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Measurement of microstructural strain in cortical bone

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Abstract

It is well known that mechanical factors affect bone remodeling such that increased mechanical demand results in net bone formation, whereas decreased demand results in net bone resorption. Current theories suggest that bone modeling and remodeling is controlled at the cellular level through signals mediated by osteocytes. The objective of this study was to investigate how macroscopically applied bone strains similar in magnitude to those that occur *in vivo* are manifest at the microscopic level in the bone matrix. Using a digital image correlation strain measurement technique, experimentally determined bone matrix strains around osteocyte lacuna resulting from macroscopic strains of approximately 2,000 microstrain (0.2%) reach levels of over 30,000 microstrain (3%) over fifteen times greater than the applied macroscopic strain. Strain patterns were highly heterogeneous and in some locations similar to observed microdamage around osteocyte lacuna indicating the resulting strains may represent the precursors to microdamage. This information may lead to a better understanding of how bone cells are affected by whole bone functional loading.

Keywords

Microdamage; bone strain; mechanotransduction

Introduction

Mechanical factors affect bone remodeling such that increased mechanical demand results in net bone formation, whereas decreased demand results in net bone resorption (Jee et al. 1983; Rubin and Lanyon 1984; Rubin and Lanyon 1985; Wronski and Morey 1983). It is believed that the coordinated actions of osteoclasts and osteoblasts control bone modeling and remodeling at the cellular level in response to mechanical signals mediated by osteocytes. This phenomenon implies that bone cells can detect and respond to their mechanical environment by altering their biological and biochemical activities (mechanotransduction). Previous studies suggest that osteocytes, due to their location within the bone matrix, are the best candidates for sensing mechanical stress (Lanyon 1993). The most prominent of the proposed mechanical signals are stress-generated fluid flow forces acting on cells, and bone matrix deformation itself. Moreover, a well-accepted current theory is that bone cells are more responsive to fluid flow than to mechanical strain. Recent experiments indirectly support this conclusion; whereby osteoblastic cells were found to increase their production of osteopontin (OPN) mRNA, prostaglandin (PGE₂), and nitric oxide (NO) in response to fluid flow in contrast to cells deformed to mechanical strain levels similar to those measured *in vivo* on a macroscopic scale (Owan et al. 1997). However, in contrast to these latter experiments, when osteoblastic cells

are subjected to substrate strains levels many times greater than those measured *in vivo*, increased biological activity again results (You et al. 2000).

Typical maximal *in vivo* strains in humans have been measured, and found to range from around 1,200 $\mu\epsilon$ (principal compressive strain) to about 1,900 $\mu\epsilon$ (maximum shear strain) (Burr et al. 1996). In these experiments, strains were measured on the surface of the tibia using a strain gage that covered an area of several square mm, which would typically encompass thousands of bone cells. The strains thus measured are only averages over the total area covered by the gage, and yield no information regarding variations in the global strain field that necessarily must result from naturally occurring microstructural inhomogeneities in the bone matrix. Such irregularities in cortical and trabecular bone constitute 'stress risers': geometric defects around which local stresses are expected to be significantly higher than the global applied stress. In bone, material irregularities such as osteons, canaliculi, and especially osteocyte lacunae, are all potential stress risers, and the strains associated with these are likely to be significantly different from average global strains. In fact, using an optical displacement mapping technique, we have demonstrated that strains in cortical bone matrix are significantly increased in the vicinity of the tip of a microcrack compared to the expected macroscopic strain (Nicolella et al. 2001). Thus, we hypothesize that local strain maxima resulting from these stress concentrations will constitute the true effective strains potentially influencing bone cells within their microstructural environments.

Recent experiments have shown that early cyclic stress-induced damage appears to localize itself around osteocyte lacunae (Reilly 2000). Interestingly, damage does not appear to initiate around all lacunae, nor is it usually symmetric about a given lacuna. Regardless, the consequences of damage, as noted by several investigators, may be significant for osteocytes residing within affected lacunae. They may in fact suffer injury, or even undergo apoptosis, if their environment is sufficiently compromised.

The foregoing observations seem to support the following hypothesis. In response to global stress (cyclic or monotonic), strain is expected to localize around natural stress concentrators, such as lacunae, in bone. Since they are asymmetric, and oriented at random with respect to any particular global stress axis, different lacunae would be expected to localize strain to different degrees. Strain levels at many lacunae should exceed the global applied strain, and if strain levels become sufficiently high, local cracking (microdamage) may occur, while some lacunae should undergo little or no strain magnification. The purpose of this paper is to investigate these hypotheses as they relate to cortical bone.

