A MONTE CARLO-BASED MODEL OF GOLD NANOPARTICLE RADIOSENSITIZATION

by

Eli Solomon Lechtman

A thesis
submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Graduate Department of Medical Biophysics
University of Toronto

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Abstract

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2013

The goal of radiotherapy is to operate within the therapeutic window - delivering doses of ionizing radiation to achieve locoregional tumour control, while minimizing normal tissue toxicity. A greater therapeutic ratio can be achieved by utilizing radiosensitizing agents designed to enhance the effects of radiation at the tumour. Gold nanoparticles (AuNP) represent a novel radiosensitizer with unique and attractive properties. AuNPs enhance local photon interactions, thereby converting photons into localized damaging electrons. Experimental reports of AuNP radiosensitization reveal this enhancement effect to be highly sensitive to irradiation source energy, cell line, and AuNP size, concentration and intracellular localization. This thesis explored the physics and some of the underlying mechanisms behind AuNP radiosensitization.

A Monte Carlo simulation approach was developed to investigate the enhanced photoelectric absorption within AuNPs, and to characterize the escaping energy and range of the photoelectric products. Simulations revealed a $10^3$ fold increase in the rate of photoelectric absorption using low-energy brachytherapy sources compared to megavolt sources. For low-energy sources, AuNPs
released electrons with ranges of only a few microns in the surrounding tissue. For higher energy sources, longer ranged photoelectric products travelled orders of magnitude farther.

A novel radiobiological model called the AuNP radiosensitization predictive (ARP) model was developed based on the unique nanoscale energy deposition pattern around AuNPs. The ARP model incorporated detailed Monte Carlo simulations with experimentally determined parameters to predict AuNP radiosensitization. This model compared well to in vitro experiments involving two cancer cell lines (PC-3 and SK-BR-3), two AuNP sizes (5 and 30 nm) and two source energies (100 and 300 kVp). The ARP model was then used to explore the effects of AuNP intracellular localization using 1.9 and 100 nm AuNPs, and 100 and 300 kVp source energies. The impact of AuNP localization was most significant for low-energy sources. At equal mass concentrations, AuNP size did not impact radiosensitization unless the AuNPs were localized in the nucleus. This novel predictive model of AuNP radiosensitization could help define the optimal use of AuNPs in potential clinical strategies by determining therapeutic AuNP concentrations, and recommending when active approaches to cellular accumulation are most beneficial.
Acknowledgements

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- Eli Lechtman
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List of Scientific Contributions

During my thesis research (Jan 2009- June 2013), I have made a number of scientific contributions, both directly and indirectly relating to the research contained in this thesis. The most significant of these contributions are listed here.

Refereed Publications

(\textit{PA} = \text{Principal Author, \textit{Co-PA} = \text{Co-Principal Author, \textit{C} = Collaborator})


Lechtman E, Chattopadhyay N, Cai Z, Mashouf S, Reilly R, and Pignol JP. Implications on clinical scenario of gold nanoparticle radiosensitization in regards to photon energy, nanoparticle size, concentration and location. *Phys. Med. Biol.* 56 4631-4647, 2011. PA. Selected by the Institute of Physics (IOP) Publishing for their “featured articles collection”, and was selected as one of 25 highlighted articles in 2011 for *Phys. Med. Biol.*. (Chapter 2 is a reprint of this manuscript with minor formatting changes)


Submitted:

Lechtman E, Lai P, Mashouf S, Cai Z, Reilly R, and Pignol JP. Influence of cancer cell type, source energy, nanoparticle size, and localization on gold nanoparticle radiosensitization. *International Journal of Radiation Oncology*Biology*Physics*. June 2013. PA. ”. (Chapter 4 is a reprint of this manuscript with some minor changes)

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<td>ARP</td>
<td>gold nanoparticle (AuNP) radiosensitization predictive (model)</td>
</tr>
<tr>
<td>AuNP</td>
<td>Au (gold) nanoparticle</td>
</tr>
<tr>
<td>BNCT</td>
<td>boron neutron capture therapy</td>
</tr>
<tr>
<td>BUDR</td>
<td>bromodeoxyuridine</td>
</tr>
<tr>
<td>c</td>
<td>speed of light (3 \times 10^8 \text{ m/s})</td>
</tr>
<tr>
<td>CTV</td>
<td>clinical target volume</td>
</tr>
<tr>
<td>DDR</td>
<td>DNA damage response</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSB</td>
<td>double strand break</td>
</tr>
<tr>
<td>E</td>
<td>energy</td>
</tr>
<tr>
<td>E\textsubscript{B}</td>
<td>binding energy of an orbital electron</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>EDPL</td>
<td>evaluated photon data library</td>
</tr>
<tr>
<td>EPR</td>
<td>enhanced permeation and retention</td>
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<tr>
<td>eV</td>
<td>electron volts</td>
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GTV  gross tumour volume

Gy  Gray - unit of absorbed dose (J/Kg)

h  Plank's constant (6.626×10-34 joule/s)

HDR  high-dose-rate (> 1200 cGy per hour)

HER-2  human epidermal growth factor receptor-2

HPV  human papillomavirus

HVL  half value layer

\(^{123}\text{I}\)  a radioactive isotope of iodine with a half-life of about 13 hours

\(^{125}\text{I}\)  a radioactive isotope of iodine with a half-life of about 59 days

\(^{192}\text{Ir}\)  a radioactive isotope of iridium with a half-life of about 74 days

ICRU  International Commission on Radiation Units

ICP-AES  inductively coupled plasma atomic emission spectroscopy

ICP-MS  inductively coupled plasma mass spectroscopy

IMRT  intensity modulated radiation therapy

IUdR  Iododeoxyuridine

J  Joule

KE  kinetic energy

keV  kilo-electron volts
kVp  kilovoltage peak
LET  linear energy transfer
LEM  local effect model
LDR  low-dose-rate (< 200 cGy per hour)
LQ   linear quadratic
LQM  linear quadratic model
m_e  rest mass of an electron
MC   Monte Carlo
MCNP Monte Carlo N-Particle code
MDR  moderate-dose-rate (200-1200 cGy per hour)
MeV  mega-electron volts
MID  mean inactivation dose
MV   megavolt
nm   nanometer
NTCP normal tissue complication probability
OER  oxygen enhancement ratio
\(^{103}\)Pd a radioactive isotope of palladium with a half-life of about 17 days
PEG  polyethylene glycol
PENELOPE  Penetration and energy loss of positrons and electrons

PTV  planned target volume

RBE  relative biological effectiveness

ROS  reactive oxygen species

SER  sensitizer enhancement ratio

$^{89}\text{Sr}$  a radioactive isotope of strontium with a half-life of about 50 days

TCP  tumour control probability

TEM  transmission electron microscopy

UV/vis  ultraviolet/visible

VEGFR  vascular endothelial growth factor receptor

$^{169}\text{Yb}$  a radioactive isotope of ytterbium with a half-life of about 32 days

$Z$  atomic number (number of protons in an atom)
Chapter 1

Introduction
1.1 Cancer

1.1.1 Incidence, mortality, and economic burden

Despite decades of modern research and advancements in treatment, cancer remains a major global health and economic burden. Consuming an estimated 1.5 percent of the world’s gross domestic product, cancer is the leading cause of death in developed countries, and is projected to become the leading cause of death worldwide [1-3]. Globally there was an estimated 12.7 million cancer cases and 7.8 million deaths in 2008. The incidence of cancer is projected to increase due to the aging world population and the increasing adoption of behaviors linked to cancer risk factors. Lung cancer is the most common cancer among males worldwide, while breast cancer is most common among women. In Canada there was an estimated 186,400 new cases of cancer and 75,700 cancer related deaths in 2012 [1]. About 40% of Canadians will develop cancer during their life, and about 25% will die of the disease [1]. Over the next 30 years, it is estimated that Canada will spend $177.5 billion in direct health care costs [4].

1.1.2 Definition, etiology, and risk factors

Cancer is characterized by an abnormal malignant proliferation of mutated cells, which grow uncontrollably and invade healthy tissues [5,6]. The word cancer is Latin for crab – an ancient analogy of how the disease spreads like a crab by reaching out with “claws” [6,7]. Malignant cells are those that have undergone genetic changes resulting in a failure to respond normally to cellular signals controlling differentiation, proliferation, and programmed cell death [7-9]. The two main types of gene alterations implicated in the development of cancer are the activation of oncogenes leading to abnormal cell growth, and the deactivation of tumour suppressor genes needed for apoptosis (programmed cell death) [6,8,9]. Cancer cells tend to have irregular shapes and
microscopic appearances compared to healthy cells. The cancer cell’s nucleus is often larger, while the cytoplasm is often smaller, lacking features of the cell of origin. At the macroscopic scale, cancers can form solid tumours that are classified as either carcinomas – cancers originating in epithelial tissue, or sarcomas – cancers originating in connective tissue [6,10]. Cancers spread throughout the body by means of direct infiltration, lymphatic drainage, or the vascular system. When a cancer forms colonies in a non-adjacent region of the body, the cancer is said to have metastasized [10,11].

The causes of cancer are diverse and still not fully understood. At the cellular level, genetic alterations including mutations and deletions, can arise spontaneously due to random errors in biological processes, or can result from exposure to chemical mutagens, viruses, and UV and ionizing radiation [11,12]. At the behavioral level there are a number of risk factors contributing to the development of cancer. Cancer risk has been correlated with increasing age, body weight, alcohol consumption, poor diet, lack of exercise, sun exposure, and stress. Tobacco smoke, a known carcinogen, is the leading cause of cancer, and estimated to be responsible for 30% of all cancer deaths [12-14]. Some other common carcinogens include acetaldehyde (from alcoholic beverages), asbestos, benzene, soot, wood dust, and human papillomavirus (HPV) [13-15].

1.1.3 Treatment and management

With advancing technologies, progress in understanding of cancer, and early detection, cancer death rates are declining in the developed world [12,15]. The majority of cancers, especially when detected early, can be cured with treatment. The aims of a given cancer treatment can be curative, disease control, or palliation, depending largely on the cancer type and stage. The main types of cancer treatments generally involve some combination of surgery, medical therapy, and radiation therapy [12]. Because of the aggressive and complex nature of cancer, a treatment plan usually
involves treating beyond the known limits of the cancer using a multimodality approach. When medical or radiation therapy are used to shrink the tumour prior to surgery, they are termed neoadjuvant therapies. When these therapies are employed after the primary surgery to reduce cancer recurrence, they are termed adjuvant [12].

1.1.3.1 Surgery

Surgery is the oldest treatment for cancer, and is still the most effective treatment of solid tumours today. More than half of all cancer treatments involve a surgical component [6,12]. The primary goal of surgery is to excise the cancer as well as surrounding tissues in which the cancer may have spread, but surgery can also be used for palliation and control of cancer symptoms to improve a patient’s quality of life. For early staged, local, non-metastasized cancers, surgery alone may offer a curative treatment. For more advanced cancers that have spread into surrounding lymph nodes, surgery would likely involve the removal of the primary cancer as well as the involved lymph nodes, preferably in one continuous excised volume, called “block dissection”.

Surgical treatment of cancer has come a long way, from the development of general anesthesia in the 19th century, to the advent of antiseptic operating conditions [6,16]. Today a surgical approach is guided by evidence-based strategies and patients’ values, with an emphasis on minimizing morbidity and aesthetic deformity. For example, the standard surgical treatment of all breast cancers over the past century was a radical mastectomy – the complete surgical removal of the breast, chest wall muscle, and block dissection of the affected lymph nodes. This approach left patients with significant deformities, impaired mobility, and a high risk of lymphedema [16,17]. Today surgical management of many early stage breast cancers is achieved using breast-conserving surgery, which involves the removal of the tumour plus at least a 1 cm margin. This procedure improves the cosmetic outcome and reduces morbidity [10,17].
1.1.3.2 Medical therapy

Medical treatments of cancer involve treating the disease with drugs such as chemotherapy, hormone therapy, and targeted therapy/immunotherapy. The goal of medical therapy is often to treat any cancer cells that have spread into healthy tissue, known as micro-metastatic disease. Medical therapies can be offered as neo-adjuvant, adjuvant, or primary treatments, but are often prescribed as a part of a combination therapeutic strategy [10,18].

Chemotherapy

Chemotherapy refers to the use of cytotoxic drugs to treat cancer. Although the term was first coined in the early 1900’s, chemotherapy would not become widely used until well into the second half of the 20\textsuperscript{th} century [10,18]. Cytotoxic drugs tend to indiscriminately kill rapidly dividing cells such as cancers, but also affect a range of healthy cells. Today there are a vast number of chemotherapy agents including antimetabolites, alkylating agents, antimicrotubule agents, cisplatin and its derivatives, and topoisomerase inhibitors. Each works through different mechanisms affecting DNA or RNA production or processes of cell division. A combination approach utilizing various chemotherapy agents is often needed to combat cancer drug resistance, and to kill cancer cells at different phases through the cell cycle [10,19].

Hormone therapy

The inception of hormone therapy came with the important discovery in 1896 by George Beatson, who observed breast cancer regression in women who had their ovaries removed [19,20]. Later in 1939, Huggins and Hodges began treating prostate cancer patients with androgen deprivation therapy [10,20,21]. Since then, hormones have been implicated in the progression of many cancers. Hormone therapy can involve inhibiting hormone production through ablation of endocrine glands,
or medically through hormone receptor antagonists that block hormone receptors or supraphysiological hormone doses intended to trigger hormonal feedback inhibition [6,10,21]. The effects of hormone therapy are generally more localized to target organs and are therefore often more tolerated compared to traditional chemotherapy [6,10].

**Targeted therapy/Immunotherapy**

Immunotherapy refers to activating the body’s immune system to treat cancer. Immunotherapy is a continually growing field with many different strategies, but attempts at developing an active cancer vaccine has proved elusive [10,22]. A common immunotherapy treatment involves the systemic delivery of monoclonal antibodies specific to overexpressed cell surface receptors in certain cancers. When these antibodies bind receptors they can inhibit cell growth and proliferation by antagonizing oncogenic pathways, and they can also opsonize cancer cells, triggering an immune response [22,23]. One such monoclonal antibody is trastuzumab, commonly prescribed for the treatment of certain breast cancers overexpressing HER-2 [23,24].

**1.1.3.3 Radiation therapy**

Radiation therapy, also known as radiotherapy, involves treating cancers with doses of ionizing radiation (in the energy range of keV and MeV) to achieve locoregional tumour control. Although unintended exposure to ionizing radiation can itself lead to cancer, a therapeutic treatment of radiation with an evidence-based selection of irradiation energy, dose, and target volume can significantly reduce a tumour’s size and ability to grow, and can improve disease symptoms, survival, and quality of life. Radiation therapy was founded on the important discoveries of x-rays by WC Röntgen [24,25], radioactive decay by Henri Becquerel, and the radioactive element radium
by Marie Curie, all in the late 19th century. Today radiation therapy is used in over 50% of treated cancers, either on its own or often in combination with surgery and/or medical therapy [6].

Radiation therapy has advanced considerably since its inception, with improved delivery techniques and image guidance. Modern radiation therapy begins with imaging of the tumour region, often acquired with computed tomography. Using this image the gross tumour volume (GTV) is contoured, comprising only the visual tumour. Next, a clinical target volume (CTV) is delineated by expanding the margins of the GTV to account for microscopic tumour spread. A final encompassing margin called the planned target volume (PTV) accounts for any uncertainties during planning and treatment delivery [25,26]. The goal of a treatment plan is to optimize the radiation delivery to achieve a homogeneous therapeutic dose throughout the PTV, while minimizing the dose to healthy tissues that could have adverse effects. In practice, normal tissue cannot be spared completely.

The delivery of radiation therapy can be broadly categorized into three main techniques – external beam therapy, brachytherapy, and unsealed radioisotope therapy.

*External beam therapy*

External beam therapy refers to the delivery of radiation using a source that is external to the patient. Conventional external radiation therapy use photons, but other methods involve electrons, protons, or heavy charged particles. Linear accelerators are the most commonly used method of producing high-energy photons called x-rays, while cobalt therapy machines are sometimes still used in developing countries [10,26]. The choice of photon energy in external beam therapy depends primarily on the tumour site relative to the skin surface, with higher-energy photons capable of deeper penetration. Linear accelerators deliver x-rays in the energy range of 6 – 18 MV and are designed to treat deep-seated tumours. Orthovoltage therapy units, delivering x-rays in the energy range of 150 - 350 kVp, are intended to treat cancers near the surface of the skin. Superficial therapy
units produce x-rays in the energy range of 80 - 150 kVp and are used to treat superficial legions [10,27].

External beam therapy is commonly delivered in a fractionated regimen in order to allow healthy tissue to heal. A fractionated treatment typically consists of 25 - 40 treatments of about 2 Gy delivered over 5 to 8 weeks [27,28]. Hyperfractionation refers to decreasing the dose per fraction and increasing the number of fractions, while hypofractionation, common in stereotactic radiation, refers to increasing the dose per fraction and reducing the number of fractions. Recent advances in external beam therapy include intensity modulated radiation therapy (IMRT), which allows for the dynamic modulation of the spatial-intensity profile of the radiation beam, improving dose conformity and homogeneity within the tumour region [28,29].

**Brachytherapy**

Brachytherapy refers to the delivery of radiation at the site of the tumour using a sealed radiation source [29]. Because the radiation does not have to traverse surrounding tissues as in external beam therapy, brachytherapy can potentially lower the adverse side effects to healthy tissue. However, due to the localized dose deposition (dose falls off from the source roughly according to an inverse square relation), proper placement of the brachytherapy source is crucial to minimize “hot” and “cold” dose regions. The radiation source can be placed interstitially for cancers such as breast and prostate, externally on the surface of the skin, or intracavity for vaginal, cervical, or anal cancer. There are two distinct delivery methods of interstitial brachytherapy: Temporary implantation and permanent implantation. These methods can also be broadly classified as either high-dose-rate (HDR) (> 1200 cGy per hour), moderate-dose-rate (MDR) (200-1200 cGy per hour), or low-dose-rate (LDR) (< 200 cGy per hour) [29-31].
Permanent seed implantation, used to treat prostate and breast cancers [29-31], utilizes LDR sources and involves surgically implanting into the tumour 50-80 metallic seeds encasing isotopes such as $^{125}$I or $^{103}$Pd. Due to the low dose rate, dose prescriptions are often higher than those prescribed for external beam therapy. Permanent brachytherapy radiation sources have a relatively low average energy below 50 keV, facilitating a highly localized dose deposition. Permanent seed implantation also offers the advantage of an outpatient procedure.

Temporary implant brachytherapy differs from permanent brachytherapy in that the radiation source, housed in a catheter, is removed after treatment. Temporary brachytherapy utilizes HDR sources and treatments are generally delivered in a small number of fractions (typically 1-4) [29,32]. Temporary brachytherapy has traditionally been delivered using isotope sources such as $^{192}$Ir (average energy $\approx$ 380 keV), but new technologies have become available offering miniature electronic x-ray brachytherapy with comparable dose rates, but at lower energies [32,33].

**Unsealed radioisotope therapy**

Unsealed radioisotopes can also be used to treat cancers from within the patient. This therapy is most often delivered systemically either orally or intravenously. $^{89}$Sr is often used for the treatment of bone metastasis where cancer cells are not well localized, while $^{123}$I is used in the treatment of thyroid cancer [33,34]. In systemic radioisotope therapy, isotopes accumulate preferentially at the cancer site either by relying on the chemical properties of the isotope, or through conjugation to cancer targeting antibodies [11,34]. There is also pilot data suggesting that unsealed sources may be effective in treating localized tumour through direct intratumoural injection.
1.2 Physics of ionizing radiation

Ionizing radiation refers to radiation that has sufficient energy to eject orbital electrons from an atom or molecule through an interaction [11,35]. The minimum energy required to eject a valence electron is between 5-25 eV equivalent to about $8\times10^{-19} - 4\times10^{-18}$ J (1 eV = $1.602\times10^{-19}$ J) [35].

There are various types of ionizing radiation including photons - electromagnetic radiation, electrons - negatively charged particles, protons - positively charged particles, neutrons, and charged atoms such as alpha particles and heavier elements. While each type has relevance to radiation therapy, the majority of radiation therapy involves photons and electrons.

