AUTOMATED QUANTITATIVE ANALYSIS OF BONE STABILITY AND TUMOUR BURDEN IN THE METASTATIC RAT SPINE

by

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Institute of Biomaterials and Biomedical Engineering
University of Toronto

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Abstract

The spine is the most common location of metastatic disease in the skeleton. The occurrence of bone metastasis can lead to severe clinical consequences and a significant decline in quality of life. The evaluation of metastatic disease in the spine has to date been mainly qualitative. More widespread access to multiple imaging modalities has motivated the development of 3D methods to quantitatively evaluate metastatic disease in the spine. Quantitative evaluation is important both in assessing stability of the metastatic spine and the progression/response of the tumour and bone to treatment over time. Previous studies quantifying stability in the metastatic spine have focused primarily on osteolytic tumours. Local and systemic treatments have impacted the nature of vertebral metastasis, increasing the occurrence of mixed osteolytic and osteoblastic disease. Thus, it is important to focus analyses on models able to accurately represent diverse distribution patterns found in bony metastasis. Preclinical models are widely used in studying the process of metastasis and are able to represent both osteolytic and osteoblastic disease. This proposal aims to establish the biomechanical implications of metastatic disease in the spine through the evaluation of stability and tumour burden in a preclinical model using a multifaceted engineering-based approach. It is hypothesized that the use of automated analysis techniques
applied to multimodality imaging will allow quantification of the impact of metastasis on biomechanical stability, tumour burden and bony architecture in the spine, and motivate prediction models that accurately reflect vertebral integrity in both osteolytic and mixed osteolytic/osteoblastic models of spinal metastasis. Specifically, this work aims to: 1) Utilize and compare µMR and µCT based radiologic methods to quantify tumour involvement and vertebral architecture in a rat model of spinal metastasis; and 2) Evaluate the ability of 2D, 3D, and continuum based methods to quantify structural integrity in vertebral metastasis. Overall, this work will focus on developing automated methods to quantify stereologic parameters, and quality in the metastatic spine and the evaluation of stability measures from 2D structural rigidity, Finite Element analysis, image registration and experimental methods. Ultimately this work will yield automated analysis techniques and evaluate the abilities of these methods to predict failure in metastatic vertebrae.
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CHAPTER 1
INTRODUCTION

1.1 Anatomy of the Spine

The human vertebral column consists of 33 vertebral levels which are divided into 4 major sections. The cervical region consists of 7 vertebrae and is the upper most region of the vertebral column. The cervical spine has great flexibility and is mechanically responsible for controlling the motion of the neck. The next region is the thoracic spine composed of 12 vertebrae. This region is rigid and attaches to the rib cage housing the heart and other important organs. Directly below the thoracic region is the lumbar spine consisting of 5 vertebrae, which allow the motion of lower back. The bottom region of the vertebral column is composed of 5 fused sacral vertebrae and 4 fused coccygeal vertebrae (Fig. 1).

Figure 1. Anatomy of human vertebral column (public image domain from Wikipedia) [1].
Each vertebra consists of the weight bearing vertebral body and the posterior elements. Their bony structure forms a protective cage around the spinal cord. The posterior elements include the pedicles, spinous process, transverse processes, and inferior and superior facets (Fig. 2). The posterior wall of the vertebral body, together with the vertebral arch (composed of pedicles and laminae), form the spinal canal. The spinous and transverse processes are attached to muscles and ligaments. These processes and their attachments support body’s motion. The inferior and superior facets limit the motion in the spine.

Connecting each vertebral level is a structure called the intervertebral disc. The intervertebral disc supports the body’s movement in all planes of motion and acts as shock absorber. A healthy disc is composed of a hydrated gelatinous nucleus pulposus surrounded by a tough multilayer annulus fibrosis. The annulus is composed of collagen fibers arranged such that the orientation of the fibers are aligned relative to the longitudinal axis of spine at +/-55 degrees and alternate their orientation in successive layers. This arrangement pattern produces maximum support against pressurization of the nucleus [2].

Figure 2. Schematic of a cervical vertebral motion segment displaying the vertebral structures and intervertebral discs (public image domain from Wikipedia) [1].
1.2 Architecture of Bone

Bones are composed of both dense compact cortical bone and porous spongy trabecular bone filled with fluid marrow. Cortical bone has an osteonal structure in human bones composed of lamellae. Lamellae surround haversian canals that house the capillaries that are responsible for the supply and removal of mineral to and from the bone (Fig. 3) [3]. The porous spongy trabecular structure is an interconnected network present in the ends of long bones and within vertebrae.

**Figure 3.** The structure of compact cortical and spongy trabecular bone (public image domain from Wikipedia) [4].

The trabecular network consists of plates and rods of varying dimensions resulting in a structure of variable porosity and density. A network that is mainly composed of rods is generally of lower density in comparison to a network of plates or plates and rods [2].

In animal models, the distribution of trabecular and cortical bone and their corresponding densities depend on the size and the type of the animal (quadrupeds vs. bipeds) and their type of motion [5]. In rodents, in particular, it has been shown that there is not a clear distinction between the trabecular and cortical regions; the cortical bone structure of rodents is non-haversian [6].
### 1.3 Bone Remodeling

There are 3 types of primary bone: circumferential lamellar bone, woven-fibered bone and primary osteons. Cortical bone at birth is composed mainly of woven-fibered bone with randomly arranged collagen bundles and vascular spaces lined with osteoblasts. The osteoblast functions to deposit layers of lamellar bone, resulting in a decrease in the vascular spaces leading to presence of primary osteons. The primary osteons contain vascular channels and are generally parallel to the long axis of the bone. The diameter of long bones increases during growth as a result of deposition of new primary lamellar bone.

Bone remodeling, or metabolism, includes resorption of mature bone tissue and formation of new bone tissue, and continues for the life of the bony structures. Bone remodeling is regulated by the activity of bone cells: osteoblasts (bone-forming), osteoclasts (bone-resorping), and osteocytes (bone-maintaining). Osteoclasts are multinucleated cells that are primarily responsible for bone resorption. They start the remodeling process by initiating resorption, which creates longitudinally oriented tubular channels. Osteoblastic cells follow by depositing lamellar bone to reduce the diameter of the channels to a small singular vascular canal. The activity of the osteoblasts and osteoclasts is regulated through signaling of osteocytes [3]. The newly formed lamellar cylinders that surround the longitudinally oriented vascular channels are called haversian systems or secondary osteons. The secondary osteons are bounded by cement lines. These cement lines are formed where osteoclastic activity stops and osteoblastic activity begins. The bone areas between secondary osteons are called interstitial bone. Interstitial bone consists of the residue of the primary bones and the secondary osteons that previously occupied that area. Normal adult cortical bone only consists of secondary osteons surrounded by circumferential lamellar bone. The only presence of woven-fiber bone occurs during rapid bone formation (i.e. fracture healing) [2].

The trabecular plates and rods are primarily developed from interstitial bone with rare presence of lamellar trabecular plates and osteonal trabecular rods. Trabecular bone can be described as a cellular structure. The density, thickness and orientation of the trabecular structure forms as a direct function of the direction and magnitude of the loads it experiences. For cases of equal loads in all directions, trabecular bone tends to form a cellular structure that is equal in all axes.
In regions of low trabecular loading, the trabeculae form a network of rods and as the loads increase the cell walls thicken and resemble a more plate-like structure [2].

1.4 Pathology of Spinal Metastasis

Breast, lung, prostate, and renal cancers are the most common primary tumours to metastasize to bone. Skeletal metastases are 40 times more common in patients when compared to all other types of bone cancer combined [7]. The spine is the most common location of metastatic disease in the skeleton [8]. Spinal metastasis can present as osteolytic (bone resorbing), osteoblastic (bone depositing) or mixed osteolytic/osteoblastic lesions (Fig. 4).


The occurrence of bone metastases has important consequences for patients in terms of both morbidity (skeletal related events) and mortality. Skeletal related events (SREs) commonly occur secondary to metastases and include pathological fracture, neurologic complications, hypercalcaemia and pain requiring surgery, radiotherapy or opioid analgesics. In particular, pathologic burst fracture in the spine has been reported to occur in 5 to 10% of all cancer patients and can result in severe clinical consequences including motor weakness (96%) pain (94%), sensory disturbance (79%) and sphincter disturbance (61%) [10–12]. SREs have been shown to significantly reduce median survival times in patients with metastatic disease [13, 14]. The high incidence of SREs (2/3 of patients with bone metastases develop at least one SRE) and their influence on rates of morbidity and mortality, motivate strategies that lead to the reduction and/or elimination of SREs [15].

The standard of care for patients with established metastases to the spine is a multimodal approach that may include radiation therapy, chemotherapy, systemic treatment with
bisphosphonates (BP), vertebroplasty / kyphoplasty and open surgical interventions. Minimally invasive local interventions, such as radiofrequency ablation, have also been utilized in treatment of the metastatic spine. More recent investigation focused on Photodynamic therapy has shown promising results in destroying tumour tissue and improving bone structure in preclinical models, in particular when combined with BP therapy [16]. A Phase I clinical trial of PDT for the treatment of spinal metastases is currently underway.

Tumour response within the spine is dependent on many factors and thus remains variable in many patients. Yet, local and systemic treatments have impacted the nature of vertebral metastasis [17]. For example, historically vertebral lesions secondary to breast cancer presented as predominantly osteolytic in nature but due to widespread adoption of systemic BP treatment, the current demographic present a majority of mixed osteolytic/ osteoblastic lesions [18]. Radiologic changes in osseous metastatic patterns as a result of ongoing treatment have been shown to influence time dependent risk for SREs [19–21]. Currently patients with metastatic disease in bone are excluded from the majority of clinical trials assessing new therapies due to a perceived inability to accurately quantify tumour burden, mechanical stability, disease progression and treatment effect in bony structures. Quantification of disease burden and mechanical stability are important parameters that may be utilized to assess the risk of pathologic fracture, better focus treatments and optimize clinical management of patients with spinal metastases.

1.5 Biomechanical Modeling of the Metastatic Spine

High incidence of SREs with demonstrated impact on morbidity and mortality has attracted researchers to develop analytical methods to better understand the mechanical behavior and structural consequences of metastatic disease in the spine. These methods include physical, computational and image-based experimental analyses [22–28].

1.5.1 Physical Models

Robust models that accurately represent metastatic disease in the skeleton are needed to provide realistic estimates of the impact of tumour burden in the spine. Both in vitro and in vivo models have been used to study the mechanics of spinal metastases.
In vitro models have utilized simulated osteolytic lesions generated through bone destruction in human and animal cadaveric vertebrae. Utilization of such models in which lesions are represented by voids (Fig. 5) have generated criteria reasonably well correlated with vertebral fracture but have not yielded a sufficiently sensitive or specific method for predicting pathological burst fracture [29–31].

Inclusion of tumour tissue in such in vitro models is necessary for intravertebral pressure generation necessary to model burst fracture [32]. As well, simulation of osteolytic lesions is generally limited to regularly shaped defects. Low numbers of metastatically involved cadaveric vertebrae available for experimental testing have limited the ability of in vitro physical models to be used to assess the complex pathology of metastatic disease in the spine.

In vivo preclinical models of skeletal metastases may better represent the pathology of spinal metastases and as such provide more realistic estimates of the impact of tumour burden and bone structure on fracture risk. In vivo models of skeletal lesions have been developed using direct injection techniques, however these injections may seriously disrupt the bony structure, particularly in the trabecular network of small animal vertebrae [33]. Generation of spinal metastases through intracardiac injection techniques have proved successful in the generation of osteolytic, mixed and osteoblastic models [34–37]. A reliable rnu/rnu athymic nude rat model of osteolytic skeletal metastases using a bioluminescent transfected MT-1 human breast cancer cell line has been successfully utilized in our laboratory to assess mechanical stability of vertebrae.
with osteolytic destruction and the effects of locally focused treatment on these vertebrae. The limitation with the athymic nude rat model is the limited life expectancy due to metastatic disease preventing longer term studies [35].

To date animal models of mixed disease have not been well studied. Koutsilieris et al. utilized PA-III cell line, derived from a spontaneous prostate adenocarcinoma in a Lobund Wistar rat, to develop osteolytic and osteoblastic reactions via implanting the cell adjacent to bone [38]. This technique however does not correspond to true metastases. The MAT-Ly-Lu subline of the rat Dunning prostate carcinoma (R33227) model also had limited success in developing mixed lesions and in many cases resulted in pure osteolytic metastases [39, 40]. Other cell lines such as the MHMX unclassified human sarcoma [34], TSU-PR1 [41], and C4-2B [42], which have been successful in developing mixed osteolytic/osteoblastic metastases, have had limited ability in rats.

1.5.2 Image Analysis
1.5.2.1 Bioluminescent Imaging
Bioluminescent imaging is a molecular imaging technique used in preclinical research. In general, bioluminescent imaging requires a reporter construct called luciferase, which reports its position through light emission. The objective in bioluminescent imaging is to capture and record the light that is emitted by luciferin. The emitted light is captured using a charge-coupled device (light sensitive camera), which is focused on an animal within a dark chamber (Xenogen Corp., Alameda, USA) [43].

Transfection of cancer cell lines with luciferase allows in vivo monitoring of metastatic tumour development in preclinical models through bioluminescent imaging. Such monitoring allows the localization of metastases (via bioluminescent and plain photograph image overlays) and semi-quantitative assessment of tumour burden throughout preclinical models. Bioluminescence imaging has been shown to be more sensitive in early detection of vertebral lesions as compared to fine-detail radiographs and has corresponded well to CT evaluation of osteolytic destruction. [35, 44]
1.5.2.2 Micro Computed Tomography

Micro Computed Tomography (µCT) is a powerful technique for 3D visualization of the bone micro-architecture. The system is composed of a stage where a sample is placed, a light source which emits x-rays at the sample, an image intensifier which intensifies the X-rays and transfers them to computer, and software that forms the final reconstruction image. The system starts by emitting one x-ray at the sample. Once the x-ray is received, intensified and recorded the sample will be rotated at a predefined angle along the axis of the x-ray tube and another x-ray will be taken. In in vivo µCT system, however, the sample is stationary and the source rotates with respect to the long axis on the sample. This process continues until 360 degrees of rotation is completed. Each x-ray that is emitted creates a shadowgraph of the sample in which the 3D structure is projected onto a 2D plane. The computer software then gathers the 2D images and based on the information about the orientation of the sample, it places each image in the correct orientation to form a 3 dimensional volume of the sample. The spatial resolution of the reconstructed volume corresponds to the ability to distinctly visualize objects that are close together. The resolution in a µCT image depends on the number of X-rays that have been taken. The intensity of each pixel in a µCT image is equal to the average attenuation of the corresponding tissues in that region of the image. This attenuation is normalized on the Hounsfield scale, which defines +3071 and -1024 as most and least attenuating respectively [45].

CT images can yield excellent visualization of skeletal tissue, including osteolytic destruction and osteoblastic bone deposition. Quantitative analyses of CT scans (qCT) in metastatically involved vertebrae have been utilized to predict mechanical stability. Based on the idea that the mechanical behaviour of a structure is governed by both material and geometrical properties, a combination of structural rigidity analysis and composite beam theory has been used to predict failure in tumour involved bone [25, 27, 29, 46]. Structural rigidity incorporates both axial rigidity (resistivity of a material to axial loading without failure) and bending rigidity (resistivity of a structure to bending without failure). Structural rigidity measurements have been used to predict failure load using composite beam theory, based on assumptions that the elastic behavior of whole bones correlates with the behavior of bone tissue at yield and that bone at a material level fails at a constant strain independent of density [29]. Using this approach, Whealan et al. demonstrated a good correlation between predicted and measured failure loads in vertebrae with simulated metastases ($r^2=0.65$ to 0.89) using qCT derived structural properties coupled with
composite beam theory [29]. Clinically, qCT based structural rigidity analysis has been shown to be highly sensitive (100% ability to predict all fractures that did occur) but only moderately specific to fracture of metastatically involved vertebrae (an error of 30 to 60% in predicting failure in vertebrae that did not fracture), with more successful application to juvenile chondrosarcomas in the femur [25, 27, 46]. The structural rigidity method has recently been applied to a preclinical study predicting failure torque in rat bone with simulated osteolytic defects and was found to correlate significantly better to mechanical testing data than dual energy X-ray absorptiometry based on bone mineral density [47].

CT imaging has also been utilized within a highly automated 3D algorithm (tracking tool) developed to quantitatively characterize focal and diffuse lytic, blastic and mixed tumour volumes, disease severity, and temporal progression / treatment effect in the bony spine through histogram based analysis of CT vertebral density distributions [32]. The algorithm uses demons deformable image registration and level set methods to accurately segment tumour-bearing vertebrae (AmiraDev; ITK, NLM, Bethesda, USA). By maintaining morphology of an atlas, the demons-level set composite algorithm is able to accurately differentiate between trans-cortical lytic tumours and surrounding soft tissue of identical intensity and blastic tumour tissue from the cortical shell [48]. This method has been successfully utilized to compare patterns of metastatic involvement in patients with spinal metastases secondary to breast cancer in two temporally distinct cohorts [18].

In atlas-based demons deformable registration, an ideal vector field \( V(X, t) \) is found that maps a moving image (the atlas) \( I_2 \) onto a fixed image \( I_1 \). \( V(X, t+1) \) is found by adding \( V(X, t) \) to \( V_t(X, t) \) until \( V(X, t) \) suitably matches \( I_2 \) to \( I_1 \) (equation set 1). The registration deforms the moving image in the direction of local normals of isointensity contours. After the completion of each resolution level, the deformation field is linearly interpolated to the resolution of the next finer level. The last level is the finest and most computationally intensive level of the pyramid with no downsampling in any direction. Between each iteration, the deformation field is smoothed by convolution with a 3D Gaussian.

\[
V_t(X, t) = \frac{[I_2(X, t) - I_1(X)]\nabla I_1(X)}{[\nabla I_1(X)]^2 + [I_2(X, t) - I_1(X)]}
\]  

(1)
The deformation field produced by demons is then applied to the atlas segmentation. This is done by wrapping the segmentation around the scan using nearest neighbor interpolation and backprojection from the output image domain to the input image domain to transform the segmentation of the atlas, producing an automated segmentation of the metastatic vertebrae. The level set method is then applied to the output of the demons segmentation to refine the boundaries of the segmentation to make sure that the algorithm accounts for all the curvature present in the vertebra.

The level set filter is applied to the output of the previous segmentation through solving the level set differential equation (equation 2) by the finite difference method.

$$\phi_t + F|\nabla \phi| = 0$$  (2)

F is called a speed function which is defined as:

$$F = \frac{\left(\mu - \lambda K\right)}{1 + \exp\left(-\left(I - \alpha\right)\beta\right)}$$  (3)

The speed function controls the flow speed of the segmentation contour. F is a sigmoid function of the pixel intensities. $\beta$ represents the pixel intensity range and represents the center of the intensity range. $K$ is the Gaussian curvature of the surface defined in equation 4.

$$K = \nabla \cdot \frac{\nabla \phi}{|\nabla \phi|}$$  (4)

In Equation 3, I is the image intensity at a specific voxel. Equation 3 controls the relative importance of pure contraction and a curvature-based evolution which are weighted by $\mu$ and $\lambda$, respectively. A higher $\mu$ results in faster contraction, while a higher $\lambda$ causes the curve to remain
smooth and more round. This method of segmentation proceeds by evolution of the surface via changing of \( \phi \) from equation 2.

Similar to the use of CT in human vertebrae, \( \mu \)CT imaging has been utilized to quantify tumour involvement in preclinical models of spinal metastasis [49]. \( \mu \)CT analysis has been compared and correlated with histologic and bioluminescent imaging techniques to assess the relative abilities of these methods to accurately quantify lytic tumour burden and bony destruction in rat vertebrae [50]. Deformable registration of 2D histological sections to 3D \( \mu \)CT volumes showed good agreement in tumour (bone loss) and bone structure in merged images.

1.5.2.2.1 Image-based Morphological Measurements

Morphological measurements defined for \( \mu \)CT volumes are widely used measures of understanding the biomechanical stability of the skeleton. The first step in such studies is to identify the region of interest (ROI) in the image where the measurement is done. This selection of the region of interest prior to calculation of morphological measures has thus far been done manually. Manual ROI selection limits the repeatability and representativeness of the analyses [35, 36] motivating automated image segmentation algorithms for widespread utilization.

The widely used plate model of Parfitt [51] estimates trabecular thickness, separation and number based upon bone volume fraction and surface area, and has been applied to study osteoporosis, osteolytic, osteosclerotic diseases and aging [52–60]. This model assumes that all the trabecular mesh consists of a series of parallel plates (although the trabecular structure actually consists of a mixture of plates and rods). This assumption results in a model-dependant bias when compared to direct 3D measures of trabecular thickness and separation [61]. A more recent model developed by Hildebrand et al. measures trabecular thickness using a model independent approach [62] in which the local thickness is defined as the diameter of the biggest sphere that fits that point without crossing the boundaries of the volume. Average thickness is then defined as the volume integral of all of local thicknesses divided by the volume of the shape of interest.

