RADIOBIOLOGICAL MODELLING OF GOLD NANOPARTICLES RADIOSENSITIZATION EFFECTS IN CONVENTIONAL AND ADVANCED RADIOTHERAPY TECHNIQUES

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by

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LIST OF ABBREVIATIONS

^{125}I	Iodine-125
^{192}Ir	Iridium-192
^{198}Au	Gold-198
2C	2-Components
Au	Gold
AuNPs	Gold nanoparticles
CO_2	Carbon dioxide
DEF	Dose Enhancement Factor
DMEM	Dulbecco's Modified Eagle's medium
DNA	Deoxyribonuclide acid
D-PBS	Dulbecco's Phosphate-Buffered Saline
DSB	Double strand breaks
EBT2	External beam therapy 2
EDTA	Ethylenedinitrilotetraacetic acid
EPR	Enhanced permeability and retention effect
FBS	Fetal bovine serum
HDR	High dose rate
IGRT	Image guided radiotherapy
IMRT	Intensity-modulated radiotherapy
KN	Kavanagh-Newman
LINAC	Linear accelerator
LET	Linear energy transfer

LQ	Linear Quadratic
MT	Multi-target
NPs	Nanoparticles
NTCP	Normal tissue complication probability
PLQ	Pade’ Linear Quadratic
TCP	Tumor control probability
SER	Sensitisation enhancement ratio
RCR	Repairable Conditionally Repairable
REF	Radiation Enhancement Factor
RER	Radiation Enhancement Ratio
RM	Repair Model
SSD	Source to skin distance
SSB	Single strand breaks

LIST OF SYMBOLS

a	The initial mean number of damage events per unit dose
α	The direct killing of cell “single hit” (linear component)
α_1	The slope of a single-hit component
α_n	The slope of a compound multi-hit component
b	The maximum amount of damage that can be repaired
β	The impact of cell killing from “double hits” (quadratic component)
c	The loss of repair due to more complex damage
cm^2	Centimeter square
D	Dose
D_1	Dose at initial slope
Dq	The size of the shoulder of the curve
D_0	Dose at final slope
Gy	Gray
g/mol	Gram per mol
kV	Kilovoltage
K_O	Linear rate
K_{OG}	Exponential rate
M	Mega
MeV	Megavoltage
mg/ml	Miligram per mililiter
mM	Milimol
Mol	Mol
Molar	Molarity

MU/min	Monitor Unit per Minutes
MV	Mega voltage
n	Number of targets / hit number
µl	Microliter
γ	The quantity lethal lesions

PEMODELAN RADIOBIOLOGI BAGI KESAN RADIOSENSITIVITI PARTIKEL NANO EMAS DALAM TEKNIK KONVENSIONAL DAN RADIOTERAPI TERMAJU

ABSTRAK

Keberkesanan strategi teknik radioterapi dengan nanoteknologi menjanjikan penyelesaian dalam mengatasi permasalahan yang timbul dalam perawatan radioterapi konvensional seperti kelemahan dalam memberi kesan radiasi secara selektif dan bersasar terhadap tumor yang bersifat kalis radiasi. Partikel nano emas telah menjadi bahan kajian yang pesat untuk bertindak sebagai penggalak kesan radiosensitiviti dalam perawatan klinikal radioterapi. Meskipun terdapat banyak kajian yang telah dijalankan, kepesatan penggunaan partikel nano emas sebagai pemeka sinaran memerlukan pendekatan kajian yang sistematik dan mendalam untuk menilai secara menyeluruh kesan radiobiologi terhadap rawatan radioterapi. Dalam penyelidikan ini, kesan radiosensitiviti telah dikaji menggunakan pelbagai parameter experimen terhadap rawatan radioterapi klinikal menggunakan tenaga radiasi yang berbeza megavolt antaranya alur foton, elektron dan juga dos tinggi brakiterapi (bahan Iridium-192). Selain radioterapi konventional, penggunaan rawatan radioterapi termaju seperti alur proton dan kilovolt monoenergi foton 'synchrotron radiation' turut dikaji. Analisa yang mendalam dengan menggunakan model-model radiobiologi dan cara-cara mengkuantifikasinya juga turut diteliti secara spesifik. Pengaruh kesan radiosensitiviti oleh partikel nano emas dari segi saiz, kepekatan dan jenis sel yang dirawat, beserta pengkajian yang meliputi internalisasi, lokalisasi dan kesan toksiknya terhadap sel turut di dikaji secara terperinci. Kebolehgunaan partikel nano emas untuk rawatan

radioterapi konvensional terbukti menghasilkan kesan radisensitiviti terutamanya dengan dos tinggi brakiterapi, di mana ianya menunjukkan kesan lebih baik berbanding radioterapi alur elektron dan foton. Impak radiosensitiviti yang berkesan terbukti lebih besar dengan perawatan alur proton dan kilovolt monoenergi foton 'synchrotron radiation' yang bersepadanan dengan ramalan teori. Pengesahan melalui model-model radiobiologi bertujuan untuk menghuraikan lekokan kemandirian sel bergantung kepada jenis kualiti alur radiasi beserta cara-cara mengkuantifikasinya. Berserta dengan itu, setiap model radiobiologi berserta parameter tersendiri turut berperanan menjadi petunjuk yang menerangkan mekanisma kejadian yang disebabkan oleh kesan radiosensitiviti. Saiz dan kepekatan partikel nano emas turut mempengaruhi kesan radiosensitiviti, bergantung juga pada perbezaan jenis sel yang dikaji. Sebelum melakukan kajian kesan radiosensitiviti radioterapi, pengunaan partikel nano emas yang optima turut diambil kira dari segi kepekatan yang tidak toksik dan intenalisasinya terhadap sel. Kesimpulannya, keberkesanan radisensitiviti partikel nano emas boleh dicapai dengan menggunakan radioterapi klinikal megavot, brakiterapi dan juga radioterapi termaju seperti terapi alur proton dan 'synchrotron' monoenergi foton radioterapi. Pemilihan secara terperinci model- model radiobiologi, mampu memberi pemahaman tentang kesan radiobiologi sel yang teraruh dari partikel nano emas dan juga piawaian cara-cara mengkuantifikasi amat penting untuk memastikan kesesuaian parameter yang terbaik bagi rawatan klinikal partikel nano emas. Cadangan yang jelas dan tertumpu amat penting dalam pengumpulan data praklinikal bagi mempercepatkan peralihan kegunaan partikel nano emas ke peringkat aplikasi klinikal kepada pesakit.

