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Abstract	Extracorporeal irradiation of an excised tumour-bearing segment of bone followed by its re-implantation is a technique used in bone sarcoma surgery for limb salvage when the bone is of reasonable quality. There is no agreement among previous studies about the dose

	<p>of irradiation to be given: up to 300 Gy have been used.</p> <p>We investigated the influence of extracorporeal irradiation on the elastic and viscoelastic properties of bone. Bone was harvested from mature cattle and subdivided into 13 groups: 12 were exposed to increasing levels of irradiation: one was not and was used as a control. The specimens, once irradiated, underwent mechanical testing in saline at 37°C.</p> <p>The mechanical properties of each group, including Young's modulus, storage modulus and loss modulus, were determined experimentally and compared with the control group.</p> <p>There were insignificant changes in all of these mechanical properties with an increasing level of irradiation.</p> <p>We conclude that the overall mechanical effect of high levels of extracorporeal irradiation (300 Gy) on bone is negligible. Consequently the dose can be maximised to reduce the risk of tumour recurrence.</p> <p>Cite this article: <i>Bone Joint J</i> 2015;97-B:??-??.</p>
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The surgical management of a primary bone tumour usually requires a wide resection to achieve local control. There are many potential methods available to fill the resultant defect and salvage the limb. These include biological reconstruction with an allograft or autograft, endoprosthetic replacement or simply leaving a pseudoarthrosis.[[1]] The last of these options has obvious biomechanical disadvantages and results in a loss of function. Endoprosthetic replacement is effective in most cases, but concerns remain about the longevity of the implants, infection and cost.[[2]] Bulk allograft has the inherent risks of infection, immunological reaction and failure to incorporate, as well as being an imperfect fit.[[3]] Furthermore, bulk bone grafts are expensive and the timely delivery of an optimally-sized bulk allograft can be a problem.

Extracorporeal irradiation (ECI) and reimplantation of bone is an alternative technique first reported in 1968.[[4]] The irradiated autograft acts as a scaffold in which living cells can replace the dead bone. The advantages of this method are that the autograft, being a perfect fit, is relatively inexpensive and avoids the complications of other modalities of treatment.

Although the technique gives good short-term results, there is no consensus about the dose of radiation to be administered to the graft. Some studies have used doses of 300 Gy to ensure that all tumour cells have been killed,[[2]] while others suggest that 50 Gy is sufficient. [[1,5]]

There are questions about the use of ECI which remain unanswered. The treatment is certainly not benign, as high rates of complication have been reported in some instances. [[6]] The principal problems concern the mechanical integrity of the bone after irradiation, infection, [[7]] avascular necrosis and graft resorption. [[8]] We hypothesised that increasing the dose of radiation applied to the autograft might have adverse effects on the collagen found within bone, causing adverse changes in its elastic and viscoelastic mechanical properties.

The principal aim of this study was to determine the effect of varying the dose of irradiation on the mechanical properties of bone. The null hypothesis was that there is no difference, irrespective of the dose of irradiation administered.

Materials and Methods

We acquired 13 freshly harvested mature bovine tibiae from an abattoir which were then immediately frozen (-17°C). Mature bone was chosen to avoid the fibrolamellar (plexiform) bone of immature animals. [[9]] Before preparing each tibia, it was thawed at room temperature and the mid-diaphysis sectioned into anterior, posterior, medial and lateral sections using a bone saw, before being cut into rectangular specimens (0.5 cm × 0.5 cm × 3 cm) with a diamond-tipped rotating blade (Smart Cut, UKAM Industrial Superhard Tools; Valencia, California).

The specimens were cut at a slow uniform speed to reduce thermal damage. The longitudinal axis of each specimen was aligned to the primary loading axis of the tibia. The specimen was then abraded, with grits from 80 to 320, to obtain the

required cross-sectional dimensions which were verified using an electronic micrometer (Mitutoyo, Absolute Digimatic; Tokyo, Japan).

A total of 12 or 13 specimens were obtained from each tibia giving a total of 164 bone samples for testing. These were wrapped in 0.9% saline soaked gauze and each group was placed within clearly marked sealable bags before being refrozen (-17°C). While refreezing has been said to damage microscopic material structures, two cycles of freezing have been found not to have any adverse effect on the structural integrity of the material.[[10]] Furthermore, all the samples underwent the same number of freeze-thaw cycles, allowing valid comparisons to be made.

Irradiation of specimens

The specimens were assigned to 12 irradiation groups and one control group. Before being irradiated, specimens were thawed at room temperature then wrapped in saline soaked gauze and placed into a sub-divided plastic container which minimised air pockets.

Irradiation was carried out using a Siemens ONCOR Impression Plus Linear Accelerator at 6MV X-ray Photon Beam in increments of 25 Gy up to a maximum of 300 Gy. The radiation was set up in an anteroposterior/posteroanterior manner, in which the gantry was rotated through 180° after half the dose had been administered. After irradiation, 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 a 450 N load cell. Specimens were placed in the grips with a 15 mm gauge length and a 1 N

preload applied (Fig. 1A). 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 (Fig. 1B). The gradient of the resulting stress-strain curve in the linear region gave the Young's modulus, E. The load was reduced to 1 N and held for one minute (Fig. 1C). After this, the specimen underwent 1 Hz ($\omega = 2\pi$) cyclical 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 (Fig. 1D). The phase lag (δ) between the stress and strain was found by best-fitting sinusoids to the stress ($\bar{\sigma}, \sigma_0$) and strain ($\bar{\varepsilon}, \varepsilon_0$) data (Equations 1 and 2) and determining the phase difference, δ , between them (Equation 3).

