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Comparison of Strain Measurement in the Mouse Forearm Using Subject Specific Finite Element Models, Strain Gaging, and Digital Image Correlation

Mark Begonia^a, Mark Dallas^b, Mark L. Johnson^b, and Ganesh Thiagarajan^a

^aUniversity of Missouri-Kansas City, Department of Civil and Mechanical Engineering, 350K Robert H. Flarsheim Hall, 5110 Rockhill Road, Kansas City, MO 64110, USA

^bUniversity of Missouri-Kansas City, School of Dentistry, Department of Oral and Craniofacial Sciences, Room 3143, 650 E 25th Street, Kansas City, MO 64108, USA

Abstract

Mechanical loading in bone leads to the activation of bone forming pathways in the osteocyte, which is most likely associated with a minimum strain threshold being experienced by the osteocyte. To investigate the correlation between cellular response and mechanical stimuli, researchers must develop accurate ways to measure/compute strain both externally on the bone surface and internally at the osteocyte level. This study investigates the use of finite element (FE) models to compute bone surface strains on the mouse forearm. Strains from three FE models were compared to data collected experimentally through strain gaging and digital image correlation (DIC). Each FE model was assigned subject-specific bone properties and consisted of one-dimensional springs representing the interosseous membrane. After three-point bending was performed on the ulnae and radii, moment of inertia was determined from microCT analysis of the bone region between the supports and then used along with standard beam analyses to calculate the Young's modulus. Non-contact strain measurements from DIC were determined to be more suitable for validating numerical results than experimental data obtained through conventional strain gaging. When comparing strain responses in the three ulnae, we observed a 3–14% difference between numerical and DIC strains while the strain gage values were 37–56% lower than numerical values. This study demonstrates a computational approach for capturing bone surface strains in the mouse forearm. Ultimately, strains from these macroscale models can be used as inputs for microscale and nanoscale FE models designed to analyze strains directly in the osteocyte lacunae.

Keywords

Strain; strain gage; forearm; mechanical loading; digital image correlation; finite element analysis

Corresponding Author: Ganesh Thiagarajan, University of Missouri-Kansas City, 350K Robert H. Flarsheim Hall, 5110 Rockhill Road, Kansas City, MO 64110, Phone: + 1 816 235 1288, ganesht@umkc.edu.

⁶Conflict of Interest

The authors declare that they have no conflict of interest.

1. Introduction

The activities of both osteoblasts and osteoclasts are regulated by osteocytes, which comprise 90–95% of all bone cells and are considered the primary mechanosensory cell in bone. Under mechanical loading conditions, osteocytes are believed to initiate cellular responses that involve the β -catenin signaling pathway (Bonewald and Johnson 2008). Recent data from mouse forearm loading experiments has shown that β -catenin signaling can be initiated within 1 hour after axial compressive loading and that subsequent to activation, the signaling from the osteocytes propagates through the dendritic network towards the bone surface where formation is predicted to occur. Interestingly, this activation occurs in a heterogeneous and random pattern only within a subpopulation of osteocytes. In other words, some osteocytes activate while others do not despite their close proximity to one another (Lara-Castillo et al. 2015).

The observed heterogeneous pattern of osteocyte activation in response to load suggests that osteocytes must experience a minimum strain threshold to initiate mechanotransduction. In order to identify the mechanism for heterogeneous osteocyte activation, researchers must develop an accurate method of measuring strains not only on the bone surface, but also internally at the osteocyte level. One method for achieving this goal is through the use of finite element (FE) models since they offer more flexibility in strain measurement compared to conventional experimental techniques such as strain gaging.

In a previous study, we simulated dynamic loading in an FE model of the mouse forearm consisting of the ulna, radius, and interosseous membrane (IOM) (Thiagarajan et al. 2014). Differences in numerical strains were determined to correspond with the experimental parameters (e.g. preload and loading frequency) and the IOM stiffness values assigned (2.5 – 15 N/mm). However, this previous work was limited by its analysis of a single FE model and its implementation of mechanical properties from literature. Therefore, the objectives of the current study were to (1) develop 3 FE models with the interosseous membrane and subject-specific properties incorporated, (2) quantify the differences in the numerical strains of each FE model based on the subject-specific properties assigned (e.g. Young's modulus), and (3) determine the extent in which the FE models are validated better through digital image correlation (DIC) versus traditional strain gaging. The eventual goal of this work is to apply the numerical strains from these macroscale FE bone models as inputs for microscale and nanoscale FE osteocyte models and subsequently identify the minimum strain threshold required for osteocyte activation.

