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'The mechanical effect of extracorporeal irradiation on bone'

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Abstract

Extracorporeal irradiation and re-implantation of a bone segment is a technique employed in bone sarcoma surgery for limb salvage in the setting of reasonable bone stock. There is neither consensus nor rationale given for the dosage of irradiation used in previous studies, with values of up to 300Gy applied. We investigated the influence of extracorporeal irradiation on the elastic and viscoelastic properties of bone. Bone specimens were extracted from mature cattle and subdivided into thirteen groups; twelve groups exposed to increasing levels of irradiation and a control group. 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, Storage Modulus, Loss Modulus and Dynamic Modulus. These were all obtained for comparison of the irradiated specimens to the control group.

We found that the overall effect of increasing irradiation doses up to 300Gy seems to present negligible change, albeit negative, on the behavior of bone. However, the increase in Poisson's ratio following extracorporeal irradiation treatment was statistically significant. Therefore, it is concluded that the overall mechanical effect of high levels of extracorporeal irradiation (300Gy) is minute, and could be administered to reduce the risk of malignancy recurrence.

Background

The surgical management of primary bone tumours frequently involves a wide resection to achieve local control. Following this, there are many potential methods available for limb salvage. These include biological reconstruction using allograft or autograft, endoprosthetic reconstruction or simply the creation of a pseudoarthrosis[1]. The latter of these options has obvious biomechanical disadvantages and leads to a loss of function. Endoprosthetic replacement is effective in the majority of cases, but longevity of the implants and costs remain a concern[2]. Bulk allograft has inherent risks of infection, immunologic reaction and failure to incorporate, as well as being an imperfect fit in terms of bony architecture[3]. Furthermore, bulk bone grafts are costly and timely delivery of optimally sized bulk allograft can be difficult.

Extracorporeal irradiation (ECI) and reimplantation of bone is an alternative technique that was first reported in 1968[4]. 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. The advantages of this method include the autograft being a perfect fit in terms of bony architecture, the fact that it is relatively inexpensive and avoids the complications described with other treatment modalities.

Although this method of treatment has good short-term results, there is no consensus on the level of radiation to be administered to the graft. Some studies have used radiation levels of 300Gy to be certain all tumour cells have been destroyed[4], while others studies suggest that 50Gy is adequate to kill all malignant cells within the autograft[3].

However, questions about the use of ECI remain unanswered. The treatment is certainly not benign, as high complication rates have been reported in some instances[7]. The principal problems relate to the mechanical integrity of the bone after irradiation and infection[8], as well as concerns about avascular necrosis and graft resorption (Davidson and Stalley 2005).

It has been hypothesised that increasing the dosage of radiation when treating the autograft may have adverse effects on the collagenous phase found within osseous tissue, causing adverse changes in the mechanical properties (elastic and viscoelastic) of bone. The principal aim of this study is to determine the effect of varying doses of radiation on the mechanical properties of bone. The null hypothesis is of no difference irrespective of the irradiation dosage.

Materials and Methods

Thirteen mature bovine tibias were freshly harvested and collected from an abattoir and frozen upon acquisition (-17˚C). Mature subjects were chosen to avoid fibrolamellar (plexiform) bone of immature specimens[5]. Prior to specimen preparation, the bone was thawed at room temperature and the mid-diaphysis sectioned into anterior, posterior, medial and lateral sections 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 thermally induced damage. This was achieved by connecting 200g to the sliding stage of the rotating blade. The longitudinal axis of the specimens was aligned with the primary loading axis of the tibia. The specimens were then abraded, with grits from 80 to 320, to obtain the required cross-sectional dimensions, verified using an electronic micrometer (Mitutoyo, Absolute Digimatic; Tokyo, Japan).

