

## Microdamage distribution in fatigue fractures of bone allografts following gamma-ray exposure

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*Purpose:* Although significant differences in bone properties have been extensively studied, results vary when bones are exposed to gamma radiation of a range usually used for sterilization purposes (25–35 kGy). Hence, the aim of this work was the study of the mechanical properties and microdamage development of human bones used as allografts following gamma-ray exposure, followed by an extensive statistical analysis of microdamage effects in fatigue behaviour.

*Methods:* Specimens of the cortical region of human femurs were exposed to 15–25 kGy and 26–30 kGy radiation levels, then they were subjected to compression fatigue tests until fracture. The fatigue life was determined in relation to the radiation level, and the evolution of microdamage was assessed using fluorescence microscopy in order to calculate characteristic lengths of microcracks.

*Results:* Significant differences in fatigue life were detected ( $p < 0.05$ ) between non-radiated (control) and radiated specimens, resulting in a drastic 89.2% fatigue life reduction of the 15–25 kGy group, and 95.3% in the 26–30 kGy group, compared to the reference. Microdamage analysis showed a considerable increase in microcrack lengths when bone was exposed to gamma radiation, which may indicate that bones used as allografts could fracture at some point when subjected to loading conditions *in vivo*.

*Conclusions:* The results of our research indicate that even if a range of 15–25 kGy is suggested to sterilize bone allografts, such practice needs to be reconsidered. In addition, using Weibull distribution, this work describes the conditions in which microcracks grow towards the fracture of bones in relation to the decrease in their mechanical properties.

*Key words:* allografts, Weibull distribution, fatigue fracture, gamma radiation, cortical bone, microdamage

### 1. Introduction

The study of the mechanical properties of bones used as allografts has gained increasing importance. Nowadays, fractures of massive allografts commonly occur in the 6% to 16% range [16], although rates as high as 42% have been reported [14]. Gamma radiation is used to inactivate bacteria, fungal spores, and viruses and has been the most accepted technique for sterilization purposes being used, as reported in [17],

by approximately 80% of the tissue banks in the United States to sterilize musculoskeletal tissue grafts.

There are two main concerns regarding the emission levels used with gamma-ray sterilization. The first one is the risk of infection. Doses of approximately 25 kGy assure the sterilization of most of the bacteria but are insufficient for inactivation of radio-resistant viruses such as HIV. The risks of implanting an allograft from an unrecognized HIV infected donor range from 1 in 1.6 million to 1 in 161 [11]. After usage of 25 kGy, the rate of infection was 13% and other

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studies concluded that a level of more than 30–34 kGy is required to inactivate the HIV in bone allografts [24]. The second concern involves the effect of gamma rays on the mechanical properties of the bones. These effects have been observed mainly through monotonic tests in trabecular as well as cortical bones.

The biomechanical effect of gamma radiation on human bones may not be evident under monotonic conditions and although clear evidence regarding significant differences in properties such as Young's modulus, yield strength, post yield plastic deformation, toughness and others has been reported for bones subjected to radiation up to 70 kGy, the reported data on significant differences for these properties varies within the sterilization range (25–35 kGy). Some of these results are shown in Table 1.

Less attention has been given to the study involving fatigue fracture of bones employed as allografts. For instance, [2] found very significant differences between control bones and bones previously exposed to 35 kGy when stress is applied (a dramatic fatigue life reduction of 99.5%). Additionally, [13] reported, with use of a rotating-bending fatigue testing apparatus under a dosage of 25–27 kGy, fatigue life reductions of 67% under 25 MPa, and 94.8% under 35 MPa.

The study of microdamage developments in bones with the use of fluorescence techniques is a method used to study the way microcracks originate and grow in bone towards the fracture by fatigue [21], [22]. Using fluorescence methods, the effects of fatigue in bones and the description of microcrack growth can be studied statistically with the use of the two-parameter Weibull distribution, in which parameters

differ according to bone type/species, and vary systematically with the number of cycles during in vitro testing [23].

In previous research [1], an explanation for microcrack behaviour under exposure of gamma radiation was provided, concluding that gamma radiation sterilization reduces the amount of overall damage, and suggesting that there may be an association between the damage process in bone tissue and the collagen structure. Nevertheless, this research also mentions that the details of these associations are “still obscure and remain to be investigated”. Hence, the aim of this work consists of the analysis of the mechanical properties of human bones used as human allografts under fatigue loads after emission of gamma radiation, considering the effect of the growth of microcracks towards their fracture. In our case, specimens of human femurs previously exposed to gamma radiation in the 15–25 kGy and 26–30 kGy ranges were put under fatigue compression stresses. The radiation ranges and the differences of fatigue resistance are reported and compared statistically. Furthermore, an extensive microdamage development analysis is included to determine the influence of gamma radiation in the way microcracks generate and grow. The Weibull distribution constants are calculated, and following the process given [23], the effect of microcracks growth is described and estimated in order to understand how they influence bone fracture.

