UNIVERSITY OF STRATHCLYDE – BIOENGINEERING UNIT

Investigating the Effects of Extra-Corporeal Irradiation on the Mechanical Properties of Bone

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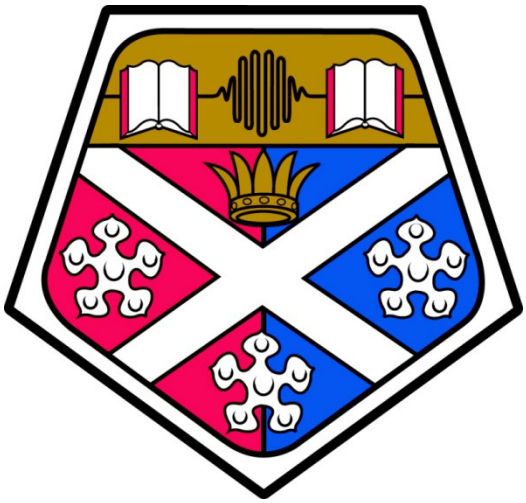

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This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed: Date:

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With utmost gratitude,

Daniel

Table of Contents

List of Tables

List of Figures

List of Equations

Nomenclature

ALP	.....	Alkaline Phosphatase
α-radiation	.....	Radiation type with alpha particles
α-value	.....	Significance factor in Statistics
BMC	.....	Bone Mineral Content
β-radiation	.....	Radiation type with beta particles
˚C	.....	Degrees Celsius
^{60}Co	.....	Cobalt^{60} Ionizing Source
CS	.....	Chondrosarcoma
DMA	.....	Dynamic Mechanical Analysis
DNA	.....	Deoxyribonucleic Acid
DVE	.....	Digital Video Extensometer
D_T	.....	Absorbed Dose in Matter
δ	.....	Overall Phase Lag
δ_1	.....	Stress Phase Lag
δ_2	.....	Strain Lag
E_{DM}	.....	Dynamic Modulus
E_{YM}	.....	Young's Modulus
$E(i\omega)$	.....	Dynamic Modulus (complex number)
E'	.....	Storage Modulus
E"	.....	Loss Modulus
ECI	.....	Extra-Corporeal Irradiation
ES	.....	Ewing's Sarcoma
ε_0	.....	Strain Amplitude
$\varepsilon(t)$	.....	Instantaneous Strain
Gy	.....	Gray
γ-radiation	.....	Radiation type with gamma particles
HPV	.....	Human Papillomavirus
H_T	.....	Absorbed Dose in Biological Matter
Hz	.....	Hertz (frequency)
i	.....	Imaginary Number
J(t)	.....	Creep Compliance Modulus
K_c	.....	Fracture Toughness Factor
LDH	.....	Lactate Dehydrogenase

MeV	.....	Mega Electronvolt
MS	.....	Muffucci Syndrome
MU	.....	Machine Units
MV	.....	Megavolts
N	.....	Newton
n	.....	Number of Specimens
n_{TOT}	.....	Total Number of Specimens
OD	.....	Ollier's Disease
OS	.....	Osteosarcoma
Pa	.....	Pascal
PD	.....	Paget's Disease
PMMA	.....	Poly(methyl) methacrylate (cement)
PTH	.....	Parathyroid Hormone
R^2	.....	Coefficient of Determination
ROS	.....	Reactive Oxygen Species
rad	.....	Rad (Unit of US Radiation Dose)
Sv	.....	Sievert
SD	.....	Standard Deviation
σ_{UTS}	.....	Ultimate Tensile Strength
σ_y	.....	Yield Strength
σ_o	.....	Stress Amplitude
$\sigma(t)$	.....	Instantaneous Stress
t	.....	Time
$\tan\delta$	.....	Dissipation Factor (Loss Tangent)
UV	.....	Ultraviolet
υ	.....	Poisson's Ratio
W_R	.....	Radiation Weighting Factor
W_T	.....	Tissue Weighting Factor
X	.....	Radiation type with X-ray particles
Y(t)	.....	Stress Relaxation Modulus

Abstract

This project investigated the influence of Extra-Corporeal Irradiation on the elastic and viscoelastic properties of bone. Bone specimens were extracted from mature cattle and subdivided into thirteen groups, including a control group with twelve groups exposed to increasing levels of irradiation. The specimens, once irradiated, underwent mechanical testing in saline at 37˚C.

Mechanical Properties were calculated by experimental means which included Young's Modulus, Poisson's Ratio, Dissipation Factor and Dynamic Modulus. These were all obtained for the comparison to the control group to the irradiated specimens.

From the results calculated in the project, it was shown that the overall effect of increasing irradiation doses up to 300Gy seems to present negligible change, albeit negative, in the behavior of bone. However, the increase in Poisson's Ratio post ECI treatment was statistically significant in relation to the dose administered to the specimen through ANOVA testing. Therefore, it is concluded that the effect that high levels of Extra-Corporeal Irradiation (300Gy) is minute, and should be administered to reduce the chances of possible recurrence of cancer.

1. Introduction

1.1 - Background

Bone is a key component in our musculoskeletal system. It serves a myriad of vital purposes for every individual including protection of organs, support for posture and storage of minerals needed for homeostasis. It is a natural composite, made up of an organic (collagen type-1) and inorganic (hydroxyapatite) phase, each contributing to the mechanical properties of bone.

Bone is a living organ within the human body, meaning that it comprises of cells which control and maintain its microscopic structure. Due to the cellular nature of all tissues and subsequently all organs, there is the possibility that these tissues can develop tumours, due to a variety of causes, both genetic and environmental.

The treatment of bone sarcomas is an inexact science. There are many forms of treatment that has been utilised in attempts to save the life of the patient. The most common form of treatment is pre and post chemotherapy with surgical excision of the cancerous lesion. However, certain bones within the skeleton do not respond well to chemotherapy, in particular the pelvis. This can lead to development of metastases both locally and systematically and would severely endanger the longevity of the patient.

Because of the poor prognoses of pelvic sarcoma patients, other treatments are available. Extra-Corporeal Irradiation is one of the treatment methods which have had excellent short-term results (1) (2). This method involves extracting either a portion or the whole bone, surgically removing the lesion, irradiating the remainder of the bone at a predetermined dosage (usually around the 50Gy mark), and reimplanting the dead autograft back into the patient. Due to the graft being the patient's own, there is no immunological response by the body, reducing complications and possible revisions. Furthermore, the patient is mobile within a week of the surgery.

Although this method of treatment has had excellent short-term results, there is no consensus on the level of radiation to be administered to the graft. Some physicians have known to go to radiation levels of 300Gy to be certain all tumour cells have been destroyed, while studies show that 50Gy is more than adequate to kill all malignant cells within the autograft (3).

1.2 - Scope of Project

It has been hypothesised that increasing the dosage of radiation when treating the autograft may have adverse affects on the collagenous phase found within osseous tissue, causing adverse changes in the mechanical properties (elastic and viscoelastic) of bone. Furthermore, increasing the dosage administered to the autograft could delay the reincorporation of the autograft due to the destruction of all cells within the bone.

The aim of this project is to quantify the damage caused by the radiation treatment on the collagenous matrix by testing specimens irradiated in increasing doses to determine the elastic and viscoelastic properties of bone. The irradiated specimens will be compared to a control group to observe any changes in the behaviour of bone.

2. Literature Review

2.1 - Musculoskeletal System & Bone Overview

The main component of the skeletal system is bone, which is aided by cartilage, ligaments, tendons and other connective tissues. The skeletal system is rudimentary in our daily lives, and bears important functions. These include physical functions like the maintenance of posture, protection of otherwise vulnerable internal organs and acting as levers in the body to create forces necessary for movement.

The skeletal system is also responsible for storage of important minerals for biological purposes, and production of different types of blood cells. In an average human adult, there are 206 bones present, although there can be discrepancies with this number due to fusion of separate bones together, seen mainly in the spine.

Bone is considered to be both an organ and a tissue. To avoid confusion, Bone (organ) is split into two separate tissues, cortical bone and cancellous bone. These two types of tissue can be distinguished both physically and mechanically. Bone accounts for approximately 20% of body weight of an average human.

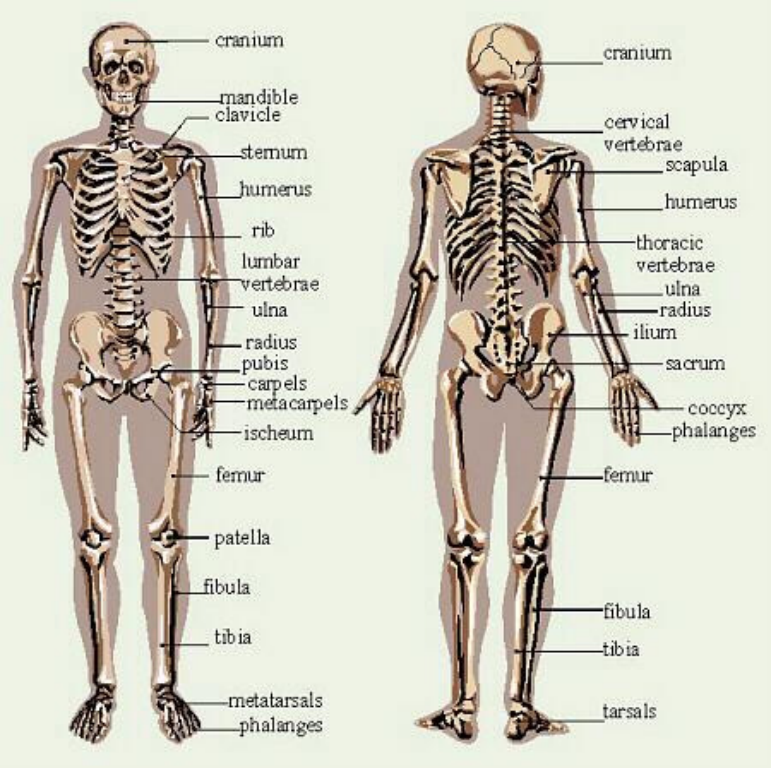

Figure 2-1 - Diagram of Human Skeletal System

2.2 - Composition of Bone

Bone is made from two key osseous tissues, cortical bone and cancellous bone. Both tissues act together to maximise the strength to weight ratio. Wolff's Law explains the shape and orientation of structures within bone optimally resist physiological loading. This explains structures found within bone such as the calcar in the femur that allows transfer of the loads from the femoral head via the distal femur to the ground.

2.2.1 Cortical Bone

Cortical bone is a hard mineralised osseous tissue. It is found on the surface of the bone, forming the cortex. Osseous material is technically a composite, consisting of two components which acts together to give bone its mechanical properties.

- The first component is Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which is the hard mineralised phase of bone. It is classified as the inorganic phase of bone. The main function of this constituent is to resist the high compressive stresses experienced by bones in routine activities (4).
- The second component of bone is Type-I Collagen. This is the organic phase of bone. Collagen has been described as the structural protein in vertebral species, being the primary protein found in the matrix (85-90%). The main function of the organic phase of bone is to resist the propagation of cracks and it contributes to the viscoelastic behaviour of bone (4).

Bone formation begins with the laying down of collagen that acts as a blueprint of the matrix. This is known as osteoid, a product formed by cells which inhabit bone known as osteoblasts. Soon, mineral is placed in the regions of the matrix where collagen fibrils terminate before another fibril begins (gap zones). The mineral is then deposited along the entire length of the fibril. The mineral tends to grow, often in the direction that the collagen is orientated in. After this, mineralisation occurs in between each neighbouring fibril to give adequate strength to the matrix (5).

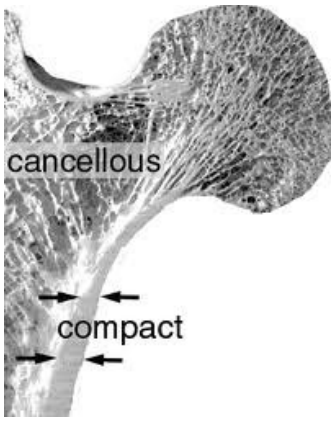

Figure 2-2 - Section through the Proximal Femur

With these two constituents acting together, bone is able to resist a variety of stress modes. When mineralisation occurs within the matrix after the osteoid is laid down by the osteoblasts, granules of hydroxyapatite begin to grow and "consume" smaller grains, up to 40nm in diameter. However, the depth of a crystal remains small due to underlying complete grains. The thicknesses of the crystals are approximately 5nm (5).

As cortical bone has a higher degree of mineralisation than cancellous bone, cortical bone will be denser than cancellous bone. A common consensus is that a bone of greater than 0.7kg/m^3 is considered cortical bone, whilst less than 0.7kg/m^3 is considered cancellous (5).

2.2.2 Cancellous Bone

Cancellous bone is less mineralised than compact bone, and forms in a honeycomb-like structure. It can be found in the ends of long bones and has two distinct functions. It firstly allows capillaries to flow easily through the porous structure, as well as accommodating bone marrow, which is essential for blood cell production. Secondly, it is considered that the small trabeculae act like support struts, allowing bones to be relatively lightweight whilst retaining their strength, which in turn reduces the overall energy expense of the individual during movement.

Currey defines cancellous bone into three distinct variations; its fine-scale structure, its large-scale structure and its porosity (5). More often than not, trabeculae are made from lamellar bone, not woven bone (See Section 2.3). Trabeculae have a mean diameter of 0.1mm, with a high level of anisotropy. As shown in Figure 2-2, these cancellous trabeculae appear to have a random orientation. The struts which have no preferred orientation are usually found deep within the bone, far from load-bearing surfaces. The more orientated struts occur near load-bearing surfaces. The porosity of bone is important in defining the tissue. If the porosity level is below 50%, it becomes challenging to identify cancellous instead of porous compact bone (5).

2.3 Structure

The internal structure of bone characterises its behaviour. Cortical and cancellous bone is constructed in one of two ways. It is either constructed with woven bone, seen in embryonic development as well as fracture healing, or else it is constructed in a lamellar structure (concentric rings of osseous tissue). Lamellar bone is created during growth of an individual, and has far superior mechanical properties to woven bone, mainly due to collagen alignment and a greater density of osteocytes, which has been proven to aid in the remodelling process seen in bone after microdamage due to physiological loading (6).

Within cortical bone there are canaliculi which run along the longitudinal axis of the bone, as well as perpendicularly. The longitudinal canaliculi are called Haversian canals, and these are inhabited by blood vessels, nerves and lymphatic vessels. The perpendicular canals are called Volkmann's canals, and serve the same purpose as the Haversian canals. Surrounding the Haversian canal are concentric rings of osseous matrix, with cement lines between neighbouring layers. Also in the concentric rings are osteocytes, located in small depressions (lacunae) which are interconnected together via their dendritic processes as seen in Figure 2-3. The overall structure is known as an Osteon. These Osteons are generally 0.2mm in diameter, and run in the direction of the long axis of the bone. A typical long bone within the body will have about 65% inorganic materials within its matrix, with the rest organic (mainly collagenous). The collagen aligns in the radial direction of the lamellae and spirals along the longitudinal axis. Necrosis and/or apoptosis of these cells cause degradation of the osseous tissue within the vicinity of an osteocyte (6).

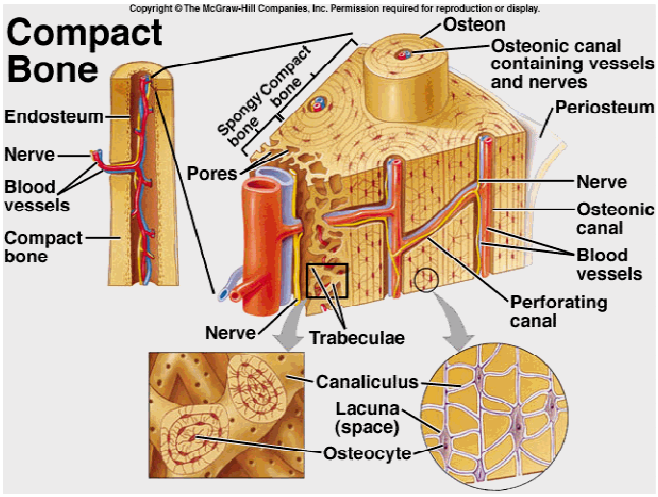

Figure2-3 – Structure of a Typical Long Bone

2.4 Mechanical Properties of Bone

Due to bone being a natural composite anisotropic material, the mechanical properties differ in relation to the anatomical position of compact bone within a bone, the orientation of the specimen, the species of bone, as well as the mineral content. The following sections try to quantify these properties in relation to the stated determining factors.

2.4.1 Ultimate and Yield Stress

The strength of a material can be measured in one of two ways. The yield stress is where the bone undergoes no plastic deformation and the ultimate stress of the material is where it reaches the maximum strength before it begins to fail. The strength of a material describes the ability of a material to withstand an applied stress without failing. These stresses applied to the material can come in a variety of modes, including compression, tension, torsion and shear. As bone is a natural composite, the different components act together to withstand these stresses as explained previously. Due to this, there are a wide range of values within the literature, and the table below shows some of these values.

	Tissue Type	Species	Loading Orientation	Testing Method	Conditions	Stress
Martin et al [1998] (7)	Cortical	Human	Longitudinal	Tensile	Wet	133MPa (Ultimate)
	Cortical	Bovine	Longitudinal	Tensile	Wet	156MPa (Ultimate)
	Cortical	Human	Longitudinal	Tensile	Wet	115MPa (Yield)
	Cortical	Bovine	Longitudinal	Tensile	Wet	141MPa (Yield)
	Cortical	Human	Transverse	Tensile	Wet	51MPa (Ultimate)
	Cortical	Bovine	Transverse	Tensile	Wet	50MPa (Ultimate)
	Cortical	Human	Longitudinal	Compressive	Wet	182MPa (Yield)
	Cortical	Bovine	Longitudinal	Compressive	Wet	196MPa (Yield)
	Cortical	Human	Transverse	Compressive	Wet	121MPa (Yield)
	Cortical	Bovine	Transverse	Compressive	Wet	150MPa (Yield)
	Cortical	Human	Longitudinal	Compressive	Wet	195MPa (Ultimate)
	Cortical	Bovine	Longitudinal	Compressive	Wet	237MPa (Ultimate
	Cortical	Human	Transverse	Compressive	Wet	133MPa (Ultimate)
	Cortical	Bovine	Transverse	Compressive	Wet	178MPa (Ultimate)

Table 2.4:1 – Experimental Values of Cortical Bone Strength

2.4.2 Stiffness

Due to the inhomogeneity of bone, there is a large variation in the Young's Modulus in both cortical and cancellous bone. Found below are values extracted from the literature;

	Tissue Type	Species	Loading Orientation	Testing Method	Conditions	Young's Modulus
Rho et al (8)	Cortical	Human Tibia	N/A	Tensile	Wet	20.7GPa
	Cortical	Human Tibia	N/A	Tensile	Dry	18.6GPa
Schaffler & Burr (9)	Cortical	Bovine Femur	Longitudinal	Compressive	Wet	22.1GPa
	Cortical	Bovine Tibia	Longitudinal	Compressive	Wet	21.4GPa
Bonfield & Tully (10)	Combined	Bovine Femur	Longitudinal	Compressive	Wet (17˚C)	23.5GPa
	Combined	Bovine Femur	Longitudinal	Compressive	Wet (41˚C)	21.8GPa
Reilly & Burnstein (11)	Cortical	Human	Radial	Tensile	N/A	12.8GPa
	Cortical	Human	Radial	Compressive	N/A	11.7GPa
	Cortical	Human	Longitudinal	Tensile	N/A	17.7GPa
	Cortical	Human	Longitudinal	Compressive	N/A	18.2GPa
	Cortical	Bovine	Longitudinal	Tensile	N/A	23.1GPa
	Cortical	Bovine	Longitudinal	Compressive	N/A	22.3GPa
Martin et al (7)	Cortical	Human	Transverse	Compressive	Wet	17.4GPa
	Cortical	Human	Longitudinal	Compressive	Wet	9.6GPa
	Cortical	Bovine	Transverse	Compressive	Wet	20.4GPa
	Cortical	Bovine	Longitudinal	Compressive	Wet	11.7GPa

Table 2.4:2 - Experimental Values of Stiffness for Bone

It is clearly seen in the table above that all the factors discussed have an effect in the recording of the mechanical properties. It was shown by Rho et al that testing bone in "wet" conditions shows a significant increase in stiffness than "dry" conditions (8). Furthermore, Bonfield & Tully show clearly that temperature also has a significant effect on the stiffness, showing creep is active in bone (10).

2.4.3 Toughness

Toughness is defined as the ability to resist the development and propagation of cracks in a material under stress. In any material, there is a trade off between stiffness, strength and toughness. Due to the large percentage of hydroxyapatite within osseous tissue, the toughness of bone is often described as the "weak-link". As a result, bone will usually fail due to fracture (excessive crack propagation), rather than yielding.

	Tissue Type	Species	Loading Orientation	Testing Method	Conditions	Toughness
Norman et al (12)	Cortical	Bovine	Longitudinal	Tensile	2mmCrack	6.29MPa$\sqrt{m}$
	Cortical	Bovine	Longitudinal	Tensile	3mm Crack	6.73MPa$\sqrt{m}$
	Cortical	Human	Longitudinal	Tensile	2mmCrack	4.32MPa$\sqrt{m}$
	Cortical	Human	Longitudinal	Tensile	3mm Crack	4.05MPa$\sqrt{m}$
Behiri & Bonfield (13)	Cortical	Bovine Tibia	Longitudinal	Tensile	1mm Crack	3.2MPa$\sqrt{m}$
	Cortical	Bovine Tibia	Transverse	Tensile	1mm Crack	6.5MPa$\sqrt{m}$
Wright & Hayes (14)	Cortical	Bovine Femur	Longitudinal	Tensile	N/A	3.04 - 3.95MPa$\sqrt{m}$
Moyle & Gravens (15)	Cortical	Bovine Tibia	Transverse	3-Point Bending	Wet	11.2MPa$\sqrt{m}$

Table 2.4:3 - Experimental Values of Cortical Bone Toughness

In the testing of fracture toughness, there is a variety of techniques which can be employed to quantify the K_c factor, including compressive, tensile, 3 and 4-point bending testing. Also the length of the original induced crack, the thickness and the orientation of the specimen is vitally important when analysing the outcome of the results.

2.4.4 Poisson's Ratio

Poisson's Ratio (υ) is an important parameter in defining the behaviour of materials as they elastically and plastically deform, and usually has a value of between 0-0.5. In regards to tensile testing, Poisson's Ratio relates the reduction in cross-sectional area with the extension seen in the axial direction. As the ratio increases towards 0.5, the material is said to become increasingly incompressible. The following table shows experimental data from studies performed on cortical bone with different testing methods and measuring techniques (5) (16).

	$\upsilon_{axial\text{-}transverse}$	±1 SD	$\upsilon_{axial\text{-}radial}$	±1 SD	Testing Method
Canine	0.325	N/A	0.3	N/A	Ultrasound
Bovine	0.29	0.08	N/A	N/A	Tensile
	0.4	0.21	N/A	N/A	Compression
Human	0.41	0.15	N/A	N/A	Tensile
	0.38	0.15	N/A	N/A	Compression
	0.295	N/A	0.285	N/A	Ultrasound
Equine	0.19	0.04	N/A	N/A	Compression

Table 2.4:4 – Experimental Values of Cortical Bone Poisson's Ratio

2.4.5 Viscoelasticity

Due to bone being a natural composite, it displays a behaviour known as viscoelasticity, including stress relaxation, creep and hysteresis. The stress in bone will be dependent on both the strain and also the time history of the strain. Creep occurs when a stress is applied to a material, and the material continues to deform gradually with increasing strain. Creep is quantified by a variable known as the Creep Compliance, J(t). Stress Relaxation is where a strain is applied to the specimen, and the stress induced by the strain gradually decreases until it reaches an equilibrium value. Relaxation is quantified in a parameter known as the Stress Relaxation Modulus, Y(t). This property comes from the fact that the hydroxyapatite constituent acts as an elastic material, having a constant stiffness.

