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Version Post-Print/Accepted Manuscript

Citation (published version) Herblum R, Beek M, Whyne CM. μFEA successfully exhibits higher stresses and strains in microdamaged regions of whole vertebrae. J Orthop Res. 2013 Oct; 31(10):1653-60. doi: 10.1002/jor.22392. PMID: 23737260

Publisher's Statement This is the peer reviewed version of the following article: Herblum R, Beek M, Whyne CM. μFEA successfully exhibits higher stresses and strains in microdamaged regions of whole vertebrae. J Orthop Res. 2013 Oct; 31(10):1653-60, which has been published in final form at https://dx.doi.org/10.1002/jor.22392. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

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μFEA Successfully Exhibits Higher Stresses and Strains in Microdamaged Regions of Whole Vertebrae

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Received 16 July 2012; accepted 28 April 2013
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.22392

ABSTRACT: Micro-finite element (μFE) modeling has shown promise in evaluating the structural integrity of trabecular bone. Histologic microcrack analyses have been compared to μFE models of trabecular bone cores to demonstrate the potential of this technique. To date this has not been achieved in whole bone structures, and comparisons of histologic microcrack and μFE results have been limited due to challenges in alignment of 2D sections with 3D data sets. The goal of this study was to ascertain if image registration can facilitate determination of a relationship between stresses and strains generated from μFE models of whole vertebral and histologically identified microdamage. μFE models of three whole vertebrae, stained sequentially with calcein and fuchsin, were generated with accurate integration of element sets representing the histologic sections based on volumetric image registration. Displacement boundary conditions were applied to the μFE models based on registration of loaded and unloaded μCT images. Histologically labeled damaged regions were found to have significantly higher von Mises stresses and principle strains in the μFE models, as compared to undamaged regions. This work provides a new robust method for generating and histologically validating μFE models of whole bones that can represent trabecular damage resulting from complex physiologic loading. © 2013 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res XX:XXX–XXX, 2013.

Keywords: microdamage; μFEA; rats; vertebrae; sequential labeling; μCT

Advancements in computational power and micro-imaging have allowed the creation of finite element models on a microstructural level that can represent complex skeletal structures. These micro-finite element (μFE) models can incorporate the micro-architecture of bone (i.e., trabecular morphology) and as such be used to analyze skeletal structural integrity down to the level of individual trabeculae. This allows evaluation of load and location of failure initiation within the physical architecture of the bone. μFE analyses of skeletal structures require an extensive amount of memory and computational time, thus they have been most commonly used to analyze trabecular structure within excised cores of bone.1–7

Experimentally, failure initiation in skeletal structures has also been studied through the examination of microdamage within trabecular and cortical bone. Microdamage in bone may present in the form of surface linear cracks, linear cracks spanning a full trabecula, diffuse damage, or complete microfracture (loss of continuity).8 Microcrack analysis has proven to be an effective method for evaluating bone biomechanical quality, alongside bone mineral density, bone histomorphometry, and mechanical testing.9 Sequential labeling has been shown to successfully identify newly formed microdamage. Microcrack identification is robust in qualitatively evaluating the presence or absence of mechanically induced bone microdamage, however dye replacement can make quantitative measurements of crack length imprecise.10

Recently, μFE has been applied to the modeling of trabecular bone cores and bones with removed endcaps and combined with microcrack analysis to evaluate skeletal structural integrity.11–15 Previous studies demonstrated success in evaluating μFE stresses, which have been shown to be capable of representing microdamage initiation in trabecular bone cores.8,12 Yet, there is a need to expand this technique beyond bone cores and apply it to trabeculae within the inherent boundary constraints that exist in whole bone structures. Whole bone analysis also allows the application of loads through joints or soft tissue attachments enabling more physiologic loading scenarios. However, quantitative comparisons of histologic microcrack and μFE results in whole bone geometry present challenges due to the requirement for accurate alignment of 2D sections with 3D data sets. As such, the goal of this study was to determine if image registration can facilitate determination of a relationship between stresses and strains generated from μFE models of whole vertebral and histologically identified microdamage. It is hypothesized μFE modeling of whole vertebrae will show elevated stresses and strains in regions containing mechanically induced microdamage.

