Predicting Distal Femur Bone Strength in a Murine Model of Tumor Osteolysis

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Abstract Predicting pathologic fractures of long bones caused by metastatic disease continues to be a challenging clinical problem. We assessed the ability of noninvasive imaging and computational techniques to predict the strength of bones with osteolytic lesions. A murine model of induced tumor osteolysis to the distal femur was used as a model system resulting in a wide range of lesion sizes. Microcomputed tomography scans were obtained and specimen-specific, voxel-based, finite element analyses were performed and results were compared with direct measurement of biomechanical strength via axial compressive loading of the distal femur. Additional indirect surrogates of bone strength included dual-energy xray absorptiometry to determine bone mineral density, radiographic scoring, and computed tomography volume/mineral estimates. Predicted bone strength was weakest ($r^2 = 0.55$) for the dual-energy xray absorptiometry measure and strongest ($r^2 = 0.91$) for the direct computed tomography voxel-based, finite element analysis. The relative success of the voxel-based, finite element modeling approach to estimate bone strength in a murine osteolytic tumor model indicates this approach, with further

development and validation, could serve as a way to non-destructively estimate bone strength in a clinical setting.

Introduction

Pathologic fracture remains an important cause of morbidity in patients with skeletal metastases from breast, prostate, renal, thyroid, and lung cancer. Currently, the most effective strategy for preventing pathologic fracture is prophylactic internal fixation, but this necessitates an invasive procedure in patients who may have important comorbidities that make them poor candidates for surgery or effective rehabilitation.

Given this clinical concern, predictive tools have been developed to discriminate between metastatic lesions that require stabilization and those that may be managed conservatively. The rating scheme published by Mirels [10] has been most widely adopted and uses information on radiographic evidence of bone destruction, anatomic location, pain, and lesion type to generate a composite score. However, Damron et al. [2] concluded that while the Mirels' rating system "would seem the best system available at this time...it is by no means a perfect solution to pathologic fracture risk prediction." The most serious problem with the Mirels system is its low specificity (33% in the original report by Mirels, 35% in the study by Damron et al. [2]). Approximately two-thirds of patients are therefore going to undergo unnecessary internal fixation procedures with strict application of these criteria.

In an effort to improve predictions of bone strength, work has been performed to advance structural-based methods, including beam theory methods [11], computed tomography (CT)-based section analysis [13], and CT-based finite element methods [5, 6]. In general, these

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approaches show promise as a means of improving our ability to estimate the strength of bones with metastatic tumors. In particular, the CT-based finite element methods (CT/FE) have the potential to directly account for changes in bone quantity, a wide distribution in bone material properties, and a wide range of loading conditions with little subjective interpretation of results.

One limitation of previous research studies is the predictive models are usually tested against experimentally created defects that probably do not accurately represent clinical tumor osteolysis [3, 8]. Many osteolytic tumors are associated with considerable trabecular bone loss before cortical bone loss; these situations are difficult to simulate in a cadaver bone. In addition, a considerable percentage of these tumors are mixed osteoblastic-lytic lesions, further complicating the relationship between lesion size and material properties. Although there have been some efforts to model and assess human bone tissue with metastatic disease [5, 6], it is difficult to procure enough specimens with the same tumor type in similar locations to fully assess predictive models. An alternative approach would be to develop and apply an animal model in which the tumor location and type can be controlled and monitored. Such a system could be used to develop improved predictive models and improve our understanding of the failure process. No such *in vivo* model has been previously reported.

We asked whether noninvasive imaging and analytic techniques, including CT-based structural modeling, could predict the strength of bones with osteolytic lesions. We raised three specific questions: (1) Can a murine model of tumor osteolysis produce a wide range of bone strengths when monitored using radiographic scoring techniques? (2) Do indirect surrogates of bone strength, based on radiographic scoring, dual-energy xray absorptiometry (DEXA), CT, and structural CT/FE analysis methodologies correlate with experimental bone strength? (3) Can a calibrated surrogate of bone strength (determined from structural CT/FE modeling) accurately predict experimental bone strength?