The influence of age in microcrack developments has been extensively studied as well. For instance, as reported in [9], microcrack density in human femoral cortical bone increases significantly with age, and is

Table 1. Summary of results concerning mechanical properties of cortical bones under gamma radiation, among emission range 25–35 kGy

Reference	Type of bone	Gamma ray [kGy]	Additional conditions	$p < 0.05$	$p > 0.05$
[2]	Cortical human femora	36.4	31 and 38 years old	Yield strain, maximum stress, fracture strain, elastic energy, post yield energy, energy to fracture, fatigue life	Modulus, yield strength
[4]	Bovine tibiae cortical bone	33	Three point bending	Work to fracture, ultimate stress, failure strain, yield strain and work-to-fracture	Modulus, yield stress, or bone mineral density
[7]	Human femora	29.5	–	Work to fracture, more brittle	Young's modulus
[14]	Cortical human femoral rings	25 and 35	–	Toughness	Maximum load, elastic limit, Young's modulus, strain in elastic region, resilience, ultimate strain, ultimate stress
[25]	Goat tibia, cortical bone	25	Torsion, three point bending, compression	Maximal shear modulus, maximal shear stress, maximal bending loading, deflection, maximal bending strain, maximal compression strain	Maximal compression loading, stress, ultimate strength, stiffness, work to failure

greater for females than males at a comparable age. In our study, we also analysed microcrack behaviour under fatigue stresses taking into consideration the varying age of donors. We specifically examined microcrack growth and development using Weibull distributions among bones obtained from young and elder donors, 32 and 55 years old, respectively, and their influence in the reduction of the mechanical properties with age.

The main goal of this study was, based on studies and experiences obtained by scientists in the field of bone allograft sterilization under gamma radiation, to determine the optimum degree of gamma ray emission in the 15–25 kGy and 26–30 kGy ranges, to achieve optimum allograft sterilization without presenting considerable risk of fracture. It is hypothesised that description of microcrack behaviour, using probabilistic analysis and the concept of a characteristic length, can be used to recognize which human bones used as allografts will be affected drastically due to aging and gamma radiation sterilization, by weakening them and causing accelerated development of microcracks.

## 2. Materials and methods

Following the standard dose range of 25–35 kGy to which musculoskeletal tissue grafts are submitted for sterilization purposes [1], [2], this research involved the development of fatigue compression tests in samples taken from cortical zones of human femurs after the exposure of gamma radiation in the 15–25 kGy and 26–30 kGy ranges.

### 2.1. Specimen preparation and radiation treatment

Thirty two and fifty five year-old male femurs were donated by the Bone and Tissue Bank of the Autonomous University of Nuevo León, Mexico. The mid-diaphysis sections were dissected, subjected to a lyophilisation procedure and kept at room temperature. The bones were cut into three segments and separated into three groups: non-radiated (reference), 15–25 kGy, and 26–30 kGy gamma radiation range. The following number of samples per group were obtained: 12 samples for reference, 32-year old group; 13 samples for 15–25 kGy range, 32 year-old group; 13 samples for 26–30 kGy range, 32-year old group; 10 samples for reference, 55-year old group; 11 samples for 15–25 kGy range, 55-year old group; and 11

samples for 26–30 kGy range, 55-year old group. The exposure of the corresponding bones to radiation was performed using a Cobalt-60 source for approximately 2 to 3 hours at the National Institute of Nuclear Research (ININ), Mexico. Following the gamma radiation exposure, the bone segments were placed in a plaster mounting, and samples of dimensions  $3 \times 3 \times 4.5$  mm were obtained for each group with the use of a CNC machine (Fig. 1a–d).

### 2.2. Dye sequence technique

The dye sequence procedure described in [21] was applied to the bone samples, in which different fluorochromes were used during a fatigue test to look for microcracks while increasing the number of cycles. Two dyes were used in our work: Alizarin complexone, which was applied to samples and left overnight in a vacuum desiccator to ensure the penetration of the stain in the samples before the tests, and calcein applied during the performance of the tests until the fracture, both in 0.0005 M of concentration. After being stained with Alizarin complexone, as shown in Fig. 1e, the samples were subjected to compressive fatigue stresses while simultaneously being stained with calcein until fracture.