MATERIALS AND METHODS

Specimen Preparation

Three spinal motion segments were excised from mature healthy Wistar rats for analysis (300–400 g, female, 1× T11–T13 and 2× L1–L3) (Fig. 1). Each intact specimen was immersed in a calcein green solution of 0.9% NaCl, 2% NaHCO3 and distilled water and placed under vacuum for 16 h at room temperature.16 The specimens were washed in distilled water for 1 h on top of a shaker to remove any excess stain within the vertebrae. The calcein green stain was used to identify preexisting microdamage and resorption.
cavities sustained in vivo prior to mechanical testing. The upper and lower vertebrae of each motion segment were potted in bone cement (PMMA) and inserted in a custom loading device in preparation for μCT scanning and mechanical testing (Fig. 2).17

μCT Acquisition and Mechanical Testing
Unloaded motion segments were scanned in a high resolution μCT scanner (14 μm³ isotropic voxel size, 90 A and 80 kVp, GE Explore Locus, GE Q3, Fairfield) adjacent to bone density phantoms (SkyScan Q4, Belgium) and reconstructed using the eXplore Reconstruction Utility. The motion segments were preconditioned under uniaxial compression for three cycles at 40 N at a quasi-static strain rate. A uniaxial compression load 100 N (predetermined to represent a load slightly below yield strength) was applied manually through the rotation of the threaded rod within a custom loading device (Fig. 2).17 Instantaneous load transmission through the load cell (Test

Figure 1. Experimental design, outlining the steps required for integrating image acquisitions, histological microdamage, and μFE analyses to determine if a relationship exists between stresses and strains generated from “physiologically” loaded μFE models and histologically identified microdamage in whole rat vertebrae.

Figure 2. Schematic of the axial compressive loading device. Load is applied through manual advancement of the screw and quantified by the load cell. Vertebrae adjacent to the level of interest are potted in bone cement and axial force is applied via the adjacent vertebrae and discs.
Resources\textsuperscript{55}, USA) was used to verify the applied load and the displacement of each specimen held constant during the loaded \(\mu\)CT scan (3 h).

**Microdamage Identification**

Following loading and imaging, the middle vertebra was isolated from each motion segment by cutting through the superior and inferior discs. The vertebrae were immersed in a 70% ethanol solution for a minimum of 24 h. Basic fuchsin hydrochloride staining was then used to label propagated microdamage in these vertebrae. Specimens were stained as whole vertebrae (endplates and posterior elements were left intact and bone marrow was not removed prior to staining). The specimens were processed through a staining/dehydration process in 1\% basic fuchsin hydrochloride (J.T. Baker) following the Burr protocol.\textsuperscript{18} The specimens were stained in 1 h steps in a series of graded alcohols (80\%, 80\%, 95\%, 95\%, 100\%, and 100\%) combined with the basic fuchsin under vacuum and embedded in MMA.

Two to three coronal sections were acquired from each vertebral body with slice thicknesses sectioned at 80, 60, and 80 \(\mu\)m. Leftover blocks were saved and the order of cuts relative to the remaining block was recorded, along with the blade thickness and amount of polishing done between each section for later use in the registration process. Each histology slide was scanned twice at 20\(\times\) optical zoom using a Mirux digital slide scanning system at the Hospital for Sick Children, Toronto, to attain plain light and green filtered fluorescence images. Locations of linear microcracks were manually identified from the basic fuchsin histology slides to evaluate the presence or absence of mechanically induced microdamage on individual trabeculae.

**Histology-\(\mu\)CT Registration**

Additional \(\mu\)CT images were acquired of each undecalcified thick section histology slide as well as the remaining block saved from the sectioning process. Thresholding bone from the glass slides yielded 3D volumetric data (slices 60–80 \(\mu\)m thick) of each bone section used for the microdamage analysis.

With the remaining block as a reference point, the \(\mu\)CT images of the slides were registered to the unloaded \(\mu\)CT data sets through initial manual alignment followed by a rigid affine registration using mutual information (Amira Dev 5.2.2 module).\textsuperscript{19} This registration allowed the sectioned histology slice to be accurately localized within the unloaded \(\mu\)CT data set, prior to \(\mu\)FE model generation. The novelty of this technique is that post analysis, the precise section of the histology section may then be extracted from the \(\mu\)FE model for direct comparison to histologically identified microdamage.