Bone is not homogeneous or isotropic, thus additional measures such as mean intercept length and volume orientation schemes have been used to describe the degree of anisotropy in bone as an orthotropic tissue [63]. Such bone quality measures have been applied to look at regional
variations of bone. [54] Kim employed a similar method and found that regions with higher bone volume fraction and density variabilities had lower yield strength [64]. It has been suggested that osteoporotic bone loss preferentially causes decreases in connectivity rather than thinning of vertebrae [59], and previous modelling has suggested that decrease in connectivity is worse mechanically than thinning [65]. In osteoarthritis, however, it has been shown that connectivity and mechanical strength have an inverse relationship due to the hypomineralized nature of the bone [66]. Cases of bone loss and trabecular thinning in the vertebra were simulated followed by the simulation of treatments that either increase trabecular thickness or trabecular number [65]. In this study it was observed that the reduction in either the Young's modulus or the strength due to simulated trabecular loss was proportional to a much higher power of reduction in bone volume fraction than trabecular thinning. This demonstrated that reductions in connectivity and trabecular number have a much higher impact on the integrity of trabecular bone than trabecular thinning.

The structural effects of osteoblastic tumour growth have previously been analyzed in a study comparing the structure of osteoblastic bone with normal tissue [28]. The trabecular bone in osteoblastic regions was observed to be highly connected and its network pattern was observed to be isotropic in comparison to normal tissue. The surface of the osteoblastic metastases was observed to be highly irregular. In spite of these surface irregularities, no significant differences in average trabecular thickness were observed between osteoblastic metastases and normal tissue. The degree of mineralization (DMB) of trabeculae in metastatic lesions was however observed to have broader range and lower mean than that of the normal tissue [28]. The architecture of complex osteolytic / osteoblastic metastatic disease patterns and their mechanical consequences have not to date been adequately characterized.

### 1.5.2.3 Magnetic Resonance Imaging

Magnetic Resonance (MR) Imaging is clinically utilized to optimally visualize spinal metastases, including localization of tumour tissue and extravasations beyond the vertebrae, which may result in neural compromise. While the quality of bone tissue visualization utilizing MR imaging is not as high as with CT imaging, MR can be used to visualize bony tissue as well [67].

MR imaging of the human body is feasible due to the body’s large composition of water molecules. Each water molecule contains two hydrogen nuclei, or protons, which align to the
magnetic field of the MR scanner. A radio frequency wave is then turned on for a short length of time causing a variation in the electromagnetic field. This results in raising the energy of the photons in the field to the point where they can be absorbed and flip the spin of the aligned protons in the body. Once the field is turned off it causes the energy absorbing protons to return to the original lower energy spin down state. This results in two opposite spins in the hydrogen dipole (1 high and 1 low). The dipole is parallel to the field at low spin but not at high spin. This results in the difference of energy being released in the form of a photon, which is detected by the scanner as an electromagnetic signal. Each of these released photons, however, has a different energy or frequency based on the location and properties of the tissue. One of these properties is the spin-spin and spin-lattice relaxation times. The spin-spin relaxation time (T2) is the time it takes for the transverse component of the magnetization vector to decay to its equilibrium value of zero. The spin-lattice relaxation time (T1) is the time it takes for the longitudinal component of the magnetization vector to come to thermodynamic equilibrium with its surrounding (lattice) (Fig. 6).

![Figure 6](image)

**Figure 6.** Signal intensity (SI) – time graph demonstrating different parametric measures in an MR sequence. The curve labeled T1 demonstrates the saturation of the signal intensity in time as the longitudinal component of the magnetization vector reaches thermodynamic equilibrium. The curve labeled T2 as well demonstrates the signal intensity during the time for the transverse component of the magnetization vector to decay to zero.

Each tissue has a specific T1 and T2 associated with it. Tissues with short T1 and T2 times (such as bone) are best visualized in T1 weighted images and tissues with long T1 and T2 times (such as soft tissue) are best visualized in T2 weighted images. The pixel intensity in an MR image is
therefore proportional to the number of protons contained within the volume element weighted by relaxation time T1 or T2 of the tissues being observed [68].

µMR imaging using a birdcage coil (39µm resolution, 4.7T Magnet, Bruker Spectrospin) has been used to yield high resolution images of spinal micro-architecture [67]. Sagittal T1 fluid attenuated inversion recovery (FLAIR), Axial Fast Relaxation Fast Spin Echo sequence (FRFSE) T2 weighted and Sagittal short inversion time inversion recovery (STIR) sequences have been used for optimal visualization of spinal metastases with MRI as per clinical MR protocols. Lytic disease has been shown to be best seen on the STIR and T2 sequences whereas sclerotic lesions appear with low signal intensity on all sequences. Trabecular architecture has been optimally imaged with µMRI using 3D spin-echo (3D SE) with 500ms repetition time, 4.08ms echo time and multi-slice multi-echo (MSME) sequences. Using these parameters, Hopper et al. were able to reconstruct a matrix size of 256 × 256 × 128 and a field of view 6 mm × 6 mm × 5 mm at the resolution of 23 µm in plane and 39 µm for slice thickness [67].

1.5.2.4 Histological Analysis

Histology is the process of analyzing the microscopic anatomy of cells of animal or plant tissues. This is done by cutting a slice from a tissue and visualizing it under a light microscope. Histology utilized for studying disease patterns in a cancerous tissue is referred to as histopathology.

Freshly excised tissues have to undergo several procedures to prepare for histological analyses. Immediately after excision the tissues are immersed in a preserving solution (commonly 10% neutral formalin). This fixation prevents decomposition of the tissue structure and preserves the cellular anatomy of the tissue’s original state. Once the tissue has been sufficiently fixed, it is then processed by dehydrating the tissue with paraffin wax. This facilitates the cutting of thin slices. The dehydrated tissue is then placed in liquid embedding material, such as agar, and is allowed to harden. Once hardened, the material can then be sectioned in slices using a microtome. The structure of interest can then be stained to enhance its contrast and improve the accuracy of its visualization under the microscope. The Haematoxylin & Eosin (H&E) is commonly used to identify live tumour cells in bony structures [69].
1.5.3 Computational Analysis

Finite element analysis (FEA) is a robust computational tool capable of modeling complex geometric and material property distributions. FEA has been used successfully to predict failure loads and fracture patterns in healthy and pathologic bone structures [32, 70–72]. Finite Element (FE) models of skeletal metastases have been validated through mechanical testing demonstrating that, given accurate geometries and material data, the FE method can be used reliably to predict the strength of bones with regular metastatic defects [32, 71]. Biomechanically based guidelines, using CT data, derived from validated FE modeling of lytic vertebral metastases has shown excellent sensitivity and specificity in application to retrospective clinical data [73]. However, the predictive ability of these quantities has not been demonstrated to date.

Whyne et al. have calculated the material properties of osteolytic tumour in a mechanical testing study [74]. They tested the osteolytic tumour under uniaxial creep and confined compression and modelled it mechanically using the linear biphasic theory. They found the osteolytic tumour to have an aggregate modulus (HA) of 3.6 +/- 1.6 kPa and a hydraulic permeability (k) of 0.59 +/- 0.46mm4 N(-1) s(-1) [74]. They used these parameters to develop representative material property models for osteolytic tumours. These material properties were used to model metastatic involvement in subsequent FE studies. These properties were utilized in a 3D parametric poroelastic FE model of the metastatic human spine that was validated experimentally and shown to be able to represent burst fracture initiation [75]. Their model yielded consistent prediction results with mechanical testing data. They found that burst fracture was highly dependent upon tumour size, magnitude of spinal loading as well as bone density [75].

Further work by O’Reilly and Whyne has demonstrated the importance of patient specific FE modeling of the metastatic spine through mesh morphing. Morphing the parametric model of Tschirhart et al. to patient CT scans allowed the capture of more accurate geometry of the vertebral body and lytic tumour and non-homogeneous bony material property assignments [74, 76]. Mesh morphing has also successfully and efficiently been applied to rat vertebrae based on μCT data [77]. To date, such specimen specific morphing algorithms have been limited to bony structures in the spine, rather than spinal motion segments (including the intervertebral discs and ligaments).
Accurate representation of load boundary conditions is essential when developing FE models for the metastatic spine. Previous parametric models of metastatic spine have mimicked physiologic loading of the spine via applying the loads on the spine through the intervertebral discs [75]. For specimen specific models, the intervertebral disc needs to be modelled separately to enable application of accurate physiological loading. In other studies, loads have been applied directly to the vertebral endplates; however this does not represent physiologic loading [78].

Specimen specific finite element models have been used to represent the mechanics of the intervertebral disc based on MR imaging [78]. The nucleus pulposus is generally modelled as a biphasic material due to its gelatinous nature and high fluid content. Two methods have commonly been used to model the behavior of annulus fibrosus: (1) fibre bundles represented as truss or cable elements within a matrix of solid elements; (2) a homogenisation approach in which anisotropic material properties have been used to represent on the macroscale the fibre alignment within extra-fibular matrix. Accurate poroelastic modeling of the disc has proven to be difficult, thus much has been done using reverse engineering and iterative model correction based on experimental results [78].

1.6 Rationale

The spine functions as a crucial support system in the body and as the protective shield of the spinal cord and nerves. Understanding the patterns of spinal metastasis, and their structural implications, is important to better the burden of disease and therapeutic outcomes. Evaluation of structural implications of metastatic disease in the spine has so far been mainly qualitative or semi quantitative. Thus, in spite of the high frequency of spinal metastasis there are no widely accepted objective criteria to evaluate ongoing research into the development of new systemic and local treatments for this pathology.

Robust preclinical models can yield insight into the behavior of healthy and diseased human vertebrae, representing an important step in the development and evaluation of new treatments. Quantifying the structural impact of metastases in the vertebrae is important in the evaluation of new and existing therapies. The ability to automate the structural assessment of metastatically involved vertebrae is crucial to the ultimate utility of such measures.
1.7 Thesis Objectives

This proposal aims to establish the biomechanical implications of metastatic disease in the spine through the evaluation of stability and tumour burden in a preclinical model. Combining recent innovations and advances in computational modeling, image analysis, and preclinical models of metastatic disease will allow quantification of tumour burden and its effect on stability in the metastatically involved spine using a multifaceted engineering-based approach.

1.7.1 Hypothesis

The use of automated analysis techniques applied to multimodality imaging will allow quantification of the impact of metastasis on biomechanical stability, tumour burden and bony architecture in the spine, and motivate prediction models that accurately reflect vertebral integrity.

1.7.2 Specific Aims

1) To utilize and compare µMR and µCT based radiologic methods to quantify tumour involvement and vertebral architecture in a rat model of spinal metastasis.

2) To evaluate the ability of 2D, 3D, and continuum based methods to quantify structural integrity in vertebral metastasis.

Radiologic changes in the presentation of spinal metastases secondary to newer systemic treatments motivate the analysis of these techniques in both osteolytic and mixed osteolytic/osteoblastic preclinical models of spinal metastasis.

1.8 Thesis Outline

This thesis examines limitations in current evaluation of spinal metastases and introduces novel methods of automatically quantifying tumour burden and mechanical stability in the metastatic rat spine. This work is comprised of a series of studies that have been published in, or will be sent for review by academic journals as partial fulfillment of the author’s doctoral dissertation.

Chapter 2 introduces a µCT based highly automated method to segment rat vertebrae. This work has been published in Journal of Neurosurgery: Spine and has been co-authored by Mr. Michael Hardisty and Dr. Cari Whyne.

Seyed-Parsa Hojjat: study design, data collection, data analysis, wrote manuscript

Michael R Hardisty: study design, sample preparation and imaging, data analysis

Cari M. Whyne: study design, data review, reviewed and revised manuscript.

Chapter 3 investigates the effect of structural model as well as spatial resolution in automated and quantitative analysis of the metastatic spine. This study has been published in the Journal of Medical Engineering Physics and has been co-authored by Dr. Cari Whyne.


Seyed-Parsa Hojjat: study design, data collection, data analysis, wrote manuscript.

Cari M. Whyne: study design, data review, reviewed and revised manuscript.

Chapter 4 discusses a multi-modal µCT/µMR based semi-automated algorithm for segmentation of rat vertebrae affected by mixed osteolytic/osteoblastic metastases. This work has been prepared for submission to the journal Medical Physics and has been co-authored by Dr. Lisa Wise-Milestone, Dr. Warren Foltz, and Dr. Cari Whyne.


Seyed-Parsa Hojjat: study design, data collection, data analysis, wrote manuscript.

Warren Foltz: µMRI sequence design, data collection, reviewed and revised manuscript.

Lisa Wise-Milestone: study design, sample preparation, reviewed and revised manuscript.

Cari M. Whyne: study design, data review, reviewed and revised manuscript.

Chapter 5 covers an investigation that evaluates the ability of µCT-based structural rigidity, image-based strain measurement, and continuum finite element analyses in predicting yield loads
in healthy and metastatically involved rat vertebrae. This work will be submitted to the journal of biomechanics co-authored by Dr. Maarten Beek, Dr. Margarete Akens, and Dr. Cari Whyne.

Seyed-Parsa Hojjat: study design, data collection, data analysis, wrote manuscript.

Maarten Beek: computational development, reviewed and revised manuscript.

Margarete Akens: study design, sample preparation, reviewed and revised manuscript.

Cari M. Whyne: study design, data review, reviewed and revised manuscript.

Chapter 6 investigates a non-destructive technique to evaluate the effect of combined bisphosphonate and photodynamic therapy on bone strain in metastatic vertebrae using Image Registration. This study has been published in the Annals of Biomedical Engineering Journal co-authored by Miss Emily Won, Mr. Michael Hardisty, Dr. Margarete Akens, Dr. Lisa Wise-Milestone and Dr. Cari Whyne.


Seyed-Parsa Hojjat: study design, data collection, data analysis, wrote manuscript.

Emily Won: study design, data collection, reviewed and revised manuscript.

Michael Hardisty: computational development, reviewed and revised manuscript.

Margarete Akens: study design, sample preparation, reviewed and revised manuscript.

Lisa Wise-Milestone: study design, sample preparation, reviewed and revised manuscript.

Cari M. Whyne: study design, data review, reviewed and revised manuscript.

Chapter 7 presents a general discussion of the major findings from our work and considers its strengths and limitations. This chapter also includes future applications and considerations towards which this work could be directed. This chapter further provides a summary of the overall significance from the present work.
1.9 References


[59]. Lane N., Yao W., Kinney J., Modin G., Balooch M., and Wronski T., “Both hpth\(1\text{-}34\) and bfgf increase trabecular bone mass in osteopenic rats but they have different effects on trabecular bone architecture.,” in J Bone Miner Res., 18(12):2105–15., 2003.


2.1 Abstract

Noninvasive evaluation of metastatic disease in the spine has generally been limited to 2D qualitative or semiquantitative analysis techniques. This study aims to develop and evaluate a highly automated micro-CT–based quantitative analysis tool that can measure the architectural impact of metastatic involvement in whole vertebrae.

Micro-CT analysis of rat whole vertebrae was conducted using a combination of demons deformable registration, level set curvature evolution, and intensity based thresholding techniques along with upsampling and edge enhancement techniques. The algorithm was applied to 6 lumbar vertebrae (L1–3) from 6 rnu/rnu rats (3 healthy rats and 3 with metastatic involvement). Osteolytic metastatic involvement was modeled via MT1 human breast cancer cells.

Excellent volumetric concurrency was achieved in comparing the automated micro-CT–based segmentations of the whole vertebrae, trabecular centrum (vertebrae excluding the cortical shell), and individual trabecular networks to manual segmentations (98.9%, 96.1% and 98.3%, respectively; 6 specimens), and the automated segmentations were achieved in a fraction of the time. The algorithm successfully accounted for discontinuities in the cortical shell caused by vasculature and osteolytic destruction.

As such, this work demonstrates the potential of this highly automated segmentation tool to permit rapid, precise quantitative structural analysis of the spine with minimum user interaction in the analysis of both healthy and pathological (metastatically involved) vertebrae. Future optimization and the incorporation of lower-resolution imaging parameters may allow automated analysis of clinical CT–based measures in addition to preclinical micro-CT–based analyses of the structural impact and progression of pathological processes in the spine.
2.2 Introduction

Metastatic involvement in bone is most frequently seen in the vertebral column [1]. Skeletal metastases are categorized as osteolytic (bone destroying), osteoblastic (bone-depositing), or mixed (osteolytic/osteoblastic) lesions. Spinal metastases can result in severe clinical consequences both in terms of morbidity and mortality [2, 3, 4]. Clinical impact is often judged in terms of skeletal-related events (SREs), which include vertebral fracture [5]. Better measures to assess clinical impact of these lesions prior to fracture would improve patient options and reduce morbidity. As such, radiological measures of metastatic disease in the spine may be used to provide important information related to disease progression as well as evaluation of treatment effects. Robust quantitative evaluation of treatment effects is important both clinically and in preclinical models used to develop and test new and existing therapeutic approaches for spinal metastases.

Noninvasive evaluation of metastatic disease in bone has generally been qualitative or semiquantitative and has historically used 2D analysis techniques [6]. A number of groups have developed quantitative methods for analyzing bony architecture using CT and micro-CT images, [7, 8] with recent work also focusing on semiautomated quantitative CT–based analysis segmenting and analyzing metastatic involvement specifically in human vertebral bodies (excluding the posterior elements) [9, 10]. However, the resolution of conventional CT precludes the analysis of trabecular architecture in the spine, and resolution issues present further challenges in segmentation of the articulations in the posterior elements (facets). Recent work in developing automated segmentation algorithms for trabecular structural analysis in the femur have been limited in their ability to function in the presence of osteolysis and vessels penetrating the cortex [11].

As such, the objective of this work was to develop and evaluate a highly automated micro-CT–based quantitative analysis tool able to measure the impact of metastatic involvement in whole vertebrae. It is hypothesized that highly automated multilevel image analysis applied to 3D micro-CT reconstructions will yield accurate and repeatable segmentation of whole vertebrae, with and without osteolytic defects, in a preclinical rat model. The development of such an
automated tool would allow for robust and repeatable measurement of the effects of metastatic disease and treatments on vertebral bone, crucial to evaluating spinal stability.

2.3 Methods

2.3.1 Image Acquisition and Automated Segmentation

A CT-based tracking tool that had been previously developed for human vertebral body images [9] was expanded and applied to micro-image analysis of rat whole vertebrae (including the posterior elements). The tool aims to automate accurate segmentation of the vertebrae at multiple levels using demons deformable registration followed by level set curvature evolution within the Amira software platform incorporating algorithms from the ITK toolkit (AmiraDEV 3.1, ITK) [12, 13].

Micro-CT scans were acquired of rat spinal motion segments (L1–3) (17.5 × 17.5 × 17.5 voxel/mm, GE Explore Locus, General Electric Co.) using an x-ray source at 90 mA and 80 kV, with 907 views covering 360° of rotation. An atlas (consisting of a segmentation and scan) was created of a single L1–3 spinal motion segment (L1–3) through manual segmentation of one individual specimen.

The automated segmentation started with registration of the previously obtained atlas scan from a corresponding spinal level to the target vertebra of interest. Prior to the registration, the vertebral bodies of the atlas and fixed target scans were manually aligned to ensure fast convergence of the algorithm. The segmentation procedure was performed in 2 steps. An automated affine registration with 12 degrees of freedom, allowing for shearing, scaling, rotation, and translation was performed to initially register the atlas to the fixed target scan followed by deformable registration. Demons deformable registration was employed using 15 resolution levels with 1000 iterations in each level to achieve a higher degree of accuracy [13]. Once one resolution level was complete, the deformation field was smoothed using a 3D Gaussian filter. The same procedure was performed on the next higher resolution level until all 15 levels were complete.

The obtained deformation field was used to deform the atlas yielding a satisfactory segmentation of the vertebral body of interest; however, this alone does not ensure full inclusion of the posterior elements in the segmentation contour. The attained segmentation was then refined...
using the level set method to ensure inclusion of high curvature areas, such as the posterior elements and to smooth boundaries [12].

The level set filter was applied in 3 steps to: 1) refine the demons segmentation of the whole vertebrae, 2) move the segmentation boundaries inward to define the cortical shell, and 3) segment the trabecular centrum (i.e. excluding the cortical shell from the vertebral segmentation). Moving the segmentation boundaries inward to be within the cortical shell was an essential intermediate step, as experience has shown that the segmentation curve does not always contract to come within the trabecular centrum without this initial push [8].

Starting with the output of the demons deformable registration, the parameters were set to cause the curve to flow outward to accurately grasp all the high curvature regions including the posterior elements. To refine the demons segmentation of the entire vertebra, the initial speed function was set to cause quick contour contraction through soft tissue (low-intensity values) and slow contour contraction through the bone (high intensity). The level set propagation term and the curvature term were used to smooth out areas of high curvature to prevent the segmentation boundaries from advancing into lytic tumours that have breached the cortical shell. The evolution ran for 60 iterations to ensure the inclusion of the posterior elements in the segmentation. In the second step of level set, the propagation term was increased and the evolution runs for 70 iterations with image intensity values set to a constant derived based on the intensity values of the scan. For the final step, the pixel intensity range was reduced and its center was increased to cause high-speed motion through high image intensity (cortical bone) and slow motion through middle and low image intensity (trabecular bone and soft tissue), again by increasing the propagation term and run for 50 additional iterations. These assignments helped to ensure the segmentation boundary did not move quickly through and hence excluded any blastic tumour tissue connected to the cortical shell when segmenting the trabecular centrum (Fig. 7).