A total of 164 bone samples were obtained with 12 to 13 specimens extracted from each tibia. The specimens were wrapped in 0.9% saline soaked gauze and each group was placed within clearly marked sealable bags before being refrozen (-17˚C). Whilst refreezing has been attributed to damage microscopic material structures, two cycles have been found not to have any implications in the structural integrity of the material[6]. Furthermore, all samples underwent the same number of freeze-thaw cycles, allowing valid comparisons to be made.

Irradiation of Specimens

The specimens were systematically assigned into twelve irradiation groups and one control group. For irradiation, specimens were thawed at room temperature before being wrapped in saline soaked gauzes, and placed into a sub-divided plastic container minimising air pockets.

Irradiated occurred using a Siemens ONCOR Impression Plus Linear Accelerator at 6MV X-ray Photon Beam in increments of 25Gy up to the maximum of 300Gy. The radiation was set up in an AP/PA manner, where the gantry was rotated through 180˚ after half the dose was administered. After the irradiation was completed the bone specimens were frozen for the final time before undergoing elastic and viscoelastic testing.

Elastic and Viscoelastic Testing

Specimens were tested in uniaxial tension using a BOSE Electroforce 3200 Material Testing Machine fitted with a temperature-controlled water bath (37°C) and 450 N load cell. Specimens were placed in the grips with a 15 mm gauge length and a 1 N preload was applied (Figure 1, A). To determine the Young's modulus, a displacement-controlled extension of 0.01 mm was applied at a rate of 0.002 mm.s^{-1} (Figure 1, B). The gradient of the resulting stress-strain curve in the linear region provided the Young's modulus, E. The load was reduced to 1N and held for one minute (Figure 1, C). After this, the specimen underwent 1 Hz cyclic tensile loading in load control, with a mean stress, $\bar{\sigma}$, of 1.2 MPa and an amplitude, σ_0, of 1 MPa for 120 cycles (Figure 1, D). The phase lag (δ) between the stress and strain was found by best-fitting sinusoids, using inbuilt Matlab routines, to the stress and strain data (Equations 1 and 2) and determining the phase difference, δ, between them (Equation 3).

$$\sigma = \sigma_0 \sin(\omega t + \delta_1) + \bar{\sigma} \qquad \text{Eq. 1}$$

$$\varepsilon = \varepsilon_0 \sin(\omega t + \delta_2) + \bar{\varepsilon} \qquad \text{Eq. 2}$$

$$\delta = \delta_2 - \delta_1 \qquad \text{Eq. 3}$$

These data were also used to determine the storage modulus (E') and loss modulus (E").

$$E' = \frac{\sigma_0}{\varepsilon_0} \cos \delta \qquad \text{Eq. 4}$$

$$E'' = \frac{\sigma_0}{\varepsilon_0} \sin \delta \qquad \text{Eq. 5}$$

ANOVA was used, adopting a 5% significance level, to determine differences with irradiation level and anatomical quadrant.