When cyclic loading is applied, hysteresis occurs. This is where there exists a certain phase lag between the applied stress and the resulting strain, and is commonly known as hysteresis. When carrying out dynamic mechanical analysis (DMA), a purely elastic material will display no phase lag, whilst a purely viscous material will display a 90˚ phase lag in strain. Bone, being viscoelastic will demonstrate a certain lag in strain. The overall result is that the behaviour of the composite of bone is considered nonlinear.

Viscoelasticity can be measured by the Dynamic Modulus of a material. This comprises of a Storage Modulus and a Loss Modulus. The Storage Modulus [E$^{'}$] represents the elastic portion of bone by measuring the stored energy in the specimen, whilst the Loss Modulus [E$^{''}$] represents the viscous portion of bone by measuring the energy dissipated by the specimen. As bone is almost completely solid, the Storage Modulus should be far larger than the Loss Modulus.

$$E(i\omega) = E' + iE''$$

Equation 2-1 – Dynamic Modulus

$$E' = \frac{\sigma_o}{\varepsilon_o}\cos\delta \;\; and \; E'' = \frac{\sigma_o}{\varepsilon_o}\sin\delta$$

Equation 2-2 – Storage and Loss Modulii

σ_o is the amplitude of the stress, ε_o is the amplitude of the strain and δ is the phase shift between stress and strain.

It has been shown that both of these properties rely heavily on the bone mineral content (BMC). As the BMC decreases, so does the Storage and Loss Modulii while the Loss Factor [tan δ] which measures the inherent dissipation of energy from the material increases (4).

It has been proposed that there were numerous physical processes occurring in bone which leads to viscoelasticity (17). Some of these processes are listed below;

- Thermoelastic Coupling
- Piezoelastic Coupling
- The Motion of Fluid in the Canal Within Bone
- Inhomogeneous Deformations in Osteons
- Cement Lines and Lamellae
- Interstitium and Fibres
- Molecular Modes in Collagen

The critical parameters of these tests are firstly the size of the specimen, and secondly the strain rate initially placed upon the specimen to reach to desired strain. Although few studies have researched this area of viscoelasticity in bone, the response of bone when subjected to these large strain rates. Table 2.4:5 below shows the response of bone after being strained to a certain level.

	Species	Loading Dir.	Testing Method	Initial Displacement	Initial Stress	Stress Relaxation (After Period)
Abdel-Wahals et al (18)	Bovine Femur	Long.	Tensile	0.15mm	21.2MPa	-17.6% (1800s)
	Bovine Femur	Long.	Tensile	0.2mm	25.6MPa	-13.4% (1800s)
	Bovine Femur	Long.	Tensile	0.255mm	29.7MPa	-14.7% (1800s)

Table 2.4:5 - Experimental Values of Stress-Relaxation of Cortical Bone

In an individual's routine life, physiological strain rates on bone are between $0.005s^{-1} – 0.08s^{-1}$ (19). As a specimen of bone is held in the straining stage; damage accumulates in bone as the residual strains increase in prolonged circumstances. This damage can be calculated by quantifying crack density (20).

The level of water content within the specimen has a direct effect on the viscoelastic behaviour according to Sasaki & Enyo (21). For this reason, nearly all testing of bone specimens are performed to mimic in-vivo conditions, usually in heated water baths. This is also important when producing the specimens in a laboratory environment; all specimens should be wrapped in saline soaked gauze before undergoing freezing to avoid mineral leaching.

2.5 Cells within Bone

Bone is a cellular tissue, with a variety of cells whose functions vary to achieve the formation and maintenance of the integrity of bone.

2.5.1 Osteocytes

Osteocytes are the main cell found in bone. They have a density of approximately 30,000 per mm^3 in bovine bone. According to Mullender et al the larger the animal, the smaller the density of osteocytes found within the bone (22). At the start of their life, these cells are osteoblasts. As the osteoblasts secrete osteoid to form the collagenous matrix, some of them are encased in the very matrix they helped create. Just before they are completely sealed into the matrix, the osteoblasts "reach out" with the aid of their dendritic processes to other similar osteoblasts. These processes inhabit minute channels (0.2μm) within the matrix, known as canaliculi. The result is a network of cells which has been argued by the scientific community to help the bone detect large regions of damage. Soon these encased osteoblasts lose many of their organelles for secreting osteoid, and differentiate into osteocytes.

It has been argued that a decrease in osteocytes leads to demineralisation of the bone, affecting its integrity and subsequently affecting its mechanical properties. Hypermineralisation can also cause the destruction of lacunae within bone, which results in a lower density of osteocytes, therefore causing bone brittleness (23). Nijweide et al propose that "*Osteocytes are mechanosensory cells of bone and play a pivotal role in functional adaptation of bone*" (6). This fact becomes increasingly important when considering the affects of Extra-Corporeal Irradiation on the dead autograft's elastic and viscoelastic properties, as will be discussed later.

2.5.2 Osteoblasts

Osteoblasts are cells derived from mesenchymal precursor cells, also known as osteoprogenitor cells, which cover the surface of all bones in a region known as the endosteum (below the periosteum). Osteoblasts are unable to divide to create new cells. Osteoblasts are responsible for creating new bone tissue. Osteoblast activity is triggered by the presence of Parathyroid Hormone (PTH). They lay down a product called osteoid, comprised of specific proteins, mainly type-I collagen which forms a collagenous matrix. There are a number of diseases caused by the abnormal activity of osteoblasts. Osteomalacia is caused by the increase accumulation of type-I collagen. The result is known as rickets,

where the bones soften and bend (24). The most likely cause of this disease is a lack of Vitamin D. Age also has an effect on osteoblasts. It has been shown in the literature that the density of osteoblasts decreases with increasing age (25).

2.5.3 Osteoclasts

Osteoclasts are responsible for the resorption of old damaged or redundant bone. The interaction of osteoclasts and osteoblasts is known as the remodelling process. Unlike the previous two types of osseous tissue cells, osteoclasts are not related to mesenchymal precursor cells. There are derived from the macrophage family, and are multinucleated (approximately 10 nuclei). These cells are quite large (approximately 40μm in diameter), and are extremely mobile. However, these cells are quite rare, with only 2-3 osteoclasts per mm^2, and are usually found in regions known as Howship's Lunacae (26). The cells will arrive at an area of damage, and begin to secret enzymes which "digest" the underlying bone. Both organic and inorganic debris from the site is collected by the osteoclast in vesicles. These vesicles are then expelled into the interstitial space (5).

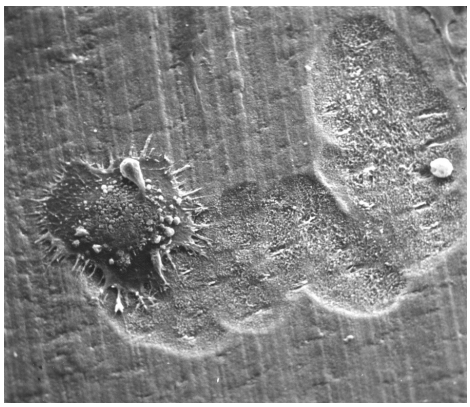

Figure 2-4 - SEM Picture of an Osteoblast moving across Bone whilst Resorbing the Organic Content (96)

Like osteoblasts, osteoclasts which display abnormal activity patterns can be linked to various diseases. If there is a decrease in osteoclast activity, osteosclerosis occurs, where excess bone is formed, causing a lower range of movement for the individual. On the other hand, an increase in osteoclast activity will result in osteoporosis, where the entire structure of bone is weakened. Osteoporosis is far more prevalent in females due to menopause; with figures of 1 in 3 women will develop osteoporosis.

The disruptions to these cells are caused due to a variety of factors. One example of how these imbalances occur is the abnormal activities of cancerous cells. These diseases can often signify larger problems, and are indications to physicians that the normal balance of bone turnover has been compromised.

2.6 Development of Sarcomas

With any biological tissue within the body, there is a risk of cells becoming cancerous due to a variety of reasons. Cancer is a disease caused by genetic mutations of cell's DNA, which cause disruption to cell growth and division. The genes that cause initial mutations are called oncogenes, and these in turn cause the formation of tumours, either benign or malignant. The difference between these two forms of tumours is that a benign tumour will remain within the epithelium or connective tissue capsule, whilst a malignant tumour will migrate to other tissues (usually via blood or lymphatic vessels) causing these cells to also develop oncogenes, resulting in secondary tumours known as metastases (27). Sarcomas can be either primary or secondary tumours. Cells affected by cancer soon begin to either lose or alter their intended functions.

Bone tumours can be subdivided into three categories; malignant, locally aggressive and benign. Shown below are a number of forms of each sub-category. In this report, the most common malignant tumours (seen in **bold**) will be discussed in Section 2.8.

Malignant	Locally Aggressive	Benign
Osteosarcoma	Giant Cell Tumour	Osteoma
Ewing's Sarcoma	Desmoplastic Fibroma	Osteoblastoma
Chrondrosarcoma	Haemangioendothelioma	Osteofibrous Dysplasia
Chondroma		Benign Fibrous Histiocytoma
Malignant Fibrous Histiocytoma		

Table 2.6:1 - Malignant, Locally Aggressive and Benign Bone Tumours

The development of sarcomas, especially the tumours that develop in bone are quite rare. Soft tissue tumours are ten-fold more likely to occur within the human population than bone tumours (28). It has been recorded that worldwide, bone tumours account for approximately 0.2% of all malignant tumours (29) (30).

Usually bone cancers are secondary tumours, most commonly derived from primary tumours of the lung, breast or prostate (28) (31). It has been shown that there are a number of factors that influence the preference of regions affected by metastases in patients, including diagnosis time from formation of tumour, age, and environmental factors. It has been reported by Kanis that there is a 70% preference of breast cancer to spread into bone compared to only 5% for gastric cancers (32).

According to research, there are two peak levels when there is an increased chance of developing bone sarcomas, being in the second and seventh decade (see Figure 2-5) (28) (33). Bone sarcomas are more common in men than women (33). However, when viewing the statistics of childhood cancers, bone sarcomas account for 4% in the UK. The main forms of bone sarcoma in children are Osteosarcoma (53%) and Ewing's Sarcoma (42%). In both forms, the main region of development is in the long bones of the leg, accounting for 84% and 38% respectively. In 2008, CancerResearchUK published that there were 2229 new cases of bone and connective tissue cancers, with 1053 patients died as a result of the disease.

In adults, Osteosarcoma is also the most frequently diagnosed, being 35.1% of total cases of bone cancers. Chondrosarcoma is 25.8%, Ewing's Sarcoma is 16%, Chondroma is 8.4% and Malignant Fibrous Histiocytoma is 5.6%, according to Dorfman & Czerniak (29).

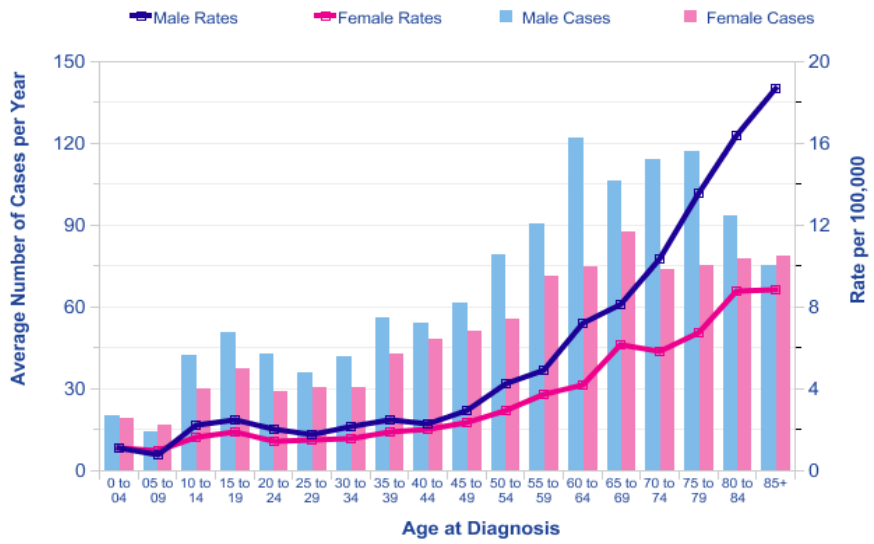

Figure 2-5 – Incidences of Bone or Connective Tissue Cancers in Relation to Age and Gender (30)

2.7 Factors Influencing Cancer in Bone

There are a range of factors which can cause the initiation of cancer in bone. Simcock & Malcolm list some reasons, including environmental carcinogens, viruses and genetics (28).

2.7.1 Environmental Carcinogens

2.7.1.1 Ionizing Radiation

Ionizing radiation is proven to be a cause of carcinogenesis in human populations. It is estimated that radiation (both ionizing and non-ionizing) accounts for 10% of environmental carcinogens. Studies performed on this subject researched populations of people with exposure to radioactive materials, such as radium dial painters and uranium miners. It is difficult to establish a difference at either the cellular or tissue level of tumours from spontaneously formed cancers. There is no specific mutation that occurs after ionizing radiation exposure, unlike UV radiation cancers. Ionizing radiation has been labelled as both an initiator and promoter of carcinogenesis (34). There is a debate as to how ionizing radiation causes cancer, with some hypothesising that either it induces mutations in key oncogenes and inhibits tumour suppressors or that it causes genetic instability with the development of free radicals.

In the case of bone, it is considered a resilient tissue when describing the sensitivity of tissues in the body to ionizing radiation. The table below shows the relative ionizing radiation sensitivity of tissues (35).

Sensitive	Moderate	Resistant
Thyroid	Lung	Kidney
Breast	Colon	**Bone**
Blood-Forming Tissues	Liver	Skin
	Pancreas	Salivary Gland
	Lymphatic System	Brain

Table 2.7:1 - Relative Ionizing Radiation Sensitivity of Tissues

The main bone tumour caused by excessive dosages of ionizing radiation is Osteosarcoma. The radiation causes cells to malfunction within the matrix of bone, which creates irregular osteoid, weakening the overall matrix and cause enlargement of the bone.

2.7.1.2 Diet and Lifestyle

It has been shown that our diets and lifestyles are major factors in determining the likelihood of developing cancer. Poor diet (and subsequent obesity) accounts for over 30% of cancer initiation, while tobacco smoke is 25%. Tobacco has been shown to cause 9 out of 10 lung cancers (36). Lack of exercise has been shown to reduce the effectiveness of an individual's immune system, therefore increasing chances of carcinogenesis. It has also been shown that populations which exercise regularly have lower levels of suspected carcinogenic hormones (37). Stress and pollutants are also contributors to cancer development (38).

2.7.1.3 Infections (Viruses & Bacteria)

Infection is second only to smoking for the leading causes of malignancies, and viruses account for approximately two-thirds of this number. Up to 26% of cancer cases were attributed to viral infections in developing countries (39). Viruses have been known to cause many cancers in the human population. The three cancers which are most generally caused by infections are stomach cancer (H.pylori bacteria), liver cancer (Hepatitis B & C viruses) and cervical cancers (Human Papillomaviruses) (40).

In relation to bone cancers, only one viral agent has been taken from a naturally occurring bone tumour, known as FBJ viral agent (named after the discoverers Finkel, Biskis & Jenkins). It has been shown to induce Osteosarcoma in rodents. The FBJ viral agent is related to a proto-oncogene (c-Fos), which has been associated with poor responses to chemotherapy in Osteosarcoma patients.

2.7.2 Genetics

Genetics accounts for up to 10% of cancers reported. With genetic cancers, the individual has an inherited gene mutation in all cells. For this reason, these tend to be more serious than acquired gene mutations. These abnormal cells can affect the cell in one of two ways;

- If the abnormal gene is an oncogene, this causes unwanted activation of the gene, resulting in unnatural activity which will lead to cancer development
- If the gene is an abnormal tumour-suppressor gene, the cell cannot repair errors in the transcription and translation stages of DNA replication, or halt cell division.

There are two classes of inherited variations in genes; high-risk mutations and low-risk genetic polymorphisms. High-risk mutations generally affect the tumour suppressor genes as stated above. These mutations have a 50% chance of being passed to offspring of the individual, explaining why many of these cancers occur in the early stages of life (41). Some of the following diseases are a direct result of inherited gene mutations:

Disease	Cancers	Gene Affected
Li-Fraumeni	Early onset of Breast Cancer Childhood Sarcomas	TP53
Neurofibromatosis Type-1	Brain Tumours and Sarcomas	NF1
Retinoblastoma*	Retina	RB1
Xeroderma Pigmentosum	Skin	XPA-XPG

Table 2.7:2 – Examples of High-Risk Mutation Cancers and Affected Genes

Low-risk genetic polymorphisms exist due to the combined effects of many gene variants. Three of the most common cancers that are caused by low-risk polymorphisms are breast, bowel and testicular cancers (42).

* Retinoblastoma is a cancer which greatly increases the chances of developing Osteosarcoma in later life and is discussed in Section 2.8.

2.7.3 Effects on Bone due to Carcinogenesis

Due to the presence of tumours in the body, there are both local and systematic effects experienced by bone. These effects are better described as bone diseases, which are either osteolytic or osteosclerotic. Osteolysis is the ongoing resorption of bone by osteoclasts, whilst osteosclerosis is the creating of excessive amounts of osteoid which will mineralise into bone. There are three ways in which these two bone disorders can occur: increased turnover, imbalance and uncoupling.

Increased turnover is the increased rate of both resorption by the osteoclasts and the formation of bone by osteoblasts. These turnovers can reach 5-fold the normal level of the bone remodelling process. It has been recorded that a 5-fold increase in turnover will cause a 20% reduction in the mass of cancellous bone (32).

Imbalance is when the volume of bone resorbed does not equal the volume of bone formed. It has been seen that with large solid tumours, there is increased resorption of bone due to a reduction in Parathyroid Hormone or Calcitrol levels. Also cytokines and interleukins are responsible of suppressing osteoblast activity. However, with the use of chemotherapy to treat cancer, levels of Calcitrol and Alkaline Phosphatase (ALP) are increased, which leads to an activation of osteoblasts, according to Kanis (32).

Uncoupling is similar to imbalance, except that the resorption and formation of bone takes place in separate regions of the bone, rather than in one specific area. In some instances, these cells accelerate skeletal loss, causing "*a destruction of bone architecture*" (32). Many cancers release a chemical known as osteoclast-activating factor (27) which results in osteoporosis. In other instances, there will be excessive deposition on the surface of the bone or in the intramedullary cavity of the bone resulting in the development of osteosclerosis.

These abnormalities are seen most readily in aggressive malignant bone sarcomas. In the following sections, the three most common bone malignancies are discussed in depth.

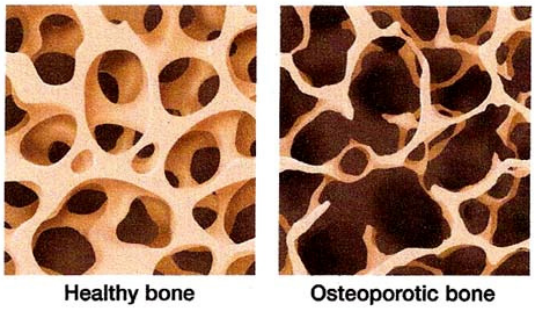

Figure 2-6 – Morphological Variation between Healthy and Osteoporotic Bone

2.8 Common Malignant Bone Sarcomas

2.8.1 Osteosarcoma

2.8.1.1 Epidemiology

Osteosarcoma is the most common form of bone tumour, accounting for 25% of malignant bone tumours and 33% of all bone tumours. It is defined as a malignant mesenchymal neoplasm (33). OS has a cumulative survival rate of 55%, taking account of all forms of the disease. It affects males to females in a ratio of 1.5:1 (43). In the UK, there are 150 newly diagnosed patients suffering from OS annually.

Literature reports clearly that up to 70% of instances of OS occur around the knee joint (distal posterior femur and proximal tibia) (43) (44). Other, less common areas where OS affects is the proximal humerus and the pelvis. OS has two peaks in the prevalence according to age; patients between the ages of 10 to 25 years old attribute to 75% of all instances and ages above 50 provide the other peak. In the younger patients, OS tumours are usually located in the regions of greatest growth, near the epiphyseal (growth) plates.

2.8.1.2 Aetiology

It is unclear how the development of OS is initiated. One theory states that the stresses applied to osteoblasts dividing within the bone matrix could cause DNA mutations, resulting in cancerous oncogene development (43). There are also genetic conditions which are said to give rise to OS. One such condition is retinoblastoma, a cancer affecting the retina cells of the eye. This disease is usually seen in children, and there is a 300-fold chance of developing OS compared to the unaffected population, bringing the risk of developing OS up to 35% during the individual's lifetime (43).

Another condition that can lead to OS is Paget's Disease (PD). PD is a disease which weakens the bone due to excessive turnover. It can cause enlargement that result in misshapen bones which can cause pain. However, unlike osteoporosis which affects all bones within the body, PD affects either one or a few bones at the most. It is very rare for patients below the age of forty to be diagnosed with PD. It can be caused either genetically or by a viral infection (e.g. the Measles). Less than 1% of patients with PD develop OS. According to Grimer et al the median survival rate of patients with OS attributed to PD is 9 months (45).

2.8.1.3 Pathology

The main occurrence of OS is in the metaphyseal region of long bones. It can also be commonly found in the pelvis, spine, craniofacial bones and the mandible. The tumour is caused by malignant mesenchymal cells. The tumour borders are poorly defined, and regions of soft tissue differentiation are usually seen in the region of the lesion, depending on the level of mineralisation, necrosis and possible haemorrhages (33).

In high-grade OS, there are a wide range of cells in the tumour, including many necrotic cells. High-grade OS is most often seen in patients of ages up to 25 years. Most patients are of grade II-b stature, meaning that the tumour is high-grade and it occurs outside the bone (44). Intermediate and low-grade OS is commonly split into parosteal and periosteal OS.

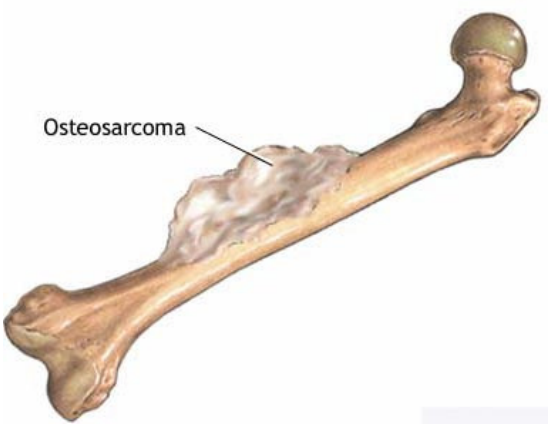

Figure 2-7 – Artist's Impression of Osteosarcoma (91)

Parosteal OS accounts for 4% of OSs. It occurs in the outer periosteal surface of long bones, in patients usually from the ages of 20-30 years old (33). Parosteal OS displays exophytic growths (tumours that grow outward) from the cortex of the bone. In most cases, these are treated with surgery, and this procedure has a survival rate of over 90% after 5 years (43). Periosteal OS accounts for 2% of OSs. This is a low/intermediate variant of OS, and occurs in the region below the periosteal surface. Periosteal OS is very rare, and is most prevalent in 10-30 year old females. Lesions appear on bony surfaces, but unlike Parosteal OS, Periosteal OS comprises of cartilaginous tissue. It is a deep developing tumour, and does not spread a great degree. This condition is treated with "*radical surgical excision*", and has a good prognosis of 70%.

2.8.1.4 Prognostic Factors

The level of biochemicals in the body can indicate OS within a patient. Chowdhry et al outline two chemicals within the body which can indicate metastases. When Alkaline Phosphatase (ALP) levels are elevated, this often indicates a pulmonary metastasis. If a patient displays raised Lactate Dehydrogenase (LDH) without metastases, there is an extremely poor prognosis for the patient (43).

Other factors that are associated with poor prognoses are the size and location of the tumour (whether it is in the axial or appendicular skeleton). However, the single greatest factor that must be considered is the maturity of the tumour at diagnosis. Chemotherapy treatment that causes a decrease in the tumour size by >95% has an excellent prognosis (43).

