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Elevated microdamage spatially correlates with stress in metastatic vertebrae

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Keywords:

Microdamage, Vertebral metastasis, Bone quality, Barium sulfate staining, Micro computed tomography, Backscatter electron imaging, Finite element analysis

Abbreviations, symbols, and terminology:

μCT	Micro computed tomography
T12, L1...	12 th thoracic and 1 st lumbar vertebrae
N	Newtons
SREs	Skeletal related events
BaSO ₄	Barium sulfate
BSE imaging	Backscatter electron imaging
μFE(A)	Micro finite element (analysis)
ANOVA	Analysis of variance
RPMI	Roswell Park Memorial Institute (RPMI) 1640 Medium
DMEM/F-12	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
CO ₂	Carbon dioxide
TBV	Trabecular bone volume
TMD	tissue mineral density
BMD	bone mineral density
TbN	trabecular number
TbS	trabecular spacing
TbTh	trabecular thickness
FHWM	full width half maximum

GLMax	grey level at the max intensity
GLAve	average grey level
GPa, MPa	GigaPascal, MegaPascal
ϵ_1 and ϵ_3	Maximum and minimum principal strains
σ_1 and σ_3	Maximum and minimum principal stress
σ_{vm}	Von-Mises stress
SD	Standard Deviation
$g(r)$	Spatial correlation

Abstract

Metastasis of cancer to the spine impacts bone quality. This study aims to characterize vertebral microdamage secondary to metastatic disease considering the pattern of damage and its relationship to stress and strain under load. Osteolytic and mixed osteolytic/osteoblastic vertebral metastases were produced in athymic rats via HeLa cervical or canine ACE-1 prostate cancer cell inoculation, respectively. After 21 days, excised motion segments (T12-L2) were μ CT scanned, stained with BaSO₄ and re-imaged. T13-L2 motion segments were loaded in axial compression to induce microdamage, re-stained and re-imaged. L1 (loaded) and T12 (unloaded) vertebrae were fixed, sample blocks cut, polished and BSE imaged. μ FE models were generated of all L1 vertebrae with displacement boundary conditions applied based on the loaded μ CT images. μ CT stereological analysis, BSE analysis and μ FE derived von Mises stress and principal strains were quantitatively compared (ANOVA), spatial correlations determined and patterns of microdamage assessed qualitatively. BaSO₄ identified microdamage was found to be spatially correlated with regions of high stress in μ FEA. Load-induced microdamage was shown to be elevated in the presence of osteolytic and mixed metastatic disease, with diffuse, crossed hatched areas of microdamage present in addition to linear microdamage and microfractures in metastatic tissue, suggesting diminished bone quality.

Conflicts of Interest: None

Introduction

Metastatic cancer accounts for the majority of tumours that occur in bone. Vertebrae are the most common site for skeletal metastases⁶⁰, which can present as bone destructive (osteolytic), bone forming (osteoblastic), or a mixture of the two. In addition to architectural changes, spinal metastasis also impacts bone quality^{24, 58, 4, 5, 6} leading to a higher incidence of skeletal related events (SREs). SREs (including pain, fracture and neurological consequences) occur in two out of three patients with vertebral bone metastases³³ and negatively affect quality of life^{50, 54}. Improvement in cancer diagnosis and treatment has increased the life expectancy of cancer patients, increasing the incidence of SREs secondary to metastatic involvement⁵⁴. Fracture risk prediction and vertebral bone strength assessment have significant clinical importance in guiding preventative strategies against SREs.

Microdamage occurs in bone due to cyclic loading and acts as a stimulus in healthy bone, initiating remodeling by triggering resorption by osteoclasts and the production of new bone matrix by osteoblasts^{15, 9, 8, 38, 1, 53, 31}. Microdamage is one of the mechanisms by which bone adapts to the mechanical loading^{16, 37}, but microdamage accumulation may lead to catastrophic bone failure. Microdamage accumulation occurs in aged bone, disease states (including osteoporosis and osteolytic metastases), and as a result of treatments that inhibit bone remodeling^{3, 36}. In conditions of low bone turnover and with little to no repair, microdamage accumulation can lead to decreased mechanical properties and ultimately fracture^{19, 7, 48, 44, 63, 23}.