Once the trabecular centrum segmentation was obtained, the scan and the segmentation were upsampled by 4 in all directions while preserving the edges of the image using the Lanczos filter (AmiraDev 3.1). This was done to separate the high-intensity voxels from low-intensity voxels as much as possible while limiting the size of the resulting file. Another 2 iterations of the Lanczos filter were applied to the upsampled image to enhance the edges that were blurred due to the upsampling. An intensity-based thresholding was then performed on the output image to
segment individual trabeculae. An intensity-based cut-off value (750 HU) was used to identify all higher-intensity voxels as bone and the lower-intensity voxels as not bone (that was, bone marrow or lytic tumour). The output of this step yields segmentation of the individual trabecular network (Fig. 7).

2.3.1 Application and Evaluation

The algorithm was applied to 6 lumbar vertebrae (L-1 to L-3) excised from 3 healthy rmu/rmu rats and 3 rats with metastatic vertebral involvement. Metastatic involvement was modeled via intracardiac injection of MT1 human breast cancer cells, which produces osteolytic vertebral lesions.

After each step of segmentation in each of the 12 specimens (whole vertebrae, trabecular centrum/cortical shell, and individual trabecular network), the algorithm was evaluated using a volumetric concurrency metric. This metric utilized the volume of the manual segmentation, the volume of the segmentation achieved by the automated algorithm, and the volume of the voxels that were present in both manual and automatic segmentations to compare the output of the algorithm to that of a manual segmentation. For assessment of the trabecular network, a volume consisting of 3 adjacent slices was manually refined for each specimen and compared with the volume segmented automatically.
Figure 7. Flowchart of the automated segmentation algorithm.
2.4 Results

A volumetric concurrency of 98.89% ± 0.85% was calculated when automated micro-CT–based segmentations of the whole vertebrae were compared with manual segmentations (6 specimens). The manual segmentations took an average of 12 hours per specimen, while the automated segmentation took on average 40 minutes to complete with minimal user interaction. Using level set curvature evolution, the algorithm was able to account for discontinuities in the cortical shell. This allowed representative volumes to be generated despite vessels and nerves entering the vertebral body and enabled segmentation of vertebrae in which lytic tumour had destroyed portions of the cortical shell (Fig. 8).

For the trabecular centrum, a volumetric concurrency of 96.08% ± 3.03% was calculated when the automated micro-CT–based segmentations were compared with the manual segmentations (6 specimens). Following the initial segmentation of the whole vertebrae, the manual segmentations of the trabecular centrum took an average of 6 hours per specimen while the automated segmentation took on average 15 minutes to complete. Volume of the cortical shell was calculated through subtraction of the trabecular segmentation from the whole body segmentation and demonstrated similar volumetric concurrency when comparing the automated versus manual analyses (96.12% ± 3.17%).

Figure 8. Representative micro-CT scans showing the segmentation of the whole vertebra (A), segmentation of the trabecular centrum (B), and segmentation of the trabeculae (C).
A volumetric concurrency of 98.25% ± 0.62% was calculated when the automated micro-CT–based segmentations of the individual trabeculae were compared with the manual segmentations (6 specimens). The manual method using intensity-based thresholding followed by manual refinement took on average 15 minutes (for the 3-slice volume) while the automated algorithm took on average 1 minute to complete. Upsampling and edge enhancement were necessary to yield accurate segmentation of the individual trabecular network.

2.5 Discussion

This work is a development of an accurate highly automated segmentation tool that enables precise quantitative structural analysis of the spine with minimum user interaction. Importantly, this tool was successful in the analysis of both healthy and pathologic (metastatically involved) vertebrae. The algorithm employs atlas-based deformable registration that is significantly faster than manual methods. The execution time of the algorithm is dependent on size of the µCT image, meaning that in applications where less accuracy is required, the speed of the algorithm could be increased by downsampling or downsizing the scanning parameters (lower resolution). Automated vertebral segmentation in reasonable analysis times will allow for widespread application of 3D spinal analysis. Automated methods remove inter-/intra-user variability and minimal user intervention reduces cost, facilitating the application of 3D quantitative measures. Reasonable analysis times and reduced costs also motivate the potential for quantitative analysis in larger research studies, increasing their potential power.

Accurate segmentation is necessary for evaluating bony architecture. Evaluating bone quality, in contrast to bone density alone, is essential in representing the structural integrity of the spine [14]. Architectural measurements allow for robust analyses to evaluate disease state, progression, and response to new or existing local or systemic therapies. The level set curvature evolution method is of major importance in achieving accurate segmentation in the metastatic spine as it prevents the segmentation from collapsing when the cortical shell is severely damaged by the tumour. The metastatically involved vertebrae analyzed varied in tumour burden, representing differing levels of metastatic involvement, from a large defect breaching the cortical shell to a smaller contained lesion within the trabecular centrum. The ability of the tool to function in segmenting structurally compromised vertebrae with defects due to osteolysis at multiple stages is essential to evaluating metastatic disease progression and the ability of treatments to
structurally improve vertebral stability. Accurate assessment of vertebral stability and fracture risk are of the utmost importance in clinical decisions related to surgical intervention.

Recent studies have demonstrated the feasibility of CT-based 3D automated segmentation methods. De Nunzio et al [15] successfully used a region-growing approach to segment the anatomy of the lung. A combination of thresholding and ellipse fitting has also been used to automatically segment the proximal femur; however, this method proved to be limited in its ability to function in the presence of discontinuities (osteoysis and vessels penetrating the cortex) [1]. In contrast, the level set method can overcome discontinuities in bony segmentation. The developed algorithm was able to use curve expansion to segment whole rat vertebrae and contraction to segment the trabecular centrum while maintaining an accurate shape by flipping the sigmoid function. The output in both cases was a smooth segmentation curve that was still able to grasp high-curvature area such as the posterior elements.

The resolution level of the images yielded clear delineation between the bony articulations at the facets, thus additional image processing was not required to ensure accurate segmentation at these sites. These segmentations, with accurate definitions of facet geometry and osteolytic defects, provide a robust platform for further mathematical modeling, such as finite element analysis. Finite element analysis represents a powerful tool to quantify mechanical stability of the spine in healthy and pathological states. The ability of the tool to yield multiple segmentations (whole bone to trabecular network) further provides a basis for the appreciation of multiscale differences in finite element analysis of vertebral bone [16].

In this work, we have presented an accurate, fast, and highly automated tool for segmentation of rat spines into whole vertebrae, cortical shells/trabecular centrums, and their individual trabecular networks. The algorithm has employed a combination of demons deformable registration, level set curvature evolution, and intensity-based thresholding techniques, along with upsampling and edge enhancement, to ensure accurate 3D segmentation of the rat spine. The automated segmentations have been evaluated in comparison with manual segmentations yielding a volumetric concurrency of approximately 95% while drastically reducing the execution time of the manual segmentations. While this tool has been developed for preclinical analysis of whole rat vertebrae, the methods employed can be translated to improve automated techniques to segment full human vertebrae (including the posterior elements) with extensive
osteolytic and/or osteoblastic disease. Future extension of this work may be used to optimize and incorporate lower resolution imaging parameters to better quantify and automate clinical CT–based measures of the structural impact and progression of pathological processes, such as metastatic disease, and associated treatments on spinal stability.

2.6 References


3.1 Abstract

Preclinical models of spinal metastases allow for the application of micro-image-based structural assessments, however, large data sets resulting from high resolution scanning motivate a need for robust automated analysis tools. Accurate assessment of changes in vertebral architecture, however may depend both on the resolution of images acquired and the models used to represent the structural data.

The objective of this work was to apply a recently developed automated µCT based analysis tool to quantify the effect of diffuse metastatic disease on rat vertebral architecture at multiple resolutions. It was hypothesized that automated methods could accurately quantify differences in vertebral micro-structure and that diffuse metastatic disease could be shown to have significant negative architectural effects on trabecular bone independent of stereologic model and resolution.

µCT images acquired at 14µm$^3$ of healthy and metastatically involved whole lumbar rat vertebrae were analyzed at high, medium and low (8.725, 17.450, and 34.900µm$^3$) resolutions using an automated algorithm to yield micro-structural measures of the trabecular centrum and cortical shell. The images analyzed at different resolutions were obtained via up/downsampling of the acquired image data. Trabecular thickness was evaluated with the Parfitt and Hildebrand models, and anisotropy was evaluated through calculation of mean intercept length.

Significant differences in micro-structural parameters measured in comparing healthy and metastatically involved vertebrae were affected by resolution, however, relative anisotropy was maintained. The Parfitt and Hildebrand models yielded similar structural differences between
healthy and metastatic vertebrae, however, the Hildebrand model was limited due to segmentation accuracy required for its automated application.

Differences in micro-structural parameters generated through automated analysis at high resolution suggest that diffuse MT1 osteolytic destruction in whole rat vertebrae results primarily in loss of trabeculae in the metastatic vertebrae, as opposed to trabecular thinning. The sensitivity of the bony architectural parameters to resolution motivates the need for high resolution scanning or post-processing of images.

3.2 Introduction

Metastatic spread of cancer to the skeleton most frequently affects the spinal column. Vertebral metastases can result in skeletal related events (SREs) such as extreme pain, pathologic fracture and neurologic compromise, and cause a significant decline in patients’ quality of life [1–5]. Clinically, accurate prognoses for spinal metastases remain a challenge [6]. This, in part, may be due to the qualitative or semi quantitative nature of currently available assessment schemes. The lack of quantitative measures for evaluation of disease in patients with spinal metastases results in difficulties related to clinical decision-making and the exclusion of these patients from many clinical trials. Considering the high occurrence of spinal metastases, there is a need for a robust quantitative method to evaluate the structural burden of spinal metastases.

Preclinical models are widely used to study spinal metastases [7]. Preclinical skeletal lesions are most commonly developed through either local or intracardiac injection of cancer cell lines. The evaluation of resultant metastatic tumour burden in bone and its effect on the skeletal microstructure has also mainly been qualitative or semi quantitative [7,8]. In semi quantitative analyses, it is common to quantify bone quality using manually selected regions of interest (ROI). This approach, however, can limit the repeatability of measurements through the introduction of inter and intraobserver errors and potential bias. These errors may be caused by the manual omission of regions that may not be visually affected by the tumour but may contribute to the mechanical integrity of the structure.

Bone quality is often assessed via architectural models that are correlated with mechanical stability. The plate model developed by Parfitt et al. [9] has long been used to describe the architecture of trabecular bone [10–18]. The Parfitt plate model calculates trabecular thickness,
separation and number through formulae derived based on bone volume and surface area. The Parfitt plate model makes the assumption that all trabeculae are plates, which is a limitation particularly in the consideration of low density trabecular bone or bone compromised by pathology such as osteolytic disease. More recently, Hildebrand and Rüegsegger [19] proposed a new method for characterizing the architecture of trabecular bone that calculates trabecular thickness without any structural assumptions. The Hildebrand method defines trabecular thickness at every point as the diameter of the biggest sphere that could perfectly fit inside the trabeculae. Using histograms, they demonstrated a range of trabecular thickness values within human bone samples, and a trabecular structure composed of both plates and rods. This work concluded that the Parfitt model over estimates trabecular thickness due to the plate assumption when rods are present within the structure.

Mechanical structure can further be described based on the degree of anisotropy in a material. Bone has been shown to have transversely isotropic material properties, with the degree of anisotropy related to bone density. The mean intercept length (MIL) method has been utilized to estimate the degree of anisotropy within trabecular bone structures [20]. MIL is defined as the average distance between the intercepts made by a line in a specific direction with the mesh of interest (i.e. trabecular mesh). The MIL for an isotropic object is the same for any line intercepting the mesh in any direction.

High resolution µCT is often used for visualization of trabecular structure in preclinical models. Depending on the size of the sample and the image resolution, a µCT scan can occupy as much as 10GB of disk space. Accurate quantitative analysis of such large image data files can be time consuming requiring a robust automated analysis tool for widespread utilization. One method of compensation is to downsample images before processing. However, downsampling can cause blurring effects in images and result in changes in measured micro-structural parameters [21, 22]. An automated quantitative analysis tool that is robust enough to function at a variety of resolutions can be utilized to determine the importance of resolution in characterizing and tracking pathological changes in bony structures. Such a tool would further facilitate analysis in preclinical studies of skeletal metastatic disease to yield accurate and precise quantification of the effects of new and existing treatments on bony architecture.
Qualitative analysis shows the apparent destruction of trabecular structure in the presence of large skeletal metastases. However, characterization of less severe more diffuse patterns of metastatic disease is more challenging. The objective of this work was to apply a recently developed automated µCT based analysis tool to quantify the effect of diffuse metastatic disease on the architecture of the rat spine at multiple resolutions. It was hypothesized that automated methods can be used to accurately quantify the micro-structural effects of metastatic disease in the rat spine and that diffuse metastatic disease can be shown to have significant negative architectural effects on trabecular bone using either the Parfitt or the Hildebrand model at different resolutions.

3.3 Materials and Methods

3.3.1 Model Development

Lumbar vertebrae from healthy (n = 6) and metastatic (n=6) rats were utilized for the study. Osteolytic spinal metastases were developed through intracardiac injection of human MT1 breast cancer cells into 4-week-old rnu/rnu rats. The MT1 cell line had previously been transfected with the luciferase gene to enable identification of tumour within the vertebrae using bioluminescent imaging. Lumbar vertebrae (L1, L2 or L3) with metastatic tumour were identified 21 days after cell inoculation with bioluminescent imaging prior to sacrifice (Fig. 9, IVIS Bioluminescent Imaging System).

Figure 9. Intracardiac injection of luciferase transfected human carcinoma cells yielded distinct vertebral metastases 2–3 weeks after cell inoculation into rnu/rnu rats as demonstrated by bioluminescent imaging.
3.3.2 Image Acquisition

Following sacrifice, the rat spines were excised and imaged using a high resolution digital μCT scanner (14μm³ resolution, GE Explore Locus, General Electric Company, Fairfield, USA). Micro-CT imaging of whole vertebrae yields 3D high resolution images of the high intensity cortical and trabecular bone tissue, with bone marrow and lytic tumour appearing as low intensity values which were not distinguishable from each other.

3.3.3 Automated Segmentation

A previously designed automated segmentation algorithm was used to segment the whole vertebrae, the trabecular centrum and the individual trabecular structure [24]. The whole vertebral images were manually aligned to a global axis prior to the automated segmentation algorithm to ensure convergence (AmiraDev) [25]. The remainder of the process was carried out in a fully automated fashion. The segmentation algorithm began with an affine registration to register a manually segmented atlas to the vertebra of interest. This was followed by a multi-resolution demons deformable registration of the atlas to the target vertebra to ensure accurate segmentation. Level set filtering was then used to refine the demons segmentation to yield a smooth segmentation curve, accurately outlining the boundaries of the whole vertebra, in spite of the discontinuities present in the shell due to metastatic destruction, as well as the nerves and vessels entering the vertebral body. The whole vertebral segmentation boundaries were then contracted while maintaining the curvature to segment the trabecular centrum. This was done using another multi-iteration application of the level set filter. Once the trabecular centrum was segmented, edge-preserving upsampling followed by intensity-based thresholding were utilized to segment the individual trabecular structure (Fig. 7).

3.3.4 Morphological Measurements

Stereologic analysis was automated within the AmiraDev 5 [25] platform to yield measures of: total vertebral volume (TVV), total volume of the trabecular centrum (TV), bone volume in the trabecular centrum (BV), trabecular bone surface area (BS), as well as the degree of anisotropy using the mean intercept length (MIL) method. Cortical bone volume (CBV) was calculated as the volume of the whole vertebrae (TVV) minus the total volume of the trabecular centrum (TV). The Parfitt plate model was employed to calculate parameters representing trabecular bone volume (TBV), trabecular thickness (TbTh), trabecular number (TbN) and trabecular separation
(TbSp) as per Eq. (5).

\[
\begin{align*}
TBV &= \frac{BV}{TV} \\
TbTh &= \frac{2}{BS/ BV} \\
TbN &= \frac{TBV}{TbTh} \\
TbSp &= \frac{1}{TbN} \cdot TbTh
\end{align*}
\] (5)

Trabecular thickness was additionally calculated using the Hildebrand model (also referred to as TbTh*). In this model, trabecular local thickness at every point is calculated as the diameter of the largest sphere that can perfectly fit inside the trabecula without crossing its borders [19]. To implement this model, we calculated the 3D float Chamfer map at every point of the generated individual trabecular segmentation, which calculated the closest distance at every point from the background. The Chamfer map is a close approximation of the Euclidean distance map and is the preferred choice for larger datasets due to its efficiency [23]. Chamfer maps are widely used in skeletonization applications (AmiraDev) [25]. The 3D center line of the distance map represents the radius of the biggest sphere that perfectly fits in each trabecula at every point. Doubling the radius value yields the local trabecular thickness at each point (i.e. the diameter of the largest sphere that could perfectly fit into the trabecula). To implement this, we constructed a 3D skeleton from the trabecular segmentation (Amira 5 Skeletonization Pack) [25] and performed an element wise multiplication with the double of the distance map matrix. The automated algorithm was validated using structures of known thickness (Fig. 10). The first and simplest test structure was a cylinder of known thickness. The second structure was composed of four parallel cylinders of the same thickness intersected by plates of the same width. The significance of the second figure was to test the ability of the algorithm to skeletonize organized connected structures of uniform thickness. The final test structure was meant to mimic the trabecular structure as it was composed of struts of variable thickness and orientation. We validated the accuracy of the algorithm by evaluating its ability to skeletonize and yield the correct thickness histogram.

Mean intercept length (MIL) was calculated in the superoinferior (X), mediolateral (Y) and dorso-frontal (Z) directions [20]. The MIL was automatically calculated by shifting the binary
image of the individual trabecular segmentation by 1 voxel in the direction of interest and subtracting the result from the original image. The number of non-zero voxels in the resulting image were counted and divided by two to yield the number of intercepts in the direction of interest. The total length in the direction of interest was calculated as the total volume divided by the average cross sectional area of the perpendicular plane. Dividing the total length by the total number of intercepts yielded the MIL measure in each direction of interest [Eq. (6)].
\[ \bar{A} = T(A) \]
\[ I_\varphi = \frac{\text{numel}(\bar{A} \cdot \varphi)}{2} \]
\[ L_\varphi = \frac{v}{CSA} \]
\[ MIL_\varphi = \frac{L_\varphi}{I_\varphi} \]

In the above equation set, \( T \) represents the transformation performed on the binary image \( (A) \) to yield the transformed image \( (\bar{A}) \). \( I_\varphi \) and \( L_\varphi \) represent the number of intercepts and the length in the orientation of interest \( (\varphi) \) respectively. Finally, \( MIL_\varphi \) corresponds to the mean intercept length in the orientation \( \varphi \).

The MIL values for an isotropic structure remain consistent in all directions. This automated approach quantifies the degree of anisotropy of the structure in a less general manner in comparison to the original work presented by Odgaard et al. [20], as it only calculates the MIL in 3 orientations.

### 3.3.5 Resolution
All of the parameters described above were calculated at \( 8.725 \times 8.725 \times 8.725 \, \mu m^3 \) (high), \( 17.450 \times 17.450 \times 17.450 \, \mu m^3 \) (medium), and \( 34.900 \times 34.900 \times 34.900 \, \mu m^3 \) (low) isotropic spatial resolutions. The data at different resolutions was obtained by resampling the image data which was acquired at \( 14 \times 14 \times 14 \, \mu m^3 \).

### 3.3.6 Statistical Analysis
Normality of the distribution of the structural measures was verified using one sample Kolmogorov-Smirnov (KS) test (SPSS statistical software) [26]. Two tailed T-tests were then used to compare the structural measures generated from the healthy and metastatically involved vertebrae. Similar analyses were applied to compare thickness values obtained using Parfitt’s plate model with the values measured using Hildebrand’s model, assuming unequal variances.
3.4 Results

Diffuse osteolytic vertebral metastases secondary to MT1 injection were confirmed through bioluminescent imaging in 6 lumbar vertebrae (2 L1, 3 L2 and 1 L3 vertebrae) generated from 4 rats. A similar cohort of vertebrae (2 L1, 3 L2 and 1 L3 vertebrae) were analyzed from 6 healthy rats.

3.4.1 Parfitt Plate Model

Using the output of the automated segmentation algorithm, the Parfitt plate model showed a significant decrease in TBV, TbN and CBV and a significant increase in TbSp in the metastatic vertebrae in comparison to the healthy group at the highest resolution (p < 0.05). There was no significant difference in TbTh (Parfitt model, p = 0.22; Hildebrand model, p = 0.20) between the healthy and metastatic groups at the highest resolution (Table 1).