2.8.1.5 Treatment

The main form of treatment that is used in healthcare nowadays is chemotherapy. This is widely used to try to salvage the limb of the patient. Before the use of chemotherapy, amputation was prescribed upon diagnosis of OS. Patients that received amputations had a grave prognosis, with 80-90% of patients developing distant metastases (44). Nowadays, pre and post-chemotherapy is used to shrink the tumour, so the limb can be salvaged (85% of patients have their limb saved (44)) and to control any possible metastases. Radiotherapy is not readily used for OS. Prostheses are used to aid the function of the limb, after surgery extraction of the tumour and surrounding bone.

However, OS mainly affects young individuals, so aggressive levels of chemotherapy are usually prescribed to patients. It has been shown that OS in patients over 40 years old have a worse prognosis than younger patients. Also in the same study, it was shown that patients with non-metastatic OS had a 46% survival rate after 5years, which is lower than the overall prognosis of the disease (45).

2.8.2 Chondrosarcoma

2.8.2.1 Epidemiology

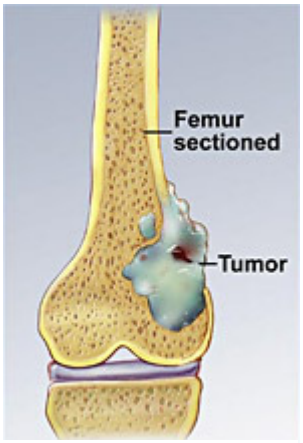

Figure 2-8 – Artist's Impression of Chondrosarcoma

Chondrosarcoma (CS) is a malignant tumour forming a hyaline cartilaginous matrix, with absence of bone formation as a result of the tumour cells. CS accounts for approximately 25% of bone tumours. Men have twice the risk of developing CS than women. CS can either be a primary tumour (85% of incidence) or a secondary tumour (15%). The main bones to be affected by these tumours are the pelvis (30%) followed by the proximal femur (20%) (33).

2.8.2.2 Aetiology

Like most bone cancers, the exact cause of the initial growth of these tumours is unknown, but many believe that it is due to either genetic or chromosomal abnormalities. Genetically, many argue that the tumour suppressant cyclin-dependent kinase inhibitor (CDKN2A) is responsible for the development of high-grade tumours.

Secondary tumours can be caused by pre-existing conditions. One example of this is Ollier's Disease (OD). This is a disorder characterised by development of benign cartilaginous tumours in or around the areas adjacent to the epiphyseal plates. This condition develops usually in childhood. 25% of OD patients subsequently develop malignancies (43).

Another condition, similar to OD is Maffucci Syndrome (MS). This is a condition involving multiple enchondromas (cartilaginous cysts) and hemangiomas (common benign endothelial cell tumours) found in the bone marrow. MS occurs in infancy, and is rarer than OD. It affects the peripheries of the body. Unfortunately for MS sufferers, there is a 100% risk of developing neoplastic cells (46), which result in malignancies. When grouped with OD, they account for 25-30% of secondary CS (43).

2.8.2.3 Pathology

There are three main types of CS, being Primary CS, Clear-cell CS and Mesenchymal CS. Each of these can form either in the medullary cavity or in the cartilage cap. CS is a slow growing tumour, and at diagnosis the tumour is relatively large (47).

2.8.2.3.1 Primary CS

All primary CS have pure hyaline cartilage differentiation. The tumours can have myxoid/mucoid material, calcium deposits and cystic degeneration. The growth of the tumour can penetrate the cortex of the bone. The tumour permeates between the trabeculae of the bone, and begins to erode the cortex. If the cortex is breached, in some instances a mass is formed surrounding the soft tissue. 90% of all CS develop in the central regions of bone. The remaining 10% are dedifferentiated tumours. These are biphasic in nature, with regions of both low-grade CS and high-grade malignant sarcomas displaying characteristics of cancers such as Fibrosarcoma, Malignant Fibrous Histiocytoma, Rhabdomyosarcoma and Osteosarcoma (46) (47).

2.8.2.3.2 Clear-cell CS

Clear-cell CS accounts for between 1-2% of all CS diagnosed. There is predominance in the male populous, and tends to be seen in the femur. There is usually a region of cell degeneration close to the articular surface of the bone. The growth may or may not have a cartilage matrix. Unlike the surrounding cartilage, the tumour has relatively large amounts of pale eosinophilic cells, surrounded with high levels of glycogen. The region around the bone will sometimes display irregular patterns of woven bone, and usually there is an absence of cartilaginous matrix (43) (46).

2.8.2.3.3 Mesenchymal CS

Mesenchymal CS accounts for 3-10% of all CS diagnosed. Unlike both Primary and Clear-cell CS, Mesenchymal CS is usually diagnosed in patients who are in their 20-30's. One in every three instances occurs in soft tissue. The region around the lesion will have cell lysis and endosteal erosions. It has a biphasic nature, with regions of hyaline cartilage and undifferentiated cells which form around vessels, according to Sumathi et al (46).

It occurs mainly in the axial skeleton, in particular the ribs and pelvis. Usually this cancer results in destruction of cortical bone, but in some cases can cause thickening of the cortex. A large proportion of cases of CS occur on or in close proximity to the periosteum of the bone. CS is diagnosed when hyaline cartilage begins to show signs of calcification, usually occurring in a process known as endochondral ossification. Again like OS, necrosis and haemorrhages can exist at the site of the lesion. The primary treatment of CS is complete surgical excision.

2.8.2.4 Prognosis

Like all cancers to affect bone and soft tissues, the size, shape, location and grade of the tumour greatly affects the prognosis of the patient. It has been reported by Sumathi et al that Grade I tumours have a survival rate of 90% after 5years, while Grade III tumours have a prognosis of 29%. Mesenchymal CS and Clear-cell CS have high instances of reoccurrences in the patient after successful treatment, and have been recorded recurring over 20 years after the initial lesion was diagnosed. These recurring lesions are generally the result of other metastases, which will lower prognosis percentages (46). Like OS, CS usually displays a painful region corresponding to the underlying bone, a certain amount of swelling and a visible mass.

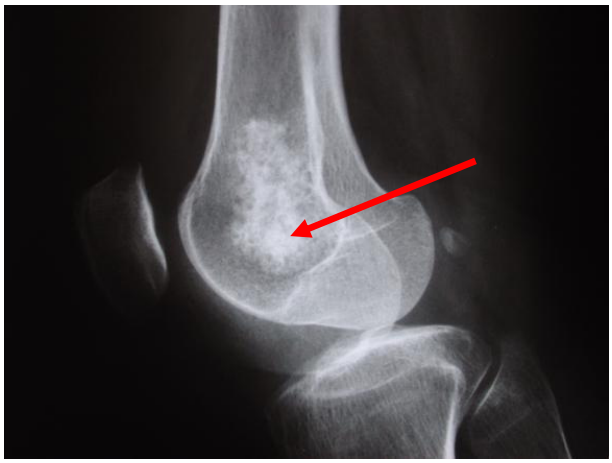

Figure 2-9 – X-Ray of Chondrosarcoma in Distal Femur (94).

2.8.2.5 Treatment

Unlike OS, CS does not respond well to chemotherapy, and like OS, radiotherapy is not a very successful method of treating CS. However, chemotherapy is highly recommended by the medical community to deal with local and systematic metastases. Radiotherapy is occasionally used for local control. The main form of dealing with CS is surgery. After the surgery, a range of procedures are used to salvage the limb, including allografts, autografts and in severe circumstances, metallic prostheses are implemented. Surgery is only avoided when confronted with low-grade tumours. In these cases, an intralesional treatment is preformed, so the affected limb is able to be salvaged, with the aid of PMMA bone cement.

2.8.3 Ewing's Sarcoma

2.8.3.1 Epidemiology

Ewing's Sarcoma (ES) can affect soft tissues as well as bone. It is usually seen in younger patients, with a reported 80% of ES affecting patients during puberty (43). The peak is seen in 10-20 year olds. It is the second most common bone cancer to affect adolescents after OS (48). The main bones to be affected by ES are the ribs, pelvis and tubular bones of the lower limbs (49). The tumour occurs in the intramedullary canal, often in the metadiaphysis, and the tumour has an extensive soft tissue component.

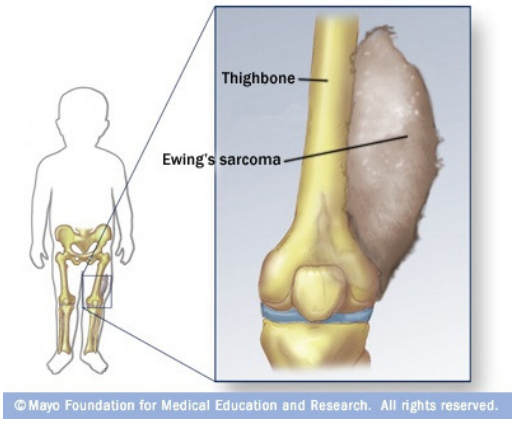

Figure 2-10 – Artist's Impression of Ewing's Sarcoma

2.8.3.2 Aetiology

The cause of ES can largely be accredited to a genetic link which accounts for 85% of all ES cases. This defect directly contributes to the development of tumours. Gene fusion (e.g. EWS-FLI1) acts as the main regulator to tumours, by becoming a transcription factor. This gene can induce IGF1 growth factors, which will turn regular mesenchymal stem-cells found in the matrix of the bone into tumour cells. These abnormalities can result in development of oncogene activation potential. Like CS, cyclin-dependent kinase inhibitors are argued to contribute to the development of ES (46) (49).

2.8.3.3 Pathology

Microscopically, ES will display abundant levels of small blue cells, which will give the tumour its blue opaque appearance. These cells produce neither osteoid nor chondroid. There are high levels of glycogen in the regions inhabited by these blue cells, and the lesion is usually highly vascularised. This region will have high proportions of necrotic cells, as well as haemorrhagic and cystic regions. These lesions can also breach the cortex of the bone, which can have severe repercussions to the patient in the form of metastases. About a quarter of patients with ES show metastasis affecting the lungs at diagnosis, with about 1 in every 20 patients have ES spread to other bones (43) (48) (49).

2.8.3.4 Prognosis

ES is a highly aggressive cancer. Without immediate treatment of chemotherapy, this cancer rapidly leads to death, with fewer than 10% surviving 5 years after diagnosis. ES has a relatively poor average prognosis, with literature reporting that the survival rate after 5 years post treatment is 41%. Prognostic factors include gender, age, size of tumour, site of lesion, if fever is present, serum Lactate Dehydrogenase (LDH) levels (due to cancer having high tissue turnover rates, the destroyed cells release LDH), anaemia, metastases and response to chemotherapy. ES affecting the pelvis has one of the worst prognoses, with the literature reporting a survival rate of 15-35% as opposed to 30-77% survival rate for a non-pelvic ES (46) (49) (50) (51).

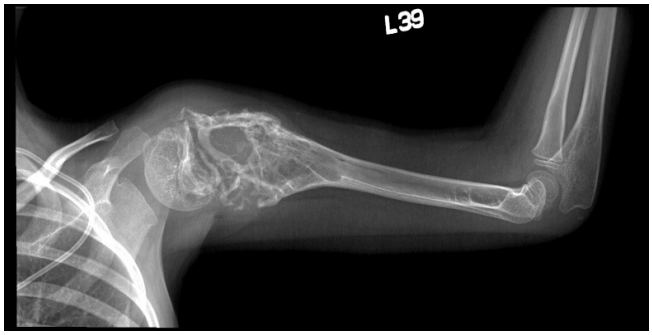

Figure 2-11 - Ewing's Sarcoma of the Proximal Humerus (95).

2.8.3.5 Treatment

With surgery alone, the vast majority of ES sufferers will not recover from the disease. The treatment of dealing with ES consists of surgery to remove the initial tumour, as well as pre and post multidrug chemotherapy and radiotherapy. Pre-multidrug chemotherapy is used to minimise the size of the tumour, so limb salvage surgery can be successful. According to a report by Jurgens & Dirksen, the most difficult primary site for treatment is the pelvis, due to at the time of diagnosis, there is a considerable bulk of tumour present, as well as presenting difficulties in the surgery and radiation required to treat the tumour. It also has the poorest histological response to chemotherapy (48) (50). If lesions occur in "non-critical" bones, such as a rib or fibula, the surgeon will simply remove the whole bone, to reduce the risk of metastasis. If the tumour affects a crucial bone, limb salvage is always recommended, to avoid amputations. Limb salvage can come in a range of methods, including endoprosthetic replacement, osteoarticular allografts, allograft composite reconstruction techniques and bone transport techniques (49).

2.9 Introduction to Radiation Terminology

As seen in the previous section discussing bone malignancies, it is apparent that pelvic tumours have a poorer prognosis than in other bones. As a result of poor responses to chemotherapy and radiotherapy, other methods of treating the tumours were proposed. The following section briefly explains the basics of radiation, the primary method in treating pelvic tumours.

When tissue is irradiated, the dose of a radiation absorbed of ionizing radiation is measured in terms of a SI unit; the gray (Gy). A Gy is the equivalent to one joule of energy per kilogram. In the US, the measurement of a rad is used mainly in industry.

$$1Gy = 1\,{}^{J}/_{kg} = 1\,{}^{m^2}/_{s^2} = 100rad$$

Equation 2-3 – Equivalent Units of 1Gy

Dosimetry is the calculation and measurement of the internal and external absorbed doses of ionizing radiation in tissues. The unit Gy refers to the absorbed dose in matter (D_T), whilst the Sievert (Sv) relates to the absorbed dose in biological matter (H_T). These values are dependent on two factors, the weighting factor and the tissue weighting factor.

The equivalent dose accounts for the different radiation types. The weighting factor (W_R) relates the radiation particles to the severity of the dose. For instance, when α-particles are absorbed by the body, a factor of 20 is given to the calculation, due to the strong ionizing nature of these particles. For X, γ and β particles, the weighting factors are 1.

$$H_T = \sum_n (W_R \,.\, D_{T,n})$$

Equation 2-4 – Biological Matter Absorbed Dose

The tissue weighting factor (W_T) takes account of the sensitivity of the tissues and organs. All tissues in the body add up to a total of 1, so 1Gy of radiation across the whole body is equivalent to 1Sv. The gonads had the largest weighting factor of any organ, with a value of 0.25 in a report dated from 1979, but this has been revised twice to a value of 0.08 in 2007. Bone has a value of 0.01 (52). Once all these factors have been taken into account, the effective dose (E) is calculated in units of Sv. It is defined in medical dictionaries as "*the measure of probabilistic effect on the whole organism due to ionizing radiation delivered non-uniformly to parts of the body*".

$$E = \sum_{n} (W_T \,.\, H_T)$$

Equation 2-5 – The Effective Dose

Tissues are continuously being bombarded by natural radiation. The two most common forms of background radiation doses are cosmic radiation (UV radiation) and naturally occurring isotopes (i.e. Radon gas). Per annum, the mean dose is between 2.5mSv-3.5mSv, with over 50% of this being attributed to Radon alone. Medical radiation accounts for approximately 12% of all radiation in the US (53) (54). When radiation is administered to biological tissues, the effects depend on a number of factors including the type and energy of radiation. For example, a single dose of 5Gy to the whole body would lead to death in 2 weeks, while doses as high as 45Gy can be tolerated if fractioned sufficiently.

In a hospital environment the dose calculated to treat the malignancy is usually determined by the use of the Monte Carlo dose calculation method, which is a method of repeated random sampling to compute results. The method usually calculated the dose as if the body was entirely made up of water, as opposed to the various tissues. In most cases, these estimations are accurate, but it has been noted that for dose calculations of bone, there can be a discrepancy of up to 10% (55).

It has been shown that radiation is one of the causes of production of reactive oxygen species (ROS), which are unstable and very reactive compounds. The other main cause of these is the body's own inflammatory response. These compounds, such as hydroxyls ($^{\bullet}OH$) and peroxyls ($ROO^{\bullet}$) have been argued to cause oxidative damage to the collagen structure, which leads to radiation-induced fibrosis (56).

2.10 Treatment of Bone Sarcomas

Currently, the most common form of treating pelvic cancer is the use of chemotherapy, surgery and radiotherapy. These have had limited success with patients. New methods were explored in the early 90's, in an attempt to increase the prognosis of pelvic and other tumours. The use of these techniques is to destroy the cancerous cells within the bone to reduce the chances of recurrence. These methods of treatment try to maintain the patient's quality of life by preserving mobility. Amputation of the diseased region of bone is not considered often due to the disability that would follow.

2.10.1 Methods Explored to Treat Bone Sarcomas

2.10.1.1 Autoclaving

To increase the prognosis of pelvic sarcomas, three methods were researched. The first method is known as autoclaving. This is a device which uses high pressure saturated steam to raise the temperature of the extracted bone for a relatively short period of time, to kill the cancer cells. However, this method was proven to be unsuccessful due to the large variations in temperature in the bone. Due to the unequalled temperature throughout the bone, some cancer cells could survive and proliferate, which would significantly reduce the prognosis of the patient. This was shown to be the case in a study carried out by Bohm & Stihler, where after an 11 minute autoclaving session with a set tumour cell killing temperature of 134˚C, some parts of the bone only reached 45˚C, far below the temperature that would destroy the cells (57).

2.10.1.2 Liquid Nitrogen Freezing

The second method used to kill tumour cells is the use of liquid nitrogen to freeze the tumour. This method has been used successfully for years to treat warts and verrucas. The diseased bone is extracted and the tumour is removed by a technique known as enucleation (the removal of a tumour lesion without dissecting it).The extracted bone is then "boiled" in liquid nitrogen at a temperature of -196˚C, for three cycles, before being re-implanted into the patient, with the aid of monocortical plates (58). The problem with this method is that the large temperature ranges have been attributed to a "high propensity for denatured collagen" (59). Furthermore, the formation of ice crystals in the bone matrix tends to damage the cells inhabiting the bone, as well as creating an imbalance in electrolytes (59).

2.10.1.3 Extra-Corporeal Irradiation

The third method for treating pelvic sarcomas is Extra-Corporeal Irradiation (ECI). The procedure essentially involves the affected area of the bone being extracted from the patient's body, irradiated and implanted back into the patient. This method, unlike autoclaving, allows a homogenous treatment through the bone. The level of irradiation is set to a level which is known to kill cancer cells (and all other cells present). The procedure to which patients undergo is shown below (60).

1. Prior to the initiation of surgery, information is collected from the patient to evaluate the size and exact location of the tumour using imaging techniques such as X-ray graphs and/or MRI scans.
2. Once the patient is in surgery, the diseased bone is extracted, usually with disease free borders unless it is not viable to do so. This usually takes up to an hour, due to gaining access to the region, and carefully disconnecting tendons and ligaments in the region of the affected bone.
3. The diseased segment of bone is placed into a plastic container. The bone is wrapped in a damp dressing (or bolus material) and placed into a sterile plastic bag. The bag is surrounded by damp material in all directions, to avoid air pockets[†].
4. Once the bone is satisfactorily sealed in the plastic box, the box is irradiated to a certain dose which is pre-determined by the physician. The irradiation occurs in a separate room to the operation, and is performed by radiologists. This takes between 45min to an hour to perform.
5. Once the bone has undergone ECI, the bone is returned to the operating room and removed from the box. The majority of the dead tumour is removed and depending on the circumstances of the borders, the bone may have to be trimmed and manipulated to fit the region. The bone, once in place, is fitted with the aid of metal plates and bone screws or PMMA bone cement. The tendons and ligaments are reconnected to the newly irradiated bone and before closing the surgical site, drains are placed in the patient to remove possible excessive fluid build-ups which occur

[†] It is important to avoid air pockets as much as possible. Radiation particles travel at different speeds through different media, so air voids would cause an inhomogeneous dose throughout the bone. Furthermore, it is important that the bone segment is completely surrounded by the damp dressing, so the bone does not receive lower than intended doses.

naturally due to inflammatory responses. This part of the surgery takes over 3 hours to complete.

6. If a small portion of bone could not be extracted for irradiation, post operative beam radiotherapy will be carried out. Usually this consists of 30 daily doses of 55-60Gy for 6 weeks, which is common practice by radiotherapists.

2.10.2 Advantages of Extra-Corporeal Irradiation

ECI is becoming increasingly common in the medical community for treatment of bone sarcomas. For example, sarcomas affecting the foot would commonly be amputated in past times, but due to the relatively simple procedure of Extra-Corporeal Irradiation, these amputation cases are now seldom (61). There are advantages of this method of treatment compared to other treatments with allografts and prostheses. Firstly, the use of the autografts preserves mobility of the patient, which is being increasingly linked to better post-operative recoveries. Due to the absence of prostheses, the problems relating to both early and late loosening of the prosthesis is avoided, as well as obvious immune system rejection problems with allografts. Finally, the irradiated autograft acts as a scaffold for the body's cells to inhabit the structure and slowly replace the dead tissue with living tissue.

It has also been questioned whether the dead tumour cells within the autograft trigger the host's immune response, which would help to lower chances of infection and subsequent revisions. In a follow-up of 17 patients by Uyttendaele et al, not one patient experienced post-operative pain (1).

At an annual ASCO (American Society of Clinical Oncology) meeting in 2005, El-Wahidi et al presented a population of patients, some which had undergone surgical excision and implantation of a prosthesis, as well as both low and high doses of Extra-Corporeal Irradiation (50Gy & 300Gy respectively). All patients, regardless of their procedure underwent neoadjuvant chemotherapy. The results from this study show the advantage of ECI compared to excision and prosthetic intervention with regards to a recurrence of the disease (8.3% to 18.7%). The rates of metastases were largely the same. In relation to the difference in the level of doses of radiation, it appears that that the higher dose of ECI will stand a better chance of eradicating the disease than the low dosage, with a reported 88% of 300Gy patients having no evidence of disease, compared to 75.1% at 50Gy. Also, fewer patients died as a result of the disease after higher doses of ECI. Finally, it was reported that the functional outcome of the patients did not change regardless of the level of ECI received (62).

ECI is a very useful method for treating bone tumours as long as there is sufficient bone remaining after the lesion has been removed. This gives the patient a lasting "biological reconstruction" with the dead autograft. It was outlined by Davidson et al that there was no risk of recurrence of the disease at the site of the autograft, a statement which has been

backed-up within literature (2). ECI is most successful in the lower limbs of the skeleton, mainly the femur and tibia.

However, there have been problems associated with ECI treatment, including symptoms such as skin necrosis, osteoradionecrosis, infection, avascular necrosis and partial resorption of the graft. These problems are most often seen in sites furthest from revascularation (humeral head and pelvis) (63) (64).

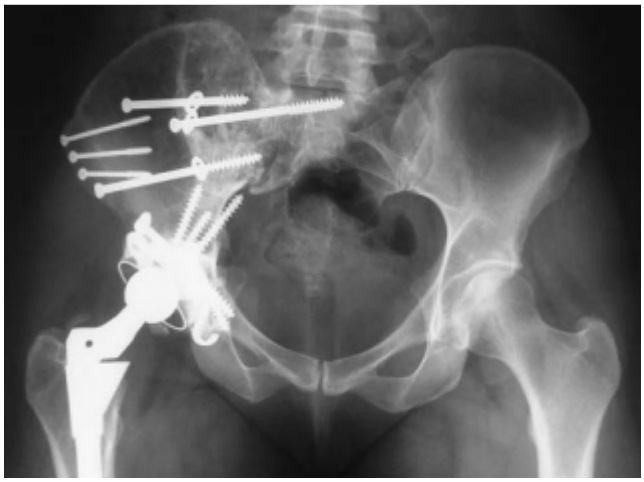

Figure 2-12 – Re-implantation of portion of pelvis after 50Gy Extra-Corporeal Irradiation (64)

2.11 Effects of Irradiation on Bone

It is difficult to quantify the effects of irradiation on bone, as bone has regions of non-growing bone (mineral cortex), growing bone (epiphyseal-diaphyseal plates) and cavities within bone (intramedullary canal). All of these regions contain cells which can become cancerous due to DNA mutations. In the treatment of bone sarcomas, both autografts and allografts undergo different radiation therapies and as a result the bone behaves differently post treatment.