Skeletal microdamage has previously been studied in transverse lesions on x-rayed tibias³⁹, “plastic slip lines” on scanning electron microscopic images¹¹, and via 2D histological analyses. While histologic analysis is the most common method to quantify microdamage, the process of staining, cutting, and grinding of thin sections is time-consuming, difficult to execute and can create damage artefacts within the specimens^{14, 9, 10}. More recently, 3D contrast-enhanced micro-computed tomography (μ CT) with barium sulfate (BaSO_4) has been demonstrated to stain microdamage accumulation in both cortical and trabecular bone samples^{32, 55, 28}. The

microdamage accumulation found in contrast-enhanced μ CT has been correlated with the histological staining, thus substantiating the method ^{28, 12}.

Micro finite element (μ FE) analysis of stress distribution has been used to understand the mechanical environment of healthy and metastatically involved trabecular bone, demonstrating agreement with mechanical testing experiments ^{25, 35, 49}. μ FE analysis has been shown to be consistent with damage labelling in healthy bone specimens, with stress concentrations occurring proximal to damaged areas ¹². More recent work has compared histologic microcracks and μ FE results by registering 2D histologic sections with 3D μ CT data sets in healthy vertebrae and vertebrae with osteolytic lesions ^{20, 12}. Elevated stresses and strains were found in damaged vs. undamaged regions of vertebral bone, with higher absolute values in damaged and undamaged regions in vertebrae with osteolytic lesions. However, it remains unclear how the type of metastatic involvement (osteolytic vs. osteoblastic) affects microdamage accumulation and its subsequent impact on whole bone mechanical behavior.

This study aims to characterize changes in vertebral microdamage due to metastatic disease (osteolytic and mixed osteolytic/osteoblastic), including the pattern of damage and the relationship that damage has with the local mechanical environment (stress/strain). It is hypothesized that microdamage will be elevated in both osteolytic and osteoblastic tissue and that stress and strain distributions from μ FE analysis will be correlated with the 3D spatial distribution of microdamage, with a dependency on disease status.

Material/Methods

Animal Model and Metastatic Inoculation: The following protocol was approved by the Ontario Cancer Institute prior to implementation. The utilized model for the physiological development of vertebral metastases within immuno-compromised rats was established in previous studies ^{13, 22, 59, 34, 12}. Briefly, 5–6 week old athymic female rnu/rnu rats were randomly assigned and inoculated with HeLa cervical cancer cells or canine ACE-1 prostate cancer cells to produce osteolytic or mixed osteolytic/osteoblastic metastases, respectively (N=5 per group). An additional 3 rats served as healthy controls. The HeLa and ACE-1 cell lines were cultured in RPMI and DMEM/F-12 media respectively at 37°C with 5% CO₂ and stably transfected with the

luciferase gene to enable bioluminescent imaging and semi-qualitative assessment of tumour growth within the rat via subcutaneous injection of luciferin. Rats were anesthetized using nose-cone inhalation of a 2% isoflurane/air mixture, and $\sim 1.5 \times 10^6$ cells (in 0.2mL of media) were injected into the left heart ventricle. After 21 days, bioluminescence imaging (Xenogen) was performed to ascertain metastatic growth throughout the rat, with a focus on the thoracolumbar spinal anatomy. After imaging, the rats were euthanized via CO₂ asphyxiation. T12 to L2 spinal motion segments were harvested and wrapped in a saline soaked pad and stored in a -20°C freezer prior to use.

μCT Imaging and BaSO₄ Staining: The spinal motion segments (T12-L2) were μCT scanned using an X-ray source of 55kV at 7.4 μm isotropic voxel size (μCT-100, Scanco Medical, Brüttisellen, Switzerland). After staining with 0.5M barium chloride followed by a 0.5M sodium sulfate solution under vacuum⁵¹, the specimens were re-imaged. A μCT compatible loading device (μCT-100, Scanco Medical, Brüttisellen, Switzerland) was then used to induce microdamage in the healthy and metastatically involved motion segments (T13 – L2) under applied axial compressive loading of 50 N for 3 hours²⁰. The loading was applied under displacement control at a rate of 35μm/s. The samples were then re-stained with BaSO₄ and re-scanned. The central L1 (loaded) and the T12 (unloaded) vertebrae were then fixed in 2% paraformaldehyde and dehydrated with rising concentrations of ethanol (70%, 90%, and 100%, each for 1 week), immersed in an Osteo-bed kit (Polysciences, Warrington, PA Cat# 17734A-900, alone, with 1gm/100ml and 2gm/100ml benzoyl peroxide (Polysciences, Warrington, PA Cat# 17734C-2, each for 1 week with the Osteo-bed exchanged once after 4 days)). Finally, the samples were polymerized by heating the blocks up to 37 °C. The sample blocks were glued to a slide with epoxy, cut to a thickness of 100-200 microns with a water-cooled precision diamond saw and a 3" Wafering Blade (Isomet® low speed saw, Buhler, USA), polished with ascending grits of silicon carbide paper (800, 1200, 2500, and 4000) and diamond paste of the following grits (9 μm, 6 μm, 3 μm, 1 μm, and 0.25 μm), and μCT scanned.