Materials and Methods

We used a validated murine model of tumor osteolysis to the distal femur [1] in which we monitored the progression of lesions using plane radiographs. Rather than euthanizing animals at fixed time points, we elected to euthanize animals based on the severity of their bone lesions to produce a heterogeneous sample population that would be expected to encompass a broad range of bone strengths. Indirect surrogates of bone strength (based on radiographic scoring, DEXA, CT-based bone volume and bone mineral content measurement, and CT/FE stiffness measures) were

compared to experimental bone strength using linear regression analysis. A final calibrated direct estimate of bone strength was based on a von Mises equivalent strain measure. In this case we used control limbs as a training data set and tumor burdened limbs as the test set (defined in detail later) and compared strength predictions with the experimental bone strength using linear regression analysis.

We anesthetized ($n = 20$) 8-week-old female NCr nude mice (Charles River, Frederick, MD) with Telazol (Wyeth, Madison, NJ) (45 mg/kg intramuscularly) and xylazine (7.5 mg/kg intramuscularly). The right femur (tumor burdened femur) was exposed through a parapatellar arthrotomy and F10 human breast cancer cells (2×10^4 cells in a volume of 20 μ L) were injected through the intercondylar fossa into the distal femoral metaphysis. The left nonoperated femur served as the control. Animals were group housed, allowed unrestricted exercise for the duration of the study, and examined twice daily for evidence of lameness, limb swelling, or systemic illness. Analgesics were not required in this study since animals were euthanized before the development of pathological fracture. At the conclusion of the study period, mice were anesthetized as for surgery and euthanized by cervical dislocation.

We obtained magnified lower limb radiographs on a weekly basis (Faxitron MX-20; Faxitron X-ray Corp, Buffalo Grove, IL) to document the progression of osteolysis. An attempt was made to euthanize the mice at points that resulted in a wide range of radiographic scores using a scoring scheme developed by Weber et al. [16]; this served as a surrogate for human clinical scoring methods. With this scheme, Grade 0 represented no lysis, Grade 1 indicated minimal but detectable lysis within the medullary canal, Grade 2 represented moderate lysis limited to the canal, Grade 3 indicated severe medullary lysis with cortical involvement, and Grade 4 indicated massive lysis with cortical destruction and tissue extension (Fig. 1).

DEXA measurements were made on the tumor burdened limbs immediately after tumor inoculation and at the time of euthanasia (PIXImus 2; GE Lunar, Madison, WI). We used the change in bone mineral density calculated as the ratio of final to original (end/start) to document changes in the amount of femoral bone.

Finite element modeling of bone requires assignment of elastic modulus properties, and this is usually assigned based on the mineralization of the bone as documented by computed tomography imaging methods. Although density-modulus relationships for human bone exist, there was a lack of data available for murine bone. Therefore, an initial nondestructive analysis of the bone mechanical response was performed to determine a density-modulus relationship for murine bone using a combination of three-point bend experiments of the control limbs and

Fig. 1A–E A full range of osteolytic lesions was achieved by radiographically monitoring the femurs on a weekly basis. Radiographic scores for the distal femur were used in which (A) Grade 0 represented no lysis, (B) Grade 1 indicated minimal but detectable lysis within medullary canal, (C) Grade 2 represented moderate lysis limited to the canal, (D) Grade 3 indicated severe medullary lysis with cortical involvement, and (E) Grade 4 indicated massive lysis with cortical destruction and tissue extension.

corresponding finite element models. A three-point bend test was chosen for two reasons. First, the compliance of the bone with a relatively long span was high relative to the test frame and support compliance, and we were able to achieve consistent compliance measurements for multiple tests of the same specimen. Second, the three-point bend test excluded the growth plate because this was shown in pilot work to confound the compliance measurements.

After euthanasia and explantation, the femoral shafts of the control (left) limbs were placed on a plexiglass custom fixture for microcomputed tomography scanning (Scanco Micro-CT 40, Bassendorf, Switzerland, 12- μ m isotropic resolution; 55 kVp/145 μ A) using the same span and orientation as used in three-point bend experimental apparatus. In this way, finite element models of bones generated from computed tomography scans were aligned in the same coordinate system as used in the experiment. We used nondestructive three-point bend tests (Fig. 2) to determine the transverse stiffness of the femoral shaft for each control femur (Q Test 1/L, MTS Systems Corp, Eden Prairie, MN). Linear elastic finite element models (Fig. 3) of the three-point bend test experiments were developed and analyzed (details below) with an initial assignment of modulus, on a voxel-by-voxel basis. Each model element voxel was assigned a gray scale that corresponded to a hydroxyapatite density, based on prior calibration of the

