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Influence of X-rays and gamma-rays on the mechanical performance of human bone factoring out intraindividual bone structure and composition indices

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ABSTRACT

Doses of irradiation above 25 kGy are known to cause irreversible mechanical decay in bone tissue. However, the impact of irradiation doses absorbed in a clinical setting on the mechanical properties of bone remains unclear. In daily clinical practice and research, patients and specimens are exposed to irradiation due to diagnostic imaging tools, with doses ranging from milligray to Gray. The aim of this study was to investigate the influence of irradiation at these doses ranges on the mechanical performance of bone independent of inter-individual bone quality indices.

Therefore, cortical bone specimens (n = 10 per group) from a selected organ donor were irradiated at doses of milligray, Gray and kilogray (graft tissue sterilization) at five different irradiation doses. Three-point bending was performed to assess mechanical properties in the study groups.

Our results show a severe reduction in mechanical performance (work to fracture: 50.29 ± 11.49 Nmm in control, 14.73 ± 1.84 Nmm at 31.2 kGy $p \leq 0.05$) at high irradiation doses of 31.2 kGy, which correspond to graft tissue sterilization or synchrotron imaging. In contrast, no reduction in mechanical properties were detected for doses below 30 Gy. These findings are further supported by fracture surface texture imaging (i.e. more brittle fracture textures above 31.2 kGy).

Our findings show that high radiation doses (≥31.2 kGy) severely alter the mechanical properties of bone. Thus, irradiation of this order of magnitude should be taken into account when mechanical analyses are planned after irradiation. However, doses of 30 Gy and below, which are common for clinical and experimental imaging (e.g., radiation therapy, DVT imaging, CT imaging, HR-pQCT imaging, DXA measurements, etc.), do not alter the mechanical bending-behavior of bone.

1. Introduction

In the field of skeletal research, a wide range of techniques is used to assess the compositional, structural and mechanical properties of bone to investigate the underlying mechanisms of fracture susceptibility in the presence of disease and aging. For this purpose, several gamma-ray-based

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and X-ray-based imaging modalities with varying irradiation doses are often applied in clinics and research. The latter modalities include high-resolution peripheral quantitative computed tomography (HR-pQCT), micro-computed tomography (μCT) and synchrotron imaging at dose ranges of mGy, cGy-Gy and kGy, respectively (Gy – Gray: unit of absorbed energy per mass). Gamma-ray based modalities include scintigraphy, SPECT (Single-photon emission computed tomography) and sterilization [1–4]. These sources of radiation result in an objects' exposure of several Grays, potentially causing mechanical deterioration in the material (cf. Fig. 1). Clinical assessments of bone health most commonly requires the use of radiation sources such as DXA (Dual-energy X-ray absorptiometry) and HR-pQCT in the range of 2–400 μGy and 3–10 mGy, respectively. Further radiological methods include clinical radiation therapy (in the range of Gy [5–7]), and irradiation sterilization of bone grafts (in the range of kGy [1,8–11]), which can affect the mechanical quality of bone depending on the irradiation dose. Therefore, the question arises, how *ex vivo* research methods utilizing X-rays (Gy to kGy) such as *ex vivo* HR-pQCT, μCT imaging, synchrotron studies for high resolution 3D-imaging [12] and crystal quantification [3]) would affect the biomechanical tissue properties. Sterilization methods for bone grafts such as gamma irradiation in the range of kGy have been known for a long time to impair mechanical competence [11,13]. Such irradiated bone grafts would not have the same mechanical properties to support the fixation of orthopedic implants as bone tissue that has not been exposed to gamma-rays. Furthermore, the current literature is still incomplete as it has not been ultimately answered whether or not there is an influence on the mechanical properties of bone caused by low doses of irradiation. Balsly et al. found no significant differences when testing the influence of irradiation dosages between 18.3 and 28.5 kGy on several tissues' mechanical competence [14]. However, Balsly et al. utilized frozen samples