Stereologic Analysis: μCT scans containing the unstained and unloaded L1 vertebra were opened in Amira 3.0 (FEI, USA). In-house, custom-built algorithms were developed to track and

segment the cortical shell and the trabecular centrum, and to assess stereological bone parameters (trabecular bone volume (TBV), tissue mineral density (TMD), bone mineral density (BMD), trabecular number (TbN), trabecular spacing (TbS), and trabecular thickness (TbTh)).

Backscatter Electron Imaging: Slides of the sagittal cross-section of the embedded vertebra were carbon-coated, and backscatter electron (BSE) imaging was completed at x150 nominal magnification (SS BSE detector, FEI) using a Philips XL30 ESEM (FEI). For each sample, the relative BSE image intensity was calibrated to silicon dioxide and magnesium fluoride standards. While the intensity of produced backscattered electrons as a function of the atomic number of interacting elements has been utilized to facilitate the analysis of mineral distribution of imaged bone in other studies ^{46, 2, 45}, the greater atomic number of Barium would shift the grey-level distribution of the analyzed image. Therefore, modifications in the features of grey-level distributions such as the full width half maximum (FWHM) of the peak, the grey level at the max intensity (GLMax), and the average grey level (GLAve) were assessed to infer the degree of BaSO₄ staining, and hence the level of microdamage observed in the cross-section within the mineralized bone tissue.

μFE Model Creation: Unstained μCT scans were segmented to separate the bone from the non-bone areas using Amira 5.4.0 (FEI, USA). The slides used for BSE analysis were registered to the respective whole vertebral unloaded μCT scans with an automated routine (Affine Registration). The registration of the slides within the whole bone prior to μFE model generation allowed extraction of μFE results for the elements corresponding to the slides, for direct comparison with BSE identified microdamage. The μFE models were generated in AmiraDEV 5.4.0 from the unloaded μCT scans of the L1 vertebra (with the slides defined as separate element sets). A voxel-based meshing algorithm (Voxelator) was implemented in AmiraDEV 5.4.0 to generate a mesh of 8-noded hexahedral elements from the segmented μCT scans. To create loading surfaces, a custom-built algorithm (GridToSurface) was used to create surfaces from the voxel-based mesh. The end plates and facet joints were manually selected as loading surfaces. The vectors from the custom-built algorithm of the deformation field corresponding to the selected surfaces were assigned as boundary conditions. An in-house

software algorithm was used to assign the displacement vectors to the appropriate nodes within the model. Abaqusinputwriter module was then used to create an Abaqus input file (ABAQUS, Pawtucket, RI) that included the generated mesh, loading surfaces, and boundary conditions. A Young's modulus of 8.5 GPa and a Poisson's ratio of 0.3 were assigned as material properties for bone tissue^{20, 26}. Abaqus Standard 6.10 was used for analysis. All the models were executed as linear, elastic models using the supercomputing facility at SciNet, University of Toronto. Maximum and minimum principal strains (ϵ_1 and ϵ_3), maximum and minimum principal stress (σ_1 and σ_3), and von-Mises stress (σ_{vm}) were obtained for each sample.

Data Analysis: The stereological analysis, backscatter electron analysis, and von Mises stress and principal strains were compared for healthy and metastatic (Hela and ACE-1) samples using analysis of variance (ANOVA) followed by Tukey post hoc testing. The mean $\pm$ standard deviation (SD) are presented. Statistical significance was set at a level of $p < 0.05$. Qualitative comparisons were also made with respect to the patterns of microdamage visualized in the backscatter electron imaging.