Fig. 2 The nondestructive experimental three-point bend tests had a span of 8.0 mm and a roller diameter of 1.0 mm.

scanner using the provided SCANCO calibration phantom. For the three-point bend models (Fig. 3), the two span roller supports were simulated with simple supports ($y = 0$ on nodes that corresponded to roller locations), and the central loading pin was simulated by a fixed transverse displacement to nodes that coincided with the central loading pin ($-y$ direction). One point on the left roller was fixed to prevent translations in the x and z directions. The corresponding vertical reaction force at the central loading

Fig. 3 Specimen-specific finite element meshes were created for each of the control bone and subjected to three-point bend loading (example shown here). These models were used to develop a relationship between elastic modulus and bone hydroxyapatite density from the CT scans. The model was simply supported with no translation ($y = 0$ mm) boundary conditions for the support rollers and fixed displacement ($-y$ direction) applied to nodes under the loading pin. A single point on the left roller was fixed to prevent translation in the x and z position ($x = z = 0$). A span of 8 mm was used.

pin was determined. By comparing the stiffness of the experiment (K_{exp}) with the stiffness of the model (K_{fea}) for the 20 linear-elastic models, we determined the scale factor ($SF = K_{\text{exp}}/K_{\text{fea}} = 12.61 \pm 1.32$ (MPa/[mg/cc HA]) for scaling modulus (E) from HA density. From this, the modulus was assigned using E (MPa) = 12.61 HA(mg/cc).

In preparation for destructive axial compressive tests, the proximal femurs of all bones (tumor burdened and control) were potted in a custom alignment fixture using dental acrylic, leaving 8 mm of the distal femur exposed. Prior to axial compression tests, we scanned femurs from the pot surface to the distal end of the femur. Each scan voxel was assigned a gray scale that corresponded to a hydroxyapatite density. We determined bone volume (mm^3) and bone mineral content (mg) for the distal 5 mm of length of bone in all of the tumor burdened bones using SCANCO bone analysis software.

Destructive axial compressive loading (Fig. 4) was performed on both control and tumor burdened bones. We cemented distal pots into a supporting chamber and a small layer of PMMA cement was used to support the condyles on the base plates. Femurs were loaded at a displacement rate of 0.1 mm/min and loading continued beyond the peak load until 50% of the peak load was reached. Displacement was determined from the cross-head displacement and force was determined from the in-line load cell. We determined bone stiffness using the ascending slope of the load-displacement response from half the peak load until the yield load (estimated using the 0.2% offset method). Peak load, for the axial compression tests, was also collected to indicate bone strength. The effect of system

Fig. 4 The axial compression tests used a small layer of PMMA cement on the axial compression base to provide support for loading to failure.

compliance was accounted for with both three-point bend and axial compression tests.

We generated voxel-based finite element meshes directly from the micro-CT scan data using custom software adapted from existing open-source software codes (Laboratorio di Tecnologia Medica, Istituti Ortopedici Rizzoli, Italy) [15]. Resulting elements were eight-noded cubes with an edge length of 24 μm . This element size was sufficient to allow three to four voxels to span the width of trabecular bone in the distal femur. A lower threshold of 400 mg/cc was used to exclude voxels with density below this level, resulting in meshes with between 700,000 and 1,000,000 elements. We used an iterative sparse solver with incomplete Choleski decomposition (MARC; MSC Software Corp, Santa Ana, CA) for all analyses on a SUN workstation (SUN Blade 1500, SUN Microsystems, Santa Clara, CA). For the axial compression tests, the models (Fig. 5) were fixed with zero displacement at the most proximal bone section, and fixed axial displacements were applied to the region corresponding to the PMMA support. As the PMMA cement was well-attached to the based loading plate, transverse displacements of the distal femur were fixed with zero displacement. All models were assumed to be linear elastic and a Poisson's ratio of 0.3 was used for all models. Material properties were assigned on an element-by-element basis using the modulus-density

Fig. 5A–B Voxel-based finite element meshes were created directly from the CT scan sets for (A) control and (B) tumor burdened bone and were subjected to axial loading. The edge length of each voxel (volumetric pixel) was 24 μm in all models.

relationship determined above. We determined the axial compressive stiffness of each model that served as an indirect surrogate of bone strength.