2. Methods

This section presents a chronological outline of both the experimental and FE modeling efforts in this study. First, we excised forearms from euthanized mice and then removed the skin and extraneous muscle tissue. Forearms were scanned via microCT and then subjected to two sets of axial loading experiments. The first series of experiments utilized the DIC system since it employed a non-contact technique for determining strains. The same forearms were then strain gaged in a second series of experiments under the same axial loading conditions as those in the previous DIC experiments. Finally, the ulna and radius

were separated and then each subjected to three-point bending tests as part of a biomechanical analysis procedure used for calculating the Young's modulus. We also conducted another series of experiments on different mouse forearms in order to determine the elastic stiffness of the interosseous membrane between the radius and the ulna.

FE modeling and simulations involved four steps. First, a 3-D solid model of the loading cap was created in Solidworks (Dassault Systemes, Waltham, MA). Second, 3-D solid models of the forearm bones were created using microCT image stacks imported into Mimics® (Mimics Research v17.0; Materialise, Plymouth, MI). Third, volume meshes were generated in 3-matic® (3-matic Research v9.0; Materialise, Plymouth, MI) using the aforementioned 3-D models. Finally, we imported the volume meshes into FEBio Software Suite (Musculoskeletal Research Laboratories, Salt Lake City, UT) to perform finite element analysis (FEA) and calculate average strains that were compared with the strain values obtained from both DIC and strain gage experiments.

2.1. Digital Image Correlation (DIC)

The procedure for performing digital image correlation (DIC) has also been reported previously (Begonia et al. 2015). Briefly, a high precision air brush (Model 200NH, Badger Air-Brush Co., Franklin Park, IL) was used to apply a speckle pattern of black, opaque, water-based paint (Createx Colors, East Granby, CT) on both the medial and lateral surfaces of each forearm. A two camera DIC system recorded high-definition (HD) videos of forearm loading experiments at 30 frames per second at lens magnifications of 2x, 3x, and 4x. Tests were conducted at a frequency of 0.2 Hz for 5 cycles to a peak load of 2.25 N using a Bose ElectroForce 3220 system. After converting videos to individual frames, MATLAB® based DIC software calculated the displacements and derived strains for regions-of-interest (ROI) located at the midshaft of both the ulna and radius. The DIC strains reported in this study represent the average peak-to-peak strain calculated from the individual peak-to-peak strains of the second, third, and fourth loading cycles.

The original code for the MATLAB® based DIC software was developed by Elizabeth Jones (Jones 2013; Jones et al. 2014) but our group has since modified the code by implementing the Savitzky-Golay filter, which smooths the displacement and strain data based on the loading frequency and number of frames per cycle. In addition to strain maps, the DIC software calculates a strain history plot with each x-value representing a single image (i.e. frame number) from the uploaded image stack and each y-value representing the average strain over the assigned ROI boundary (i.e. spatial average). We apply the Savitzky-Golay filter to smooth the displacement history plot in order to facilitate the numerical computation of the peak-to-peak strain response which uses the displacement history data.

2.2. Strain Gage Experiments

The procedure for strain gaging mouse bones has been reported previously (Begonia et al. 2015). Briefly, forearm specimens were harvested from 4.5 month old female TOPGAL mice ($n=3$). After mice were euthanized, the skin and muscle tissue were removed from both the ulna and radius. Each forearm was wrapped in PBS-soaked gauze and then stored at -20°C . Prior to testing, forearms were thawed to room temperature while rehydrating in

PBS. Experimental strain measurements were collected from strain gages (EA-06–015DJ-120/LE, Vishay Precision Group, Malvern, PA) attached to the medial surface (Robling et al. 2003). Strain gages were positioned approximately 1–2 mm proximal to the midshaft of each bone. Each strain gage was trimmed down using a new razor blade under a binocular microscope. A multimeter was used to check the resistance before and after testing. After the muscle tissue was removed using micro scissors and forceps, a cotton swab dipped in acetone was used to remove any remaining soft tissue from the bone. This step was taken to ensure that the gage adhered properly and tightly to the curved bone surfaces. Before the gage was attached, a small bead of glue was placed on the bone region. Preliminary experiments revealed that applying too much glue can yield strain results that may be more indicative of the glue properties rather than the bone underneath it. Although other strain gaging protocols recommend using polyurethane coating, we elected not to use it because strain gages in past experiments did not work properly after applying the coating. A cotton swab dipped in PBS was used to moisten the lateral bone surface prior to collecting strain data. Axial compressive loading experiments were performed using a Bose ElectroForce 3220 system (TA Instruments, ElectroForce Systems Group, Eden Prairie, MN). The test protocol included a preload of 0.3 N followed by a maximum applied load of 2.25 N for 5 cycles at a frequency of 0.2 Hz.

