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μFE models can represent microdamaged regions of healthy and metastatically involved whole vertebrae identified through histology and contrast enhanced μCT imaging

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ABSTRACT

Micro-damage formation within the skeleton is an important stimulant for bone remodeling, however abnormal build-up of micro-damage can lead to skeletal fragility. In this study, μCT imaging based micro finite element (μFE) models were used to evaluate tissue level damage criteria in whole healthy and metastatically-involved vertebrae. T13-L2 spinal segments were excised from osteolytic ($n=3$) and healthy ($n=3$) female athymic rnu/rnu rats. Osteolytic metastasis was generated by intercardiac injection of HeLa cancer cells. Micro-mechanical axial loading was applied to the spinal motion segments under μCT imaging. Vertebral samples underwent $BaSO_4$ staining and sequential calcein/fuchsin staining to identify load induced micro-damage. μCT imaging was used generate specimen specific μFE models of the healthy and osteolytic whole rat vertebrae. Model boundary conditions were generated through deformable image registration of loaded and unloaded scans. Elevated stresses and strains were detected in regions of micro-damage identified through histological and $BaSO_4$ staining within healthy and osteolytic vertebral models, as compared to undamaged regions. Additionally, damaged regions of metastatic vertebrae experienced significantly higher local stresses and strains than those in the damaged regions of healthy specimens. Areas identified by $BaSO_4$ staining, however, yielded lower levels of stress and strain in damaged and undamaged regions of healthy and metastatic vertebrae as compared to fuschin staining. The multimodal (experimental, image-based and computational) techniques used in this study demonstrated the ability of local stresses and strains computed through μFE analysis to identify trabecular micro-damage, that can be applied to biomechanical analyses of healthy and diseased whole bones.

1. Introduction

Physiological micro-damage formation within the skeleton serves as a stimulant for bone remodeling. Accumulation of unrepaired micro-damage in trabecular bone can result from inferior bone quality due to age-related changes or skeletal pathology (Iwata et al., 2014). Despite the importance of micro-damage to the mechanical properties of bone, the trabecular stresses and strains experienced at its initiation are not well characterized. Evaluation of stresses and strains associated with micro-damage initiation at the local level may enable improvement of fracture risk assessment techniques and the development of therapeutic methods for treatment of skeletal fragility diseases such as metastasis.

Micro-damage sites can be experimentally identified through sequential staining and histomorphometry (Lee et al., 2000). Sequential histologic staining (i.e. calcein green and fuchsin) has been utilized to differentiate pre-existing and test induced micro-damage (Lee et al., 2000, Herblum et al., 2013). Barium sulfate ($BaSO_4$) contrast enhanced μCT imaging has been used as a non-destructive 3D alternative to conventional histology to detect accumulation of micro-damage in trabecular and cortical bone (Landrigan et al., 2011, Wang et al., 2007).

Micro-finite element analysis (μFEA) has been utilized to study local damage initiation properties of trabecular bone (Herblum et al., 2013, Nagaraja et al., 2005). This technique converts segmented μCT voxels representing bone directly into finite elements, yielding large models which can represent complex structural

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tissue organization. μFEA has the capability to model the trabecular morphology, allowing calculation of stresses and strains at histologically identified damage sites (Keaveny et al., 2001). Nagaraja et al. used this approach to determine variations in micro-damage initiation parameters in response to age-related changes in human and bovine trabecular bone cores (Nagaraja et al., 2005, 2007, 2011, Green et al., 2011). μFEA of whole bones allows loading through joints/soft tissues, simulating more physiological loading conditions. Herblum et al. recently demonstrated successful application of μFEA to show elevated stresses and strains in regions containing mechanically induced micro-damage within whole healthy rat vertebrae (Herblum et al., 2013).

Spinal metastasis progressively degrades the trabecular architecture of the vertebral body, leading to an increased risk of fracture (Kurth and Muller, 2001). Previous studies have demonstrated significant differences in microstructural parameters (i.e. trabecular thickness, trabecular number, etc.) between healthy and metastatically involved vertebrae (Hojjat and Whyne, 2011). The microstructural parameters do affect the distribution of stresses and strains across the trabecular micro-architecture, but the distribution of bone tissue (changes in trabecular architecture) is not sufficient to explain all the difference (Hojjat et al., 2012). It is not clear if the initiation and propagation of unrepaired micro-damage, which precedes fracture, is distinct in metastatic spines. This study aims to generate μFE models that accurately represent damage initiation of whole healthy and osteolytic vertebrae based on histological and contrast enhanced μCT damage quantification and to determine a range of values at which damage initiates based on these models. It is hypothesized that μFEA will yield a consistent range of values for damage initiation in healthy and osteolytic vertebrae.