1.2.1 Interactions of ionizing radiation with matter

Ionizing radiation is often categorized as either directly ionizing or indirectly ionizing. Directly ionizing radiation refers to charged particles that cause ionizations along their path within matter through small Coulomb-force interactions [35,36]. Indirectly ionizing radiation refers to uncharged particles such as photons and neutrons, which transfer their energy to directly ionizing charged particles in the medium. Interaction mechanisms are governed both by the energy and type of radiation, as well as the properties of the material [36,37]. All radiation interactions occur stochastically, meaning they are governed by probability distributions. It is therefore impossible to determine the precise interactions of an individual particle; rather, the various types of interactions can be examined along with their associated probabilities and expectation values [11,37].

1.2.1.1 Photon interactions

Photons travel at the speed of light, c, corresponding to $3\times10^8$ m/s in a vacuum. Photons can be conceptualized as both a particle, and a wave with a frequency, $f$, and wavelength, $\lambda$ [11,38]. A photon’s energy can be determined from its frequency or wavelength with a simple relation:
\[ E = hf = \frac{hc}{\lambda} \]

Where \( E \) is the photon’s energy in J, and \( h \) is Plank’s constant \((6.626 \times 10^{-34} \text{ joule/s})\). Photons are called gamma rays when they are emitted from an atomic nucleus, and x-rays when they are produced electronically. Typical photon energies used in radiation therapy are between 10 keV - 10 MeV. At these energies photons are scattered by Rayleigh scattering, and impart energy to matter through three main interactions: photoelectric absorption, incoherent Compton scattering, and pair production. Each interaction is characterized by unique mechanisms and probabilities. When a narrow beam of photons are incident on a medium, they each may travel distances without interactions, and are attenuated along their path according to an exponential relationship:

\[ N = N_0 e^{-(\mu_{\text{tot}} + \mu_{\text{ph}} + \mu_{\text{pp}} + \mu_{\text{ra}})L} \]

Where \( N_0 \) is the initial number of incoming photons, \( \mu_x \) represents the linear attenuation coefficients, or interaction probabilities of a given reaction type, \( L \) is the depth that the photons have traversed in the material, and \( N \) is the number of photons that have not yet interacted [38,39].

**Photoelectric absorption**

Photoelectric absorption involves the complete absorption of a photon by an atom in the medium. The total energy absorbed is then transferred to ejecting an orbital electron [35]. The kinetic energy, \( KE \), of the ejected electron, called a photoelectron, is given by:

\[ KE = hf - E_B \]

Where \( hf \) is the energy of the absorbed photon and \( E_B \) is the binding energy of the orbiting electron shell. For this process to occur, the photon’s energy must therefore be greater than \( E_B \) of a given atomic orbital. The highest probability of photoelectric absorption occurs for photons with energies
just slightly greater than $E_B$, with k-shell electrons being the most likely ejected electrons. The probability of photoelectric absorption is proportional to the atomic number, $Z$, of the medium raised to the $3^{\text{rd}}$ or $4^{\text{th}}$ power, and inversely proportional to the photon’s energy raised to roughly the $3^{\text{rd}}$ power. The photoelectric effect becomes increasingly probable below about 0.1 MeV, and is the dominant interaction in high-Z materials at low-energies [39].

Subsequent to a photoelectric absorption, the ejected photoelectron can go on to directly ionize the surrounding matter, and the absorbing atom undergoes orbital relaxation. During orbital relaxation, an electron from an outer shell fills the vacancy in the inner shell left by the ejected electron. As the vacancy migrates to outer shells, this process is repeated until all electrons have been brought to their lowest possible energetic state [35]. Each of these transitions produces an amount of energy equal to the difference in potential energy of the donor and recipient orbitals:

\[(1.4) \quad E = E_{B2} - E_{B1}\]

This energy can either be used in the production of a characteristic x-ray, called fluorescence yield, or alternatively it can be imparted to ejecting a bound electron, termed an Auger electron, resulting in another orbital vacancy. A single photoelectric absorption can therefore result in the production of characteristic x-rays as well as a cascade of low-energy Auger electrons [40].

**Compton scattering**

Compton scattering involves the interaction of a photon with a loosely bound electron with a negligible binding energy. The probability of a Compton interaction is related to the electron density of the material, and the incoming photon energy. Compton interactions are the dominant interaction type in the energy range of 20 keV – 30 MeV, in low-Z materials such as human tissues. This type of interaction can be likened to a billiard ball interaction where energy and momentum are
conserved. An incoming photon knocks an electron, transferring kinetic energy to the electron and scattering the photon. The direction of scatter and the amount of energy transferred in a Compton collision are related to the incoming photon energy, and is described by differential cross-sections defining the probability distribution function of such events [35,39].

Pair production

In electron-positron pair production, a photon is completely absorbed in an atom, converting into an electron-positron pair. The photon’s energy is used in the creation of the electron-positron pair, with the remaining energy converted into kinetic energy [35,41]. This conversion can take place when a photon of energy greater than 1.022 MeV (2m_e c^2) interacts with the Coulomb field of an atomic nucleus. Alternatively, photons with energies greater than 2.044 MeV (4m_e c^2) can interact with atomic electron fields, undergoing so called triplet production resulting in an electron-positron pair, and an ejected electron. The probability of pair production is proportional to Z^{2-3}, and increases at higher incoming photon energies [41,42]. Because pair production interactions dominate above 10 MeV, this interaction type is less relevant for the low-energy radiation therapy sources used in brachytherapy.

1.2.1.1 Electron interactions

In contrast to photons, which are sparsely ionizing and can travel relatively large distances without an interaction, electrons and other charged particles continuously interact with the material they traverse through Coulomb forces. While many of the interactions electrons undergo are elastic, and therefore impart a negligible amount of energy to the medium, fast electrons can ionize and excite matter through inelastic orbital collisions [37,38,42], and can generate photons through Bremsstrahlung emissions.
**Inelastic orbital collisions**

When an electron passes within a close distance to an orbital electron, it can ionize the atom by ejecting the orbital electron. This process is known as a knock-on interaction, and the ejected electron is termed a delta ray. If the ejected electron was from an inner shell, this will result in atomic relaxation, releasing characteristic x-rays and Auger electrons [37,38,43].

**Bremsstrahlung emission**

Bremsstrahlung emission can occur when an incoming electron passes within close proximity of the nucleus of an atom. In most of these cases, the electron is scattered elastically, but occasionally a photon is created from the energy transferred in slowing down the electron. The photon’s energy can be between $0 - E$, where $E$ is the energy of the incoming electron. The probability of Bremsstrahlung emission is proportional to $Z^2$ of the material. Bremsstrahlung emission is exploited in the production of high-energy photons in linear accelerators. Electrons are accelerated through an accelerator tube and interact with a high-Z target, usually tungsten, to produce a photon beam through Bremsstrahlung interactions [35,43].

**1.2.2 Absorbed dose**

There are various metrics used to quantify the interaction of ionizing radiation with matter [35]. In the field of radiation therapy, the absorbed dose in matter is an important metric used in the prescription, planning, and delivery of a radiation treatment. The integral or average absorbed dose in a volume is defined as the energy imparted to that volume (the energy deposited in the volume minus the energy leaving the volume) divided by the mass of the volume. The absorbed dose at a point is therefore the expectation value of the energy imparted to an infinitesimally small volume
divided by the infinitesimal mass of this volume [35,44]. The SI unit of absorbed dose is the Gray (Gy), equivalent to J/kg.

### 1.2.3 Monte Carlo simulation

#### 1.2.3.1 Overview of Monte Carlo simulation

Monte Carlo (MC) simulation is a computational approach to study stochastic events, such as radiation transport in matter [37,45]. As opposed to deterministic numerical approaches, which produce average values for a given stochastic phenomena, the MC approach uses pseudo random numbers to sample known probability distributions producing “histories” of individual events. Average quantities can then be determined through the central limit theorem by simulating many such random events. Due to its reliance on random numbers, the name “Monte Carlo” - first coined in the 1940’s during the nuclear weapon project in Los Alamos - is a reference to the famous Monte Carlo Casino in Monaco. In 1954, Hayward and Hubbell were the first to use MC simulations to transport photons, generating 67 histories on a desk calculator. With modern day computing power, Monte Carlo simulation has emerged as a gold standard in the study of radiation transport in matter.

A Monte Carlo simulation of radiation transport follows individual radiation particles (photons, electrons, etc.) through specified materials and geometries by assuming that the history of a particle through matter consists of a Markovian (future and past events are independent) series of free flights, each followed by an interaction. Pseudo random numbers are generated to sample numerical or analytical probability distributions based on empirical data or model calculations describing such interaction events [44-50]. The history of a radiation particle is simulated in a series of steps:

1) The distance of free flight before an interaction is randomly simulated based on the total interaction cross-section.
2) The type of interaction is randomly sampled based on the probabilities of each individual interaction type.

3) The energy deposited in the material, the angle of scatter, and the production of progeny particles are randomly sampled from differential cross-sections.

4) Steps 1-3 are repeated for the primary particle and all progeny particles put into motion (known as a particle shower) as the initial energy is imparted to the material, until the kinetic energy of each particle has reached a lower limit.

By repeating this process for many source particles, the expectation value of metrics such as absorbed dose can be tallied. The variance of average quantities tallied in Monte Carlo simulations is inversely proportional to the square root of the number of histories simulated. The major advantages of the MC approach over deterministic solutions such as the Boltzman transport equation, are the ability to simulate radiation transport through complex geometries, and the ability to interrogate the transport process and study the characteristics and contributions of specific particle types and mechanisms [45].

1.2.3.2 Variance reduction

One of the major obstacles of the Monte Carlo approach is the simulation time required to reduce to the variance of tallied quantities. There are a number of variance reduction techniques available in Monte Carlo simulations that can significantly reduce simulation time, but these techniques can introduce systematic errors if not used in the proper context.
Condensed history

While some simulations are quite straightforward, the simulation of high-energy particles and their progeny in large volumes can require many simulated interaction events. In particular, the simulation of charged particles can be onerous on computation. For example, an electron will experience over $10^5$ interactions in aluminum while slowing down from a kinetic energy of 0.5 MeV to 0.0625 MeV. For this reason, many general purpose Monte Carlo radiation transport codes (MCNP, ETRAN, GEANT, ITS, EGSnrc, FLUKA) use a so called condensed history approach to simulate electron transport [45-53]. Condensed history algorithms, based on multiple-scattering theories of radiation interactions, allow for the representation of a large number of interactions by a global estimation [44,51-53]. In this approach, a step length is chosen to encompass many small interactions, which together deposit a relatively small amount energy. Instead of each interaction being simulated individually, the average total energy loss and angular deflection are sampled based on multiple scattering theories. While the condensed history approach can significantly improve the efficiency of MC simulation, it can introduce large systematic errors in the investigation of radiation transport at small physical scales. For this reason, more specialized track-structure Monte Carlo codes, designed to examine radiation tracks at the microscopic scale use an event-by-event interaction approach [44,54,55]. The general purpose MC simulation code PENELOPE, uses a mixed approach to electron simulation, allowing the user to adjust the level of simulation detail from a condensed history approach to an event-by-event approach [37].

Other variance reduction techniques

There are various other techniques used in Monte Carlo simulation to speed up simulation time and reduce the variance of tallied quantities [45]. An energy cutoff specifies the threshold energy below which to terminate the particle without further simulation, locally depositing its remaining energy.
MCNP uses a built in cutoff of 1 keV for all particles whereas PENELOPE uses a minimum cutoff of 50 eV [37,45]. At the level of geometry description, the clever user will exploit geometric symmetry to increase the number of interactions in a region of interest, and will truncate materials of little significance to the simulation. Advanced nonanalog variance reduction techniques include interaction forcing, particle splitting, and Russian roulette, which involve artificially biasing certain events or regions of interest, and manipulating the statistical weights of particles to compensate.

1.2.3.1 limitations of Monte Carlo

Although MC codes continue to develop and advance, there are still a number of limitations that exist in such simulation approaches, most notably in the description of low-energy and nanoscale interactions [38,56]. The main factors contributing to these limitations are simplifications made regarding the absorbing matter and the inherent uncertainty in low-energy interaction cross sections.

For most general purpose MC transport codes, an assumption is made that the absorbing material consists of homogeneously distributed independent atoms as the targets of radiation interactions. At the macroscopic level, this assumption works well, but at the molecular level, this assumption does not hold. Because atoms are considered independent of each other, MC models tend to ignore effects such as multiple coherent scattering and atomic coupling of excited states, which become relevant at small physical scales and as the wavelength of an electron becomes comparable to the distance between molecules [54,55]. Furthermore, as electrons slow down, atomic polarization creates an electric field which significantly effects the trajectory of the incident electron [54,56,57]. Other physics phenomena are often omitted where the error in simulation is seen to balance the savings in computation. As an example, in PENELOPE detailed atomic relaxation is only simulated until the K, L, and M shells have been filled, and any energy given to outer shell relaxation is transferred
entirely to the kinetic energy of an ejected electron. While this simplification has no bearing on macroscopic quantities, it can cause simulation artifacts at the nanoscale.

The uncertainty in available cross section data for radiation interactions with condensed material is perhaps the biggest limitation to low-energy MC transport codes [54,56-58]. Empirical data and models of radiation interactions are continuously being updated, but they still contain large uncertainty in the low-energy range. For example, D.E. Cullen, the author of the current Evaluated Photon Data Library (EPDL) stated, “Between 100 eV and 1 keV you can still safely use this data, but your results could be limited by the uncertainty in the data” [37,58]. For photoelectric absorption below 100 eV, uncertainties in data have been estimated to be on the order of 1000% in solid matter [37,56,58]. Furthermore, differential cross sections available for low energy electron elastic scattering in condensed material can only be considered semi-quantitative below 1 keV [37,56,59-61]. Knowledge of these limitations is important when interpreting MC simulation results.

1.3 Radiobiology

Radiobiology is the study of the effects of ionizing radiation on biological tissues [27]. Radiation damage begins at the smallest physical and temporal scales, but its effects can be far reaching, lasting from seconds to years, and impacting functioning at the cellular to the systemic level. Of particular importance is the damage process that occurs at the cellular level.

1.3.1 Radiation damage to cells

Ionizing radiation can cause damage throughout a cell, but the current understanding of radiobiology suggests that deoxyribonucleic acid (DNA) is the critical cellular target, and if damaged, cell functioning and survival could be compromised [27,59-61]. Radiation induced cellular damage can be thought of as comprising three phases: the physical phase, the chemical phase, and the biological
phase. The physical phase occurs during irradiation, and involves ionization events within the cell either directly from charged particles or from indirectly ionizing radiation such as photons. An absorbed dose of 1 Gy can lead to over $10^5$ ionizations per cell [27,62,63]. The chemical phase, lasting on the order of seconds, refers to the breakage of molecular bonds and the cascading chemical reactions subsequent to ionizations. The biological phase, spans seconds to days or even years, and refers to cellular process that respond to DNA damage. These can include the activation of DNA damage response (DDR) pathways triggering repair mechanisms, cell cycle arrest, or programmed cell death [11,62-64].

1.3.1.1 DNA damage

Ionizing radiation causes DNA strand breaks and base damage through two main mechanisms known as direct action and indirect action (not to be confused with directly ionizing and indirectly ionizing radiation). Direct action refers to DNA damage that occurs when radiation interacts directly with the DNA molecule. For x-ray therapy using photons, direct action is most likely to occur when a photon produces a secondary electron that goes on to interact with the DNA. Indirect action refers to DNA damage caused by reactive oxygen species (ROS), also known as free radicals (atoms or molecules with an unpaired electron in the outer shell), such as hydroxyl radicals, produced near the DNA by radiation ionizations [11,64]. Hydroxyl radicals are produced by ionizations in water in two steps. In the first step, a water molecule is ionized:

$$H_2O \rightarrow H_2O^+ + e^-$$  (1.5)

Where $H_2O^+$ is a water ion, and $e^-$ signifies a free electron. The water ion, itself a free radical, quickly reacts with another water molecule (within $10^{-10}$ seconds) to form an hydroxyl radical:
The hydroxyl radical, $\text{OH}^\cdot$ is highly reactive because it desires an electron pair. It can diffuse short distances on the order of 2-4 nm, and can attack DNA molecules causing damage [11,64,65]. It is estimated that for sparsely ionizing radiation, indirect action accounts for up to 66 % of the total DNA damage [11,65].

Not all DNA damage has the same effect on cells. Isolated damage on a single strand of DNA is readily repairable by cellular repair mechanisms using the complementary strand as a template, and is not likely to result in permanent damage. However, multiple damage to the DNA in close proximity can have a more devastating effect on the cell [65,66]. When opposite strands of DNA are damaged in close proximity ($< 20$ nm, or a few tens of base-pairs) this is known a double strand break (DSB) [64,66,67]. Double strand breaks can result from independent interactions, but are more likely the result of associated ionizations such as from densely ionizing radiation tracks, or multiple radical attack [64,67,68]. It is estimated that for typical therapeutic x-ray sources, an absorbed dose of 1 Gy will result in about 2000 isolated DNA damages (including single strand breaks and base damage), and about 40 double strand breaks per cell [68,69]. Although DSBs are considered the critical type of DNA damage leading to chromosome aberrations, cells may still repair such damage. As a cell incurs multiple DSBs, repair becomes more difficult.

1.3.1.2 Cell survival

Just as radiation interactions are stochastic in nature, cellular response to radiation involves stochastic processes [27,69,70]. A population of individual cells irradiated under similar conditions may exhibit various outcomes. Subsequent to radiation damage, cells may experience no effect (where full repair is possible), or a number of different fates including bystander signaling,
reproduction delay, mutation, or cell death by necrosis, apoptosis or reproductive failure [27,61,62,70-72]. There are various methods to quantify cellular response to radiation damage [61,62,71-73], but for the purposes of radiation therapy, where the goal of treatment is to stop the growth of cancer cells, an important measure of cell response is reproductive integrity. Clonogenicity, a surrogate of reproductive integrity, refers to the ability of a cell to multiply indefinitely forming a cell colony, practically of 50 cells or more.

**Clonogenic formation assay**

Clonogenic survival can be assessed using the colony formation assay, which involves irradiating cells *in vitro* at various doses, and observing the number of colonies that form from a known number of treated cells [11,73]. As the radiation dose increases, the surviving fraction decreases. **Figure 1.1** shows the results of a colony formation assay plotted as the surviving cell fraction (on a log scale) as a function of the absorbed dose. Notwithstanding the differences in the cellular environment between *in vitro* and *in vivo* cell conditions, the cell formation assay has proven informative to predict the effect of radiation on a range of cell types [11,27].
Clonogenic survival differs with varying cell type, cell culture conditions and irradiation conditions. It is therefore of much interest in radiobiology to fit a curve to clonogenic survival as a function of dose. This allows for the characterization of the cellular response to radiation based on the fitting parameters. There are generally two main features of mammalian cell response to radiation. In the low dose region, the natural logarithm of cell death seems to be linearly related to dose. Next there is often a so called “shouldered response” when transitioning from low to higher doses, but the size of this shoulder varies wildly with different conditions [11,27,69]. There have been a number of

**Figure 1.1:** The colony formation assay characterizes cell survival as a function of dose. The linear quadratic model (LQ) can be used to fit a curve to the data and characterize the cell’s radiosensitivity.

*Cell survival curves*

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models developed over the years to account for the observed relationship between clonogenicity and radiation dose, each with its own “philosophy” and assumptions of radiation induced cell death [69,74]. The earliest models developed are known as target models [69,74]. In the multi-target model, an assumption is made that there are a number of targets (≥ 1) in a cell that need to be critically damaged in order to cause cell death. Using Poisson statistics to described the occurrence of lethal events in the cell, the multi-target model in its basic form can be written as:

\[ S = 1 - (1 - e^{(-D/D_0)})^n \]  

Where \( S \) is the surviving fraction, \( D \) is the absorbed dose, \( n \) is the number of targets, and \( D_0 \) is the dose required to produce an average of one hit per target. By adjusting these values, a best fit can be obtained for experimental clonogenic survival. Although target models enjoyed wide spread use up until the 1980’s [27,69,75], the major shortcomings of such models were the need for additional parameters to fit low dose survival, and the elusiveness of the targets being described.

Today, the linear quadratic model (LQM) is the most widely used cell survival model [27,69,75-77]. The linear quadratic model was originally developed as an empirical model, but mechanistic interpretations have since been suggested [76,77]. The model uses two free parameters and employs Poisson statistics for the occurrence of lethal events to arrive at a survival model given by:

\[ S = e^{-(\alpha D + \beta D^2)} \]  

Where \( \alpha \) is the linear dose sensitivity parameter and \( \beta \) is the quadratic dose sensitivity parameter.