The whole vertebral bodies excluding the loading surfaces (endplates and facet joints) and growth plates were used to quantify the maximum and minimum values of principal stresses and strains and the percentage of elements with stresses exceeding a damage criterion. The damage criterion (a von Mises stress over 90 MPa) was used to label elements within the model that likely would have been damaged. This damage criterion was chosen based on a value one SD below the mean stress in damaged regions identified in previously tested healthy rnu/rnu rat vertebrae¹². For consistency (and based on the high variance in stress levels identified in metastatic bone tissue) the 90MPa von Mises stress criteria was also used as the damage criterion in the metastatic vertebrae. Previously we found the ability of the von Mises criterion to best represent difference between the damaged and undamaged regions of bone²⁰. The corresponding slices within each μ FE models were isolated for comparison with the associated BSE images and μ CT images. Calculation of the percentage of elements exceeding the damage criterion was also quantified within the elements representing each slide. Spatial correlation

$g(r)$,¹⁷ specifically the “inflate Algorithm”, was used to look at agreement between damage within the μ FE results and the BSE images on a voxelwise basis (Amira 5.4.0, FEI, USA).

$$g(r) = \frac{\text{Mean}(\text{volume of Ba labeled voxels within } r \text{ distance of FE high stress regions})}{\text{Mean}(\text{volume of Ba labeled voxels within randomly assigned voxels})}$$

$$= \frac{\text{mean}_{\text{voxels labeled } a}(\text{conv}(S, L_B))}{\text{mean}_{\text{voxels randomly labeled}}(\text{conv}(S, L_B))}$$

$$S = \begin{cases} 1 & , \sqrt{x^2 + y^2 + z^2} < r \\ 0 & \text{otherwise} \end{cases}$$

Where L_B is a label field with voxels labeled with 1 where BSE detected damage. S is a spherical kernel for convolution with the label field to create the inflated region. r is the distance from each voxel considered as correlating. $g(r) = 1$ means there is no correlation, while $g(r) > 1$ means that objects of each type (labelled microdamage vs. μ FE damaged elements) are found nearer to each other than would occur by simple chance. A value of 0.125mm was chosen for r to represent the distance at which two points are no longer considered to be correlated. r was chosen based upon scan resolution and inherent errors in the registration of the μ FE models to the labeled damage images.

Results

Metastatic Involvement and Stereological Analysis: Metastatic disease including involvement in the heart, lungs, and skeleton was found in all rats injected with HeLa and ACE-1 cells. The stereological analysis demonstrated significant changes in bone morphometry parameters between the metastatic groups and the healthy group (Table 1).

BaSO₄ Staining and Backscatter Electron Analysis: μ CT images show the presence of the microdamage regions highlighted by BaSO₄ in sagittal slices (Figure 1 D, H and L). Back scattering electron analysis (Figure 1 C, G and K) shows the BaSO₄ staining in areas of microdamage at higher resolution. There was a significant widening of the FWHM after loading in the metastatic groups (HeLa, ACE-1) in comparison to the healthy group (Table 2). There was also a trend found with respect to an increase in the difference between loaded and unloaded

GLMax in the metastatic groups (HeLa, ACE-1) vs. the healthy group. In both osteolytic and mixed samples there were significantly higher values for Loaded vs Unloaded GLMax and FWHM, but no difference for GLAve.

BSE imaging quantifies the intensity level of the pixels within a given image which is used to reflect the calcium levels within a given bone sample. Staining with BaSO₄ impacts the gray level distribution within the bone tissue; more BaSO₄ causes an increase of FWHM, GLmax and GLav measures. As such it is possible to compare the amount of load induced microdamage between healthy and metastatically involved vertebrae through the change in the BSE measures in a BaSO₄ stained specimens before and after loading. No significant change was seen in GLav in all groups. Differences between loaded and unloaded specimens for FWHM and GLmax were 1.5x to over 2x larger in the metastatically involved vertebrae as compared to healthy controls. This indicates a much larger level of load induced microdamage in the metastatic vertebrae. Note that while the absolute values of GLav and GLmax are lower in the metastatic vertebrae this does not indicate a lower level of initial microdamage as these measures represent a combination of the calcium in the bone (which has been reported to be lower in metastatically involved vertebral bone⁴ and the BaSO₄ stain.