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ABSTRACT

Improvement of radiotherapy techniques with nanotechnology is a promising strategy to overcome the limitations arises in conventional treatment such as lack of eradication selectivity and radioresistance characteristic of targeted tumour. Gold nanoparticles (AuNPs) is a subject of growing interest to induce radiosensitization effects with potential application in clinical radiotherapy. Despite numerous evidences, development and application of AuNPs as radiosensitizer require systematic and comprehensive experimental approaches to fully evaluate the radiobiological impact in radiotherapy. In this study, multiparametric investigations on the radiosensitization effects were conducted using different energies of clinical megavoltage photon, electron, and high-dose rate (HDR) gamma rays from Ir-192 source. In addition to conventional radiotherapy, advanced techniques using proton beam and monoenergetic synchrotron photon beam of kilovoltage energies were also employed. Comprehensive analysis using different radiobiological models and quantification methods were specifically examined. Influence of AuNPs size, concentration and types of cells on the radiosensitization effects were also elucidated including internalization, localization and cytotoxicity of AuNPs. Applicability of AuNPs for clinical conventional radiotherapy have been proven where radiosensitization effects have been observed especially for HDR brachytherapy that show better effects compare to electron and photon beam therapies. Substantially bigger impact of AuNPs radiosensitization have been confirmed for proton beam and

monoenergetic kilovoltage synchrotron photon beam in concurrently with theoretical prediction. Validity of radiobiological models to describe the cell survival curve is dependent on the beam quality as well as quantification methods. Radiobiological models and their parameters also could be adopted as indicator to explain the mechanistic events in the AuNPs' radiosensitization effects. The AuNPs size and concentration are found to influence the radiosensitization effects and different types of cells exhibit different radiosensitivity responses. The radiosensitization impacts of AuNPs with radiotherapy beams were considered with prior indication on the cellular internalization of AuNPs and the non-toxic concentration for optimal AuNPs application. As conclusion, effective radiosensitization by AuNPs could be achieved for megavoltage clinical radiotherapy, brachytherapy and advanced radiotherapy such as proton beam therapy and monoenergetic synchrotron beam radiotherapy. Precise radiobiological characterization drawn from radiobiological models may provide insight towards radiobiological impacts induced by AuNPs and standardization of quantification methods is crucial for highlighting suitable parameters for AuNPs clinical application. Clear directive recommendation from comprehensive preclinical data provided in this study may expedite the clinical translation of AuNPs for human application.

CHAPTER 1
INTRODUCTION

1.1 Modern radiotherapy in cancer treatment

Millions of people globally are dying with life-threatening cancer diseases for decades. However, radiation therapy (RT) leads in cancer treatment by delivering higher tumour doses while preserving healthy tissues aims for organ preservation, an abiding principle of radiotherapy, thus resulting in increasing cancer cures with the least morbidity. Radiotherapy aims to encompass all cancer cells with sufficient therapeutic doses of radiation that aim on inhibition of cancer cells proliferating capacity, eliminate the malignant cells, reduce the risk of recurrence while simultaneously sparing surrounding normal tissues and ultimately to improve survival.

Enormous progress has been made on the development of precision RT treatments using linear accelerators that pave towards current advanced technologies. All efforts in understanding RT limitations and improved its ability to treat tumours have been done through extensive research and development. Advances in technologies specialized in radiation devices to improve RT such as dynamic multi-leaf collimators, automated treatment planning, new imaging modalities, powerful software operations and various treatment delivery methods help to achieve the conformal radiation dose.

The improvement of radiotherapy techniques is routing through several approaches. Firstly, the standard of prescribed dose and delivery at the target cancer region were elevated. Secondly, introduction of the new method beyond the conventional radiation techniques such as volumetric modulated arc therapy (VMAT) and particles beam therapy. Thirdly, the combination of image-guidance and radiotherapy enable dose monitoring delivery at daily treatment. Last and most

innovative outlook are exploring the radiobiological initiatives by manipulating the nature of cancer cells behavior to become more radiosensitive towards the radiation imposed (Joiner et al., 2009).

Figure 1.1 shows the evolution of the radiotherapy technique from standard conventional treatment to conformal radiotherapy and currently the emerging of particles beam therapy. A preclinical study on the synchrotron-based treatment is currently undergoing and could be available for patients in the near future.

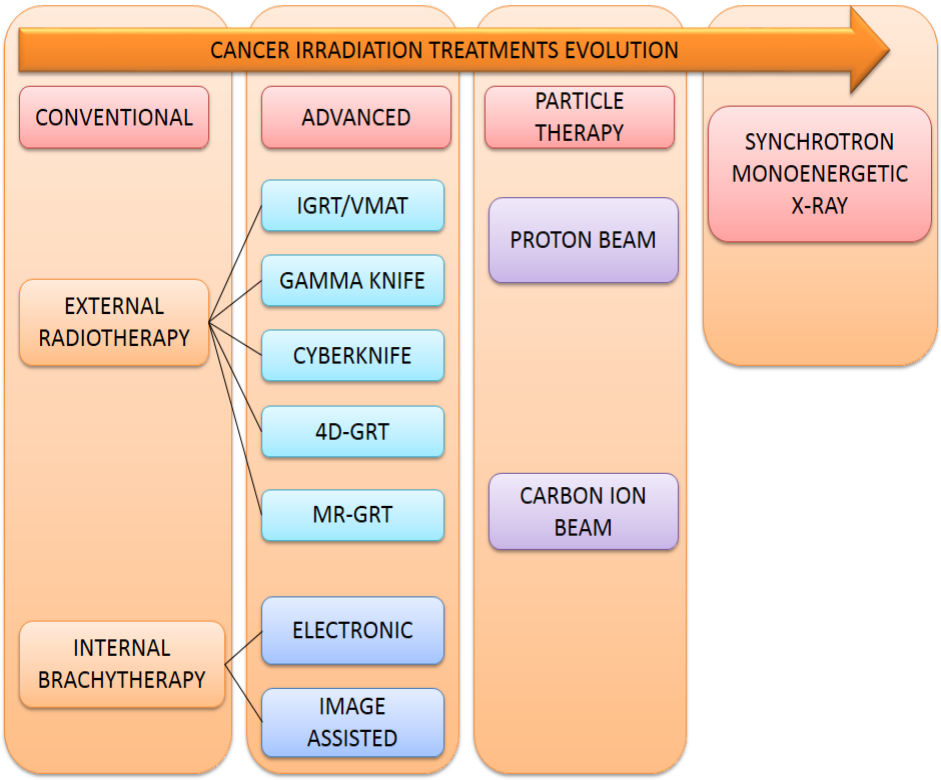

Figure 1.1 The evolution of cancer radiotherapy treatments

1.2 Radiobiology in radiotherapy

The theoretical and experimental radiobiology in radiotherapy offer strategies and techniques from the most basic conventional therapy to specific treatment directly on the manipulating radiobiological cellular environment. There are three different levels on radiotherapy development as shown in Figure 1.2.