**Figure 1.1** shows the LQM fit to experimental cell survival. The mechanistic description that has evolved is that the \( \alpha \) component of cell survival represents DNA DSBs resulting from single radiation tracks, while the \( \beta \) component of cell survival represents DSBs resulting from two or
more radiation tracks [77,78]. Although the mechanism is still disputed, the LQM allows for the characterization of radiation response for a wide range of cells and conditions, especially in the dose ranges of fractionated radiotherapy. Its success as a model prompted Fowler to remark rhetorically, “Was it a coincidence that the current successes of modern radiotherapy schedules should have arisen soon after the linear-quadratic (LQ) formula came into widespread use in early 1980s? [27,69,77-79]”

Other models focusing on the stochastic mechanisms of radiation damage and repair include the lethal-potentially lethal model and the repair saturation model [27,69,77,79,80]. While these models are gaining traction, they often involve many parameters and produce results similar to the linear quadratic model.

1.3.2 Radiosensitivity

Radiation sensitivity, also known as radiosensitivity, refers to the degree of biological susceptibility of cells to ionizing radiation, and can be characterized by the sensitivity parameters, \( \alpha \) and \( \beta \) of the LQM [80-82]. Radiosensitivity varies widely with intrinsic biological factors such as cell line, cell cycle phase, and oxygen concentration, as well as the irradiation energy, particle type, and delivery method [11,80-82]. Due to this wide variation, and the implications for radiation therapy, it is important to understand the factors effecting radiosensitivity.

1.3.2.1 Intrinsic Radiosensitivity

DNA content/configuration and cell cycle

Because DNA is the critical target for radiation damage, DNA content is often correlated with radiosensitivity [11,83]. Furthermore, DNA configuration can alter radiosensitivity by changing the
physical cross-sectional area of the DNA target. DNA content and configuration are both intimately related to the mitotic cell cycle; therefore cell cycle can have a large effect on the radiosensitivity of cells. The mitotic cell cycle consists of two phases known as interphase and mitosis. During mitosis, as the cells divides, the chromosomes condense. It is during this phase, where the DNA is configured in condensed chromosomes, that cells experience increased radiosensitivity. The interphase can further be subdivided into the first growth phase \( G_1 \), the DNA synthesis phase \( S \), and the second growth phase, \( G_2 \). Cells exhibit another radiosensitive period towards the end of the \( G_1 \) phase. Different cell lines spend varying amounts of time in each phase of the cell cycle, resulting in varying radiosensitivity. When cells experience radiation damage, they may halt the mitotic cycle to check and repair damages. This process can itself decrease sensitivity by preventing cells from entering the radiosensitive mitotic phase. Rapidly dividing cells, including endothelial cells and certain cancers, are observed to be more radiosensitive than slowly diving cells due to the increased contribution of radiation induced mitotic cell death [63,83].

**Gene expression and cell signaling**

Subsequent to radiation, cells may repair the damage, delay their growth, or commit suicide through apoptosis. Each of these fates rely on signaling pathways, which if compromised, could effect radiosensitivity [11,63,84,85]. There are a number of genes involved in DNA DSB repair including XRCC5 Ku 80, XRCC6 Ku 70, and XRCC7. Cells with mutated, overexpressed, or under-expressed repair genes can exhibit varying degrees of radiosensitivity, or its antonym, radioresistance. Some examples of inherited syndromes in humans associated with radiosensitivity include Down’s syndrome, and ataxia telangiectasia [11,81,84-87]. Apoptosis is a major pathway of cell death following radiation damage. Some cancer cells exhibit increased radioresistance due to mutations resulting in ill functioning apoptotic and growth-suppressing pathways such as Fas-mediated,
ceramide-mediated, or p53-dependant pathways [81,86-88]. Radioresistance is characterized by a more pronounced shoulder on a cell survival curve. Various other genes including oncogenes have been studied in terms of their relationship to radiosensitivity, but results are inconsistent [88,89].

1.3.2.2 Oxygenation and hypoxia

The presence of oxygen can significantly effect the resulting damage from free radicals produced during irradiation [89-91]. Oxygen reacts with the free radical damage on the DNA resulting in a less repairable lesion [11,90,91]. Therefore, when free radical damage is a significant contribution to radiation induced DNA damage, as is the case for sparsely ionizing radiation, the presence of oxygen can increase radiosensitivity. Oxygen concentration has a strong influence on radiosensitivity up until atmospheric concentrations, where additional oxygen has little affect [11,92,93]. An understanding of how oxygen concentration affects radiosensitivity is important in radiotherapy, where many tumours exhibit hypoxia (oxygen deficiency), due to irregular vascularization, and are therefore radioresistant [92-95]. The oxygen enhancement ratio (OER) is defined as the ratio of doses required to produce a given cell survival in hypoxic and oxygenated conditions.

1.3.2.3 Dose rate

As discussed earlier in the chapter (Chapter 1.1.3.3), radiation treatments differ in delivery technique. Whereas fractionation refers to the delivery of radiation doses in separate treatments, dose rate refers to the rate at which dose is delivered during a treatment. Both fractionation and dose rate affect cell radiosensitivity by modulating how DNA damage is spread over time [94-96]. In most cases, especially with photon irradiation sources, a lower dose rate results in an increased resistance to radiation, as cellular repair mechanisms do not become over saturated with damage,
and multiple sub-lethal damages are less likely to compound [11,96,97]. A lower dose rate results in a more pronounced shoulder on a cell survival curve, meaning that a higher total dose is needed to achieve the same biological response compared to radiation delivered at higher dose rates. For these reasons, radiation doses prescribed in LDR brachytherapy are typically higher than external beam treatments [11,97,98].

1.3.2.4 Radiation quality

Radiation quality refers to the energy and type (photons, electrons, heavy ions, etc.) of radiation. For a given dose and dose rate, radiation of different qualities can result in very different biological responses. The underlying mechanism relates to the varied spatial distributions of ionizations for radiation of different qualities. Whereas sparsely ionizing photons produce only a few spread out ionizations, densely ionizing radiation, such as heavy charged particles, can produce complex “local multiply damaged sites” along their path [98,99]. As an example, an absorbed dose of 1 Gy to a cell nucleus would require the interaction of about 1000 individual photons, distributing ionizations randomly throughout the nucleus. In comparison, the same absorbed dose would be deposited by approximately four alpha particles, resulting in a much more localized and heterogeneous distribution of ionization events [99,100].

Linear energy transfer (LET) is often used to characterize radiation quality [100,101]. While there are various methods to calculate LET, this microscopic metric quantifies the average energy, dE, imparted to the medium by a particle traveling an infinitesimally small distance, dl [27,70,101,102]:

\[
LET = \frac{dE}{dl}
\]

Where LET is usually given in units of keV/µm. LET values range from 0.2 – 2 keV/µm for clinical x-rays, 0.3 – 30 for electrons of varying energies, and 100-200 keV/µm for heavy charged particles
The majority of high-LET DNA damage occurs through direct action, rather than through indirect production of free radicals. For this reason, oxygen concentration plays less of a role in high-LET radiation damage [11,103,104]. Similarly, due to the complex and devastating nature of the DNA damage from high-LET particles, cell survival depends less on repair mechanisms, dose rate, and cell cycle [11,104].

Whereas LET is a physical metric characterizing different radiation qualities, the relative biological effectiveness (RBE) characterizes the cellular response to different radiation qualities. RBE is a ratio of the dose required to achieve a given biological effect using a standard photon source, divided by the dose required for the same effect from a test radiation source [11,105,106]. RBE increases to a factor of around 8 as LET increases to 100 keV/µm, but at higher LET, the RBE drops. An explanation for this is that an LET of 100 keV/µm corresponds to ionization events that are approximately 2 nm apart – the diameter of double stranded DNA. This represents the optimal spacing to cause DSB per ionizations. Above this value, additional ionization can be thought of as overkill [105-107]. While RBE has traditionally been measured experimentally through clonogenic survival, a recently developed model called the local effect model (LEM), attempts to predict the RBE of heavy ions based on the linear quadratic cell sensitivity parameters from sparsely ionizing radiation, and a description of nanoscale energy deposition pattern of heavy ions [107-110].

1.3.3 Radiosensitization

The therapeutic ratio of a cancer treatment can be defined as the ratio of tumour control probability (TCP) divided by the normal tissue complication probability (NTCP) for a given dose [110]. The goal of radiation therapy is to operate within the therapeutic window – delivering doses of radiation to achieve locoregional control, while minimizing adverse effects to healthy tissue. Despite modern radiotherapy techniques designed to deliver a conformal dose to the tumour region, inevitably as
radiation doses are escalated, normal tissue damage ensues. In cases of radioresistant tumours, like glioblastoma, the therapeutic window may be limited, and treatment would therefore involve a tradeoff between tumour control and normal tissue complications.

Radiosensitization refers to a therapeutic intervention designed to radiosensitize the tumour, thereby widening the therapeutic window [27,110]. Just as there are a range of factors effecting radiosensitivity, there are a number of approaches to radiosensitization. No matter the mechanism, an ideal radiosensitizing agent is one that is tumour cell specific either in function or localization, persistent during the duration of treatment, and biocompatible – exhibiting low systemic toxicity. Preferential tumour localization can be achieved either through passive means – relying on the enhanced permeation and retention (EPR) effect of some tumours exhibiting leaky vasculature [111,112], or through active strategies involving radiosensitizers conjugated to tumour targeting moieties [113].

1.3.3.1 Cell response sensitizers

Some radiosensitizers come in the form of agents that modulate cellular response to radiation damage, such as targeted chemotherapy agents or anti-inflammatory agents, which can disrupt cellular DNA damage repair mechanisms [11,108-110]. Many of these types of drugs are used today, but often have systemic cytotoxic effects.

1.3.3.2 Hypoxic cell sensitizers

Because many tumours may be hypoxic, or contain hypoxic regions, hypoxic radiosensitizing agents are desirable. The most obvious hypoxic radiosensitizing agent is oxygen. Increased oxygenation can be achieved through blood transfusion prior to radiation therapy [11,92,93]. However, oxygen is quickly metabolized and therefore not retained in the tumour region for an extended time. Other
investigated strategies involving compounds such as Misonidazole, Etanidazole, and Nimorazole designed to mimic the reducing characteristics of oxygen, have met limited success [11,27,92,93,110]. A more advanced type of oxygen mimicking hypoxic radiosensitizers have been developed, including Tirapazamine, which become activated in hypoxic conditions [11,27,110,114-116].

1.3.3.3 Radiation modulating radiosensitizers

Another class of radiosensitizers are designed to modulate the radiation beam in the tumour region. These physical radiosensitizers have an increased interaction cross-section compared to biological tissue and can therefore increase the local absorption of radiation resulting in a localized dose enhancement, and a modification in the local radiation quality.

Boron neutron capture therapy

Boron neutron capture therapy (BNCT) is a technique developed to treat hypoxic glioblastoma multiform with the use of neutron radiation [114-118]. In this technique the radiosensitizer $^{10}$B, an isotope of boron that exhibits a high interaction cross section with thermal neutrons, must be accumulated in the tumour region. When thermal neutrons interact with $^{10}$B this results in a fission reaction yielding high-LET helium and lithium particles, which in turn, deposit their energy over a very short range (about 5-9 µm). A difficulty of this technique is the selective accumulation of $^{10}$B in the tumour region. Furthermore, neutron therapy requires a reactor for the production of a neutron beam. In Canada, the majority of radiation therapy uses photons.

High-Z radiosensitizers

High atomic number, or high-Z radiosensitizers, such as iodine ($Z=53$), gadolinium ($Z=64$), platinum ($Z=78$) and gold ($Z=79$), exploit the increased photoelectric cross-section of low energy
photons in high-Z materials, resulting in a localized production of low energy Auger and photoelectrons, which leads to a localized dose enhancement [117-119]. Furthermore, the conversion of photons into low energy electrons, effectively modifies the local radiation quality, as these electrons have an increased LET [119,120]. The influence of high-Z materials on dose modification has been studied for over 50 years [120,121]. Dose enhancement was first observed in the form of normal tissue necrosis at the interface between tissue and metal implants [121,122]. While the use of high-Z foils results in dose enhancement at the interface with tissue, it is not a practical method to achieve clinical radiosensitization [117,122,123].

A more elegant technique using halogenated pyrimidines - a nucleic acid analog labeled with a single high-Z atom such as Iododeoxyuridine (I UdR) and bromodeoxyuridine (BU dR) - involves the incorporation of these molecules into cancer cell DNA [117,123,124]. The appeal of these radiosensitizers is the proximity to the DNA target. Tumour specificity of halogenated pyrimidines relies on the increased metabolism of rapidly dividing cancer cells. Such techniques met limited success due to the limited amount of high-Z atoms incorporated into cancer cells. Only a 5-10% DNA substitution was clinically achievable, while it was estimated that significant radiosensitization would require 22–45% DNA substitution [123-125].

Because metal foils are bulky, and individual high-Z atoms are hard to accumulate in cells at significant concentrations, Herold et al experimented with gold microspheres as radiosensitizers and observed significant effects in vitro, but reported difficulty in obtaining homogeneous microsphere distributions for in vivo tumour models [125,126]. With the advent of nanotechnology, and the nanoparticle as a vehicle to accumulate a large number of high-Z atoms within cells, a novel gold nanoparticle radiosensitizer has sparked much interest.
1.4 Gold nanoparticles

The use of gold for medical treatments dates back millennia [126,127]. Gold is known to be well tolerated at relatively high doses, and has been used to treat rheumatoid arthritis for decades in the form of gold thiolates [127-129]. Gold nanoparticles (AuNPs) (defined as gold particles with nanometer dimensions) possess many unique properties such as a large surface-to-volume ratio, quantum size effects, and multiple surface sites for chemical binding [111,128,129]. In recent years there have been many investigations on the use of nanoparticles for medical applications such as radiosensitization [111,130-132], infrared and radiofrequency activated thermal therapy [130-135], diagnostic imaging [129,133-138], and targeted drug and gene delivery [129,136-139].

1.4.1 Preparation and characterization

1.4.1.1 Synthesis

AuNPs can be synthesized easily. AuNPs are most commonly synthesized through the reduction of chloroauric acid (HAuCl₄) by sodium citrate, resulting in an aqueous AuNP colloid [139-141]. AuNP size (often measured in terms of diameter) can be modified by adjusting the chloroauric acid to sodium citrate ratio. A typical 30 nm AuNP contains about $8 \times 10^5$ gold (Au) atoms.

1.4.1.2 Imaging and quantification

AuNPs can be characterized using various techniques, each taking advantage of unique properties of AuNPs. AuNPs can be imaged using transmission electron microscopy (TEM), providing high resolution images of AuNPs at the nanoscale [140-143]. Dark field microscopy, confocal microscopy and other microscopy techniques can also be used to visualize clusters of AuNPs, by exploiting their light scattering and absorbing properties [140,142-144].
Common methods of quantifying AuNP concentration include inductively coupled plasma mass spectroscopy (ICP-MS) [140,141,144], or inductively coupled plasma atomic emission spectroscopy (ICP-AES) [141]. Owning to the characteristic ultraviolet/visible (UV/vis) absorption of AuNPs around 520 nm, UV/vis spectrophotometry can measure relative concentrations of AuNPs [113,141]. Another quantitative approach involves radiolabeling AuNPs with radioactive isotopes and then measuring radioactivity as a surrogate for AuNP concentration [113,136,140,145,146].

1.4.1.2 Conjugation and functionalization

AuNPs have a surface well suited for conjugation to various antibodies, oligonucleotides, or nuclear localizing peptides through either non-covalent physical adsorption, or gold-thiol covalent bonding [136,140,145-149]. AuNPs have been functionalized with antibodies targeting various cancer biomarkers including the epidermal growth factor receptor (EGFR), the human epidermal growth factor receptor-2 (HER-2) and angiogenesis markers such as the vascular endothelial growth factor receptor (VEGFR) [140,147-152]. These AuNP conjugates show increased cancer cell accumulation. AuNPs modified with nuclear localizing peptides can achieve nuclear localization provided they are small enough to enter nuclear pores [150-152].

1.4.1.3 Cellular uptake, biodistribution and toxicity

Cellular uptake of AuNPs is understood to occur mainly through energy dependent receptor-mediated endocytosis resulting in AuNPs accumulated in endosome – lysosomes [150,153,154]. Alternative pathways have been reported for AuNPs modified with cell penetrating peptides or targeting antibodies. Uptake is known to be AuNP size dependent, with 50 nm AuNPs exhibiting the most efficient cellular accumulation and retention. Successful accumulation in the cell nucleus has also been achieved using nuclear localizing peptides [150,152].
Numerous studies have been published examining the biodistribution and cytotoxicity of AuNPs [133,142,144,155-158]. In vitro cytotoxicity varies with cell line and AuNP size, concentration, and surface modification [142,159-164]. In vivo studies show that AuNP are generally biocompatible and inert, being cleared from the body through the kidneys [111,142], although other studies warn of AuNP size dependent cytotoxicity and accumulation in the liver [156,157,165,166]. In a mouse study by Hainfeld et al, an injected concentration at 2.7 g of gold per kg of tissue, resulted in the preferential accumulation of non-targeted AuNPs in the leaky vasculature of malignant tumours compared to normal tissue (8:1 ratio), and no toxicity was observed [111]. AuNP surface modification with polyethylene glycol (PEG) chains can dramatically alter its pharmacokinetic properties. PEGylation of AuNPs prevents AuNP self-aggregation and limits opsonization and subsequent sequestration by the reticuloendothelial system. This increases AuNPs’ blood circulation half-life, and gives AuNPs more time to reach the tumour target before phagocytosis occurs [155].

1.4.2 AuNPs as radiosensitizers

1.4.2.1 Why AuNPs?

AuNPs represent an appealing radiosensitizer due to a number of characteristics:

1) With an atomic number of 79, and a density of 19.3 g/cm³ gold exhibits a significantly increased photoelectric absorption cross-section compared to biological tissue especially around the k-edge of gold (80.7 keV) and at lower energies (≈ 20 keV) [167].

2) AuNPs are biocompatible, and at reasonable concentrations, exhibit acceptable toxicity levels.

3) AuNPs strike a balance in size; they are large enough to carry many high-Z atoms within each particle, but they are small enough to easily permeate tumour vasculature, diffuse
through tight junctions between endothelial cells of capillaries, and enter cancer cells, providing the potential for a more uniform radiosensitizing distribution compared to larger particles.

4) AuNPs are amenable to surface modification and conjugation with tumour targeting moieties, increasing the potential for tumour targeting, and cellular accumulation and retention [140].

1.4.2.2 Experimental evidence

In 2004 Hainfeld et al. demonstrated the proof-of-principle of AuNP radiosensitization in vivo [111]. Mice bearing subcutaneous EMT-6 mammary carcinomas were irradiated with 250 kVp photons after intravenous administration of non-targeted 1.9 nm gold nanoparticles at a concentration of 2.7 g Au/kg. The mouse survival rate at one year was 86% with radiation and AuNPs, versus 20% with radiation alone. This seminal study sparked much research interest in AuNP radiosensitization over the past years. In vitro AuNP radiosensitization has been examined and demonstrated with plasmid and supercoiled DNA [168-171], bacteria [172], and a range of cell lines [140,158,173-177].

Radiation source energy

Initial experiments focused on photon source energies around and above the k-edge of gold (80.7 keV) to take advantage of the sharp absorption peak from k-shell ionizations. However, a greater effect was observed using lower photon energy sources [158]. Even for a given energy, AuNP radiosensitization has been observed to vary widely with cell type [175].

AuNP size

AuNP size has been shown to be a critical factor effecting radiosensitization [177]. Some experiments have reported that smaller AuNPs are more effective [168], while others suggest the
opposite [171]. Cellular uptake of AuNPs depends on AuNP size, and uptake concentration can significantly effect the degree of radiosensitization [141,177].

To summarize, experimental results reveal that AuNP radiosensitization varies widely in different conditions. The main factors implicated in effecting radiosensitization are, i) radiation source energy, ii) cell type, iii) AuNP size, iv) AuNP intracellular accumulation and localization, and v) AuNP concentration. However, it is not fully understood how and to what extent each factor effects radiositivity. Furthermore these factors can also interact with each other (e.g. AuNP size can effect localization), making it difficult to isolate their contributions to radiosensitization. In order to translate AuNP radiosensitization from the bench to the bedside, an accurate picture of AuNP radiation enhancement is need.