2.3. Biomechanical Analysis

Three-point bending experiments were performed to determine the Young's modulus separately for the radii ($n=3$) and ulnae ($n=3$) of the TOPGAL mice that were previously scanned by microCT and then used to develop the FE models. Each bone was positioned across two supports, which were spaced 7.65 mm apart, and with the medial surface facing upward. After detecting a preload of 0.5 N, the crosshead of the Bose system travelled at a displacement rate of 0.1 mm/s until the bone fractured. After obtaining the stiffness from the resulting load-displacement curves, we calculated Young's Modulus (E) using the following equation:

$$E = \frac{SI^3}{48I}$$

where S represents the stiffness, I represents the span (i.e. distance between the supports), and I represents the area moment of inertia about the anterior-posterior axis.

The BoneJ plug-in for ImageJ (National Institutes of Health, Bethesda, MD) was used to realign the μ CT scans with respect to the longitudinal axis of the bone and to calculate the area moments of inertia for multiple microCT slices across the span (Doubé et al. 2010; Javaheri et al. 2014; Schneider et al. 2012). This realignment procedure was performed for each bone to ensure that the cross-sectional slices were perpendicular to the longitudinal axis and thus did not alter the moment of inertia calculation. We used the average moment of inertia of eleven slices near the midshaft rather than a single microCT slice at the midshaft. The average moment of inertia was calculated from the moment of inertia at the mid-shaft as well as ten moment of inertia values that correspond to five microCT slices distal and five microCT slices proximal to the mid-shaft. These ten additional microCT slices were spaced

out equally across the span. We believe that this procedure produces a more suitable average moment of inertia since it represents the behavior of a larger proportion of the bone during testing.

2.4. Finite Element Modeling

FE models of forearms were built from microCT images of 4.5 month old female TOPGAL mice ($n=3$). TOPGAL mice (DasGupta and Fuchs 1999) were selected due to their ability to exhibit activation of the β -catenin signaling pathway through histological staining with β -galactosidase. These mice were maintained in our colony on a mixed C57Bl/6 X CD1 background. As a first step, microCT images were collected at an in-plane resolution of 10.5 μm with axial slices spaced 10.5 μm apart (vivaCT 40, SCANCO Medical AG, Zurich, Switzerland). The microCT image set was then imported into Mimics® (Mimics Research v17.0; Materialise, Plymouth, MI) where the cortical and trabecular regions of the radius and ulna were segmented using the thresholding option. Masks of the marrow cavities were also generated for each bone and a three-dimensional model of the forearm was generated. The entire 3-D forearm model was imported as an STL format file into 3-matic® (3-matic Research v9.0; Materialise, Plymouth, MI), where the corresponding surface meshes were smoothed and converted to volume meshes. The loading cap was created in Solidworks (Dassault Systemes, Waltham, MA) and also imported into 3-matic as an STL format file. The positioning options in 3-matic were used to accurately position the loading cap with respect to the bone. A volume mesh was also created for the loading cap, and all volume meshes were composed of 10-node tetrahedral elements. FE models of Forearm #1, #2, and #3 consisted of 135,870, 200,734, and 194,796 elements, respectively. In addition, Forearm #1, #2, and #3 consisted of 224,603, 329,187, and 322,220 nodes, respectively. Other studies have demonstrated the effectiveness of using tetrahedral elements in FE simulations of bone (Ramos and Simões 2006). In a previous work (Lu et al. 2012), mesh sensitivity analysis showed convergence for mesh sizes that were similar to those in this study.

Each forearm model was realigned with the longitudinal axes of the bones oriented vertically along the z-axis and with the distal end positioned underneath the concave surface of the loading cap. The volume meshes of both the forearm and the loading cap were then imported into the FEBio Software Suite (Musculoskeletal Research Laboratories, Salt Lake City, UT) for assignment of loading parameters, material properties, and boundary conditions. A fixed boundary condition was applied to selected nodes on the proximal end of the forearm model, and one-dimensional springs were used to simulate the interosseous membrane connecting the ulna and radius. Two springs (1400 N/mm) were inserted at the distal and proximal ends of the forearm while a series of 18 additional springs were inserted along the midshaft. Stiffness values ranging from 2.5 N/mm to 15 N/mm have been used in previous studies (Lu et al. 2012; Tejwani et al. 2005; Thiagarajan et al. 2014), but the IOM for all three FE models was assigned a stiffness of 15 N/mm. Therefore, each IOM spring was assigned an individual stiffness of 0.83 N/mm. Although experiments were performed with a preload of 0.3 N and maximum load of 2.25 N at a frequency of 0.2 Hz for 5 cycles, FE simulations were set up to analyze only one-half of a single loading cycle to reduce computational time.