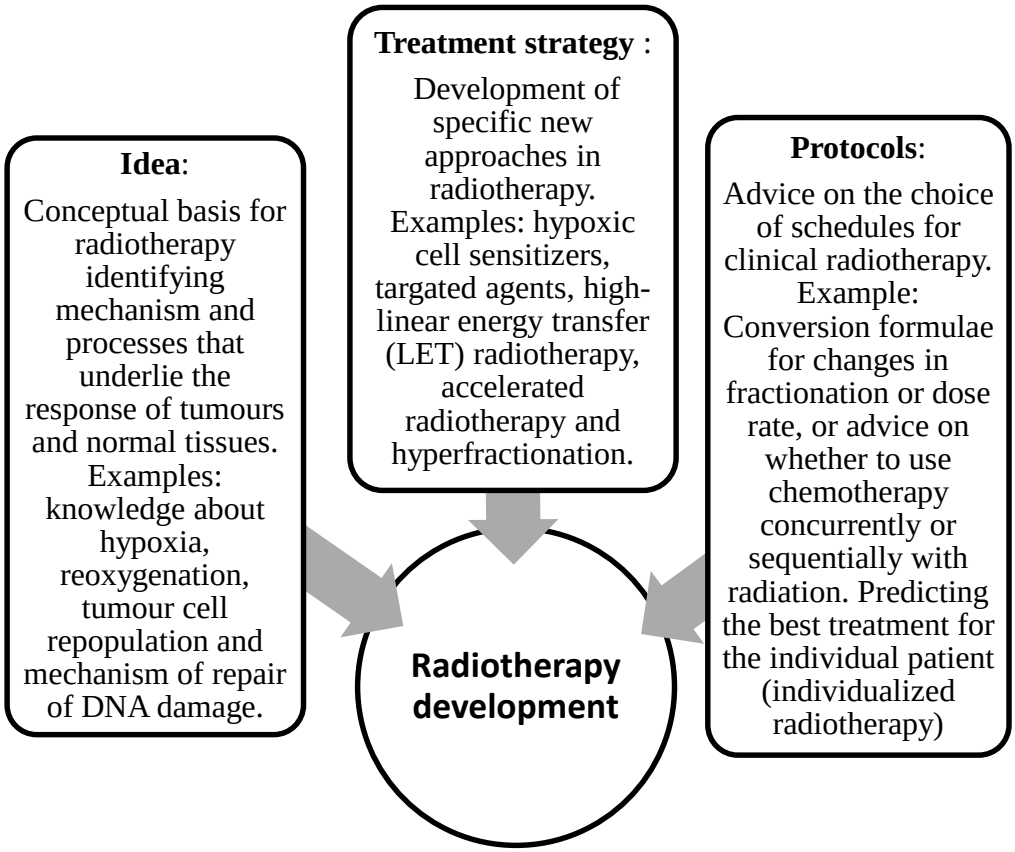

Figure 1.2 The different levels of radiotherapy development. Reproduced from Joiner et al., 2009.

1.2.1 Radiation induced cellular damage

Ionizing radiation causes atomic excitation and ionization of electron from the molecules of cells that will induce biological damages and cell death. This effect occurs in three phases which the physical phase, chemical phase and biological phase as described in the following section.

1.2.1(a) The time scale of radiation effects

1.2.1(a)(i) Physical phase

This phase portrays the mechanisms between charged particles and the atoms within the tissue composition. The light speed of electron takes about 10^{-18} second to pass over the DNA strands and about 10^{-14} second to go across a mammalian cell. The mechanism occurs when the external particles hit the orbital electrons resulting some of the electron from atoms being expelled (ionization) and elevating others to higher energy levels within an atom or molecule (ex citation). The secondary electrons with sufficient energies may excite or ionize other atoms near their path. For example, 1 Gy of absorbed radiation dose will cause in excess of 10^5 ionizations within the volume of every cell in diameter of 10 um.

1.2.1(a)(ii) Chemical phase

The chemical phase illustrates the process of atoms and molecules impaired recurred with other cellular components in accelerated chemical interactions. The formation of broken chemical bonds results from the processes of ionization and excitation direct to the production of 'free radicals'. These are highly potent with the characteristics which were a highly reactive need to the restoration of electronic charge equilibrium, which is accomplished within approximately 1 millisecond of radiation exposure. The important characteristic of the chemical phase is the competition between scavenging reactions, for example with sulphydryl compounds that

inactivated the free radicals and fixation reactions that lead to stable chemical changes in biologically important molecules.

1.2.1(a)(iii) Biological Phase

The end stage of biological phase determines all the subsequent processes of cell death. DNA lesions induced by radiation caused base damage, single-strand breaks (SSBs) and double-strand breaks (DSBs) are aforethought to be most lethal. In the case of SSBs damage, precision removal damaged base carried out by glycosylases, forming damaged base or an abasic sites, which leads to fork collapse and DSB formation.

DSBs are identified by specific protein regulator that works for cell apoptosis responses or cell cycle arrest, thus stopping the proliferation of tumour cells with damaged DNA and inhibiting tumorigenesis. The time–scale of the observable effects of ionizing radiation may thus extend up to many years after exposure (Begg, Stewart & Vens, 2011). Figure 1.3 shows the time-scale of effects of radiation interaction with biological materials.