1.4.2.2 Radiosensitization models

Due to the large variations in experimental results, there have been various models developed in an attempt to describe the radiation enhancement and to quantify and predict the extent of AuNP radiosensitization [128,178-184].

Macroscopic dose enhancement

Early theoretical calculations and Monte Carlo simulations of AuNP radiosensitization quantified the macroscopic dose enhancement [178,181,184]. The geometries in these models were simplified to assume a homogeneous concentration of AuNPs or Au atoms throughout the tumour region. An average dose enhancement was then calculated based on the increased photoelectric cross section of gold. Although dose enhancement is a simple metric with which to make cell survival predictions, macroscopic models tend to underestimate the radiobiological effect by ignoring the local
modification in radiation quality. Furthermore these models are limited in exploring the radiobiological effects of AuNP size, and intracellular location.

*Nanoscale radial dose enhancement*

An alternative approach to characterize AuNP radiosensitization involves examining the radial dose enhancement around AuNPs at the nanoscale [180,183,185]. This model, first reported in a Monte Carlo study by Carter et al, evaluates the dose absorbed in concentric spheres around the AuNP [128]. Carter predicted that the AuNPs must be within 5 nm of the DNA to produce any biological radiosensitization. While this model overcomes some of the shortcomings of a macroscopic model, and incorporates AuNP localization, the results suggesting that AuNPs must be localized in the nucleus are not representative of experimental findings [158,177]. Furthermore, translating a nanoscale radial dose profile into a cell survival prediction is not straightforward [186].

In summary, the lack of predictive capability in the current AuNP radiosensitization models suggests a subtly in the mechanism of radiosensitization that requires further elucidation. There is therefore a current need for a predictive radiobiological model of AuNP radiosensitization that is able to effectively account for the unique dose heterogeneities in the vicinity of AuNPs due to escaping Auger cascades and low energy photoelectrons.

### 1.5 Thesis overview

#### 1.5.1 Motivation

The research in this thesis is motivated by the widely variable effects of AuNP radiosensitization reported, the lack of a comprehensive AuNP radiosensitization predictive model, and a desire to elucidate some of the mechanisms behind AuNP radiosensitization.
The guiding premise of this thesis is that the highly localized and heterogeneous energy deposition at the subcellular scale is the driving mechanism behind AuNP radiosensitization. Hence, understanding and accurately modeling these interactions is required to further elucidate this mechanism and predict the radiobiological effects with respect to varying physics and pharmacological parameters.

1.5.2 Hypothesis

The working hypothesis of this thesis is that the development of a model of the energy deposition around AuNPs at the subcellular scale, based on Monte Carlo simulations, can be used both to predict the radiobiological effect of AuNP radiosensitization, and to help define the optimal design and use of AuNPs as radiosensitizers.

1.5.3 Objectives and outline

With respect to the thesis hypothesis, there are four main objectives of this thesis:

1) Develop a Monte Carlo approach to study the physics of AuNP radiation modification, exploring metrics such as the rate of radiation absorption in AuNPs, and the range of escaping secondary radiation.

2) Develop of a Monte Carlo-based radiobiological model to predict the extent of AuNP radiosensitization with respect to various parameters such as cancer cell line, photon energy, and AuNP size, concentration, and intracellular localization.

3) Validate the radiobiological model through the comparison of predicted and experimental cell survival for various cell lines, photon source energies, AuNP sizes, and concentrations
4) Employ the model to make predictions of AuNP radiosensitization, and discuss the optimal use of AuNPs as radiosensitizers in potential clinical scenarios.

The methodology, results, and discussions related to these objectives are described in Chapters 2, 3, and 4 of this thesis. Chapter 2 explores the first objective, while Chapters 3 and 4 explore the three remaining objectives. Chapter 5 provides an overall discussion of this research as it relates to the hypothesis as well as possible future directions.
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Chapter 2

A Monte Carlo approach to study the physics of gold nanoparticle radiosensitization
This chapter represents a reprint of “Lechtman E, Chattopadhyay N, Cai Z, Mashouf S, Reilly R and Pignol JP 2011 Implications on clinical scenario of gold nanoparticle radiosensitization in regards to photon energy, nanoparticle size, concentration and location. Phys. Med. Biol. 56 4631–47” Copyright 2011 IOP Publishing Ltd. Minor formatting modifications have been made to maintain consistency throughout this thesis.

2.1 Abstract

Gold nanoparticle (AuNP) radiosensitization represents a novel approach to enhance the effectiveness of ionizing radiation. Its efficiency varies widely with photon source energy and AuNP size, concentration, and intracellular localization. In this Monte Carlo study we explored the effects of those parameters to define the optimal clinical use of AuNPs. Photon sources included $^{103}$Pd and $^{125}$I brachytherapy seeds; $^{169}$Yb, $^{192}$Ir high dose rate sources; and external beam sources 300 kVp, and 6 MV. AuNP sizes were 1.9, 5, 30, and 100 nm. We observed a $10^3$ fold increase in the rate of photoelectric absorption using $^{125}$I compared to 6 MV. For a $^{125}$I source, to double the delivered dose required concentrations of 5.33–6.26 mg/g of Au or $7.10 \times 10^4$ 30 nm AuNPs per tumour cell. For the 6 MV source, concentrations of 1560–1760 mg/g or $2.17 \times 10^7$ 30 nm AuNPs per cell were needed, which is not clinically achievable. Examining the proportion of energy transferred to escaping particles or internally absorbed in the nanoparticle suggests two clinical strategies: The first strategy uses photon energies below the k-edge and takes advantage of the extremely localized Auger cascade. It requires small AuNPs conjugated to tumour targeted moieties and nuclear localising sequences. The second strategy, using photon sources above the k-edge, requires a higher gold concentration in the tumour region. In this approach, energy deposited by photoelectrons is the main contribution to radiosensitization, and AuNP size and cellular localization are less relevant.
2.2 Introduction

In recent years, Gold nanoparticles (AuNPs) have been actively investigated as a novel high-Z radiosensitizing agent [1-3]. The premise of AuNP radiosensitization relies on gold’s increased photoelectric absorption cross-section relative to tissue [3,4]. Upon irradiation, this increased photon absorption results in a highly conformal energy deposition around the AuNPs, caused by the localized spray of escaping photoelectric products (photoelectrons, Auger electrons, and characteristic x-rays) [5,6]. The appeal of the AuNP is enhanced by the large number of atoms per particle (ranging from 250 for a 2 nm AuNP to 835,000 for a 30 nm AuNP), a surface well suited for conjugation to tumour targeting moieties [7,8], and gold’s general biocompatibility [9]. These features of AuNPs offer support to the notion that AuNP radiosensitization, as part of a future clinical strategy, might be both robust and tumour specific.

AuNP radiosensitization was first demonstrated in vivo by Hainfeld et al who showed a significant increase in long term survival of mice bearing EMT-6 mammary carcinomas irradiated with 250 kVp photons after intravenous injection of non-targeted 1.9 nm AuNPs [2]. Subsequently, AuNP radiosensitization has been observed in more controlled in vitro irradiation of cells and plasmid DNA [6,10-13]. Butterworth et al showed single strand and double strand break enhancement of 2.29 and 1.25 respectively when irradiating plasmid DNA in the presence of 5 nm AuNPs [10]. Rahman et al observed a biological dose enhancement factor of up to 24.6 when irradiating bovine aortic endothelial cells with 80 kVp photons in the presence of 1.9 nm AuNPs [12].

While demonstrating the potential efficacy of AuNP radiosensitization, the large variations in these experimental results revealed AuNP radiosensitization to be highly sensitive to a number of physics and pharmacological parameters including irradiation energy and AuNP size, concentration, and intracellular localization [2,10,12].
Towards understanding and predicting the effects of these parameters there have been a number of Monte Carlo simulation studies exploring AuNP dose enhancement at the macro, micro, and nano-scales [5,14-20]. Carter’s simulation study showed that the dose deposited by the Auger cascade escaping a single nanoparticle fell below the background dose beyond a distance of 2 nm from the AuNP surface, suggesting that significant radiosensitization could only occur if nanoparticles are localized very close to the DNA inside the cell nucleus [5]. However, these findings seem to contradict in vitro data where significant radiosensitization has been noted for AuNPs localized far from the cell nucleus [12]. On the other hand, Leung reported that photoelectrons and Auger electrons escaping AuNPs can travel distances as far as 3 µm to 1 mm from the AuNP, sufficient to reach the cell nucleus from endosomes, and even crossfire between cells [16]. Similar results were produced by Jones who examined various photon sources (\(^{125}\)I, \(^{103}\)Pd, \(^{169}\)Yb, \(^{192}\)Ir, 50 kVp, and 6 MV x-rays), and simulated the dose distribution around clusters of gold inside cell endosomes. Jones observed a limited dose enhancement beyond distances of a few microns [20].

The experimental and simulation studies published thus far have helped provide insight into the mechanism of radiosensitization, but they leave lingering questions as to which set of parameters define the optimal use of AuNPs for radiosensitization from a clinical paradigm. These clinical questions include: (1) how much gold, both in terms of the number of AuNPs per cell, and in mg of AuNPs per g of the tumour is required for a significant dose enhancement? (2) How does the photon source energy and AuNP size influence the spatial distribution of the energy deposited around AuNPs? (3) When is active cell/nucleus targeting of AuNPs most beneficial? In this Monte Carlo simulation study we explored the interplay of photon source energy, nanoparticle size, and intracellular location on AuNP radiosensitization in order to address these questions and to define the optimal use of AuNPs as radiosensitizers as part of a potential clinical strategy.
2.3 Materials and Methods

2.3.1 Parameters investigated and metrics evaluated

The metrics studied here included the rate of photoelectric absorption in AuNPs of various sizes (1.9, 5, 30, and 100 nm diameter), the subsequent dose enhancement in the surrounding medium, and the energy and range of the escaping electron cascade. These metrics were expressed in terms of number of gold atoms, number of AuNPs and also milligrams of AuNPs in order to comprehensively compare the effects of various AuNP sizes. The metrics were evaluated either per photoelectric absorption, or per 2 Gy delivered dose to a tumour in order to facilitate clinical evaluation.

Clinical photon sources were simulated in this investigation including two external beam sources - the Gulmay D3300 (Gulmay Ltd., Chertsey, UK) 300 kVp kilovoltage X-ray therapy unit (average energy $\approx$ 100 keV, HVL=3.1 mm Cu, filter: 1.5 mm Al + 0.25 mm Cu + 0.5 mm Sn) and the Philips SL 20 6 MV (Philips, The Netherlands) accelerator unit (average energy $\approx$ 2 MeV, percent depth dose at 10 cm = 66.7%) [21,22], two high dose rate brachytherapy sources - the $^{192}$Ir microSelectron-HDR (Nucletron, Neenendaal, The Netherlands) (average energy $\approx$ 395 keV) and the $^{169}$YbHDR 4140 (Implant Sciences Corporation) (average energy $\approx$ 93 keV) [23,24], and two low dose rate brachytherapy seed sources - the $^{125}$I IsoAid ADVANTAGE model IAI-125 (IsoAid LLC., Port Richey, Florida) (average energy $\approx$ 28 keV) and the $^{103}$Pd IsoAID ADVANTAGE (IsoAid LLC., Port Richey, Florida) (average energy $\approx$ 21 keV) [25,26].

2.3.2 Monte Carlo Simulation

Monte Carlo transport codes employed in this study included MCNP-5 (Los Alamos National Laboratory) with the ENDF/B-VII cross-section library and PENELOPE 2008.1 (Barcelona,
Catalonia) [27,28]. Both of these codes were used to take advantage of their respective strengths. Briefly, MCNP-5 contains powerful variance reduction methods that allow for efficient calculation of macroscopic tallies. However, MCNP-5 is of limited use in nano-scale transport as it has a default energy cutoff below 1 keV and uses a condensed history algorithm for the transport of electrons. PENELOPE on the other hand can simulate in a detailed way, the transport of electrons down to 50 eV, corresponding to electron ranges of about 2-4 nm [29]. PENELOPE was therefore used to simulate the production and transport of Auger cascades as they escaped the AuNPs after a photoelectric event, and to evaluate the subsequent dose deposited around AuNPs.

For a more illustrative depiction of AuNP radiosensitization, an in house visualization tool was developed in Matlab (Natick, Massachusetts) to import the track histories from PENELOPE simulations and plot the paths of escaping particles around the AuNP in 3D. This was achieved by modifying the PENELOPE file PENMAIN.F, and recompiling the program. Flag variables were created to print the energy, position, and production information of all particles escaping the AuNP after a photoelectric event. Escaping particles were followed event by event until termination due to the energy cutoff.

2.3.3 Simulation Geometry and Tallies

The first set of simulations evaluated the photon energy spectra after tissue penetration (ICRU four component soft tissue [30]), as well as the rate of photoelectric absorption in AuNPs within the tissue. These simulations were carried out using MCNP in photon mode. Photon showers were simulated penetrating a tissue depth of 1 cm and 5 cm for brachytherapy sources and external beam sources respectively. Brachytherapy sources were described in detail including seed casings [21-26]. The generation of the photon phase-space for the 6 MV and 300 kVp external beam sources has been described in detail in a previous publication [21]. Due to the low probability of photons
interacting with individual nanoparticles imbedded at a depth in tissue, AuNPs were not modeled in this simulation geometry. Rather a variance reduction approach using MCNP’s FM function tally multiplier was utilized in the tally volume to predict the AuNP photoelectric absorption within this region. The FM function allows for the calculation of tallies in materials that are not present in the simulation volume and hence do not influence the particle transport. A 1 mm thick tally volume consisting of tissue was used to score the photon flux using the F4 tally. This flux tally was used to calculate the rate of photoelectric absorption in gold with the FM multiplier tally. The rate of photoelectric absorption was then normalized to the volume of a single AuNP and per 2 Gy delivered dose to the tally volume using the *F8 tally function. $1 \times 10^9 - 2 \times 10^{10}$ source particles were simulated for each calculation maintaining a standard error of less than 0.1%.

The second set of simulations examined the characteristics of the photoelectric absorptions, the subsequent spray of the photoelectric products escaping AuNPs, and the dose enhancement in the surrounding tissue due to the enhanced photoelectric absorption in AuNPs. Detailed event-by-event MC simulations were performed in PENELOPE using the following parameters: EABS(1:3)=50 50 50; C1=0; C2=0; WCC=0; WCR=-100. The geometry consisted of a single AuNP (1.9, 5, 30 and 100 nm) embedded in a large volume of tissue (10 cm radius). Photons were initiated adjacent the AuNP directed towards it from a disk with radius equal to the AuNP radius. The input photon source energy spectra were obtained from the tissue-penetrated photon energy spectra collected from the first set of simulations. A customized main steering program in PENELOPE based on PENMAIN.F was developed to include unique tallies. These customized tallies measured the relative rate of absorption in each orbital of the gold atom, the number and energy of electrons escaping the AuNP per photoelectric absorption, the dose deposited to a 1 cm radius sphere of tissue surrounding the AuNP, and the distance traveled by Auger and photoelectrons in the surrounding tissue after escaping the AuNP. The distance or range was defined here as the maximum distance from the
surface of the AuNP reached by each escaping electron. To reduce simulation time and to avoid following primary photons that did not interact with the AuNP, each PENELOPE simulation was split into two components. The first component included only the AuNP with no surrounding medium. A customized phase space file recorded the energy, position, and direction of the photoelectric products as they escaped the surface of the AuNP. Over $1 \times 10^6$ escaping particles were recorded for each simulation and the numbers of photoelectric absorptions were recorded for normalization. The second component of the simulation used this phase space file as the source input to follow the particles in the surrounding tissue volume. Tallies were normalized by the number of primary photoelectric absorptions. The dose was further normalized to a 2 Gy delivered dose to the tissue (using the rate of photoelectric absorption in the AuNP per 2 Gy from the MCNP simulation results). The dose enhancement per AuNP was finally expressed both in terms of the number of AuNPs per cell (cell mass estimated at $3.37 \times 10^{-12}$ kg), and the mg of AuNPs per g of tumour required to double the delivered dose to the tumour region. $7 \times 10^7 - 3 \times 10^{11}$ source particles were simulated for each configuration (depending on both the AuNP size and photon source energy) maintaining an average uncertainty of less than 1% for all tallies.

2.4 Results

2.4.1 Rate of Photoelectric Absorption

Results from the first MC simulation reveal a large increase in photoelectric absorption in the AuNPs for the lower energy sources and larger AuNP diameters (Table 2.1). Specifically, the number of 30nm AuNPs required to generate a single photoelectric event per cell with the 6MV source was about 39,500 AuNPs, corresponding to $3.29 \times 10^{10}$ Au atoms. In contrast, the number of 30 nm AuNPs required for a single photoelectric absorption with the $^{125}$I source was about 39 AuNPs, corresponding to $3.28 \times 10^7$ Au atoms. This represents an increase in photoelectric
absorption by three orders of magnitude. The rate of photoelectric absorption as a function of AuNP size simply follows a radius-cubed relation, and therefore nearly a four orders of magnitude increase in photoelectric absorption was observed between a 5 nm AuNP and a 100 nm AuNP.

**Table 2.1:** Number of photoelectric absorptions per AuNP per 2 Gy delivered dose at a tissue depth of 1 cm and 5 cm for brachytherapy sources and external beam sources respectively.

<table>
<thead>
<tr>
<th>AuNP diameter</th>
<th>Pd-103 (20.6 keV)</th>
<th>I-125 (27.0 keV)</th>
<th>Yb-169 (100.7 keV)</th>
<th>300 kVp (127.1 keV)</th>
<th>Ir-192 (324.3 keV)</th>
<th>6 MV (1861 keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 nm</td>
<td>7.38 × 10⁻⁶</td>
<td>6.47 × 10⁻⁶</td>
<td>1.29 × 10⁻⁶</td>
<td>7.62 × 10⁻⁷</td>
<td>5.28 × 10⁻⁸</td>
<td>6.43 × 10⁻⁹</td>
</tr>
<tr>
<td>5 nm</td>
<td>1.34 × 10⁻⁴</td>
<td>1.18 × 10⁻⁴</td>
<td>2.34 × 10⁻⁵</td>
<td>1.39 × 10⁻⁵</td>
<td>9.61 × 10⁻⁷</td>
<td>1.17 × 10⁻⁷</td>
</tr>
<tr>
<td>30 nm</td>
<td>2.90 × 10⁻²</td>
<td>2.55 × 10⁻²</td>
<td>5.06 × 10⁻³</td>
<td>3.00 × 10⁻³</td>
<td>2.08 × 10⁻⁴</td>
<td>2.53 × 10⁻⁵</td>
</tr>
<tr>
<td>100 nm</td>
<td>1.08</td>
<td>9.43 × 10⁻¹</td>
<td>1.87 × 10⁻¹</td>
<td>1.11 × 10⁻¹</td>
<td>7.69 × 10⁻³</td>
<td>9.38 × 10⁻⁴</td>
</tr>
<tr>
<td>Per mg AuNP</td>
<td>1.06 × 10¹¹</td>
<td>9.33 × 10¹⁰</td>
<td>1.85 × 10¹⁰</td>
<td>1.10 × 10¹⁰</td>
<td>7.61 × 10⁸</td>
<td>9.28 × 10⁷</td>
</tr>
<tr>
<td>Per Au atom</td>
<td>3.48 × 10⁻⁰⁸</td>
<td>3.05 × 10⁻⁰⁸</td>
<td>6.06 × 10⁻⁰⁹</td>
<td>3.60 × 10⁻⁰⁹</td>
<td>2.49 × 10⁻¹⁰</td>
<td>3.04 × 10⁻¹¹</td>
</tr>
</tbody>
</table>

*Average photon energy at tissue depth of 1 cm and 5 cm for brachytherapy sources and external beam sources respectively.

Comparing the relative rate of photoelectric absorption in each orbital provides insight into the resulting relaxation cascade and therefore the resulting energy and range of the photoelectric products released from such absorptions. These results, normalized per photoelectric absorption are summarized in Table 2.2. At energies below the k-edge of gold (80.7 keV) photoelectric absorption can only occur through outer atomic shells (L, M, N, etc.). With the 300 kVp source, the ¹⁹²Ir source, and the 6 MV source we observed a significant absorption in the K-shell. These inner-shell absorptions transfer more energy into the surrounding medium compared to an outer-shell
absorption. So while there is a higher overall rate of photoelectric absorption from lower energy sources, the energy released per absorption increases with increasing photon source energy.