Strains from both DIC and finite element analysis (FEA) were calculated by taking a spatial average from a region of interest (ROI) that was located on the medial surface of the bone and covered a cross-sectional area of approximately 0.52 mm x 0.38 mm (i.e. size of sensing grid on strain gage). The spatial average was calculated by computing the average strain value from all the individual strains observed within the ROI boundary selected in either the DIC software or the FE model. The same technician who performed the strain gaging procedure assisted with specifying the location and size of the ROIs during DIC and FEA. Before DIC analysis was performed, videos of mouse bone experiments were converted into image stacks using a custom MATLAB script, and the technician marked the ROI boundaries on the subsequent image stacks. Before the strain response in the FE models was analyzed, the technician marked the approximate location for the sensing grid on the model and then we used the Pt. Distance tool in PostView (FEBio Software Suite, Musculoskeletal Research Laboratories, Salt Lake City, UT) to outline the ROI and measure the distances between nodes thus ensuring that all the selected elements were within the 0.52 mm x 0.38 mm ROI boundary. These tasks were carried out to enable a suitable comparison between the average strain values obtained from strain gaging, DIC, and FEA.

The interosseous membrane (IOM) between the ulna and radius has been represented as elastic springs in this study. A spring sensitivity study was also performed to analyze the change in the numerical strain as a function of the number of IOM springs used. Four variations of each FE model were developed using 18, 24, 30, or 36 1-D springs to represent the interosseous membrane. The purpose was to determine if increasing the number of 1-D springs substantially altered the numerical strains of the ROIs on the medial surfaces of both the ulna and radius.

3. Results

3.1. Biomechanical Analysis

Figure 1 shows the area moment of inertia about the anterior-posterior axis for the ulnae and radii of forearms that were selected for microCT and FE modeling. Differences in moment of inertia were observed based on the forearm, bone, and distance in relation to the midshaft. For example, Forearm #1 produced a 1.5x higher moment of inertia at the ulna midshaft compared to Forearm #3, which exhibited the lowest moment of inertia ($\sim 0.004 \text{ mm}^4$). At the radius midshaft, Forearm #2 produced a 1.4x higher moment of inertia compared to Forearm #3, which yielded the lowest moment of inertia ($\sim 0.007 \text{ mm}^4$). Moment of inertia values also ranged from 0.003 to 0.005 mm^4 at the distal support and from 0.023 to 0.034 mm^4 at the proximal support for the ulna. In addition, biomechanical analysis of the radius led to moment of inertia values ranging from 0.008 to 0.01 mm^4 at the distal support and from 0.002 to 0.003 mm^4 at the proximal support.

Table 1 shows the subject-specific material properties that were assigned to the ulna and radius of each FE model. The Young's modulus for each bone was obtained from the biomechanical analysis procedure that utilized three-point bending, microCT, and BoneJ. Poisson's ratios of 0.30 and 0.33 were assigned to the ulna and radius, respectively, for all 3 FE models.

3.2. FE Modeling – Comparison with DIC and Strain Gaging

Figure 2a shows the haversine waveform for one cycle of loading applied to each FE model while Figure 2b and Figure 2c show the digital image correlation (DIC) strain contours on the medial surfaces of the radius and ulna, respectively. The non-uniform DIC strain maps were relatively heterogeneous compared to the predominantly homogenous strain distributions observed in the FE models. Figure 3 shows the medial surface for all three FE forearm models at one-half of a loading cycle. In contrast to the DIC strain maps, the corresponding FE strain maps were more uniform for both the ulna and radius. Furthermore, midshaft ROIs (0.52 mm x 0.38 mm) showed differences in the average peak-to-peak strain among all 3 FE models.

Figure 4 shows the average peak-to-peak strains obtained from strain gaging, DIC, and FEA. After comparing both strain measurement techniques, we found that the strain gage method yielded lower standard deviations ($\pm 30 \mu\epsilon$) compared to those from the DIC method ($\pm 500 \mu\epsilon$). However, we also found that the average FE strains were better matched with DIC strain measurements than with strain gage measurements. The differences between FE and DIC average strains were only 14.2%, 10.7%, and 3.3% for the ulnas of Forearms #1, #2, and #3, respectively. Similarly, the radius of Forearm #3 exhibited only a 0.6% difference between FE and DIC strains. Conversely, the radii of Forearms #1 and #2 produced FE strains that were 38% and 31% lower than the corresponding DIC strains, respectively.

