostics imaging agent or therapeutic agent. Gold have high atomic number (Z=79) and very inert to

tissue interaction. These characteristics makes gold ideal for photoelectric interaction. The interaction of x-rays with high Z nanoparticles results in the development of the concept where the efficiency for radiation mediated cellular damage could be elevated. In one of the study conducted using AuNPs, a significant dose enhancement effect upon irradiation were observed in cell culture. In another the study using AuNPs with ~ 3 μm sizes, less cell growth were observed after irradiation. Dose enhancement effects however depend on many factors such as particles size and localisation inside cells. Larger particles have problem with lack of diffusion in cancer tissue. Small size nanoparticles have been extensively optimized and utilized in various cancers treatment (Kwatra, et al., 2013).

AuNPs have many beneficial attributes such as easy to synthesis, functionalization and shape control that frame this type of nanoparticle as an attractive target for further development (Thakor, et al., 2011). Gold are very inert and highly biocompatible. The AuNPs are well absorbed into the systemic circulations and tumour tissue due to the AuNPs properties that have low systemic clearance compared to low molecular contrast agent such as iodine. AuNPs also could be specifically delivered to the tumour tissue by attaching the nanoparticles to the targeting moieties such as antibodies.

In contrast to the bulk materials, nanoparticles can be designed to contain thousands of atom in one particles. This important property potentially could increase the radiation interaction cross section at the target. Therefore, AuNPs of various sizes and shapes can deliver optimum dose to the tumour tissue based on the delivery requirements such as the tumour size and location (Kwatra, et al., 2013).

Other than AuNPs, metal nanoparticles such as gadolinium nanoparticles are also identified as one of the radiation sensitizers. Gadolinium nanoparticles are very practical because they could be easily view *in vivo* through magnetic resonance imaging (MRI). Gadolinium also can generate long-lived pi-radical cations during exposure to hydrated electrons (Kwatra, et al., 2013). An *in vitro* study on the effectiveness of Gadolinium on HT-29 cells found that the Gadoliniums are effective either *in vivo* or *in vitro* study. A new class of radiosensitizer detectable by MRI, Gadolinium (III) texaphyrin is being developed for imaging and therapeutic purpose (Young, et al., 1996).

Material such as titanium dioxide (TiO_2) is a common semiconductor material that is widely used for oxygen detection in environmental gas sensor. This TiO_2 material is somehow found it usefulness in killing cancer cells via photocatalytic chemistry. The activation of the TiO_2 by x-rays resulted in the formation of reactive oxygen species (ROS) which enhanced photoelectric effects *in vivo*. TiO_2 nanotubes were formulated and tested for their radiosensitization effects of glioblastoma because the elongated organic nanoparticles are internalize into the cells more effectively compare to spherical shapes. A study done by Mirjolet, et al., (2013) found that the TiO_2 nanotubes are very effective radiosensitizers in SNB-19 and U87MG cells by enhancing the DNA damage and retarding the DNA repair. Petkovic, et al., (2011) has conducted a study on pre-photoactivated TiO_2 nanoparticles using HepG2 cells and observe the cytotoxic effects are enhanced by induction of double stranded breaks.

Silver nanoparticles (AgNPs) have similar radiosensitizing and physicochemical properties as AuNPs and hence utilized similar mechanisms for radiosensitization effects like other high Z-number atoms. AgNPs are more cost

effective compare to AuNPs and relatively less biocompatible (Coulter, et al., 2013; Kwatra, et al., 2013). Previous studies confirmed that AgNPs had obvious anti-tumour capabilities when tested *in-vitro* which make it attractive for radiotherapy application. Different types of AgNPs have been synthesis and studied for their radiosensitizing activity. Multiple different coatings AgNPs combined with radiations in glioma cell lines shows the possession of anticancer properties (Xu, et al., 2009; Kwatra, et al., 2013). For examples, chitosan-coated triangular AgNPs shows better radiosensitizing effects compare to conventional poly-(ethylene) glycolcapped (PEG) coated AuNPs on human non-small lung cancer cells (Boca, et al., 2011; Kwatra, et al., 2013).