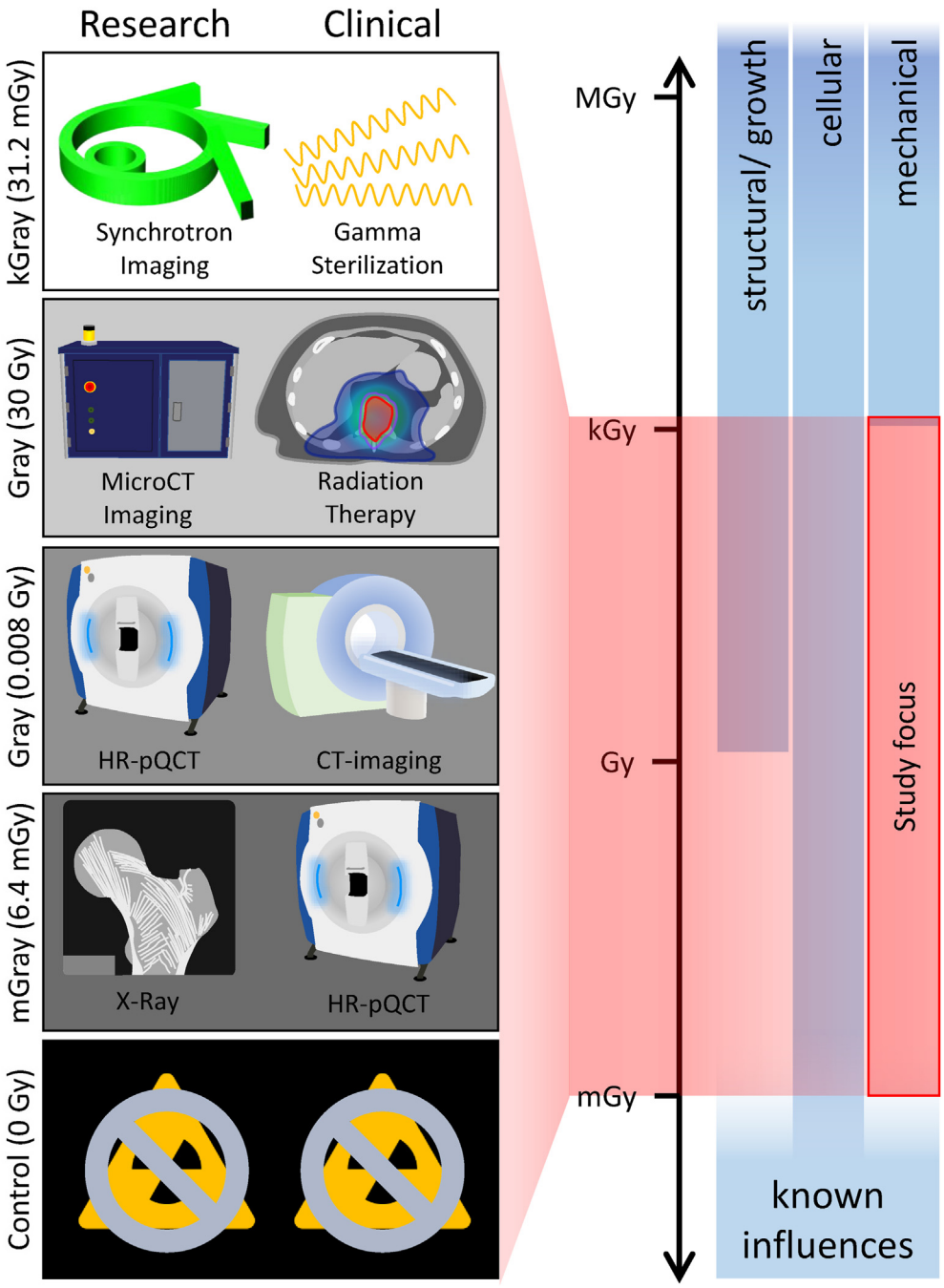

Fig. 1. *Sources of irradiation in clinics and research:* Bone can experience multiple doses of irradiation. Clinical imaging methods such as virtual bone biopsies in HR-qQCT expose the bone to doses of mGy, such as X-ray scans for research studies. Clinical standard CT imaging and large volume HR-pQCT expose the body to larger doses than does clinical HR-pQCT imaging. Regarding radiation therapy in clinical applications and microCT imaging in research applications, the samples are exposed to doses in the range of several dozen Gray. Regarding gamma irradiation for bone tissue sterilization and synchrotron imaging, the doses involved are of several kGy. Low doses have been shown to severely impact growth throughout embryonic development [20]. Cellular damage is caused by either stochastic damage due to irradiation without a threshold value or deterministic damage with a radiation threshold [21].

that were subject to sterilization processes, while it remains unanswered if sub-zero temperatures may protect against radiation damage [14]. Here, further studies are needed. Several studies have revealed both an influence of irradiation at very high doses (i.e. dozens of kGy) on the mechanical competence of bone [1,2,8] as well as on the chemical integrity [1,9] of the proteins with respect to the chemical bonds. Barth et al. reported a reduction in ultimate bending strength and strain, as well as reductions in crack initiation and growth toughness for doses ranging from 630 kGy to 70 kGy and did not detect effects at 50 Gy [2]. Currey et al. demonstrated that irradiation at doses of 17–94.7 kGy have a severe influence on the bending strength and work to fracture [8]. Akkus and coworkers revealed information on possible mechanisms of destruction of collagen molecules at an irradiation dose of 35 kGy [1]. Several studies have been undertaken to address the influence of irradiation on bone cells, bone marrow [15,16], bone growth [17], demineralized tissue [18] as well as in repeated μCT-scans in mice [19]. However, potential radiation-dependent changes of mechanical properties caused by clinically relevant dosages (cf. Fig. 1) are scarce. Therefore, in this study, irradiated human bone samples were subject to experimental bending tests to unravel how bone's biomechanical behavior is affected. Here, we investigated the direct influence of different clinically relevant irradiation doses on the mechanical performance of human bone.

The aim of this study is to examine the influence of irradiation at clinically relevant doses including higher doses of kGy that are common in bone graft sterilization. The presence of interindividual differences such as differences in the degree of tissue mineralization or compositional peculiarities remain a substantial problem in studies of human bone in terms of sample size and statistical power. However, samples from animal models have also been tested, where tissue characteristics show in general less inter-individual differences in bone quality. The composition and structure as well as the age of animal bones differ substantially from human bone. To cope with these problems and exclude influencing interindividual factors, we conducted our study using exclusively bone specimen from one single donor. Therefore, intra-individual bone structure and composition indices were factored out, while at the same time a precise preparation of idealized beams to achieve sufficient statistical power was possible. Additionally, the factor porosity, which influences the cross-sectional area, was quantified; the porosity data were normalized to an equal cross-sectional area.