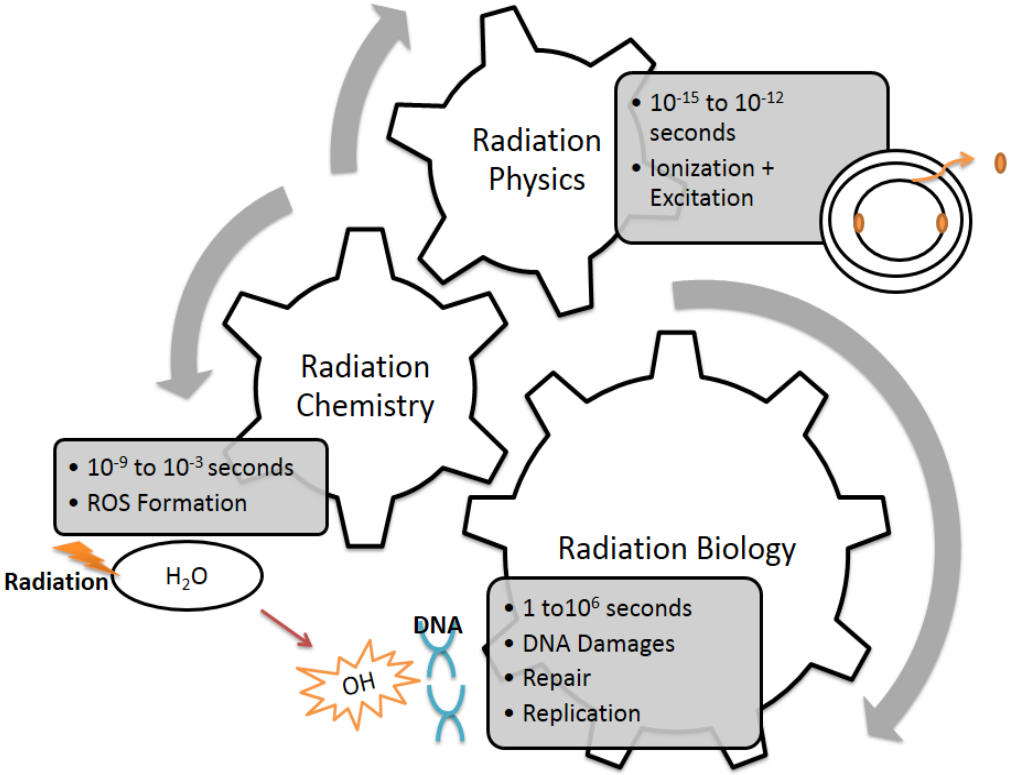

Figure 1.3 The time-scale of effects of radiation interaction with biological materials.

1.2.1(b) DNA damage mechanism

Clusters of energy deposition events (ionization and excitations) occur at the track of secondary electrons resulting in multiple closely-spaced lesions (multiply damaged sites) within a range of 20 nm. This process has been recognized as important for cell killing and in regard to the ability of cells to repair such lesions.

Irradiation generates damage in various patterns of lesions in DNA molecules such as strand rupture either single or double strand breaks, base alteration, sugars destruction and cross-links involving nuclear proteins such as histones and non-histones form of dimers. Altogether the number of DNA lesions produced by irradiation is about 100 distinct patterns. Alteration induced in the DNA of a cell by a dose of 1-2 Gy is approximately: modified base by the formation of radical

hydroperoxide > 1000; single strand breaks (SSB) ~1000; double-strand breaks (DSB) ~40 (Wouters & Begg, 2009).

The indirect effect of strand break plays a prevalent role in cell killing, the most frequent DNA lesion for in vitro study with the highest radiochemical yield. Single-strand break (SSB) produced a large portion of hydroperoxide radicals. Following breakage of the phosphodiester bond, the two strands separate.

Other experimental data show that low dose radiation produced early radiosensitivity DSB, which is a critical indicator for cell killing. While for higher doses, cell deaths are a consequence of unrepaired or mis-repaired DSB. Tremendous effect is observed with high LET irradiation, resulting in the formation of complex DSB lesions. Figure 1.4 shows the DNA damages mechanism of single and double strand break.

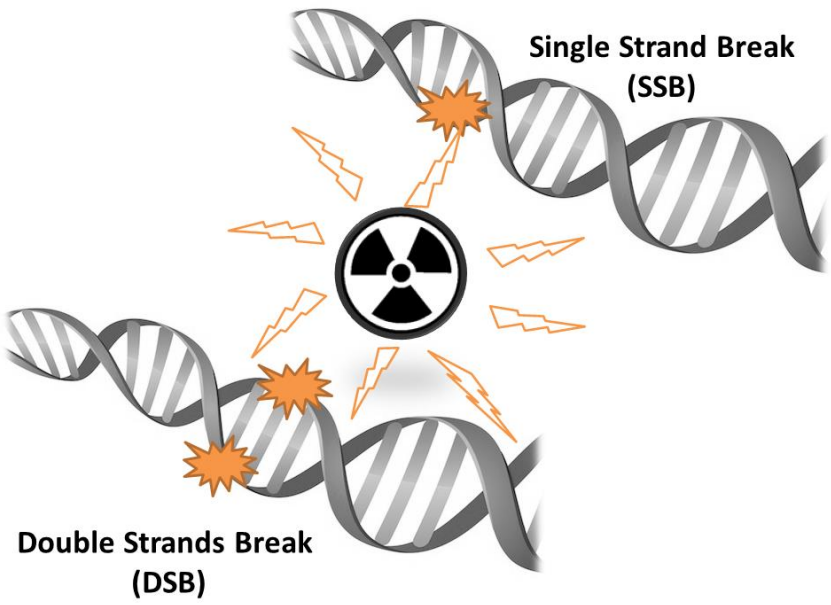

Figure 1.4 DNA damages mechanism of single and double strand breaks.

1.2.2 Radiation cell survival and damage

The success of radiotherapy depends on the ability to cause damage to the tumour's cells. Quantification of the damage can be done by using the different biological technique. However, clonogenic assay and cell survival curves are the basis for understanding radiation cell survival and damages.

1.2.2(a) Cell survival curves

The first cell survival curve used for quantitative evaluation of radiobiological was practised by Puck and Markus, 1956. Then in 2006, Nicolaas *et al*. had published the protocol for clonogenic assay as the assessment for in-vitro techniques, based on the ability of the cell considered to be survived then gives rise to a colony formation. The colonies containing more than 50 cells were counted and depicted in graphically by cell survival curve (Tubiana et al., 1990). Radiation sensitivity evaluation using this protocol of different adherent cell type had become the gold standard for radiation doses response.

The outcome of colonies formation will be presented in the so-called survival curve, which is consists of survival fraction versus dose. The common pattern shows the proportion of surviving cells (Sf) decreases as the dose (D) increases. The curve was plotted in semi-logarithmic coordinates (log Sf as a function of D). This type of plot emphasizes very small values of S at high doses but may sometimes less appreciate survival after low doses. Thus, it is crucial to manipulate curves response by presenting in mathematical functions. These are based on the hypothetical mechanisms of cell lethality or acute damages, which are always emphasized by radiobiological modelling (Tubiana et al., 1990). Figure 1.5 shows the survival curve of HeLa cells proliferation versus dose (Puck and Marcus, 1956). The survival curve

for single cells of the HeLa cells line depicted the formation of the shoulder of the curve is unequivocal and constitutes evidence for a multiple hit killing mechanism.