[[Fig 1]]

[[FigCap]]Diagrammatic representation of tensile testing protocol.

$$\sigma = \sigma_0 \sin(\omega t + \delta_1) + \bar{\sigma}$$

$$\varepsilon = \varepsilon_0 \sin(\omega t + \delta_2) + \bar{\varepsilon}$$

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

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

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

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

Analysis of variance was used, adopting a 5% significance level, to determine differences between dose of radiation and anatomical quadrant.

Results

While there were significant differences between individual irradiation groups, there was no discernible relationship between the dose of irradiation and Young's modulus (Fig. 2), $\tan(\delta)$ (Fig. 3) and storage and loss moduli (Fig. 4).

[[Fig 2]]

[[FigCap]]Graph showing the mean Young's Modulus (GPa) for each group of specimens according to irradiation intensity (Gy).

[[Fig 3]]

[[FigCap]]Graph showing the variation in mean $\tan(\delta)$ for each group of specimens according to irradiation intensity (Gy).

[[Fig 4]]

[[FigCap]]Graph showing the mean storage and mean loss moduli variation (GPa) with irradiation intensity (Gy).

There was no effect of anatomical quadrant on E and $\tan(\delta)$, although the storage and loss modulus showed significant variation ($p < 0.001$); anterior and lateral quadrants having higher moduli than medial and posterior quadrants (Fig. 5).

[[Fig 5]]

[[FigCap]]Diagram showing a) mean storage modulus (GPa) and b) mean loss modulus (GPa) variation around the cortex.

Discussion

Barth et al[[11]] showed that the plastic and elastic properties of bone are unaffected by doses of irradiation below 35 000 Gy: our findings agree entirely with these data. Above these levels, bone stiffness and strength are adversely affected [[12]].

Pathological fractures occurred in 5 out of 7rat tibiae irradiated at 50 000 Gy while

those that underwent 25 000 Gy irradiation showed signs of delayed healing and, at the end of the experiment, incorporation of the graft was reduced by a mean 50%.^{[[12]]} However, 35 000 Gy is significantly above the level of irradiation which is used clinically and we therefore felt it important to investigate fully the mechanical properties of bone in this region and to reaffirm that clinical irradiation of autografts does not adversely affect bone quality. Moreover, Barth et al^{[[11]]} did not investigate the viscoelastic properties of bone, which are more likely to be affected by a small change in collagen degradation than the elastic and plastic properties.

Our results on bovine bone indicate that at the irradiation levels used in this study, increasing the dose of irradiation does not affect the stiffness of the bone, with both E and E' showing no consistent trend with an increase in the intensity of irradiation. Since the mineral phase of bone is primarily responsible for the stiffness of the bone, it is largely unaffected by the development of free radicals during irradiation.^{[[13]]} We suggest that the statistical variation seen between irradiation groups may be more closely associated with inherent biological variation than with the irradiation itself. Furthermore, increasing the irradiation dose does not affect the viscoelastic properties of bone. The loss modulus, E'' , and $\tan(\delta)$ did not exhibit consistent trends across the irradiation intensities which would be indicative of changes to the mechanical behaviour of the collagen component. Our values for $\tan(\delta)$ and storage and loss moduli are consistent with recent and past literature,^{[[13,14]]} the differences being attributable primarily to the different experimental and testing methods used.

We have argued that neither the elastic nor viscoelastic properties of bone vary with irradiation. As it has previously been shown that the plastic properties of bone are also insensitive to irradiation,^{[[11]]} it follows that other mechanical

behaviour, such as fatigue behaviour, may also be assumed to be insensitive. The increased stiffness in the anterior and lateral quadrants is consistent with previous data on the microhardness of the ovine radius, explained by a higher mineral content in these quadrants. This is a result of these regions being under more longitudinal tension than their opposite quadrants.[[15,16]] The large number of samples used in our tests, combined with agreement from the literature, gives rise to confidence that we had sufficient power in our experiment to ascertain differences because of irradiation. Therefore, evidence from this study, backed by that of Barth et al,[[11]] confirms that levels of irradiation of the order of 300 Gy do not affect the elastic, viscoelastic, plastic and ultimate mechanical properties of bone and that ECI of this intensity does not cause a decrease in the mechanical properties of the reimplanted bone.

The reported level of irradiation at which tumour cells are killed varies between studies[[17,18]] but 300 Gy appears to be more consistently successful than lower doses. Furthermore, the level of autograft incorporation does not vary between different levels of irradiation.[[19,20]]

In conclusion, from the literature and our animal model the limiting factor in choosing the radiation dose is most likely to be the effectiveness of the irradiation in causing tumour cell death. We report that the mechanical integrity of the sample after irradiation within clinically relevant ranges is unchanged.

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