2. Materials & methods

2.1. Bone samples

Bone samples were taken from the femoral diaphysis of a 39-year-old, skeletally healthy female organ donor. The cause of death was accidental, while no signs of metabolic bone diseases were found following full autopsy. The organ donor was not tested for monogenetic diseases. Beams for three-point bending tests were cut fresh and non-fixed in the longitudinal direction of the bone using a diamond-band-saw (EXAKT Advanced Technologies GmbH, Norderstedt, Germany) to ensure that the Haversian System is oriented in the same longitudinal direction including the collagen, and mineral alignment. Subsequently, the beams were ground to the same width (2.021 ± 0.062 mm) and height (3.001 ± 0.056 mm) representing the same cross-sectional shape using a water-cooled disc grinding machine (EXAKT Advanced Technologies GmbH, Norderstedt, Germany). The length of the beams was assured to be minimum 30 mm in length. A total of 50 beams were prepared and assigned to five groups, including groups with ascending irradiation doses and a control group. The beams were randomly assigned to the groups (n = 10 each) to prevent the results from being confounded by different structural features. The samples were kept hydrated for the full preparation process and frozen until the irradiation event and subsequent mechanical testing. The experiments and tissue extraction were conducted according to local laws of the city of Hamburg, Germany [22].

2.2. Irradiation of bone-beams

To simulate the exposure of bone to different irradiation doses, the following study groups were chosen. All specimens were placed side-by-side to prevent beam hardening or shrinkage and to ensure uniform irradiation. The specimens were exposed to the beam with their largest surface area. In HR-pQCT the specimens were placed horizontally side-by-side:

(1) The 6.4 ± 0.87 mGy group, in which HR-pQCT 1st generation (Scanco, Brüttisellen, Switzerland) was used to simulate a virtual bone biopsy.
(2) The 0.008 Gy group, in which a gamma irradiation device BIOBEAM GM 2000 (Gamma-Service Medical GmbH, Leipzig, Germany) was used to apply a dose resembling that of a clinical CT scan. Dose was calculated by multiplying the dose rate (2.64 Gy/min) of the gamma source times the exposure time.
(3) The 30 Gy group, in which the BIOBEAM GM 2000 (2.64 Gy/min) was used to apply irradiation to a third group at a dose corresponding to radiotherapy in clinical practice and high dose micro-CT imaging (often ranging in the area of cGy depending on resolution, integration time and other scanning parameters) in research applications. Dose was calculated according to the aforementioned point.
(4) The 31.2 kGy group, in which to simulate the gamma sterilization of bone grafts or synchrotron experiments, an irradiation sterilization facility (bbf Sterilisationsservice GmbH, Kernen-Rommelshausen, Deutschland) was used. Dose calculation was carried out by bbf Sterilisationsservice GmbH and certified. Validation was done using a photometric measurement.
(5) A control group that was not subject to any kind of irradiation.

All groups underwent the same number of freeze-thaw cycles, including the control group, since freeze-thaw cycles are known to may have influence on the mechanical competence of bone [23].

2.3. Three-point bending tests

Three-point bending tests were carried out with a universal material testing machine, Z2.5/TN1S (Zwick GmbH & Co. KG, Ulm, Deutschland) on non-fixed fresh samples that have been once frozen for storage and thawed prior to testing. The bearing distance was 20 mm with a span-to-height-ratio of 20/3 indicating lower apparent Young's modulus than at higher rates [24]. The pre-force was set to 0.2 N and approached the sample at a displacement rate of 0.5 mm/min. The load was applied at the center between the mountings, therefore mainly provoking mode I fractures [25]. The testing protocol was conducted at a displacement rate of 0.5 mm/min with a bending modulus calculation in the elastic region of 15–25 N in the force-displacement diagram. By the chosen parameter, this test qualifies as a quasi-static mechanical test. After mechanical testing, the yield point ($R_{p0.2}$), maximum and fracture stress ($\sigma_{max}/\sigma_{fracture}$) and strain ($\varepsilon_{max}/\varepsilon_{fracture}$) were calculated. The dissipated energy until maximum strain (W_{max}) and that until fracture ($W_{fracture}$) were calculated by integrating the area under the curve (AUC) of the force displacement curve using the manufacturers software textXpert v 10.1 (Zwick GmbH & Co. KG, Ulm, Deutschland). Three-point-bending tests do create compression above the neutral plane of the beam and tension beneath the neutral plane. Withstanding loads associated with bending is important with special regard to fracture risk [26]. Mechanical parameters such as stresses, which include the beam cross-section or any volumetric information for calculation, were corrected for the porosity values evaluated using μCT after mechanical testing. The beam-specific porosity was assumed to be evenly distributed to the beam cross-section. Structural stiffness loss was calculated according to the methods described by Tang et al.; the slope of the elastic curve was divided by the slope between the origin and the fracture force of the force displacement curve [27].