2.11.1 Autograft Irradiation

2.11.1.1 Effects on Osteogenic Cells

Till & Meyer, supported by other researchers in the field declared in their work that bone is not radiosensitive (34) (65). In general, the mineral phase of bone is considered to be of minimal or no risk of carcinogenesis, when exposed to environmental radiation. However, both the marrow and the osteogenic cells are susceptible to developing forms of cancer (leukemia and bone sarcomas respectively).

It was demonstrated by Casarett in a study performed on rats that radiation affects cellular processes, which in turn affects the performance of tissues. Radiation was given to rats' knees and the cellular response was recorded. It was shown after 2-3days mitosis ceased, and the cells present began to swell and degenerate. Within a week, all cells were destroyed. Systematically, all tissues surrounding the irradiated knee (bone, cartilage, muscle, fat, blood vessels, etc.) showed morphological changes. Cartilage had one of the most pronounced changes, with its structure becoming irregular and eventually it degenerated. Osteoblasts were destroyed following the dose of radiation; demonstrating osteogenic cells are prone to radiation damage. The cartilage (found in growth plates) was replaced with a non-cellular bone-like substance, resulting in impeded limb growth of the rats (66). Exposure to a single dose of 40Gy can cause a considerable reduction in osteoblast activity, as well as inhibiting cell growth and ALP activity (63).

Radiation may cause a disruption to the remodelling process seen in bone, causing osteolysis (excessive resorption) or osteosclerosis (bone overgrowth). In irradiated bone, fractures are common due to either deposition of radioactive isotopes which localise within

the bone or due to localised doses of radiation therapy (67). Single doses of 20Gy or greater have been shown to cause osteopenia‡ in 8-23% of patients in various studies (63).

The prescribed level of radiation to kill tumour cells completely within the resected bone varies within the literature. Many studies claim 50Gy is more than sufficient to kill the cancerous cells, with 0% recurrence rate at the site of the graft. However, some studies lean on the side of caution, stating that only doses of ≥80Gy are required to eradicate tumour cells, or even higher doses are needed if the graft is in an anoxic environment (3). It has been widely reported and confirmed in clinical studies that ECI of a bone near the subchondral region in bones (osteoarticular grafts) will eventually cause bone disintegration, leading to joint degeneration. Sabo et al demonstrated that subchondral irradiation at a relatively low dose of 25Gy using a 15MeV linear accelerator caused cartilage degeneration in the form of superficial clefts and hypercellularity (3) (68). This report directly disputed claims that doses under 50Gy showed "no obvious degeneration changes" (68), and has been backed up with other studies using doses of 10Gy and 40Gy.

Tests were performed on mice to see whether low amounts of irradiation (5 and 20Gy) would show morphological and material property changes. The hind limbs of the mice underwent focal irradiation. The limbs were harvested after a predetermined period and the distal femora were firstly inspected and secondly underwent an axial compression test to account for the strength of the bone. The results show that cortical bone increases in mass, whilst trabecular bone decreases. After a prolonged period in-vivo, the strength decreased (69).

The production of Reactive Oxygen Species (ROS) in the irradiation treatment causes damage to the microstructural hierarchy of bone. The ROS attack the extra-cellular matrix molecules, which in turn disturb the structure of collagen permanently. This is due to uncontrolled fibroblast activation and irregular collagen accumulation. A test carried out showed that after relatively small amounts of irradiation (0-30Gy), protein synthesis was reduced in all irradiated cases, whilst the relative amount of collagen increased, proving that irradiation causes excessive fibroblast activity (56).

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‡ Osteopenia is a condition where the bone mineral density is lower than usual. Many argue that this condition is a precursor to Osteoporosis, but remains disputed.

2.11.1.2 Effects on Varying Maturity of the Tissue

The level of maturity of the tissue is a factor when assessing the effects of radiation on connective tissues. For a child, a dose of 1Gy may be enough to cause growth issues of the limb, and in doses above 20Gy, other skeletal changes are noticeable. For mature adults, cartilage can withstand 40Gy fractioned over 4 weeks, or in excess of 70Gy fractioned over 10-12weeks. Bone has been known to withstand 65Gy fractioned over 6-8weeks (70).

2.11.1.3Effects on Re-incorporation of Autografts

A study was carried out in Mumbai, India, where 12 patients with Ewing's Sarcoma were treated using Extra-Corporeal Irradiation. Not one of the 12 patients had metastases detected. The diseased portion of bone (8 femora, 2 tibias, 2 humerii) was removed and treated with 50Gy, before being re-implanted with the aid of plates and bone screws. Two patients suffered from post-operative infections and were discounted from the subsequent study. Of the remaining 10 patients, a follow-up was preformed after a length of time (mean 2 years). 84% of the osteotomy regions displayed a healthy union between the surfaces of the irradiated autograft and the rest of the limb bone, with metaphyseal union occurring after a mean of 6 months and diaphyseal union taking 8 months. The main conclusions drawn from this study was that 50Gy was adequate to kill tumour cells within the bone, whilst avoiding severe damage to both the biomechanical and biological properties of bone (71).

In Kyoto University, a study was carried out on rabbits to show how autografts, resected from the animal and undergone ECI at various doses (0Gy, 50Gy, 100Gy, 200Gy), incorporated after 6 months (72). The incorporation of the autograft was measured with a variety of techniques; roentgenography, histology and histomorphometry. Both the roentgenography and histology techniques showed no difference in incorporation between the control and ECI autografts. In relation to histomorphology, it is shown that the control has a lower mean unresorbed fraction than the irradiated specimens, but there does not appear to be any difference in incorporation with the different doses of irradiation. This has been seen in another study, where autografts receiving 250Gy displayed excellent healing at osteotomy junctions (2). The study concludes that the ECI treatment possibly affects the contribution of the grafted marrow to the incorporation process, but does not affect the matrix contribution (72). In all studies carried out, it has been discussed that ECI is an extremely useful method for treating sarcomas within bone, but this relies heavily upon the

state of the bone after resection. If the bone has been sufficiently compromised by osteolytic destruction, ECI is not a viable treatment (2).

It has been seen in experimental research and in literature reviews that the dosage of radiation administered to sections of bone has an adverse effect on the healing process of autograft reimplantation. In 1996, a study was carried out on Wistar rats, where diaphyseal tibia were extracted and irradiated to give effective doses of 0Gy, 1kGy, 5kGy, 25kGy & 50kGy from a ^{60}Co source. The healing process was then evaluated every three weeks for a period of twelve weeks. Three weeks after reimplantation, most (>66%) of the 50kGy specimens suffered from pathological fractures. The bones which underwent 25kGy irradiation displayed delayed healing and at the end of the experiment, they had a mean of 50% reduction in the incorporation of the graft. There was no statistical difference seen between the 1 and 5kGy specimens, with the un-resorbed fraction of the incorporation being 16% and 24% respectively, compared to the control group (73). These levels of irradiation far surpassed that which would be administered to a patient suffering from bone sarcomas, but this study does show that there is a certain adverse effect that increasing dosages of radiation has on bone. It was also shown that the density of osteocytes within the autograft dropped significantly after irradiation compared to control samples.

2.11.1.4Effects of Chemotherapy on Re-incorporation of the Autograft

Chemotherapy is an extremely important factor when trying to destroy all traces of cancer within the body. However, chemotherapy has been shown to prolong the reincorporation of the graft. Once the graft has successfully incorporated, the graft functions the same as normal, disease free bone. It has been outlined that unless it is absolutely necessary, secondary interventions must be avoided, due to the risk of post-operative infections. Secondary interventions are usually carried out to speed up graft healing with the aid of a metallic prosthesis. The prosthesis will have no effect on prolonging the graft healing. The pelvis is the site in the body which has the lowest graft survival rate. One major factor that causes this is that many pelvic resections also include the hip joint (Example seen in Figure 2-12). The hip joint is replaced with a synthetic hip joint, which has been proven (once load is applied through the region) to disrupt the osteosynthesis of the graft (3).

2.11.2 Allograft Sterilisation

Donor allografts have to undergo high levels of radiation to rid them of any trace of their previous host. Otherwise, the immune system could be triggered causing swelling, pain and possibly lead to infections. Due to the high levels of radiation bombarding the allograft, there are important effects that irradiation cause in bone to report.

2.11.2.1 Effects on Fracture Toughness

Tests were carried out on researchers on the effects of gamma radiation on the mechanical properties of bone. One such test explored the effect of gamma sterilisation on allografts. It has long been recognised that the degeneration of the mechanical properties of bone come second to the functionality of the implanted allografts. An allograft irradiated at 27.5kGy (standard sterilisation dose) was compared to an un-irradiated allograft. The irradiated allograft had poorer fracture toughness, but more notably was the reduction in the fracture energy required to propagate a fracture (74). This signifies that the collagen component was damaged somewhat, as collagen is known to impede crack propagation by a process known as crack-tip blunting.

2.11.2.2 Effects on Bending Strength and Elastic Modulus

With the degradation of collagen within the matrix due to radiation bombardment, a reduction in the bending strength has been observed. This directly ties in with the loss of collagen that opposes the tensile stresses induced. It was shown that there was no evidence that doses lower than 35kGy disrupt the strength of cortical bone. However, above an effective dose of 70kGy, the material displays a complete absence of post-yield plastic deformation and as the doses increase, there is a clear reduction in the critical fracture load and toughness. The Young's Modulus is not affected by damage to the collagen component within bone, indicating that the Young's Modulus is determined solely by the mineral phase of bone (75) (76).

2.11.2.3 Effects on Fatigue Life

Like the previous study, gamma irradiation was studied to see whether it caused major reductions in the mechanical properties of bone. Bone undergoes repetitious loadings every day, known as physiological loading. These include activities such as walking, running and breathing. Cortical bone was irradiated at a received dose of 36.4kGy and compared to a

control group. These groups were then fatigued and the data was reviewed. The result from these tests was that γ-radiation greatly reduces the fatigue life of the bone, with a reported two magnitude decrease in longevity (77).

2.11.2.4 Effects of Dosage on Collagen Components

The effects of irradiation on collagen have been studied in depth in both biomedical tissue engineering and biomedical material engineering. A study by Cheung et al demonstrated that at a dose of radiation of 1Mrad (10kGy) for sterilisation purposes did not cause the collagen peptide backbone to degrade (although other cross-links were disrupted, approximately 5%), but with higher levels of irradiation, degradation was apparent with levels of over 40% of collagen cross-link degraded (78).

A study was performed to show if there is any distinguishable effects between low and moderate absorbed doses received. Two groups of both soft and bone tissues were tested at low doses (18.3-21.8kGy) and moderate doses (24-28.5kGy). The results conclude that in both groups, there were no effects seen in either the strength or the elastic modulus of the tissue (79). These concur with other experiments on allografts, including no changes in screw pullout strength to that of non-irradiated allografts (80).

High levels of received irradiation (≥35kGy) cause severely disrupt the collagen fibres which make up a large proportion of the extracellular matrix in bone. This is caused by the disruption of the peptide bonds in collagen's atomic structure. It was shown that the irradiation affects the mature cross-links of collagen, whilst causing little or no disruption to the immature cross-links. Compared to a control specimen, a 70kGy specimen's ratio of mature to immature cross-links decreased by over 66% after irradiation (75).

2.12 Methods to Prevent Collagen Damage caused by Radiation

Collagen within bone is degraded as a result of the bombardment of radiation particles throughout the matrix. These particles cause water-derived free radicals to interfere with the microstructure of collagen, disrupting the fibres and increasing the cross-links. The increasing development of cross-links causes a complete loss in toughening, which leads to a reduction in ductility and strength according to studies (81). A method to prevent the disruption of collagen cross-links during ECI treatment is to deep freeze the autograft/ allograft before the treatment. This has been shown by Hamer et al to "inhibit water-derived free radicals" (82).

2.13 Study Rationale

Although some studies have been carried out on the basic mechanical properties of bone, there has been little research in terms of the viscoelastic properties once a bone undergoes irradiation. Also the tests that have been carried out have performed at substantially higher doses than autografts would experience during ECI treatment. Due to the lack of research, there is no agreed dose of radiation set in the treatment of bone sarcomas. Countries around the globe used different levels; with the highest recorded dose administered to a patient's autograft is a un-fractioned 300Gy irradiation. Many dispute this level, claiming its unnecessarily high, but this has not been backed up with research.

ECI shows excellent short-term results, and is increasingly used within the medical community to treat life-threatening sarcomas of the bone (83). The aim of this project is to hopefully show the effects of increasing levels of Extra-Corporeal Irradiation doses has on the collagenous network present within bone. This may help find the optimum level of irradiation which preserves the autograft's mechanical properties whilst also providing sufficient levels of irradiation to eradicate the tumour cells. If some of the mechanical properties show similarities to healthy bone, less time will be required for recovery and integration of the graft, reducing prolonged periods of immobility and hence increasing the quality (and hopefully the longevity) of the patient's life. The aims of this project are listed on the next page;

1. Acquire fresh bovine tibia from an abattoir.
2. Extract rectangular specimens (5x5x30mm) from the mid-diaphysis of the tibia and label them with respect to both the individual bone and the anatomical position of the specimen.
3. Irradiate the specimens in increasing doses of radiation from 25Gy up to the maximum 300Gy limit.
4. Test specimens in Bose Electroforce Testing Machine with tensile loads to explore the elastic and viscoelastic behaviour of the different groups when compared to the control group.
5. Quantify each data group statistically to the control group using T-tests and ANOVA to find statistical trends.

3. Methodology

3.1 Materials

Thirteen mature bovine tibias were freshly harvested and collected from an abattoir. Mature subjects were chosen to avoid fibrolamellar (plexiform) bone of immature specimens (5). No ethical considerations were required due to the animal being directly from the human food chain. The fresh bones were immediately frozen upon acquisition (-17˚C). After several weeks, the mid-diaphysis was extracted with the aid of a bone saw.

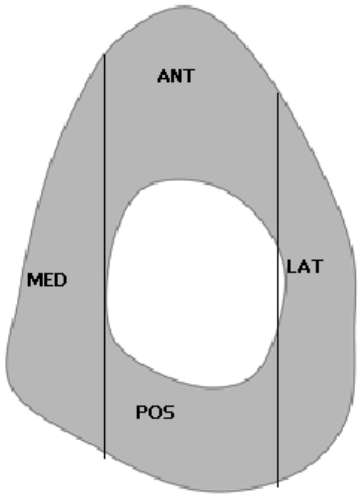

Figure 3-1- Cross-Section Displaying Sectioning of the Mid-Diaphysis of a Tibia

The mid-diaphyses were thawed at room temperature (25˚C) and sectioned into anterior, posterior, medial and lateral sections (see Figure 3-1) with the use of a bone saw, before being cut with a diamond tipped rotating blade (Smart Cut, UKAM Industrial Superhard Tools; Valencia, CA, USA) into rectangular specimens (0.5cmx0.5cmx3cm).

The specimens were cut at a slow uniform speed to reduce any thermally induced damage. This was achieved by placing weights (2x100g) which were connected to the holding stage of the rotating blade. The specimens were cut along the primary loading axis of the tibia. The specimens were then sanded down to obtain the specific cross-sectional measurement using firstly an ISO Grit Specification of P80 to remove the bulk of the excess bone. For fine sanding and polishing of the specimen, a grit of P320 was utilised. The specimen's measurements were checked using an electronic micrometer (Mitutoyo, Absolute Digimatic; Tokyo, Japan). A tolerance of $\pm 0.04 mm^2$ was allowed for the cross-sectional area.

Each set of specimens were separated with respect to the position and also the individual bone, to try minimise statistical differences of individual properties of bone. On average, 12-13 specimens were extracted from each bone, giving a total of 164 specimens for testing. The specimens were carefully wrapped in 0.9% saline soaked gauze and each group was placed within clearly marked sealable bags before being refrozen (-17˚C).

Ideally, all the bone preparation would be carried out on a single day with fresh bones, to reduce the amount of times that bone had to undergo refreezing. Refreezing has been attributed to damage to the microscopic material structures. It has been suggested that freezing a specimen twice before testing does not have any implications in the structural integrity of the material, but higher cycles of refreezing should be avoided in experimental methodology (84). However, due to time constraints this was unfortunately inevitable. Furthermore, as all specimens have undergone the same refreezing process, the specimens should not present unusual results when being compared to their counterparts.

During the harvesting of specimens, a problem arose with the refreezing process where a bottle was incorrectly labelled. As a result, some bone specimens were frozen in impure water. As a result, all such specimens were removed from subsequent testing due to not adhering strictly to preparation and testing protocol. As a result, there were some groups (75Gy, 250Gy, 275Gy, 300Gy) that only had three sets of specimens as opposed to four sets (See Table 3.2:1).

3.2 Irradiation of Specimens

The specimens were split up into thirteen groups, to undergo the irradiation in the University of Glasgow Small Animal Hospital. The grouping of specimens attempted to include all anatomical locations from different bones to avoid complications of abnormal individualistic parameters.

Dose (Gy)	Sub Groups (No. Indicates Bone and Letter Indicates Anatomical Position in Tibia)				n_{TOT}
0 (Control)	1M	4L	7A	10P	13
25	1L	4A	7P	11M	11
50	1A	4P	8M	11L	15
75	1P	N/A	8L	11A	12
100	2M	5L	8A	11P	11
125	2L	5A	8P	12M	11
150	2A	5P	9M	12L	16
175	2P	6M	9L	12A	17
200	3M	6L	9A	12P	13
225	3L	6A	9P	5M	11
250	3A	6P	10M	N/A	11
275	3P	7M	10L	N/A	11
300	4M	7L	10A	N/A	12
Total					**164**

Table3.2:1 – Format of the Thirteen Test Groups

Each group of specimens were allowed to thaw to room temperature before being wrapped in saline soaked gauzes, and placed into a sub-divided plastic container. The sub-divided container had measurements of 19cmx17cmx0.4cm.The specimens were placed close to the midpoint of the container as instructed by the oncologist expert, with the use of 1cm of moist bolus material on the bottom and on top of the specimens. This prevents the specimens from encountering a build up effect and a fall-off of radiation to the midplane according to Veterinarian Dr. J. Morris (through personal communication).

It is important to have no air pockets within the plastic containers, as the radiation particles travel at different speeds through different media. If this precaution was not taken, there is a large probability that there would be an irregular dose given to the specimens therefore corrupting subsequent data from the testing stages.

One group acted as a control group, where no irradiation was administered. The other twelve groups were irradiated on a Siemens ONCOR Impression Plus Linear Accelerator at 6MV X-ray Photon Beam in increments of 25Gy up to the maximum of 300Gy under the supervision of Radiation Therapist Mrs. S. Burnside. This was achieved by having all twelve groups within the beams focus. The radiation was set up in an AP/PA manner, where the gantry was rotated through 180 after half the dose was administered. The dose was calculated in terms of Machine Units (MUs) (See Appendix 9.2).

Figure 3-2 - Siemens ONCOR Impression Plus 6-10MVLinear Accelerator

Once the 25Gy dose was administered to the whole group, one group removed before the subsequent irradiation. This group was labelled accordingly with respect to the dose it had received. After the irradiation was completed, the bone specimens were returned into individual containers and frozen for the final time before undergoing elastic and viscoelastic testing.

3.3 Elastic and Viscoelastic Testing

For the testing stage, the specimens were tested in the BOSE Electroforce 3200 Material Testing Machine. This testing machine was opted for instead of the Instron Material Testing Machine as a heated bath could be used in conjunction with the BOSE. The specimens were marked with five black dots in the shape of an X to allow the Bose Digital Video Extensometer (DVE) to determine displacements at the points to calculate the axial and lateral displacement of all specimens. The distances between each of the corners of the X were 4mm (See Figure 8-3).

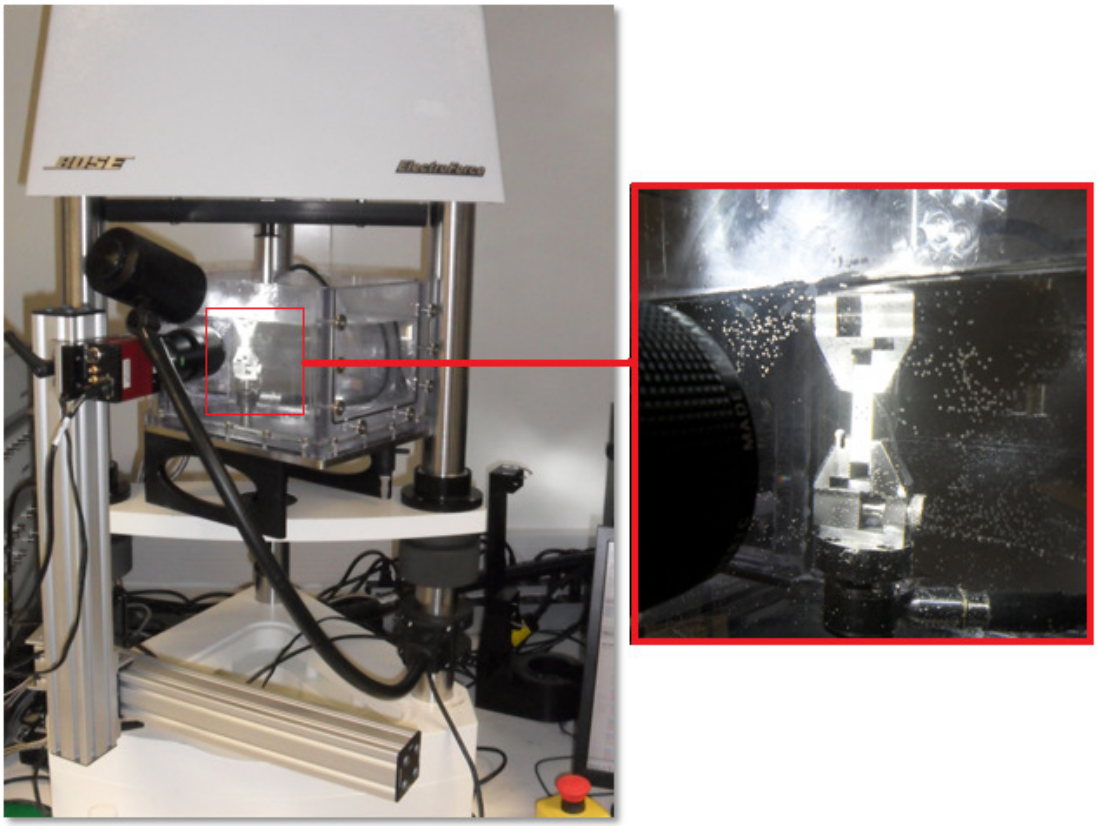

Figure 3-3 – Image of the Experimental Apparatus

The specimens were clamped firmly in place, and the gauge length measured (15mm). The specimens were then submersed in a water bath and allowed to equilibrate as the bath reached the standard in vivo temperature (37˚C). The effective gauge length of the specimens was 15mm, and this was measured using a measurement technique with a minimum measurement unit of 0.5mm. A tensile preload of 1N (40kPa) was applied before zeroing the measurements to confirm the clamps were adequately tightened.

The BOSE testing machine then performed a ramped strain, with an extension of 0.01mm at a displacement rate of 0.002mm/s. These values were chosen as 0.01mm is less than 0.1% extension of the specimen so no yielding should be present. Currey declared that bones rarely exceed strains of $0.005s^{-1}$ in-vivo. $0.002s^{-1}$ is amongst the average strain rates seen during physiological loading, as well as in experimental testing (5) (85). It has been shown that strain rates within the range $0.002\text{-}0.009s^{-1}$are shown to display viscoelastic behaviour in bone (86). The subsequent strain rate chosen was far below the maximum in-vivo strain rates, where vigorous activity can produce strain rates of up to $0.03s^{-1}$ (5).

After the ramp peaked, the load was reduced to a load of 1N. After this, the specimen underwent cyclic tensile loading, where the specimen was subjected to a stress range of 2MPa at a frequency of 1Hz for 120 cycles. Frequencies of between 0.02-2Hz are usually used to inspect changes seen in cyclic loading (86). The mean stress experienced was 1.2MPa.