Table 1. Micro-structural measures of the healthy and metastatic vertebrae at high (8.725µm³), medium (17.450µm³) and low (34.900µm³) resolution.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Healthy (n=6)</th>
<th>Metastatic (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8725 µm³</td>
<td>17.5 µm³</td>
</tr>
<tr>
<td>TBV (%)</td>
<td>55.92 ± 32</td>
<td>56.12 ± 3.4</td>
</tr>
<tr>
<td>TbN (unit/µm³)</td>
<td>5.79 ± 0.77</td>
<td>3.04 ± 0.11</td>
</tr>
<tr>
<td>TbSp (µm)</td>
<td>78 ± 13</td>
<td>144 ± 11</td>
</tr>
<tr>
<td>Parfitt’s TbTh (µm)</td>
<td>100 ± 17</td>
<td>184 ± 14</td>
</tr>
<tr>
<td>Hildebrand’s TbTh (µm)</td>
<td>87 ± 21</td>
<td>104 ± 25</td>
</tr>
<tr>
<td>CBV (cm³)</td>
<td>0.0411 ± 0.0006</td>
<td>0.0412 ± 0.0095</td>
</tr>
</tbody>
</table>

3.4.2 Hildebrand Model

The automated thickness calculation method using Hildebrand model yielded accurate results when applied to objects of known thickness (exact match between histogram of input data and resultant histogram from Hildebrand model) (Fig. 10). Fully automated calculation of trabecular thickness based on the generated individual trabecular segmentation using the algorithm proposed by Hildebrand et al. was limited due to the presence of a low number of small islands created in the thresholding stage of the automated segmentation. As the Hildebrand model assigns a thickness to every structure segmented as bone, presence of the islands of negligible thickness drastically reduces the average trabecular thickness measured. Time consuming manual island removal was required to ensure appropriate removal of islands without removing any of the trabecular bone structure. Once the islands were manually removed, similar results to the Parfitt model were found demonstrating no significant change in trabecular thickness in
comparing the healthy and metastatically involved vertebrae at the highest resolution (Table 1). While the average trabecular thickness measures were lower using the Hildebrand model as compared to the Parfitt model for both the healthy and metastatically involved vertebrae, this result was not significant (p = 0.09 for high, 0.09 for medium, and 0.2 for low resolution levels).

3.4.3 Mean Intercept Length

Transverse isotropy of the trabecular structure was found in both the healthy and metastatic vertebrae, demonstrating significantly higher MIL values in the axial (Z) direction as compared to the transverse (X and Y) directions. As expected, the MIL values were found to be higher in the metastatic group in all directions due to tumour destruction; however no difference in the relative degree of anisotropy was observed, as the ratio of MIL values in the axial direction to the values in the transverse directions remained consistent (~1.5:1). (Table 2)

Table 2. Mean intercept length (\( \gamma \) m) of the healthy and metastatically involved vertebrae measured high (8.725µm\(^3\)), medium (17.450µm\(^3\)) and low (34.900µm\(^3\)) resolution.

<table>
<thead>
<tr>
<th>Direction</th>
<th>Healthy ( 8.725 \mu m^3 )</th>
<th>Healthy ( 17.45 \mu m^3 )</th>
<th>Healthy ( 34.9 \mu m^3 )</th>
<th>Metastatic ( 8.725 \mu m^3 )</th>
<th>Metastatic ( 17.45 \mu m^3 )</th>
<th>Metastatic ( 34.9 \mu m^3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>66 ± 14</td>
<td>64 ± 12</td>
<td>76 ± 14</td>
<td>88 ± 26</td>
<td>97 ± 10</td>
<td>105 ± 15</td>
</tr>
<tr>
<td>Y</td>
<td>67 ± 12</td>
<td>66 ± 10</td>
<td>79 ± 12</td>
<td>53 ± 28</td>
<td>100 ± 10</td>
<td>108 ± 13</td>
</tr>
<tr>
<td>Z</td>
<td>90 ± 19</td>
<td>89 ± 18</td>
<td>111 ± 20</td>
<td>133 ± 39</td>
<td>145 ± 20</td>
<td>157 ± 26</td>
</tr>
</tbody>
</table>

3.4.4 Resolution

All of the micro-structural parameters in the healthy and metastatically involved vertebrae were measured at 17.450 × 17.450 × 17.450 \( \mu m^3 \) and 34.900 × 34.900 × 34.900 \( \mu m^3 \) isotropic resolution levels and compared to the highest resolution (8.725 × 8.725 × 8.725 \( \mu m^3 \)) results. TBV at all resolutions was higher in the healthy group. TbTh values were significantly affected by resolution. In comparison to the lack of a difference measured at the highest resolution, larger TbTh values were calculated in the healthy vs. the metastatic group at both the medium and low resolutions for both the Parfitt and Hildebrand models. TbN decreased significantly as the resolution was lowered. In direct contrast to the highest resolution, TbN values were found to be significantly larger in metastatically involved vertebrae at medium and low resolutions. TbSp increased significantly as the resolution was lowered and, again in contrast to findings at the highest resolution, no significant differences were found in TbSp values at medium and low resolutions (Table 1). At all resolutions, MIL values remained consistent with lower values but
similar amounts of anisotropy in comparing the healthy and metastatically involved vertebrae (Table 2).

3.5 Discussion

This work demonstrates the ability of an automated algorithm to quantify micro-structural differences in healthy vertebrae vs. vertebrae with diffuse osteolytic metastatic disease based on µCT image data at multiple resolutions. The accurate automated method enables objective and repeatable analysis of multiple morphologic measures, and allows the comparison of two distinct models for describing trabecular thickness (Parfitt and Hildebrand).

The automated segmentation method used in this study has been previously validated against manual segmented data [24]. Excellent volumetric concurrency was demonstrated between the automated and manual segmentation techniques for whole vertebrae, trabecular centrums and individual trabecular networks (95 to 98%).

The morphologic measurements generated using the automated algorithm demonstrated expected significant differences between micro-structural bone measures in comparing the healthy and metastatic vertebrae. Significant decreases in CBV, TBV, TbN and a significant increase in TbSp were observed in the metastatic group when compared to the healthy group at the highest resolution. Both the widely used Parfitt plate model and the Hildebrand model demonstrated no significant difference in TbTh between the two groups at the highest resolution. This supports the idea that the osteolytic destruction results primarily in loss of trabeculae as opposed to trabecular thinning [16].

The Hildebrand model required further manual refinement of the automated segmentation to yield correct results. This was due to the presence of limited number of isolated islands, which were assigned an extremely low thickness value resulting in a considerable drop in the average trabecular thickness. The Parfitt plate model was not as sensitive to such errors in the segmentation, as it defines the micro-structural parameters as a function of total bone volume and surface area. Regardless of the accuracy of trabecular thickness measurements generated from the Hildebrand model due to its model independent nature, its sensitivity to perfect segmentation limits its use in fully automated applications. Automated image denoising algorithms could be used to make Hildebrand model more robust, however the application of
such algorithms to diseased bone could result in wrongful exclusion of some of the bony structures.

Parfitt’s plate model makes the assumption that all trabeculae are plates, which leads to overestimation of mean trabecular thickness in our samples. Other works, however, have shown that the bias introduced by the Parfitt’s model is dependant upon the ratio of plates to rods [27]. Day et al. have shown that Parfitt’s plate model results in an overestimation of the trabecular thickness when the structure is primarily composed of plates, while in rod-like structures this model results in an underestimation of the trabecular thickness values due to the larger surface areas of rods [27]. In our study, however, we did not observe any difference in trabecular thickness values of the healthy and osteolytic groups. As such, in this application represented Parfitt’s model as a promising option as it enabled robust automated analysis. The automated Mean Intercept Length (MIL) method showed that the degree of anisotropy is consistent for both healthy and metastatic groups. This suggests that the MT1 osteolytic destruction in these rats does not have any directional preference. However, the loss of bone in all directions in these vertebrae, as measured through the increased MIL values, indicates that the mechanical integrity in these metastatic rat spines may be compromised due to overall bone loss as opposed to change in the directional pattern of the micro-structure.

Image resolution is an important factor in micro-structural analysis as trade-offs are made in reducing image acquisition and computational analysis time while maintaining accuracy. Both Parfitt’s plate model and the refined Hildebrand model calculated increased trabecular thickness as the resolution was lowered. These increased thickness measures were consistent with image blurring that results following the reduction of resolution. No significant differences were seen in TbTh between the healthy and metastatic group at the highest resolution, in contrast to differences measured at the lower resolutions. Similarly, significant differences in TbSp and TbN seen at the highest resolution were not observed at the medium and low resolutions. The above findings are consistent with image blurring and the melding of individual trabeculae into single structures. This suggests that sufficient resolution is essential not only to accurately describe the absolute values of structural measures [22] but also to represent structural differences in vertebrae due to the presence of diffuse osteolytic tumour. However, more global measures of bone volume (both cortical and trabecular), MIL and degree of anisotropy remained consistent at all resolutions.
The processing time for segmentation and the micro-structural calculation at the lowest resolution averaged 56 minutes per specimen. This processing time increased to 111 and 221 minutes for the medium and high resolutions respectively. At medium and high resolutions, the size of the structure became larger than the limitation of our software and thus the vertebra had to be cropped to smaller pieces and analyzed in steps. Comparative global measures of bone volume and the degree of anisotropy remained consistent at all resolutions. As such, these measures may be sufficiently robust to allow the comparison of bone architecture in applications where high-resolution imaging is not accessible, or storage size and processing time are of importance.

In resolution studies, optimal modification of the resolution would occur when the images are initially acquired. However, limited access to higher resolution scanners, and added times/costs associated with acquiring multiple images, have led to the utilization of images generated through the simulation of resolution differences via image processing. In this work different resolution levels were obtained via data upsampling and downsampling. While edge preserved upsampling did not create new data, it yielded sharper images, which resulted in a smoother trabecular segmentation that more closely represented the bony surface (Fig. 11). Future work could include imaging these samples at higher resolutions and comparing the quantification results to the current work. The downsampling process, however, would potentially be equivalent to acquiring the images at lower resolutions as it is done by disregarding some of the voxels from the acquired image rather than creating new image data. Downsampling of the image data resulted in moderate blurring of the edges and merging of structures, which ultimately resulted in an increase in trabecular thickness and a decrease in trabecular number. This implies, as expected, that as the resolution is lowered thinner trabeculae merge together to form thicker ones and result in a drop in the total number of trabeculae.
Figure 11. Selected trabecular region from a coronal µCT slice acquired at 14 µm³ isotropic resolution demonstrating trabecular segmentation at (a) 8.725 µm³, (b) 17.450 µm³, (c) 34.900 µm³ isotropic resolutions.

The segmentations are equally accurate relative to the spatial resolution however the segmentation obtained at higher resolution is smoother due to the presence of more voxels across a trabecula.

Image-based structural analytic techniques, including the Parfitt model and MIL measures have previously been applied to the metastatically involved spine, however the evaluation of disease burden has been limited to selected regions of interest [28]. In their work, Tamada et al. found a significant difference in the degree of anisotropy between non-pathologic regions and regions with osteoblastic metastasis. In contrast, using our automated algorithm to quantify the structural effects of osteolytic metastasis throughout the entire vertebra, we found a consistent overall degree of anisotropy. Future work quantifying differences in anisotropy in osteolytic, osteoblastic and mixed osteolytic/osteoblastic lesions, regionally and in whole vertebrae may yield additional insight into the varied fracture risks and patterns produced by the presence of cancer in the spine.

A previous study examining the effects of blurring due to limited resolution on thickness measurements demonstrated that blurring results in an increase in measured thicknesses [21, 22]. We have confirmed this finding, demonstrating an increase in measured trabecular thickness, using both the Parfitt and Hildebrand models, as resolution is reduced. Structural differences in comparing normal vertebrae to those with diffuse metastatic involvement were affected by
resolution. However, as the severity of the metastatic involvement increases, significant structural differences may be more consistently identified at lower resolutions.

Other works have looked at determining an optimum resolution for imaging trabecular bone for different applications [29, 30]. Peyrin et al. acquired µCT images from human vertebrae at 2, 7, and 14 µm isotropic voxel size and noted that 14 µm provided sufficient structural detail [29]. This was, however, concluded via qualitative observation. Müller et al. [22], in studying the effects of resolution on quantification of the micro-structure of the human iliac crest, also concluded that isotropic resolution of 14 µm yielded correct values for the micro-structural parameters. They noted that micro-structural parameters increase or decrease monotonously as a function of resolution, and thus commented that a suitable calibration procedure could be used to restore the correct data. We also observed this monotonous behavior in the micro-structural parameters analyzed at different resolutions in our data. Niebur et al. performed a convergence study to determine the optimal resolution for micro finite element modeling and have concluded that the optimum acquisition resolution should be about ¼ of average trabecular thickness [30]. Resolution studies in preclinical models are more limited, however Bouxsein et al. [31] have performed a comprehensive study on micro-CT based assessment of micro-structure in rodents. While the minimum ratio of voxels to object size is reported as 2, the authors noted that this ratio should ideally be higher (3 to 4 voxels) for accurate morphologic measurements and finite element model generation. The trabecular dimensions in rat vertebrae have been described to be in the range of 60µm, similar to the findings at the highest resolution for the healthy rat vertebrae in this study (87±21µm). As such, utilization of an isotropic voxel size of 14 µm during image acquisition is acceptable based on the 3 to 4 voxel per trabecula guideline. However, we found that further upsampling of the acquired data to obtain a sharper image enabled us to more smoothly segment the trabecular structure.

Robust automated micro-structural analyses can be used to facilitate accurate evaluation of the effects of new and existing treatments aimed at spinal lesions in preclinical models. Overall, the presented work suggests that automated use of Parfitt’s plate model along with the MIL method can be used to yield quantitative analyses demonstrating differences in vertebral micro-structure due to moderate metastatic involvement. However, the sensitivity of many of the architectural parameters to resolution motivates the need for high resolution scanning or post-processing of images to reduce blurring in future preclinical applications.
3.6 References


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CHAPTER 4
MULTI-MODAL µCT / µMR BASED SEMI-AUTOMATED SEGMENTATION OF RAT VERTEBRA AFFECTED BY MIXED OSTEOLYTIC / OSTEOBLASTIC METASTASES

4.1 Abstract

Skeletal metastases most frequently happen in vertebral column. Spinal metastases lead to severe clinical consequences significantly reducing patients’ survival time. Widespread use of new therapies aimed at skeletal lesions has resulted in a change in the pattern of spinal metastases. Historically, spinal lesions appeared as distinct focal osteolytic lesions, while in recent years the occurrence of mixed osteolytic/osteoblastic tumours has significantly risen. Multimodal micro imaging techniques in preclinical models are widely used to look at the effect of spinal metastases on bony structure, however the evaluation of tumour burden and its effect on the micro-structure has thus far been qualitative or semi quantitative. The quantification of the analysis of such imaging modalities is a time consuming task motivating automated methods. In this work we have developed a low complexity semi-automated multimodal µCT / µMR based approach to segment rat vertebral structure affected by mixed osteolytic / osteoblastic destruction. Accurate registration of µCT and µMRI modalities yielded accurate and precise segmentation of different structures. This was done via utilizing a bone µMRI as the localizer to register the µCT to the contrast µMRI. Our method was able to utilize the µCT to accurately segment the whole vertebra, trabecular centrum, and individual trabeculae, as well as the osteoblastic tumour attached to the outside of the vertebra. The algorithm was however limited in segmenting the osteoblastic tumour cells inside the trabecular centrum on µCT since they appeared to be identical to newly formed bone, and they were not as populated inside the trabecular centrum to result in detectable deformations. Our algorithm was able to accurately segment the osteolytic tumour in the vertebra using the contrast µMRI. This semi-automated segmentation method could readily be used in preclinical applications evaluating new and existing treatment effects on tumour burden and the micro-structure. As well, our algorithm could be used to develop mathematical models analyzing the mechanical stability of vertebra under load to accurately predict the ultimate fracture load and location in metastatic vertebra.
4.2 Introduction

The skeleton is one of the most common sites of metastatic disease; bone metastasis accounts for skeletal lesions in 40 times as many patients as those affected by all other forms of bone cancer combined [1]. Skeletal lesions most commonly appear in the spine [2] and can present as osteolytic (bone destroying), osteoblastic (excess bone deposition), or mixed osteolytic/osteoblastic. Modern cancer therapies have resulted in a significant increase in the occurrence of mixed vertebral lesions with more diffuse patterns of involvement [3, 4]. Understanding the structural implications of complex patterns of metastatic involvement in the spine is an important step towards the development and appropriate use of interventions to prevent fracture and associated neurologic complications [5, 6].

Preclinical models are commonly used to represent metastatic disease in the skeleton [7, 19, 21, 22, 23]. Widespread access to high resolution imaging modalities has made it possible to visualize the pattern of metastatic disease within the skeleton in preclinical models on a microscale. Such image data can be used towards building accurate mathematical models to quantify the impact of bone quality (including architecture and material properties), and tumour burden on bone stability, and to predict the risk and location of fracture. Development of such models requires accurate segmentation of the vertebral structure including bone, marrow and both osteolytic and osteoblastic tumour tissue. However, the amount of data contained in these high resolution scans motivates the development of automated segmentation methods.

In previous works [8, 9], semi-automated CT-based methods have been developed for quantification of metastatic disease in human vertebral bodies. In this work, the quantification scheme was based on conventional CT in which osteolytic destruction and osteoblastic deposition appear as low and high intensity voxels respectively. Conventional CT is, however, not suitable for the examination of micro-structural effects. We extended this work and developed a µCT based automated method to segment the full healthy vertebrae (including the posterior elements) and vertebrae with osteolytic metastatic involvement in a rat model (as presented in chapter 2, [10]). This approach was able to accurately segment whole vertebrae, the trabecular centrum, and the individual trabecular mesh. In this work, tumour burden was evaluated as the volume of osteolytic destruction. However, both tumour tissue and bone marrow
appear as low intensity voxels in the µCT images, and thus, the true tumour burden was not quantified.

A solution to determine the true extent of tumour burden within bone is to employ multi-modal imaging. MRI yields robust images of soft tissue structures and is able to differentiate tumour burden (higher intensity) from bone marrow. However, registration of this data with the bone information generated from the µCT image data is necessary to spatially resolve the relative distributions of the soft and hard tissues.

Previous works have introduced different methods for multi-modal registration of medical images [11 – 14]. All of these works use a mutual information metric to perform the registration, however the techniques used to assure accuracy in the registration differ. Chen et al. employ extensive preprocessing to eliminate noise and enhance the quality of the images before starting the registration process [11], while Lu et al. use the method of joint histogram estimation to predict the distribution of the fused image [12]. Other work performed by Eldib et al. uses the concept of surface matching to register the two image modalities [13], and finally, Piella et al. [14] use a sophisticated multi-resolution wavelet-based approach to register two images. None of the mentioned algorithms, however, have been employed on high resolution images. The utilization of high resolution data may greatly affect the computational time and memory requirements considering the extensive computations used in all of these algorithms.

The objective of this study was to develop a low complexity, highly-automated multimodal approach to segment the rat vertebral structure and quantify metastatic tumour burden within bone using a combination of µCT and µMR modalities. We hypothesize that semi-automated multi-modal analysis applied to 3D µCT and µMRI reconstructions will allow registration of whole vertebrae affected by mixed osteolytic/osteoblastic metastases and yield accurate and repeatable segmentation of both bone and soft tissue elements within the bone.
4.3 Materials and Methods

4.3.1 Animal Model

Osteolytic/osteoblastic spinal metastasis were developed using canine Ace-1 prostate cancer cells in one 3 week old rnu/rnu rat. Ace-1 cells were previously transfected with the luciferase gene to enable bioluminescent image monitoring of tumour growth within the rodent model. Cells were cultured for 7 days using a standard protocol (DMEM/F12 media with 10% FBS and 1% antibiotics). One and a half million cells in 200µl of media were injected into the left ventricle of anaesthetized rats (2% isoflurane/oxygen). All rats were weighed, marked and monitored until fully awake.

Bioluminescence imaging (IVIS Imaging System, Caliper Life Science, Hopkinton, MA) was performed on day 14 following intracardiac cancer cell injection to confirm the establishment of metastases (Fig. 12A). Luciferin (Xenogen Corp., Alameda, CA) was dissolved in 0.9 % NaCl solution at a concentration of 30mg/ml and 60 mg/kg injected intra-peritoneally to anaesthetized animal (2% isoflurane/oxygen). After five minutes, the bioluminescent signal was acquired with a 1 minute integration time. The signal was captured as the absolute total flux ( # of photons emitted per steradian cm$^2$) and analyzed using Living image software with the bioluminescence image overlaid on a plain photograph. Following subsequent bioluminescent confirmation of vertebral metastatic involvement at day 21 (Fig. 12B), the rat was sacrificed and L1-L3 were excised and immediately fixed in 4% paraformaldehyde [18].

![Figure 12](image_url)

**Figure 12.** Example bioluminescence image taken from the rat (a) 14 days and (b) 21 days post injection, displaying the tumourous regions in bright colors.
4.3.2 Image Acquisition

4.3.2.1 Micro-CT

First to third lumbar vertebrae were dissected and scanned at 14 µm³ isotropic resolution using a digital µCT scanner (GE Explore Locus, General Electric Company, Fairfield, USA). The 3D volume was reconstructed using the GE Explore Reconstruction Utility. The µCT was able to display cortical and trabecular bone as well as osteoblastic tumour as high intensity voxels. Bone marrow and osteolytic tumour appeared as low intensity values, which were not distinguishable from each other (Fig. 14a).

4.3.2.2 Micro-MR

MRI used a 7 tesla preclinical Biospec system (Bruker, Ettlingen, Germany) with a quadrature birdcage coil tuned to the 1H frequency (~300.3 MHz) which was custom-built by Stark Contrast MRI Research, Germany. This coil was specifically designed for good signal to noise ratio in imaging ex vivo bone. The coil was fixed tuned to an average load as load change is not essential for small sizes. The inner diameter of the coil was 17mm, to fit a 15 cc Falcon tube, and the outer diameter was set to 60mm, to fit the B-GA6S gradient coil insert of the Bruker system.