2.4. μCT analysis

After mechanical testing, one piece of each beam (half beam length equals 15 mm) was fixed in 3.5% formalin for two days. After fixation, the beams were stored at room temperature for drying. Subsequently, the samples were scanned with a μCT 40 scanner (Scanco, Brüttisellen, Switzerland) at an isometric voxel size of 10 μm at 55kV$_p$ and additionally at 8 μm isometric voxel size. Next, each sample was analyzed to calculate the BV/TV (bone volume to tissue volume), representing the porosity caused by the Haversian system of each beam, being potentially different thus influencing the stress calculations. Any inter-individual differences determining the base line bone quality have been ruled out. Degree and heterogeneity of mineralization, collagen quality, and matrix characteristics are similar for all 50 samples as they have specifically obtained from one single individual. For porosity calculation, a contour was drawn inside of the beam volume at both ends of the beam. Subsequently the contour was morphed in between. It was assured that a minimum distance of 4 pixels was set to be inside of the beam volume and to prevent partial volume effects.

Additionally, data sets from 8 μm resolution scans were evaluated using XamFlow 1.7.3.1 (Lucid Concepts AG, Zurich, Switzerland). A closing procedure was carried out after thresholding the μCT images measured at 8 μm isometric voxel size. Subsequently the resulting mask was eroded by 7 pixels. Afterwards the mask was applied to the originally thresholded image. Within the mask, the mean value was calculated per slice and for the whole volume. Background is presented by a pixel value of 0 (black) and bone by 1. Therefore, the mean value per slice or volume indicates the exact porosity within the mask/slice. Porosity was calculated in 3D for each beam and per slice (Suppl. Fig. 2 and 3). The same threshold was applied to all beams (734.7mgHA/cm^3). The stress and strain results were corrected according to the BV/TV (1 - porosity) measured in this analysis decreasing the respective cross-section accordingly. Therefore, the cross-sectional area was multiplied by the BV/TV. Additionally, the fracture characteristics were assessed by means of surface analyses. Here, the volume of the fracture affected beam was imaged and the haversian system was analyzed to ensure a longitudinal direction of the canals with respect to the beam geometry, to guarantee the longitudinal orientation of the beams. The volume of the fracture comprises the mineralized material between the non-affected beam-volume and the highest fracture surface peak.

2.5. Crack surface analysis

The crack surfaces of beams from the control and 31.2 kGy groups were imaged using an opto-digital reflection microscope (DSX500 3D, Olympus, Japan). The beams were mounted orthogonal to the microscope stage with respect to their longitudinal axis. Subsequently, the projected surface (cross section of the beam) of the region of interest (ROI) as well as the fracture surface area were assessed (Fig. 2e). The fracture surface (topological profile) was normalized to the 2D-ROI (cross-sectional area). The ratio of the ROI and the fracture surface area represents a measure of the deflection of the crack and thus the type of fracture (brittle or ductile). Measurement of the surface topography offers the opportunity to assess the fracture pattern quantitatively. The higher the ratio, the more crack deflection occurred during the fracture. Additionally, the fracture surface area of beams was imaged using scanning electron microscopy to visualize and magnify specific aspects of ductile and brittle fracture characteristics (i.e. crack deflection, crack twisting, microcracks, roughness).

2.6. Statistical analysis

For analysis, SPSS 22 was used. The normality of the data for the groups was tested using the Kolmogorov-Smirnov test and homoscedasticity was checked. Since normal distribution and equal homoscedasticity was given, ANOVA was carried out with a Bonferroni *post-hoc* test with correction for multiple tests. No outliers were detected. *P*-values ≤ 0.05 were considered to represent a significant difference. Presented values are porosity corrected if applicable.

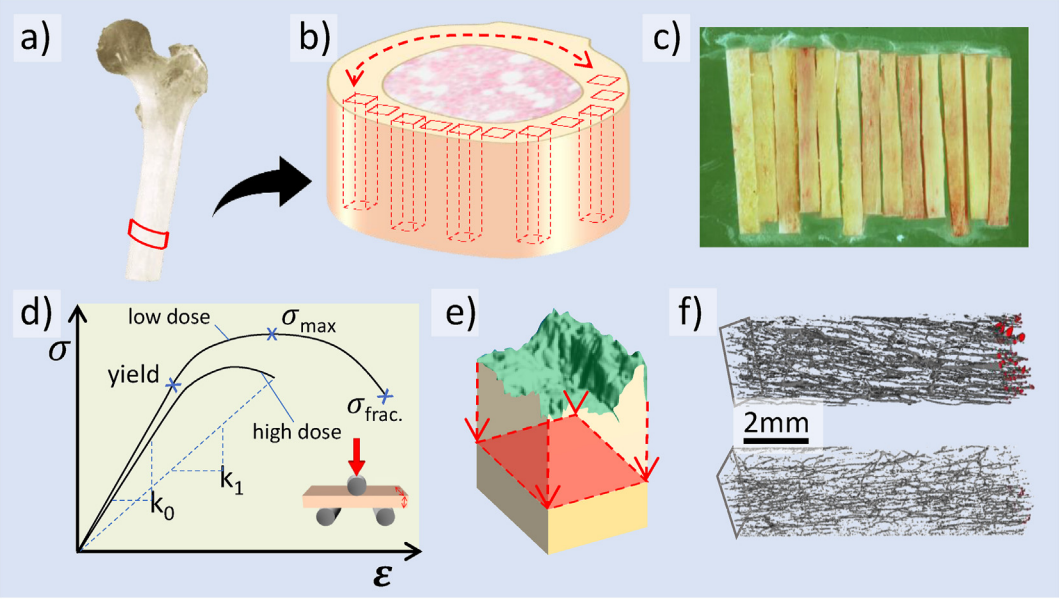

Fig. 2. *Methodological Approaches:* The samples were taken from one femur diaphysis (a) to prevent interindividual differences from confounding the results. The beams were extracted longitudinally to the long axis of the bone and polished to the exact same size. (b and c) c) Beam specimens cut from the diaphysis and prior to grinding. A three-point bending test was performed (inset d). In addition to stress-strain parameters (upper curve), the structural stiffness loss (lower curve) was calculated by dividing k_0 by k_1. Here the two curves represent exemplary high mechanical competence (upper) and reduced competence (lower) (d). Using opto-digital microscopy (e), the surface of the crack was measured (e, green). This surface was compared to the cross-section (e, red) of the respective sample (e) by dividing the fracture surface by the cross-section. To correct the stresses measured during three-point bending test, the porosity of each sample was measured by μCT. (f) The upper panel shows a representative beam with high porosity, and the lower panel shows a representative beam with low porosity.