3.3. FE Modeling – Spring Sensitivity Analysis

Figure 5 shows the average FE strains for ROIs on the (a) medial ulna and (b) medial radius of Forearm #3 according to the number of 1-D springs used to simulate the interosseous membrane. Based on the spring sensitivity analysis, we found that steadily increasing the number of springs led to minimal changes in strains at the midshaft of either bone. In Forearm #1, doubling the number of springs from 18 to 36 yielded numerical strains that varied by only 0.8% in the ulna and 2.8% in the radius. Forearm #2 and Forearm #3 also exhibited similar trends in which the numerical strains varied minimally for each bone. Forearm #2 showed only a 1.6% change in the ulna and a 1.2% change in the radius whereas Forearm #3 produced decreased strains of 0.6% and 0.8% for the ulna and radius, respectively.

4. Discussion and Conclusions

4.1. Biomechanical Analysis

Figure 1 shows that the area moments of inertia differed among forearms at the midshaft of each bone. The area moments of inertia were also region-specific since the value changed depending on whether the bone regions near the distal support or proximal support were analyzed. These variations were incorporated into an average moment of inertia calculation that accounted for the entire span (i.e. 7.65 mm) rather than a single moment of inertia value corresponding to the center of the bone where the load was applied. As shown previously in Equation (1), these variations in the area moments of inertia can influence the Young's modulus, and any changes to this parameter would subsequently affect the strain response as well.

As shown in Table 2, FEA of all three forearm models confirmed that decreases in strain were due to increases in the Young's modulus. Compared to Forearm #1, Forearm #2 was assigned higher modulus values for the ulna (1.5%) and radius (17.3%), which led to numerical strains that were subsequently lower for the ulna (5.1%) and (6.4%) radius. Similarly, Forearm #3 was assigned even higher modulus values for the ulna (14.6%) and radius (27.2%), which yielded numerical strains that were lower for the ulna (17.5%) and radius (12.3%). Although these results were expected since a linear analysis was applied, it was important to quantify the extent in which the average strain changed due to the subject-specific properties assigned to each FE model.

4.2. FE Modeling – Comparison with DIC and Strain Gaging

Figure 5 shows that numerical strains from the FE models were in better agreement with DIC strains versus strain gage readings for most cases. As demonstrated in our previous study (Begonia et al. 2015), the underestimates of strain associated with the strain gage method could be attributed primarily to the process of strain gage attachment, which artificially stiffens the bone and restricts its deformation to a small extent. Meanwhile, the non-contact feature of the DIC technique allows the bones to deform naturally under mechanical loading conditions. DIC and FE strains matched well for most cases. In two cases, however, the radii produced average DIC strains that were higher than average FE strains by approximately 1180 $\mu\epsilon$ in Forearm #1 and 820 $\mu\epsilon$ in Forearm #2. One potential reason for the larger differences between the FE and DIC radial strains of Forearms #1 and #2 is the absence of the paw, which may have caused the force from the loading cap to be transmitted primarily to the ulnas rather than the radii. Overall, comparisons among the three strain measurement methods confirm that the FE approach is better validated experimentally through DIC as opposed to strain gages.

To demonstrate how strain gage placement could affect the strains measured, we performed DIC analyses on additional ROIs aside from the original ROIs located at the midshaft of the ulna and radius. The additional ROIs were located approximately 0.5 mm distal and 0.5 proximal to the midshaft ROI. Table 3 provides a summary of the DIC analysis performed on the three ROIs for each bone.

4.3. FE Modeling – Spring Sensitivity Analysis

Multiple FE simulations were performed to determine if the numerical strains on the medial bone surfaces were influenced by the number of 1-D springs used to represent the interosseous membrane. We determined that steadily increasing the number of springs from 18 to 36 altered the numerical strains by no more than 1.4% in the ulnae and 1.5% in the radii. These minimal changes in strain demonstrate that fewer springs are required to simulate the interosseous membrane when compared to previous work (Thiagarajan et al. 2014). Furthermore, assigning springs in a non-crosshatch pattern was determined to be more time-efficient compared to the procedure used in the previous study, which applied a crosshatch pattern of over 80 springs. Since the number of springs representing the interosseous membrane had a minimal effect on the numerical strains, we concluded that differences between the three FE forearm models were attributed primarily to variations in bone geometries as well as the material properties assigned.