**Table 2.2:** Relative rate of photoelectric absorption (in %) in the atomic orbitals of gold per photoelectric event.

<table>
<thead>
<tr>
<th>Orbital</th>
<th>Pd-103 (20.48 keV)</th>
<th>I-125 (26.07 keV)</th>
<th>Yb-169 (62.11 keV)</th>
<th>300 kVp (97.85 keV)</th>
<th>Ir-192 (157.0 keV)</th>
<th>6 MV (99.85 keV)</th>
</tr>
</thead>
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<td>K</td>
<td>-</td>
<td>-</td>
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<td>56.69</td>
<td>52.97</td>
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</tr>
<tr>
<td>L1</td>
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<td>15.23</td>
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<td>L2</td>
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<td>8.64</td>
<td>8.89</td>
<td>12.87</td>
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<td>31.35</td>
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<td>3.36</td>
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<td>3.51</td>
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</tr>
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<td>M4</td>
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<td>0.41</td>
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<td>3.01</td>
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<td>Outer Shells</td>
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<td>5.29</td>
<td>4.53</td>
<td>2.31</td>
<td>2.69</td>
<td>5.55</td>
</tr>
</tbody>
</table>

2.4.2 Dose enhancement

Results from the second set of simulations exploring dose enhancement reveal a general trend of increasing dose enhancement using lower energy sources (**Figure 2.1**). However, as the source energy becomes too low (as with $^{103}$Pd) this trend breaks down.
Figure 2.1 expresses the amount of AuNPs required to double the delivered dose to the tumour both in terms of number of AuNPs per tumour cell (Fig 2.1 a), and in milligrams of AuNPs per gram of tumour (Fig 2.1 b). Examining the number of AuNPs per cell required to double the delivered dose, in the worst-case scenario, with a 6MV source and 1.9 nm AuNPs, one would require $7.59 \times 10^{10}$ AuNPs per cell. In the most efficient scenario, with a $^{125}$I seed source and 100 nm AuNPs, only $1.83 \times 10^3$ AuNPs per cell would be needed to double the dose. Examining the results in terms of AuNP mass concentration, the worst-case scenario would use the 6 MV source and 100 nm AuNPs, and would require 1760 mg of AuNPs per g of tumour. This concentration would be clinically infeasible to accomplish. The most efficient scenario using a $^{125}$I brachytherapy source and 1.9 nm AuNPs, would require 5.33 mg AuNP per g of tumour. Dose enhancement as a function of AuNP size was not observed to be strictly proportional to the AuNP radius-cubed. This can be attributed to low energy Auger and delta electrons being absorbed more readily within AuNP of increasing size.

**Figure 2.1**: Simulation results of amount of AuNPs required to double the delivered dose to the tumour as a function of different clinical photon sources and AuNP size. Expressed as both the number of AuNPs per tumour cell (a) and milligrams of AuNPs per gram of tumour (b).
Figure 2.2: Pie charts representing the percentage of energy converted into escaping particles or internally absorbed per photoelectric absorption in a AuNP.
2.4.3 Photoelectric Energy Conversion

**Figure 2.2** summarizes the percentage of energy from an absorbed photon transferred to escaping Auger and delta electrons, photoelectrons, characteristic x-rays, or internally absorbed within the nanoparticle. As the AuNP size is increased, energy from Auger and delta electrons is increasingly internally absorbed in the AuNP. Conversely, the percentage of energy escaping as photoelectrons and characteristic x-rays remains mostly unchanged for the AuNP sizes investigated in this study. As the primary photon energy is increased, the percentage of escaping energy from photoelectrons and characteristic x-rays increases due to K-shell ionizations. **Figure 2.3** shows the photon energy spectrum escaping a 30 nm AuNP from the 300 kVp source and the $^{103}$Pd source illustrating the contribution of k-shell ionizations. Understanding how the energy is distributed after a photoelectric absorption also sheds light on the spatial distribution of the energy that leaves the AuNPs. Auger and delta electrons have a very short range (less than one micron), photoelectrons can travel much further (up to hundreds of microns) and characteristic x-rays can travel as far as centimeters.

2.4.4 Characteristics of escaping electrons

**Figure 2.4** depicts the range of escaping electrons. These plots consist of logarithmically spaced bins normalized per photoelectric event and to the bin width. It can be observed that each curve contains at least two distinct relative peaks - the first located around 0.02 - 0.1 µm and the second around 0.25 - 1.5 µm corresponding to electron energies of approximately 0.8 - 2.5 keV and 4.5 - 12 keV respectively. These electron energies correspond to Auger electrons emitted from non-radiative transitions from shells L and M. Another subtle relative peak can be observed for the 1.9 nm AuNP for all energies, located around 0.004 - 0.01 µm corresponding to energies of 60 – 200 eV. While this demonstrates the release of very low electrons from the 1.9 nm AuNP, the precise interpretation of this peak is likely confounded by the inherent uncertainty of low-energy Monte Carlo simulation
[28]. Other unique humps on certain range spectrums can be traced back to relative peaks in their respective incoming photon spectrums.

As a function of AuNP size, one can observe the effects of internal absorption with larger AuNP sizes. The range plots depict more electrons with very short ranges for smaller AuNPs, due to the fact that low energy electrons are able to escape the AuNP. Specifically, about 2.6 low energy Auger and delta electrons escape per photoelectric event from the 1.9 nm AuNP, while less than one Auger and delta electron was observed to escape for the 100 nm AuNP. This result was similar for all photon energies investigated.

**Figure 2.3:** Characteristic x-ray spectrum escaping a 30 nm AuNP from the 300 kVp source and the $^{103}$Pd source, illustrating the contribution of k-shell ionizations.
Figure 2.4: The range of escaping electrons from each photon source as a function of AuNP size. Range curves consist of logarithmically spaced bins normalized per photoelectric event and to the bin width.
**Figure 2.5** displays the electron ranges for a 30 nm AuNP for all photon sources in relation to the size of a hypothetical 10 μm cell radius indicated by a vertical marker. Another vertical marker represents the maximum range of escaping Auger and delta electrons. The maximum range was defined here as the range beyond which there is a 95% probability that any one Auger or delta electron will not exceed. This plot offers insight into the magnitude of ‘cross-firing’ that can be expected if AuNPs accumulate in some cells but not in others. For lower energy sources such as $^{103}$Pd and $^{125}$I, most electron ranges are confined to a single cell, while for higher source energies, photoelectrons can cross-fire between cells. **Figure 2.6** provides a 3D visualization of the typical difference in distance traveled by the escaping electrons from a low-energy source and a high-energy source. This figure also includes the tracking of electrons liberated in the surrounding water from electrons that originated in the AuNP.

![Range of Electrons Escaping a 30 nm AuNP](image)

**Figure 2.5:** The range of electron escaping a 30 nm AuNP from each photon source relative to the dimensions of a cell.
Figure 2.6: A zoom-out visualization of the photoelectric products produced from a single random photoelectric absorption in a single 30 nm AuNP. Electrons are represented in red. Fig. 6-a to 6-d depicts the cascade of photoelectric products from a 6 MV source. Fig. 6-e to 6-h depicts the cascade of photoelectric products from an I-125 source. These image sequences zoom out from the nano-scale (Fig. 6-a and Fig. 6-e) to 100 µm (Fig. 6-d and Fig. 6-h), contrasting the relative distance traveled by the escaping electrons from a low energy source and a high-energy source.
Table 2.3 presents the mean energy divided by the mean range of the escaping electrons. This quantity provides some insight into the relative biological effectiveness of the escaping electrons. The trend demonstrates that lower energy electrons deposit their energy within proportionally shorter ranges than higher energy electrons.

Table 2.3. Ratio of mean energy over mean range of escaping electrons (keV/µm).

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<th>AuNP diameter</th>
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Yb-169 300 kVp

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Ir-192 6 MV

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2.5 Discussion

The results presented in this study provide clinically relevant insight as to the effects of photon source energy and AuNP size on the rate of photoelectric absorption and the subsequent spatial distribution of secondary radiation emitted from the AuNPs. Compared to previously published work, our simulations explored AuNP radiosensitization in clinically relevant configurations including the simulation of primary photon tissue penetration, results were normalized to 2 Gy delivered dose, and concentration of gold was expressed in terms of number of AuNPs per cancer cell, number Au atoms per cell, and mg of AuNPs per g of tumour. We have also uniquely explored in detail the percentage of energy converted to Auger and delta electrons, photoelectrons, characteristic x-rays, or internally absorbed in AuNPs per photoelectric event. Our results offer further support of a pronounced AuNP radiosensitization effect in conjunction with photon energies below the k-edge of gold, as was shown in previous publications by Cho, Montenegro, Brun, Jones, and Leung [11,15,16,19,20]. The pronounced dose enhancement for energies below the k-edge (Figure 2.1) can be explained by the increased photoelectric absorption associated with lower energy sources (Table 2.1). However, we will also discuss the potential clinical use of AuNPs in conjunction with energies above the k-edge.

(1) How much gold, both in terms of the number of AuNPs per cell, and the mg of AuNPs per g of the tumour is required for a significant dose enhancement?

Our results indicate that to achieve a doubling of the delivered dose in a tumour, the amount AuNPs required would need to be approximately 300 times greater for the 6 MV source compared to the lower energy brachytherapy sources. We therefore conclude that AuNP radiosensitization using a 6 MV photon source is not clinically feasible. These results of dose enhancement are comparable to the findings of Cho’s 2009 Monte Carlo study, as well as other theoretical estimates [15,31]. We
have shown that in terms of the number of AuNPs per cell, larger particles produce significantly more dose enhancement than smaller particles due to the increased number of Au atoms. However, in terms of mg of AuNPs per g of the tumour, smaller particles produce a greater dose enhancement allowing more low energy electrons to escape into the surrounding tissue. Both metrics - the number of AuNPs per cell, and the mg of AuNPs per g of tumour are useful in assessing AuNP radiosensitization from a clinical paradigm.

Previously published AuNP dosimetry papers have revealed the difficulty in applying the metric of dose to AuNP radiosensitization [5,14-17,20]. This is because the Auger cascades create uniquely localized dose heterogeneities at the nano-scale, depositing clusters of energy along the tracks of Auger electrons. The traditional concept of dose alone cannot properly treat the important spatial component of this energy distribution. A number of authors have examined the radial dose enhancement around AuNPs by defining concentric spheres to tally dose [5,16,20]. One issue with this approach may be the variation in scale associated with the increasingly large concentric volumes further away from the AuNP. While escaping electrons may travel many microns as we have shown, their contribution to the radial dose profile is not represented. These longer ranged electrons may still be effective in inducing biological lesions including single and double DNA strand breaks, especially towards the end of their track. In this study we have therefore purposely avoided simulating radial dose enhancement, and instead focused on electron range as a metric of the spatial energy distribution.

(2) How does the photon source and AuNP size influence the spatial distribution of the energy deposited around AuNPs?

Looking more carefully at the energy distribution in the surrounding medium from AuNP radiosensitization three distinct spatial zones can be identified. The first zone, comprising about 3 -
32% of the total escaping energy (depending on the AuNP size and photon source), and extending about 2 µm from the AuNP surface, is due to escaping Auger and delta electrons. The energy deposited in this zone has an increased relative biological effect due to the high linear energy transfer (LET) of these particles. Larger AuNP sizes tend to internally absorb these low energy electrons and therefore reduce the relative energy contribution in this zone. The second zone, comprising about 42 - 69% of the total escaping energy, and extending up to hundreds of microns or beyond from the AuNP surface, is due to photoelectrons. These electrons lose their energy over a larger distance and can cross fire between cells, but can still cause significant DNA damage at the ends of their tracks. The contribution of these electrons depends mostly on the incoming photon spectrum, with more energetic photon sources producing higher energy and longer ranged photoelectrons. The third zone, comprising about 11 - 42% of the total escaping energy comes from escaping photons that can travel much larger distances from the AuNP. The conversion of an incoming photon into a characteristic x-ray will generally not significantly change its relative biological effect, and therefore this type of escaping energy may not contribute to AuNP radiosensitization.

Comparing our results of electrons ranges with those of Leung [16], there are some similarities, but also a large discrepancy especially for the 6 MV source. For example, the average range of electrons escaping a 100 nm AuNP using a 6 MV source was 1090 µm as reported by Leung, whereas we found this range to be 81 µm. This difference can be attributed to the fact that our simulations did not include Compton interactions within AuNPs, whereas Leung’s study did include Compton interactions. We decided to exclude Compton interactions because AuNP radiosensitization results from gold’s increased photoelectric absorption, and gold does not exhibit an enhanced Compton cross-section compared to tissue. Leung’s electron range results using a 6 MV source therefore reflect the large distances travelled by Compton electrons set in to motion by high energy photons.
For lower energies, where Compton interactions are less frequent, our results are in better agreement with those of Leung.

(3) When is active cell/nucleus targeting of AuNPs most beneficial?

Our results reveal that as the photon source energy is reduced below the k-edge, AuNP localization becomes an increasingly important parameter. With the permanent seed sources, $^{103}$Pd or $^{125}$I, electrons escaping the AuNPs travel on the order of a micron on average, suggesting that AuNP cell internalization may be necessary for these Auger cascades to reach the DNA targets. Furthermore, to make use of the increased number of escaping Auger electrons as AuNP size is decreased, the AuNPs would need to be sufficiently close to the DNA. On the other hand for higher energy photon sources, where photoelectrons account for most of the escaping energy and significant cross firing occurs, AuNPs may not need to be precisely localized in the tumour cells to have a radiosensitizing effect.

With these results in mind, we can outline two potential clinical approaches employing AuNPs as radiosensitizers. The first potential approach involves clinical photon source energies below the k-edge where photoelectric absorption is most efficient. In this approach, a large number of small AuNPs must be accumulated in tumour cells, and localized within 2 microns of the cell nucleus to exploit the low energy escaping Auger and delta electrons. AuNPs would therefore need to be conjugated to tumour specific cell surface markers and possibly nuclear localizing sequences [32-36]. Small AuNPs are required here to avoid internal absorption of the low energy Auger cascade, and for possible infiltration through the nuclear pore, which can allow translocation of particles as large as 30 nm [37]. While the macroscopic dose enhancement with this strategy may not be profound, due to the difficulty of accumulating a very large number of individually targeted particles in the nucleus, smaller AuNPs release proportionally more low energy Auger electrons in the
immediate vicinity of the AuNPs. These Auger electrons are characterized by a higher linear energy transfer. Increased LET has been shown to correlate well with an enhanced relative biological effectiveness up to LET values of about 100 keV.µm⁻¹ [38]. This radiosensitization approach can therefore be characterized by intense dose heterogeneities at the nano-scale. Thus, the number of AuNPs required per cell for a clinically significant effect using this approach may be significantly lower than we have predicted using a macroscopic dose metric. Taken as an upper limit, our results suggest that to achieve a doubling of the delivered dose, \(1.44 \times 10^7\) 5 nm AuNPs would be required per cell (corresponding to 5.39 mg of AuNPs per g of tumour).

In regards to the photon source energy, the clinical challenge with permanent brachytherapy seeds is that the delivered dose is delivered continuously over the span of weeks. In this case it would be necessary to understand the full pharmacokinetic implications of different sized AuNPs with various conjugations to ensure they remain in the cancer cells for extended periods. With a half-life of 17 days, \(^{103}\)Pd may be a more practical source than \(^{125}\)I, which exhibits a half-life of 59.4 days. A more clinically relevant scenario could involve novel miniature electronic 50 kVp x-ray source in conjunction with a balloon catheter.

The second clinical strategy, involving higher energy photon sources above the k-edge such as the high dose rate \(^{169}\)Yb source or the 300 kVp orthovoltage source, would constitute a bulk approach to AuNP radiosensitization. This strategy would exploit the more energetic photoelectrons escaping the AuNPs, which travel on the order of tens of microns and can easily reach the cell nucleus from outside the cell. AuNPs would not necessarily need to be internalized in the cancer cells because the relative distance from the DNA is not as crucial using higher energy sources. Accumulation could therefore be achieved passively by relying on the increased permeation and retention of the leaky vasculature of certain tumours. However, targeted AuNPs would still be beneficial in this case to
ensure retention of nanoparticles in the tumour for the duration of the radiotherapy [8,40]. In this approach the size of AuNPs is less crucial as the main benefit of smaller particles is only realized at very small distances from the AuNPs.

Rather than recommending the number of AuNPs required per cell, a more fitting metric for this approach would be the mg of AuNPs per g of tumour. Based on our calculations the concentration of AuNPs in the tumour region required to double the delivered dose would be between 12.1 -13.7 mg AuNP per g for the $^{169}$Yb HDR source and 15.4 - 20.2 mg AuNP per g for the 300 kVp source. This approach requires significantly higher concentrations of AuNPs but does not necessitate the production of cellular or nuclear targeting AuNPs.

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Chapter 3

A Monte Carlo-based radiobiological predictive model of gold nanoparticle radiosensitization

### 3.1 Abstract

Radiosensitization using gold nanoparticles (AuNPs) has been shown to vary widely with cell line, irradiation energy, AuNP size, concentration and intracellular localization. We developed a Monte Carlo-based AuNP radiosensitization predictive model (ARP), which takes into account the detailed energy deposition at the nano-scale. This model was compared to experimental cell survival and macroscopic dose enhancement predictions. PC-3 prostate cancer cell survival was characterized after irradiation using a 300 kVp photon source with and without AuNPs present in the cell culture media. Detailed Monte Carlo simulations were conducted, producing individual tracks of photoelectric products escaping AuNPs and energy deposition was scored in nano-scale voxels in a model cell nucleus. Cell survival in our predictive model was calculated by integrating the radiation induced lethal event density over the nucleus volume. Experimental AuNP radiosensitization was observed with a sensitizer enhancement ratio (SER) of $1.21 \pm 0.13$. SERs estimated using the ARP model and the macroscopic enhancement model were $1.20 \pm 0.12$ and $1.07 \pm 0.10$ respectively. In the hypothetical case of AuNPs localized within the nucleus, the ARP model predicted a SER of $1.29 \pm 0.13$, demonstrating the influence of AuNP intracellular localization on radiosensitization.
3.2 Introduction

Gold nanoparticle (AuNP) radiosensitization represents a novel approach to enhance the local effects of radiation. The proof of principle of AuNP radiosensitization was first demonstrated by Hainfeld et al, who effectively treated mammary carcinoma xenografts in mice with radiation after in vivo administration of 1.9 nm AuNPs [1]. The mechanism of AuNP radiosensitization relies on the enhanced attenuation of low-energy photons by high-atomic number (high-Z) materials through the photoelectric effect. Photoelectric absorptions within Au atoms result in the release of a localized spray of photoelectric products comprising characteristic photons, photoelectrons and Auger electron cascades. The conversion of photons into short-ranged Auger electrons exhibiting a potentially higher linear energy transfer (LET) can alter the local radiation quality [2,3]. Previous designs of high-Z radiosensitizers including halogenated pyrimidines such as iododeoxyuridine (IUdR) and bromodeoxyuridine (B UdR), relied on the incorporation of individual high-Z atoms into cellular DNA [4]. These strategies failed to produce clinically positive results due to the limited accumulation of high-Z atoms into cancer cells [4,5]. With the advent of nanotechnologies and the nanoparticle as a vehicle to accumulate a large number of high-Z atoms within a specific target, novel research opportunities have emerged in the field of radiation dose enhancement. However, whereas halogenated pyrimidines are incorporated directly into the DNA, the sub-cellular location of nanoparticles within various cell compartment may place the DNA out of reach from the low energy products of the photoelectric cascade. In recent years gold nanoparticles (AuNPs) have been extensively studied for radiosensitization due to gold’s high atomic number, general biocompatibility [6], and a surface well suited for conjugation to tumour targeting moieties [7,8].

Experiments have revealed that AuNP radiosensitization is highly sensitive to cancer cell type [9], photon source energy [10], and AuNP size [11], concentration [12], and localization relative to
cellular DNA [13]. Various metrics of AuNP radiosensitization have been evaluated including the macroscopic dose enhancement to the tumour [14-16], and the radial dose enhancement around AuNPs at the nanoscale [17-19]. While informative, translating these metrics directly into a radiobiological effect is not straightforward and generally underestimates experimental findings [9,10,12].