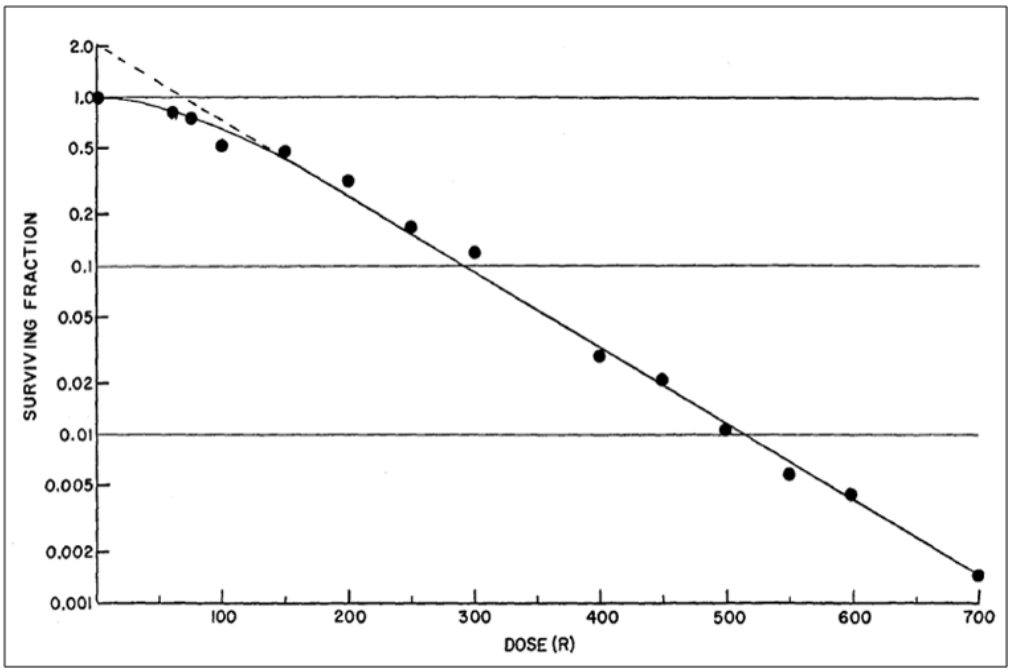

Figure 1.5 Survival curve of HeLa cells proliferation versus dose (Puck and Marcus, 1956).

1.3 Radiobiological model

Radiobiological modelling is used for quantitative radiation biology that will explain both dose-response and time-dose relationships.

1.3.1 Application of the LQ model

Radiobiological models primarily LQ model has been implemented over more than a decade in clinical radiotherapy. The quantitative mathematical model has been adequately simulated the shape of cell-survival curves for mammalian cells and was applied to assess the optimum clinical outcome from conventional therapy to sophisticated therapeutic approaches.

LQ model has been classified as the most common fitted radiobiological model to the survival dose curve. The LQ model describes the relationship between total isoeffective dose and the dose per fraction in fractionated radiotherapy. This model

forms the robust quantitative environment for considering the balance between acute and late reactions (and effect on the tumour) as dose per fraction and total dose changed. This is one of the most important developments in radiobiology application to radiotherapy. In the present time, it is strongly recommended that LQ model should always be used, with correctly chosen α/β ratio, to describe isoeffect dose relationships at least over the range of doses per fraction between 1 and 5 Gy. The α/β ratio describes the shape of the fractionation response: a low (0.5-6 Gy) is usually characteristic of late-responding normal tissues and indicates a rapid increase of total dose, with decreasing dose per fraction and a survival curve for the putative target cells that are significantly curved. While for high ratio (7-20 Gy) represents the condition of the early–responding normal tissues and rapidly-proliferating carcinomas; it indicates a less curved cell-survival response for putative target cells. The LQ model is an appropriate way to be used in clinical calculations and comparisons or in the change in total dose for an altered dose per fraction either old or new treatments. For late reactions, it is usually unnecessary to modify total dose in response to a change in overall time, but for early reactions (and for tumour response) a correction for overall treatment time should be included. Although the effect of time on biological effect is complex, the simple linear correction has been shown to be of some values.

1.3.1(a) The validity of the LQ model

The question regarding the validity of the LQ approach can only be addressed on an endpoint-by-endpoint basis. The LQ model has been applied to datasets from quite a few clinical studies, and there has in many cases been good agreement between the predicted and observed study outcome. This gives some confidence in using the model to estimate the effects of changed dose fractionation in a situation where there are clinical parameter estimates.

Data has supported that the implementation of the LQ model is reliable for dose or dose per fraction ranges from about 1 Gy to 5 Gy. However, as the dose range of the radiotherapy widens, the classical LQ model becomes less and less accurate (Brahme, 2011). According to Andisheh, et al., (2013) LQ model is not suitable in the high-dose region where it underestimates the surviving fraction in the high dose range. Its parameters are essential but to describe the more complex treatment, a realistic model is more desirable. Limitation of the LQ model in clinical radiotherapy could be listed as follow:

1. LQ model does not accurately explain the observed (in-vivo) clinical data
2. LQ model was derived largely from in-vitro study and does neglect the side effect irradiation on the neighbouring normal tissues.
3. LQ model does not consider the effect towards radioresistant cells which is also known as cancer stem cells response. (Kirkpatrick et al. 2008).

1.4 Problem statement and rationale of the study

Ideally, the behavior shown by AuNPs as biocompatible radiosensitizer had been proven by many studies. The highly productive of photoelectric interaction between AuNPs and x-ray especially at kilovoltage energy caused simultaneously increasing of secondary radiation beneficial to induce cell death significantly. However, the nature of kilovoltage x-ray beam was very strongly attenuated by soft tissues in which treatment limited only for superficial tumours. Therefore, megavoltage radiation used in clinical radiotherapy will be more promising as it has superior penetration that allows for greater dose uniformity and reduced the dose delivered to surrounding healthy tissues. The application of AuNPs with megavoltage radiotherapy could produce radiosensitization or dose enhancement as better as kilovoltage x-ray beam (McMahon et al. 2011, Liu et al., 2018). Radiosensitization effects have also been explored for advanced radiotherapy modalities using proton and synchrotron-based therapy (Cho et al., 2016, Enferadi et al., 2017). However, the data reported are still limited and identification on further parameters for quantifying AuNPs radiosensitization effects is extremely important for clinical stage.