4.4. Effect of Variation of Bone Geometry

To examine the effects of the bone geometry on the strain response of each FE model, we performed additional simulations by assigning the same properties (e.g. densities and Young's moduli) to all three FE models. Forearm #1 and Forearm #2 were given the same properties as Forearm #3. Forearm #3 had the highest Young's modulus for both the ulna and radius. Table 4 shows the medial strain responses of each bone when mouse-specific properties were assigned and after identical properties were reassigned. Strains were measured at the midshaft of each forearm and bone. In the 'Identical Properties Assigned' section of the table the order of the Moment of Inertia (MOI) for each bone value is ordered based on the midsection value shown in Figure 1. The letter 'H' indicates that the forearm had the highest moment of inertia (MOI) value, 'I' indicates that it has an intermediate value and 'L' indicates that it had the lowest MOI value for that particular bone.

4.5. Concluding Remarks

Previous research involving the mouse ulna (Lara-Castillo et al. 2015) has shown that mechanical loading leads to activation of the β -catenin signaling pathway (i.e. osteocyte activation) in some osteocytes but not all those adjacent to the activated cells. This observation led to the hypothesis that the osteocyte possesses a minimum strain threshold that it must experience before it can activate various pathways associated with biological responses to mechanical loading. Before developing complex FE models that incorporate osteocytes as inclusions and therefore are more likely to produce heterogeneous and random strain fields, we performed this macromodel study with homogeneous, linear elastic, isotropic models to gain a sense of how other factors (e.g. subject-specific material properties) affect the strain response. The eventual goal is to develop micro FE models that predict strains in the osteocyte lacunae whereas the focus of the current study is on the development and validation of macro FE models that possess unique features such as the interosseous membrane and individualized material properties.

This study also highlights the benefits of utilizing both FE modeling and DIC for strain measurement in the mouse forearm instead of the widely established strain gage technique. Similar studies have been performed to explore viable alternatives to measuring strains in mouse bones. One group recently developed a technique for analyzing subject-specific strains by using only microCT data, thus avoiding the typical requirements of forearm loading studies such as a load-strain calibration or a finite element model (Wagner et al. 2013). This same group then performed a follow-up study to develop an image-based computational technique for measuring ulna strains and to confirm that the strain gage method underestimates the surface strains generated during mechanical loading (Norman et al. 2015). Other studies have also acknowledged the limitations of strain gaging and subsequently utilized digital image correlation to measure bone surface strains in mouse loading experiments involving the forearm (Begonia et al. 2015) and the tibia (Carriero et al. 2014; Sztefek et al. 2010).

Table 3 shows that even a 0.5 mm offset from the targeted region of analysis (i.e. midshaft ROI) on the mouse forearm yields different DIC strain results. The differences observed among the three forearms analyzed in this study can be attributed to the inherent variations

in their bone geometry as well as the heterogeneous properties of each bone. In some cases, the distal ROIs exhibited the highest DIC strain while other cases showed the midshaft ROIs producing the highest DIC strain. These observations could be attributed not only to the bone structure and properties, but also to the subtle differences in the orientation of the flexed paw when it experiences the cyclic loading. The DIC analysis data in Table 3 demonstrates the practical benefits of the DIC method over the traditional strain gaging method. One major advantage is the ability to analyze bone strains through a non-contact approach, which facilitates multiple strain measurements on the same specimen rather than requiring a series of strain gage experiments to be performed on the same specimen and inevitably stiffening the bones in the process. Another major advantage of the DIC technique is its ability to validate the strain response in multiple regions of an FE model rather than being restricted to the midshaft of the bones, where most strain gages are typically applied.

Based on the results in Table 4, we concluded that the differences in FE strain could be attributed to the variations in bone geometry. In addition, the curvatures of the ulna and radius could slightly alter the loading experienced by each forearm. Even though properties such as the Young's modulus affect the strains generated by each FE model, the data shown in Table 4 confirms that the unique bone structure of each FE model can also influence the strain response of both the ulna and radius. Furthermore, if distinctive bone geometries are to be included when analyzing multiple FE models, it may be advantageous for researchers to consider also incorporating properties specific to each specimen from which the models are based. Although the variation in microstrain for the three FE models was only 550 $\mu\epsilon$ for the ulnae and only 230 $\mu\epsilon$ for the radii, adopting this approach is recommended since a multiscale FE modeling scheme would utilize outputs from macroscale FE models as inputs for microscale and nanoscale FE models. Previous studies have used idealized FE osteocyte models to examine the strains generated in osteocyte structures such as the lacunae and canaliculi as well as the perilacunar region (Nicolella et al. 2006; Rath Bonivitch et al. 2007; Wang et al. 2015). These studies concluded that the strain magnification factor (i.e. local maximum strain $\div$ applied global strain) correlated with various factors such as the perilacunar tissue modulus and loading frequency. However, the accuracy of the microstructural tissue strains predicted in the lacunae would ultimately be dependent on the accuracy of the applied macroscopic deformations, which are influenced by the subject-specific heterogeneous properties assigned to each bone.