Recent radiobiological predictive models of AuNP radiosensitization [20,21] have been adapted from the Local Effect Model (LEM) used for heavy-ion therapy treatment planning [22]. The basic theory of the LEM proposes that cell survival can be better predicted by accurately accounting for the microscopic spatial energy deposition of a given radiation treatment. This paper describes a Monte Carlo-based AuNP-tailored adaptation of the LEM, called hereafter the AuNP Radiosensitization Predictive (ARP) model, to estimate cell survival within the parameter space of a given cancer cell type and radiation sensitivity, photon source energy, as well as AuNP size, concentration, and intracellular location. The current study provides an alternative adaptation of the LEM for AuNP radiosensitization presented by McMahon [21], by building upon a previously described Monte Carlo approach to investigate photoelectric absorptions within AuNPs and the subsequent spatial distribution of escaping energy presented by Lechtman et al [16].

3.3 Materials and methods

3.3.1 Cell experimentation

3.3.1.1 Cell culture and characterization

In vitro experiments were carried out on PC-3 human prostate adenocarcinoma (American Type Culture Collection, Manassas, VA). Cells were cultured in RPMI 1640, with L-glutamine and sodium bicarbonate supplemented with 10% fetal bovine serum (Cellgro laboratories, Manassas.
VA), and 5% penicillin and streptomycin (Invitrogen, Carlsbad, CA). Exponentially growing cells were seeded in 35 mm treated culture dishes with 2 ml cell culture media and grown to 80% confluence. To characterize cellular volumes, live cells were imaged using confocal microscopy, and cell nucleus and cytoplasm volumes were measured using ImageJ as described by Cai et al [23].

3.3.1.2 AuNP preparation

30 nm diameter AuNP colloids (Ted Pella Inc., Redding, CA) were PEGylated by reaction with 0.235 g MeO-PEG-SH (IRIS Biotech GmbH, Marktredwitz, Germany) at a molecular weight of 2000 daltons per 500 ml of AuNPs, and then concentrated through centrifugation. AuNP concentration was verified by UV-Vis absorption using the Nanodrop 2000 (Thermo Scientific, Wilmington, DE). Highly concentrated AuNPs were re-suspended in cell culture media at a concentration of 2 mg/ml. Cell cultures were incubated with the final AuNP/cell media mixture 24 hours prior to irradiation and analysis.

3.3.1.3 AuNP concentration and localization

After incubation with AuNPs, dishes were thoroughly washed 4 times with PBS to remove any AuNPs not taken up in cells. Cells were detached using 0.25% trypsin/EDTA, buffered in cell culture media, counted, and pelleted for analysis. AuNP cellular uptake was quantified using inductively coupled plasma mass spectroscopy (ICP-MS) after pellets were digested in 10 ml of concentrated trace-metal analysis grade HCl (SeaStar Chemicals, Sidney, BC), and then further diluted (~450x) with 3% HCl and 1% thiourea. Results were compared to an Au standard (Inorganic Ventures, VA), and measurements were converted to AuNPs per cell, and mg of gold per ml of cells. Nine ICPMS trials were conducted per point. AuNP intracellular localization was visualized at various magnifications using transmission electron microscopy (TEM) after cell pellets were fixed
with Karnovsky’s fixative, thin sectioned (60-70 nm) and stained. TEM imaging was conducted using a H7000 transmission electron microscope (Hitachi Corp, Tokyo, Japan) at various magnifications.

3.3.1.4 Radiobiological experiments

Irradiation was carried out on a clinical Gulmay D3300 (Chertsey, UK) 300 kVp x-ray therapy unit (average energy \( \approx 100 \) keV, HVL=3.1 mm Cu, filter: 1.5 mm Al, 0.25 mm Cu, and 0.5 mm Sn) using a 10 cm diameter cone collimator. Radiation was delivered from above the culture dishes penetrating 4 mm of cell culture media. Cell cultures with no gold in the media were irradiated at 0, 1, 2, 4, and 8 Gy. Cell cultures incubated with gold for 24 hours were irradiated at 0, 2, 4, and 8 Gy with AuNPs still present in the media. Experiments were repeated three times to obtain the average cell survival with standard deviations.

Immediately after irradiation, culture dishes were washed four times with PBS, trypsinized, counted, and plated into 60 mm cultured dishes producing 100 ± 50 colonies (three dishes per point). Colonies were fixed and stained with methylene blue after 11-13 days. Colonies of 50 cells or more were counted, and cell survival was calculated relative to the plating efficiency of controls receiving no radiation.

Clonogenic survival as a function of dose with and without AuNP present was fit to the linear-quadratic cell survival model \( S = e^{-(\alpha D + \beta D^2)} \). Alpha and beta parameters were extracted from the data using non-linear least-squares regression analysis in Matlab and expressed with 95% confidence intervals.
3.3.2 ARP model simulations

3.3.2.1 Monte Carlo simulation of AuNP radiosensitization

A detailed account of the sub-cellular spatial energy deposition around AuNPs was required as input for the ARP model. Monte Carlo simulation of this energy deposition was performed in steps to reduce computation time.

First, the 300 kVp source photon phase-space was simulated in air using the MCNP-5 code [24] as described by Keller et al [25]. The rate of AuNP photoelectric absorption was calculated from a simulation of photons penetrating 4 mm of cell culture media, as described by Lechtman et al [16]. Second, the PENELOPE code version 2008.1 [26], was used to calculate the macroscopic dose enhancement due to AuNPs, and to perform detailed event-by-event simulations of the energy deposition around AuNPs from escaping electron and photon tracks [16]. For each simulated photoelectric absorption within a AuNP, the escaping electron and photon tracks were followed in 3D and the local nanoscale energy deposition was recorded using a customized tally. Although PENELOPE has limitations with regards to very low-energy electron inelastic scattering simulation and track structure calculations [27,28], we chose this code due to its comprehensive coupled electron/photon transport simulation through various mediums including tissue and gold, and flexibility of its geometry package [29]. Furthermore, PENELOPE offers a highly customizable code allowing for user defined tallies that were required for this study [26].

3.3.2.2 The ARP model

The ARP model incorporated a three-compartment spherical cell model comprising a cytoplasm, a radiosensitive nucleus region, and an extracellular region. For each simulation AuNPs were randomly distributed within the three regions of the ARP cell model based on input concentrations.
For computational efficiency, nanoparticles beyond a cutoff distance from the cell, corresponding to the maximum range of escaping photoelectrons, were not considered in the simulations [16]. Simulated energy deposition tracks were randomly selected, and their spatial coordinates were transposed in Matlab to represent individual photoelectric interactions with AuNPs. The number of photoelectric events was determined based on the calculated rate of photoelectric absorption. The energy released from AuNPs and deposited within nanometric nucleus voxels was then scored. Background dose delivered by photons was assumed homogeneously distributed throughout the nucleus. Each simulation was repeated eleven times with random AuNP locations, producing an average survival and maintaining a standard error of less than 1%. Cell survival was determined similarly to the local effect model [30]:

\[
S = e^{-N_{\text{lethal}}} ; \quad \text{Where} \quad N_{\text{lethal}} = \int \nu(D_{\text{local}}) \, dV
\]

\(S\) is the surviving fraction, \(N_{\text{lethal}}\) is the average number of lethal events per cell, and \(dV\) is the differential voxel element. In our implementation of this model, we assumed voxel sizes of finite volume and therefore the integral was carried out as a Riemann sum. \(\nu(D_{\text{local}})\) is the lethal event density, which is a function of \(D_{\text{local}}\), the local dose absorbed within voxels of the nucleus. In theory, the lethal event density can be formulated as any function of the local energy distribution. We followed Elsässer’s two component formulation based on the well-known linear-quadratic cell survival model for local doses below a threshold value, \(D_{t}\), and a purely exponential survival model for local doses above the threshold to account for the limitation of the linear quadratic model at very high energy densities [31]:
With $\alpha$ and $\beta$ being the cell radiosensitivity parameters of the linear-quadratic model for low-LET photon radiation, and $S_{max} = \alpha + 2\beta D_t$. The threshold dose - an empirical parameter - has been shown to produce more accurate predictions of cell survival at increasingly high doses [32].

ARP model survival calculated at delivered doses from 0 to 8 Gy was then fit to the linear quadratic model in order to compare against experimental results.

3.3.2.3 Macroscopic dose enhancement model

Another prediction of cell survival was modeled from the estimated macroscopic dose enhancement given by:

\[
S = e^{-\left[\alpha(D + D_{\text{enhance}}) + \beta(D + D_{\text{enhance}})^2\right]}
\]

Where $D$ is the dose delivered by the photon source, and $D_{\text{enhance}}$ is the macroscopic dose enhancement predicted from simulations [16]. The macroscopic dose enhancement survival at delivered doses from 0 to 8 Gy was fit to the linear quadratic model to compare against experimental results. Table 3.1 highlights the similarities and main differences between the macroscopic dose enhancement model and the ARP model.

3.3.2.4 ARP validation and predictions

In order to quantify AuNP radiosensitization and to compare experimental and predicted survival, the mean inactivation dose (MID), represented by the area under the survival curves was calculated.
The MID is a useful metric, as it represents radiosensitivity over the whole survival curve with a single parameter [33]. Sensitizer enhancement ratios (SER) were calculated by dividing the MID without AuNPs by the MID with AuNPs [9]. MID and SER values were presented along with standard deviations. Predicted SER values were compared to experimental results and the effect of AuNP intracellular localization was explored using ARP.

### Table 3.1: A comparison of two models used to predict AuNP radiosensitization

<table>
<thead>
<tr>
<th>Macroscopic enhancement model</th>
<th>ARP model</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCNP Monte Carlo simulation to determine the rate of photoelectric absorption within AuNPs</td>
<td>Randomly selected tracks of escaping radiation simulated to interact with a model cell. Dose enhancement scored in nanoscale voxels of the nucleus</td>
</tr>
<tr>
<td>PENELOPE Monte Carlo simulation of secondary energy escaping AuNPs</td>
<td>Cell survival estimated by integrating the local lethal event density over the cell nucleus as described by equations (3.1) and (3.2)</td>
</tr>
<tr>
<td>Macroscopic dose enhancement calculated in a volume around the AuNPs corresponding to the cell culture media</td>
<td></td>
</tr>
<tr>
<td>Cell survival estimated using the linear quadratic model as described by equation (3.3)</td>
<td></td>
</tr>
</tbody>
</table>
3.4 Results

3.4.1 PC-3 cell characteristics and AuNP accumulation

The average cell volume and nucleus volume were measured from confocal images to be 2290 ± 1480 µm$^3$ (n=26) and 9480 ± 6040 µm$^3$ (n=27) respectively (Figure 3.1). Therefore, the average cytoplasmic volume ignoring cytoplasmic organelles was estimated to be ~7190 µm$^3$.

![Figure 3.1: Confocal images of live PC-3 cells. Cell surfaces (red) were stained by wheat germ agglutinin-Alexa Fluor 594 conjugate, whereas cell nuclei (blue) were stained by Hoechst 33342 dye. Average cell and nucleus radius were measured to be 13.1 ± 2.5 µm and 8.2 ± 2.1 µm respectively.](image)

The amount of gold per PC-3 cell, as measured using ICP-MS was 2.27×10$^4$ ± 1.47×10$^4$ AuNPs per cell. TEM imaging (Figure 3.2) revealed 30 nm AuNPs to be randomly distributed within cytoplasmic vesicles, but were not observed to enter the cell nucleus. Given this observation, the concentration of gold within the cytoplasm was estimated to be 0.84 mg of gold per ml, resulting in
a concentration uptake ratio (AuNP concentration inside the cells divided by the concentration in the surrounding media) of 0.42.

Figure 3.2: TEM images reveal 30nm AuNPs accumulate in cytoplasmic vesicles both individually and in groups within vesicles. Nanoparticles of these sizes were not observed to enter the nucleus.

3.4.2 Clonogenic survival of PC-3 cells

PC-3 cells incubated with AuNPs at 2 mg/ml without irradiation did not show loss of clonogenicity. Figure 3.3 shows the clonogenic survival of cells irradiated without and without AuNPs. Radiosensitivity of PC-3 cells was observed to have a shouldered response, but this shoulder was less pronounced when irradiation was carried out with AuNPs in the media. Table 2 shows the linear quadratic parameters fitted to the experimental data.
3.4.3 ARP model parameters and comparison to clonogenic assays

For the 300 kVp source, the rate of photoelectric absorption within a 30 nm AuNP was calculated from simulations to be $1.00 \times 10^{-3}$ per AuNP per Gy absorbed dose. The maximum range of photoelectrons escaping AuNPs was determined from simulations to be 147 µm, and this value was used as the cutoff distance for simulating AuNPs in the media surrounding the ARP model cell. Outside the cell AuNPs were assumed randomly distributed at a concentration of 2 mg/ml. The ARP tally voxel size was set to $20 \times 20 \times 20$ nm$^3$. The threshold dose was set to 23.9 Gy – a value that fit...
the ARP model well to experimental cell survival at 2 Gy with 2 mg/ml AuNPs. At AuNP concentrations of 2 mg/ml, macroscopic dose enhancement was calculated to be $6.45 \times 10^{-2}$ Gy per Gy delivered.

**Table 3.2:** Parameters of cell survival.

<table>
<thead>
<tr>
<th>Sensitizer enhancement ratio ± SD</th>
<th>Mean inactivation dose ± SD [Gy]</th>
<th>β (confidence bounds) [Gy⁻²]</th>
<th>α (confidence bounds) [Gy⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AuNPs (experimental)</td>
<td>2.56 ± 0.25</td>
<td>0.044 (0.016, 0.072)</td>
<td>0.217 (0.141, 0.293)</td>
</tr>
<tr>
<td>2mg/ml AuNPs (experimental)</td>
<td>2.11 ± 0.10</td>
<td>0.037 (0.017, 0.057)</td>
<td>0.345 (0.288, 0.402)</td>
</tr>
<tr>
<td>Macroscopic dose model</td>
<td>2.40 ± 0.00</td>
<td>0.050 (0.050, 0.050)</td>
<td>0.231 (0.231, 0.231)</td>
</tr>
<tr>
<td>ARP model</td>
<td>2.13 ± 0.02</td>
<td>0.045 (0.045, 0.046)</td>
<td>0.315 (0.314, 0.316)</td>
</tr>
</tbody>
</table>

**Figure 3.4** shows a good agreement between experimental survival and the ARP model survival curves, while the macroscopic dose enhancement survival model underestimates AuNP radiosensitization. **Table 3.2** shows the extracted linear quadratic parameters of both models compared to experimental results along with the MID and SER values. The SER obtained by the ARP model is in close agreement with experimental results, while the macroscopic model underestimates sensitization by 12%. It should also be noted that the macroscopic model assumed a uniform concentration of 2 mg/ml AuNP concentration both inside and outside the cell, but we observed a lower concentration within the cells experimentally. While the macroscopic dose enhancement model underestimates the radiobiological effect of short ranged Auger electrons, in this case it overestimated the intracellular AuNP concentration, and therefore appears to show better agreement than the model warrants.
Figure 3.4: Comparing the ARP model and the macroscopic model with experimental results. The ARP model fits experimental data more closely by taking into account energy deposition at the nanoscale, as well as experimental details such as cell dimension, and intracellular AuNP concentration.

Figure 3.5 shows ARP survival for a 2 Gy delivered dose with varying AuNP concentrations and distributions within the cell. Three hypothetical cases were considered: i) 2 mg/ml AuNPs distributed in the media only, ii) 2 mg/ml AuNPs distributed in the media and the cytoplasm, iii) 2 mg/ml AuNPs distributed in the media, cytoplasm and nucleus. The ARP model was compared to the macroscopic dose enhancement prediction, which did not incorporate AuNP localization and assumed a homogeneously distributed AuNP concentration of 2 mg/ml.
Based on the experimental data presented, the ARP model provides a more accurate prediction of AuNP radiosensitization - with a predicted SER in close agreement to the experimental value - compared to macroscopic dose enhancement model predictions. Furthermore, as opposed to macroscopic dose enhancement predictions [14-16], the ARP model incorporates detailed information regarding the radiation source, cell line, and characteristics of AuNPs including size, concentration, and cellular distribution to predict the extent of radiosensitization. This model builds

**Figure 3.5:** The predicted effects of AuNP cellular and nuclear accumulation. The ARP model shows a strong influence of AuNP intracellular distribution on radiosensitization. These effects are not taken into account in the macroscopic dose enhancement model.

### 3.5 Discussion

Based on the experimental data presented, the ARP model provides a more accurate prediction of AuNP radiosensitization - with a predicted SER in close agreement to the experimental value - compared to macroscopic dose enhancement model predictions. Furthermore, as opposed to macroscopic dose enhancement predictions [14-16], the ARP model incorporates detailed information regarding the radiation source, cell line, and characteristics of AuNPs including size, concentration, and cellular distribution to predict the extent of radiosensitization. This model builds
on the local effect model adapted for AuNPs proposed by McMahon, by incorporating structural compartments of the cell and AuNP localization as input parameters [21]. This gives the ARP model flexibility to examine radiosensitization through a vast parameter space of pharmacological and cell modelization. With increasing efforts to conjugate AuNPs to targeted moieties, towards the aim improving cellular and possibly nuclear incorporation [8,34-36], the ARP model can help provide a framework for predicting the benefits of such targeting strategies.

Figure 3.5 reveals the radiobiological benefit of high-LET Auger and delta electrons as predicted by the ARP model. These escaping electrons travel an average distance of 1.06 µm from the AuNP surface, as calculated from PENELOPE simulations, and therefore only contribute to cell killing when AuNPs are sufficiently close to the radiosensitive DNA. Significant radiosensitization is observed when AuNPs are localized in the cytoplasm, resulting in a predicted sensitizer enhancement ratio of 1.23 ± 0.11 at an AuNP concentration of 2 mg/ml. The largest effect is predicted when AuNPs are located in the nucleus, resulting in a sensitizer enhancement ratio of 1.29 ± 0.13 at an AuNP concentration of 2 mg/ml. When AuNPs are exclusively located in the surrounding cell culture media, the ARP model still predicts significant, though less substantial radiosensitization. This effect is likely due to longer ranged photoelectrons traveling 40 microns on average that can reach the nucleus from outside the cell.

The influence of AuNP localization is expected to be most pronounced for photon sources with average energies below the k-edge, at which escaping photoelectrons have shorter average ranges and Auger and delta electrons comprise a significant percentage of the total escaping energy. For higher energy photon sources, longer ranged photoelectrons are the main contributor to radiosensitization [16].
Comparing the extracted linear quadratic parameters from experimental survival with and without AuNPs, we observed an increase in the $\alpha$ component but only a small change in the $\beta$ component. This suggests that AuNP radiosensitization involves radiobiological mechanisms similar to that of high-LET radiation, which exhibits a predominantly linear dose response [37]. This finding agrees with other experimental findings of AuNP radiosensitization [10], and demonstrates that the ARP model is able to effectively represent this feature of AuNP radiosensitization.

In our development of the ARP model, we have incorporated two fundamental distinctions from the LEM model. The first involves a more detailed simulation of the energy deposition at the subcellular scale, rather than representing the energy deposition as a radial dose function [30], or scoring dose in radial bins [21]. This provides additional information of the Auger cascade, and avoids variations in the calculation of dose associated with the increasingly large concentric volumes further away from the AuNP [16]. Another distinction of the ARP model is the calculation of lethal event density within finite sized voxel elements. This method, rather than a point response approach, may be a better representation of radiobiological mechanisms that occur over finite distances, such as diffusion of free radicals, double strand breaks, multiply damaged sites, and clustered effects [38,39]. At the smallest relevant scale, the DNA helix is 2.2-2.6 nm wide and each base 0.33-0.34 nm long [40]. The maximum distance of double strand breaks has been reported to lie between 20-43 base pairs, corresponding to a distance of 6.6-13.2 nm, with some reported distances beyond 20 nm [41]. Radical diffusion, which further extends the distance of radiobiological action, is known to occur within 4 nm of DNA molecules [42]. We therefore chose a voxel dimension of 20 nm in order to encompass these features. Although PENELOPE’s low-energy simulations differ from more accurate models below a few hundred eV [29,43,44], and furthermore the transport of electrons below a few hundred eV is still poorly understood [45-48], the ARP model voxel dimension of 20 nm, corresponding to the average path length of $\approx 500$ eV electrons [49],
minimizes the inherent uncertainty in transporting electrons below this energy. It should also be noted that the ARP survival predictions do not change significantly as a function of voxel size below \( \approx 30 \) nm because of the purely exponential survival function above the threshold dose.