Therefore, the novelty of this study is to determine the radiosensitization effects by AuNPs for different radiotherapy modalities: megavoltage photon beam, electron beam and high-dose rate (HDR) gamma rays f rom Ir-192 source available in HUSM (convebtional radiotherapy). For advanced radiotherapy, we had achanced to investigate proton beam and monoenergetic synchrotron photon beam of kilovoltage energies from our international research collaboration team. The radiosensitization response is simultaneously manifested with various radiobiological model and quantification methods. Such efforts had never been done from other reserachers in full detail, thus the results obtained from this thesis will contribute to a more

comprehensive data in unravelling the mechanisms that influence the tissue's radiobiological response and cells' reaction systems treated with AuNPs as radiosensitizer across multiple radiotherapy beams. This is extremely important when considering the wide range of various radiotherapy beams combined with AuNPs treatments; as such huge data range will encompass a great understanding of the complexity and specificity of identifying the ideal treatment selection and formulation when optimizing the radiation dose given for clinical translations.

1.5 General objective

To characterize the radiosensitization effects by gold nanoparticles (AuNPs) for advanced radiotherapy using multiple radiobiological models.

1.6 Specific objectives

1. To investigate the cytotoxicity, cellular uptake and intercellular localization of AuNPs.
2. To quantify the radiosensitization effects by AuNPs for photon beam, electron beam and HDR brachytherapy as well as proton beam and monoenergetic synchrotron beam.
3. To analyze the validity of different radiobiological models and quantification methods in characterizing the radiosensitization effects by AuNPs.
4. To evaluate parameters that influence the radiosensitization effects by AuNPs such as cell types, beam energy, nanoparticles size and concentration.

1.7 Thesis outlines

The thesis is divided into two parts. Part 1 is the study on conventional clinical radiotherapy which as follow photon beam, electron beam and HDR brachytherapy. The study was conducted at Hospital Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan.

Part II consists of the study conducted using specialized beam of the proton beam and monochromatic synchrotron photon beam. The study on proton beam was conducted at Hyogo Ion Beam Medical Center in Hyogo, Japan from the period of 2016-2018. The study using monochromatic synchrotron beam was conducted at Australian Synchrotron in Clayton Melbourne Australia in which the beam time was acquired in December 2015.

The chapters of the thesis:

Chapter 1: The introduction describes the modern radiotherapy approaches, the specification on radiobiological effects and the innovation of nanomedicine applications. The objectives of study are also emphasized.

Chapter 2: The literature review of the study details on gold nanoparticles application with radiotherapy, the mechanism of the interactions and the pre-clinical test of previous study.

Chapter 3: The methodology of the study was initially specified on cell culture maintenance, gold nanoparticles preparation, cellular localization, uptake and cytotoxicity assay protocol. The cell irradiations setup, radiation dosimetry and clonogenic assay were illustrated. Radiobiological models applied for cell survival curve and quantification methods of radiosensitization effects were then described.

Chapter 4: The description of the results begins with gold nanoparticles uptake, localization and cytotoxicity. The cell survival analysis using different radiobiological models were then presented. Linear Quadratic (LQ), Multi-Target (MT), Repairable Conditionally Repair (RCR), Pade' Linear Quadratic (PLQ), Repair (RM), Kavanagh and Newman (KN) and Two Components (2C) Models are validated. The survival curve of different beam was compared and analysed. Radiosensitization impact were quantified using different methods (DEF, SER, RER, REF). The radiosensitization effects due to AuNPs size, concentration and cell types are also examined.

Chapter 5: The findings of gold nanoparticles uptake, localization and cytotoxicity was discussed. The detailed-on the radiosensitization impact for conventional radiotherapy are explained. The influence of AuNPs sizes, concentrations and different cells response were justified.

Chapter 6: Introduction and literature on proton beam therapy was briefly described. The cell survival experimental work for proton beam was illustrated. The study was also supported by additional measurement of reactive oxygen species (ROS) due to AuNPs interaction with proton beam. All those findings were discussed in detail accordingly.

Chapter 7: A brief introduction on synchrotron radiation production and its applications is described. Literature review on previous challenge in overcome dose limitation and optimization in radiotherapy were also discussed. In this study, the experimental methods were explained, and results were analyzed using similar radiobiological models approaches. The quantification of radiosensitization effects has

also been evaluated. The experimental data were also supported by theoretical dose enhancement calculation. All the corresponding results were discussed.

Chapter 8: The conclusion of the study was reported by each main chapter, respectively. Limitations and future recommendations were also discussed briefly.

CHAPTER 2
LITERATURE REVIEW

2.1 Nanomedicine in radiotherapy

Nanomedicine is an active research area that holds great future to intercede with cancer treatment at the molecular level and deliver effective doses to targeted cancer cells with improved selectivity and lessened toxicities towards neighbour normal tissues. The core of nanomedicine is the development of nanoparticles, materials that hold unique distinctions such as nanoscale size, high surface-to-volume ratio and suitable physicochemical characteristics. Nanoparticles have wide range of types are able to act as a multifunctional agent that incorporates with the specific binding of drugs to targets in cancer cells or the tumour microenvironment, simultaneous visualization of tumours using innovative imaging techniques, prolonged drug-circulation times, controlled drug-release kinetics, and superior dose scheduling for improved patient compliance. In an example, the carbon nanotubes have future usefulness in cancer thermal ablation therapy, because of their electrical and thermal conductivity. However, their properties such as length and diameter might cause inflammatory and toxic effects (Markman et al., 2013).

Novel metal nanoparticles in particular gold nanoparticles are also capable as a therapeutic agent, imaging contrast media and nanocarrier of such proteins, DNA or RNA. The gold core is considered to be non-toxic and the therapeutic payload can be forced to be released from the conjugate due to their photo-physical properties (Webster et al., 2013). In radiotherapy, nanoparticles possess properties as a radiosensitizer to enhance radiation dose to the target by increasing the probability of radiation interaction and production of the secondary electron that will cause DNA damage. Examples of nanoparticles based radiosensitizer that has the potential to be

applied in radiotherapy are metallic nanoparticles (Au, Bi, Pt, Gd), superparamagnetic iron oxide nanoparticles, quantum dots and various hybrid nanoparticles.