μFE Analysis and Backscatter Electron Imaging: μFE images of a sample from each group showing the von Mises stress distribution are shown in Figure 1 (whole vertebrae A, E and I; sagittal slices B, F and J). As depicted, the vertebral regions of elevated Von Mises stress are aligned with the presence of the microdamage regions highlighted by BaSO₄ in BSE images. Differences were also seen in the types of microdamage in the metastatic (HeLa and ACE-1) and the healthy bone. The microdamage visualized in the metastatic groups is linear, diffusive, and crossed hatched, whereas under the applied loading conditions the healthy vertebral bone exhibits only linear damage. From the μFE models, a significant increase in the percentage of damaged elements were found within the metastatically involved vertebrae (Table 3). The percentage measure is utilized for comparison as there are significantly fewer elements in the metastatic models due to bone loss. Significant increases in the minimum principal stress and maximum principal strain were found between the mixed metastatic ACE-1 group and the

healthy controls (Table 3). No significant differences in stress and strain were found comparing the osteolytic (HeLa) and healthy groups.

Spatial Correlation: Significant spatial correlation ($g(r) = 5.63 - 8.86$) was found between the preexisting (stain 1) and the load induced (stain 2) labeled damage and the regions of high stress in μ FEA (Table 4). No differences in spatial correlation were detected between the different groups.

Discussion

In this study load-induced microdamage was shown to be elevated in the presence of both osteolytic and mixed metastatic disease. Qualitative differences were observed visually indicating a difference in the nature of the microdamage. Pathologic involvement yielded an altered pattern of load induced microdamage in vertebral bone with diffuse, crossed hatched areas of microdamage present in addition to linear microdamage and microfractures. This is consistent with reported reductions in the quality of metastatically involved bone^{4, 5, 6}.

BaSO₄ identified microdamage was found to be spatially correlated with regions of high stress in μ FEA. This is consistent with previous 2D histologic analyses in which microdamage was shown to correlate to an elevation in tissue level stresses and strains in damaged regions as measured in μ FE models of trabecular bone cores and whole vertebrae^{41, 20, 12}. The strength of the spatial correlations between the μ FEA stress/strain and experimental BaSO₄ staining were not different based on disease status.

Sequential staining with BaSO₄ and μ CT imaging enables clear distinction between pre-existing and load-induced microdamage. However, the high magnification BSE imaging of the BaSO₄ stained vertebrae was essential in order to distinguish microdamage patterns. The pattern in both osteolytic and mixed samples is of much more extensive diffuse microdamage with parallel cracks along lamellar surfaces or cross-hatched patterns across the lamellae. In the healthy vertebrae, the microdamage is confined to parallel cracks. This is consistent with metastatic trabeculae being subjected to bending and/or buckling, leading to both tensile and compressive loading, in contrast to the primarily compressive loads experienced by the healthy

trabeculae^{56, 52}. Complete fractures which occurred in some of the metastatic trabeculae are the end result of the load induced microdamage across the trabecular thickness, as previously described⁶¹.

BSE image analysis also revealed quantitative differences in load induced microdamage in metastatic vs. healthy vertebral bone as indicated by greater relative increases in FWHM and GLMax between loaded and unloaded specimens. Bone plasticity and deformation are associated with features of the tissue matrix such as the orientation of collagen fibrils^{64, 29} intrafibrillar collagen cross-linking⁴⁷, as well as fibril-fibril and fibril-mineral interactions^{62, 30}. Microdamage is a consequence of bone plasticity, indicating slip at the collagen/mineral interface and fibrillar sliding. The observed increases in microdamage in metastatic bone indicate a modification in the formation of these sacrificial bonds allowing for more fibrillar sliding and this microscale deformation prior to critical crack propagation^{62, 30}. Other work has shown that metastatic involvement can modify features of the tissue such as a reduction in collagen fibril organization and tissue mineralization^{4, 5, 6}. Such changes could potentially diminish the degree of fibril-fibril interactions (due to reduced parallel alignment) and the mineral-fibril interactions (as there is less tissue mineral), modifying the ease of deformation and observed microdamage.