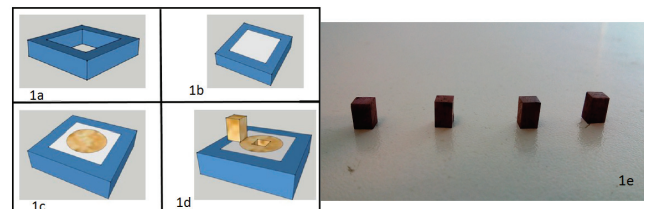


Fig. 1. Machining and staining process of cortical bone samples: (a) Image of stainless steel frame; (b) Addition of plaster and water mixture before the integration of bone segments; (c) Integration of bone segments in the plaster and water mixture; (d), Extraction of cortical samples from the plaster and water mixture with the use of a CNC machine to be subjected to fatigue compression tests; (e) Samples of cortical segments of human femurs after being stained with alizarin complexone

### 2.3. Fatigue mechanical tests procedure

A FIME-MASTIC I chewing simulator and a MTS-858 servo-hydraulic testing machine were used to apply cyclic axial compressive fatigue stresses to the samples (even though two machines were employed, application of the stress took place under the exact same conditions). The samples were introduced into a custom-made mounting (Fig. 2), which kept them in

a proper placement for the tests while being stained with calcein. The fatigue compression tests were performed under low cycle fatigue, at a frequency of 3 Hz, under sinusoidal waveforms with an applied force of 850 N in order to apply a stress of 94 MPa, cycling between 11 and 105 MPa. This stress was applied until fracture of the samples.

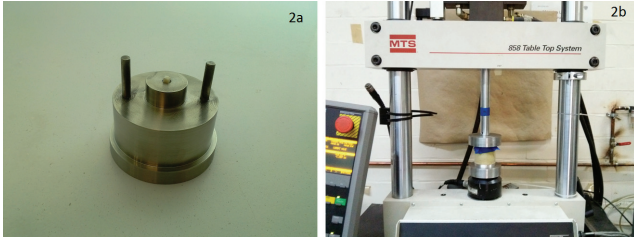


Fig. 2. (a) A cortical human bone sample placed in the custom-made mounting before performance of the fatigue compression tests; (b) Cortical human bone sample placed in the custom-made mounting during the fatigue tests

## 2.4. Microdamage observation

Compact bone ground sections were prepared following the procedure used by [10], where samples were placed in a small clamp and slices of approximately 250  $\mu\text{m}$  were cut using a diamond saw. Slides were cut transversally for each sample. Sheets of No. 1200 silicon carbide paper were used to manually thin down the slices to a thickness between 100 and 150  $\mu\text{m}$ . The slices were agitated in 0.01% washing up liquid and cleaned with de-ionized water to remove debris. Finally, the slides were air-dried and placed in a mounting medium (DPX) under a glass cover slip. Microdamage was observed using fluorescence microscopy at  $\times 125$  magnification. In a manner similar to the one used by [21], the microcracks were measured in terms of mean number of cracks occurring per section, crack numerical density (which was the number of counted microcracks occurring per area ( $\text{mm}^2$ )), crack surface density (total measured length of microcracks occurring per area ( $\mu\text{m}/\text{mm}^2$ )), and mean crack length (total length of microcracks divided by the number of microcracks per group, measured in  $\mu\text{m}$ ). Very important variables, which were called Weibull characteristic length and variability, were determined as well with the microdamage measurements. These variables are presented in the following section.

## 2.5. Statistical analysis

Statistical analysis involving number of cycles to failure between differing ages and gamma radia-

tion ranges was performed, using unpaired *t*-tests with a significance level of 0.05 to look for significant differences (MINITAB<sup>®</sup> Release 14.1). Similarly, this statistical analysis was used in a point-by-point basis for the microcrack developments specified in the previous section (crack numerical density, crack surface density, mean crack length). Following the studies done by [23], microcracks could be accurately described by the two-parameter Weibull distribution through the following equation:

$$P = 1 - e^{-\left(\frac{a}{a_0}\right)^\alpha} \quad (1)$$

which relates the cumulative probability  $P$  with microcrack length.

The microcrack length data were plotted in terms of the cumulative probability, as a function of microcrack length. The cumulative probability, which varies from 0 to 1, is the probability that a crack will be equal to or less than the specified length. For each set of data, theoretical curves were fitted assuming a Weibull distribution.

The two-parameter Weibull distribution analyses, is a method which involves the linearization of eq. (1) as follows:

$$\ln[-\ln(1-P)] = \alpha[\ln a - \ln a_0] \quad (2)$$

By plotting the left side of this equation  $\ln[-\ln(1-P)]$  against  $\ln a$ , the corresponding Weibull variables ( $a_0$  and  $\alpha$ ) were obtained. The parameters  $a_0$  and  $\alpha$  in (1) are then the characteristic length and variability constants, respectively. It was expected for  $a_0$  to increase and  $\alpha$  to decrease in proportion to bone aging, and a similar effect was suspected in terms of gamma radiation emission ranges. Therefore, these constants were good candidates to characterize the threshold levels of microcrack lengths which tend to cause bone fracture. An additional variable,  $a_{\text{max}}$  ( $P = 0.9$ ), which represents the length below which 90% of all cracks are found, and considered a measure of the largest crack present, was measured as well and was expected to increase in proportion to aging and to the increase in gamma radiation emission ranges.