The current study also possesses a few limitations that should be acknowledged. One experimental limitation is the lack of multiple strain gage attachment sites, which would have provided additional strain measurements for regions distal and proximal to the midshaft of each bone. However, this approach was not employed due to the surface coverage of a single strain gage, which would prevent the proper attachment of three strain gages since a portion of one strain gage would inevitably be attached to a portion of another strain gage instead of the bone surface. Furthermore, rather than collecting all strain measurements simultaneously, we would be limited to running three separate experiments on each forearm specimen in a process that would likely stiffen the bones due to the constant attachment and detachment of strain gages. One modeling limitation worth noting is that the three FE forearm models did not incorporate the osteocyte lacunae distribution thereby not fully capturing the heterogeneous properties of the bone matrix. Since future nanoscale and

microscale FE models will incorporate structural features of the lacunae, the macroscale FE models of the forearms in this study were simplified to volume meshes comprising only bone and marrow cavities. There were no distinctions among cortical bone, trabecular bone, and osteocytes embedded within the bone matrix. However, these models could be further enhanced through the assignment of material properties that were based on bone density or voxel intensity information provided by microCT. This approach would not only incorporate heterogeneous properties into the FE forearm models, but also potentially yield non-uniform strain contours that would coincide with those captured through DIC.

In this study, we performed *ex vivo* cyclic loading tests on mouse forearms and then compared experimental strains obtained from DIC and strain gaging methods with numerical strains observed in the corresponding FE models. Perhaps not too surprisingly, given the known material heterogeneity in bone and geometry differences from one region of bone to another, we observed regional differences in modulus detectable by DIC that were not discernable from strain gage methods. A biomechanical analysis technique that features three-point bending, microCT, and ImageJ was utilized to analyze differences in the area moment of inertia for each bone and to calculate the Young's moduli that was assigned to the FE models. Our results showed that the FE models, which were each assigned distinctive properties at first, were better validated through the DIC methodology than through strain gaging. A separate DIC analysis confirmed that relocating the ROI either 0.5 mm distal or 0.5 mm proximal to the original midshaft ROI will alter the DIC strain measurement, thus providing an indication of how variable strain gage placement could affect the bone surface strains measured. A secondary analysis of the FE models revealed that steadily increasing the number of 1-D springs, which collectively represented the interosseous membrane, did not appreciably affect the strain response. Lastly, a third series of simulations was performed with all three FE models possessing identical properties, and we determined that the strain response was influenced by the variations in their bone geometries. The objective of the current study was to develop macromodels and validate them experimentally to gain a better understanding of the strain magnitudes that should be applied eventually to micromodels that are currently under development and will be used to predict strain levels in the osteocyte lacunae. Future studies will aim to correlate the surface strains from these macroscale FE models of forearms with the lacunae strains observed in both microscale and nanoscale FE models of osteocytes. By utilizing this multiscale FE modeling approach, we can identify the minimum strain threshold that osteocyte lacunae must experience to initiate mechanotransduction and then determine if osteocytes gradually lose their mechanosensitivity with aging.

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Fig. 1. Tension experiments performed on a separate set of forearms ($n=3$) yielded stiffness values of 12.1, 7.1, and 9.6 N/mm in Forearm #1, #2, and #3, respectively. These experimental stiffness values were within a range that was similar to previous work (Thiagarajan et al. 2014)

Fig. 2.
Comparison of area moments of inertia about the anterior-posterior (A/P) axis for (a) ulnae and (b) radii as a function of distance from the midshaft. Negative distances denote distal locations whereas positive distances denote proximal locations

Fig. 3. Comparison of strain contours observed on the medial bone surfaces of FE models of the mouse forearm. The negative signs denote compression while the legends show strain values ranging between 0 and -0.004 (i.e. between 0 and $-4000 \mu\epsilon$). Strain contours are shown at one-half of a loading cycle for (a) Forearm #1, (b) Forearm #2, and (c) Forearm #3