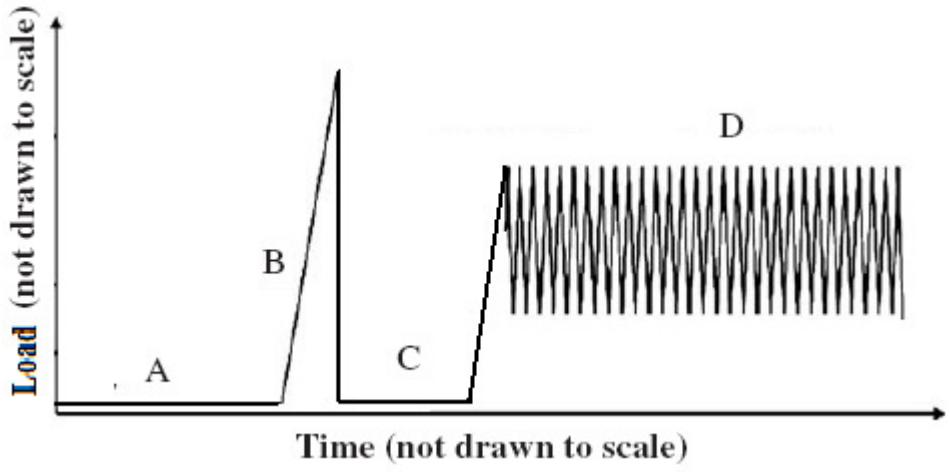

Figure 3-4 – Schematic of Tensile Testing Protocol

Segment A refers to the 15minute dwell period before the testing began with a preload of 1N. B refers to the ramping at a displacement rate of 0.002mm/s to reach an extension of 0.01mm. C is another dwelling period for 1minute to allow relaxation of the specimen. D is the cyclic loading at 1Hz with a stress range of approximately 2MPa

All the collected data was compiled together in each of the thirteen groups were compared to the control group and each other. Values from each specimen were statistically analysed to their own group specimens with the use of t-tests. An alpha value of 0.05 was

utilised to determine statistical significance. Then the mean of each group will be statistically tested against the other groups using t-tests and ANOVA.

Altogether, five parameters were collected from each specimen to help characterise the behaviour of bone after irradiation. These were as follows;

- Young's Modulus [E] – This describes the stiffness of the material.
- Storage Modulus [$E^{'}$] – This represents the elastic portion of bone by measuring the stored energy in the specimen.
- Loss Modulus [$E^{''}$] – This represents the viscous portion of bone by measuring the energy dissipated by the specimen.
- Loss Tangent (also known as Dissipation Factor) [tan δ] – This is the measure of the inherent dissipation of energy from the material
- Poisson's Ratio [υ] – This relationship is the ratio of longitudinal expansion divided by the ratio of transverse compression.

3.3.1 Calculating the Phase Lag and Dissipation Factor

The dissipation factor (tanδ) quantifies the loss of energy in a system after mechanical oscillations. The energy lost is usually in the form of heat. This parameter can be quantified by implementing the following equations;

The time-dependent sinusoidal stress is defined as

$$\sigma(t) = \sigma_o . \sin(\omega t + \delta)$$

And the time-dependent sinusoidal strain is

$$\varepsilon(t) = \varepsilon_o . \sin(\omega t + \delta)$$

Rearranging

$$\omega t + \delta_2 = \sin^{-1} \frac{\varepsilon(t)}{\varepsilon_o}$$

$$\omega t + \delta_1 = \sin^{-1} \frac{\sigma(t)}{\sigma_o}$$

Finally the Strain Phase Lag can be extracted from the previous equations to give

$$\delta_1 = \sin^{-1} \frac{\sigma(t)}{\sigma_o} - \omega t$$

$$\delta_2 = \sin^{-1} \frac{\varepsilon(t)}{\varepsilon_o} - \omega t$$

$$\delta = \delta_2 - \delta_1$$

With the phase lag, the dissipation factor as well as the Storage and Loss Modulii can be calculated from experimental data with the following equations;

$$E' = \frac{\sigma_0}{\varepsilon_0} \cos\delta$$

$$E'' = \frac{\sigma_0}{\varepsilon_0} \sin\delta$$

Where σ_0 is the applied stress amplitude and ε_0 is the resulting strain amplitude. Finally, the Dynamic Modulus can be obtained by using the Storage and Loss Modulii in the following equation

$$E_{DM} = \sqrt{\left(\left(E'^2\right) + \left(E''^2\right)\right)}$$

4. Results

4.1 Effect of Radiation on Young's Modulus

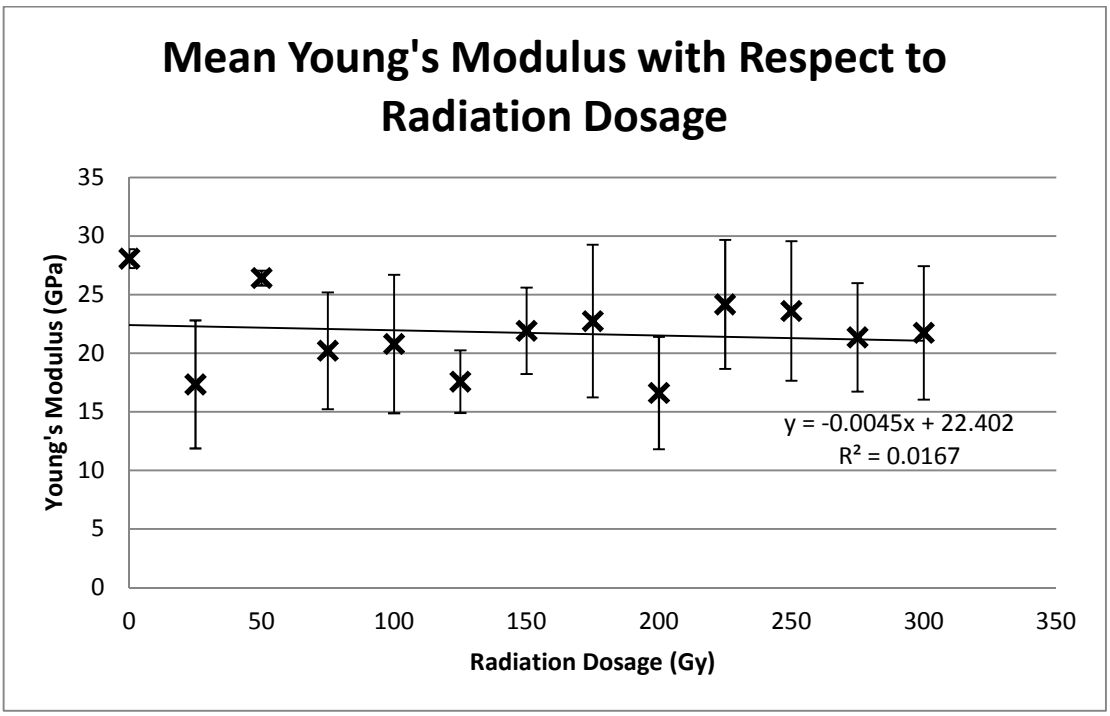

Figure 4-1 – Mean Young's Modulus with Respect to Radiation Dosage

Figure 4-1displays the Young's Modulus (E_{YM}) of cortical bone as radiation is increased. It is quite clear that the there appears to be very little change in the Young's Modulus throughout the entire spectrum of specimens, although a small linear decrease has been shown in the diagram.

Dose (Gy)	Medial	±1SD	n	Lateral	±1SD	n	Anterior	±1SD	n	Posterior	±1SD	n	Mean	±1SD	n_{TOT}
0	29.025	2.440	2	28.000	2.821	5	27.050	2.334	2	28.200	1.577	4	28.069	0.811	13
25	17.340	6.316	3	12.840	N/A	1	14.120	1.926	3	25.020	2.969	4	17.330	5.465	11
50	27.240	0.719	3	26.410	0.460	2	25.700	1.895	2	26.280	1.983	4	26.410	0.634	11
75	N/A	N/A	0	22.603	1.638	6	23.540	2.115	4	14.470	3.917	2	20.204	4.988	12
100	21.060	2.971	4	24.640	0.721	2	25.070	1.092	4	12.340	N/A	1	20.778	5.905	11
125	14.970	5.875	3	18.060	0.191	2	16.150	1.542	2	21.100	1.915	3	17.570	2.676	10
150	17.890	2.004	4	25.470	1.232	6	24.570	2.186	4	19.700	2.793	2	21.908	3.688	16
175	27.930	1.794	5	23.770	5.503	4	13.290	0.948	2	25.950	2.478	6	22.735	6.522	17
200	15.150	0.657	2	22.080	2.070	4	18.360	4.016	3	10.790	0.368	2	16.595	4.795	11
225	23.980	6.480	3	19.100	2.871	2	21.690	7.500	4	31.870	3.041	2	24.160	5.513	11
250	27.290	1.548	6	N/A	N/A	0	26.770	2.592	3	16.720	7.354	2	23.593	5.958	11
275	22.030	3.137	5	25.600	3.382	3	N/A	N/A	0	16.410	6.117	2	21.347	4.633	10
300	21.840	6.196	4	27.380	1.442	4	15.980	1.280	4	N/A	N/A	0	21.733	5.701	12

Table 4.1:1 – Results of Mechanical Testing of Young's Modulus with Respect to Radiation Dosage

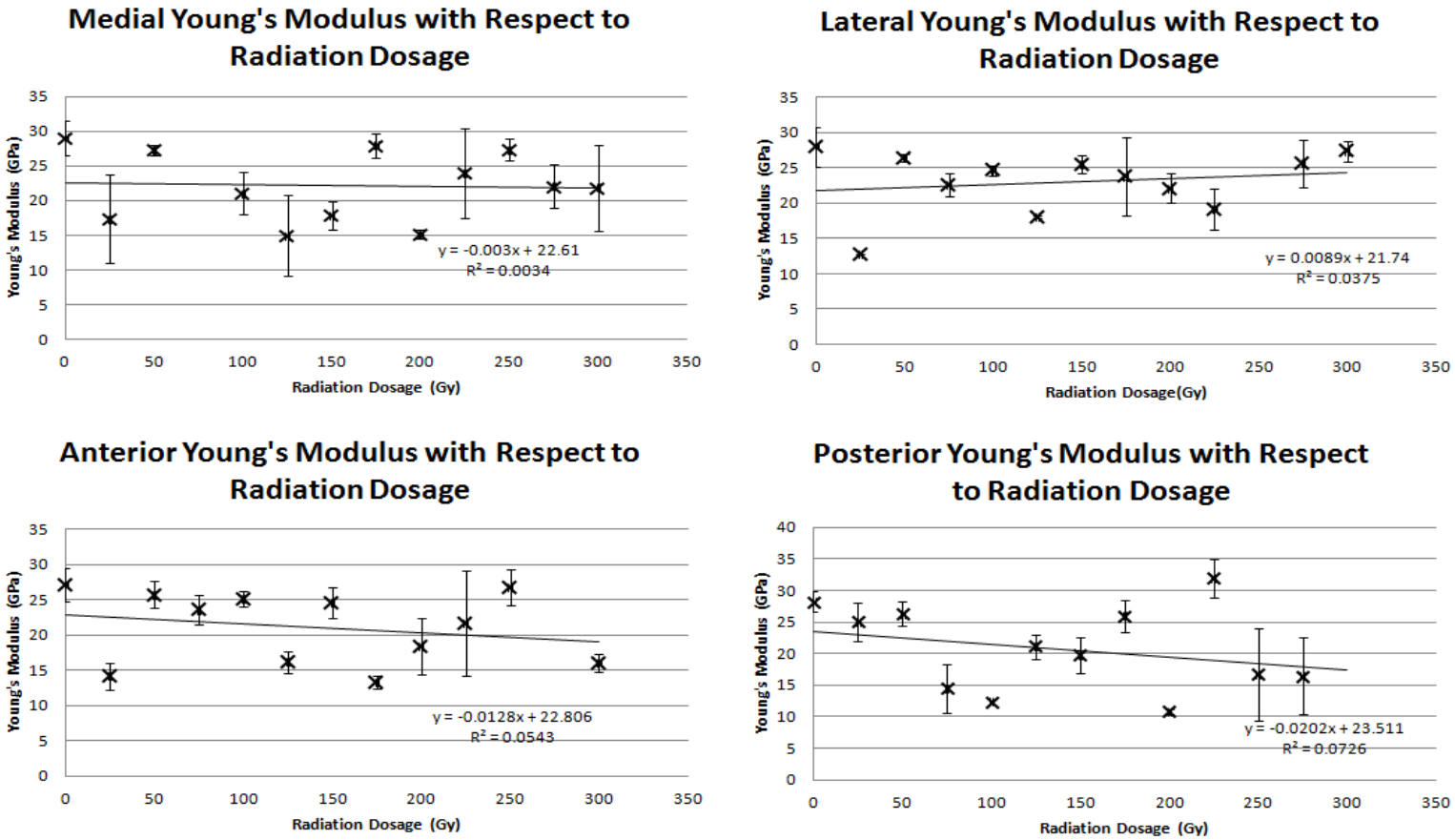

Figure 4-2– Medial, Lateral, Anterior and Posterior Graphs of Young's Modulus with Respect to Radiation Dosage

A linear decrease is seen in three of the four anatomical groups, with only lateral specimens showing an increase in Young's Modulus as doses of Radiation increase. On average, the lateral portion of bone appeared to be the stiffest, with posterior being least stiff as shown in Figure 4-3. The four groups (Medial (n=46), Lateral (n=43), Anterior (n=40) and Posterior (n=35)) were summed together to give the experiment 164 specimens.

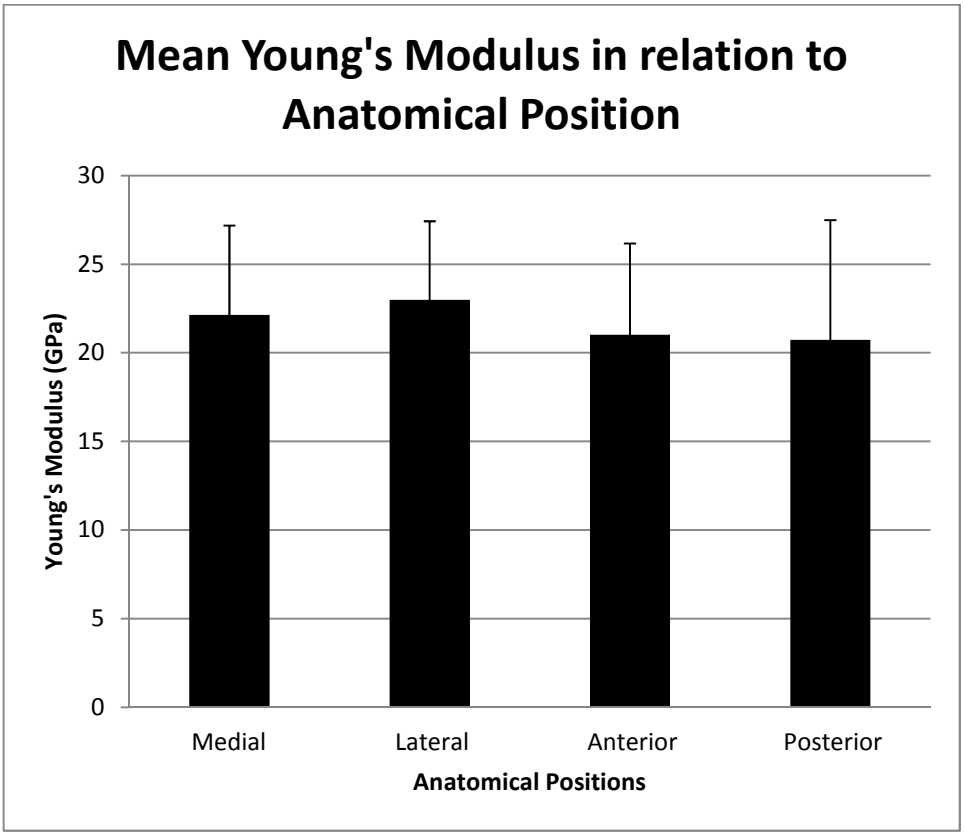

Figure 4-3 – Young's Modulus in Relation to Anatomical Position

4.1.1 T-Test Analysis of Young's Modulus

T-tests were carried out on the specimens to see whether the values obtained were statistically different to the control group. A p-value of 0.05 was used to determine statistical significance. Shown in the following table, less than half of the groups were statistically different from the control group.

Dose (Gy)	Mean	±1SD	P-Values in Relation to Non-irradiated Group
0	28.069	0.811	N/A
25	17.330	5.465	**0.03**
50	26.410	0.634	**0.02**
75	20.204	4.988	0.11
100	20.778	5.905	0.09
125	17.570	2.676	**0.00**
150	21.908	3.688	**0.04**
175	22.735	6.522	0.20
200	16.595	4.795	**0.02**
225	24.160	5.513	0.25
250	23.593	5.958	0.32
275	21.347	4.633	0.13
300	21.733	5.701	0.19

Table 4.1:2 – T-Test Results for Young's Modulus with Respect to Control

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4.1.2 ANOVA Results of Young's Modulus

An ANOVA test was carried out on the data for Young's Modulus (please find all ANOVA test results in Appendices (Section 9.1)) to see whether the results in the t-test portrayed any alteration in the mechanical properties of bone. The anatomical positions were inspected to see whether there was any statistical difference between the means of the Young's Modulus of each group. As bone is a naturally occurring material, different individuals will display different properties, such as strength, stiffness and mineral content.

This fact was taken into account when analyzing the data using ANOVA also. Finally, the dose received was also taken into account to see whether the dose caused any significant change in the data. Therefore, a three-factor ANOVA was performed using Minitab software (v16.0.0), taking into account the dose received, the individual bone and the anatomical position. Also the interactions between each of these factors were inspected.

	Degrees of Freedom	F-Value	P-Value
Main Effects	3	0.39	0.758
Dose	1	0.36	0.553
Bone	1	0.01	0.907
Position	1	0.76	0.387
2-way Interactions	3	0.19	0.904
Dose & Bone	1	0.02	0.898
Dose & Position	1	0.55	0.461
Bone & Position	1	0.01	0.927

Table 4.1:3 – ANOVA results for single and two-way effects

From the results shown in Table 4.1:3, is can be shown that ANOVA demonstrates that there is no statistical significance seen in any of these factors or any of the possible interactions between these factors. Therefore the trends shown in the previous graphs have to be taken to have occurred due to random variability, as well as the t-test results.

4.2 Effects of Radiation on Poisson's Ratio

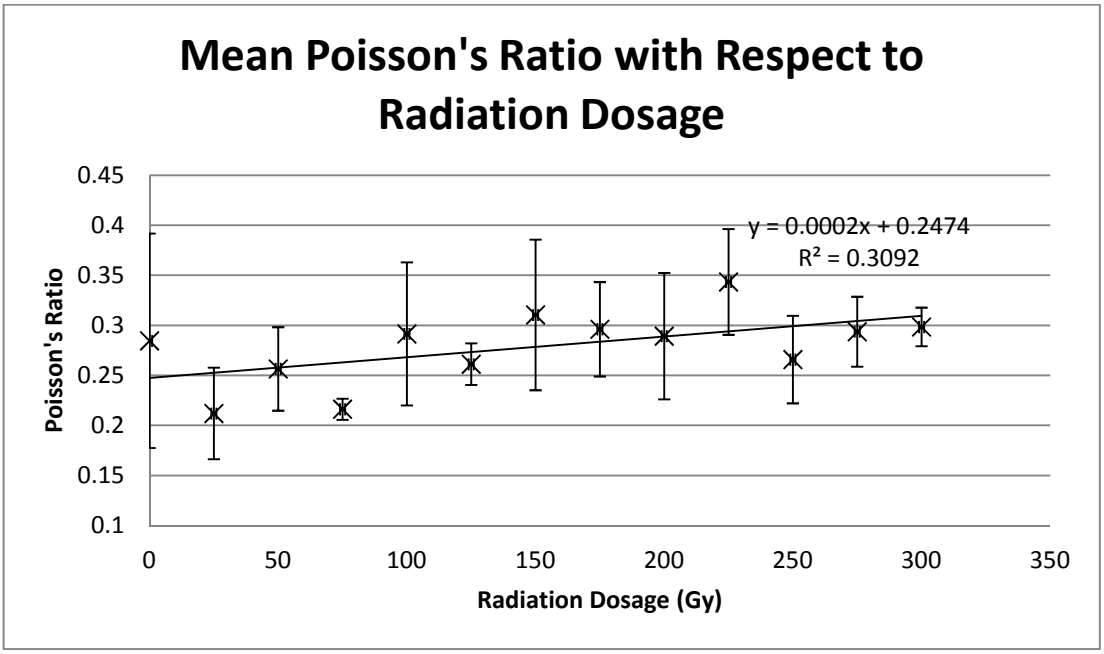

Figure 4-4 – Mean Poisson's Ratio with Respect to Radiation Dosage

The effect of irradiation of the Poisson's Ratio of bone appears to be that Poisson's Ratio increases with increasing radiation. T-tests were carried out on the results which are found in Table 4.2:1 on the following page.

Dosage	Mean	±1SD	n	P-Values from T-test compared to Control
0	0.285	0.107	12	N/A
25	0.212	0.046	10	0.28
50	0.257	0.042	11	0.65
75	0.216	0.011	12	0.29
100	0.292	0.072	11	0.92
125	0.261	0.021	11	0.70
150	0.310	0.075	15	0.71
175	0.296	0.047	14	0.85
200	0.289	0.063	11	0.94
225	0.343	0.053	11	0.38
250	0.266	0.044	11	0.77
275	0.294	0.035	9	0.88
300	0.298	0.019	11	0.82

Table 4.2:1 – T-Test Results for Poisson's Ratio

As all the P-values are above the significance factor (p=0.05), all groups are statistically similar to the control group. To further explore the effects that the radiation had on bone, ANOVA was utilized. In each of the four anatomical positions there appears to be an increasing trend in Poisson's Ratio, except with the anterior specimens.