## 3. Results

### 3.1. Fatigue tests

After performing the compression tests under a constant stress of 94 MPa, the mean number of cycles to

Table 2. Number of cycles to failure ( $N_f$ ) of control, group radiated under gamma ray at 15–25 kGy emission range, and group radiated under gamma ray at 26–30 kGy emission range. Results are shown for both 32- and 55-year-old human femurs. Also shown is the percentage (%) of fatigue life reduction after gamma-ray emission, in relation to the non-radiated group (reference)

32 years	Control ( $N_f$ )	15–25 kGy ( $N_f$ )	26–30 kGy ( $N_f$ )
Average number of cycles to failure ( $N_f$ )	5089.4	548.8	240.2
stdev	1284.5	180.0	87.6
% Fatigue life reduction with control		89.2%	95.3%
55 yrs.	Control ( $N_f$ )	15–25 kGy ( $N_f$ )	26–30 kGy ( $N_f$ )
Average number of cycles to failure ( $N_f$ )	2373.80	292.7	96.2
stdev	1239.85	138.9	47.4
% Fatigue life reduction with control		87.7%	96%

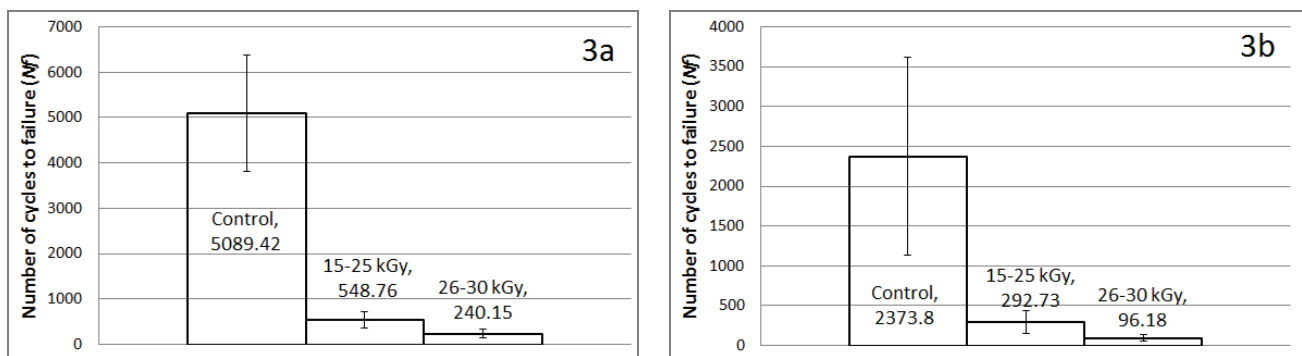


Fig. 3. (a) Mean number of cycles to failure between control, gamma ray 15–25 kGy emission range group, and gamma ray 26–30 kGy emission range group. All the cases concern the 32-year-old human femurs; (b) Mean number of cycles to failure between control, gamma ray 15–25 kGy emission range group, and gamma ray 26–30 kGy emission range group. All the cases concern the 55-year-old human femurs

fracture for the 32 year-old control group samples was  $5089.4 \pm 1284.5$  cycles, whereas for the 55-year-old control group the number was  $2373.8 \pm 1228.0$ , resulting in a significant difference between them ( $p = 0.004$ ). In addition, for the radiated groups in the 32-year-old samples a significant difference was found between the control group and those radiated at range 15–25 kGy ( $p < 0.001$ ) as well as between the control group and the group radiated at range 26–30 kGy ( $p < 0.001$ ). Furthermore, there was a drastic fatigue-life reduction in both radiated groups of 89% and 95%, respectively. Similarly, in the case of the 55-year-old samples, a significant difference was found between the control group and the 15–25 kGy group ( $p = 0.001$ ) as well as between the control group and the 26–30 kGy group ( $p < 0.001$ ). In this case, the fatigue-life reduction was of 88% and 96%, respectively. In Table 2 the results of number of cycles to failure of the 32- and 55-year-old human femurs are summarized.

The differences on the mean number of cycles to failure are shown graphically in Fig. 3.

### 3.2. Microdamage analysis

Following the fatigue tests, observations under fluorescence microscopy revealed significant differences between reference and radiated groups: a basic comparison of the maximum microcrack length per group revealed a huge difference between the longest microcrack of the 32-year-control group (350  $\mu\text{m}$ ) and

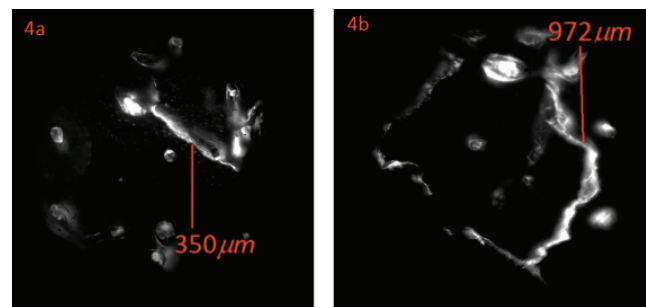


Fig. 4. Longest microcrack lengths measured for (a) control group, and (b) gamma ray 26–30 kGy emission range group. Both microcrack lengths concerning the case of 32-year-old samples