Metallic nanoparticles composite a packed of metal particles which are functionalized as scatter medium when interact with high energy radiation (megavoltage) to produce secondary radiation. The secondary radiation directly localized into tumours cells in large amount at a short distance. This mechanism gives therapeutic radiation in tumours area at the same time despair neighbour of healthy tissues. The concept of using high Z materials incorporated in cells was initially investigated using iodine which presented higher efficiency for secondary radiation production (Matsudaira et al., 1980). This study was also supported by a separate study using gold films that indicated multifold and significant dose enhancement effect upon irradiation (Regulla et al., 1998). Dose enhancement effects induced by high Z materials are believed to be more efficient by resizing the gold or others types of metal into nanoparticles.

Several types of metallic nanoparticles have been introduced as radiosensitizer show similar significant dose enhancement effects when tested both *in-vitro* and *in-vivo*. Metallic nanoparticles such as gadolinium, iron oxide, titanium and silver nanoparticles provide similar outcome of dose enhancement or radiosensitization effects (Tokumitsu et al., 2000; Xu et al., 2009; Khoei et al., 2014, Nakayama et al., 2016). Superparamagnetic iron oxide nanoparticles for examples withhold special properties of highly biocompatible while collaterally induced cytotoxic effects when interact with radiation (Klein et al., 2013). In some cases, these nanoparticles are also combined with chemotherapeutic agents or nanocomposite metal based like (Fe_2O_4/Ag) for increased efficiency (Huang et al., 2012) (Zhao et al., 2012).

Other than metal-based nanoparticles, the non-metal nanoparticles also exhibit potential to acts as radiosensitizer. Klein et al. (2012) have investigated the ultrasmall uncapped and aminosilanized oxidized silicon nanoparticles to treat breast cancer cells with X-rays. Quantum dots which made of semiconductor (CaF, LaF, ZnS or ZnO) have been developed as photosensitizer capable in producing radicals upon absorption of visible light. This type of light however gives less penetration and suitable specifically for superficial cancer treatments (Shao et al., 2011).

Nanomedicine in general occupied various applications in radiotherapy. Summary most of all classification of nanomedicine applied in elevating radiation efficiency is presented in Figure 1.6.

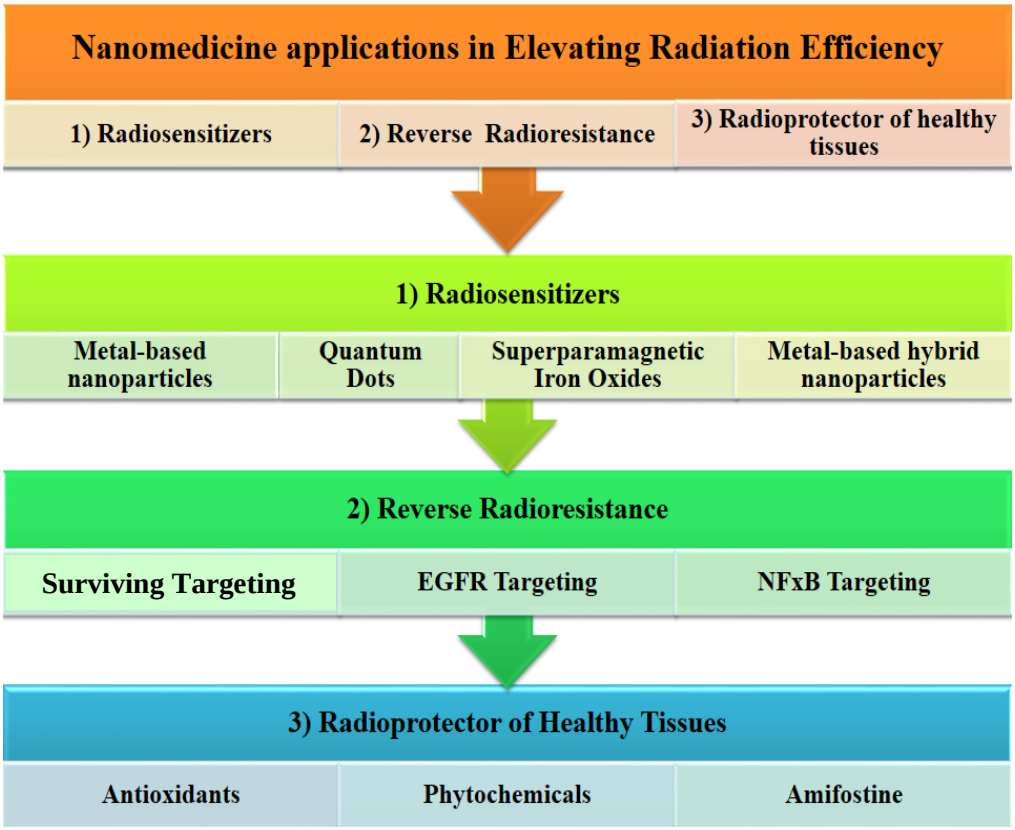

Figure 2.1 Nanomedicine in radiotherapy

2.2 Application of Gold Nanoparticles (AuNPs) in radiotherapy

The development of potential high Z materials as radiosensitizers in recent years becomes more interesting. The basic principle of primary radiation collide with high Z elements deposited secondary radiation locally surrounding targeted cancer area may improve radiotherapy efficiency in treating malignant diseases. Pioneer studies have proven the presence of iodine in mammalian cells in culture indeed induced radiosensitization or dose enhancement effects that enhance cell killing when combined with radiation. Subsequent studies with other types high Z matters had been reported such as gold (Au), silver (Ag), hafnium oxide, platinum (Pt), gadolinium, iron oxide (Fe_3O_4) and quantum dots (QDs). The most promising high Z elements which is gold, tuned into nanoparticles scale given efficiency distribution throughout the specific target. Gold nanoparticles (AuNPs) features of synthetic versatility, capable for size, shape and surface characteristics modification.

Regulla et al. (2000) were first verified the dose enhancement effects using monolayer culture of C3H/10T1/2 mouse embryo fibroblasts irradiated with 48 keV X-ray on gold foil. This is followed by seminal work by Hainfeld et al. (2010) on nano-small sized 1.9 nm AuNPs that were injected into the mice bearing SCCV11 squamous cell carcinomas. The results show improvement in the survival days by the increment from 53 days (-gold) to 76 days (+gold). The promising results of AuNPs are due to the higher mass attenuation coefficient of AuNPs in which at 100 keV of photon energy, gold could provide 2.7 times better sensitivity per unit weight than iodine and gadolinium. This indication picturized the existence of AuNPs in the targeted tumour may cause the dose enhancement effects in radiotherapy (Goswami et al., 2017). Improvised gold nanoparticles i.e: AuNP-dual peptide has been investigated to show that at 4 Gy, dose enhancement factor (DEF) up to 3.2 had been produced (Nicol et al.,

2017). AuNPs in combination with radiation also delayed the tumour growth around 10.2 days instead of 3.4 day with irradiation alone (Nicol et al., 2018).