**\(\mu\)FE Model Generation**

The \(\mu\)FE models were generated based on the unloaded \(\mu\)CT images (AmiraDev 5.2.2). A threshold was applied to each data set (at the 14 \(\mu\)m\(^3\) voxel size) to initially segment the whole vertebrae. A value equal to the average intensity of the histogram of each segmentation was then used to threshold the bone from the surrounding non-bone tissue. Following this, the images were down-sampled to 33 \(\mu\)m\(^3\). A voxel based meshing algorithm was used to convert the segmented isotropic bone voxels directly into eight-noded hexahedral elements of edge length 33 \(\mu\)m. (\(\sim\frac{1}{2}\) the mean trabecular thickness, as stipulated by convergence studies), to reduce computational requirements while maintaining sufficient accuracy.\textsuperscript{11–13,20–24}

**\(\mu\)FE Load/Boundary Conditions**

Deformable image registration analysis was implemented to provide the vector displacement of the selected boundary surfaces (bony endplates and facet joints) of the central vertebra of the loaded motion segment to each \(\mu\)FE model.\textsuperscript{19} Each set of loaded-unloaded \(\mu\)CT images was initially manually aligned to a principal axis. A rigid affine registration using a quasi Newton optimizer was applied to each image set followed by a multi-resolution deformable registra-
tion algorithm. The method optimizes an affine mapping in 12 degrees of freedom to ideally match up the unloaded to the loaded scan. A four level pyramid scheme was used in which the unloaded scan is partitioned and the pieces individually registered using the affine transform found for registering the previous level as its initial guess. The output of this analysis yields an axial displacement field determined from the deformation of the unloaded scan with respect to the loaded scan. Based on this output, an axial displacement vector was assigned to each node of the identified loading surfaces (50,000–80,000 nodes representing the endplate and facets) from corresponding locations within the displacement field.

Analysis
Abaqus Standard v6.10-1 (ABAQUS) was used for the μFE analysis. Each analysis was executed as a linear, static model on a supercomputer at the Centre for Computational Biology High Performance Facility, Toronto. The output parameters extracted from the μFE models were stress (von Mises, maximum principal and minimum principal) and strain (maximum principal, minimum principal, and axial (zz)).

The maximum and average stress and strain values over the span of elements for locations containing fuchsin stained trabecular microdamage ($n = 20$) and adjacent locations lacking microdamage ($n = 20$) were recorded.8,12 Approximately 8–15 elements were selected that corresponded to the area spanning the damaged region, with a similar number of elements selected in close proximity for the neighboring undamaged bone. A One-Sample Kolmogorov–Smirnov Test was performed to determine whether the stress and strain data were normally distributed. A series of independent $t$-tests were then applied to compare the μFE results (stresses and strains) in all the damaged regions to all the undamaged regions.

Figure 4. (a) Pre-existing microdamage labeled with calcein (white arrow), with no mechanically induced damage present (below, yellow arrow) following basic fuchsin staining. (b) Conversely, some damage exists as labeled with calcein green (white arrow), with damage propagated, as evident on the basic fuchsin staining following mechanical compression (yellow arrow).

Figure 5. (a) A single surface generated from the coronal section histology μCT scan perpendicular to the transverse μCT of the unloaded data demonstrating the alignment of trabeculae at the intersection of the two planes. (b) Three sections aligned to the unloaded μCT data.
regions. Paired \( t \)-tests were performed to assess the differences in stress and strain between damaged and undamaged bone in local regions (i.e., along a single trabecula).

**RESULTS**

**Microdamage Identification**

Mechanically induced microdamage was labeled within whole vertebrae using the described sequential labeling technique. The stained images show the ability of both the calcein and basic fuchsin stains to penetrate the cortical shell of the vertebral body and label damage of the most interior trabeculae (Fig. 4). The fuchsin stain identifies damage initiated and propagated as a result of the applied uniaxial compression.

**Histology to \( \mu \)CT Registration**

The surface matching algorithm was able to align the volumetric \( \mu \)CT images of the histology block and slides with the intact unloaded scans based on their outer surface geometry and curvature. Recording the order of slides during the sectioning process and an assumption of parallel slices with similar separation distances allowed the alignment of multiple slices (two to three sections per vertebra, Fig. 5). Following the registration of all slices within the 3D \( \mu \)CT scan, individual trabeculae visible in the histology were easily identified within the \( \mu \)CT data sets. Quantitatively, an average volumetric concurrency of 73\% (range: 60–84\%) was found between the \( \mu \)CTs of the histology slides and the extracted equivalent sections from the whole \( \mu \)CT data sets (\( n = 8 \) sets).