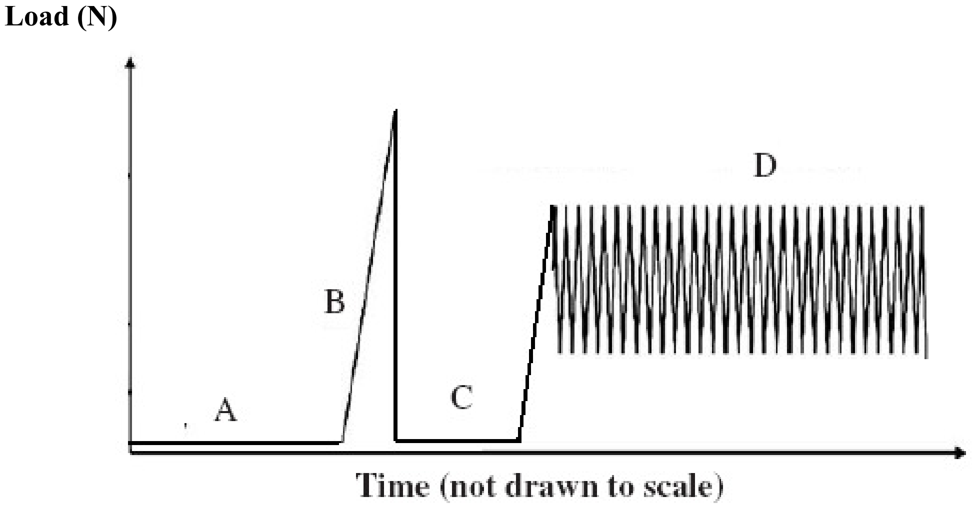

Figure 1 – Schematic representation of tensile testing protocol

Results

Whilst there may be significant statistical differences between individual irradiation groups, there appear to be no discernable trend associated with irradiation intensity with Young's modulus (Figure 2), tan(δ) (Figure 3) and storage and loss moduli (Figure 4).