Other promising study using multifunction AuNPs-formulation (Chitosan-capped-AuNPs-Doxorubicin) with MCF-7 cells irradiated using 6 MV photon beam exhibited potent radiosensitization effects up to 2 fold compared to irradiation only (Fathy et al. 2018). Another latest investigation reported that 20 nm of AuNPs-PEG-anti-human epidermal growth factor receptor type 2 (HER2) antibody showed a significant dose enhancement at only 3 Gy of radiation dose (Hatoyama et al., 2019). Shreds of evidence clearly potrayed the potential of the AuNPs application as radiosensitizer on top of others different promising usage of AuNPs in many areas.

2.2.1 Characteristic and properties of AuNPs

Gold nanoparticles have been reported to show potential as radiosensitizers for radiotherapy. This mainly due to its special characteristic and properties in Table 2.1.

Table 2.1 The characteristic and properties of AuNPs

Characteristics	✓ High-Z atomic number (Z=79), an ideal material for radiosensitization reactions when combining with radiotherapy (Hainfeld & Slatkin, 2008). ✓ Gold being very inert, it is highly biocompatible which issues need to be considered as in vivo inflammatory effect would be resulted (Shukla et al., 2005). ✓ Nanoparticles surface ratio able to enhance the effect of the radiation over a large area of tumour thus eliminating the need of the nanoparticles to be delivered to all the cells of the tumour tissues (Jain et al., 2007).

Properties	✓ Nanoparticles are known to have low systemic clearance as compared to low molecular contrast agents such as iodine allowing the photosensitizing material enough time to get absorbed into the tumour tissue (Longmire et al., 2008). ✓ Nanoparticles are known to be well absorbed into the systemic circulation, better permeation into the tumour tissue. This along with lower clearance rate results in the enhanced permeation and retention (EPR) effect (Iyer et al., 2006). ✓ Better attaching target with antibodies or other targeting moieties, given a good specifically delivered to the tumour cells location (Hainfeld et al., 2008). ✓ The feasible nano shape based on delivery requirements of the tumours tissues (such as its size and location) so as to achieve optimum delivery and effects (Chithrani et al., 2010). ✓ Improving specific tissue pharmacokinetics, cause them easy to image and quantify by using optimum dose level for best result (Xia et al., 2016).

2.2.2 Radiosensitization Effects by AuNPs

2.2.2(a) Theoretical dose enhancement by high Z materials

A non-Monte Carlo method also known as a systematic analysis of mass attenuation coefficient in different energies of photon had been simulated by Corde et al. (2004) for dose enhancement produced by iodine. This theoretical model-based calculation was also been used by Rahman et al. (2009) on AuNPs. The theoretically expected dose enhancement ratio or factor was calculated from the variation of the mass energy-absorption coefficient of the target due to the presence of high Z matter such as AuNPs. The dose enhancement factor (DEF) calculation is shown in the Equation 2.1:

$$DEF = \frac{\left(\frac{\mu_{en}}{\rho}\right)_E^{water+\,Au}}{\left(\frac{\mu_{en}}{\rho}\right)_E^{water}} = \frac{w_{Au}\left(\frac{\mu_{en}}{\rho}\right)_E^{Au} + (1-w_{Au})\left(\frac{\mu_{en}}{\rho}\right)_E^{water}}{\left(\frac{\mu_{en}}{\rho}\right)_E^{water}} \qquad (2.1)$$

The mass-energy absorption coefficient for the considered compound were simulated with monochromatic x-rays beam (energy: E) and in the fraction by weight of Au in the mixture. Figure 2.1 shows the theoretical DEF for several Au aqueous mixtures.

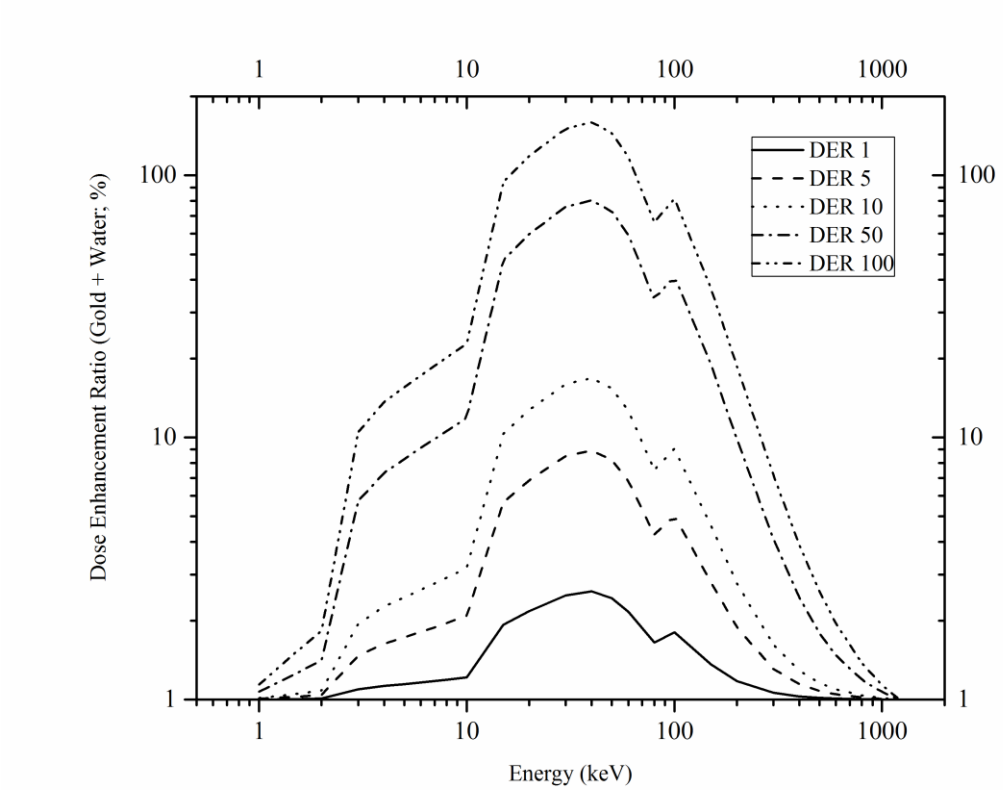

Figure 2.2 Energy dependence of the theoretical of DEF for several Au aqueous mixtures (from bottom to top, the mass proportion of Au in water, w_{Au} range from 0.01 to 1.