Table 3. Results of the statistical analysis for microdamage developments.  
The way microdamage development differences increase between in vivo and after fracture conditions as well as the manner in which microdamage developments increase as gamma ray emission rise are clearly shown for both the 32 and 55 year-old samples

	Number of samples per group		Number of cracks	Total crack length [ $\mu\text{m}$ ]	Numerical crack density [ $\#/\text{mm}^2$ ]	Surface crack density [ $\mu\text{m}/\text{mm}^2$ ]	Total mean length [ $\mu\text{m}$ ]
32 yrs Control	12	<i>in vivo</i>	12	905.68	0.03	2.17	75.47
		fracture	153	18810.47	0.37	45.06	122.94
32 yrs 15–25 kGy	13	<i>in vivo</i>	23	1575.59	0.07	4.91	68.50
		fracture	286	45782.33	0.89	142.66	160.08
32 yrs 26–30 kGy	13	<i>in vivo</i>	25	2423.95	0.10	9.87	96.96
		fracture	276	65033.24	1.12	264.88	235.63
55 yrs Control	10	<i>in vivo</i>	38	5389.68	0.16	22.12	141.83
		fracture	504	93394.41	2.07	383.37	185.31
55 yrs 15–25 kGy	11	<i>in vivo</i>	5	571.36	0.04	4.81	114.27
		fracture	307	79329.99	2.58	667.35	258.40
55 yrs 26–30 kGy	11	<i>in vivo</i>	18	1951.53	0.17	18.27	108.42
		fracture	284	90475.75	2.66	847.10	318.58

Table 4. *p*-values from the statistical analysis of surface crack densities.  
Significant differences ( $p < 0.05$ ) among reference and radiated samples, among 32- and 55-year-old samples, as well as between *in vivo* and fractured samples are clearly observed

		<i>p</i> value			<i>p</i> value
Control 32 yrs	<i>in vivo</i>	0.001 < 0.05	Control 32 yrs	fracture	0.014 < 0.05
	fracture		15–25 kGy 32 yrs	fracture	
15–25k Gy 32 yrs	<i>in vivo</i>	0.002 < 0.05	Control 32 yrs	fracture	0.001 < 0.05
	fracture		26–30 kGy 32 yrs	fracture	
26–30 kGy 32 yrs	<i>in vivo</i>	0.000 $\ll$ 0.05	15–25 kGy 32 yrs	fracture	0.037 < 0.05
	fracture		26–30 kGy 32 yrs	fracture	
Control 55 yrs	<i>in vivo</i>	0.001 < 0.05	Control 55 yrs	fracture	0.038 < 0.05
	fracture		15–25 kGy 55 yrs	fracture	
15–25 kGy 55 yrs	<i>in vivo</i>	0.000 $\ll$ 0.05	Control 55 yrs	fracture	0.027 < 0.05
	fracture		26–30 kGy 55 yrs	fracture	
26–30 kGy 55 yrs	<i>in vivo</i>	0.001 < 0.05	15–25 kGy 55 yrs	fracture	0.179 > 0.05
	fracture		26–30 kGy 55 yrs	fracture	

the longest one in the same age group but with the highest radiation exposure (972  $\mu\text{m}$ ) (Fig. 4). Similar differences appeared for the case of the 55-year-old samples: 1013  $\mu\text{m}$  maximum microcrack length of control group against 1588  $\mu\text{m}$  maximum microcrack length of the group radiated under 26–30 kGy.

The complete results of the statistical analysis for microdamage developments, including the mean number of cracks, numerical crack densities ( $\#/\text{mm}^2$ ), surface crack densities ( $\mu\text{m}/\text{mm}^2$ ), and total mean lengths ( $\mu\text{m}$ ), all of them for both the control and radiated groups and for microcracks formed *in vivo* and after fracture, are shown in Table 3.

Table 3 shows that for every increase in gamma radiation range, bone age, and fatigue life, there was considerable increase in the development of microcracks. In particular, in the case of surface crack densities, Table 4 shows significant differences between *in vivo* and fractured samples, as well as between fractured samples without (reference) and with radiation exposure.

Figure 5 shows the case of the 55-year-old samples submitted to a 26–30 kGy gamma radiation range.