3. Results

3.1. Three-point bending

No significant ($p > 0.05$) difference in the Youngs modulus was detected (Fig. 3a) among the groups. Significant differences ($p < 0.05$) were found between the control and 31.2 kGy group (Fig. 3 b-e), presented in the suppl. Table 1, specifically changes in maximum-stress and -strain and work-to-maximum-stress as well as fracture-stress, -strain and work-to-fracture. No differences were found between the control group and the 6.4 mGy, 0.008 Gy and 30 Gy groups or between these groups with respect to the parameters assessed. A 17.24% lower yield point ($Rp_{0.2}$) was detected in the group treated with an irradiation dosage of 31.2 kGy than in the control group (Fig. 3b, $p \leq 0.05$). For the plastic region, all parameters of the 31.2 kGy (cf. supplemental Table 1) did exhibit significantly lower values than the control group ($p \leq 0.05$). The structural stiffness loss [27] increased by 11.31% (Fig. 3f). The dissipated energy until σ_{max} (W_{max}) was quantified to be 68.11% lower than that in the control group (suppl. Tab. 1), and the dissipated energy until fracture ($W_{fracture}$) was 70.71% lower than that in the control group.

3.2. μCT-analysis

No significant differences in porosity (%) were found between the groups (4.73 ± 1.56, 4.87 ± 1.81, 3.58 ± 1.3, 4.45 ± 0.76, 4.16 ± 1.25 for control, 6.4 mGy, 0.008 Gy, 30 Gy and 31.2 kGy, respectively).

3.3. Crack surface analysis

Opto-digital analysis of the crack surfaces revealed a significantly ($p < 0.005$) smaller crack surface in the 31.2 kGy group than in the control group (Fig. 4). Here, the control group exhibited a crack-surface/ROI ratio of 1.51 ± 0.15, whereas the high irradiation dose group exhibited a ratio of 1.310 ± 0.086 (Fig. 4 a), indicating a smooth fracture surface in the 31.2 kGy group. Fig. 4 b-f.