**Stresses and Strains Corresponding to Histological Microdamage**

Following completion of the \( \mu \)FE analysis, the elements corresponding to the individual histology slices were successfully extracted from each whole \( \mu \)FE mesh (Fig. 6). The One-Sample Kolmogorov–Smirnov Test showed stress and strain values from the \( \mu \)FE models satisfied the normal distribution assumption justifying the use of the independent and paired \( t \)-tests for the statistical analyses. In comparing histologically damaged to undamaged regions in the \( \mu \)FE models, significantly higher von Mises stress (max) (153\%), von Mises stress (avg) (150\%), and maximum and minimum principal strains (max) (156\% and 142\%, respectively) were found in the damaged versus un-
damaged regions \( (p < 0.05 \text{ for all}) \) (Table 1). Similarly, in paired \( t \)-tests (comparing adjacent regions) von Mises stress (max) (207\%), von Mises stress (avg) (184\%), and maximum and minimum principal strains (max) (192\% and 233\%, respectively) were significantly elevated in the damaged versus undamaged regions \( (p < 0.05 \text{ for all}) \). The paired \( t \)-test also indicated significantly higher axial (zz) average strain (100\%) in the damaged areas. (Table 1).

**DISCUSSION**

This work has demonstrated that physiologically load-
ed \( \mu \)FE models of whole vertebrae preferentially exhib-
it higher stresses and strains in areas of histologically
identified microdamage. In previous work on bovine
trabecular bone cores subjected to uniaxial compres-
sion, Nagaraja et al. reported elevated maximum
principal stresses and strains in comparing damaged to
undamaged bone (max principal stress—damaged:
144–219 MPa, undamaged: 89–100 MPa; max principal
strain—damaged: 0.86–1.24\%, undamaged: 0.34–
0.86\%).12 In the present study, similar differences were
found between the average values of the maximum
principal stress in damaged and undamaged bone (74
and 42 MPa, respectively) and maximum principal
strains in damaged and undamaged bone (0.9\% and
0.6\%, respectively).12 \( \mu \)FE work assessing overall maxi-
mum principal stress in rat vertebral trabecular bone
by Boyd et al., yielded a similar mean value of
122 MPa for boundary conditions simulating a 1% applied strain.29 In the histology, there was a greater
amount of damage located at the endplate regions,
corresponding to larger areas of high stress and strain
in the \( \mu \)FE models, similar to previous findings in the
literature.23 Though this study does not present a
novel finding in that \( \mu \)FE stresses and strains are
higher in regions of damaged bone, it expands the
application of \( \mu \)FE-histology analyses beyond simple
trabecular cores, demonstrating that such findings can
be extended to trabeculae in whole bone samples.

The present study did not attempt to predict the
value at which damage initiation occurs in rat verte-
brae, but rather quantified stresses and strains from
\( \mu \)FEA in damaged versus undamaged bone. The ability
of the von Mises criterion to best represent difference
between the damaged and undamaged regions of bone
aligns with the work of Peterlik et al.30 who elegantly
demonstrated that bone tissue is a composite material
that exhibits ductile fracture behavior. The values at
which damage initiates may lie somewhere between

Table 1. Average Stress and Strain Results From the \( \mu \)FEA for Damaged and Undamaged Bone Regions

<table>
<thead>
<tr>
<th>Stress</th>
<th>Damaged (MPa)</th>
<th>Undamaged (MPa)</th>
<th>Independent t-test</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Mises</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max:</td>
<td>263 ± 94</td>
<td>172 ± 79</td>
<td>0.002*</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Avg:</td>
<td>173 ± 71</td>
<td>115 ± 53</td>
<td>0.006*</td>
<td>0.0006*</td>
</tr>
<tr>
<td>Max principal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max:</td>
<td>128 ± 134</td>
<td>85 ± 64</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>Avg:</td>
<td>74 ± 87</td>
<td>42 ± 39</td>
<td>0.14</td>
<td>0.05*</td>
</tr>
<tr>
<td>Min principal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max:</td>
<td>−207 ± 104</td>
<td>−160 ± 102</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>Avg:</td>
<td>−108 ± 77</td>
<td>−96 ± 67</td>
<td>0.62</td>
<td>0.50</td>
</tr>
<tr>
<td>ZZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max:</td>
<td>−1.1 ± 1.0%</td>
<td>−0.8 ± 0.9%</td>
<td>0.32</td>
<td>0.087</td>
</tr>
<tr>
<td>Avg:</td>
<td>−0.7 ± 0.6%</td>
<td>−0.5 ± 0.5%</td>
<td>0.23</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

\( \pm \), represents one standard deviation from the mean for stress and strain.