Applying the *t*-test with a limit of  $p = 0.05$ , highly statistically significant differences were found in mi-

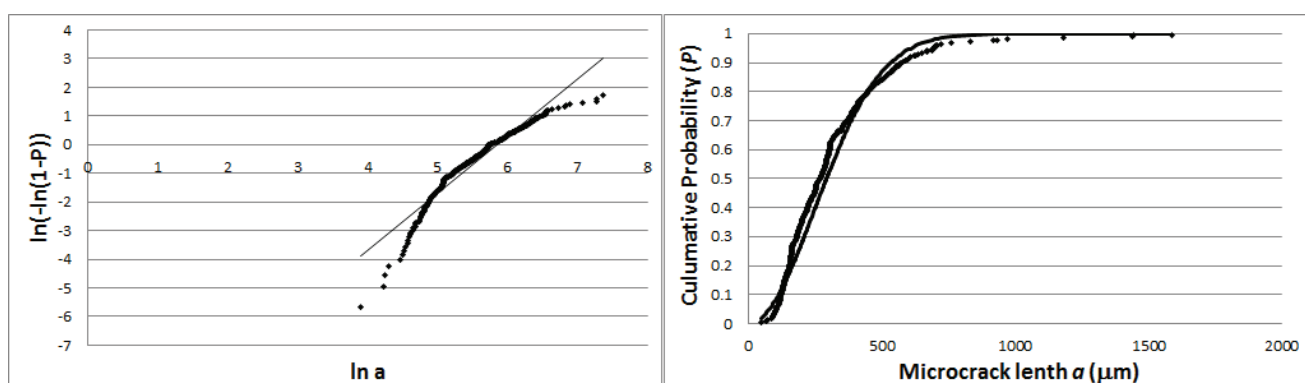


Fig. 5. Example of the linear replotting (on the left) and the original plot  $P$  a (on the right) for the Weibull equation for the 55-year-old samples exposed under gamma ray 26–30 kGy emission range

Table 5. Weibull constants of microdamage developments.  
 $\alpha$  decreases while  $a_0$  and  $a_{\max}$  ( $P = 0.9$ ) increase  
as the mechanical properties of the bones diminish

		$\alpha$	$a_0$	$a_{\max}$ ( $P = 0.9$ )
Control 32 yrs	<i>in vivo</i>	3.83	83.66	104.01
	fracture	3.06	136.99	179.91
15–25 kGy 32 yrs	<i>in vivo</i>	3.45	76.37	97.26
	fracture	2.16	177.39	260.99
26–30 kGy 32 yrs	<i>in vivo</i>	3.06	108.94	143.07
	fracture	2.00	259.51	393.73
Control 55 yrs	<i>in vivo</i>	2.39	159.72	226.43
	fracture	2.16	205.37	302.07
15–25 kGy 55 yrs	<i>in vivo</i>	2.01	134.38	203.59
	fracture	2.12	288.72	428.23
26–30 kGy 55 yrs	<i>in vivo</i>	2.53	123.02	171.06
	fracture	2.00	352.14	535.13