Methods

Cortical bone test specimens were prepared from fresh bovine tibial bone. Tensile specimens were fabricated by pinning and clamping milled longitudinal sections between two hardened steel patterns, and milling by hand the final specimen geometry. Specimens were machined using a miniature milling machine and were continuously irrigated during the machining process. After machining, the surface of each specimen was polished with successively finer grit paper beginning with 500 grit, using a 30 N normal force for 15 s; 1200 grit, using a 30 N normal force for 30 s; and 4000 grit, using a 30 N normal force for 15 s. Next, specimens were polished twice with 3 μm diamond paste on a polishing cloth for 30 s with a 30 N normal force. The finished samples were wrapped in gauze, stored in calcium-buffered saline, and frozen at approximately -21°C until tested. Immediately prior to testing, a single strain gage (EA-06-062AQ-350, Measurements Group Inc., Raleigh, NC) was applied to one surface of the sample on centerline and oriented longitudinally; this strain gage was used to measure the global, macroscopic strain applied to the specimen. Since the gage area was approximately $1.5 \times 1.5\text{-mm}$, the reported (strain gage) strain is an average over an area of 2.25 mm^2 . The

specimen was loaded in tension using a custom microscopy loading frame (Kammrath and Weiss, Germany) under displacement control to increasing levels of macroscopic strain as determined by the mounted strain gage. During loading, the specimen surface opposite the gage was imaged at either $\times 200$ or $\times 500$ total magnification using an optical microscope (Zeiss Universal Model 24, Carl Zeiss Inc, Thornwood, NY); images were acquired using a digital camera (Kodak MegaPlus 4i, Eastman Kodak Company, Rochester, NY) at 1312×1032 pixel resolution.

The captured unloaded versus loaded (captured at various global strains up to approximately 2,000 to 3,000 $\mu\epsilon$) image pairs were analyzed using a digital stereoimaging technique (Nicolella et al. 2001; Williams et al. 1980), which quantified the local deformation and strain fields. The displacement search procedure is controlled by a normalized image correlation algorithm

$$r(u, v) = \frac{N \sum_i I_i M_i - \left(\sum_i I_i \right) \left(\sum_i M_i \right)}{\sqrt{\left[N \sum_i I_i^2 - \left(\sum_i I_i \right)^2 \right] \left[N \sum_i M_i^2 - \left(\sum_i M_i \right)^2 \right]}}$$

where $r(u, v)$ defines the degree of correlation between the image areas, I is the target (stressed) image region, M is the model (reference) image region, M_i is the model pixel location (x_i, y_i), I_i is the target pixel location ($u + x_i, v + y_i$), N is the number of pixels in the model image where i ranges from 1 to N . The trained image area is a square array of pixels (with sides N) around each measurement grid point. A correlation value of $r = 1$ indicates a perfect match, $r = 0$ no correlation, and $r = -1$ is a perfect mismatch (Franke et al. 1991). This algorithm determines the position of the deformed material points with respect to the undeformed image. Rotations are not accounted for with this algorithm, however, the images are aligned manually to remove rigid body rotations prior to analysis. All image processing functions are performed with custom software running on a personal computer.

Regions selected for analysis consisted of osteocyte lacunae and other microstructural features, as well as areas of otherwise flawless bone matrix. The digital stereoimaging parameters were determined for each specimen imaged to ensure optimal displacement and strain resolution. A local image model size (M) of 51×51 pixels was used for the image based displacement and strain measurement and resulted in a strain resolution of approximately 35 $\mu\epsilon$ (Nicolella et al. 2001). Strain fields were measured over the field of view of the image and, more locally, at specific microstructural features (Figure 1).

Results

Osteocyte lacunae that were sectioned during preparation of the specimen are clearly visible on the surface of the bone specimen (Figure 1). Also visible are unsectioned osteocyte lacunae that lie slightly below the surface of the specimen and therefore appear light in color compared to the surrounding bone matrix.

The average microstructural strain measured over the images area was consistent with the average macroscopic specimen strain measured with the strain gage. At several load points, the average microstructural strain is greater than the macroscopic strain, possibly due to local inhomogeneities in the bone matrix and strain concentrating effects of the local microstructure. The local microstructural strain was measured over an image area representing approximately 400 μm^2 while the strain gage measured strain is an average over 2.25 mm^2 (Figure 2).

Bone matrix strain around osteocyte lacunae on the surface, as well as near lacunae visible below the surface, typically reached levels of approximately 12,000 – 16,000 $\mu\epsilon$ and peak local strains exceeded 30,000 $\mu\epsilon$ within one lacuna diameter of an osteocyte lacuna (Figure 3A –

C). Measured strain fields were similar for both lacunae cut on the surface as well as intact lacunae below the surface. A region of strain localization that bridges two lacunae illustrates flaw interaction effects (Figure 3A). In this region, strains are on the order of 16,000 $\mu\epsilon$, while strains in the surrounding area are significantly lower (0–5,000 $\mu\epsilon$). In a relatively homogeneous area of the micrograph, the bone matrix strain is similar to the global strain except for regions where a few lacunae are present. It should be noted that strain magnitudes around some lacunae were not significantly elevated above the average matrix strain.