For imaging with this coil, the spine was embedded in agarose gel within a 15 cc Falcon tube. Imaging then proceeded using 30.8 mm field-of-view along the spine length, and 15.4 mm field-of-view in each transverse plane. Spine anatomy was then resolved to 60 µm³, using a 512x256x256 matrix, with 13.5 hour acquisitions.

The first scan presented a T1-weighting to accentuate bone ultrastructure and ensure accurate µCT and µMRI registration. Data acquisition parameters included an echo time (TE) of 7.6 ms, repetition time (TR) of 500 ms, RARE factor of 2, and 3 signal averages. Using these parameters, the boundaries of the trabecular structure were clearly visible, though bone marrow and osteolytic tumour could not be visualized distinctly (Fig. 14b).

The second scan presented a T2-weighting to accentuate soft tissue contrast and clearly differentiate lower intensity bone marrow from higher intensity tumour tissue (Fig 13C). Data acquisition parameters included a TE of 48 ms, TR of 3000 ms, RARE factor of 4, and 1 signal average.
4.3.2.3 Histological Confirmation of Osteolytic/ Osteoblastic Tumours

Following µCT / µMR imaging, the sample was processed for undecalcified histology. Briefly, the sample was sagitally cut and then dehydrated in increasing concentrations of acetone (70%, 90%, 100%, 100%) followed by infiltration with increasing concentrations of spurr/acetone solutions (50%, 80%, 100%, 100%). The sample was then embedded undecalcified in polymerized plastic spurr blocks and sectioned on a rotary microtome (Reichert-Jung 2050). Five µm sections were subsequently stained using Goldner’s Trichrome, which differentiates between mineralized bone (green/blue) and unmineralized new bone formation (osteoid; red/orange) (Fig. 13).

![Figure 13](image)

**Figure 13.** (a) Sagital histology slice of L1 vertebra stained with Goldner’s Trichrome, which differentiates between mineralized bone (green/blue) and unmineralized, new bone formation (osteoid; red/orange) (b) Inset shown at 6.8x magnification.

In histological analysis osteoblastic tumour can be qualitatively characterized as a disorganized arrangement of osteoblastic cells (indicated in the rectangle in figure 12), however, in µCT images the intensity of the voxels corresponding to osteoblastic tumour is equivalent to that of non-pathologic newly formed bone.
4.3.3 Semi-Automated Multimodal Segmentation

4.3.3.1 Rigid Registration

All µMR and µCT images were resampled to 34.9 µm^3 and manually aligned to a global axis (AmiraDEV 3.1, Visage Imaging, USA). The aligned µCT scan was further registered to the bone µMRI data using a rigid 3D registration algorithm (AmiraDEV 3.1). In this registration, the µCT was chosen as the moving image and the µMRI was chosen as the fixed image. A quasi newton optimizer was used with an initial optimizer step of (10^3 × bounding box) and final optimizer step of (10^7 × voxel size) for translation. The values for rotation, scaling and shearing were chosen appropriately by the algorithm. A normalized mutual information metric was utilized. The histogram range for both the fixed and the moving image were chosen as the full range of gray level values for each image. The contrast µMRI was then registered to the bone µMRI using the rigid registration algorithm with the same parameters. For this registration, bone µMRI was used as the fixed image and the contrast µMRI was used as the moving image. This resulted in accurate registration of all 3 scans (Fig. 14).

![Figure 14](image)

**Figure 14.** (a) Sagittal view of a µCT slice of a rat spine with mixed osteolytic/osteoblastic metastatic involvement secondary to Ace-1 cancer cell injection, (b) Corresponding slice in the bone µMR after the rigid registration, (c) Corresponding slice in the contrast µMR after the rigid registration.
4.3.3.2 Segmentation

4.3.3.2.1 Micro Computed Tomography

An extended version of a previously developed algorithm [10] was used to segment the whole vertebra of interest from the μCT scan. The algorithm uses atlas-based demons deformable registration along with level set curvature evolutions to segment the whole vertebra. Mixed metastatic involvement in the vertebra resulted in growth of osteoblastic tumour on the outer cortical shell of the vertebra along with large osteolytic lesions within the trabecular centrum and compromising the cortical shell. This heterogeneous distribution of bone required an increase in the number of pyramid levels of the demons deformable registration (from 15 to 30) in order to achieve good accuracy in the segmentation of the pathologic vertebrae (Fig. 15a). Additional iterations of the level set algorithm were also used with the inverse speed image to contract/smooth the segmentation boundaries yielding a clear segmentation of the trabecular centrum (Fig. 15b).

![Figure 15](image)

**Figure 15.** (a) Axial view of a segmented whole vertebra using the automated segmentation algorithm, (b) Axial view of the segmented trabecular centrum using the automated segmentation algorithm.

As validated by histology, the osteoblastic destruction was predominantly attached to the periosteal surface of the cortical shell (Fig. 15). To segment the osteoblastic deposition, the
trabecular centrum was removed from the whole vertebral segmentation, leaving the outside band of the vertebra including the cortical shell and osteoblastic tumour. Intensity-based histogram analysis was performed on this region. Based on concurrency with manual segmentation, a threshold was chosen as a function of the mean (µ) and the standard deviation (σ) to segment the osteoblastic tissue, with an upper bound of (µ + σ) and a lower bound of (µ - σ/4) for L1, (µ - σ/2) for L2 and L3. (Fig. 16).

![Figure 16](image)

**Figure 16.** (a) Axial view of the μCT image with the cortical shell segmented using the automated segmentation algorithm. (b) Segmentation of the osteoblastic tumour growth on the outside of the vertebra.

The image was edge enhanced, and resampled to 8.725 × 8.725 × 8.725 μm³ prior to the next step of the segmentation. In contrast to earlier work done on rat vertebrae affected by osteolytic destruction alone [10], the presence of osteoblastic tumour resulted in a larger variation in intensity values within the trabecular centrum. This prevented the utilization of a constant intensity based thresholding technique to segment the individual trabecular structure. Rather, a threshold value equal to the mean intensity value of all the voxels present in the trabecular centrum was employed to effectively segment the complex trabecular structure within these rat vertebrae (Fig. 17).
Figure 17. (a) Histogram of the intensity values in the µCT of the trabecular centrum with the mean of the distribution indicated, (b) Axial view of the segmentation of the trabecular mesh from the µCT image.

4.3.3.2.2 Micro Magnetic Resonance Images

The whole vertebral segmentation output from µCT scan was further masked around the contrast µMR which was previously registered to the µCT scan. An intensity-based threshold equal to the mean of the segmented region plus half of one standard deviation ($\mu + \frac{\sigma}{2}$) was chosen to define the osteolytic tissue (Fig. 18).

Figure 18. (a) Axial view of the µMR image segmented using the segmentation boundaries obtained from the µCT image. (b) Segmentation of the osteolytic tumour within the whole vertebra.
4.4 Results

The automated segmentation of the whole vertebrae yielded 91% concurrency in L1, 94% concurrency in L2 and 92% concurrency in L3 when compared to manually refined segmentations. The segmentation of the trabecular centrum yielded 93%, 95%, and 92% concurrency in L1, L2 and L3 respectively when compared to the manually refined segmentations. The output of the segmentation of the individual trabecular network did not require any manual refinement. The osteoblastic tissue segmentation on the outside of the cortical shell yielded 80% to 86% concurrency when compared to manually refined segmentations. Although based on histological analysis osteoblastic tumour was primarily concentrated on the outside of the trabecular centrum, in a few slices they were also detected inside the trabecular centrum. Despite several attempts, including histogram based approaches applied inside the trabecular centrum (with and without the growth plate), we were not able to distinguish newly formed healthy bone from osteoblastic tumour tissue in these vertebrae using the µCT or µMR image data, as they possess the same tomographical attitude.

The customized µMR coil enabled good quality image acquisition of the bony structure as well as good quality images visualizing the non-bone soft tissue structures (bone marrow and osteolytic tumour). Using the contrast µMRI, we were able to accurately segment the osteolytic tumour with no need for further manual refinement.

4.5 Discussion

In this work we have presented a semi-automated multimodal approach to segment rat vertebrae affected by mixed osteolytic / osteoblastic tumour. The whole bone vertebral segmentation algorithm was able to accurately segment metastatic vertebra despite extensive osteolytic destruction and osteoblastic deposition caused by the aggressive behavior of tumour cells. The algorithm was also able to accurately segment the trabecular centrum, and individual trabecular network clearly accounting for the boundaries of the bone and non bone structures.

The algorithm was limited in segmenting the osteoblastic voxels (lower volumetric concurrency ~80%) as they appeared as the same intensity as newly formed bone. Future work on older rats may provide a more sensible osteoblastic deposition pattern that would be more amenable to histogram or feature based segmentation. In applications such as finite element analysis,
however, it is not necessary to differentiate newly formed bone from osteoblastic tumour, as they possess the same mechanical properties.

A customized µMR coil was necessary to achieve sufficient resolution in imaging the bony vertebral structure. With a quick change of parameters, the coil was also capable of generating robust soft tissue images to allow clear differentiation between bone marrow and osteolytic tumour tissue within the bone.

Except for the initial manual alignment of the images to the global axes, the remainder of the algorithm was executed with minimum user interaction, making it a precise and repeatable algorithm. Rigid registration was shown to be a fast and easy way to register multimodal vertebral images. It was able to accurately register the µCT and µMR volumes despite the resampling carried out prior to the registration.

Bone µMRI was observed to be required as a localizer for accurate registration of the µCT to the contrast µMRI, however it did not yield sufficient resolution and contrast (in comparison to the µCT data) to be used for accurate quantification of the bony structure. Future work post-processing such images may be undertaken in an effort to obtain similar bone and soft tissue segmentation using µMRI alone.

Achieving sufficient resolution on images remains a concern when attempting to accurately quantify micro-structure. We have previously shown that sufficient resolution for accurate segmentation/analysis of micro-structure of 21 day old rnu/rnu rats is $8.725 \times 8.725 \times 8.725 \mu m^3$ [15]. Upsampling of µCT images prior to trabecular segmentation can provide sufficient contrast from lower resolution scans ($14 \times 14 \times 14 \mu m^3$). In other applications, however, such as finite element and micro finite element analysis (FEA and µFEA), lower resolutions have been shown to be sufficient to yield accurate analysis of vertebral biomechanical behaviour under load [16, 17].

The highly automated registration and segmentation algorithm presented in this study has demonstrated its ability to accurately quantify skeletal structure and metastatic involvement in the spine. Such information could be used to develop mathematical models of vertebrae in order to analyze strain patterns generated through finite element modeling or the comparison of loaded and unloaded 3D images, to estimate ultimate failure loads and to predict fracture locations.
Moreover, it could readily be used to analyze micro-structural parameters of metastatic vertebra in preclinical applications looking at quantitative evaluation of new and existing treatments aimed at spinal lesions.

4.6 References


5.1 Abstract

The metastasis of cancer to bone most frequently occurs in the spine [1]. Spinal metastases can result in significant declines in patients’ quality of life [1]. Spinal metastases can appear as osteolytic (bone resorping), osteoblastic (bone depositing) or mixed osteolytic/osteoblastic. Robust quantitative methods to evaluate the impact of complex patterns of spinal metastasis on the biomechanical stability of the spine remain limited. The objective of this work was to evaluate the ability of 2D and 3D image-based analytical methods to characterize the structural stability of the metastatic spine.

\(\mu\)CT and \(\mu\)MR images were acquired of second lumbar vertebrae from 15 rnu/rnu rats: 5 healthy, 5 with osteolytic lesions secondary to MT1 human breast cancer cell injection, and 5 with mixed osteolytic/osteoblastic lesions secondary to Ace-1 canine prostate cancer cell injection. The structural integrity of the vertebrae were analyzed using 2D structural rigidity analyses [2], a 3D image-based strain measurement algorithm [3] and specimen specific continuum finite element modeling. All samples were then loaded to failure under axial compressive loading. Pearson correlations were employed to evaluate the ability of each of these methods to predict experimental yield loads and to compare image-based strain measurement Finite Element Analysis (FEA) derived strains.

The image-based strain measurement algorithm demonstrated high strain regions at the growth plates and at areas with osteolytic destruction. Significant negative correlations were found between mean strains and experimental yield load in the healthy and osteolytic groups (\(r = -0.809, -0.832\) respectively) as well as for median strain in the osteolytic group (\(r = -0.84\)).
While the structural rigidity based predicted failure load did not correlate with experimental yield load for the combined group, significant correlations were found with the experimentally measured yield load results in the mixed osteolytic/osteoblastic group \( (r = 0.946) \) and a similar trend was observed in the osteolytic group \( (r = 0.788, p = 0.057) \). The absolute value of structural rigidity based yield loads were on average 1.5 to 2 times the experimental results.

Qualitatively, good agreement was observed between the strain patterns in the vertebral bodies using the image-based strain measurement and continuum finite element analyses (FEA) with a significant correlation \( (p < 0.05) \) found between the mean strains in the healthy group \( (r = 0.893) \). The FEA based 10\textsuperscript{th} percentile strains in the combined group showed a significant correlation \( (p < 0.05) \) with experimental yield load \( (r = -0.502) \).

Large structural differences leading to varied modes of failure and differences in material properties in the healthy, osteolytic and mixed osteolytic/osteoblastic vertebrae may limit the ability of structural rigidity and FEA to accurately represent the moduli and failure load, in comparison to studies focused on simulated defects [4, 5]. The complexity of experimental loading through the intervertebral discs may have introduced higher variability into the load to failure measurements which may not have been represented in the deformation fields applied to the image-based strain and FEMs [3]. Lower strain resolution based on the image-based strain approach may also limit quantitative comparisons with higher resolution FE results. Quantitative correlations may also be improved through improved modeling and material property representation in the growth plates and metastatically involved tissue in the FEMs. Yet qualitatively, good agreement between these two 3D image-based methods motivates further development of these 3D techniques in non-destructive evaluation of structural stability of the metastatic spine.

5.2 Introduction

Skeletal metastases most commonly affect the vertebral column [1]. Spinal metastases can present as osteolytic, osteoblastic or with a mixed osteolytic/osteoblastic pattern of involvement. Non-invasive quantitative evaluation of the effect of spinal metastases on biomechanical stability of vertebral column may aid in accurate management of patients with established spinal metastases. Specimen specific \( \mu \)-imaging based analysis allows for the representation of complex patterns of bone and tumour that occur in the metastatic spine. Continuum level finite element
(FE) modeling [3, 6, 7, 8] and image-based strain registration [3] have been used to quantify biomechanical effects of osteolytic metastatic involvement in the spine. However, to date, limited analysis has been conducted on vertebrae with more complex mixed metastatic disease.

A µCT based strain measurement algorithm has previously been developed as a non-destructive method to quantify the strain patterns of metastatic rat vertebrae under load. This algorithm uses registration of an unloaded µCT scan of a rat vertebra to a µCT scan of the same vertebra under an axial compressive load to quantify deformation and strain within the vertebral body [3]. This method has been applied to demonstrate structural effects of local and systemic therapies in metastatic rat vertebra [9]. Robust validation of the image-based strain measurement algorithm has been performed including comparisons with finite element analysis (FEA) of a rat tail vertebra [3].

Two-dimensional CT-based structural rigidity has also been used to assess mechanical stability in human whole vertebrae with metastatic involvement [2]. This approach defines axial/bending rigidity, the resistivity of a material to axial loading/bending, in every 2D axial CT slice as a function of elastic modulus (E) based on apparent density ($\rho_{\text{app}}$). Composite beam theory using axial and bending rigidities is then used to predict failure load of a given material [2]. Cory et al. have later determined density modulus relationships to allow adaptation of this 2D method to µCT-based analyses of rat bones [10]. The CT based and µCT based structural rigidity algorithms have been used in a number of studies to quantify mechanical stability in human and rat bones [2, 5]. The structural rigidity method was observed to be 100% sensitive, and 44% - 70% specific, in prediction of fracture risk in patients [2]. Their prediction of rat failure torque was also observed to correlate well with mechanical testing results ($r^2 = 0.85$) [5].

FEA is a powerful tool that has been used previously in evaluation of mechanical stability in metastatic spine [3, 4, 6, 7, 8]. Metastatic modeling has been limited, however, to osteolytic involvement and, in general, has focused on simulated defects with regular geometries [4]. Poroelastic parametric FE modeling has been used to evaluate the stability of metastatically involved human spinal motion segments [4], and more recent work has demonstrated the importance of specimen specific modeling and the application of CT intensity based material properties in determining strains in metastatically involved vertebrae [11].
The objective of this work is to compare the ability of 2D structural rigidity analyses, multimodal continuum-level FE modeling and µCT image registration in the evaluation mechanical stability of healthy and metastatically involved rat vertebrae. It is hypothesized that these methods could be used to accurately demonstrate the biomechanical stability of rat vertebrae regardless of the presence or pattern of the metastatic disease. Non-invasive image-based techniques that accurately reflect vertebral integrity are essential in better predicting fracture risk and treatment effect in both clinical and preclinical scenarios, and could ultimately improve the management and outcome of patients with established spinal metastases.

5.3 Materials and Methods

5.3.1 Animal Model

Fifteen 4 week old rnu/ rnu rats were utilized for this study. The animals were divided into 3 groups based on pathologic involvement of the spine: healthy, osteolytic metastases, and mixed osteolytic/ osteoblastic metastases. Osteolytic lesions were developed via intracardiac injection of MT1 human breast cancer cells [12], and mixed osteolytic/ osteoblastic lesions were developed via intracardiac injection of Ace-1 canine prostate cancer cells [13]. Three weeks post injection the presence of vertebral metastases was confirmed via bioluminescent imaging prior to sacrifice. Motion segments composed of the first to third lumbar vertebral levels were then dissected for further analyses.

5.3.2 Image Acquisition

5.3.2.1 Micro Magnetic Resonant Imaging (µMRI)

µMRI images were acquired of all specimens with metastatic involvement (n=10) initially post sacrifice, as excised bone loses its magnetic resonant capabilities quickly due to dehydration. The excised motion segments were immersed in agarose gel within a 15ml falcon tube. T1 and T2 weighted µMR scans were acquired at a 60µm³ isotropic voxel size in a 7 tesla preclinical Biospec system (Bruker, Ettlingen, Germany). The images were acquired using a custom built quadrature birdcage coil (Stark Contrast MRI Research, Germany) tuned to the 1H frequency (~300.3 MHz).
The T1-weighted µMRI was utilized to accentuate bone ultrastructure enabling accurate µCT and µMRI registration of the vertebrae. Data acquisition parameters included an echo time (TE) of 7.6 ms, repetition time (TR) of 500 ms, RARE factor of 2, and 3 signal averages. Using these parameters, the boundaries of the trabecular structure were clearly visible, though bone marrow and osteolytic tumor could not be visualized distinctly (Fig. 23b).

The T2-weighted µMRI images were acquired to accentuate soft tissue contrast and clearly differentiate lower intensity bone marrow from higher intensity tumor tissue (Fig. 23c). Data acquisition parameters included a TE of 48 ms, TR of 3000 ms, RARE factor of 4, and 1 signal average.

5.3.2.2 Micro Computed Tomography

5.3.2.2.1 Unloaded Configuration

Each sample (n=15) was µCT scanned in an unloaded configuration adjacent to bone calibration phantoms (Skyscan Corp) at a 14\(\mu\)m\(^3\) isotropic voxel size. This was done using an x-ray source at 90 mA and 80 kV, with 900 views covering 360° of rotation (GE Explore Locus, General Electric Co.). GE commercialized software was further used to reconstruct the acquired frames into a µCT volume (Explore Reconstruction Utility, General Electric Co.).

5.3.2.2.2 Loaded Configuration

The L1 and L3 vertebrae of all spinal motion segments were then potted in bone cement and placed into a calibrated customized axial loading device (Fig. 19) [9]. The device allowed for the application of known axial compressive loads to the L2 vertebra through the intervertebral discs. µCT images of the samples were acquired with the L2 vertebra under static axial loads (45N to 65N). The applied axial loads were chosen to yield sufficient deformation to produce detectable strain in the L2 vertebra (based on the image registration algorithm [3]) without causing fracture. As such, the loading applied was varied based on qualitative assessment of the level of metastatic destruction visible within the unloaded scans. As in the unloaded images, the µCT scans were acquired adjacent to calibration phantoms at a 14\(\mu\)m\(^3\) isotropic voxel size (Explore Reconstruction Utility, General Electric Co.).
**Figure 19.** Schematic and photograph of the tubular axial compressive loading device. Load is applied by turning the screw and is measured by the load cell. The vertebrae adjacent to the level of interest were fixed to the loading device with bone cement (polymethyl methacrylate). Force was applied to the vertebra of interest via the adjacent vertebral bodies and intervertebral discs.
5.3.3 Image-based Strain Measurement

All scans were aligned to a global axis in Amira visualization software (AmiraDev). Due to computational cost limitations, only the vertebral body of the scans were considered for this part of the study. The cropped vertebral body from the unloaded scan was then registered to the corresponding loaded vertebral body using a rigid registration (AmiraDev). For this registration a quasi newton optimizer was utilized with initial and final step sizes of $10^3 \times$ voxel size and $10^7 \times$ voxel size respectively. A normalized mutual information metric was implemented to account for intensity differences between the scans.