Dosage	Medial	±1SD	n	Lateral	±1SD	n	Anterior	±1SD	n	Posterior	±1SD	n	Mean	±1SD	n_{TOT}
0	0.301	0.231	2	0.242	0.210	5	0.424	N/A	1	0.171	0.176	4	0.285	0.107	12
25	0.223	0.075	3	0.183	N/A	1	0.272	0.042	2	0.170	0.130	4	0.212	0.046	10
50	0.312	0.132	3	0.259	0.058	2	0.244	0.070	2	0.212	0.053	4	0.257	0.042	11
75	N/A	N/A	0	0.213	0.066	6	0.208	0.062	4	0.228	0.139	2	0.216	0.011	12
100	0.332	0.008	4	0.307	0.065	2	0.186	0.082	4	0.341	N/A	1	0.292	0.072	11
125	0.271	0.059	3	0.243	0.046	2	0.286	0.057	2	0.245	0.063	4	0.261	0.021	11
150	0.306	0.103	4	0.220	0.061	6	0.404	0.047	4	0.312	N/A	1	0.310	0.075	15
175	0.308	0.099	3	0.279	0.075	4	0.355	0.024	2	0.243	0.063	5	0.296	0.047	14
200	0.294	0.138	2	0.238	0.087	4	0.248	0.100	3	0.377	0.066	2	0.289	0.063	11
225	0.343	0.048	3	0.346	0.014	2	0.278	0.071	4	0.407	0.026	2	0.343	0.053	11
250	0.285	0.090	6	N/A	N/A	0	0.216	0.106	3	0.297	0.188	2	0.266	0.044	11
275	0.255	0.091	4	0.305	0.042	4	N/A	N/A	0	0.322	N/A	1	0.294	0.035	9
300	0.278	0.107	4	0.316	0.049	4	0.302	0.010	3	N/A	N/A	0	0.298	0.019	11

Table 4.2:2 – Results of Mechanical Testing for Poisson's Ratio in Bone Specimens

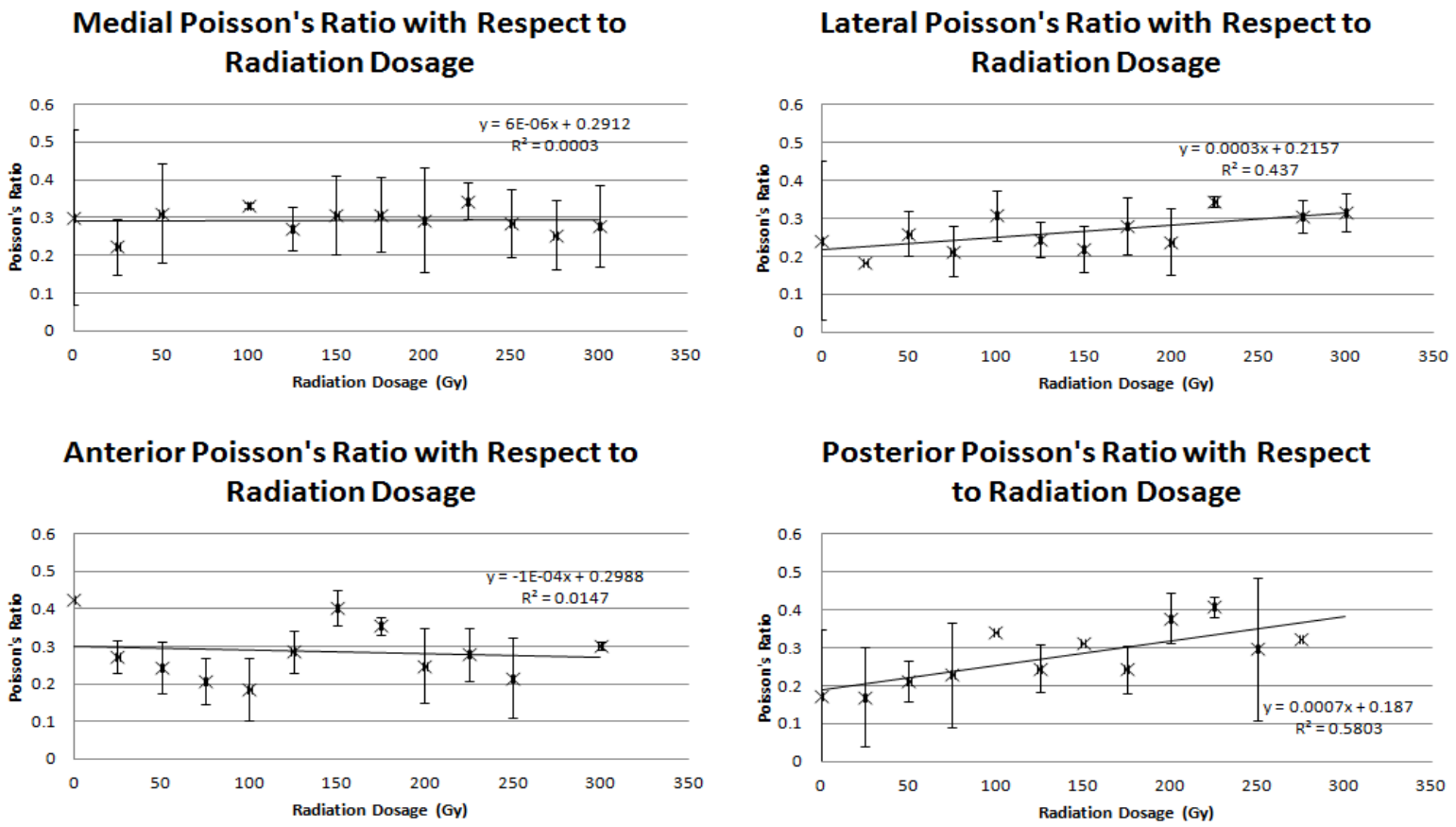

Figure 4-5 – Medial, Lateral, Anterior and Posterior Graphs of Poisson's Ratio with Respect to Radiation Dosage

4.2.1 ANOVA Results of Poisson's Ratio

	Degrees of Freedom	F-Value	P-Value
Main Effects	3	1.58	0.207
Dose	1	4.66	**0.036**
Bone	1	0.09	0.763
Position	1	0.07	0.797
2-way Interactions	3	1.34	0.274
Dose & Bone	1	0.19	0.664
Dose & Position	1	2.25	0.140
Bone & Position	1	1.74	0.194

Table 4.2:3 – ANOVA Results from Single and Two-Way Effects

In Table 4.2:3 shown above, all factors surpass the significance factor of 0.05, with the exception of the dosage administered. This suggests that there is a significant difference that occurs as the dose in increased when taking into account the individual bone as well as the anatomical position.

4.3 Effects of Irradiation on the Dissipation Factor

After analysing the phase shift between the applied stress and the resulting strain, it was found that the phase shift increased by a small degree from the control to the irradiated specimens. The dissipation factor grew gradually as shown in Figure 4-6, marking an increase in the internal friction of bone.

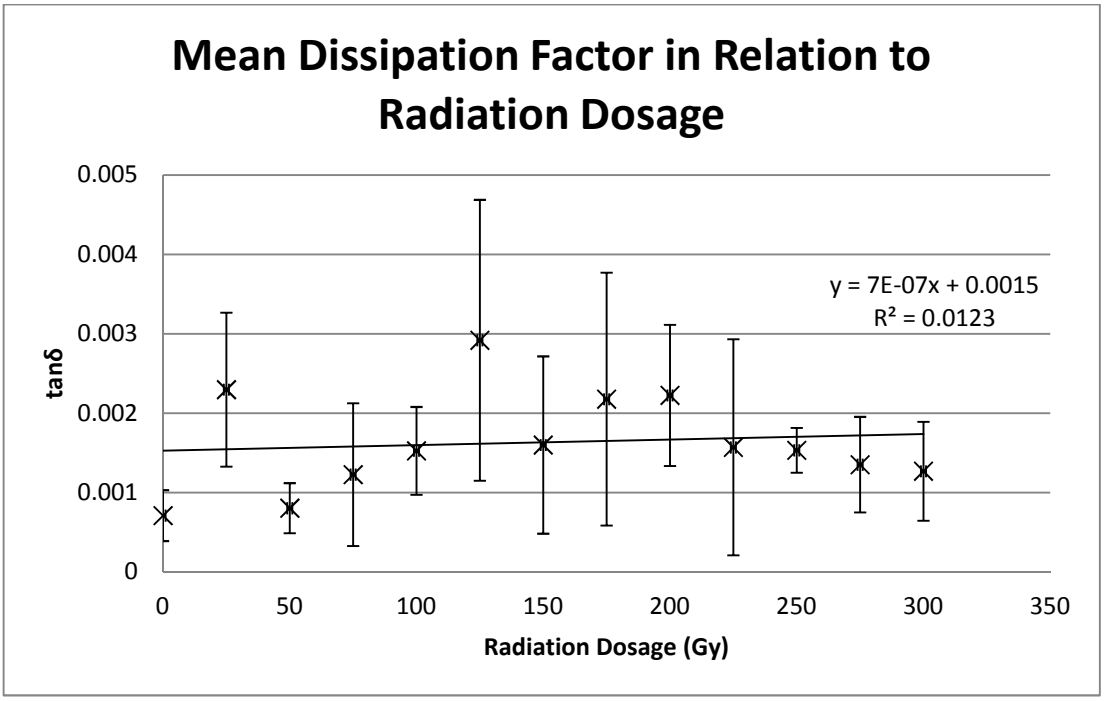

Figure 4-6 – Mean Dissipation Factor with Respect to Radiation Dosage

Dose (Gy)	Mean (10^{-3})	±1SD (10^{-3})	P-Values in Relation to Non-irradiated Group
0	0.710	0.321	N/A
25	2.297	0.971	**0.041**
50	0.804	0.316	0.253
75	1.236	0.900	0.428
100	1.526	0.553	0.053
125	2.920	1.769	**0.038**
150	1.600	1.117	0.211
175	2.177	1.593	0.162
200	2.225	0.889	**0.036**
225	1.570	1.361	0.298
250	1.532	0.281	**0.017**
275	1.350	0.603	0.197
300	1.296	0.624	0.257

Table 4.3:1 – T-Test Results of Dissipation Factor with Respect to the Control Group

T-tests were performed to determine whether the values were statistically similar or different compared to the control specimen. Like before, an α-value of 0.05 was chosen to clarify statistical significance. It was found that there were a number of the groups which were statistically different to the control group.

4.3.1 ANOVA Results of Dissipation Factor

	Degrees of Freedom	F-Value	P-Value
Main Effects	3	1.97	0.131
Dose	1	2.56	0.116
Bone	1	0.82	0.370
Position	1	1.9	0.175
2-way Interactions	3	0.54	0.655
Dose & Bone	1	0.59	0.446
Dose & Position	1	0.5	0.485
Bone & Position	1	0.08	0.780

Table 4.3:2 – ANOVA Results for Single and Two-Way Effects

Similarly to the analysis of Young's Modulus, there appears to be no statistical significance within the data corresponding to any of the factors shown in the Table above, although the dose administered approaches statistical significance. This would indicate that the trends in Figures 4.8 & 4.9 are merely due to random variation within the data.

Dose (Gy)	Medial (10^{-3})	±1SD (10^{-3})	N	Lateral (10^{-3})	±1SD (10^{-3})	N	Anterior (10^{-3})	±1SD (10^{-3})	N	Posterior (10^{-3})	±1SD (10^{-3})	N	Mean (10^{-3})	±1SD (10^{-3})	N_{TOT}
0	0.336	3.646	2	0.903	0.310	5	0.560	0.610	2	1.040	0.285	4	0.710	0.321	13
25	2.998	0.975	3	2.930	N/A	1	2.353	0.229	3	0.907	0.392	4	2.297	0.971	11
50	0.545	0.437	3	0.604	0.347	3	1.242	0.578	4	0.823	0.160	4	0.804	0.316	11
75	N/A	N/A	0	2.033	0.758	6	1.389	0.507	4	0.256	0.232	2	1.226	0.900	12
100	1.193	0.281	4	1.225	0.086	2	1.337	0.874	4	2.350	N/A	1	1.526	0.553	11
125	2.259	1.369	3	2.378	0.077	2	1.529	1.551	2	5.513	4.211	3	2.920	1.769	10
150	3.143	2.021	4	1.033	0.126	6	0.581	0.151	4	1.641	0.347	2	1.600	1.117	16
175	1.283	1.588	5	1.795	3.112	4	4.526	2.368	2	1.104	2.070	6	2.177	1.593	17
200	1.342	1.163	3	1.607	0.254	4	2.778	1.732	4	3.175	0.297	2	2.225	0.889	11
225	1.246	1.653	3	3.453	3.122	2	1.378	0.566	4	0.203	0.174	2	1.570	1.361	11
250	1.600	2.028	6	N/A	N/A	0	1.223	1.182	3	1.774	1.055	2	1.532	0.281	11
275	1.220	0.531	5	0.826	0.809	3	N/A	N/A	0	2.009	1.198	2	1.352	0.603	10
300	1.065	1.222	4	1.970	2.922	4	0.773	1.781	4	N/A	N/A	0	1.269	0.624	12

Table 4.3:3 – Results of Mechanical Testing for Dissipation Factor

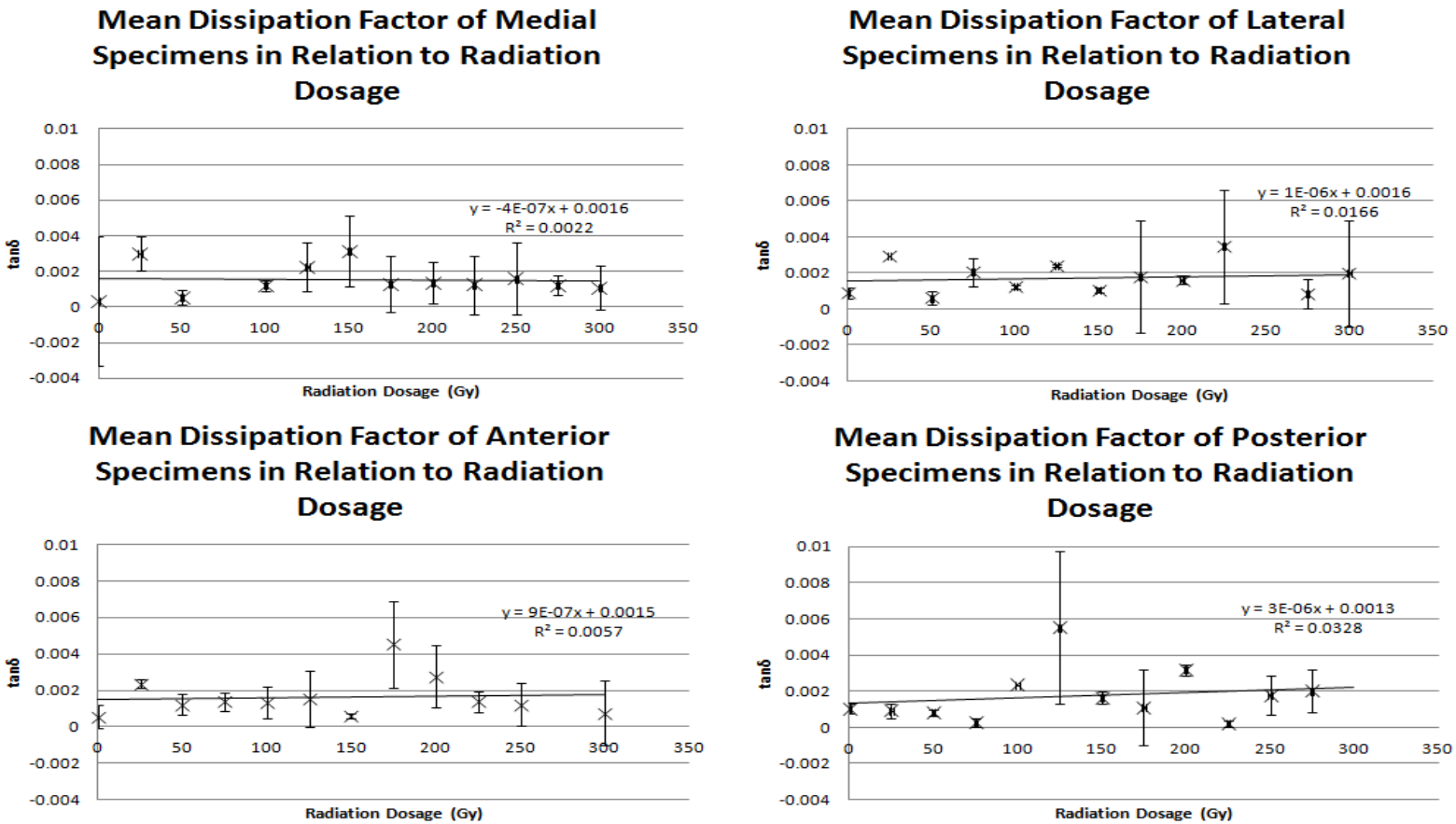

Figure 4-7 – Medial, Lateral, Anterior and Posterior Dissipation Factors with Respect to Radiation Dosage

4.4 Effect of Radiation on the Storage and Loss Modulii

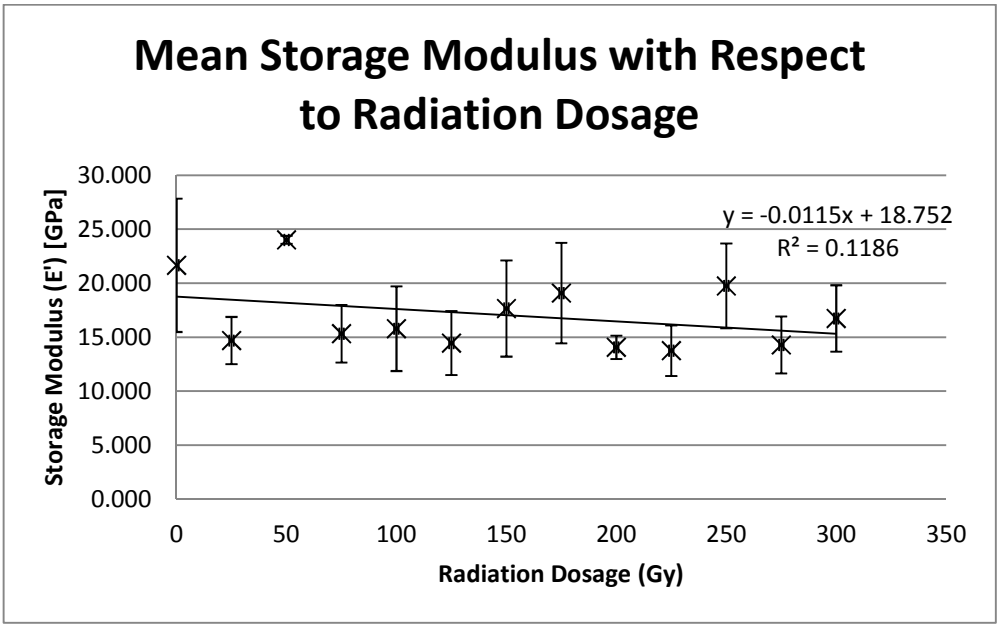

Figure 4-8 – Mean Storage Modulus with Respect to Radiation Dosage

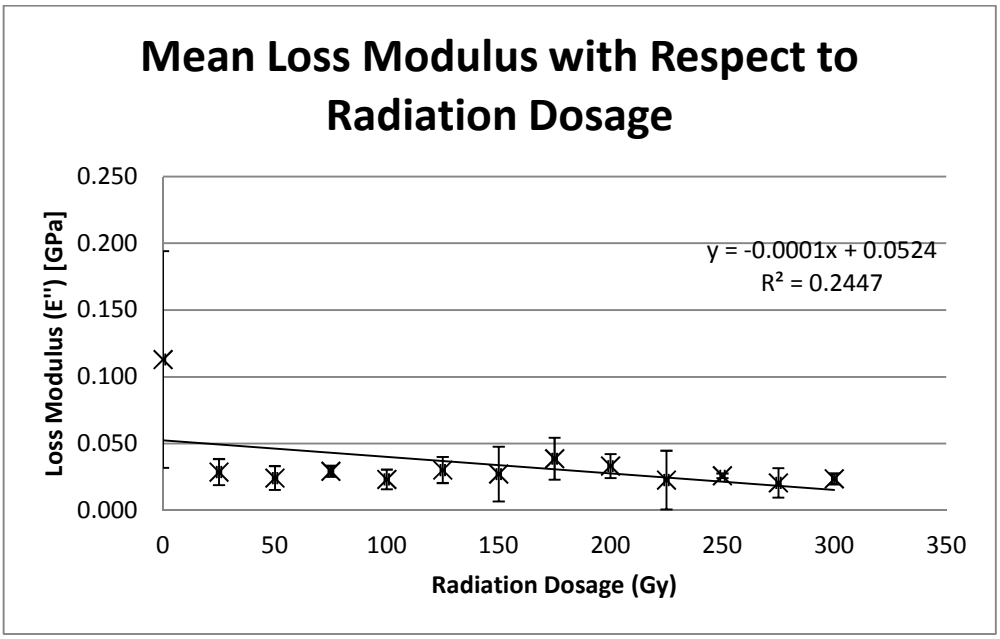

Figure 4-9 – Mean Loss Modulus with Respect to Radiation Dosage

Radiation Dosage (Gy)	Storage Modulus (E') [GPa]	±1S.D.	Loss Modulus (E'') [GPa]	±1S.D.	Phase Lag (δ˚)	n	P-value with Respect to Control
0	21.674	6.179	0.113	0.081	0.012	13	N/A
25	14.702	2.192	0.029	0.010	0.040	11	0.515
50	24.027	0.373	0.024	0.009	0.014	11	0.387
75	15.340	2.664	0.029	0.004	0.021	12	0.320
100	15.796	3.918	0.023	0.007	0.027	11	0.499
125	14.461	2.962	0.030	0.010	0.051	10	0.512
150	17.671	4.453	0.027	0.020	0.028	16	0.806
175	19.098	4.662	0.039	0.016	0.038	17	0.839
200	14.079	1.085	0.033	0.009	0.039	11	0.479
225	13.769	2.340	0.023	0.022	0.027	11	0.831
250	19.763	3.921	0.026	0.002	0.027	11	0.206
275	14.299	2.643	0.021	0.011	0.024	10	0.272
300	16.747	3.076	0.024	0.004	0.022	12	0.567

Table 4.4:1 – Storage and Loss Modulii, as well as the Phase Lag with respect to Radiation Dosage

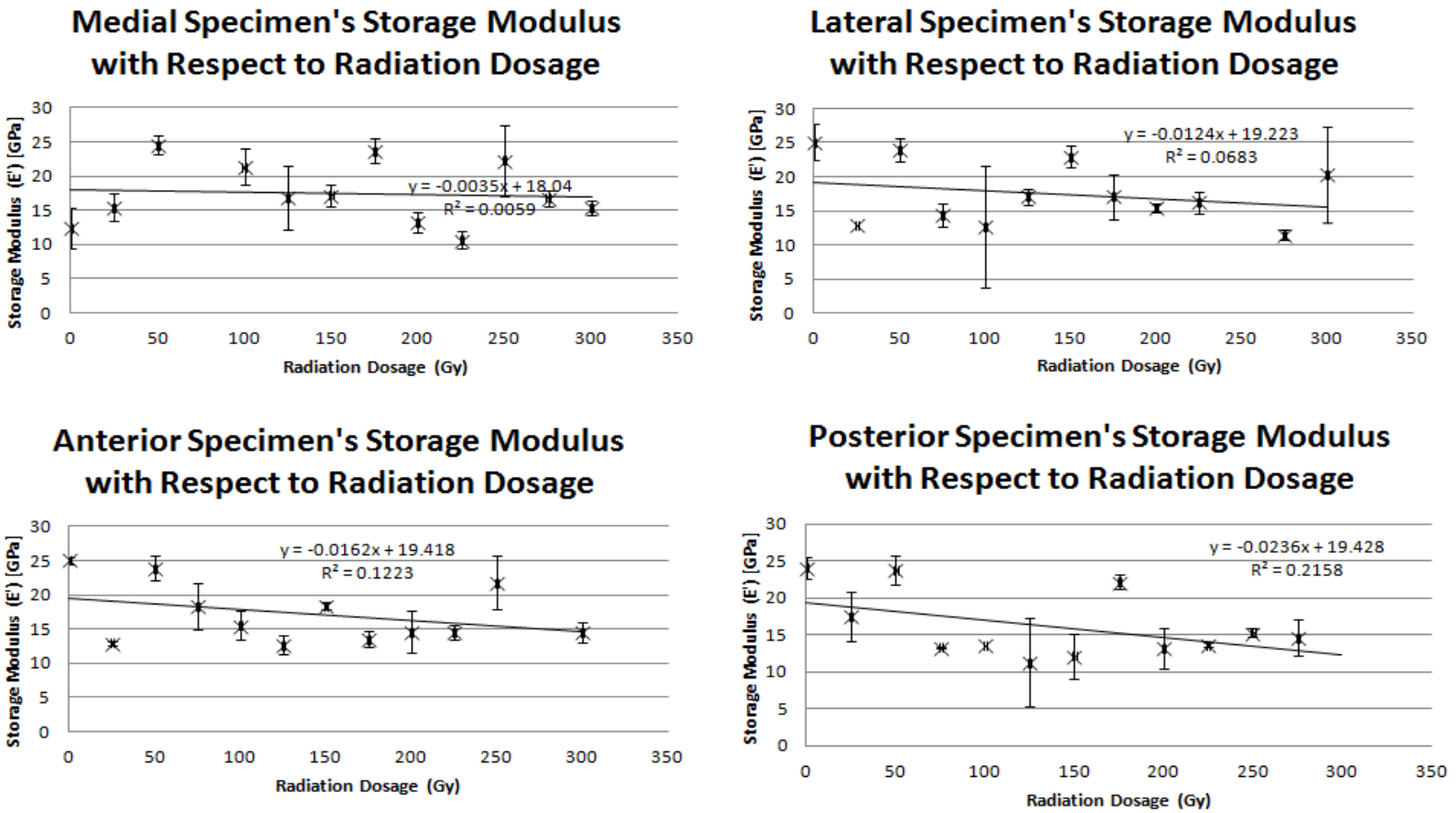

Figure 4-10 – Medial, Lateral, Anterior and Posterior Storage Modulii with Respect to Radiation Dosage