The ARP model also includes several simplifications. First it assumes that cancer cells have a spherical shape, with a nucleus that is centered in the middle of the cytoplasm. While this assumption is somewhat acceptable for undifferentiated cancer cells, the shape of the cell may have an impact on the biological efficiency of AuNP radiosensitization. Second, the ARP model assumes a random distribution of AuNPs inside the cytoplasm instead of an accumulation into the phagolysosomes – an observation noted in several studies \([9,10,12,50]\). While this assumption may result in AuNPs closer or further away from the nucleus compared to reality, it is not yet understood where the nanoparticle would be localized at the sub-cellular scale \textit{in vivo} during irradiation, as AuNPs have been shown to exhibit complex trajectories once inside cells \([51]\). Finally, similar to the LEM model developed by Kraft, the ARP model includes the concept of a threshold dose, beyond which the cell survival response is expected to be purely exponential \([31]\). While there is evidence that survival takes on a purely exponential shape after a threshold dose \([32,52]\), extracting this value from experimental results may be difficult. All these assumptions may limit the use of the ARP model as an ‘absolute’ radiobiological predictive tool. However, this limitation should not impact its capacity to provide relative comparisons between different clinically relevant uses of AuNPs; for example comparing different AuNP sizes, concentrations, intracellular localizations, and various beam energies, radiation doses, and cancer cell radiation sensitivities. The ARP model therefore remains a useful tool to define the best scenario for a clinical application of AuNP radiosensitization.
Acknowledgements: This research was supported by a grant from the Canadian Breast Cancer Research Alliance (Grant 019374) and the Canadian Institute for Health Research Terry Fox New Frontiers Program Project in Ultrasound for Cancer Therapy.

Conflict of interest: The authors have no conflict of interest to disclose.
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Chapter 4

Investigating AuNP radiosensitization with a Monte Carlo-based nanoscale predictive model
This chapter represents the manuscript in preparation: “Lechtman E, Lai P, Mashouf S, Reilly R, and Pignol JP 2013 A model of gold nanoparticle radiosensitization to evaluate cell survival as a function of cancer cell type, source energy, nanoparticle size, and localization".

4.1 Abstract

Gold nanoparticles (AuNPs) act as radiosensitizing agents through enhanced photoelectric conversion, resulting in a localized spray of low-energy electrons. AuNP radiosensitization varies widely with cell line, radiation source energy, and AuNP size, concentration, and intracellular localization. A Monte Carlo-based AuNP predictive model (ARP) has been developed taking into account the nanoscale radiation modification around AuNPs. ARP model simulations were compared to in vitro experimental AuNP radiosensitization using two photon source energies - 100 kVp and 300 kVp, two cancer cell lines - PC-3 and SK-BR-3, and two AuNP sizes - 5 nm and 30 nm. Experimental radiosensitization ranged from enhancement factors of 1.05 to 1.51. Model predictions closely described experimental results with the proportional scaling of a model sensitivity parameter. Hypothetical simulations were performed, exploring the effects of various intracellular distributions of AuNPs using two source energies - 100 and 300 kVp, and two AuNP sizes - 1.9 nm and 100 nm. The most pronounced effect of AuNP localization was observed with the 100 kVp source in conjunction with 1.9 nm AuNPs. Under these conditions enhancement varied from a factor of 1.34 when AuNPs were not accumulated in the cell, to 2.56 when AuNPs were simulated localized in the cytoplasm and nucleus. The ARP model thus provides a framework for continued investigation of AuNP radiosensitization.
4.2 Introduction

In recent years, there has been increased interest in the use of gold nanoparticles to enhance the therapeutic effects of radiation therapy. The efficacy of AuNP radiosensitization has been demonstrated \textit{in vitro} in DNA and cell models [1-5], and \textit{in vivo} in mice models [6,7]. The premise of gold nanoparticle (AuNP) radiosensitization as it is currently understood involves three basic principles: i/- the selective accumulation of AuNPs in the tumour region; ii/- the enhanced attenuation of low-energy photons within AuNPs at the tumour site through photoelectric absorption; iii/- the release of photoelectric products such as low-energy Auger and photoelectrons from within AuNPs and the interaction of this secondary radiation with sensitive targets in the cancer cells [8]. These processes, and therefore the extent of radiosensitization, are highly sensitive to physical and pharmacological parameters including the photon source energy spectrum, the AuNP size, and the achievable tumour concentration and intracellular localization. Furthermore, because AuNP radiosensitization involves a modification of the local radiation quality, resulting in an increased linear energy transfer (LET) and a more heterogeneous energy deposition profile at the nanoscale, traditional metrics of the macroscopic dose enhancement do not adequately predict the effects of AuNP aided radiotherapy on cell survival [9-12].

Recently, AuNP radiosensitization predictive models have been adapted from the local effect model (LEM) – a model employed to predict the increased relative biological effectiveness of heavy ion therapy [12-14]. Both heavy ion therapy and AuNP radiation enhancement are characterized by localized dose deposition and increased LET. The premise of the LEM proposes that cellular response to sparsely ionizing radiation can be applied to densely ionizing radiation with an appropriate description of the spatial energy deposition pattern [14]. Therefore, the LEM and its adaptations can provide a framework to explore the radiobiological effects of high-LET radiation.
using the cell sensitivity parameters of low-LET sources. A recently published adaptation of the LEM called the AuNP radiosensitization predictive (ARP) model has been developed, based on the Monte Carlo simulation approach of Lechtman et al, to study the physics of AuNP photon absorption and the release of secondary radiation [11,12]. The ARP model incorporates information of the photon source spectrum, cancer cell type, AuNP size, concentration, and intracellular localization, with detailed event-by-event simulations of the localized spray of photoelectric products and the associated energy deposition at the nanoscale. In the previous publication, the ARP model was validated by comparison to radiobiological assays using one cell line, one AuNP size, and one photon source. In this paper, we further explored the predictive capabilities of the ARP model by comparison to radiobiological experiments varying photon energy, AuNP size and cancer cell type. The ARP model was then used to explore the effects of various targeting strategies with respect to the photon source energy and AuNP size, and the clinical implications were discussed.

4.3 Methods

The experimental and simulation methods build upon previously published methods. For further details please refer to [11,12].

4.3.1 Cell cultures

*In vitro* experiments were carried out on two cell lines - PC-3 human prostate adenocarcinoma and SK-BR-3 human breast adenocarcinoma (American Type Culture Collection, Manassas, VA). Prior to experimentation, exponentially growing cells were seeded in 35 mm culture plates and grown to 80% confluence.
4.3.2 AuNP preparation, concentration and localization

AuNP colloids of 5 nm and 30 nm diameter AuNPs (Ted Pella Inc., Redding, CA) were PEGylated to prevent aggregation and then concentrated through centrifugation. AuNP concentration was verified by UV-Vis absorption using the Nanodrop 2000 (Thermo Scientific, Wilmington, DE). Highly concentrated AuNPs were re-suspended in cell culture media at a concentration of 0.2 or 2 mg/ml. Cell cultures were incubated with the AuNP/cell media mixture 24 hours prior to irradiation and analysis.

To analyze AuNP cellular uptake, cell culture dishes were thoroughly washed with PBS after incubation with AuNPs, to remove any AuNPs not taken up in cells. AuNP cellular uptake was quantified using inductively coupled plasma mass spectroscopy and AuNP intracellular localization was visualized using transmission electron microscopy (TEM).

4.3.3 Radiobiological experiments

Irradiation was carried out on a clinical Gulmay D3300 (Chertsey, UK) x-ray therapy unit at energies of 300 kVp (average energy ≈ 100 keV, HVL = 3.1 mm Cu, filter: 1.5 mm Al, 0.25 mm Cu, and 0.5 mm Sn) and 100 kVp (average energy ≈ 33 keV, HVL = 2.7 mm Al, filter: 2.0 mm Al) using a 10 cm diameter cone collimator. Radiation was delivered from above the culture dishes penetrating 4 mm of cell culture media. Cultures of PC-3 and SK-BR-3 cells with no gold in the media were irradiated at 0, 1, 2, 4, and 8 Gy. Cell cultures incubated with gold for 24 hours were irradiated with AuNPs still present in the media. Table 4.1 summarizes the radiosensitization experiments conducted. Average cell survival was assessed using the clonogenic assay and normalized relative to the plating efficiency of controls receiving no radiation. Where applicable, clonogenic survival as a function of dose with and without AuNP present was fit to the linear-
quadratic cell survival model \( S = e^{-(\alpha D + \beta D^2)} \). Alpha and beta parameters were extracted from the data using non-linear least-squares regression analysis in Matlab and expressed with 95% confidence intervals.

<table>
<thead>
<tr>
<th>Table 4.1: Radiobiological Experiments and model predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Line</strong></td>
</tr>
<tr>
<td>Energy (kVp)</td>
</tr>
<tr>
<td>AuNP size (nm)</td>
</tr>
<tr>
<td>AuNP concentration (mg/ml)</td>
</tr>
<tr>
<td>Dose (Gy)</td>
</tr>
<tr>
<td>Experimental enhancement ± standard deviation</td>
</tr>
<tr>
<td>ARP model Enhancement ± standard deviation</td>
</tr>
</tbody>
</table>

4.3.4 ARP model simulations

4.3.4.1 Monte Carlo simulation of AuNP radiosensitization

The ARP model relies on a detailed account of the nanoscale energy deposition of photoelectric products escaping individual AuNPs. To model this phenomenon while reducing computation time, Monte Carlo simulations were performed in steps using both the MCNP-5 and PENELOPE Monte Carlo codes. The choice of these codes as well as a detailed discussion of the limitations of Monte Carlo radiation transport codes can be found elsewhere [11,12,15,16]. In this study 100 kVp and 300 kVp source photons were simulated penetrating 4 mm of cell culture media. At this depth photons were simulated to interact with AuNPs of 1.9, 5, 30, and 100 nm diameter. The rate of photoelectric absorption per AuNP was calculated, and for each simulated photon interaction within a AuNP, the
escaping electron and photon tracks were followed and the local nanoscale energy deposition was recorded using a customized tally.

4.3.4.2 The ARP model

The ARP model incorporated a three-compartment spherical cell model comprising a cytoplasm region, a radiosensitive nucleus region, and an extracellular region. PC-3 and SK-BR-3 nucleus and cytoplasm dimensions were taken from previous studies [12,17]. AuNP concentrations and distributions were varied within these compartments based on experimental observations and hypothetical cases. Radiation tracks corresponding to individual interactions of photons within AuNPs were applied and the energy deposited within nanometric nucleus voxels was scored. The voxel size was set to 20×20×20 nm as previously described by Lechtman et al [12]. The background dose delivered by photons was assumed homogeneously distributed throughout the nucleus. Cell survival was determined by integrating the local dose response, or lethal event density, in each voxel over the nucleus volume.

\[
S = e^{-\int \nu(D_{local}) dV}
\]

The lethal event density was defined by a two component formulation based on the linear-quadratic cell survival model for local doses below a threshold value, \(D_t\), and a purely exponential survival model for local doses above the threshold to account for the limitation of the linear quadratic model at very high energy densities:

\[
\nu(D_{local}) = \begin{cases} 
\frac{\alpha D_{local} + \beta D_{local}^2}{V_{nucleus}} & \text{if } D_{local} \leq D_t \\
\nu(D_t) + \frac{S_{max} (D_{local} - D_t)}{V_{nucleus}} & \text{if } D_{local} > D_t
\end{cases}
\]
Where $\alpha$ and $\beta$ represent the cell radiosensitivity parameters of the linear-quadratic model for low-LET photon radiation without AuNPs present, $D_{\text{local}}$ is the sum of the dose deposited from AuNPs and the background radiation, and $S_{\text{max}} = \alpha + 2\beta D_t$. The threshold dose for PC-3 cells has been empirically determined for the ARP model to be 23.9 Gy [12]. Following previous reports that the threshold dose is proportional to $\alpha / \beta$, the threshold dose for SK-BR-3 was scaled accordingly [18,19].

4.3.5 Metrics of comparison

Where applicable, the mean inactivation dose (MID) was obtained from experimental and simulated survival, represented by the area under the survival curves. Sensitizer enhancement ratios (SER) were calculated by dividing the MID without AuNPs by the MID with AuNPs [1]. For radiosensitization conditions with only few dose points, extracting MID values was not possible, and therefore SER was defined as the ratio of the doses needed to produce the same cell survival (corresponding to 2 Gy with AuNPs) with and without AuNPs.

4.4 Results

4.4.1 Cellular uptake of AuNP

Figure 4.1 shows TEM images of thin sections of cells after overnight incubation with either 5 nm or 30 nm AuNPs. AuNPs of these sizes were not observed to localize in the nucleus. Table 4.2 presents the cellular dimensions obtained from previous publications, and summarizes our results of ICPMS analysis quantifying AuNP cellular uptake. Because AuNPs were not found in the nucleus, the estimated concentration of gold in the cells was expressed per ml of cytoplasmic volume.
Figure 4.1: TEM images reveal AuNPs accumulate in cytoplasmic vesicles. a) PC-3 cells incubated with 5 nm AuNPs at 2 mg/ml. b) PC-3 cells incubated with 30 nm AuNPs at 2 mg/ml. SK-BR-3 cells incubated with 30 nm AuNPs at 2 mg/ml. AuNPs of 5 nm and 30 nm were not observed to enter the nucleus.
### Table 4.2: Cell dimensions and AuNP cellular uptake

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>PC-3</th>
<th>SK-BR-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus radius ± SD (µm)</td>
<td>13.1 ± 2.5</td>
<td>9.3 ± 2.9</td>
</tr>
<tr>
<td>Cytoplasm radius ± SD (µm)</td>
<td>8.2 ± 2.1</td>
<td>6.3 ± 1.8</td>
</tr>
<tr>
<td>AuNP size (nm)</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>AuNP concentration in media (mg/ml)</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>3.69×10^5 ± 2.15×10^6</td>
<td>1.47×10^4 ± 2.27×10^4</td>
</tr>
<tr>
<td></td>
<td>0.84</td>
<td>0.38</td>
</tr>
<tr>
<td>AuNP concentration in cytoplasm (mg/ml)</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>2.07×10^3 ± 1.02×10^4</td>
<td>1.19×10^3 ± 0.274×10^3</td>
</tr>
</tbody>
</table>

#### 4.4.2 Comparing radiobiological experiments with the ARP model

Without the presence of AuNPs, experimental PC-3 cell survival as a function of dose did not significantly differ between the 100 kVp and 300 kVp source. Therefore, PC-3 cell sensitivity parameters without AuNPs for both energies were taken from Lechtman et al to be \( \alpha = 0.217 \) (0.141, 0.293) and \( \beta = 0.044 \) (0.016, 0.072). For SK-BR-3 cells, irradiated with 300kVp photons, cell sensitivity parameters were estimated from radiobiological assays to be \( \alpha = 0.274 \) (0.125, 0.423) and \( \beta = 0.035 \) (-0.023, 0.092). Cells incubated with AuNPs of 5 nm and 30 nm at a maximum concentration of 2 mg/ml did exhibit a loss of clonogenicity. The extent of experimental radiosensitization is summarized in the **table 4.1**.

Using a 100 kVp photon source penetrating 4 mm of cell culture media, Monte Carlo simulations estimated the rate of photoelectric absorption in a 30 nm AuNP to be \( 9.05\times10^{-3} \) absorptions per AuNP per Gy delivered. For a 300 kVp source interacting with 30 nm AuNPs, the photoelectric absorption rate was estimated to be \( 1.00\times10^{-3} \) absorptions per AuNP per Gy delivered. For a 300 kVp source interacting with 5 nm AuNPs, \( 4.63\times10^{-6} \) absorptions per AuNP per Gy delivered was
These values were used as input for the ARP model.

Figure 4.2 shows PC-3 experimental cell survival as a function of dose with and without 30 nm AuNPs incubated at 2 mg/ml using the 100 kVp photon source energy, as well as the ARP model predictions of AuNP radiosensitization. Figure 4.3 shows PC-3 cell survival with and without 5 nm AuNPs as a function of dose. For all PC-3 ARP results, the threshold value was set to 23.9 Gy.

**Figure 4.2:** PC-3 cell survival was quantified as a function of delivered dose, with and without the presence of 30 nm AuNPs. The ARP model shows close agreement with experimental results.
Figure 4.3: PC-3 cell survival was quantified as a function of delivered dose, with and without the presence of 5 nm AuNPs. The ARP model shows close agreement with experimental results.
**Figure 4.4** shows SK-BR-3 cell survival as a function of AuNP concentration in the cell culture media. For the ARP model, the threshold dose was scaled to 38.7 Gy, proportionate to the SK-BR-3 $\alpha/\beta$ ratio. The last rows of **table 4.1** summarize the metrics of assessment comparing the ARP model with experimental results.

![SK-BR-3 Survival with 30 nm AuNPs at 2 Gy - 300 kVp source](image)

**Figure 4.4:** SK-BR-3 cell survival was quantified as a function of AuNP concentration in the cell culture media. The ARP model shows close agreement with experimental results.
4.4.3 ARP model predictions

Figure 4.5 shows ARP model predictions for a 2 Gy delivered dose using 100 or 300 kVp sources and varying distributions of 1.9 nm or 100 nm AuNPs. Three AuNP distributions were considered for each case: 1) AuNPs localized in the media only at 2 mg/ml, 2) AuNPs localized in the media and cell cytoplasm at 2 mg/ml, 3) AuNPs localized in the media, cytoplasm and nucleus at 2 mg/ml. The effect of AuNP localization is observed to be most pronounced in the case of the 100 kVp source in conjunction with 1.9 nm AuNPs. For this case an SER of 1.34 is predicted when AuNPs are localized only in the media, while an SER of 2.56 is predicted when AuNPs are distributed in the cytoplasm and nucleus. For the 300 kVp source, a less pronounced dependence on AuNP localization is observed.
Figure 4.5: The ARP model predicted effects of varying AuNP cellular and nuclear accumulation. When AuNPs were simulated in a region, the concentration was set to 2 mg/ml. a) 100 kVp source and 1.9 nm AuNPs. b) 100 kVp source and 100 nm AuNPs. c) 300 kVp source and 1.9 nm AuNPs. d) 300 kVp source and 100 nm AuNPs.
4.5 Discussion

In this study, the scope of the ARP model’s predictive capabilities was explored by comparison to radiobiological experiments using two cell lines, two photon energies, and two AuNP sizes. Our model compared well to experimental results, and required only the proportional adjustment of the threshold dose to accurately predict AuNP radiosensitization for cells with different low-LET $\alpha/\beta$ ratios.

The ARP model combines experimentally measured cell parameters with a detailed simulation of the AuNP radiation enhancement at the nanoscale. Previous predictions of cell survival based on macroscopic dose enhancement measurements generally underestimate the radiobiological effect of AuNP radiosensitization, as these measurements do not account for AuNP intracellular localization, or the modified range and LET of secondary electrons [20]. The ARP model builds upon previous adaptations of the LEM [21], by defining cellular compartments and including AuNP intracellular location as an input parameter.

Other than the experimentally determined parameters such as cell size, cell sensitivity to sparsely ionizing radiation, and AuNP concentration, the ARP model includes two free parameters - namely, the voxel dimension, and the threshold dose. Both parameters can effect the predicted cell survival. In McMahon’s adaptation of local effect model, using a point dose response, the voxel size is implicitly defined within the radial dose function, and varies as a cubic function of the radius [21]. In our model, individual tracks of radiation are simulated interacting with the nucleus, and therefore the voxel size must be specified explicitly. The use of nanoscale voxels is a defining feature of the ARP model; if the voxel size were set to macroscopic dimensions, the ARP model would produce macroscopic dose enhancement predictions. A 20 nm voxel dimension was chosen to balance the inclusion of radiobiological mechanisms that occur over finite distances, such as radical diffusion.
and double strand breaks [22,23], with the limits of Monte Carlo simulation accuracy. It should also be noted that the ARP model survival predictions do not change significantly below a voxel dimension of ≈ 30 nm due to the purely exponential survival function for doses beyond the threshold dose.