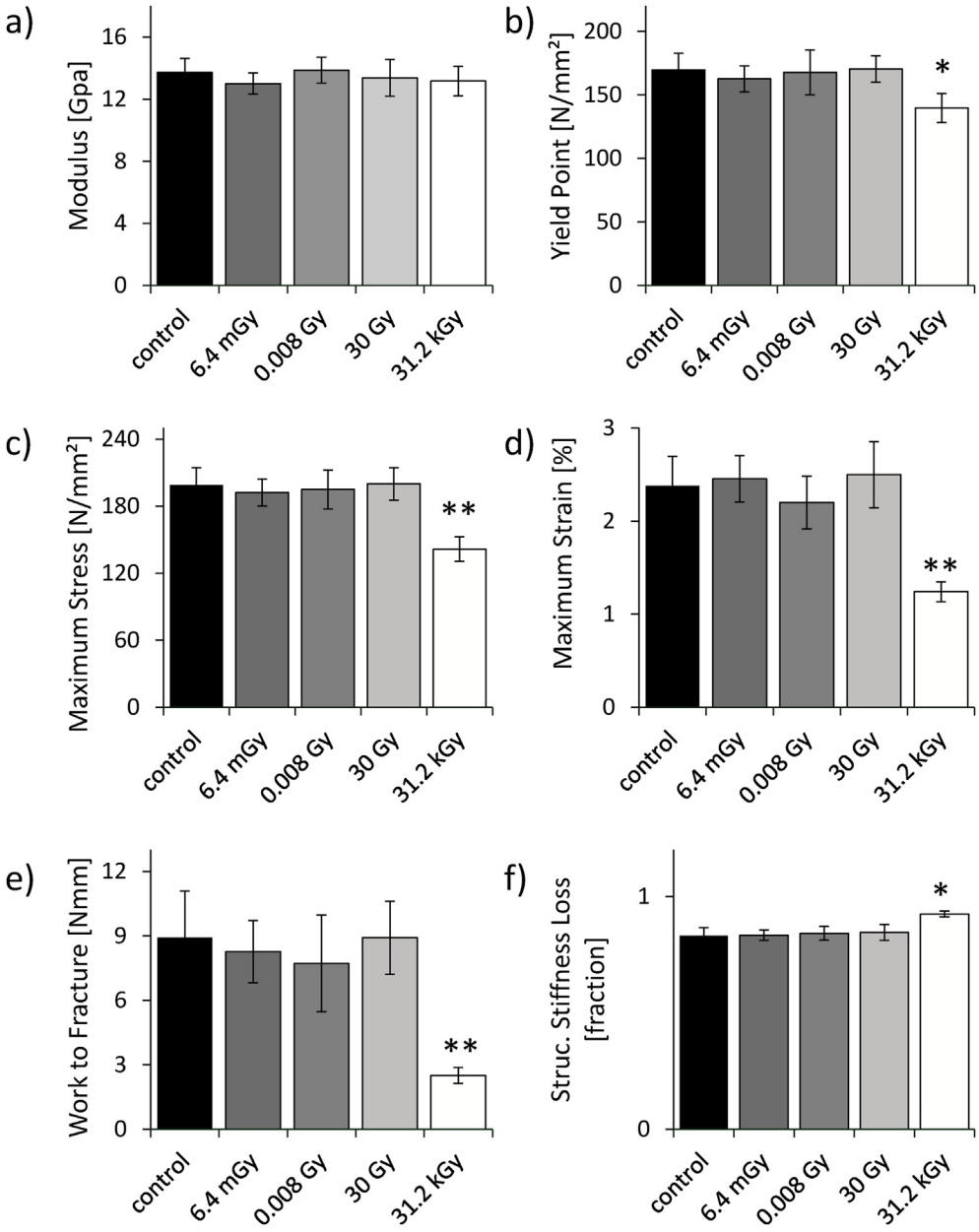

Fig. 3. *Mechanical testing results:* No differences were detected regarding the Young's modulus (a). However, a 17.24% lower yield point ($Rp_{0.2}$) was detected in the group treated with an irradiation dosage of 31.2 kGy than in the control group ($p \leq 0.05$) (b). The maximum stress (σ_{max}) was 28.38% lower in the high dosage group (c), and the strain (ε_{max}) at σ_{max} was 47.68% lower (d). The fracture parameters $\sigma_{fracture}$ and $\varepsilon_{fracture}$ decreased by 23.31% and 52.13%, respectively. Accordingly, the work to fracture was significantly lower ($p \leq 0.01$) for the 31.2 kGy irradiation dose (e). The extent of structural stiffness loss was higher in the group with 31.2 kGy irradiation dose compared to the control group (f). *$p \leq 0.05$, **$p \leq 0.01$.