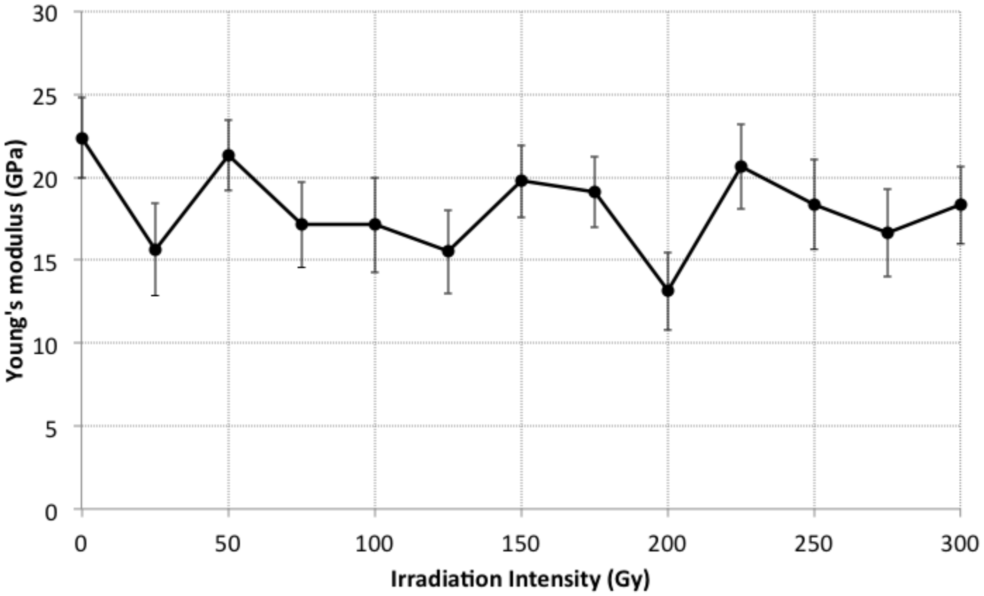

Figure 2 - Young's Modulus with respect to irradiation intensity

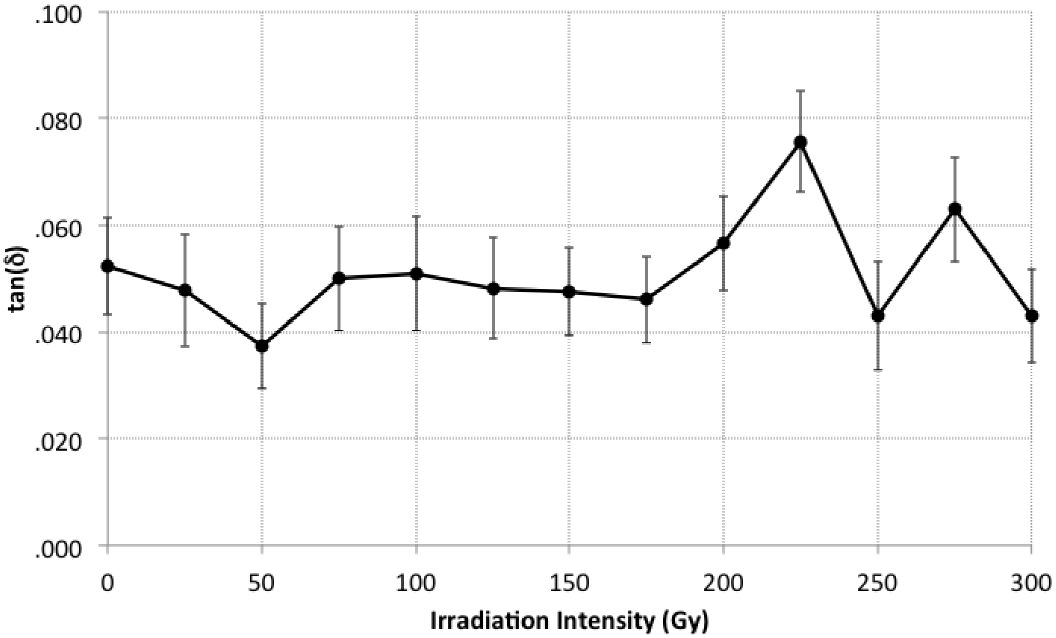

Figure 3: Variation in tan(δ) with irradiation

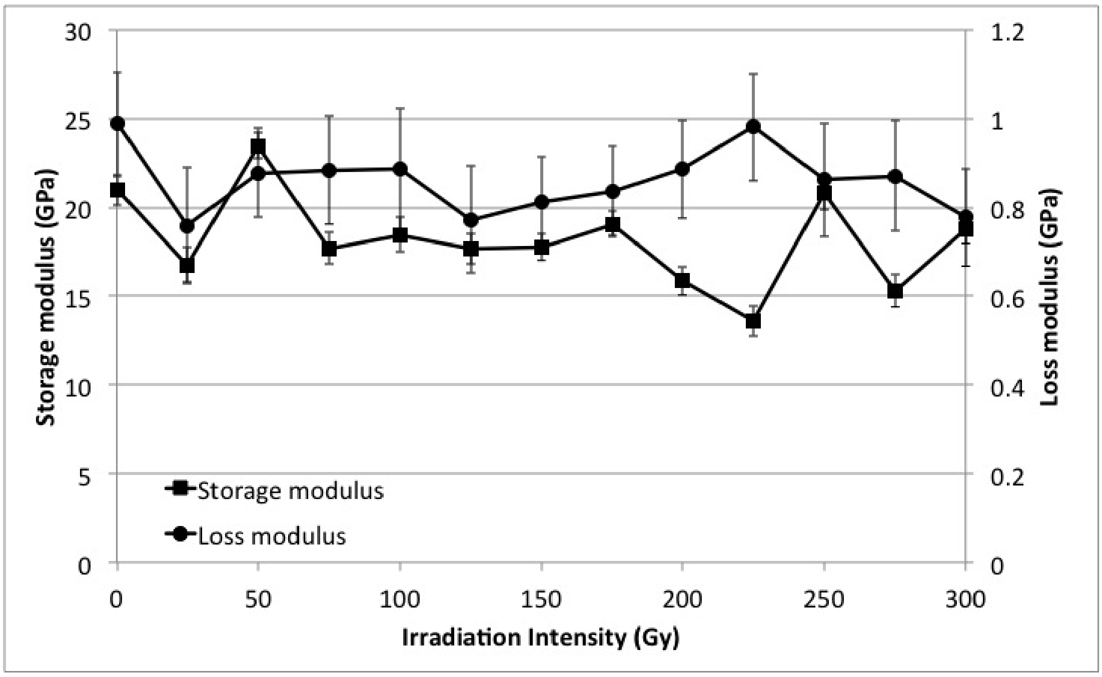

Figure 4: Storage and loss moduli variation with irradiation intensity

There was no effect of anatomical quadrant on E and tan(δ), although the storage and loss modulus demonstrated a significant variation ($p < 0.01$), with anterior and lateral quadrants having higher moduli than medial and posterior quadrants (Figure 5a and b).