The initial rigid vertebral registration was then improved using a multi-resolution deformable registration algorithm, implemented as plug in to Amira from the Insight Tool Kit data base [3]. The deformable registration started by dividing the unloaded scan into 8 regions. Each of these regions was then registered to the corresponding region in the loaded scan using an affine transform which allowed for translation, rotation, scaling and shearing. Each of the 8 regions was subsequently divided into 8 smaller subregions and registered to the corresponding subregion in the loaded scan for 2 more iterations to achieve the final level of analysis (Fig. 20) [9].

The result of the final registration was the overall deformation field, which was used to calculate the strain in each of the image blocks (Equation set 7) [3, 9].

$$\tilde{\mathbf{A}}(x, y, z) = \tilde{T}P(x, y, z) - P(x, y, z)$$

$$e = \frac{1}{2} \left( \nabla \tilde{\mathbf{A}}^T + \nabla \tilde{\mathbf{A}} \right)$$  \hspace{1cm} (7)

The transformation matrix (T) based on this final level of analysis was used to calculate the displacement matrix (A).

The axial strain values calculated from spatial displacement of the µCT voxels were quantified through the calculation of mean, median, 10th percentile, and 90th percentile strains with negative values corresponding to compression.
Figure 20. Scheme of the multiresolution image-based strain measurement algorithm [9]. The loaded and unloaded vertebral body images are first registered using subvoxel interpolation, and are then deformably registered using the multiresolution deformable registration algorithm which yields the deformation field and the average strain in each image block.
5.3.4 2D Assessment of Vertebral Stability

5.3.4.1 Image Segmentation

The µCT images of whole L2 vertebra of the unloaded scans were segmented using atlas-based demons deformable registration and levelset curvature evolution [14]. The whole vertebrae were manually aligned to a global axis (amiraDev). A manually segmented atlas was then deformed using multiresolution demons deformable registration algorithm to register to the target vertebra. The output of this deformation field was then masked around the segmentation boundaries of the atlas yielding the segmentation of the whole target vertebra. The segmentation boundaries were further smoothed while maintaining the curvature, particularly around the posterior elements. The bony structure was then segmented using intensity based threshold equal to the mean value of the histogram of the whole vertebra (Fig. 21).

Figure 21. Representative axial slice of the L2 rat vertebrae with the bony structure segmented using the automated segmentation algorithm.
5.3.4.2 2D Structural Rigidity

The segmented whole vertebrae were converted to equivalent density ($\rho_{EQ}$) images using the data obtained from the calibration phantoms. The elastic modulus ($E$) was calculated for each voxel using the relationship derived by Cory et al. [10] (equation 8).

$$E = 8362.8 \ (\rho_{EQ})^{2.56}$$  \hspace{1cm} (8)

The axial rigidity ($EA$) was further calculated for each slice based on the relationship derived by Snyder et al [2] (equation 9).

$$EA = \int E(\rho_{EQ}) da$$  \hspace{1cm} (9)

The design of the tubular loading device limited loading to the axial direction and, consequently, the failure load was defined as a product of the yield strain and the axial rigidity (equation 10).

$$F_z = \varepsilon \times EA$$  \hspace{1cm} (10)

Where $F_z$ represents the failure load, $\varepsilon$ is the theoretical yield strain and $EA$ represents the axial rigidity. Yield strain for our samples was chosen to be 0.96 % [15]. Once $F_z$ was calculated for all axial slices, the lowest value of the $F_z$ was identified as the predicted failure load.
Yield strain ($\varepsilon$) for our samples was chosen to be 0.96 % (Fig. 22) [15]. Once $F_z$ was calculated for all axial slices, the lowest value of the $F_z$ was identified as the predicted failure load.

\begin{align*}
\text{Elastic Modulus:} & \quad E = 3362.8 (\rho_{EQ})^{2.56} \\
\text{Axial Rigidity:} & \quad EA = \int E(\rho_{EQ}) \, da \\
\text{Yield Load:} & \quad F_z = \varepsilon \times EA
\end{align*}

\textbf{Figure 22.} Schematic of the structural rigidity based prediction of failure load. $da$ represents a single pixel in an axial slice with dimensions $14 \times 14 \, \mu m$. The density of each pixel is converted to elastic modulus ($E$) which in turn is used to calculate axial rigidity ($EA$) and predict failure load ($F_z$) [2].

\textbf{5.3.5 Continuum Finite Element Analyses}

\textbf{5.3.5.1 Image Segmentation for Healthy Rats}

The healthy L2 whole vertebrae were segmented using atlas-based demons deformable registration and levelset curvature evolution algorithm [14]. The whole vertebrae were first manually aligned to a global axis (AmiraDev). The manually segmented atlas was then used to segment the whole vertebrae using demons deformable registration (Insight Tool Kit). The segmentation boundaries were further smoothed out and contracted using another iteration of the
levelset curvature evolution. Separate segmentation of the cortical shell was necessary to reduce edge effects in material property assignments required for the finite element analyses.

5.3.5.2 Image Segmentation for Metastatic Rats

5.3.5.2.1 Rigid Registration

The unloaded μCT scans and the T1 and T2 weighted μMR images were resampled to 35μm³ isotropic voxel size to enable accurate multimodal registration. The μCT scans and T2 weighted μMR images were registered independently to the T1 weighted μMR images using a rigid registration to ensure accurate 3D overlap of all three image sets. A quasi newton optimizer with initial step size of $10^3 \times$ voxel size and a final step size of $10^7 \times$ voxel size was used for this registration. A normalized mutual information metric was utilized to account for intensity variations between the μCT and μMR scans (Fig. 23).

![Figure 23](image)

**Figure 23.** (a) μCT image, (b) T1 weighted μMR image, (c) T2 weighted μMR image of the L1-L3 motion segment with mixed osteolytic/osteoblastic metastatic involvement following rigid registration.
5.3.5.2.2 Multimodal Image Segmentation

The whole vertebrae from the rigidly registered μCT scans were segmented using atlas-based demons deformable registration and level set curvature evolution. The cortical shell was segmented out using an additional iteration of levelset curvature evolution (Fig. 24a) (Insight Tool Kit, AmiraDev). The whole vertebra segmentation boundaries from the μCT data set were masked around the T2 weighted μMR image to segment out the ROI for each vertebra. An intensity-based threshold equal to the mean of the segmented region plus half of one standard deviation (μ + σ/2) was chosen by observation to define the osteolytic tissue intensity (Fig. 24b).

Figure 24. Segmentation of a vertebra with mixed osteolytic/osteoblastic metastatic disease: (a) the trabecular centrum and cortical shell on an axial μCT slice and (b) the osteolytic tumour based on an equivalent T2 weighted μMR axial slice.
5.3.5.3 Mesh Generation

Refined and simplified triangular surfaces were generated from the segmentations of the vertebral body, cortical shell and tumour volumes. Tetrahedral meshes were automatically generated from the surfaces in AmiraDev. The number of tetrahedral elements was optimized to ~60000. The meshes were then imported into Abaqus 6.10 (Simulia, Pawtucket, RI) (Fig. 25a).

5.3.5.4 Material Properties

Bone elements were assigned elastic isotropic material properties with a Poisson’s ratio of 0.33 [16]. The cortical shell was considered as a separate material, based on the work of Pahr et al., and assigned a constant elastic modulus (E) of 15GPa [16]. The equivalent density values were derived for the other bone elements based on the µCT intensity values. The µCT intensity values were converted to density using the density-intensity relationship information derived from the calibration phantoms within each scan. The equivalent density was then converted to elastic modulus based on the relationship derived by Cory et al. for rat bone [10], (equation 8).

\[ E = 8362.8 (\rho_{EQ})^{2.56} \]  (8)

The osteolytic tumour was modelled as an elastic isotropic material with a Poisson’s ratio of 0.4995 and an elastic modulus of 0.0036MPa [17]. Poroelastic material properties were not in the model based on the quasi-static loading scenario [17].

5.3.5.5 Boundary Conditions

Displacement boundary conditions were applied to the end plates and the facets of each vertebra. The displacement vectors applied at each node were obtained from the registration of the unloaded and loaded µCT scans (Fig. 25b).
Figure 25. (a) The automatically generated tetrahedral mesh, (b) The displacement boundary conditions applied to the endplates and facets of the finite element model.

The final mesh was analyzed in Abaqus 6.10 using a high performance Opteron Cluster Supercomputer (Centre For Computational Biology High Performance Facility, Toronto).

Upon completion of finite element analyses, the result file was visualized in Abaqus CAE 6.10. The strain patterns were qualitatively analyzed and compared to the results obtained from image-based strain measurement method. Mean, median, 10th percentile and 90th percentile strain values were also quantified for the whole vertebrae as well as the vertebral bodies alone. Average values for the maximum principle strain and stress values and Von Mises stress values were also calculated for the whole vertebrae.

5.3.6 Destructive Mechanical Testing

The first and third lumbar vertebrae were potted in bone cement and the L2 vertebra was loaded in axial compression to failure through the intervertebral discs at a loading rate of 1mm/min on an MTS servo-hydraulic testing system [18] (MTS Bionix 858, Eden Prairie, USA). Failure load was identified as the value at the end of the linear region of the load vs. deformation plot, which was monitored during loading (Fig. 26).
5.3.7 Statistical Analyses

Normality of the distribution of the quantitative measures was confirmed using 1 sample kolmogorov-Smirnov (KS) tests (SPSS statistical analysis software). Pearson correlation coefficients were used to quantitatively compare the mean, median, 10\textsuperscript{th} percentile and 90\textsuperscript{th} percentile vertebral body strain values obtained from image-based strain measurement with equivalent measures obtained from the continuum finite element analyses. The comparison was focused to vertebral body as the image-based strain measurement algorithm was performed solely on the vertebral body.

Pearson correlations were also used to evaluate the relationship between the image-based measures (the rigidity based predicted yield load, the quantitative strain values obtained from the image-based strain measurement algorithm and the quantitative strain and stress values obtained from continuum finite element analyses of the whole vertebrae) and the experimentally measured yield load. Results were obtained grouping all 15 samples and subsequently considering each group separately (healthy, osteolytic and mixed osteolytic/ osteoblastic).

Figure 26. Load- deformation plot of a spinal motion segment tested to failure under axial compressive loading at a rate of 1mm/min.
Post hoc power analyses were carried out to determine the probability of observing a moderate Pearson correlation coefficient of 0.7 for the combined and individual groups [19]. This Pearson correlation coefficient was chosen based on an earlier study evaluating the ability of structural rigidity analyses to predict experimental yield load in metastatic vertebrae [2].

5.4 Results

Qualitatively, the image-based strain measurement method was able to resolve the strain patterns throughout the healthy and metastatically involved vertebral bodies. As expected, the results showed high strains around areas of osteolytic destruction in the metastatic samples as well as areas of high strain at the growth plates of all vertebrae (Fig. 27). Quantitatively, the strain values for the metastatic samples were higher than those found in the healthy vertebral bodies suggesting a reduction in mechanical stability.

Figure 27. Qualitative first principal strain patterns (a) a vertebral body with mixed osteolytic/osteoblastic metastases (osteolytic metastases indicated), and (b) a healthy vertebral body. Red indicates a compressive strain $\geq 7\%$ and higher and blue indicating low compressive strain (or tension).
Quantitatively, in considering all samples, only a relationship between median strain and experimental yield load demonstrated a trend toward a weak negative correlation ($r = -0.405$, $p = 0.067$). A significant negative correlation between the experimental yield load and the median strain was found separately in the healthy and osteolytic groups ($r = -0.809$, -0.832 respectively). A significant negative correlation was also observed between the mean strain in the healthy group and the experimental yield load ($r = -0.840$). The relationship between 10th percentile strain and experimental yield load in the osteolytic group demonstrated a trend toward a negative correlation but was not significant ($r = -0.749$, $p = 0.072$). No other correlations were detected (Table 3).

Table 3 Pearson correlation coefficients (R), and the respective p-values of the bivariate correlations between quantitative strain measures obtained using image registration and the experimental yield load (significant relationships, $p<0.05$, are highlighted in bold text)

<table>
<thead>
<tr>
<th>Analysis Group</th>
<th>Strain Quantity</th>
<th>Combined</th>
<th>Healthy</th>
<th>Osteolytic</th>
<th>Mixed Osteolytic/Osteoblastic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>-0.349</td>
<td>0.101</td>
<td>-0.270</td>
<td>0.33</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>-0.405</td>
<td>0.067</td>
<td><strong>-0.809</strong></td>
<td><strong>0.049</strong></td>
</tr>
<tr>
<td>10th Percentile</td>
<td></td>
<td>-0.005</td>
<td>0.493</td>
<td>-0.104</td>
<td>0.434</td>
</tr>
<tr>
<td>90th Percentile</td>
<td></td>
<td>-0.340</td>
<td>0.108</td>
<td>-0.363</td>
<td>0.274</td>
</tr>
</tbody>
</table>

The rigidity-based predicted yield load demonstrated significant ($p<0.01$) positive correlation with the experimental yield load in the mixed metastatic group ($r=0.946$) and a trend towards positive correlation with the experimental yield load in the osteolytic group ($r=0.788$, $p=0.057$). We did not observe any other significant correlations (Table 4). The absolute value of the predicted yield load was, however, observed to be 1.5 to 2 times higher than the experimental yield load.
Table 4 Pearson correlation coefficients (R), and the p-value of the bivariate correlations between rigidity based predicted yield load and the corresponding experimental yield load (significant relationships, p<0.05, are highlighted in bold text)

<table>
<thead>
<tr>
<th></th>
<th>Combined</th>
<th>Healthy</th>
<th>Osteolytic</th>
<th>Mixed Osteolytic/Osteoblastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>0.195</td>
<td>0.593</td>
<td>0.788</td>
<td><strong>0.946</strong></td>
</tr>
<tr>
<td>P</td>
<td>0.243</td>
<td>0.146</td>
<td>0.057</td>
<td><strong>0.007</strong></td>
</tr>
</tbody>
</table>

Qualitatively, the strain patterns obtained from the finite element analyses matched well with the strain patterns obtained from image-based strain measurement algorithm (Fig. 28). Quantitatively, a significant positive correlation was found in comparing results for mean vertebral body strain generated based on the image-based strain measurement and the FEA methods in the healthy group (r=0.893). No other significant correlations were observed in any of the quantitative strain values in any of the study groups (Table 5).

**Figure 28.** First principal strain patterns in a vertebral body with mixed osteolytic/osteoblastic metastatic involvement generated via (a) image-registration, (b) FEA and (c) FEA with osteolytic tumour elements removed (bone only). The strain patterns results based on the two techniques yield similar patterns as shown in the 2D coronal slice.
Table 5 Pearson correlation coefficient (R), and the p-value of the bivariate correlation between quantitative strain measures obtained using FEA and image-based strain measurement (significant relationships, p<0.05, are highlighted in bold text)

<table>
<thead>
<tr>
<th>Analysis Group</th>
<th>Strain Quantity</th>
<th>Combined</th>
<th>Healthy</th>
<th>Osteolytic</th>
<th>Mixed Osteolytic/Osteoblastic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>Mean</td>
<td>0.309</td>
<td>0.131</td>
<td>0.893</td>
<td>0.021</td>
<td>0.305</td>
</tr>
<tr>
<td>Median</td>
<td>0.299</td>
<td>0.140</td>
<td>0.03</td>
<td>0.481</td>
<td>0.467</td>
</tr>
<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt; Percentile</td>
<td>0.2</td>
<td>0.238</td>
<td>0.663</td>
<td>0.111</td>
<td>0.620</td>
</tr>
<tr>
<td>90&lt;sup&gt;th&lt;/sup&gt; Percentile</td>
<td>0.216</td>
<td>0.220</td>
<td>0.628</td>
<td>0.128</td>
<td>0.387</td>
</tr>
</tbody>
</table>

Table 6 Pearson correlation (R) coefficient, and the p-value of the bivariate correlation between quantitative strain measures obtained using FEA and the experimental yield load (significant relationships, p<0.05, are highlighted in bold text)

<table>
<thead>
<tr>
<th>Analysis Group</th>
<th>Strain Quantity</th>
<th>Combined</th>
<th>Healthy</th>
<th>Osteolytic</th>
<th>Mixed Osteolytic/Osteoblastic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>Mean</td>
<td>-0.331</td>
<td>0.114</td>
<td>-0.311</td>
<td>0.305</td>
<td>-0.4</td>
</tr>
<tr>
<td>Median</td>
<td>-0.387</td>
<td>0.154</td>
<td>-0.249</td>
<td>0.343</td>
<td>-0.635</td>
</tr>
<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt; Percentile</td>
<td>-0.502</td>
<td>0.028</td>
<td>-0.123</td>
<td>0.422</td>
<td>-0.354</td>
</tr>
<tr>
<td>90&lt;sup&gt;th&lt;/sup&gt; Percentile</td>
<td>-0.166</td>
<td>0.277</td>
<td>-0.282</td>
<td>0.323</td>
<td>-0.506</td>
</tr>
<tr>
<td>Max Prin. Strain</td>
<td>-0.3</td>
<td>0.139</td>
<td>-0.286</td>
<td>0.311</td>
<td>-0.241</td>
</tr>
<tr>
<td>Max Prin. Stress</td>
<td>-0.197</td>
<td>0.241</td>
<td>-0.528</td>
<td>0.180</td>
<td>-0.41</td>
</tr>
<tr>
<td>Von Mises Stress</td>
<td>0.058</td>
<td>0.419</td>
<td>-0.249</td>
<td>0.343</td>
<td>0.074</td>
</tr>
</tbody>
</table>
In comparing the results of the FEA to the experimental yield load, a significant negative correlation was found with 10\textsuperscript{th} percentile strain in the combined group ($r = -0.502$). A similar trend was found in comparing the maximum principle stress in the mixed osteolytic/osteoblastic group to the experimental yield load ($r = -0.698$, $p=0.095$). No other significant correlations were observed between FEA based measures and experimental yield load (Table 6).

Based on the post hoc power calculation the probability of observing a significant correlation with a Pearson coefficient of 0.7 was measured to be 94 \% for the combined group and 33\% for the individual groups.

5.5 Discussion

The objective of this work was to compare the ability of three computational based approaches, 3D µCT image registration, 2D structural rigidity analyses and 3D multimodal continuum-level FE modeling, in the evaluation of mechanical stability of healthy and metastatically involved rat vertebrae. Overall, the ability of each of the three methods to represent experimental load to failure in both healthy and metastatically involved vertebrae was limited.

The image, based strain measurement algorithm demonstrated high strains at the growth plates in all vertebrae due to the relative compliance of this soft tissue, as noted in previous application of this algorithm [3, 9]. As expected, the quantitative compressive strain values were higher in the metastatic cases in comparison to the healthy vertebral bodies. Quantitative strain values did not correlate significantly with experimental yield load in the combined group, but significant correlations were observed between strains in the healthy (median) and osteolytic (mean and median) groups and the experimental yield load. Incremental loading within a µCT scanner, may yield a better representation of loading applied to the vertebral body immediately prior to failure and improve correlations of image-based strain with failure load.

Image registration was able to provide full field deformation and strain information in the vertebrae without any assumptions as to force models or applied loading. However, the image-based strain measurement algorithm was limited in its application to the vertebral body alone. The large size of the files, due to the high resolution needed to resolve the strains, limits the region of interest that can be accommodated in the analyses. Larger models incorporating the whole vertebrae were unable to run. As well the spatial resolution of the deformation and strain
results is limited based on the multi-resolution deformable registration algorithm utilized, which requires a sufficient pattern (i.e. trabecular network) to be matched between images. Image registration can only calculate average strain for regions that are individually registered. As such, the limited strain resolution tends to smooth the strain field, moving maximums and minimums towards the centre of the range [3].

Previously, structural rigidity analyses have demonstrated moderate correlations in predicting failure based on mechanical testing of single cadaveric vertebrae with simulated and naturally occurring osteolytic metastases. [20, 21]. Post fracture stability of spinal motion segments with simulated metastatic defects yielded more modest correlations with axial rigidity measures [22]. Moreover, structural rigidity based analysis yielded excellent sensitivity with more limited specificity (44% to 70%) when applied to non-invasively predict fracture of vertebrae with metastatic involvement in a clinical scenario [2]. Structural rigidity analyses have also been applied in the non-invasive evaluation of failure torque in rat long bones, yielding a strong correlation between predicted and experimental results ($r^2 = 0.85$) [5].

In contrast, in our study, when comparing the 2D structural rigidity predicted yield load to the experimental yield load, no significant correlations were observed in the combined group. The correlations, however, were considerably improved when each group was analyzed separately. As such, it may be that metastatic disease may change the failure behavior of the vertebrae, limiting the ability of a single structural rigidity – yield load relationship to represent failure risk. Additional factors related to the 3D trabecular structure (i.e. the relationship between trabecular length and propensity to bending or buckling failure) may be required to yield a more general relationship. As well, the absolute value of the structural rigidity predicted yield load was considerably higher than the experimental yield load. Improvement of the density-modulus relationship specific to anatomical site or the type of bone disease present may be required to allow accurate predictions of yield load values.