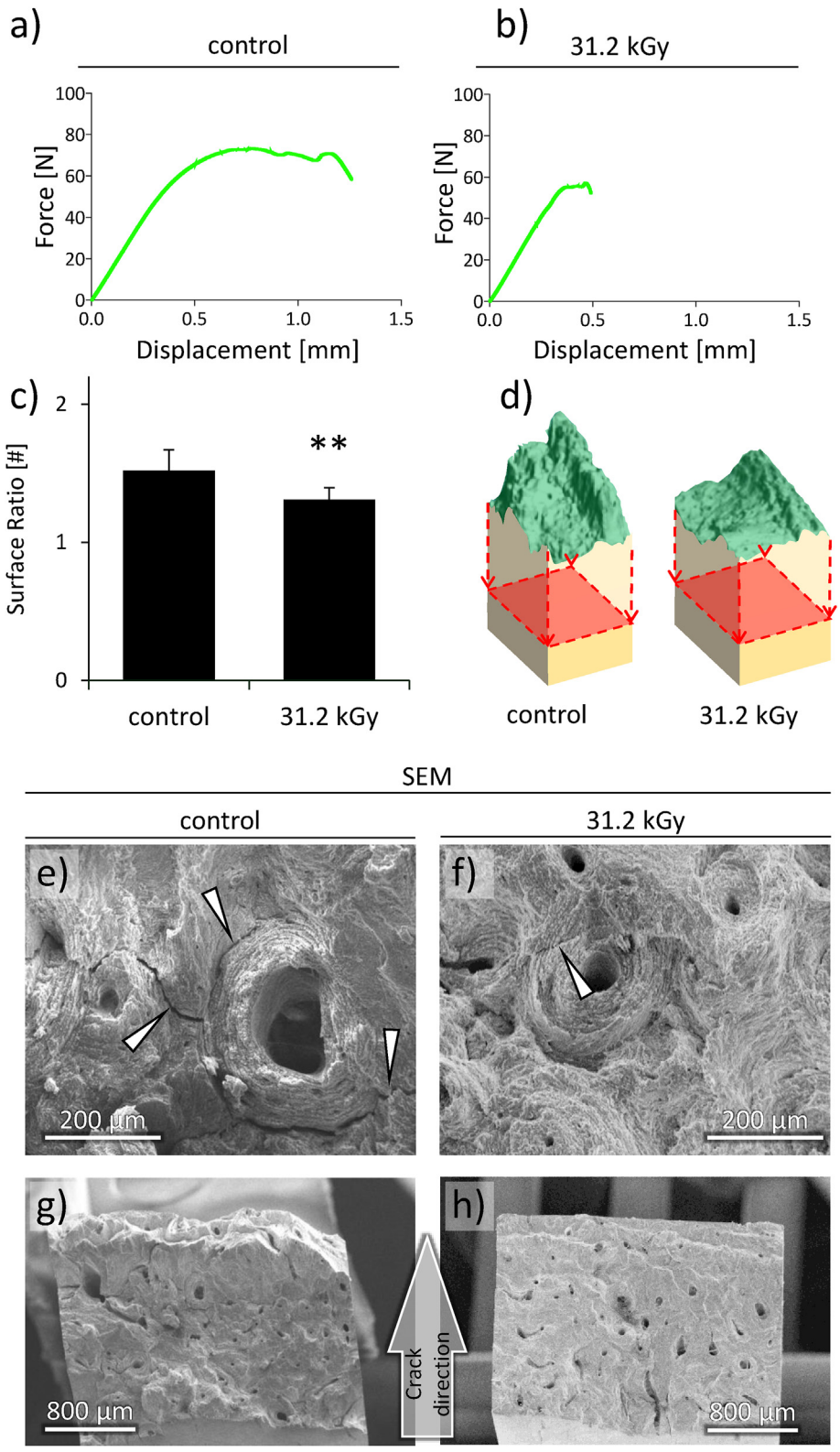

Fig. 4. Fracture characteristics. a-b) Depicted are the individual load-displacement curves obtained by three-point-bending (blue = control, pink = 31.2 kGy). The green lines represent the averaged load-displacement curves. The control group clearly indicates a larger plastic region with higher displacement and higher maximum forces (a). In contrast, the 31.2 kGy group exhibits very little plastic deformation (b). c) The control group exhibited a higher ratio of crack surface area (green labeling) to beam cross-sectional area (red labeling). e-h) Differences in fracture characteristics are visualized via SEM imaging. The control group showed a much more unsteady and rougher fracture surface (e, g) than the 31.2 kGy group (f, h), which is most likely caused by an intact collagen phase promoting ductility. **$p < 0.01$.

4. Discussion

4.1. Sources of irradiation and their known influence

Bone is subjected to different doses of irradiation, ranging from mGy to kGy, in both clinical [28–30] and research applications [2,3,8,9,31]. Although the risk of radiation to the human body is well documented, the benefits of techniques requiring radiation in clinical imaging outweigh the potential risks. Thus, the human body is exposed to irradiation doses of mGy in clinical applications such as CT imaging scans [32–34]. Beyond imaging purposes, radiation therapy is applied in clinical settings, being a source of irradiation at a dose of several units of Gray [35,36]. With regards to clinical tissue sterilization utilizing gamma irradiation, doses of several units of kGy can be reached [9,37], however, mechanical

competence of the bone graft is of primary importance only, since bone grafts are supposed to be replaced by vital bone over time. Similarly, radiation is needed in research on imaging techniques utilizing HR-pQCT (several units of mGy) and μCT (several units of cGy-Gy) to assess the structure and mineral content of bone specimens and in studies on synchrotron imaging (range of kGy) to assess bone structure and composition [2,31].

The effects of irradiation on bone mechanics have to be considered for both clinical and research purposes since mechanical competence is one of the main factors of bone survival and one of the main parameters used in bone research. High-dosage synchrotron imaging has been shown to severely impact the mechanical performance of bone at doses ranging from above 25 kGy–630 kGy by decreasing the plastic competence of the bone tissue [2,38]. The data obtained in the 31.2 kGy group proves that the impairment of mechanical properties is in the order of magnitude that was reported by other groups [1,2,9,39]. In this context any effect on bone tissue properties should become evident with the selected experimental testing set-up when focusing on lower radiation dosages.

4.2. Doses without direct mechanical influence

Importantly the presented results do not show a primary influence on the bone mechanics for doses of 30 Gy and below in bending experiments *ex vivo*. This result is of great importance for lab-based CT imaging in the micrometer range. Workflows such as μCT imaging prior to and after mechanical testing do not influence the mechanical behavior of the bone according to the three-point bending test results. However, as a secondary effects of radiation, cell death has been shown in multiple cases [15,40] and may affect bone cells *in vivo* with subsequent mechanical deterioration [41,42] by a changed metabolism.

4.3. Loss of mechanical competence after irradiation

In the present study, the three-point bending experiments did not show any significant differences in the Young's modulus among all five groups. These results, acknowledging mineral content to be the main driver of the linear elastic mechanical behavior (Young's modulus) of bone [37,43,44], indicate, that changes of the bone tissue are mainly induced to the collagenous phase. It is most likely that Hydroxyapatite may not change structurally or mechanically due to high-energy irradiation as it is in a crystal phase and therefore differently structured and bounded than proteins. In contrast, the organic collagenous phase is very susceptible to energy from irradiation due to its protein nature and the respective chemical bindings. Thus, the elastic region with respect to the bending stiffness described by the Young's modulus, which varies by the mineral, is maintained at very high irradiation doses, as shown in previous studies [8]. However, the yield point was lower in the 31.2 kGy group than in the control group, reflecting the interaction of the two components of bone, namely, the collagen and mineral in a composite material, to transfer load [3,12]. The decreased yield point and drastic decrease in the plastic region reflects impaired load transfer between minerals and collagen [45] when the plastic behavior of the bone is reached. Here, the 47.68% decrease in ε_{max}, 28.38% decrease in σ_{max} and 70.71% decrease in $W_{fracture}$ reflect a severe effect of irradiation on the collagen of bone, as well as an increased loss in structural stiffness. Since the plastic behavior is highly dependent on the collagen of the bone [44,46,47], these results do suggest that irradiation influences the organic component of bone [9], which is in line with prior findings [9,13,18,38].