Figure 4 shows the (areal) frequency of strains measured over an area of $200 \times 200 \mu\text{m}$, irrespective of defects that clearly are responsible for most of the variations observed. The mean strain measured is approximately 7,900 $\mu\epsilon$, a factor of 3.95 greater than the global applied strain (2,000 $\mu\epsilon$) and a likely measure of the average lacunar ‘strain magnification’ effect. The largest strains measured (39,000 $\mu\epsilon$), exceed the average deformation by a factor of nearly 20 times.

Discussion

The local displacement and strain fields in cortical bone subjected to global macroscopic strains (typical of *in vivo* measured strains in humans) were measured using a digital image correlation technique. Digital micrographs included osteocyte lacunae visible both on and directly below the prepared surface. Local strains in the bone matrix surrounding lacunae were highly inhomogeneous and reached levels of up to 30,000 microstrain (3%); these were caused by a macroscopic average strain of only approximately 2,000 microstrain (0.2%).

The high levels of strain measured in bone matrix associated with the osteocyte lacuna are consistent with analytical estimates for strain concentration factors for elliptical defects in a material. The estimated strain concentration factor for an elliptical defect in an infinite plate is given by (Peterson 1974;

$$\epsilon_L = \epsilon_G \left(1 + 2 \left(\frac{a}{b} \right) \right)$$

where ϵ_L is the local strain, ϵ_G is the globally applied strain, a is the minor axis of the ellipse, and b is the major axis of the ellipse. Using an average minor axis dimension of 3.86 μm and an average major axis dimension of 8.86 μm , the strain concentration factor is 1.88. The variability in the size and shape of each osteocyte lacuna will contribute to the variability in the measured strain magnification. Furthermore, the presence of canaliculi radiating from the lacuna will alter the apparent tissue strain (and therefore the strain concentration factor) as compared to the analytical estimate since the analytical estimate assumes the material is a continuum.

There are several points to be made that may affect the interpretation of the analytical predicted strain concentration factors and the measured microstructural strains. First, lacunae are not purely ellipsoidal; their shapes vary and the lacunar walls are not smooth; canaliculi emanating from the lacunar wall will result in additional discontinuities. The resolution of our optical microscopy imaging system does not allow individual canaliculi to be resolved. Thus, a deformation of the canaliculi (e.g., increasing the diameter of the canaliculi), is interpreted as a continuum deformation (and strain), which will misleadingly increase the measured bone matrix strain around a lacunae. Although we have not explicitly measured the effect of a lacunae being within the ‘influence zone’ of a larger microstructural feature such as a Haversian or Volkmann canal, one could hypothesize that the global strain is multiplied twice: once by the Volkmann canal resulting in an increased global strain acting on the local lacunae, which acts as a local stress riser.

The detection of strain concentrations around osteocyte lacunae in the present investigation is consistent with the observation of microdamage in the form of microcrack clusters associated with lacunae generated under equivalent loading conditions. Reilly (2000) has shown that for bovine, equine, and human bone subject to similar monotonic loading conditions, microcrack clusters tend to be associated with lacunae, although could not conclude that lacunae caused the microcracks to form. The cracks tend to orient themselves normal to the tensile stress axis, and perpendicular to the long axis of the lacunae. Cracks were observed to be produced (by a few cycles of loading) at global strains as low as 2,500 $\mu\epsilon$, similar to the present monotonic global strain level (2,000 $\mu\epsilon$).

Similarly, Burr et al. (1985) observed that after 10,000 cycles at a global strain of 1500 $\mu\epsilon$, highly significant ($P < 0.005$) differences were observed in microdamage levels in canine radii and ulnae as compared with uncycled controls. On the other hand, for an applied strain of 625 $\mu\epsilon$, no statistically significant difference in the level of microdamage was observed.

If the 'strain multiplier' relating global strain to mean lacunar strain is taken to be 3.95, then it can be estimated, based on the results of Burr et al. (1985), that essentially no microcracks are produced at lacunar cyclic strains as high as 2469 $\mu\epsilon$ ($3.95 \times 625 \mu\epsilon$). Via the same reasoning, microcracks would be expected at lacunar strains on the order of 10,000 $\mu\epsilon$. Thus, the present mean lacunar strain of 7900 $\mu\epsilon$ must be close to, or even slightly above, the strain threshold for low cycle ($< 10,000$ cycles) fatigue microcracking.

This means that microfracture occurs within a local 'plastic zone' whose size should scale with that of the individual lacunae. This is directly analogous to traditional considerations of the plastic zone associated with a notch or hole in an elastic plate, wherein the size of the yielded region is usually a significant fraction of the hole diameter or notch root radius.