The generation of the finite element models analyzed in this study utilized a novel multi-step image-based approach. The multimodal imaging, segmentation and registration algorithms allowed for construction of vertebral models representative of the complex distribution of tumour in bone that results in metastatic disease. Utilization of the deformation field obtained from the loaded-unloaded µCT-based image registration as the displacement boundary condition for the
FEA enabled a consistent simulation of the loading scenario between the FEA and image-based strain techniques. Moreover, this approach allowed simulation of non-uniform loading that occurs *in vivo* via the intervertebral disc and facets without the computational time and expense required to model complex soft tissue structures and contact. This is increasingly important as the size of models generated through high resolution µ-imaging continues to grow along with the computational costs required to analyze them. It also enables more accurate modeling of complex loading scenarios without the need for assumptions about the material properties/behaviour of soft tissue structures or load transfer.

Qualitatively, there was good agreement between the strain patterns obtained from FEA and the image-based strain measurement algorithm in all 15 samples. Quantitatively, only the mean strain values in the healthy vertebral bodies from the two analysis techniques demonstrated a significant correlation. The finite element analyses did not have the same computational limitations as the image-based strain measurement algorithm and thus yielded strains at a higher resolution, limiting the direct comparison of strain values between the two methods. The mismatch may also be due to high strains present in the osteolytic tumour elements. The inability to detect significant correlations could also be caused by inaccuracies of the density-modulus relationship used to assign material properties in the FEMs. Quantitative correlations may also be improved through improved modeling and material property representation in the growth plates in the FEMs.

In comparing the FEA results for the whole vertebrae with mechanical testing, we observed a significant correlation between the 10th percentile strain and the experimental yield load in combined group as well as a trend towards a significant correlation between maximum principle stress and the experimental yield load in the mixed osteolytic/osteoblastic group. The inability of the FEA to yield strong correlations with the mechanical testing results may again be due to imperfections in the material property assignments or a mismatch between the µCT based loading conditions and the loading conditions applied in the experimental testing on the MTS.

Furthermore, post hoc power analyses demonstrated sufficient power for detection of moderate correlations in the combined group. This suggests that a linear relationship may not exist between the image-based parameters and experimental yield load for the combined group of healthy, osteolytic, and mixed osteolytic/osteoblastic samples. As described earlier, this may be
due to structural and/or material differences between groups which may alter their ultimate failure behavior. This is supported by observations of significant correlations between some image-based parameters and mechanical testing results (in spite of low sample sizes) when the groups were analyzed separately.

Mechanical testing of rodent spinal motion segments is challenging. As such, many studies have conducted load to failure testing on isolated vertebrae [18, 23]. This study attempted to acquire load to failure data of spinal motions segments in order to better represent physiologic loading as applied through the adjacent intervertebral discs. The scheme of the mechanical testing as loading the L2 vertebra through the adjacent intervertebral discs, ensured close correspondence to the design of image-based strain measurement and continuum finite element analyses methods. This more complex loading may introduce higher variability into the load to failure measurements as shear of the intervertebral discs, which may have occurred during load to failure testing, would alter the boundary conditions. This fact, however, would not have been represented in the image-based deformation fields.

Parametric FE modeling has demonstrated the importance of tumour size, bone density and the magnitude of load applied to the spine in predicting burst fracture in vertebrae with simulated osteolytic defects [4]. In our specimen specific FE models, accurate representation of tumour involvement was achieved through µMR based segmentation, heterogeneous distributions of bone density were included based on µCT intensity and calibration phantoms [10], and registration of loaded and unloaded µCT images yielded accurate boundary conditions representing loading through the intervertebral discs and facets. Yet, large structural differences in the healthy, osteolytic and mixed osteolytic/osteoblastic vertebrae may lead to varied modes of failure. As well, differences in material properties may limit the ability of structural rigidity and FEA to accurately represent moduli and failure loads, in comparison to studies focused on simulated defects [4, 20]. The use of spinal motions segments rather than isolated vertebrae introduces much complexity to experimental loads applied to the central vertebrae. Although more physiologic, this loading may have introduced higher variability into the yield load measurements, which may not have been represented in the deformation fields applied to the image-based strain and FEMs at reduced strain levels [3].
Qualitatively, good agreement between the 3D image-based registration and FE methods motivates further development of these 3D techniques in non-destructive evaluation of structural stability of the metastatic spine. The lower resolution strain results, based on the image-based strain approach, may limit quantitative comparisons with higher resolution FE results. Quantitative correlations may be enhanced through improved modeling and material property representation in the growth plates and metastatically involved tissue in the FEMs. Ultimately, the availability of 3D non-destructive techniques that can quantify structural stability may improve the efficiency (reduce sample sizes) of preclinical studies and allow temporal mechanical evaluation of tumour growth and treatment effects over time.

5.6 References


CHAPTER 6

Non-Destructive Evaluation of the Effects of Combined Bisphosphonate and Photodynamic Therapy on Bone Strain in Metastatic Vertebrae Using Image Registration

6.1 Abstract

Skeletal metastases most frequently affect the vertebral column and may lead to severe consequences including fracture. Clinical management of skeletal metastases often utilizes a multimodal treatment approach, including bisphosphonates (BPs). Previous work has demonstrated the synergistic potential of photodynamic therapy (PDT) in combination with BP in treating osteolytic disease through structural, histologic, and destructive mechanical testing analyses. Recent work has developed and validated image-based methods that may be used to non-destructively determine mechanical stability in whole bones, and enable their use for additional (i.e. histologic) analysis. In this work we use an intensity-based 3D image registration technique to compare the strain patterns throughout untreated control and BP + PDT treated rnu/rnu rat spinal motion segments with osteolytic metastases. It was hypothesized that the combination treatment will reduce average and maximum strain values and restore the pattern of strain to that of healthy vertebrae. Mean, median, and 90th percentile strains in the control group were significantly higher than the treatment group. High strain areas in both groups were observed around the endplates; in the control group, large areas of high strains were also observed around the lesions and adjacent to the dorsal wall. Absence of high strains adjacent to the dorsal wall (similar to healthy vertebrae) may correspond to a reduced risk of burst fracture following BP + PDT therapy. This study demonstrates the application of non-destructive image analysis to quantify the positive mechanical effects of combined BP + PDT treatment in the metastatic spine.

6.2 Introduction

The spinal column is the most common site of metastatic involvement in the skeleton, often resulting in mechanical instability leading to vertebral fractures that can significantly impact
patients’ quality of life [1, 2, 3]. Traditional management of patients is through a multimodal approach that includes bisphosphonates (BPs) and radiation therapy. The response of each patient to current treatment approaches remains variable, motivating further research on new local methods to treat spinal lesions [4].

Photodynamic therapy (PDT), consisting of the administration of a photosensitizer that becomes activated by light at a specific wavelength, has been used to locally treat cancer in various soft tissues, due to preferential uptake of the photosensitizer by tumour cells. In the presence of oxygen, activation of the photosensitizer leads to generation of highly reactive singlet oxygen resulting in cell toxicity and tissue necrosis. PDT has been shown to be a good local treatment for spinal metastases in preclinical studies, both killing tumour cells and improving skeletal architecture. In particular, Won et al. have shown that PDT, while destroying the tumour tissue, results in significant increases in bone volume fraction and trabecular thickness, and a significant decrease in trabecular separation [5, 6]. They have further demonstrated that PDT treated vertebrae become stiffer and stronger through destructive mechanical testing [5, 6].

Treatment with PDT following pre-treatment with BPs has been shown to result in further improvements in structural properties of the metastatically involved bone, restoring its mechanical behavior to levels similar to non-pathologically involved vertebrae [6]. However, in such studies that attempt to perform both histological analyses and mechanical testing, destructive protocols require independent specimens for each test.

Hardisty et al. have developed a 3D intensity-based image registration technique, which uses the deformation of the trabecular structure under load to calculate regional strain within vertebrae with low error [7].

Using image-based methods to analyze strain patterns throughout whole vertebral bones provides additional information to destructive mechanical testing, indicating specific locations at high risk for fracture initiation. Such information can aid in improving the understanding of destructive patterns resulting from metastatic involvement in vertebral bone and in the ability to evaluate new and existing treatments aimed at spinal lesions without destructive testing. This study aims to apply this validated image-based measurement tool [7] to accurately measure strain in vertebral bodies with metastatic disease, treated with a combination of BP and PDT, and
compare the results to both untreated metastatically involved vertebrae and untreated healthy non-pathologic vertebrae. We hypothesize that the combination treatment will reduce both average and maximum strain values as compared to untreated metastatically involved vertebrae and restore the pattern of strain to that seen in untreated healthy nonpathologic vertebrae.

### 6.3 Materials and Methods

Ten 5- to 6-week-old athymic nude (rnu/rnu) female rats (Harlan Sprague–Dawley, Indianapolis, USA) were randomly assigned to control (n = 5) and BP + PDT treated (n = 5) groups. Approval for all procedures was obtained from University Health Network, Toronto prior to the initiation of the experiments.

#### 6.3.1 Model Development

Osteolytic metastatic involvement in both groups was developed via intracardiac injection of luciferase transfected human breast cancer cells (MT1) with rats under general anesthesia (2% isofluorane/oxygen) [8].

Seven days after tumour injection the rats in the BP + PDT group were injected subcutaneously with the bisphosphonate zoledronic acid (Zometa; Novartis, Dorval, Canada) at a dose of 60 lg/kg. This dose represents the dose currently used clinically to treat patients with metastatic bone disease. The presence of metastatic involvement in the vertebral column was confirmed using bioluminescence imaging at 13.5 µm pixel size on day 14 (IVIS Imaging System, Caliper Life Science, Hopkinton, MA). This confirmation was followed by the administration of PDT to the BP + PDT group. Under general anesthesia, animals were first injected intravenously with 1.0 mg/kg Benzoporphyrin derivative monoacid (BPD-MA) photosensitizer (verteporfin, Visudyne; Novartis, Dorval, Canada) dissolved in 200 IL of 5%dextrose. A flat cut optical fiber was then inserted percutaneously under fluoroscopic imaging adjacent to the L2 vertebra (the chosen target level for this study). A 15-min drug-light interval was followed by the delivery of 75 J of light energy from a 690 nm diode laser inserted through the optical fiber. The power output for the total 12.5 min treatment time was 100 mW [6].

On day 21 all animals were once again imaged using bioluminescence imaging to qualitatively assess the final metastatic tumour burden. Animals were then sacrificed and their L1–L3 vertebral levels harvested, wrapped in saline-soaked gauze, and stored at -20 ºC.
6.3.2 Load Application

A customized loading device was used to apply axial loading to the individual L1–L3 spinal motion segments (Fig. 19). The load cell was calibrated prior to testing using standard calibration weights (STO-AWEIGH Ohaus Scale Corporation, NEWARK, NJ).

The distal parts of the posterior elements of the adjacent vertebrae were trimmed to remove facet joint loading. Prior to placing the motion segments in the device, the L1 and L3 vertebral levels were potted in bone cement. This allowed for strain patterns in L2 vertebrae to be assessed under a relatively physiologic loading applied through the adjacent intervertebral discs. Axial load was ensured by the tubular design in which specimens were fixed to acetal rings that slide within an outer polycarbonate tube. Each spinal motion segment was axially loaded to 65 N, previously calculated to be the optimum load to cause detectable strain in metastatically involved vertebrae in this preclinical model without causing fracture [7]. Application of the axial force on the sample was performed via manual advancement of the load-calibrated threaded rod. Load application for each motion segment on average took 4 min. There was no preloading. The specimens were maintained under the static 65 N load during imaging (~2.5 h).

6.3.3 Image Acquisition

Micro-CT images were acquired from all spinal motion segments in loaded and unloaded configurations (Fig. 29) (GE Explore Locus, General Electric Company, Fairfield, USA). The imaging was done using a tungsten X-ray source at 90 mA and 80 kV, with 900 orientations covering a full 360° rotation. There were 905 views taken. A single exposure took 2 s, and each frame was an average of three exposures.

The X-ray tube was mounted with its long axis inline with the sample. The projections were reconstructed using the commercial GE reconstruction software to obtain a 3D volume at 14 µm isotropic resolution.
Figure 29. Sagittal slice of micro-CT image of a metastatically involved untreated L2 vertebral body in (a) unloaded and (b) loaded configurations.

6.3.4 Image-based Strain Measurement

Amira visualization software was used to implement the strain-based image registration algorithm (Amira- Dev 3.0 and 4.1). All the images were first manually aligned with Amira’s global axes. Once aligned, the images were cropped so that only the body of the target L2 vertebra remained. Analysis was confined to the vertebral body, as it bears the primary load and contains the majority of trabecular structure needed for image correlation [7].

A 3D rigid registration was used to register the loaded vertebral body image data to the unloaded image data (AmiraDev 4.1). A Quasi Newton optimizer was used for this registration with initial and final optimizer steps set to 0.001, and 0.0000001 respectively. The tolerance of the gradient optimizer was set to 0.000001 to ensure accurate optimization. A Normalized Mutual Information metric was used for this registration to account for intensity variations between the scans.
The two rigidly registered vertebral bodies were further deformably registered using an algorithm developed by Hardisty et al. [7], which utilizes a mutual information metric to ideally match the image blocks of the unloaded image to those of the loaded image.

The deformable registration starts by dividing the unloaded scan into eight regions and registering each region to the corresponding region in the loaded scan. This is done using an affine transform that allows for 12 degrees of freedom through translation, rotation, scaling, and shearing. Subsequent division of each of the eight regions into a further eight subregions was then done for two more iterations to achieve the final level of analysis (Fig. 19).

The transformation matrix (T) based on this final level of analysis was used to calculate the displacement matrix (A). The axial displacement was used to the calculation of the axial strain (c).

\[
\bar{A}(x, y, z) = \tilde{T}P(x, y, z) - P(x, y, z)
\]

\[
e = \frac{1}{2} \left( \nabla \bar{A}^T + \nabla \bar{A} \right)
\]

The axial strain values calculated from spatial displacement of the micro-CT voxels were quantified through the calculation of mean, median, 10th percentile, and 90th percentile strains with negative values corresponding to compression. Paired t tests were used to compare the axial strains developed under load in the treated and untreated groups (Microsoft Office Excel). Vertebral axial strains in the treated metastatically involved vertebrae were further compared to axial strains generated in previously analyzed healthy (non-pathologically involved) vertebrae.

6.4 Results

Micro-CT images were successfully acquired for all motion segments in loaded and unloaded configurations (Fig. 28) and analyzed using the strain-based image registration algorithm. Compressive strain values representative of the mean, median, and 90th percentile values were found to be significantly higher (~2x) in the untreated metastatically involved vertebrae as compared to the BP + PDT treated group (Table 7). No difference was seen in the 10th percentile
strain values, which were tensile in both the metastatically involved untreated and BP + PDT treated groups. This suggests that some degree of bending was present in the trabeculae under load. The direction of bending is dependent on the three-dimensional orientation of individual trabeculae.

Qualitative evaluation of the strain measurement data showed large areas of high strain in the untreated metastatically involved vertebrae adjacent to the endplates, the dorsal wall, and surrounding areas of osteolytic destruction (Fig. 30a). In contrast, the high strain areas in the treated group were generally confined to the area around the growth plates, as expected due to the compliance of this tissue in comparison to the surrounding bone (Fig. 30b).

Table 7 Strain calculation (%) for control and treated groups with * indicating a statistically significant difference between groups (p < 0.05).

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Tumor-bearing control L2 vertebrae (n = 5)</th>
<th>Tumor-bearing L2 vertebrae—combined BP + PDT treatment (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median strain*</td>
<td>-4.9 ± 1.8</td>
<td>-2.4 ± 0.9</td>
</tr>
<tr>
<td>Mean strain*</td>
<td>-8.6 ± 1.1</td>
<td>-3.6 ± 1.8</td>
</tr>
<tr>
<td>10th Percentile</td>
<td>5.3 ± 4.5</td>
<td>5.9 ± 2.6</td>
</tr>
<tr>
<td>90th Percentile*</td>
<td>-28.0 ± 4.7</td>
<td>-16.7 ± 4.4</td>
</tr>
</tbody>
</table>
In areas without large regions of osteolytic destruction similar patterns were observed in treated and control specimens. However, the magnitudes of strain in these regions of the treated vertebral body were lower than that seen in the control vertebrae.

6.5 Discussion

In this work, a validated 3D image-based technique for measuring vertebral body strains [7] was used to compare strain in vertebral bodies with metastatic involvement, untreated to those treated with a combination of BP + PDT. The analyses yielded full field strain patterns within the metastatically involved vertebral bodies. Large areas of high strains were generated in the untreated metastatic vertebral bodies around the osteolytic lesions and adjacent to the vertebral wall, indicating potential mechanical instability and susceptibility to fracture. The spatial distribution of this strain adjacent to the dorsal wall in the tumour bearing vertebrae may be
reflective of increased clinical risk of burst fracture (fracture of the posterior vertebral body wall) seen clinically in individuals with osteolytic spinal metastases [9, 10]. Additional presence of high strain regions at the growth plates was expected due to their limited calcification in these young rats [11]. In contrast, the high strain areas in the treated group were limited to the growth plates alone.

Quantitatively, the treated group demonstrated significantly lower mean, median and 90th percentile strains as compared to the control group with untreated metastases, indicating a reduced risk of fracture initiation under equivalent loading. In the control group, the strain patterns in the areas without large osteolytic destruction were similar to those of the treated group, but with lower strain magnitudes. Combined BP + PDT treatment has been shown to augment vertebral structure via bone deposition as early as 1 week post-PDT [6]. This structural augmentation occurs in all areas independent of metastatic osteolytic destruction, explaining the overall increases in stability when compared to the untreated metastatically involved vertebrae. In support of these structural changes, the biomechanical benefit of this treatment can be demonstrated through this non-destructive image-based analysis.

Axial strain patterns generated in the BP + PDT treated metastatically involved vertebrae were found to be similar to patterns exhibited in healthy (non-pathologically involved) vertebrae (T12 to L4) [12]. Mean strain levels were similar in the previously analyzed healthy L2 vertebrae (22.34 ± 0.9%; n = 2) and the PDT + BP treated L2 levels analyzed in this study. However, 90th percentile (26.9 ± 4.7%) and median strains (20.5 ± 0.2%) were lower in the healthy L2 vertebrae.

Combined BP + PDT treatment has been shown to destroy tumour tissue, increase bone density, and strength in this preclinical skeletal metastasis model [6]. The reduction in tumour burden has been qualitatively observed in our work through bioluminescence images of the treated metastatically involved vertebrae compared to untreated metastatically involved vertebrae [6].

Won et al. have also shown that combined BP + PDT treatment results in a 76% increase in bone volume fraction, a 26% increase in trabecular thickness, a 43% increase in trabecular number, an 85% increase in trabecular volumetric bone mineral density, and a 52% decrease in trabecular
separation. This improvement in bone structural properties was significantly greater than that observed in rats treated with BP or PDT alone [6].

While the BP + PDT treatment was shown in previous work to result in a significant increase in ultimate force, stress and stiffness when compared to the untreated metastatically involved group, its effect on these mechanical parameters was not significantly different from the BP only or PDT only groups [6].

Stability in the setup of the motion segments within the loading chamber was essential to allow the application of sufficiently high non-destructive mechanical loads to the spinal motion segments necessary to resolve axial strain. A certain minimum amount of strain is needed based on the pattern of the structure to allow resolution of the strain using this algorithm. As such, this work focused on axial strain alone and did not analyze the smaller magnitude sagittal, coronal, or shear strains occurring in the vertebrae.

Motion segments were utilized as the intervertebral discs are important in generating physiologic loading patterns. Digital image correlation is sensitive to subtle changes in boundary conditions that can lead to large differences in strain fields [13]. Replication of complex physiologic load and boundary conditions using other analytical methods can be difficult to achieve. The image-based strain measurement method does not make any assumption about the distribution of load or the material properties of the structure (bone or soft tissues) making it robust approach for strain calculation within complex structures. In contrast, other analytical methods such as finite element analysis (FEA) are limited by material and loading assumptions [7]. Physical strain measurement techniques (i.e., using strain gages) are also often unable to generate full field information, but rather yield averaged surface strains at a limited number of locations. Moreover, the relatively small size of rat vertebrae makes the application of strain gages difficult. The spatial resolution of strain measurement possible with the presented image registration technique is limited, however, based on the texture of the material being analyzed [12, 14]. The deformable registration algorithm requires high contrast to accurately register the trabecular structure. At the current resolution, there is not sufficient contrast to properly resolve strain in the bony areas adjacent to the growth plate. As such, the bone adjacent to the soft tissue of the growth plate appears overly strained; this phenomenon is referred to as a “bleeding effect” [14]. As well, the
spatial resolution limits the ability to precisely localize areas of maximum strain, thereby limiting the accurate identification of specific locations at elevated risk for failure.

The utilization of strain as a surrogate for failure of bone has been widely accepted as bone has been demonstrated at a continuum level to fail at constant strain (~1%) independent of bone density [15, 16]. However, at a micro-structural level, individual trabeculae are able to bear significantly higher strains before failure. The length scale on which the strains are measured in this study lie between apparent and tissue level behavior. The apparent axial strains measured within this study are on the order of previously found apparent level strains in trabecular bone and strains measured under failure of individual trabeculae [11, 14, 17].