2. Methods

2.1. Animal models

The workflow of this study is included as Fig. 1. A previously described rat tumor model was used to generate osteolytic metastasis in rat spines through intra-cardiac injection of HeLa cells (Burch et al., 2007). Three osteolytic and three healthy rnu/rnu female rats of similar age (7–8 weeks) and weight (160–170 g) were sacrificed and T13-L2 spinal motion segments were extracted.

2.2. Loading and μCT imaging of spinal motion segments

A μCT compatible loading device (Fig. 2) was used to induce micro-damage in the healthy and metastatically-involved spine samples. The loading device allows for μCT scanning of the samples while under load. The top and bottom vertebrae of each 3 level motion segment were potted in a custom jig with PMMA to prevent lateral movement of the sample while loading. The motion segments of the healthy and osteolytic rats were preconditioned under uniaxial compression for 3 cycles at 40 N at a constant strain rate of 3 μm/s. Axial compressive loads of 100 N and 50 N

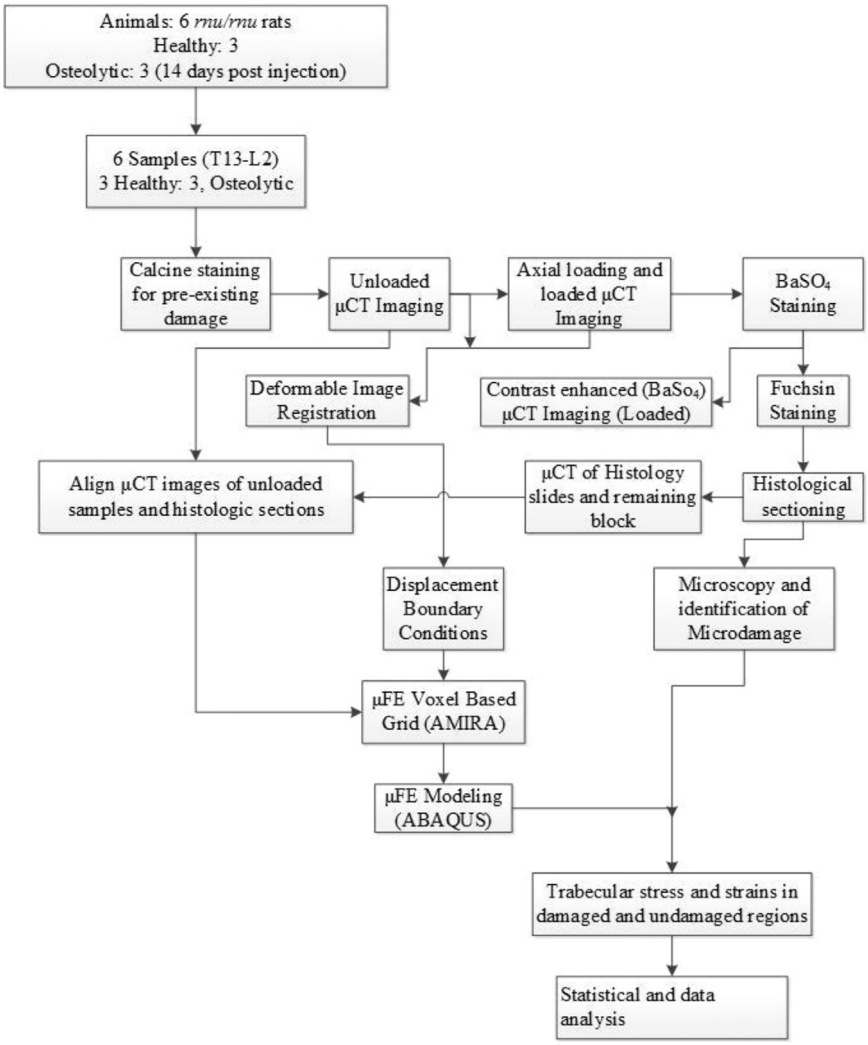

Fig. 1. Experimental design.