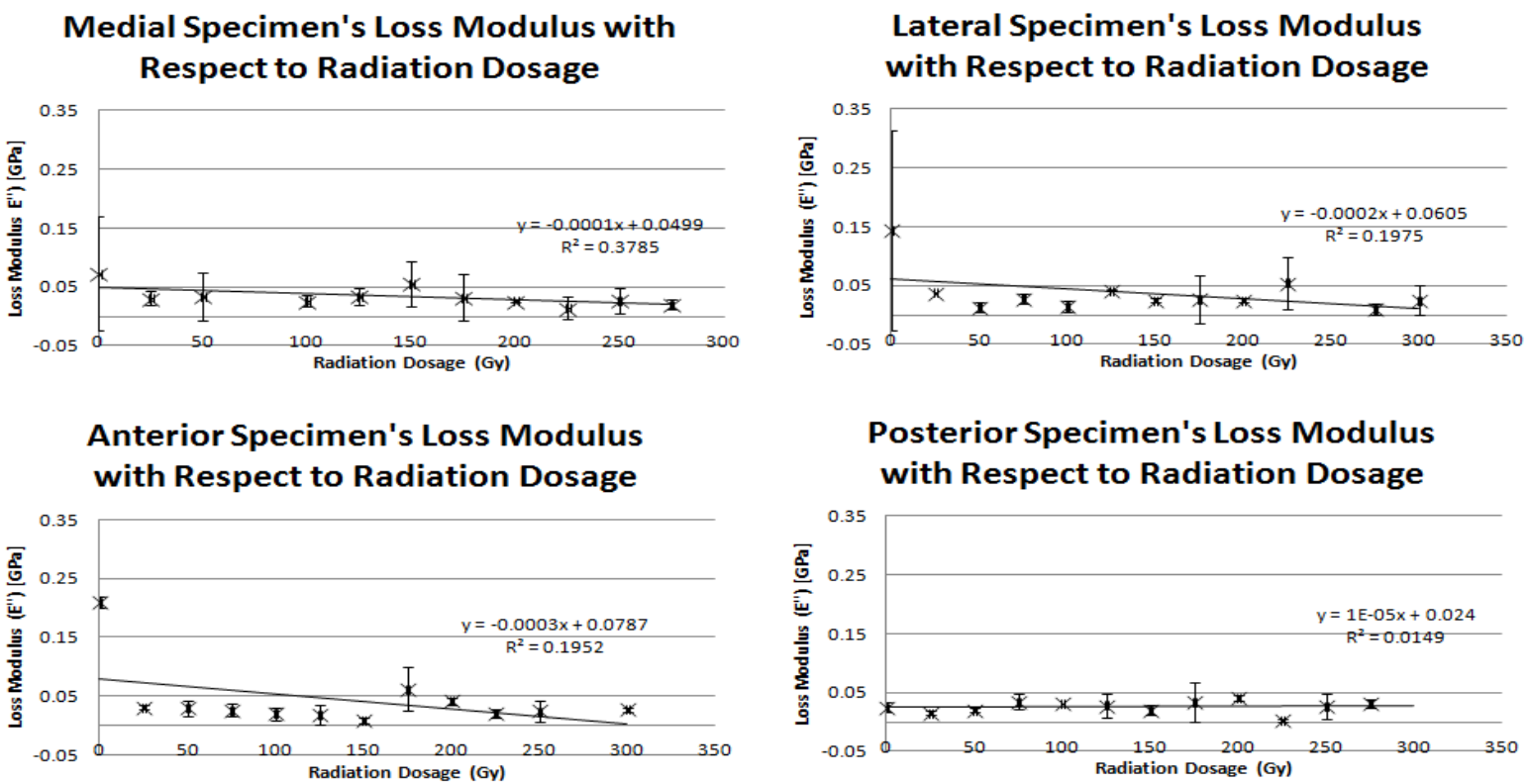

Figure 4-11 - Medial, Lateral, Anterior and Posterior Loss Modulii with Respect to Radiation Dosage

From data in Table 4.4:1, the mean Dynamic Modulus was calculated as demonstrated in Section 3.4.1. This was plotted against the Young's Modulus obtained in Table 4.1:1. The findings show that on average, the specimens are less stiff during the sinusoidal loading. However, the Dynamic Modulus does show a greater decrease as the irradiation dosage is increased shown below in Figure 4-12.

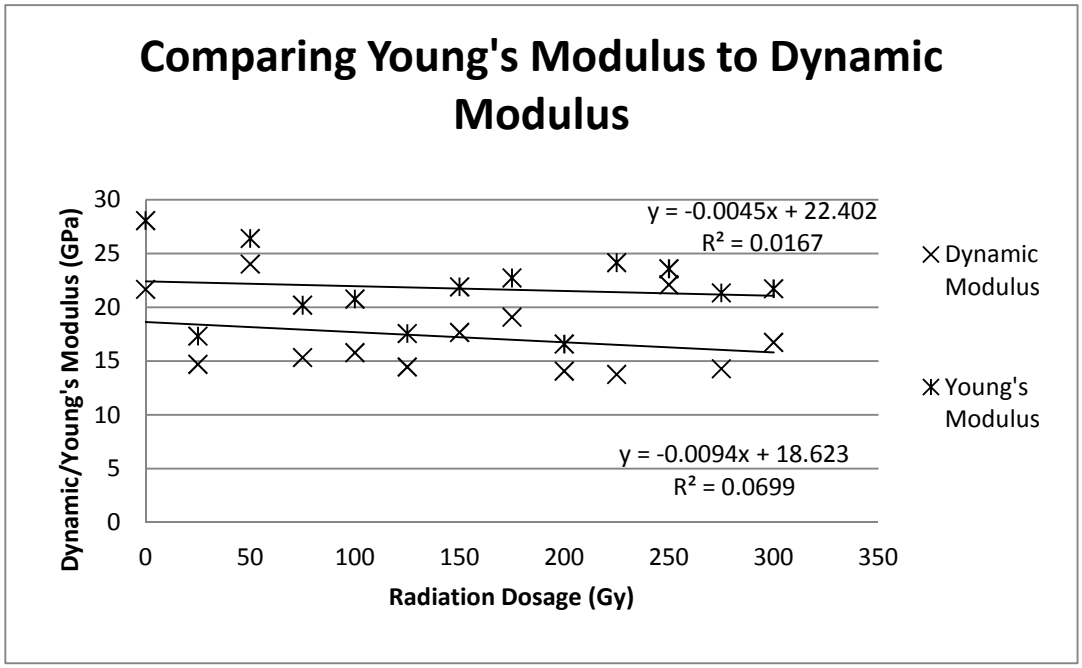

Figure 4-12 – Comparison between Young's Modulus and Dynamic Modulus

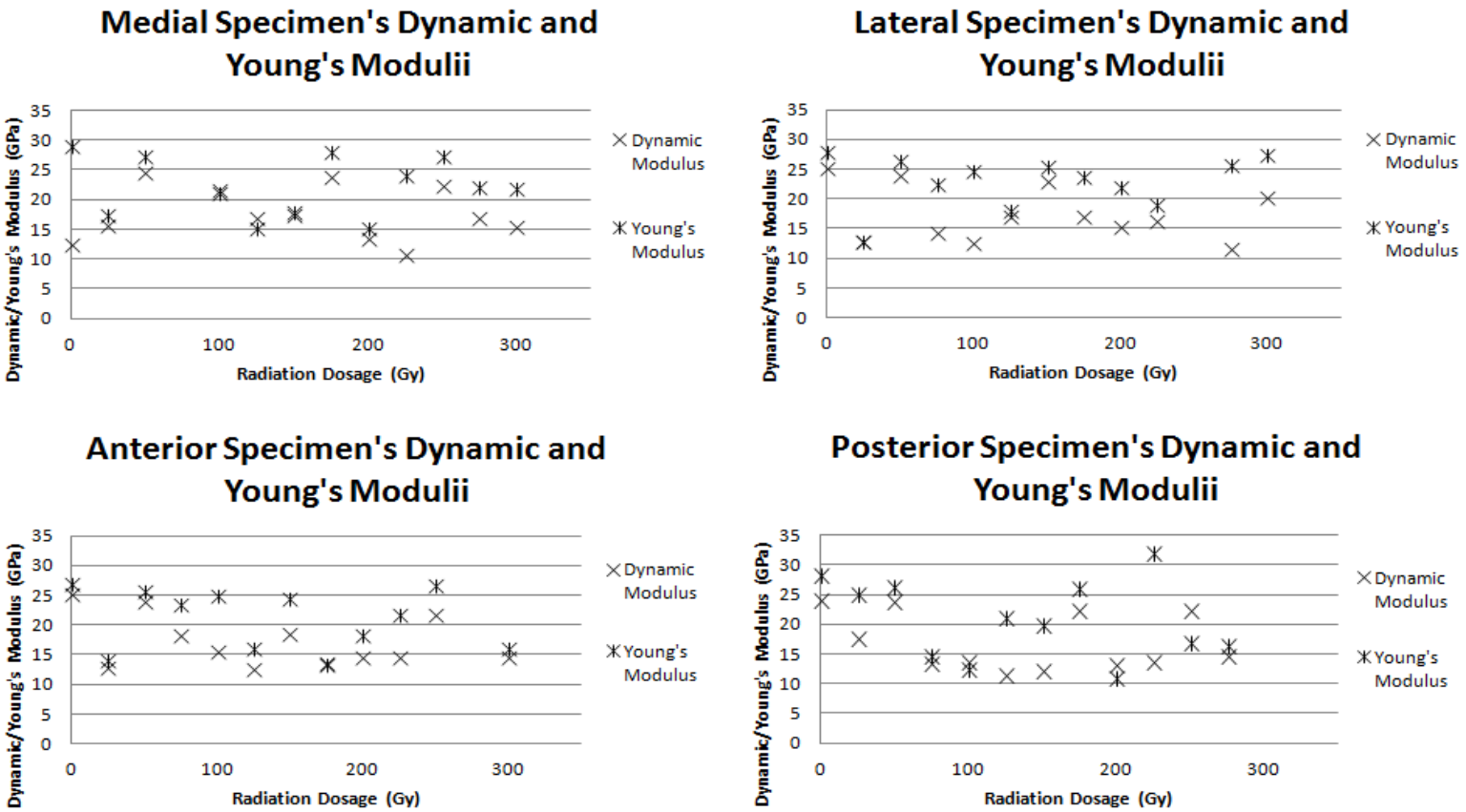

Figure 4-13 - Medial, Lateral, Anterior and Posterior Comparisons of Dynamic and Young's Modulus with Respect to Radiation Dosage

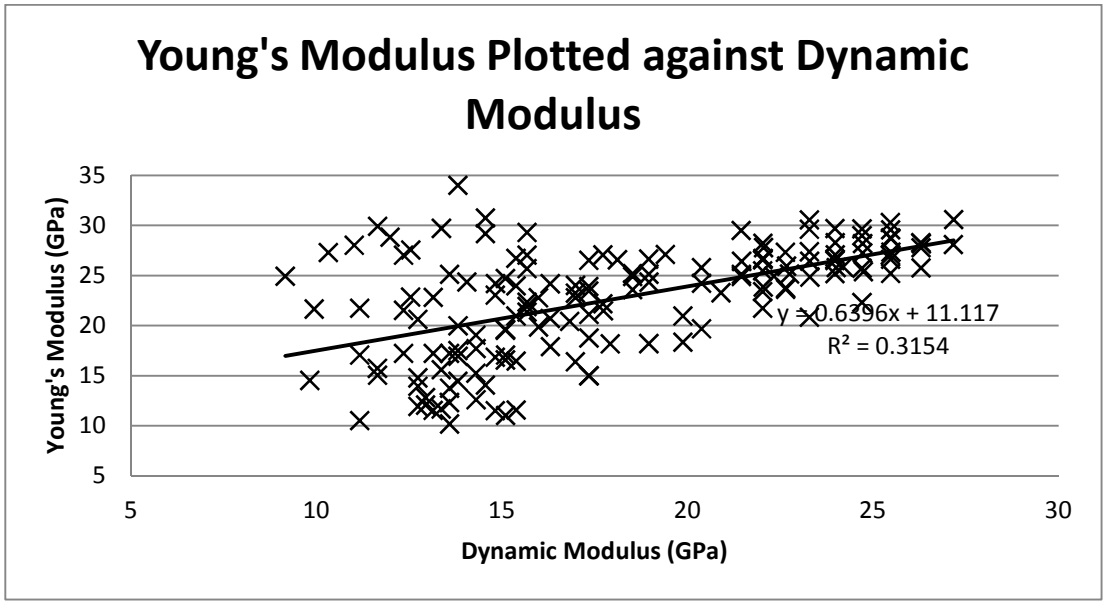

Figure 4-14 – Young's Modulus plotted against Dynamic Modulus

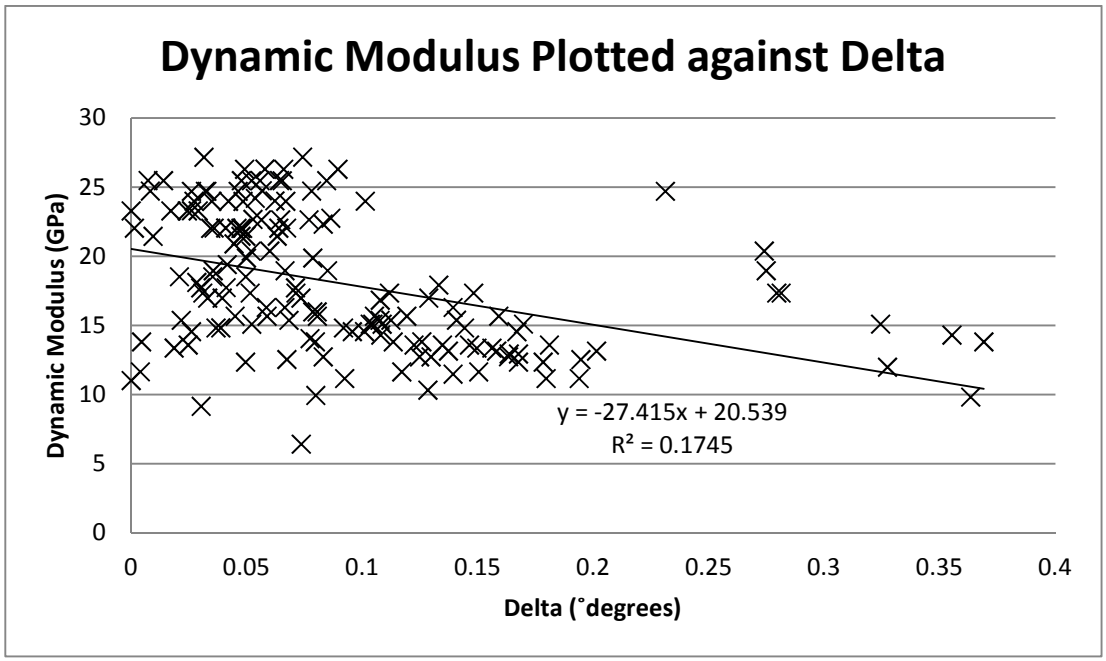

Figure 4-15 – Dynamic Modulus Plotted against Delta

5. Discussion

5.1 Young's Modulus Analysis

In Figure 4-1, the Young's Modulus displays a negligible trend as bone is subjected to increasing doses of irradiation. This would agree with literature which states that the mineral phase of bone (hydroxyapatite) is responsible for the stiffness of the bone, and that the mineral content is largely unaffected by irradiation from the subsequent development of free radicals (4).

All of the bones displayed a Young's Modulus in the region of 20-25GPa except for Bone 12, which is considerably lower than the mean. Regardless of this fact, all values of Young's Modulus concur with the literature (See Table 2.4:2). This shows that there were no instances of individual bones possessing abnormal values of Young's Modulus before undergoing irradiation.

From Table 4.1:2, it can be deduced that there is no difference between the control and irradiated groups. Therefore, the trends seen in the bone in relation to Young's Modulus occur due to random variability. This statement agrees with some of the literature, where states that damage to the collagen network decrease both strength and toughness, but not the stiffness according to JD Currey (76).

5.2 Poisson's Ratio Analysis

In Figure 4-4 there is a clear behavioral difference in the specimens as the irradiation dosage is increased. This shows that the bone shows signs of becoming more incompressible. This may show that as the collagen is damaged, there are less dampening mechanisms in place which causes the specimen to become stronger, increasing the level of post-yield deformation. Due to the collagen damage, the bone experiences less viscoelastic behavior.

With regards to literature on bovine bone's Poisson's Ratio, it appears that the values obtained experimentally are similar to that recorded in previously recorded experiments. Although few comprehensive bovine results could be found, bovine, canine and human bone possess similar ranges, whilst equine appeared to be below the range seen in the experimental data (See Table 2.4:3).

In analyzing the data through AVOVA, it showed that the trend seen in the specimens was statistically significant between Poisson's Ratio and the received dose of irradiation. This supports the discussion previously that the damage to the collagen causes the bone to become more incompressible. In regards to the other factors explored, the ANOVA shows no statistical significance within the data.

5.3 Dissipation Factor Analysis

In Figure 4-6, a trend emerges from the data. However, as seen in Table 4.3:1, the increase in the internal friction of bone is minute which may have been caused by random variation in bone specimens. It appears that the increasing doses of irradiation had a negligible effect of the strain lag. The tanδ of the experiment appears to conform to other experimental data, with the total mean phase shift is 0.028˚. In comparison to literature, Wang & Feng demonstrated in their experiments that the control group of bovine femurs (n=7) had a tanδ=0.02˚ at 37˚C (4). Lakes and Katz showed that the Dissipation Factor remained at 0.01 from angular velocities from 10^3 – 1 rad/s (88). Therefore it has been clarified that the experimental data from this project directly fits in with previous literature.

In regards to the trend of the experimental data seen in Figures 4-8 and 4-9, it has been shown that demineralisation of bone, the temperature that bone is exposed to and the frequency of testing significantly affects the Storage and Loss Modulii as well as the Loss Tangent. As frequency increases, both the Storage and Loss Modulii increase, but subsequently cause the Dissipation Factor to decrease (89). The data shows a decrease in the Storage and Loss Modulus, but in the case of the Loss Modulus, the control specimens are quite high when compared to irradiated specimens, which exaggerates the trend seen in Figure 4-9. This was caused by obtaining one positive and one negative phase lag and getting the absolute value of each and then getting the mean. However, after statistical analysis in Table 4.4:1, these phenomena are the result of random variance in the specimens.

5.4 Comparison of Dynamic Modulus to Young's Modulus

When the Dynamic Modulus was compared to Young's Modulus in Figure 4-12, there was a notable difference. In nearly all sub-groups of the experiment, the Young's Modulus, calculated from the initial ramping of the specimens was higher than the Dynamic Modulus calculated from the cyclic load controlled test. This may show that the collagen

within the matrix of the bone has become damaged as a result of the cyclic loading as well as the radiation.

The Young's Modulus was then plotted against the Dynamic Modulus seen in Figure 4-14. This data behaviour is known as systematic heterogeneity, or also known as a funnel shape graph. There is a clear increasing trend in the data, and shows that errors are more expected at low values of a Dynamic Modulus.

This systematic heterogeneity was also seen in the plot of Dynamic Modulus against the Phase Shift of every individual specimen in Figure 4-15. However, unlike the previous example, this graph displays a decreasing trend. Due to the small number of points beyond a value of δ=0.2˚, one must be sceptical about the behaviour of the data. Errors are more likely to occur at low levels of phase change angles. Therefore, as δ increase, the Dynamic Modulus decreases.

5.5 Final Remarks

In all tests, it had been shown that there is a small change in the properties of bone after increasing doses of irradiation. However, when sterilising allografts for pelvic replacement/reconstruction, these undergo large levels of irradiation compared to Autografts due to sterilisation purposes (average sterilisation dose is 27.5Gy) (74). It has been shown that these natural scaffolds usually survive in vivo and cells will migrate into the allograft. Due to this fact, it seems unlikely that small levels of irradiation to autografts will cause severe damage to bone and alter the behaviour of bone significantly.

6. Conclusions

A number of conclusions have been drawn up from the influences of Irradiation Dosage on the Elastic and Viscoelastic Properties of Bone. They are found below;

1. In regards to the effects of Irradiation on the Elastic Properties of Bone it was shown that there was a small decreasing trend in Young's Modulus as the Irradiation Dose increases. In the literature it has been stated that hydroxyapatite is solely responsible for the stiffness of bone. However, the experimental data presented in this report show that collagen may have a small influence on the stiffness of bone. T-tests and ANOVA was performed on all groups, but proved to be largely inconclusive to show statistical significance.

2. Poisson's Ratio was also observed to show how Irradiation Doses affect the elastic properties of bone. Poisson's Ratio significantly increased, which may show that as bone undergoes radiation therapy, the specimen becomes stronger. This may increase the post-yield deformation level, causing the bone to become more incompressible due to a greater density of collagen cross-links according to Barth et al (81). A t-test was performed on all groups in relation to the control group and the results show that all groups are statistically similar to the control group. This conclusion should be carefully interpreted due to the large range of problems encountered during testing with the DVE.

3. To examine the effects of increasing Irradiation Doses on the viscoelastic properties of bone the dissipation factor ($\tan\delta$) and phase lag of strain to stress were evaluated. In relation to the dissipation factor, there was a very small increase from the control to the maximum irradiation dose (0.002 ± 0.002). This small increase is almost negligible and has to be evaluated to three decimal places. All anatomical positions of bone show the small trend. The phase lag is directly related to the dissipation factor and changed by a negligible degree as well ($0.28^{\circ}\pm0.15^{\circ}$). These results show that there appears to be very little influence of radiation on the internal friction and dampening mechanism of bone.

4. To evaluate the effect of irradiation of bone on the viscoelastic properties of bone, both the Storage (E') and Loss Modulii (E'') were inspected. In the Storage Modulus, there was a slight decrease in all four anatomical positions and in the Loss Modulus, there was the same decreasing trend seen in the Storage Modulus specimens except for the posterior group. T-tests confirm that all results obtained are statistically similar. These trends appear to be negligible, and can be accredited to the inhomogeneity in the property of bones.

5. Through experimental trial and error, a number of conclusions were drawn up on how to improve testing protocol in this area of research.
    a. Firstly, specimens should avoid being refrigerated for any length of time before testing, as it has been shown to decrease the stiffness of the material by the action of leaching. It is the belief of the author, and other researchers in the area that if the specimens were soaked with water instead of 0.9% saline solution, the leaching effect would have been more pronounced.
    b. As previously mentioned some of the specimens slipped when in the grips during testing and subsequently corrupted the data measured. To avoid problems like this in future, "dumb-bell" specimens should be created to firstly allow a greater surface area for gripping, but also to reduce stress concentrations that build up in the proximity of the grips.
    c. In using the DVE for determining the surface strain of specimens, there were a number of problems encountered as explained in the previous section. When using the water bath, it is important to use distilled water to reduce the formation of dissolved gas bubbles with have been shown to disrupt the tracking system.
    d. The Indian ink used for the markers was not extremely effective due to the fact that bone does not readily absorb the ink into its surface. This form of marking is better used for softer, more permeable materials, such as muscle or cartilage.

In conclusion, the clinical relevance of the findings of this project it can be stated that from low doses of irradiation (25Gy) up to relatively high doses of irradiation (300Gy) there appears to be a small (albeit negative) influence on the elastic and viscoelastic behaviour of bone. Many of the trends presented in this report are as a result of random variation in the groups of specimens, and only the analysis of Poisson's Ratio showed that the irradiation dose did have a statistical impact on the bone.

Barth et al have shown in carefully controlled studies that there is no mechanical property degradation in doses below 35kGy, which suggests that 300Gy is far too low to have any great impact on bone (75).Bohm et al showed that autografts irradiated at relatively high levels (250Gy) are reincorporated, and have a healthy unison with bone at the same rate as a 50Gy group of specimens (2).