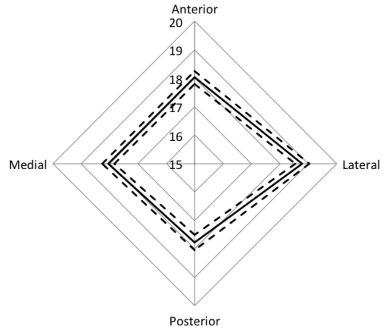

Figure 5a: Storage modulus (GPa) variation around the cortex.

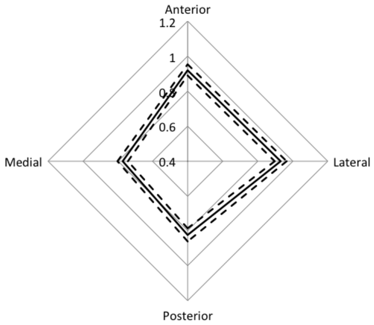

Figure 5b: Loss modulus (GPa) variation around the cortex.

Discussion

Barth et al[7] demonstrated that the elastic and plastic properties of bone are unaffected with irradiation levels below 35kGy, and our findings are fully consistent with these data. Above these levels, it has been found that bone stiffness and strength is adversely affected. 66% of rat tibiae irradiated at 50kGy suffered from pathological fractures, whilst samples 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[8]. However, 35kGy is significantly above the level of irradiation used for autografts and therefore we felt it important to fully investigate the mechanical properties of bone in this region and to reaffirm that irradiation of autografts does not deteriorate bone quality. Moreover, Barth et al[7] did not investigate the viscoelastic properties of bone, which may be more likely to be affected by a small change in collagen degradation than the elastic and plastic properties.

Our results indicate that at irradiation levels used in this study, increasing the dose of irradiation does not affect the elastic stiffness of the bone, with both E and E' showing no consistent trend with irradiation intensity. Since the mineral phase of bone is primarily responsible for the stiffness of the bone it is largely unaffected by irradiation from the subsequent development of free radicals[13]. We propose that statistical variation seen between irradiation groups may be more associated with inherent biological variation than the irradiation itself. Furthermore, increasing the irradiation dose does not affect the viscoelastic properties of bone. The loss modulus, E'', and tan(δ) do not exhibit consistent trends across the irradiation intensities, indicative of changes to the mechanical behaviour of the collagen component. Our values for tan(δ) and storage and loss moduli are consistent with recent and past literature[13,14], with the differences primarily being attributed to the different experimental and testing modalities adopted. The increased stiffness in the anterior and lateral quadrants are consistent with previous data on the microhardness of the ovine radius, explained by a higher mineral content in these quadrants which is a result of these regions being more in longitudinal tension than their opposite quadrants[15, 16]. The large number of samples used in our tests, combined with literature agreements, gives rise to confidence that we had sufficient power in our experiment to ascertain differences due to irradiation. Therefore, evidence from this study, backed by that of Barth et al, confirm that levels of irradiation of the order of 300Gy do not affect the elastic, viscoelastic, plastic and ultimate mechanical properties of bone and that ECI at this intensity should not be concerned with a loss of bone mechanical quality upon reimplantation.

The reported irradiation level at which tumour cells are killed varies between studies[9, 10] but 300Gy consistently appears to be more successful than lower doses. Furthermore, the level of autograft incorporation does not vary between irradiation levels[11,12]. Therefore, in conclusion, the limiting factor in choosing an irradiation level is most likely to be the effectiveness of the irradiation in causing tumour cell death, and not the mechanical integrity of the sample post irradiation or the efficacy of subsequent autograft incorporation.

Acknowledgements

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