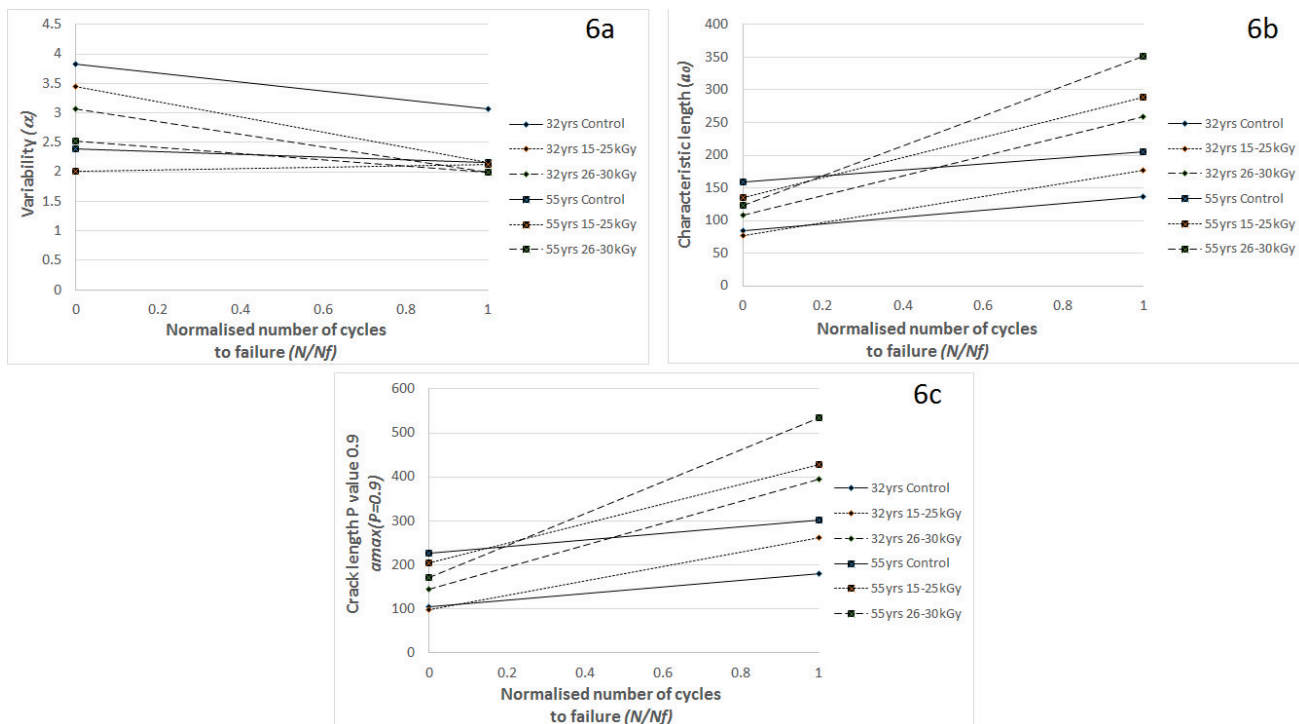


Fig. 6. (a) Variability ( $\alpha$ ) reduction tendency as a function of normalised number of cycles to failure. Reference, gamma ray 15–25 kGy emission range and gamma ray 26–30 kGy emission range; for 32- and 55-year-old samples (diamond and square markings, respectively); (b) Characteristic length ( $a_0$ ) increase tendency as a function of normalised number of cycles to failure. Reference, gamma ray 15–25 kGy emission range and gamma ray 26–30 kGy emission range; for 32- and 55-year-old samples (diamond and square markings, respectively); (c)  $a_{\max}$  ( $P = 0.9$ ) increase tendency as a function of normalised number of cycles to failure. Reference, gamma ray 15–25 kGy emission range and gamma ray 26–30 kGy emission range; for 32- and 55-year-old samples (diamond and square markings, respectively)

crocrack length distributions between reference and the groups of both gamma radiation levels as well as same fatigue instances between differing ages. To support these affirmations, the values of the Weibull variables are shown in Table 5 where a clear decrease in  $\alpha$ , with a tendency towards reaching a value of 2.00, as well as an increase in  $a_0$  and  $a_{\max}$  ( $P = 0.9$ ) as gamma radiation ranges and age increase are observed. The same tendency can be noted in the case of microdamage developments *in vivo* and in fractured samples.

In order to highlight the results shown in Table 5, we were able to plot the three Weibull variables as a function of number of cycles  $N$  normalized by the number of cycles to failure  $N_f$ , where the *in vivo* data corresponds to a value  $N/N_f = 0$  and the factured data to  $N/N_f = 1$  (Fig. 6).

## 4. Discussion

One of the main reasons for the extreme importance of the usage of bones as allografts is to avoid

using the patient's own bones which on many occasions are insufficient for the different types of lesions and fractures. Sterilization is necessary for their proper usage, and several types of bone allograft sterilization methods have been studied. The authors of these studies have striven to give convincing conclusions about their effectiveness, a few examples were the ones given by [18], [20]. Regarding infection risks, HIV resides in bone and the reduction in the transmission risk of HIV by a femoral head allograft was calculated to be only 5% when submitted to 25 kGy radiation [12]. Consequently, two emission ranges were selected for this study: 15–25 and 26–30 kGy. These are slightly higher than the previously mentioned disinfection level, and lower than 31–33 kGy in which gamma radiation is known to considerably reduce fatigue crack propagation resistance [19], and where dramatic loss of collagen network connectivity occurs due to  $\alpha$ -chain fracture [4].

Previous tests with the use of monotonic loads found significant reductions in the post-yield (plastic) properties of cortical bones, while pre-yield (elastic) properties were unaffected under 28 kGy [1]. The fatigue compression tests of our work support [2] the



conclusion of “a dramatic fatigue life reduction after emission of gamma radiation”. Our work supports also the recent results presented by [13], in which with the use of rotating-bending fatigue testing apparatus, the authors determined high significant fatigue life reductions with the application of emission in a range of 25–27 kGy (95% under 35 MPa). Our fatigue life compression results for the case of samples from 32- and 55-year-old produced fatigue reductions between 88% and 96% (Table 2 and Figure 3). We are aware that 94 MPa is an elevated range of stress compared to the one developed during normal running (850–1200  $\mu\epsilon$ , approx. 20–30 MPa) [3]. The selection of 94 MPa was determined as the FIME-MASTIC I chewing simulator was limited towards the submission of 850 N, not allowing feasible changes towards the stresses to be applied according to its design.

Regarding lyophilisation of cortical bones, except for the results presented by Cornu et al. [5], who found that this procedure resulted in just a slight reduction in the ultimate compressive strength and stiffness but did not affect the work to failure, in the last few years it has been extensively concluded that it has detrimental effects on the mechanical behaviour of cortical bones. Some of these studies were the ones presented by [8]. In our case, the lyophilisation procedure was done to facilitate bone handling and sending to the National Institute of Nuclear Research (ININ), Mexico, for the sterilization process.

Results of our study indicated that extremely high microcrack developments tend to appear with increased age, fatigue life, and gamma radiation emission levels, as indicated in Table 3. Regarding the influence of gamma radiation, significant differences in microcracking developments were observed when comparing fracture lapses between the control and radiated bones (Table 4). These differences were caused as gamma radiation altered collagen structure by producing radicals by radiolysis of water within the bone tissue. This led to the cleavage of peptide bonds in collagen [1], embrittling the bone and making it more susceptible to significant growth in microcracks.