Unique high Z-elements such as hafnium oxide (HfO_2) are uncommon but some studies show potential of this type of nanoparticles as dose enhancer agent in radiotherapy. HfO_2 nanoparticles have been tested in HCT116 cell lines *in vitro* and it show reaction towards photo-luminescent properties which cause thermal induced stress damage to cellular components. Significant radiosensitization effects have been observed with HfO_2 nanoparticles as well as good biodistribution properties and excellent biocompatibility (Maggiorella, et al., 2012; Kwatra, et al., 2013).

Iron oxide nanoparticles (IONPs) have been traditionally used for disease imaging and it is recently used for cellular-specific targeting, drug delivery and multi-modal imaging (Veiseh, et al., 2010). IONPs are a magnetic nanoparticles that represent a class of non-invasive imaging agents that have been developed for magnetic resonance imaging (MRI) and currently the only types of nanoparticles that have been approved for clinical study (Mahmoudi, et al., 2009). IONPs with appropriate surface chemistry have been widely used for in vivo applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoassay,

hyperthermia, drug delivery and radiotherapeutic purpose. Details of IONPs properties will be discussed in the next section.

2.2 Iron Oxide Nanoparticles (IONPs)

Among all types of nanoparticles, IONPs are special in their superparamagnetic properties which allow them to be directed and localized to a particular organ using external magnetic force. IONPs with proper surface architecture and conjugated targeting ligands/proteins have attracted a great deal of attention for drug delivery applications (Mahmoudi, et al., 2010). IONPs are highly biocompatible with negligible toxicity to healthy tissues which make them suitable for therapy application (Kwatra, et al., 2013).

Naked IONPs are quite unstable and even form bulk aggregates in biological fluids. So, appropriate design of core and shell of IONPs is extremely important for medical applications (Li, et al., 2013). Plunoric F-127-coated iron oxide nanoparticles have been developed for application as MRI contrast agent and also anti cancer therapies. These NPs have relatively higher T2 relaxivity and capable to accommodate high payloads of multiple drugs. The drug released from iron oxide NPs is effective in killing cancer cells (Wajadkar, et al., 2013). Studies also found out that IONPs are able to inhibit the function of the receptor to induce apoptosis in glioma cells *in vitro* (Kievit, et al., 2011).

2.2.1 Types of IONPs

Iron Oxide nanoparticles (IONPs) exist in variety of structures and characteristic. Ferrous and ferric iron oxides were presented in seven phases of crystalline. The most common were hematite, maghemite, magnetite and wustite. IONPs have been used in many applications. In examples, magnetite NPS have been used in cancer diagnosis and therapy and maghemite is used in magnetic resonance imaging (MRI) (Martinez, et al., 2009).

2.2.2 Properties of IONPs

i. Magnetic properties

Iron oxide nanoparticles exhibit paramagnetism and diamagnetism properties and are classified by their response to an external magnetic field (Gupta, et al., 2005). Different types of magnetism can be identified based on the orientation of magnetic moments in a particle which depend on the magnetic induction; *B* on the magnetic field, *H*. Iron oxide nanoparticles also shows ferromagnetism properties in which it can be permanently magnetized.

One of the important advantages of magnetic nanoparticles is their superparamagnetism. Superparamagnetic iron oxide nanoparticles (SPIONs) are potentially applied for biomedical applications such as magnetic drug targeting, enhanced resolution magnetic resonance imaging and cell targeting. SPIONs are useful as drug delivery agents because the SPION can be transported by magnetic field to desired site (Mahmoudi, et al., 2009).The SPION will remains at the target for a certain period of time once the external magnetic field is removed (Gupta, et al., 2005; Mahmoudi, et al., 2009).