The qualitative and quantitative strain patterns in our study together with the morphological measurements conducted in previous work [5, 6] suggest that combined BP + PDT treatment is able to reduce the structural deficit that occurs during rat vertebral metastatic involvement with human MT1 breast cancer cells. Thus, in addition to destroying tumour tissue, combined BP + PDT is able to maintain/restore the structural integrity of the rat spine. Moreover, this algorithm is able to demonstrate both qualitatively and quantitatively this biomechanical restoration without destructive testing. This enables both biomechanical and histological analyses to be performed on the same specimens; potentially halving sample sizes in future investigations on metastatically involved trabecular bone structures requiring both types of information.

6.6 References


The high incidence of spinal metastasis and its impact on quality of life in patients living with cancer motivates the development of robust image-based measures to quantify the structural effects of tumours in bone and the implications of these changes on biomechanical stability [1, 2, 3]. Preclinical models are widely used in cancer research, including in the evaluation of skeletal metastases. Preclinical models allow for in vitro (and in vivo) visualization of the microstructural effects of the metastatic disease in bone micro-architecture and can be used to evaluate the effects of new and existing treatments aimed at spinal lesions [4, 5]. Analyzing high-resolution images of these structures is, however, a time consuming task thus motivating automated methods for widespread utilizations. The purpose of this work was to develop automated methods to firstly quantify the destructive effect of osteolytic and mixed osteolytic/osteoblastic disease on vertebral micro-architecture and secondly to evaluate the ability of 2D, 3D, and continuum based methods to quantify the biomechanical stability of rat spine with or without metastatic destruction.

7.1 Preclinical modeling

Four-week old rnu/ rnu rats were used for this project, with osteolytic and mixed osteolytic/osteoblastic metastases developed via intracardiac injection of MT1 human breast cancer cells and Ace-1 canine prostate cancer cells, respectively. Analyses of metastatic disease occurred three weeks post injection, as the presence of metastatic disease throughout these animal prevented longer term evaluation of this pathology or any treatment effects.

The preclinical models were good representations of the metastatic disease in the spine as the cells were delivered through intracardiac injection and were allowed to metastasize through the blood stream. However, the use of immune compromised animals may have affected disease progression, the response of bone to the tumour cells and therapeutic efficacy.

The success rate of vertebral metastasis development in these models is highly dependent upon the level of expertise and experience of the individual performing the cell injection as well as the
vendor from which the rats were purchased. Tumour burden was highly variable from rat to rat and bone to bone. This prevented us from being able to apply consistent loads to all L2 rat vertebrae in the study presented in chapter 5. Most samples in chapter 5 were axially loaded with a force of 65N. This force was, however, too large for samples with large osteolytic lesions and resulted in fractures in 3 specimens (1 osteolytic and 2 mixed osteolytic/osteoblastic). The samples that broke under 65N of axial loading were replaced as accurate yield load data and loaded µCT images, which could be utilized in the image-based strain algorithm, were not available for these specimens. Based on these fractures, for some subsequent specimens (including replacement specimens) the force was lowered based on qualitative observation of unloaded µCT and µMR scans.

7.2 Micro-MR and micro-CT based radiologic methods to quantify tumour involvement and vertebral architecture in a rat model of spinal metastasis

Micro-CT imaging was acquired of all specimens to be able to visualize the destructive effects of metastatic disease on the vertebral micro-structure. Chapter 2 of this thesis presented a highly automated µCT based algorithm which was used to segment whole vertebrae, trabecular centrums and individual trabeculae in healthy and metastatically involved rats. The automated segmentation algorithm was able to accurately segment these structures accounting for gaps and breaches through the cortical shell. It also enabled repeatability and objectivity in the subsequent analyses performed on these images throughout this thesis.

The segmentations were used in chapter 3 to quantify the destructive effects of osteolytic disease in whole vertebrae. The parameters used for this quantification included trabecular bone volume (TBV), cortical bone volume (CBV), trabecular thickness (TbTh), trabecular separation (TbSp) and trabecular number (TbN) [6, 7] along with the degree of anisotropy through the mean intercept length method (MIL) [8]. These parameters have been used in the field of biomechanics and are well known identifiers of structural stability. The specific purpose of chapter 3 was to examine the effect of a stereologic model and image resolution on automated quantification of stereologic parameters in whole vertebrae with osteolytic metastases. The two stereologic models used for this automated quantification were the well known Parfitt’s plate model [6] and the more recent Hildebrand model [7]. Parfitt’s plate model makes the assumption that all
trabeculae are plates and based on that assumption it uses measures of volume and surface area to calculate trabecular thickness, number and separation [6]. Hildebrand et al. showed that this assumption is not true and it in fact results in an overestimation of the trabecular thickness values. They defined trabecular thickness as the diameter of the largest sphere that could fit inside a given trabecula [7]. Despite of the over estimation in the value of trabecular thickness due to the plate assumption for the trabecular structure, we found Parfitt’s model to be a better candidate for automated relative quantification of the micro-structure. Specifically, we observed that both models yield the same trend in relative values of trabecular thickness, however, the Hildebrand model required manual refinement of the segmentations as it was extremely sensitive to small islands present within the segmentations. These islands were partially due to the presence of noise signal in the µCT images. We also observed that the stereologic parameters (with the exceptions of TBV, CBV, and MIL) were highly sensitive to resolution. These finding are extremely important as they provide valuable information that can be utilized to optimize the stereologic model and acquisition resolution for future applications of quantitative structural analyses.

We observed a significant decrease in TBV, TbN, and CBV and a significant increase in TbSp in the osteolytic rats when compared with healthy rats, and noted no significant differences in TbTh. This suggests that the loss of whole trabeculae dominates the structural deficit imposed by the severe osteolytic involvement found in these vertebrae. This approach may be valuable to quantify the progression of disease from earlier stages of involvement, in particular when the presence of diffuse tumour tissue can be localized based on the registration of µMR with µCT imaging. The same automated approach was used to quantify the destructive effect of the mixed metastatic disease on 4 week old rnu/rnu rats. [9] This work showed a significant decrease in TBV, trends towards a reduced TbN and increased TbSp in mixed osteolytic/ osteoblastic metastatically involved vertebrae compared to the healthy controls. As such, these stereologic parameters can help us to better understand the relative destructive effects of the different patterns of metastatic disease on the biomechanical stability of the spine.

In µCT images one cannot distinguish between bone marrow and osteolytic tumour as they both appear as low intensity voxels. Yet visualizing osteolytic tumour is important in accurately evaluating the volume of tumour within vertebrae (initially and post treatment) as tumour can be present in a diffuse pattern within the trabecular network. Localizing the osteolytic tumour is
also important in the development of accurate finite element models of the metastatic spine in order to accurately assign material properties. This is particularly important in μFEA which, in contrast to continuum modeling, models the individual trabeculae within a bone structure separately from the marrow or tumour tissue present within the trabecular network. Chapter 4 of this thesis presented a highly automated μCT/μMR based approach to segment bone and tumour in a rat model affected by mixed osteolytic/osteoblastic metastases. The algorithm yielded accurate segmentation of whole vertebrae, trabecular centrums, individual trabeculae and the osteolytic tumour. Segmentation of the osteoblastic tumour was however a challenging task yielding lower volumetric concurrencies when compared to manual segmentations. The tumour segmentations were qualitatively compared to histological images of the same rat. Such segmentations were utilized in the development of the FE models used in chapter 5.

The automated algorithms used in chapters 2, 3, and 4 enable objective and robust quantification of the vertebral architecture with or without metastases. Automated quantitative evaluation of spinal metastases is necessary to better understand the destructive effects of metastatic disease on the micro-architecture and its potential correspondence to biomechanical stability.

The performance of such algorithms is highly dependent on image resolution. As the image resolution lowered, accuracy in some parameters was lost; however, at high resolutions the processing time was considerably slower and required more powerful computer hardware. This dependency could be a limitation for study designs, which require the analysis of large numbers of specimens. Further investigation towards less computationally expensive algorithms enabling faster execution time for similar high resolution analyses would be beneficial for the wider application of these techniques. Although Parfitt’s plate model was found to be the method of choice for relative quantification of micro-structural parameters, it did result in an overestimation of the absolute values of the trabecular thickness values [7]. As such, in studies where the objective is automated absolute quantification of the micro-structural parameters, better image quality, noise reduction algorithms or validation of techniques to convert measured parameters to absolute values would be required.

In chapter 4 we developed a highly automated μCT/μMR based segmentation algorithm to segment the bony and tumour structure in mixed model of spinal metastasis. We verified the tumour segmentations using histological analysis; however this was performed only on a single
sample. Our multimodal segmentation algorithm was also limited in segmenting the osteoblastic tumour. This was due to the highly disorganized arrangement of the tissue which made accurate segmentation difficult (even in using manual techniques). In our Ace-1 model of mixed osteolytic/osteoblastic metastases, the osteoblastic tumour was primarily present on the outer surface of the cortical shell, but more limited amounts of osteoblastic bone were found within the trabecular centrum in this model as well [9]. Evaluation of different time-points in the Ace-1 tumour development in the rat may change the balance between osteolytic and osteoblastic involvement and other models of mixed osteolytic/osteoblastic and pure osteoblastic metastasis may present distinct patterns of bone deposition. Improved automated segmentation of osteoblastic disease may be facilitated at different time-points or in different models and will be important in studies examining treatment effects and bone quality in mixed osteolytic/osteoblastic and pure osteoblastic metastases.

7.3 Evaluation of the ability of 2D, 3D, and continuum based methods to quantify structural integrity in vertebral metastasis

In this work we evaluated the predictive ability of 3 non-destructive image-based algorithms, 3D image-based strain measurement, 2D structural rigidity and 3D continuum FE analysis, in representing the biomechanical stability of rat spine.

The image-based strain measurement algorithm has been previously used to quantify the strain pattern throughout rat vertebral bodies with and without metastatic destruction [15]. It has also been applied to demonstrate differences in growth plate behavior in rat vertebrae [21] and the effect of combined treatment with BP+PDT in rat vertebrae with osteolytic metastases, as presented in chapter 6 [11]. The previously validated image-based strain measurement algorithm displayed expected patterns of strain in vertebral bodies with and without metastatic disease. The quantified strain values demonstrated significant correlations of moderate strength with mechanical testing results for the healthy and osteolytic groups independently. The lack of a relationship between the image-based strain measurement algorithm results in the mixed osteolytic/osteoblastic model and failure load could be due to the computational restriction of the algorithm, which limited the analyses to vertebral body, as in this group in particular, the osteolytic destruction heavily affected the posterior elements. As well, the limited resolution of
strain possible with this technique may limit the ability of the algorithm to detect peak strains where fracture initiates.

Clinically, 2D structural rigidity analyses have been shown to correlate extremely well to fracture risk in juvenile chondrosarcomas and with excellent sensitivity (100%) but more limited specificity (44% to 70%) in spinal metastases [12, 19, 20]. These structural rigidity analyses have also been applied in a preclinical scenario looking at failure torque in rat bone with simulated osteolytic defects and have been shown to be a good representation of the mechanical testing results \( r^2 = 0.85 \) [14].

In our work we used the equivalent model developed by Cory et al [13] to predict fracture load in rat spines with or without metastatic destruction based on µCT intensity. In considering the combined group of all 15 specimens, the rigidity based predicted yield load did not yield significant correlations with the experimental yield load. However, strong significant correlation was found between structural rigidity and experimental yield load in the mixed osteolytic/osteoblastic group with a similar trend in the osteolytic group. The absolute value of the predicted yield loads were however 1.5 to two times higher than the experimental yield loads suggesting that the density-modulus relationship utilized may be overestimating the elastic modulus for the calcified tissue in the metastatically involved vertebrae. Surprisingly, no relationship was found in the healthy vertebrae. In contrast to preclinical models, where loading is applied directly to the bone, the present study applied loads to the L2 vertebrae through the intervertebral discs and facets. This more complex, albeit axial compressive loading scenario may not be represented by considering axial rigidity alone. Sequential µCT imaging of the specimens, as they approach the failure load, may shed light on the load distribution applied to L2 at failure and enable identification of the site of failure initiation.

The FE method has been utilized in studies examining fracture prediction in vertebrae affected by osteolytic destruction [16, 22, 23, 24]. To date, however, FEA of the metastatic spine has not considered mixed osteolytic/osteoblastic metastases, nor has it employed the use of multimodality imaging in order to accurately represent tumour infiltration within the trabecular structure. This has been accomplished in this study by integrating metastatic infiltration from segmentations of tumour tissue based on µMR imaging into the generation of the FEMs. Despite limited, albeit significant correlations, between FE derived mean and median strains and yield
load, further study is needed to validate these models. Information from sequential µCT imaging of the specimens, as they approach failure, may be preferential to single value load to failure mechanical testing data for FE model optimization and validation. FE simulations have been used to validate the strain patterns obtained in skeletal structures under simplified loading conditions using image-registration [15], however determining gold standards for validation purposes is difficult in considering complex loading of multifaceted biological structures such as the spinal column. The continuum FEA approach utilized was novel in that it represented complex loading of a spinal motion segment through intervertebral discs and facets without making any assumptions about the soft tissue behavior or the modeling of contact surfaces. Yet, this limited the analysis of the models to a quasi-static loading, and as such, poroelastic properties were not included in the models despite the presence of osteolytic tumour tissue. The fluid phase, however, may have influenced the vertebral behavior in the experimental destructive testing. The linear elastic modeling approach allows for rapid FEA, but requires additional (loaded) µCT imaging data and application of the image-based registration to yield the deformation fields.

The automatically generated FE models and image-based strain measurement algorithm demonstrated qualitatively similar strain patterns throughout the vertebral body in each of the 15 specimens. Yet, the mean strain values of the two methods significantly correlated only for the healthy group. Similarly the quantitative strain and stress parameters did not correlate well with the mechanical testing results. Limitations in the assignment of material properties, (i.e. incorrect estimation of the elastic modulus as a result of the density-modulus relationship used) can greatly affect FE modeling results. This includes inaccurate modeling of the growth plate. As well, more research is needed to determine the mechanical behavior of tumour involved bone at a tissue level, particularly in considering regions of osteoblastic disease. Even with better material property assumptions for tumour involved bone, the difficulties in accurate segmentation of osteoblastic tumour based on µCT imaging remains a challenge. This would represent a particular challenge to µFE models in which a single material property assignment has generally been applied to represent bone tissue. A specific set of material properties representative of osteoblastic elements (and perhaps also for bone adjacent to osteolytic tumour tissue) may be required.
The 15 vertebral motion segments were axially loaded to failure to determine their experimental yield loads. The quantitative parameters obtained from image-based strain measurement, 2D structural rigidity based prediction of failure load and continuum FEA were evaluated against experimental yield load to determine their ability to represent the structural stability of the L2 vertebrae. As a whole, correlations between the image-based parameters and the experimental yield load were limited. Yet, relationships within individual groups did reveal some stronger relationships. However, the low sample sizes in the healthy, osteolytic and mixed osteolytic/osteoblastic groups individually, limit the power of these results. These observed differences between groups were beneficial as they highlighted the effect of the metastatic disease on the structural and material properties of the vertebrae and its complex consequences.

Image-based strain measurement was successfully applied to evaluate the strain patterns in metastatic rat vertebrae treated with combined BP+PDT. This work demonstrated, through non-destructive mechanical testing, the positive structural effects of BP+PDT on structural stability. These observations were in agreement with previous studies evaluating the effects of the combined treatment through morphological measurements, histological analyses and destructive mechanical testing [10]. The indication from this study that based on strain patterns this combined therapy was able to restore the mechanical stability of metastatically involved vertebrae, provides additional basis for clinical translation of PDT for the treatment of spinal metastases.

7.4 Future Direction

This work demonstrated the use of automated techniques in quantification of tumour burden and mechanical stability of the metastatic rat spine. The first step of the project was the generation of the spinal metastases within the preclinical rat model. Although all animals were the same age/strain and received similar intracardiac injections, there was considerable variation in the severity of the metastases. Future work may focus on quantifying the temporal progression of metastatic disease in the rat models using multimodality imaging.

Automated image segmentation was another important step in this project. The combination of atlas-based demons deformable registration and levelset curvature evolution yielded accurate segmentation of the whole rat vertebrae. The computational cost of this segmentation method however, is highly dependent on image size and resolution, limiting its desirability in real time or
near real-time high resolution applications. Future work may focus on the development of resolution independent algorithms, such as landmark or surface based registration [17, 18], and applying them to similar automated quantification applications.

Automated quantification of stereological parameters and imaged based strain measures have been used successfully in comparative evaluations of the effect of PDT and combined treatments applied to the metastatic rat spine [10, 11]. As such, future work could further utilize these automated quantification methods to better evaluate both local and systemic treatment effects aimed at spinal and other skeletal lesions. Ultimately, larger preclinical models of metastatic disease (i.e. a mini-pig) would be extremely beneficial, especially in the evaluation of local minimally invasive (i.e. cannula based) therapies. However, to date, no such robust models exist.

A novel aspect of this project was the use of multimodal image analyses in quantitative analyses of spinal metastases. The use of $\mu$MR/$\mu$CT based image processing algorithms enabled accurate visualization and quantification of bone and osteolytic tumour, which in turn enabled development of automated continuum FE models incorporating these complex tissue distributions. The automated $\mu$MR/$\mu$CT based quantification algorithms could be used in future studies looking at ablative treatments aimed at skeletal lesions, to be able to accurately quantify the tumour ablation effect as well as possible damage to the surrounding bony tissue. Such multimodal analyses could potentially be used in place of histological analyses to enable quantification of tumour burden and compilation of mechanical testing on the same set of samples increasing the statistical power of such analyses by potentially doubling the sample size.

The automated 2D axial rigidity analysis was not able to predict the yield load in the whole rat vertebrae in the combined group of samples, but its performance improved considerably in considering the groups individually. Yet, in all cases the predicted failure load was over estimated. Future work optimizing the density-modulus relationships for tumour involved bone, that includes factors that could account for the presence and type of bony disease as well as the skeletal site, may improve the overall performance of this technique.

Image-based strain measurement method uses a multiresolution deformable registration algorithm. This algorithm is an intensity based algorithm and thus is sensitive to blurring which results at lower resolutions. The multiresolution aspect of the algorithm also made the analysis
computationally expensive, limiting the analyses to the vertebral body. Utilization of a registration algorithm that is not purely intensity based may be considered in future work as such an approach may be able to perform well at lower resolutions.

The image-based strain measurement algorithm could also be used as a replacement of destructive mechanical testing in studies where the yield load is not the necessary outcome. This could in turn allow quantification of mechanical stability as well as histological analyses to be done on the same set of samples potentially halving the sample size. The ability of this non-destructive technique to highlight the positive biomechanical effect of photodynamic therapy in combination with bisphosphonates in rat spinal metastases [11], motivates its application in studies wishing to conduct histological and biomechanical evaluation of other new and existing treatments for spinal metastases or other skeletal pathologies.

The automatically generated µMR/µCT based segmentations were used to generate continuum FE models of the metastatic rat spine. Using the deformation field generated from the image-based strain measurement algorithm as the displacement boundary condition along with material properties from the literature resulted in models, which demonstrated qualitatively similar strain patterns to those generated by the image-based strain measurement algorithm. The FEA based measures however did not correspond well with the experimental yield loads, potentially due to limitations in material property assumptions. Future studies may consider further characterizing the density-modulus relationship of skeletal tissue to account for different skeletal sites and disease. Future work may also consider utilizing image-based deformable registration as a quasi-static validation tool for modeling of the intervertebral discs. Removing the need for loaded µCT images allows the FEA to perform as a stand-alone tool. Multimodality FEA may have ultimate application as an accurate non-invasive image-based method for quantification of mechanical stability in preclinical or clinical applications.

Overall, future studies can use the findings from the current project in further optimizing automated image-based techniques to allow rapid disease quantification, assessment of fracture risk and the evaluation of new and existing treatments aimed at the metastatic spine or other pathologically involved skeletal sites.
7.5 Significance

Patients with metastatic disease are living longer as a result of current treatments, as such, the impact of metastatic disease in the spine on quality of life has expanded. This has motivated researchers to develop methods to better understand the destructive effects of metastatic disease in bone and has been facilitated by widespread access to 3D digital imaging techniques. Preclinical models have also been widely used in evaluation of spinal metastases, however evaluation of tumour burden and its biomechanical implications have thus far been mainly qualitative or semi-quantitative [4, 5]. Quantitative assessment of 3D high resolution images is a time consuming task motivating the use of automated techniques for widespread utilization.

In this work we have presented a comprehensive multi-modal approach to understand the biomechanical implications of spinal metastases. This has been done through combining recent advances and innovations in computational modeling, image analyses and preclinical model developments, which allow quantification of complex patterns of tumour involvement in the spine and their effects on biomechanical stability. This work developed automated techniques that quantify the micro-structural parameters and biomechanical behaviour of rat vertebrae affected by osteolytic and mixed osteolytic/osteoblastic metastases. These methods can be applied to quantify the impact of disease, track disease progression and evaluate the structural impact of new and existing, local and systemic therapeutic approaches to treating the metastatic spine. A better understanding of the strengths and limitations of structural models, stereologic parameters, µCT and µMR imaging (i.e. resolution), and computational modelling approaches (including FEA) is important in future study design and in the ultimate clinical translation of preclinical knowledge toward improving quality of life for patients with spinal metastases.

7.6 References