It is important to mention that the case presented in Table 4, between ranges 15–25 and 26–30 kGy for samples taken from 55-year-old appeared as non-significant. From this result it was observed that, regardless of the radiation level to which bones are exposed, significant differences are perceived in the growth of microcracks compared to un-irradiated bones. Still, significant differences might not be found among radiated bones, yet the results suggest that any level of radiation may affect the bone to the point of rendering it unsuited for allograft usage.

The use of the two-parameter Weibull distribution helps to determine the growth of microcracks towards the fracture of bones. Changes in the microcrack lengths distributions are given in the Weibull parameters. In our previous work [23], the test data comparing the samples that fractured and those that did not, showed that the value of characteristic lengths was greater in the fractured group. In the case of the current work, as shown in Figure 6,  $\alpha$  decreased towards a value of 2.00 while characteristic lengths ( $a_0$ ) and  $a_{\max}$  ( $P = 0.9$ ) increased. The decrease or increase tendencies of the three variables were clearly maintained, both for the aging and gamma radiation range variations. Increase of characteristic lengths between *in vivo* and fractured samples highlight the existence of drastic microdamage developments throughout the process of fatigue until fracture (Table 5).

As also indicated in Table 5, the values of characteristic lengths for fractured bones when gamma-radiation levels increased, it highlighted and confirmed the notable development of microcracks in relation to the exposure to gamma radiation. Regarding characteristic lengths when considering age difference of donors, the existence of drastic development of microdamage as bone gets older was also observed. These results provide the necessary evidence to elucidate which processes occurring inside the bone microstructure cause the observed weakening in the mechanical properties of bones, as previously shown with the variables studied and reported in Table 2.

Henceforth, it is extremely important to reaffirm that the microdamage results of this study indicate there is a clear increase in microcrack characteristic lengths when exposed under gamma radiation, which may result in the fracturing of bones used as allografts at some point while loaded under *in vivo* conditions. Although suggested range of emission to sterilize bone allografts is 15–25 kGy, in view of these results the effectiveness of gamma radiation for sterilization purposes of bone allografts should be studied even further, moreover, HIV protocols as well as donor tests need to be developed in order to improve this restorative technique.

Utilizing information on the characteristic lengths presented in this work can be useful in discussing the mechanisms to predict the instances in which bones of certain athletes, members of the military as well as elderly people suffering from osteoporosis will fracture by fatigue. With this method the way the microcracks grow as the intrinsic properties of bones are reduced can be quantified and thresholds towards fracture can be established. Depending on external factors, the decrease of the bone's organic composi-

tions will have a direct effect on its microcrack characteristic lengths. Considerable increase in these characteristic lengths, occurring mainly due to radiation exposure, aging, excessive training regimes and even osteoporosis, set the conditions in which bones will fracture at some point when loaded under *in vivo* conditions stresses.

It is extremely important to focus on the use of microdamage characteristic lengths in devising ways to predict and prevent the generation of fractures of bones by fatigue. As by Kanis et al. [15] who said that more women died from osteoporosis fractures than breast, cervical and uterine cancer combined, osteoporosis is one of the major health challenges for all countries in the western world. As senile fractures in osteoporotic patients are due to fatigue rather than the impact of a fall [6], the results of this research may contribute towards the prevention of these types of fractures in elderly people.

Regarding the limitations of our study, it is necessary to underline the inability to reproduce stresses comparable to those that bones are submitted under *in vivo* conditions (30–40 MPa). Concerning these statements, the machine used for the fatigue compression tests were limited to the submission of stresses of approximately 94 MPa. Even so, the objectives of our study were fully accomplished as the influence of microcracks due to aging and gamma radiation leading to the tendency to cause fracture by fatigue was determined. Additionally, regarding the images of Figure 4, the original objective of our investigation was to obtain images in colour format, however and unfortunately, the available microscope was not equipped properly to obtain pictures with such characteristics. We, therefore, managed to take black and white images, considering that the purpose of this pictures was to show the differences in the lengths of the longer microfractures, in both the control group and the group under the emission of gamma radiation, and that was shown sufficiently using the available equipment.

## 5. Conclusions

In effect, our results show that bone properties are drastically diminished after being exposed to gamma radiation in ranges comparable to those used for sterilization purposes. Under those conditions, we show how microcrack distributions increase along with bone aging, which is another indication of the intrinsic mechanical properties of the bones being reduced by the exposure to gamma radiation.

Our analysis of the values of Weibull parameters show different fracture instances according to age and gamma radiation range. Furthermore, characteristic lengths increase and variabilities decrease along with increasing gamma radiation intensities and bone aging. The Weibull distribution is useful in determining characteristic lengths and can be used to describe the tendencies of growth of long microcracks to cause the final fracture of bones. Fatigue theoretical models may be developed with the use of Weibull parameters at fracture instances, depending on the physical properties of the bone.

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