Fig. 4. Comparison of strains measured from finite element (FE) models, strain gaging and digital image correlation (DIC). In general, average FE strains from the FE models were better matched with the ulna strains measured by DIC than by strain gaging

Fig. 5. Comparison of average FE strains for ROIs located at the midshaft on the medial surfaces of the (a) ulna and (b) radius after performing a spring sensitivity analysis of Forearm #3

Table 1

Summary of subject-specific properties assigned to each bone in all 3 FE models

Forearm No.	Bone Selection	Young's modulus (GPa)
1	Ulna	19.1
2	Ulna	19.4
3	Ulna	21.9
1	Radius	12.1
2	Radius	14.2
3	Radius	15.4

Table 2

Summary of the Young's modulus assigned and numerical strain measured at the midshaft in the bones of each forearm. The negative signs indicate compression.

Forearm No.	Bone Selection	Young's Modulus (GPa)	Numerical Strain ($\mu\epsilon$)
1	Ulna	19.1	-3185
2	Ulna	19.4	-3021
3	Ulna	21.9	-2627
1	Radius	12.1	-1856
2	Radius	14.2	-1736
3	Radius	15.4	-1626

Table 3

Comparison of DIC strains in each forearm and each bone based on the ROI location analyzed. All strain values are shown in microstrain ($\mu\epsilon$) and the negative signs indicate compression.

Bone	Forearm #1			Forearm #2			Forearm #3		
	Midshaft	Distal	Proximal	Midshaft	Distal	Proximal	Midshaft	Distal	Proximal
Ulna	-3730	-3924	-2774	-3382	-2918	-2908	-2475	-3362	-2806
Radius	-3116	-3720	-2907	-2501	-3554	-2929	1744	-3453	-2264

Table 3 shows that both the ulna and radius in Forearm #1 produced DIC strains that were higher at the distal ROIs and lower at the proximal ROIs when compared to the midshaft ROI. Forearm #2 also showed a higher DIC strain at the distal ROI on the radius but not in the ulna. The highest DIC strain in Forearm #2 was observed at the midshaft ROI instead. Although Forearm #3 generated its highest DIC strains at the distal ROIs of both the ulna and radius, the lowest DIC strains were observed at the midshaft ROIs rather than the proximal ROIs.

Table 4

Comparison of FE strains on the medial ulna and medial radius based on the Young's moduli assigned to each bone. All strain values are shown in microstrain ($\mu\epsilon$) and the negative signs indicate compression.

Bone	Mouse-Specific Properties Assigned			Identical Properties Assigned		
	Forearm 1	Forearm 2	Forearm 3	Forearm 1	Forearm 2	Forearm 3
Ulna	-3185	-3021	-2627	-2528 (H)	-2639 (I)	-2627 (L)
Radius	-1856	-1790	-1626	-1417 (I)	-1522 (H)	-1626 (L)

Table 4 shows that Forearm #1 and Forearm #2 experienced lower FE strains after they were assigned the same properties as those given to Forearm #3. In Forearm #1, the FE strain of the medial ulna decreased by 20% while the medial radius decreased by 23%. Similarly, Forearm #2 exhibited FE strains that were 12% lower in the medial ulna and 15% lower in the medial radius. From the 'Identical Properties Assigned' section of the data, the lower strain can be explained by an increased MOI values for all cases except the radius of Forearm 'I'. The disproportional decreases in strain across each forearm and bone could be attributed to other factors such as differences in the bone cross-sectional areas among forearm specimens. Table 5 summarizes the cross-sectional areas for each bone and forearm. In a similar format as Table 4, Table 5 also shows the letters 'H', 'I', and 'L' to indicate which strain values corresponded with the highest, intermediate, and lowest cross-sectional areas, respectively. As evidenced by the differences in MOI values and bone cross section measurements, variations in bone geometry can also contribute to the strain responses observed in each forearm model.

Table 5

Comparison of cross-sectional areas in the midshaft regions of each bone and forearm. Negative signs indicate compression.

Bone	Cross-Sectional Area (mm ²)			Strains ($\mu\epsilon$) with Identical Model Properties Assigned		
	Forearm 1	Forearm 1	Forearm 1	Forearm 1	Forearm 2	Forearm 3
Ulna	0.2830	0.2795	0.2669	−2528 (H)	−2639 (I)	−2627 (L)
Radius	0.2839	0.2687	0.2554	−1417 (H)	−1522 (I)	−1626 (L)