Damaged regions in healthy bone have been shown to experience higher stresses when loaded to strains closer to the yield strain⁴⁰ consistent with our spatial correlations between microdamage and stress in healthy and metastatic bone. In this current work, stresses and strains in the metastatic models were significantly higher than those in healthy models under equivalent loading (50N). As in previous studies, stereologic analysis of both osteolytic and mixed samples demonstrated a lower trabecular thickness and trabecular number²² than healthy vertebrae. As such, at an equivalent load, the bone tissue within metastatic spines will exhibit higher stresses in comparison to healthy spines. This is consistent with previous work in which damaged regions in metastatic vertebral bone have been shown to exhibit higher stresses and strains with more variability (higher standard deviations) than seen in healthy vertebrae¹². The metastatic vertebrae have a fragile structure, leading to an elevated risk of bulk yield at “physiological loads”⁵⁷. In this study, the ACE-1 cells yielded a mixed phenotype

that was predominantly osteolytic. As such, no significant differences were found in the morphometric properties between the osteolytic and mixed specimens.

The use of a linear elastic homogenous material model to represent both the metastatic and healthy vertebral bone is a limitation of this study. Voxlated μ FEA models of bone have historically utilized this approach, however, the elastic modulus of metastatic vertebral bone has been shown to be lower than that of normal bone^{42, 4}. Underestimation of local strains in the metastatic models may result when utilizing a healthy bone modulus. In future models, utilizing nonlinear μ FE modeling, with damage initiation and evolution criteria, may give more accurate results by taking into account material softening during damage initiation and stress redistribution to the surrounding undamaged elements⁴¹.

The growth plates in the rat remain open throughout life and as such exhibit high deformation under load^{18, 20, 21}. As such, in comparing skeletal microdamage to maximum and minimum stresses and strains in this study, the growth plates were excluded. Loading surfaces (endplates and facet joints) were also excluded in order to avoid stress concentrations associated with the application of the displacement boundary conditions on these surfaces generated through deformable registration.

The resolution of the μ FE models used in this study was 35 μ m. This element size has been shown to be sufficient to represent rat vertebral architecture^{27, 43}. However, the vertebral end plates, which consist of a high trabecular number and low trabecular thickness, are consolidated at that resolution, which makes elucidating the microdamage within them inefficacious. This further justifies the exclusion of the endplates/growth plates from the region of interest used in our analyses. While higher resolution μ CT imaging is possible, the size of higher resolution models remains a computational challenge. A practical tactic of reduction of computational cost has been to decrease the resolution of μ FE models in non-critical areas, as used in rat vertebrae by Herblum (2013). However, when assessing microdamage in the whole bone, different resolutions may impact spatial correlations and the nature of damage captured by the model.

BaSO₄ staining is a powerful tool that yields 3D volumetric information on microdamage. Moreover, this technique when combined with BSE imaging yields high resolution images capable of discerning the nature of the microdamage (diffuse and/or linear cracks). Further work in nonlinear modeling incorporating altered material models in the presence of microdamage, modeling damage progression to fracture, and the redistribution of load to surrounding undamaged elements will enable extension of this work to model post-yield response of the bone and catastrophic failure. Ultimately a better understanding of microdamage can enable better fracture risk prediction models and bone strength assessment in patients with vertebral bone metastases.

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Tables

	HeLa (n=5)	Ace-1 (n=5)	Healthy (n=3)
Trabecular Bone Volume (TBV) [%]	0.402 ± 0.008 [*]	0.404 ± 0.019 [*]	0.451 ± 0.014
Trabecular Number (TbN) [#/mm²]	5.242 ± 0.331	4.613 ± 0.556 [*]	5.844 ± 0.275
Trabecular Spacing (TbS) [mm]	0.114 ± 0.009	0.126 ± 0.02 [*]	0.09 ± 0.0035
Trabecular Thickness (TbTh) [mm]	0.075 ± 0.0059 [*]	0.078 ± 0.0096 [*]	0.095 ± 0.0038
Bone Mineral Density (BMD) [mg/cm³]	445.45 ± 29.848 [*]	412.892 ± 33.32 [*]	541.467 ± 40.129
Tissue Mineral Density (TMD) [mg/cm³]	868.37 ± 35.771 [*]	812.57 ± 29.456 [*]	966.94 ± 8.665

Table 1: Comparison of morphometric properties of metastatic vertebrae (HeLa and Ace-1) to healthy controls (mean ± SD, * indicates significant (p<0.05) differences between metastatic and healthy groups).