These zones may constitute the basis of the observation by Burr et al. (1997), that bones during flexure lose significant stiffness prior to observation of so-called 'linear' microcracks. This stiffness loss has been interpreted in terms of finer-scale levels of matrix damage that must pre-exist the cracks. As noted by Bentolila et al. (1998), both Boyce et al. (1998) and Schaffler et al. (1996) showed that fatigue of bone produces focal patches characterized by increased permeability to fuchsin stain, apparently due to the observed fact that they are composed of very fine-scale sublamellar matrix damage. According to Burr et al. (1997), such diffuse damage would correspond to collagen fiber-matrix disbonding, disruption of the mineral collagen aggregate, and collagen fiber failure; i.e. ultra-structural pre-crack damage.

Eventually (with fatigue cycles) or at a critical strain (under monotonic loading) microcracks will nucleate within the plastic damage zone attending a lacuna (Prendergast & Huiskes 1996). The present work suggests that this occurs at a critical local strain on the order of 8000 $\mu\epsilon$. Reilly (2000) has noted that such microcracks do not tend to grow; instead other (likewise non-growing) cracks nucleate within the same plastic damage zone, and eventually form a microcrack cluster near the lacuna. As these clusters develop, they may begin to interact and linkup, a process that clearly would be facilitated by the linkup of local strain enclaves.

It is not surprising that a crack that nucleates within the lacuna 'plastic zone' might tend not to grow. During its nucleation, the crack is dominated by the plastic strain associated with the lacuna itself. As the tip of an embryonic microcrack moves away from the lacuna, the crack opening plastic strain contribution from the plastic zone itself drops off, so that the principal crack driving force becomes the stress intensity of the microcrack tip, which is proportional to the square root of the length of the microcrack. Since the latter is extremely small, the resulting stress intensity may be insufficient to generate the crack opening (plastic displacement) required to produce further increments of crack growth.

In a similar vein, the maximum strains that were measured in association with lacunae were so large (39,000 $\mu\epsilon$) that they merit consideration as to their actual physical meaning. That is, these may not be true matrix strains, but rather a strain-representation of discrete displacements generated by the opening(s) of a microcrack network within a local damage zone. The combined compliance of this synergistic set would tend to reduce the crack opening requirement for any one crack, and to stabilize them all against individual crack growth (Tada et al. 1985). It also would exceed the true ductility of uncracked bone matrix.

It is worth noting that despite the fact that the strains induced near lacunae in bone clearly have profound influence on local damage development, their magnitudes really are not large compared to the deformation levels required to affect cellular expression. During fluid-flow experiments designed to measure such expression in MLO-Y4 osteocytic cells, for example, much larger cellular deformations, on the order of 25% (Nicolella et al. 1999), typically are generated. In addition, matrix strain transmission to osteocytes would be attenuated by the compliant nature of the cellular attachments to the wall of the lacuna. On this basis, it might be anticipated that matrix strain, as such, may play a secondary role at best in affecting osteocyte signaling.

However, it has been noted (Noble & Reeve 2000) that strain-nucleated microdamage may well cause cell injury, or even cause the osteocyte to undergo apoptosis (Qui et al. 1997). Either or both of these events may be a critical step in the process by which osteocytes signal for damaged bone removal by osteoclastic resorption (Verborgt et al. 2000). It also has been suggested (Prendergast & Huiskes 1996) that rather than damaging the osteocytes, local microcracking may relieve local strains (via the compliant crack-opening scenario discussed earlier), and thereby trigger remodeling signals from the resident osteocytes.

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Figure 1.

Optical micrograph of the surface of a cortical bone specimen imaged at a total magnification of $\times 500$. Osteocyte lacunas that have been sectioned during specimen preparation are visible on the surface as well as lacuna that lie just below the specimen surface. Superimposed on the image is the grid at which displacement and strain measurements were made. This coarse measurement grid was used to measure the average strain in the imaged area. Original magnification, $\times 500$.

Figure 2. Macroscopic stress – strain behavior versus the local microscopic strain response. Good agreement is seen between the macroscopic and the microstructural strain response. The microstructural strain response was calculated using the measurement grid shown in Figure 1.

Figure 3.

Microstructural strain field overlaid on a digital micrograph of a cortical bone specimen that was loaded to a global strain of 2,095 $\mu\epsilon$ in the horizontal direction. Each color represents a specific level of maximum principal strain as indicated by the legend. These contours of equal maximum principal strain indicate the complexity of the resulting microstructural strain field. Local peri-lacunar strain reaches peaks of over 35,000 $\mu\epsilon$. Original magnification, $\times 500$.

Figure 4.

The distribution of microstructural maximum principal strain in bone varies considerably with respect to the applied macroscopic strain. The average maximum principle strain over the area measured in Figure 3A is approximately 7,900 $\mu\epsilon$ and ranges to over 37,000 $\mu\epsilon$.