For the CT/FE calibrated surrogate of bone strength, the finite element analysis was modified to include von Mises equivalent strain [9] as a yield criteria based on the work of Niebur et al. [12]. In their work, tensile and compressive failure strains of 0.78% and 1.06%, respectively, were determined for human trabecular bone. Although a corresponding trabecular yield strain is not known for murine trabecular bone, we assumed a 1% strain level guided by the work of Niebur et al. [12]. To apply this yield criterion to the murine model, we determined the volume fraction of bone in each of the control bone FE models that had a von Mises effective strain at or above 1% when the corresponding experimental peak load was applied. The average volume fraction from all of the control bones was used to define the “yielded volume fraction of bone.” It is noted that with these linear elastic models, true yielding of the bone elements was not implemented in the formulation. The predicted strength of each of the tumor burdened femurs was then calculated by determining the axial force required to produce a yield volume fraction of the same magnitude as found for the control bones. To do this, we take advantage of the proportionality in von Mises strain and applied load. With this approach, the control femurs could be considered the “training” femurs, whereas the tumor burdened femurs could be considered as the “test” femurs for this calibrated surrogate measure of bone strength.

Model validation is often a concern with finite element models. In this study, model validation was performed (by design) via specimen-specific finite element analysis where each model was directly compared via linear regression to the corresponding experimental response. Limitations of the models are explored in the discussion.

We used linear regression analysis to determine relationships between bone strength and the various radiographic, imaging, and FE outcome measures. Standard error of the estimate (SEE) and maximum residual error were determined for each regression model using the difference between

predicted and measured strength. We applied Bonferroni corrections in cases in which there were multiple comparisons. The radiographic scoring measure (0 to 4) was assumed a continuous variable for purposes of the regression analysis.

Results

The longitudinal sampling of osteolysis progression was generally successful in terms of generating femurs with a full range of radiographic scores for osteolysis and a wide range of mechanical strengths (Table 1). Four animals were removed at Week 0, one at Week 1, two at Week 2, four at Week 3, seven at Week 4, and one at Week 5, resulting in a distribution of radiographic scores (RS) with four at RS0, seven at RS1, two at RS2, three at RS3, and four at RS4. One femur had a complete fracture on explant leaving 19 tumor burdened and 20 control femora for analysis and testing.

The finite element model axial stiffness for control (1232 ± 167 N/mm) and tumor burdened bones (880 ± 484 N/mm) was much higher than the corresponding experimental stiffness measurements for control (351 ± 78 N/mm) and tumor burdened bones (193 ± 127 N/mm), even after accounting for system compliance. The indirect surrogates of axial compressive bone strength (Table 2) all correlated positively with actual experimental bone strength for the tumor burdened bones. Normalized bone mineral density (by DEXA) had the largest standard error of the estimate, followed by radiographic scoring, CT bone mineral content and volume. The voxel-based FE stiffness was the best indirect predictor with a standard error of the estimate of 5.66 N and a comparatively high correlation ($r^2 = 0.89$).

The volume fraction of bone above the 1% von Mises equivalent strain level in the control bones was $0.77\% \pm 0.26\%$. The highest von Mises equivalent strains were observed in focal areas of trabecular bone within the metaphyseal and epiphyseal regions of the distal femur. For the tumor burdened bones, the CT/FE calibrated surrogate

Table 1. Distribution of biomechanical properties (peak strength, initial stiffness), DEXA bone density, and CT-based bone volume and bone mineral content for control and tumor-burdened limbs (n = 19 pairs)

Factor	Limb	Mean	Standard Deviation	Range
Biomechanical peak strength (N)	Control	45.2	8.9	34.8–61.2
	Tumor	28.6	17.7	0.19–52.7
Biomechanical initial stiffness (N/mm)	Control	351	78.3	203–495
	Tumor	193	128	2.5–346
DEXA normalized BMD (end/start)	Control	1.15	0.09	1.00–1.29
	Tumor	1.21	0.15	0.93–1.47
CT bone volume (mm ³)	Control	9.01	0.94	7.62–10.77
	Tumor	8.97	1.43	6.45–11.27
CT bone mineral content (mg)	Control	8.96	0.96	7.51–10.69
	Tumor	8.99	1.54	6.09–11.26

DEXA = dual-energy xray absorptiometry; CT = computed tomography; BMD = bone mineral density.