The described loss of mechanical competence in the plastic region is strongly associated with impaired collagen quality since the plastic behavior of bone mainly depends on its ability to deform by means of sliding collagen fibers [47,48] and effectively transfer load to mineral particles [45]. For each tested beam the obtained porosity value was used to correct the measured mechanical properties to account for possible variations (Suppl. Fig. 2). Of note, physiological fractures are not specifically mode I but also include mode II. Mixed mode fractures do rather present common fracture types in patients. However, we have mainly addressed the influence of irradiation by three-point-bending with a fracture located at the point of the highest momentum.

4.4. How irradiation alters mechanical performance

Several factors can explain the diminished collagen quality. Irradiation has been shown to increase non-enzymatic crosslinking (NEC) in bone [9] and other tissues [49]. NECs have been shown to influence the mechanical behavior of bone and to render the material more brittle [50]. Ribose protectants such as a ribose pre-treatment [39] can protect human cortical bone from loss of mechanical competence by irradiation [10,39,51]. Also, irradiation priorly has been shown [18] to fragment collagen at high irradiation doses implicating the loss of plastic competence to be driven by a decreased collagen quality. However, this study is not directly accessing the damage mechanism within the mineral-collagen-interface but rather highlights the mechanical damages on the tissue level. The mechanism associated with the cause of the fracture following irradiation may not be a single one. Therefore, future studies are needed to address the fraction of each and its contribution to the overall damage of the bone tissue.

Decreased mechanical bone competence is also reflected in the crack path pattern, as measured by the fracture surface area indicating a more brittle crack behaviour for the 31.2 kGy group. The effect of irradiation on crack appearance has formerly been shown after irradiation and cyclic loading at a dose of approximately 230 kGy, however not in case of approximately 33 kGy [38]. One of the very strong energy dissipation mechanisms is crack deflection [47]. The more crack deflection there is, the rougher the surface of a crack. In our study, the significantly lower ratio of the crack surface with respect to the projected ROI implicates a decreased ability to dissipate energy. Thus, our results indicate brittle fractures occur already at doses in the range of dozens of kilogray, even without cyclic loading when the interaction of collagen and mineral platelets [45] is disturbed. These contrasting results in comparison to Fernández et al. [38] may be explained by the reduced image quality of the CT-imaging approach of the fracture surface in contrast to SEM-imaging in our case. This finding implicates that not only the mineral and its distribution [3] but also the collagen mineral interaction has a severe impact on the fracture behavior. This interaction is hampered in the case of 31.2 kGy due to collagen destruction by irradiation.

Taken together, our results emphasize that gamma sterilization and synchrotron imaging at high doses (several kGy) have a severe effect on bone mechanical properties [2,13,38] and therefore point to the necessity to account for the effects of irradiation on bone when experimental studies are designed. Clearly, mechanical experiments performed in combination with synchrotron imaging and gamma-rayed allografts for primary bone stabilization are subject to harmful irradiation. Alternative methods for imaging and sterilization need to be sought to prevent irradiation damages.

This study has some limitations. We did not examine the long-term effects of irradiation on bone of living species with respect to mechanical parameters or cellular viability. However, osteocyte death may also increase remodeling [52] if other cells are still viable to migrate. Additionally, no toughness tests or NEC quantification assessments was carried out in this study. We used X-ray and gamma irradiation to quantify the influence of radiation on bone mechanics. However, these two radiation types have similar characteristics, and the range of applications in medicine in research is broader when both versus only one of these types are considered. This manuscript presents data of $n = 50$ beams all from one organ donor. Thereby this study design cannot address effects of irradiation on various bone quality conditions (i.e. aged bone, osteoporosis, etc.), however, using exclusively one organ donor does rule out possible interindividual differences causing variability of the results. This study mainly addresses to what extent irradiation can affect bones' mechanical competence, but not explicitly which mechanism of fracture is occurring in relation to the tested irradiation dosages.

Using only one organ donor, this study has reduced the variability of our results for a higher sensitivity. Yet, the absolute values cannot be used as an exact reference because variations in the population are not covered. Pathologies were excluded forensically. Potential rare genetic mutations, which would be subclinical in their expression, were not detected. Furthermore, this study has not addressed the exact order of magnitude what irradiation dosage leads to early mechanical decay following irradiation. Future studies are needed to address this point which is needed to determine the maximum irradiation dosage that can be used without harming the mechanical performance of bone tissue. Referring to the applied quasi-static testing, an influence on the dynamic behavior of bone tissue cannot be determined.

5. Conclusion

In conclusion, we observed that irradiation doses of 30 Gy and below have no significant effect on the bending properties of bone. In case lab-based CT imaging of tissue samples are planned, researchers can plan mechanical testing of samples at any time point of the study as only above 30 Gy detrimental effects on the mechanical performance of bone were identified. In addition, this study clearly shows a severe effect of 31.2 kGy on the mechanical competence of bone, strongly affecting the plastic behavior of bone. This highlights the importance of considering radiation damage in synchrotron imaging and allograft sterilization applications.

Credit author statement

Conceptualization: FNS, BB. Data curation: FNS, MH, TR, TK, BB. Formal analysis: FNS, BB. Funding acquisition: BB. Investigation: FNS, MH, BB. Methodology: FNS, MH, BB. Project administration: KP, MA, BB. Resources: ChS, KP, MA, BB Software: FNS Supervision: BB. Validation: FNS, KES, BB. Visualization: FNS, CoS. Roles/Writing – original draft: FNS, KES, BB. Writing – review & editing: FNS, MH, EKS, TR, CoS, TK, ChS, KP, MA, BB.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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