The concept of a threshold dose is supported by radiobiological experiments which suggest that the linear quadratic model is accurate at low doses, but tends to underestimate survival at higher dose due to the increasing slope cause by the quadratic term [24,25]. Therefore, when it is necessary to explore cell survival at higher doses, a threshold dose is introduced, resulting in a so called linear-quadratic-linear model. Because of the high local doses simulated with the local effect models, a linear-quadratic-linear model is therefore appropriate [26]. However, extracting the threshold dose from radiobiological experiments may be difficult due to the statistical variation in cell survival at large doses. Beuve has therefore suggested that a cell specific threshold dose must be empirically determined for the local effect model by fitting the model to one set of radiobiological experiments involving high-LET radiation [27,28]. The need to empirically determine the threshold dose for each cell type, may limit the utility of such models when comparing the effect of AuNP radiosensitization across various cancer cells lines. Instead, we sought to vary the threshold dose proportionally to the cell specific $\alpha/\beta$ ratio after empirically determining the threshold dose for a single cell line. The $\alpha/\beta$ ratio represents the dose where the $\alpha D$ and $\beta D^2$ components of cell killing are equal. This proportionate scaling was discussed by Astrahan [18], and is observed in the relationship between the $\alpha/\beta$ ratio and threshold doses used in the standard formulation of the LEM shown in Figure 4.6 [19]. Proportionate scaling of the threshold dose may extend the scope and predictive power of the ARP model, but experiments with additional cell lines are needed to confirm this result. It should also be noted that the threshold dose is not an integral parameter of the ARP model. In theory, the lethal event density can be defined by an alternative radiation response function such as target
models, or damage-repair models [29]. The linear quadratic model was chosen here due to the widely available data, and the simplicity of the few number of parameters.

**Figure 4.5** demonstrates the relationship between AuNP size, intracellular location and source energy on radiosensitization. Monte Carlo simulations predict an increased photoelectric absorption within AuNPs of \( \approx 9 \) times when using the 100 kVp source compared to the 300 kVp source. However, in the case of AuNPs localized exclusively outside the cell, there is only a small increase.
in radiosensitization using the 100 kVp source compared to the 300 kVp source, with predicted SER of \( \approx 1.3 \) and 1.2, respectively. The ARP model suggests that for photon sources with average energies below the k-edge of gold (80.7 keV), the majority of radiosensitization results from low-energy Auger electrons escaping AuNPs located inside the cell. For higher energies sources, AuNPs outside the cell have a significant impact on radiosensitization because of the longer ranges of escaping photoelectrons.

These results offer insight into the potential uses of AuNPs. In a clinical situation involving low-energy sources such as permanent seed brachytherapy treatments or miniature electronic x-ray sources, radiosensitization could be dramatically enhanced by actively accumulating AuNPs into cells through conjugation with tumour targeting moieties [30-32]. Further enhancement could be achieved through conjugation with nuclear localizing sequences [33,34], but this would require AuNPs small enough to pass through nuclear pores [35,36].

In a clinical situation involving higher-energy sources such as those used in high-dose-rate brachytherapy, or external beam orthovoltage X-rays, AuNPs would need to be loaded into tumours at relatively high concentrations of about 2 mg/ml, possibly through direct tumoural injection [37]. In this strategy, where radiosensitization is attributed mostly to longer ranged photoelectrons, AuNP intracellular localization would be less crucial and therefore a passive approach to cellular uptake could be employed.

Looking again at Figure 4.5, AuNP size appears to have a less pronounced effect on radiosensitization compared to the source energy and AuNP localization. Smaller AuNPs allow proportionally more low-energy Auger electrons to escape, but these electrons have very short ranges. Therefore AuNP size is only observed to effect radiosensitization when AuNPs are localized in the nucleus, and only at low photon source energies, where Auger electrons comprise a significant
portion of the escaping energy. However, AuNP size can strongly influence radiosensitization due to the size dependence on cellular uptake. We observed a higher cellular uptake by mass of 30 nm AuNPs compared to 5 nm AuNPs. Similarly Chithrani et al found that larger AuNPs of 50 nm were an ideal size for accumulation and retention within HeLa cells [38]. AuNP size is also an important parameter influencing the pharmacokinetics, cytotoxicity, and biodistribution of AuNPs [39,40].

The findings of the ARP model can also help explain the varied reports of AuNP radiosensitization. For example, Chithrani, et al, explored AuNP radiosensitization on HeLa cells [2]. After 24 hour incubation with 50 nm AuNPs at a concentration of $8.8 \times 10^{-3}$ mg/ml cells were irradiated using a 105 kVp photon source and cell survival was assessed. They observed comparable radiosensitization to our experimental results at a similar energy, but at an incubated AuNP concentration $\approx 225$ times less than our methods. An explanation to this discrepancy is that while the concentration in the media was different, both groups reported a similar gold concentration inside the cells (between 6.3 -7.6 ng). At these energies, the ARP model predicts that intracellular AuNP concentration is the critical parameter.

The ARP model has been validated against a range of in vitro radiobiological experiments, and hypothetical scenarios of AuNP radiosensitization have been explored. Although current limitations in low-energy Monte Carlo simulation accuracy and scientific understanding of nano-scale radiobiological events may limit the ARP model as an “absolute” mechanistic predictive model, it remains a useful tool to explore AuNP radiosensitization within a clinically relevant parameter space.
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Chapter 5

Discussion and conclusions
5.1 Thesis summary

The focus of this thesis was to study the physics and radiobiology of AuNP radiosensitization. The main objectives were completed and the hypothesis was confirmed that a Monte Carlo-based nanoscale radiobiological model could be used to predict the radiosensitizing effect of AuNPs. However, the scope of the model’s predictive capabilities could be further investigated in the future. Monte Carlo simulations were conducted, producing a library of event-by-event interactions representing the escaping radiation from individual AuNP photoelectric absorption products. The range and energy of this escaping radiation was studied and the library served as the input for a novel Monte Carlo-based nanoscale radiobiological predictive model of AuNP radiosensitization. The radiobiological model was validated against in vitro AuNP radiosensitization, and further predictions were explored.

The physical mechanisms of AuNP dose modification were investigated through a Monte Carlo approach described in Chapter 2. This novel approach used two available MC codes, standard and in-house macroscopic and microscopic tallies, and simulation partitioning as a variance reduction technique. Where applicable, results compared well to other simulation studies and theoretical calculations [1-3]. The rate of photoelectric absorption in AuNPs of different sizes was calculated for clinical photon sources of varying energies. The highest rate of photoelectric absorption was observed using low-energy brachytherapy sources, which exhibited a $10^3$ fold increase in absorption compared to a 6 MV source. The percentage of energy released as Auger electrons was inversely related to the AuNP size and photon source energy, while internal reabsorption of Auger and delta electrons was most significant for low energies and large nanoparticles. A significant finding in this chapter was the range of escaping radiation from AuNPs and the implications for AuNP radiosensitization. Electrons escaping AuNPs from brachytherapy sources had maximum ranges on
the order of a cell dimension, with most of the energy traveling much shorter distances. For higher energy sources, escaping energy was observed to travel orders of magnitude farther. This suggests that AuNP intracellular accumulation has a more profound effect on AuNP radiosensitization using lower energies (below the k-edge of gold).

In Chapter 3 a radiobiological model called the AuNP radiosensitization predictive (ARP) model, was adapted from a previously developed model used in heavy-ion therapy called the LEM (local effect model) [4]. The premise of the ARP model was that cellular response to radiation depends on the local pattern of energy absorption. The implication of this premise is that the LQM cell sensitivity parameters of cell survival curves from sparsely ionizing radiation could be applied to predict the effects of AuNP radiosensitization with an accurate description of the radiation modification. The ARP model employed the MC approach developed in Chapter 2 to create a library of event-by-event interactions representing the escaping radiation from individual AuNP photoelectric absorption products. Other input parameters were obtained experimentally or chosen to represent hypothetical scenarios. Due to limitations in the LQM at high doses, a threshold dose was empirically chosen to allow the ARP model to make reasonable predictions. The ARP model was then compared to *in vitro* cell survival and macroscopic dose enhancement predictions. The ARP model showed good agreement to experimental radiosensitization and was able to reproduce features of the cell survival curve more accurately compared to macroscopic predictions.

In Chapter 4 the scope of the predictive capabilities of ARP was explored. *In vitro* AuNP radiosensitization was assessed for two cancer cell lines (PC-3 and SK-BR-3), two AuNP sizes (5 and 30 nm) and two source energies (100 and 300 kVp). The ARP model demonstrated close agreement to experimental results as the energy and AuNP size was varied. In order to extend the ARP model to produce accurate predictions for various cell types, it was necessary to scale the
threshold dose proportionately to the cell specific $\alpha/\beta$ ratio. The ARP model was also used to simulate AuNP radiosensitization for a range of AuNP intracellular distributions using 1.9 and 100 nm AuNPs, and 100 and 300 kVp source energies. These results provided insight into the interdependent effects of AuNP size, localization, and source energy. In this simulation, it was observed that AuNP localization dramatically affects the radiobiological outcome using energies below the k-edge. In contrast, AuNP localization has only small effect on the outcome for higher energies. Because of this, similar radiosensitization was observed for both energies when AuNPs were not simulated within the cell. At equal mass concentrations, AuNP size did not impact radiosensitization unless the AuNPs were localized in the nucleus.

5.2 Thesis conclusions

The overall conclusions of this thesis relate to both the development of the ARP model, and the insight gained from model predictions:

General conclusions

1) AuNP radiosensitization involves a local modification of the radiation quality that must be taken into account to predict the radiobiological effect.

2) Monte Carlo simulations provide an effective method to investigate AuNP radiosensitization, but are limited at low-energies ($< 100$ eV).

3) A Monte Carlo-based nanoscale radiobiological predictive model of AuNP radiosensitization has been developed and validated against \textit{in vitro} experiments. This model accounts for the effects of source energy, cell line, and AuNP size, concentration and intracellular location.
**ARP model insights**

1) **Energy**: In contrast to initial predictions, radiosensitization does not correlate directly with photoelectric enhancement. Instead, the effects of source energy on AuNP radiosensitization are observed to be dependent on AuNP size, and location relative to the nucleus. Furthermore the modification in radiation quality caused by AuNPs varies with source energy, with higher energies imparting a higher percentage of energy to photoelectrons. The findings of this research suggest that radiosensitization in megavolt range is infeasible due to the high concentration of AuNPs needed.

2) **AuNP size**: From a physical perspective, AuNP size has little effect on the resulting radiation modulation except at very close proximity to the AuNP surface. Inconsistent reports of the optimal AuNP size seem to be explained by normalizing AuNP concentrations to number of Au atoms rather than number of AuNPs. It is likely that AuNP size is a much more critical factor from a pharmacokinetic, cellular uptake, and cytotoxic perspective.

3) **Intracellular localization**: Based on the premise that DNA is the radiosensitive region in the cells, localization of AuNPs in or close to the nucleus is expected to enhance radiosensitization. However, for higher energy sources, where long ranging photoelectrons comprise the majority of the converted energy from a photoelectric absorption, AuNP localization is less important. For source energies below the k-edge, cellular and even nuclear accumulation of AuNPs would be needed to fully realize the potential for radiosensitization.
5.3 Discussion and future directions

5.3.1 Theory vs. reality

This thesis focused on the physics and radiobiology of AuNPs. The developed ARP model can be utilized to define the optimal combination of source energy, AuNP size, and intracellular localization. However, a successful approach to AuNP radiosensitization must encompass the broader constraints and limitations of such a medical intervention. Pharmacokinetics, biodistribution, cellular uptake, and toxicity are all strongly dependent on AuNP size and surface coating [5-9]. These factors are likely to take precedent in the design of a targeted AuNP radiosensitizer. In a recent study, Chattopadhyay et al found that intravenous injection of HER-2 targeted AuNPs did not improve in vivo tumour uptake of AuNPs. Instead, they reported a high tumour retention and low systemic exposure after direct intratumoural injection of HER-2 targeted AuNPs [8].

The radiation source energy is also governed by clinical constraints. Low-energy brachytherapy is used most often for tumours that are accessible through cavities or through minimally invasive procedures. Higher energy external radiation is often prescribed for deep-seated tumours.

Based on clinical constraints and ARP model predictions, an optimal strategy for AuNP radiosensitization might be in conjunction with permanent seed brachytherapy sources or electronic brachytherapy with average energies below 50 keV. In this approach active cancer cell targeting could greatly enhance cell uptake and retention thereby exploiting the low-energy Auger and photoelectrons. A direct tumoural injection of AuNPs during the brachytherapy procedure could avoid systemic exposure and increase AuNP concentration at the tumour site. In this strategy, AuNP tumour concentrations of 0.2 mg/ml would be expected to have a significant and localized radiosensitizing effect on the tumour.
Current research aims to utilize the unique properties of AuNPs to target cancer cells, deliver therapeutic agents, and radiosensitize the tumour. A novel nanotherapeutic involves radiolabeled AuNPs as a combination unsealed radiation source and radiosensitizer. In a novel pilot study by Dr. Reilly and Dr. Chattopadhyay at the University of Toronto, intratumouraly injected, HER-2-targeted, $^{111}$In-labeled AuNPs showed strong anti-tumour effects with no apparent toxicity in athymic CD-1 mice bearing MDA-MB-361 tumours.

5.3.2 Further validation

The findings of this thesis suggest that the most significant parameters effecting radiosensitization, aside from AuNP concentration, are photon source energy, and AuNP localization. However, the radiobiological experiments conducted in this research did not fully explore these parameters. A relatively narrow range of source energies was used (100 – 300 kVp), and AuNP localization was not purposely varied. In order to comprehensively explore the scope of the ARP model, future experiments could be carried out on a broader range of energies and AuNP localization could be varied through a number of approaches such using AuNP-conjugates to increase cell uptake.

5.3.3 ARP model assumptions and simplifications

The ARP model relies on a number of assumptions and simplifications to provide useful insight into AuNP radiosensitization. These assumptions and simplifications are worthy of discussion and warrant possible future study.

The nucleus is the only critical target

The observed impact of AuNP localization on radiosensitization in the ARP model is based in part on the premise that the cell nucleus is the only radiosensitive region of the cell. While the prevailing
understanding of radiation damage to cells supports the DNA as the critical target [10-13], there are reports of cell death from radiation damage induced to cytoplasmic organelles [14]. Furthermore, the ARP model assumes that the entire nucleus is homogeneously sensitive. This situation may resemble the interphase of the cell cycle, when DNA is dispersed throughout the nucleus. It is not immediately clear how a more accurate account of the DNA configuration would effect the model predictions, but it should be noted that in a recent improvement of the local effect model (LEM) the physical nucleus was replaced by an effective nucleus of about 80% the volume to represent the DNA content. [15].

*Cells are simple concentric spheres*

The ARP model assumed a two-component spherical cell model with a nucleus at the center. While this assumption is somewhat acceptable for some undifferentiated cancer cells, cancer cells can have highly irregular shapes as seen in the TEM images in Chapters 3 and 4. Furthermore, *in vitro* monolayers tend to take on a shape resembling a sunny-side-up egg. The relative location of the nucleus can affect the predicted impact of AuNP localization; if the nucleus is very close to the edge of the cell, AuNP enhancement from outside the cell could be within close proximity. While a spherical cell provides a simple geometry, future studies could incorporate cells with sizes and shape dispersity.

*AuNPs are randomly dispersed within cellular compartments*

The ARP model used experimentally determined intracellular concentrations as an input parameter. However, while TEM images revealed AuNPs to be accumulated in groups within cytoplasmic vesicles, the ARP model assumed AuNPs to be randomly dispersed throughout the cytoplasm. Although it has not been explored in this study, if a large number of AuNPs are clumped together,
there is a possibility of energy reabsorption between AuNPs. AuNP-antibody conjugates may enter cells through alternative processes providing a more homogeneous AuNP distribution [16]. While a random AuNP distribution seems appropriate to make relative comparisons of AuNP radiosensitization, future studies of ARP could examine the effects of a clumped versus random cytoplasmic distribution.

*AuNPs radiosensitization relies only on increased photoelectric absorption*

It is well established that gold and other high-Z material exhibit an increased photoelectric cross-section compared to tissue especially at low energies. This feature provides the basis for high-Z contrast enhanced x-ray imaging [17]. For AuNP radiosensitization, it appears to be the dominant mechanism responsible for radiation modification. For this reason, photoelectric absorptions and photoelectric products were used as the basic input for the ARP model. However, there is also evidence that enhanced electron attenuation plays a role in AuNP radiosensitization [18,19]. Furthermore, in a recent study using an alternative adaptation of the LEM, McMahon postulated that electron attenuation in AuNPs may be responsible for reported AuNP radiosensitization with MV photon sources [20]. The ARP model did not simulate electrons incident on AuNPs. The justification to ignore incident electrons is that at low energies, their interaction with AuNPs results in the production of knock on electrons of similar quality. A future study with AuNP - electron interaction enhancement is warranted.

*AuNPs radiosensitize cells only through radiation damage*

A fundamental premise of the ARP model is that radiation modification is the mechanism of AuNP radiosensitization. The evidence for this mechanism is the strong dependence on source energy observed [19]. While radiation modification is certainly a mechanism, it may not be the only
mechanism behind radiosensitization. An alternative mechanism has been reported involving AuNP regulation of cell cycle [21]. To incorporate such mechanism into the ARP model, additional sensitivity parameters would have to be introduced. It should be noted that while the ARP model assumes radiation modification to be the driving mechanism, secondary effects of radiation such as free radical damage, cellular repair mechanisms, and even the bystander effect are implicitly included in the linear quadratic model, and by extension the ARP model.

*AuNP radiosensitization acts primarily at the nanoscale*

The current understanding of high-LET radiation damage postulates that the increased relative biological effectiveness results from a dense ionization pattern at the nanoscale that creates local multiply damaged sites on DNA [22]. The evidence for this is that high-LET radiation tends to be less dependent on oxygenation, dose rate, cell cycle, and cellular repair mechanisms [23-25]. There is some evidence that multiple distant damages can interact by stressing the cells repair mechanisms, but this relates more to low-LET radiation [26]. In the ARP model AuNP radiosensitization is assumed to act primarily on the scale of 20×20×20 nm voxels. Due to the formulation of the ARP model (equations 3.1 and 3.2), the quadratic component, $\beta$, of the lethal event density contributes only to local energy deposition effects. On the other hand, the linear component, $\alpha$, does not distinguish the spatial pattern of energy deposition. In this sense, the ARP model implicitly allows for distant radiation damage to have an additive effect but not a multiplicative effect.

*Sparsely ionizing photon radiation deposits dose homogeneously*

Both the ARP model and the local effect model attempt to predict the effects of high-LET radiation using low-LET cell sensitivity parameters. Implicit in this approach is the assumption that low-LET sparsely ionizing radiation deposits dose homogeneously [4]. While this is true at macroscopic scale,
evidence suggests that at the scale of DNA, sparsely ionizing radiation deposits energy in quite a heterogeneous fashion [27]. In fact, this realization has lead to a reexamination of the quadratic component of the LQM [28]. Although sparsely ionizing radiation is nonhomogeneous at the nanoscale, the energy deposition is random and featureless, compared to the localized energy deposition pattern around tracks of high-LET radiation and AuNPs. Because the cell sensitivity parameters of LQM are determined using the macroscopic absorbed dose, it is therefore reasonable to assume that the dose was absorbed homogeneously. However it is worth exploring the energy deposition pattern of sparely ionizing radiation in the context of the ARP model. This could provide a more accurate picture of the degree to which AuNPs modify the radiation quality.

The linear quadratic model can be extrapolated to high dose ranges

In order to calculate the lethal event density (equations 3.1 and 3.2), the ARP model must correlate the local energy absorbed to a probability of lethal event. While in theory any survival model could be used, the LQM was chosen due to its simplicity (only two parameters) and predictive capability. The LQM is known to fit survival well for doses in the range of 0-10 Gy. Due to the quadratic term, the LQM continually bends at increasing dose, whereas experimental evidence suggests that survival becomes linear at high doses [29,30]. In the ARP model, local doses could exceed 10,000 Gy and therefore, a threshold dose was introduced to provide reasonable lethal probabilities at these high doses. Due to the difficulty of experimentally determining the threshold dose, future studies of the ARP model could replace the linear quadratic model with alternative more stable models, such as target models [10]. Other nanoscale metrics that could be used to predict survival are ionization density and LET [22,31].
5.4 Concluding remarks

Gold nanoparticles represent an exciting new agent for use in medicine, but in order to translate AuNP radiosensitization to the clinical, extensive multidisciplinary research must be conducted. The goal of this thesis was to investigate the parameters effecting gold nanoparticle radiosensitization with the hope that the methods, findings, and discussion would elucidate some of the mystery behind their effects. The Monte Carlo based radiobiological predictive model developed in thesis provides a framework for continued research into AuNP radiosensitization.
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