Table 2. Indirect surrogates of bone strength for the tumor-burdened bones (n = 19)*

Parameter	Standard error of estimate (n)	Maximum residual error (n)	Correlation (r ²)	p Value
DEXA normalized BMD (end/start)	11.52	24.2	0.55	0.0015
Radiographic score	10.93	20.96	0.62	0.0005
CT bone volume (mm ³)	9.82	19.4	0.67	0.0005
CT bone mineral content (mg)	9.19	18.1	0.71	0.0005
CT FE stiffness (n/mm)	5.66	10.7	0.89	0.0005

* A Bonferroni correction was performed to account for multiple comparisons; DEXA = dual-energy xray absorptiometry; BMD = bone mineral density; CT = computed tomography; FE = finite element.

Fig. 6 Direct prediction of biomechanical strength for tumor burdened bones using a voxel-based finite element model (CT/FE) was made using a von Mises equivalent strain criterion ($r^2 = 0.91$, slope = 1.02, $p < 0.0001$). An exact one-to-one correspondence between the model and the experiment is indicated by a dashed line.

correlated strongly ($r^2 = 0.91$) with measured strength (Fig. 6) when the load at which yield volume fraction of 0.77% was used in the models. However, there was a mean offset in predicted strength (shift to the right when compared to ideal one-to-one correspondence, Fig. 6) on the order of 6.5 N (x-intercept) with a slope (1.02) that was very close to the ideal (1.0). The resulting standard error of the estimate in the predictions was 7.80 N with a maximum residual error of 17 N.

Discussion

Predicting the fracture risk of bones with lytic defects caused by metastatic disease is a challenging clinical problem. We performed a laboratory study using a murine model of tumor osteolysis to determine if noninvasive imaging and computational structural modeling techniques could predict the strength of bones with osteolytic lesions. We wanted to determine if a murine model of tumor osteolysis could produce a wide range of bone strengths when monitored using a weekly radiographic scoring protocol.

We also wanted to determine if indirect surrogates of bone strength, based on radiographic scoring, DXA, CT, and structural CT/FE analysis would correlate with experimental bone strength. Finally, we wanted to know if a calibrated estimate of bone strength using CT/FE analysis could accurately predict experimental bone strength.

Although we allowed for a direct comparison between the model and the experiment for bones with osteolytic tumors in terms of estimating bone strength, there were several limitations. The loading condition chosen was fairly simple (axial compression) and the risk or probability of fracture for each animal was not specifically addressed. In translation for human clinical use, the fracture risk would need to be assessed for a range of loading conditions. In addition, the threshold at which surgical intervention would be required was not directly assessed; this would require further investigation of fracture risk. Only one anatomic site (the distal femur) was considered in this study with the tumor localized to the distal diaphyseal, metaphyseal, and epiphyseal regions. While the bone strengths found experimentally spanned a wide range of magnitudes from very weak to normal, there was a lack of bones with strengths between 10 and 25 N despite our efforts to cull bones with moderate to severe amounts of osteolysis.

The finite element models developed for this study were also quite large (on the order of one million elements), requiring a substantial amount of processing time (12 hours on an engineering workstation) to estimate bone strength. As such, rapid estimation of bone strength in a time scale similar to the CT acquisition was not achieved. The use of coarser meshes with fewer elements [14] could be an alternative approach, although there is likely to be a tradeoff between the coarseness of the FE mesh and the predictive power of the models. Use of this animal model system would certainly be useful for assessing the effects of reduction in mesh density, as the computational results could be directly compared to results of experiments from which the models are derived. Although micro-CT cannot be routinely performed on human patients, high-resolution peripheral CT scan systems capable of resolving trabecular architecture have recently been developed. It would be expected that larger-dimension elements could be used for human application with equivalent results to the present murine model as long as the number of elements that represented equivalent structures such as individual trabeculae were preserved. Although the acquisition of these scans would involve exposure to relatively high levels of radiation (as compared with diagnostic radiography or CT), this would likely be considered inconsequential in patients who would go on to receive much higher doses of radiation as palliative therapy for their bone disease.