Treatment	Loaded vertebrae (GLMax)	Unloaded vertebrae (GLMax)	Difference loaded - unloaded vertebrae (GLMax)	Loaded vertebrae (GLAve)	Unloaded vertebrae (GLAve)	Loaded vertebrae (FWHM)	Unloaded vertebrae (FWHM)	Difference loaded - unloaded vertebrae (FWHM)
Healthy	193.11 ± 7.56	185.85 ± 5.23	6.11 ± 9.08	176.59 ± 6.99	175.29 ± 2.13	53.33 ± 6.10	44.43 ± 7.02	10.9
HeLa	186.19 ± 2.31	173.17 ± 6.09 [*]	13.08 ± 7.07 [#]	163.04 ± 8.31	158.43 ± 5.38	62.9 ± 3.75 [*]	48.55 ± 4.32	14.35
Ace-1	187.25 ± 4.55	176 ± 8.32 [*]	13.00 ± 6.32 [#]	165.87 ± 4.76	164.43 ± 7.41	68.67 ± 6.98 [*]	51.71 ± 7.17	16.96

Table 2: Comparison of GLMax, GLAve and FWHM of loaded and unloaded stained vertebral bone in the Healthy, Osteolytic (HeLa) and Mixed (Ace-1) groups (mean ± SD, * indicates significant (p<0.05) differences; # indicates a trend (p= 0.07) between the metastatic group and the healthy controls).

Mean $\pm$ SD	ϵ_1	ϵ_3	σ_1 (MPa)	σ_3 (MPa)	σ_{vm} (MPa)	% of damaged elements
Healthy (n=3)	0.513 $\pm$ 0.047	-0.012 $\pm$ 0.012	38.27 $\pm$ 5.38	-33.60 $\pm$ 2.09	78.13 $\pm$ 29.37	46.19 $\pm$ 6.11
HeLa (n=5)	0.839 $\pm$ 0.109	-0.019 $\pm$ 0.011	55.75 $\pm$ 11.84	-97.82 $\pm$ 1.71	93.67 $\pm$ 40.31	67.92 $\pm$ 4.35*
Ace-1 (n=5)	1.174 $\pm$ 0.46 *	-0.022 $\pm$ 0.015	121.72 $\pm$ 22.91*	-116.62 $\pm$ 6.94 *	131.06 $\pm$ 70.68	79.61 $\pm$ 17.06*

Table 3: μ FE results from the healthy, Ace-1 and HeLa models (mean $\pm$ SD). Mechanical metrics (ϵ_1 , ϵ_3 , σ_1 , σ_3) were calculated within each sample. The standard deviations were then calculated by sample (* indicates significant ($p < 0.05$) differences between metastatic groups (HeLa or Ace-1) and healthy controls).

	G(r)- Spatial correlation between pre-existing stained micro damage and failed finite elements (Mean $\pm$ SD)	G(r)- Spatial correlation between load induced stained micro damage and failed finite elements (Mean $\pm$ SD)
Healthy	8.86 $\pm$ 2.10	6.29 $\pm$ 0.75
HeLa	6.44 $\pm$ 1.24	7.12 $\pm$ 1.82
Ace-1	5.63 $\pm$ 0.03	5.8 $\pm$ 0.01

Table 4: Spatial correlation G(r) results from the healthy, Ace-1 and HeLa models (mean $\pm$ SD), between pre-existing microdamage (middle column) and load induced microdamage (right column) and failed elements (>90 MPa) as determined by μ FEA.

Figure 1. FEA model image showing the Von Mises stress on the whole and in a slice from the whole and a correlating BSE images showing bright BaSO₄ staining after axial compressive in loaded metastatically involved HeLa (A- whole model B- Slice from the whole and C- BaSO₄ correlating image) metastatically involved Ace-1 (D- whole model E- Slice from the whole and F- BaSO₄ correlating image), in healthy (G - whole model H- Slice from the whole and I- BaSO₄ correlating image)(scale bar 500 micron), vertebral trabecular bone samples (scale bar 20 micron). The amount of staining is significantly increased in the metastatically involved bone and the pattern demonstrates more interconnected and congregated defects.