With these factors and the results compiled from this study, it is of the opinion that the irradiation dosage appears to have a negligible effect initially on the mechanical properties of the autograft. It has been shown that generally higher doses of Extra-Corporeal Irradiation have lower rates of disease recurrence (62). Therefore, irradiating at 300Gy should have no immediate adverse effects on the mechanical properties of the autograft.

7. Recommendations for Further Work

1. Due to the problems encountered with gripping the specimens as well as the size of the specimens, the specimens could not be tested to failure. Therefore, both the yield stress and ultimate tensile stress were unattainable. These parameters could be useful in determining the effects of irradiation on the mechanical properties of bone. These parameters could help explain the phenomenon of the increasing Poisson's Ratio after increasing the irradiation dose. Both the yield stress and ultimate tensile stress of bone has been shown to depend on the collagenous phase, and these properties could indicate any changes present in the behavior of bone (76).

2. The same tests described in this report should be carried out on specimens that have been subjected to large levels of irradiation as seen in allografts. If the results in this report are accurate, the same trends should be seen in larger irradiation groups. With these two data sets, researchers could extrapolate between the two data sets to show the overall trends of how bone properties are altered post-irradiation.

3. It has been shown that the phase lag in strain compared from stress and subsequently the dissipation factor ($\tan\delta$) is greatly influenced by the frequency of the testing. Testing should be carried out in the range of commonly experienced physiological frequencies (0.02-2Hz) (86).

4. Due to the problems encountered with the DVE, more tests should be carried out on the analysis of Poisson's Ratio to clarify if the dose administered does indeed cause a change in the incompressibility of bone. Furthermore, all testing should be performed with either fresh specimens or specimens that have underwent one cycle of freezing to minimize the disruption caused by the refreezing process.

5. Due to Extra-Corporeal Irradiation being used to treat bones such as the pelvis which respond poorly to radiation therapy, tests should be carried out on specimens derived from the pelvis. The pelvis is known as a flat bone, and is different in structure to long bones, with a thinner cortex and greater amount of trabecular bone. As parts of the pelvis are not necessarily load-bearing unlike long bones, the structure may be altered greater post ECI treatment. Tests should be carried out on human pelvis specimens, both cortical and cancellous bone to determine a closer response to the

treatment that patients undergo. It has been shown that mechanically, cortical and cancellous bone behaves differently (69). Testing may show that cancellous bone has a far greater probability of behavioural change post ECI treatment.

6. Viscoelastic models should be created and compared to experimental to attempt to find an accurate portrayal of the viscoelastic model shown in experimental data. Factors should be added in to try to quantify the effects of irradiation dosage has on the mechanical properties of bone, to help physicians determine the optimum level of irradiation needed to treat bone sarcomas.

7. Due to problems with the DVE system and the gripping issues explained in Section 5, as well as human errors encountered during the preparation of the specimens, the groups were unfortunately uneven. Also some bones yielded more specimens than others, as well as with some anatomical positions, it was easier to extract more specimens. Finally, the skill of extracting correctly sized specimens gradually increased, with the first bones having fewer specimens than the final lot (See Table 7:1-1). These factors were for the most part unavoidable and may have had an effect on some of the results obtained. For future work, same size groups should be experimented with to rule out the large deviations in the data.

Bone	M	L	A	P	Total
1	2	1	4	2	9
2	4	2	4	6	16
3	3	2	3	2	10
4	4	5	3	4	16
5	3	2	2	2	9
6	5	4	4	2	15
7	5	4	2	4	15
8	4	6	4	4	18
9	4	4	4	2	14
10	6	4	4	4	18
11	3	3	4	1	11
12	3	6	2	2	13
Total	**46**	**43**	**40**	**35**	**164**

Table 7:1-1 – Bovine Specimens Extracted from Each of the Twelve Bones

8. During the acquisition of the projects data, little attention was paid to the fracture toughness and fatigue strength of the bone. Clinicians have reported “brittle bones”

in patients who have undergone Extra-Corporeal Irradiation. If irradiation does indeed cause damage to the collagen constituent within bone, the fatigue strength could be a clear indication of the overall effects of irradiation. As of yet, no analysis have been performed on the fracture toughness on specimens which received low doses of irradiation.

9. An extensive study should be carried out on how the loss of bone cell, in particular osteocytes, within the bone matrix affects the mechanical properties of bone. This study attempted to quantify the immediate effects post irradiation treatment. Osteocytes have been shown to be vital in the integrity of bone over long periods (6). Therefore, tests should be carried on animal models to attempt to quantify the change in bone morphology and mechanical properties at different stages of recovery.

8. Problems Encountered During Testing

A problem faced by many engineers attempting to quantify the material properties and behavior is the subject of gripping specimens. This was a problem that was encountered in this experiment. Ultimately the loads that were required to grip the specimen in place sufficiently were unrealistic with the available apparatus. Due to this, the original testing procedure had to be altered to accommodate the gripping problem. Originally, the stress range was set at 8MPa with a mean stress of 5MPa. However, there were gripping issues and as a result, the loads were reduced significantly to a maximum of 55N which delivered a stress range of 2MPa.This was not the only solution available for the testing phase. Another solution would be to use another material in the region of the grips to give the specimen a larger surface area, such as dental stone. However, due to time constraints this solution was not opted for.

The problems with the gripping lead to a discovery with respect to the Young's Modulus. Seven randomly chosen groups of specimens were chosen to be the first candidates for testing. After the problems encountered with the gripping, these specimens were stored in a refrigerator for one week as the testing protocol was changed and confirmed with the project's supervisor. The refrigerator was chosen as another cycle of refreezing was undesirable. When the specimens were eventually tested, all specimens displayed below average Young's Modulii, leading to conclusions to be made that refrigeration causes significant decrease in Young's Modulus. In regards to the overview of Young's Modulus being affected by Irradiation, these specimens did not seem to disturb the statistical trends.

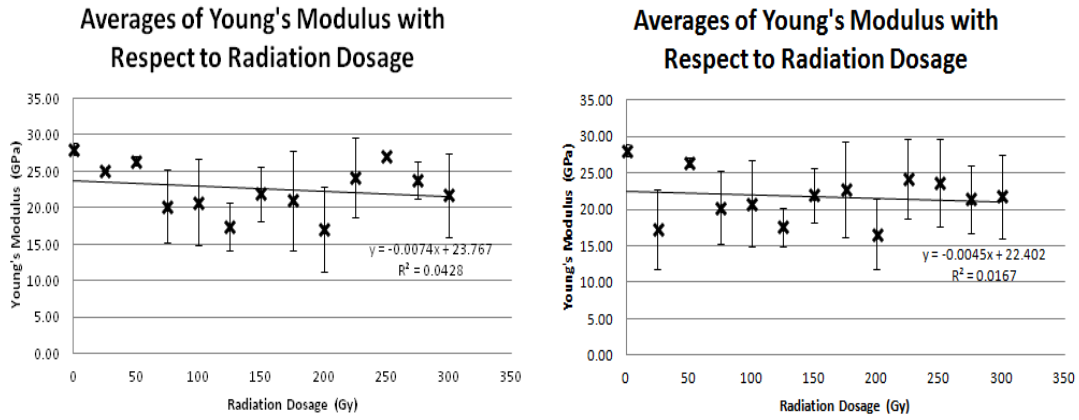

Figure 8-1 – Post Removal of Refrigerated Specimens and Pre Removal of Refrigerated Specimens

When calculating the Young's Modulus, on a few occasions, some of the specimens displayed lower than usual values. On secondary inspection, the strain rate appears normal, but the load that the load cell recorded was far below the usual loads seen. This indicates that these specimens slipped to a certain degree, and then settled, so the displacement limit was not triggered. These values were not included in the analysis of the Young's Modulus of bone.

When using the BOSE Digital Video Extensometer (DVE) for tracking the axial and transverse strains, there were various problems with during the testing. The camera seemed to experience noise disturbances, causing the marker points to migrate slightly. These migrations distorted the results to a considerable degree, and made accurate calculations of Poisson's Ratio extremely difficult. Due to moving parts within the testing machine, a vibration was induced across the surface of the water. Regardless of the placement of the light source, some of these vibrations were reflected off the surface of the water which disrupted the tracking system.

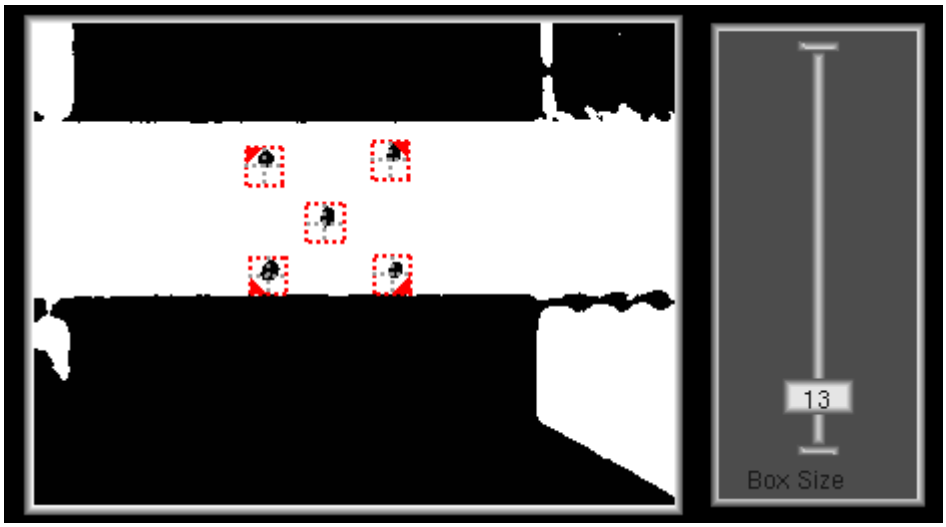

Figure 8-2 – Image taken of the Markers (Red) being placed on the Black Reference Points.

Another problem found was the development of bubbles as the water bath heated up and maintained a temperature of 37.1±0.2˚C. These bubbles arise from dissolved gases in the water, and made clear pictures difficult to obtain, and interfered with the tracking system of the DVE. To combat this problem, double distilled pure water was used for the water bath to remove the formation of dissolved gases found in normal water. This reduced the quantity of bubbles within the bath, but did not cease the formation of all of them as atmospheric gases diffused back into the water bath during experiments.

A third problem was the development of adequate contrast between the black ink (Indian ink) dots and the white of the bone. The problem presented itself in the form of the light source, as it was very directional in nature. As there was insufficient light, the edges of the dots became difficult to contrast for the software. This problem, along with the effect of noise on the specimen, caused the results of the axial and transverse strains to be corrupted with deviations even during the dwelling period. To try and rectify this problem, an extra light was used in conjunction with the normal light. This light was a surgeon's overhead light, to attempt to shed light on the whole specimen to increase the contrast of the markers. However, after various positions, the extra light did not prove to make any difference for contrasting purposes.

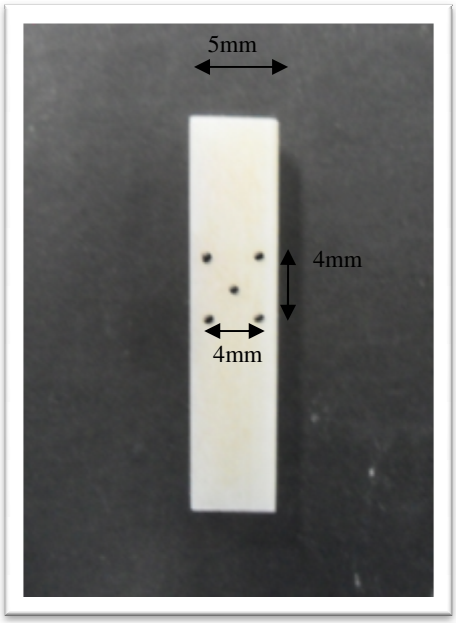

Figure 8-3 – Example of Markers Placed on a Specimen

The size of the markers was a considerable issue with the tracking of axial and transverse strains. If the markers were too small, the problems with the contrast made it very difficult to track. However, if the markers were too large, there were two problems associated with this; the size of the dots reduce the length between the points, causing slightly incorrect recordings, and due to the size of the dots, the ink sometimes became unstable in the water bath, corrupting the strain readings. To combat the problem, the dots were applied to the specimens over an hour before testing, and allowed to dry. This however caused another problem, as bone dries out rapidly in room temperature. The solution to this problem was to allow 10minutes for the dots to adequately dry, before being half submerged

in a saline solution for the remaining time before testing. If the specimens were partially submerged in water, there is the tendency for leaching of the mineral content to occur.

Due to these problems with the extensometer, there were a number of tests that were corrupted with unusable data. As a result, fewer specimens were analyzed which may have an effect on the overall trends seen in Section 5.

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10. Appendices

10.1 ANOVA Results derived from Data

All P-values were declared statistically significant if they were below the significance factor α which had a value of 0.05. The F-Value corresponds to the variance between treatments divided by the variance within treatments.

In each of the three ANOVA Tests, the Radiation Groups that contained specimens from only three anatomical positions (75, 250, 275, 300Gy) a fictitious bone (Bone 13) was created to normalize the data. The values placed in these positions was the groups previous mean, to try minimize the disturbance felt in the analysis.

In the Analysis, the three-way interaction between dose received, the individual bone and the anatomical position was omitted. This was due to the fact that ANOVA would then have zero degrees of freedom error and would not be able to perform further analysis. However, after inspection, this interaction would seem to have no statistical significance in any case.

10.1.1 ANOVA Results for Young's Modulus

Young's Modulus versus Dose, Bone, Position

Estimated Effects and Coefficients for Value (coded units)

Term	Effect	Coef	SE Coef	T	P
Constant		21.707	0.7526	28.84	0.000
Dose	-1.448	-0.724	1.2099	-0.60	0.553
Bone	-0.286	-0.143	1.2145	-0.12	0.907
Position	-1.739	-0.870	0.9951	-0.87	0.387
Dose*Bone	0.527	0.263	2.0355	0.13	0.898
Dose*Position	-2.415	-1.208	1.6230	-0.74	0.461
Bone*Position	-0.308	-0.154	1.6623	-0.09	0.927

S = 5.32475 PRESS = 1795.60
R-Sq = 3.77% R-Sq(pred) = 0.00% R-Sq(adj) = 0.00%

Analysis of Variance for Value (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	3	34.02	33.46	11.1518	0.39	0.758
Dose	1	11.69	10.15	10.1473	0.36	0.553
Bone	1	1.08	0.39	0.3938	0.01	0.907
Position	1	21.24	21.65	21.6548	0.76	0.387
2-Way Interactions	3	15.95	15.95	5.3155	0.19	0.904
Dose*Bone	1	0.03	0.47	0.4745	0.02	0.898
Dose*Position	1	15.68	15.70	15.6962	0.55	0.461
Bone*Position	1	0.24	0.24	0.2431	0.01	0.927
Residual Error	45	1275.88	1275.88	28.3529		
Total	51	1325.85				

Unusual Observations for Value

Obs	StdOrder	Value	Fit	SE Fit	Residual	St Resid
15	15	12.8400	22.5764	2.1922	-9.7364	-2.01R
49	49	31.8700	19.8170	1.6680	12.0530	2.38R

R denotes an observation with a large standardized residual.

Estimated Coefficients for Value using data in uncoded units

Term	Coef
Constant	22.0431
Dose	0.0065437
Bone	-0.024987
Position	0.34497
Dose*Bone	0.00029259
Dose* Position	-0.00536688
Bone* Position	-0.017103

10.1.2 ANOVA Results for Poisson's Ratio

Poisson's Ratio versus Dose, Bone, Position

Estimated Effects and Coefficients for Value (coded units)

Term	Effect	Coef	SE Coef	T	P
Constant		0.279994	0.007961	35.17	0.000
Dose	0.055275	0.027638	0.012798	2.16	0.036
Bone	-0.007779	-0.003889	0.012847	-0.30	0.763
Position	-0.005457	-0.002729	0.010526	-0.26	0.797
Dose*Bone	-0.018839	-0.009419	0.021531	-0.44	0.664
Dose*Position	0.051533	0.025766	0.017167	1.50	0.140
Bone*Position	0.046392	0.023196	0.017584	1.32	0.194

S = 0.0563248 PRESS = 0.185197
R-Sq = 17.11% R-Sq(pred) = 0.00% R-Sq(adj) = 6.06%

Analysis of Variance for Value (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	3	0.016736	0.015034	0.0050112	1.58	0.207
Dose	1	0.016369	0.014795	0.0147948	4.66	0.036
Bone	1	0.000078	0.000291	0.0002907	0.09	0.763
Position	1	0.000289	0.000213	0.0002132	0.07	0.797
2-Way Interactions	3	0.012742	0.012742	0.0042474	1.34	0.274
Dose*Bone	1	0.000134	0.000607	0.0006071	0.19	0.664
Dose*Position	1	0.007088	0.007146	0.0071464	2.25	0.140
Bone*Position	1	0.005521	0.005521	0.0055207	1.74	0.194
Residual Error	45	0.142762	0.142762	0.0031725		
Total	51	0.172240				

Unusual Observations for Value

Obs	StdOrder	Value	Fit	SE Fit	Residual	St Resid
27	27	0.424200	0.242858	0.016512	0.181342	3.37R
33	33	0.404050	0.275883	0.014600	0.128167	2.36R

R denotes an observation with a large standardized residual.

Estimated Coefficients for Value using data in uncoded units

Term	Coef
Constant	0.338500
Dose	-2.87797E-05
Bone	-0.00552162
Position	-0.0370378
Dose*Bone	-1.04659E-05
Dose*Position	0.000114517
Bone*Position	0.00257731

10.1.3 ANOVA Results for Phase Lag

Phase Lag (x10000)[‡‡] versus Dose, Bone, Position

Estimated Effects and Coefficients for Value*10000 (coded units)

Term	Effect	Coef	SE Coef	T	P
Constant		98.3984	0.07047	1396.25	0.000
Dose	0.3626	0.1813	0.11329	1.60	0.116
Bone	0.2058	0.1029	0.11372	0.90	0.370
Position	-0.2570	-0.1285	0.09318	-1.38	0.175
Dose*Bone	-0.2928	-0.1464	0.19059	-0.77	0.446
Dose*Position	-0.2141	-0.1070	0.15196	-0.70	0.485
Bone*Position	-0.0877	-0.0438	0.15565	-0.28	0.780

S = 0.498576 PRESS = 14.1628
R-Sq = 14.70% R-Sq(pred) = 0.00% R-Sq(adj) = 3.33%

Analysis of Variance for Value*10000 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	3	1.5228	1.4720	0.49068	1.97	0.131
Dose	1	0.7878	0.6368	0.63681	2.56	0.116
Bone	1	0.2065	0.2036	0.20357	0.82	0.370
Position	1	0.5284	0.4727	0.47271	1.90	0.175
2-Way Interactions	3	0.4054	0.4054	0.13513	0.54	0.655
Dose*Bone	1	0.2628	0.1466	0.14664	0.59	0.446
Dose* Position	1	0.1229	0.1233	0.12331	0.50	0.485
Bone* Position	1	0.0197	0.0197	0.01972	0.08	0.780
Residual Error	45	11.1860	11.1860	0.24858		
Total	51	13.1141				

Unusual Observations for Value*10000

Obs	StdOrder	Value*100	Fit	SE Fit	Residual	St Resid
7	7	99.8580	98.5758	0.1318	1.2822	2.67R
12	12	99.7216	98.7671	0.1941	0.9545	2.08R
23	23	99.8950	98.5201	0.1565	1.3749	2.90R
34	34	99.8870	98.4331	0.1265	1.4539	3.01R

R denotes an observation with a large standardized residual.

Estimated Coefficients for Value*10000 using data in uncoded units

Term	Coef
Constant	97.8767
Dose	0.00353658
Bone	0.0537262
Position	0.019788
Dose*Bone	-1.62648E-04
Dose* Position	-4.75690E-04
Bone* Position	-0.0048708

[‡‡] - This value had to be multiplied by a factor of 10^4 as the values were too small to have been accurately analysed. The multiplication did not alter the P or F-values

10.2 Radiotherapy Sheets

SIEMENSONCOR IMPRESSION + (plus) 6MV (Mega Volts) Photons

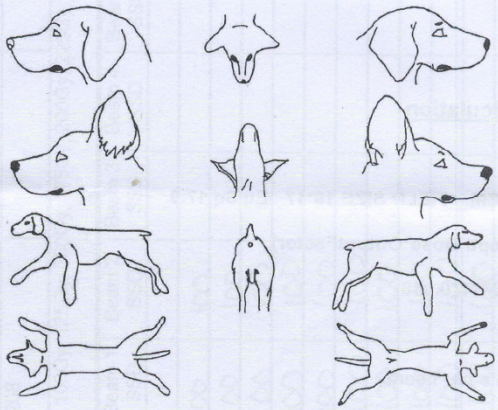
University of Glasgow | Small Animal Hospital

RADIOTHERAPY SHEET

Hospital Number: Sticker Here **Animal:** **Owner** **Species:** **Breed:** **Age:** **Sex:**	**Diagnosis:** **Treatment Site:** **Start Date:**

Treatment Site	Energy	Dose	Fractionation	Signature
1. BONES	6MV	UP TO 300Gy	1	
2.				
3.				

TUMOUR MAP



Treatment Summary

COMPLETED AS PRESCRIBED? YES/NO

SIDE EFFECTS:

PATIENT POSITIONING (NOTES AND COMMENTS)

Groups of bones in a box filled with water, 1cm bolus in the bottom of the box.

Box size 19cm*17cm
Box depth 4cm

AP/PA set up

Manual Calculation

Photons

6MV SEP 4CM FIELD SIZE 19*17 Eq Sq 17.9

M = Dose/(DepthDose*OutputFactor)

= 2500/(1.0476*0.988)

= 2415/2

= 1208 Mu's per beam

Working

Equivalent Square 14.5

Percentage Depth Dose 17 = 98.8
18 = 98.8

PDD = 98.8

Output Factor 17 = 1.044
18 = 1.048
Dif = 0.004/10 = 0.0004*9 = 0.0036
1.044 + 0.0036 = 1.0476

Output Factor = 1.0476

Field ID	1. Ant				2. Post				3				4				5				Amendments to plan
Energy	6MV				6MV																
Field size	X1 9.5		X2 9.5		X1 9.5		X2 9.5		X1		X2		X1		X2		X1		X2		
	Y1 8.5		Y2 8.5		Y1 8.5		Y2 8.5		Y1		Y2		Y1		Y2		Y1		Y2		
Angles	Gan	Coll	Tab		Gan	Coll	Tab		Gan	Coll	Tab		Gan	Coll	Tab		Gan	Coll	Tab		
	0	0	0		180	0	0														
Beam Modifiers	Bo	We	MLC		Bo	We	MLC		Bo	We	MLC		Bo	We	MLC		Bo	We	MLC		
	No	No	No		No	No	No														
SSD	100				100																
MU's	1208				1208															Calc by: Date:	
Repeat for each group 25Gy, 50Gy, 75Gy, 100Gy, 125Gy, 150Gy, 175Gy, 200Gy, 225Gy, 250Gy, 275Gy and 300Gy																				2nd check: Date:	

Date	#	Cum dose	Portal Imaging	Beam 1 SSD	Beam 2 SSD	Beam 3 SSD	Beam 4 SSD	Beam 5 SSD	Treated By	Weight	Bloods	Comments
28/08/2012												
25Gy	1	2500		100	100				SB			
50Gy	2	5000		100	100				SB			
75Gy	3	7500		100	100				SB			
100Gy	4	10000		100	100				SB			
125Gy	5	12500		100	100				SB			
150Gy	6	15000		100	100				SB			
175Gy	7	17500		100	100				SB			
200Gy	8	20000		100	100				SB			
225Gy	9	22500		100	100				SB			
250Gy	10	25000		100	100				SB			
275Gy	11	27500		100	100				SB			
300Gy	12	30000		100	100				SB			
	13											
	14											
	15											