\*Indicates \( p < 0.05 \).
the damaged and undamaged ranges (i.e., von Mises stress range of 172–263 MPa or a maximum principal strain range of 0.9–1.4%). There are however limitations in determining such a cutoff with the use of this elastic model, as the incorporation of a damage initiation threshold may lead to significantly larger/smaller stresses. Further analysis could focus on the identification of a cut-off based on stress and/or strain values or a damage criterion from μFE analyses that best represents the initiation of histological damage. As well, while pre-existing microdamage was visualized in the calcein staining of the vertebrae, the presence of this microdamage was not incorporated into the current μFE models. Although the amount of pre-existing microdamage was not quantified, qualitatively it appeared to be much lower than the amount of load induced microdamage present in the vertebrae. Its impact on model behavior would be important to evaluate in future work.

This research presents a robust method for validating the ability of μFE modeling to quantify trabecular damage in whole rat vertebral bodies. The sequential labeling process with the use of calcein green followed by basic fuchsin histologically identified mechanically induced damage in whole rat vertebrae. A novel aspect of this research is the adaptation of previously developed staining procedures to whole vertebrae as opposed to trabecular cores or vertebrae with removed endplates or posterior elements. This staining method may be used in combination with mechanical testing and image-based models to evaluate identification of microdamage in whole bones. Using the described technique, there is no need to modify the sample prior to staining, enabling more “physiologic” loading (i.e., through the intervertebral discs). Physiologic loading however presents challenges in the quasi-static analyses required for sequential μCT imaging as stress relaxation, particularly in the intervertebral discs, may alter the quasi-static loading applied to the central vertebra. Larger amounts of displacement may have been applied to the vertebral endplates than were represented by the loaded μCT images.

As well, it is important to note that although stain penetration was complete, it was not totally homogeneous throughout each sample, making it difficult to count and categorize overall damage morphology or measure crack length. While it is recognized that diffuse damage is an important mechanism in microdamage, linear microcracks, the most severe form of microdamage, were most evident in the histology slides, and as such were utilized for the comparisons. While diffuse damaged can be observed in this work, further optimization of this staining procedure may allow for improved visibility of damage and quantification of microdamage.

Previous studies have described challenges in aligning 2D histologic sections to 3D μCT trabecular bone data sets using 2D registration algorithms. μCT imaging of undecalcified thick sections allowed accurate registration of the histology and μCT data through 3D volumetric registration in six degrees of freedom. Accurate registration of histology to the full sample was essential to allow direct comparison of the histologically labeled damage with the μFE results.

Acquiring μCT images of the histology slides allowed registration to the unloaded models using equivalent imaging parameters. This greatly increases the amount of data that can be used and facilitates the registration process. The volumetric concurrency achieved, ranging from 60% to 84%, was sufficient to identify common features (trabeculae) present in both the histology and μFE models needed to perform the microdamage—stress/strain comparisons. Errors in the volumetric concurrency measure may have arisen due to inaccuracies in processing of the segmentation of the thick sections (i.e., differentiation of bone from the glass and glue of each slide). Future work with higher resolution μCT images could help to improve this registration technique, as a greater number of elements would be acquired that span the thickness of the slide.

Despite the successful utilization of the image registration code for the determination of the loading conditions, it was not possible to analyze image based strain patterns in individual trabeculae using this approach. The current code could not be adapted to utilize enough pyramid levels to break the image up into the number of blocks required to attain trabecular level strains. Improvement to the registration technique to evaluate image-based strains may incorporate feature-based algorithms that determine the junction point of diverging trabeculae in an unloaded scan and find these corresponding points in the loaded scan. Image registration of unloaded to loaded scans can represent true skeletal deformation and has the potential to act as the gold standard in the validation of trabecular level deformation and strain in μFE models.

Overall, the μFE models generated higher stresses and strains in damaged regions of vertebral bone across the full sections and locally along individual trabeculae. This information may be important both in determining weak points in a given bone and in predicting the location of failure on individual trabeculae. The accumulation of microdamage results in an overall weakness of bone, and a cascade effect of trabecular microfractures may ultimately lead to whole bone failure. The ability of these μFE models to localize high stresses and strains in damaged trabecular bone and evaluate the structural integrity of individual trabeculae may motivate future utilization of computational modeling to study failure mechanisms. μFE modeling may further enable analysis with respect to specimen specific structural differences that occur within healthy and pathologic vertebrae under complex loading scenarios.
ACKNOWLEDGMENTS
This study was supported by the Canadian Institutes for Health Research and the Ontario Graduate Scholarships in Science and Technology. The authors thank the Burr lab for help with the fuchsin staining protocol, the University of Toronto Dentistry histology lab for histologic sectioning and the High Performance Facility at the Centre for Computational Biology at the Hospital for Sick Children for the computational μFE analyses.

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