The CT/FE model could not adequately account for the growth plate region in terms of experimental deformations

and, as a consequence, the models were consistently too stiff. Pilot work using micro-CT imaging of femurs that were subjected to axial compressive loads in the CT scanner indicated there was greater deformation in the growth plate region than was determined in the finite element models. Further work is needed to improve material property assignment to this region. The inclusion of a true failure model would also be expected to improve predictions as the nonlinear response after yield to the bone material would likely more closely simulate the actual mechanical response. However, there would be a substantial increase in computational cost for these large scale models.

In this laboratory study, we determined the predictive capability of imaging/structural assessment modalities to determine whole bone strength for a murine model of tumor osteolysis. We found that by monitoring radiographic changes, a wide assortment of axial compressive bone strengths could be achieved, ranging from small lytic lesions with trabecular bone loss in the metaphyseal region to extensive lesions with trabecular and cortical wall involvement. All of the indirect surrogates of bone strength, including radiographic scoring, DEXA (BMD), CT (bone volume and bone mineral contact), and structural CT/FE stiffness, correlated with experimental bone strength. However, the correlation was strongest for the CT/FE stiffness surrogate, most likely because this approach accounts for the quantity and distribution of bone, and has a loading condition applied to simulate the experimental conditions. A calibrated surrogate of bone strength using CT/FE analysis had regression errors similar to the CT/FE stiffness surrogate but also had the added benefit of providing results in force units. It is also noted the radiographic scoring technique assumed score was a continuous variable (0 to 4). This presumed equal spacing and ordering of the radiographic scores that may not be correct. It is possible calibration of radiographic score with bone strength could improve this indirect surrogate, but this would require a separate set of calibration runs. Radiographic score is also subject to interobserver variation; this was not explored here.

Several different approaches have been used to develop and assess predictions of bone fractures for experimental and clinical cases of skeletal lesions. A comparatively simple and computationally efficient approach to combine imaging and structural analysis components in bone strength prediction is to determine structural stiffness of CT cross-sections. This is reportedly successful for vertebral bodies [17] with simulated osteolytic lesions ($r^2 = 0.69$ for predicting vertebral body strength) and for isolated cylindrical trabecular cores with defects ($r^2 = 0.85\text{--}0.95$) [3]. The primary limitation of this approach is the deformed shape of the bone (through beam

theory) is assumed and may not apply equally well to all types and locations of bone tumors. However, for cases in which the tumor is reasonably far from the joint and in which geometry changes are limited such as the diaphysis of long bones, this structural analysis approach could improve predictive capability substantially over existing radiographic scoring schemes [3].

Keyak and coauthors [4–7] have championed the development of CT/FE modeling of tumor burdened bone using continuum-based models in which element size is on the order of 3 mm. In contrast to the micro-CT modeling performed in this study, continuum-based models do not directly model the trabecular structure. Rather, they rely on modulus-density relationships developed from independent experiments to determine the average structural response of prepared bone samples with length scales on the order of 5 to 10 mm. Using this approach in a small series of human cadaveric specimens with and without metastatic lesions in the proximal femur, Keyak et al. [6] reported a strong linear relationship ($r^2 = 0.83$) between predicted and actual strength, which is similar to that found in this study ($r^2 = 0.91$). The mean error determined in the Keyak study (444 N) was a small fraction (5.7%) of the average bone strength of the human proximal femur [6]. In contrast, in our study of mouse bones the average error (6.65 N) was a larger fraction (14.7%) of normal distal femur strength.

One interesting finding of the present study that was also noted by Keyak et al. [6] was the general overprediction of bone strength for tumor burdened bones. It is possible the tumor burdened bones could have already been damaged from in vivo loading, something that may not be captured adequately in the models either in terms of structural connectivity or in terms of the way in which the bone fails. Alternatively, it may be that the density-modulus relationship for tumor burdened bone differs from that of healthy bone perhaps as a result of subtle changes in the bone matrix. Further work is needed to clarify this and to determine the underlying cause for this discrepancy.

This animal model of tumor osteolysis provides a test system in which to perform investigations into the effects of CT resolution, model size, and loading conditions on the prediction of bone strength using a combined imaging-structural modeling approach. From an ethical and cost perspective, such an approach is appealing because serial measurements could be made over time in the same animal, thereby reducing animal use and improving statistical power through the use of repeated measures testing. Although clinical application of this technique may be some time off, there are a range of preclinical applications for this methodology, including investigations into the effects of radiation or drug therapy on bone destruction, bone strength, and fracture risk.

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