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Microstructural strain near osteocyte lacuna in cortical bone *in vitro*

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Abstract

Mechanical factors affect bone remodeling such that increased mechanical demand results in net bone formation, whereas decreased demand results in net bone resorption. Two proposed mechanical signals are stress-generated fluid flow forces acting on cells and bone matrix deformation itself. A prominent current theory is that bone cells are more responsive to fluid flow than to mechanical strain. Recent experiments support this conclusion: bone cells increase their production of osteopontin (OPN) mRNA, prostaglandin (PGE_2), and nitric oxide (NO) in response to fluid flow in contrast to cells stimulated by mechanical strain levels similar to those measured *in vivo*. However, when cells are subjected to substrate strains levels many times greater than those measured *in vivo*, increased biological activity again results. We assert that it is neither fluid flow nor matrix deformation per se, but rather the resulting cell deformation that causes cell biological response. Machined specimens of undamaged bovine cortical bone were subjected to increasing levels of macroscopic strain while observed under an optical microscope at 220X. Continuum level strain was measured using a standard foil strain gauge attached to the back of the specimen and ranged from 500 to 6,000 microstrain. Images of the specimen surface at each strain level were captured. To determine the level of osteocyte deformation that results from fluid flow *in vitro*, MLO-Y4 cells were cultured on collagen coated 190 cm^2 plastic sheets and subjected to steady fluid flow at 16 dynes/cm^2. Images representing the initial undisturbed cell configuration and the configuration of the cells after ten minutes of fluid flow were acquired from a videotape of the flow experiment. The captured unloaded vs. loaded image pairs were analyzed to determine the local deformation and strain fields using a digital stereoimaging system. When subjected to a nominal continuum strain level approximately equal to that measured in humans *in vivo* during rigorous activity (2,000 microstrain), the local, osteocyte level strains can be as high as 12,000 to 15,000 microstrain (1.2% to 1.5%). Average osteocyte strains due to fluid flow *in vitro* increase from 7,972 microstrains after 16 seconds of flow to 22,856 microstrains after 64 seconds of flow. In contrast, maximum strains measured *in vivo* are approximately 1,800 microstrain in humans and up to 3,000 microstrain in other species. These data may help to explain why bone cells are more sensitive to fluid flow than substrate strain; fluid forces result in cell deformations much higher than those considered to be "physiological".

Keywords: Bone Mechanics, Bone Strain, Micromechanics, Osteocyte Lacuna

Introduction

Mechanical factors affect bone remodeling such that, for example, increased mechanical demand results in net bone formation, whereas decreased demand results in net bone resorption[1-4]. It is believed that the coordinated actions of osteoclasts, osteoblasts, and osteocytes control bone modeling and remodeling at the cellular level in response to mechanical signals. Due to their location within bone matrix, previous studies suggest that the osteocytes are the best candidates for sensing mechanical stress[5,6]. Two proposed mechanical signals are stress-generated fluid flow forces acting on cells and bone matrix deformation itself. A prominent current theory is that bone cells are more responsive to fluid flow than to mechanical strain. Recent experiments support this conclusion: bone cells increase their production of osteopontin (OPN) mRNA, prostaglandin (PGE_2), and nitric oxide (NO) in response to fluid flow in contrast to cells stimulated by mechanical strain levels similar to those measured *in vivo*[7-9]. However, when cells are subjected to substrate strains levels many times greater than those measured *in vivo*, increased biological activity again results.

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In vivo, the osteocyte deformation resulting from bone matrix deformation is unknown. Similarly, while the forces resulting from fluid flow across a cell necessarily cause the cell to deform, this deformation likewise is unknown. We assert that it is neither fluid flow nor matrix deformation per se, but rather the resulting cell deformation that causes cell biological response. Our hypothesis is that strain at the microstructural level in cortical bone varies significantly over macroscopically measured strains and specifically that in general, bone matrix strains around osteocyte lacunae are much greater than the average, macroscopically measured strain.

Methods

Machined and polished specimens of undamaged bovine cortical bone were subjected to increasing levels of macroscopic (continuum level) strain while observed under an optical microscope (Zeiss Model 24, Carl Zeiss Inc, Thornwood, NY) at 220X magnification. Continuum level strain was measured using a standard foil type strain gauge attached to the back of the specimen and ranged from 500 to 6000 microstrain. Images of the specimen surface at each strain level were captured using a CCD camera (DXC 151, Sony Electronics) and a microcomputer (Power Macintosh, model 8600, Apple Computer) running NIH image. To investigate the effects of bone damage (microcracks) on the deformation and strain fields near osteocytes, bovine cortical bone specimens containing microcracks were prepared. The initial crack was created by pre-cracking a flat, edge-notched longitudinally oriented piece of cortical bone with a razor blade; the razor blade was slowly inserted into the notch until a crack approximately 250 μm in length was created at the notch tip. To determine the level of osteocyte deformation that results from fluid flow *in vitro*, MLO-Y4 cells were cultured on collagen coated 190 cm^2 plastic sheets and subjected to steady fluid flow at 16 dynes/cm^2. Images representing the initial undisturbed cell configuration and the configuration of the cells after ten minutes of fluid flow were acquired from a videotape of the flow experiment.

The captured unloaded vs. loaded image pairs were analyzed to determine the local deformation and strain fields using a digital stereoimaging system[10,11]. The optimal digital stereoimaging parameters were determined for each specimen imaged, ensuring optimal displacement and strain resolution. Using a local strain size of 51 X 51 pixels, a strain resolution of approximately 35 με was achieved[10].

Results

In vitro cortical bone microstructural strains

When subjected to a nominal continuum strain level approximately equal to that measured in humans *in vivo* during rigorous activity (2,000 microstrain or 0.2%), the local, osteocyte level strains can be as high as 12,000 to 15,000 microstrain (1.2 to 1.5%). Furthermore, the magnitude of the local tissue strains on a cellular scale vary widely about the average globally applied strain, ranging from –6,000 microstrains (compression) to over 16,000 microstrains (tension). As the magnitude of the globally applied strain is increased, the distribution peak shifts towards higher strains while the magnitude of the strain variability increases.

Effect of micro damage on micro structural strains

Strains at the tips of the microcrack reached 30,000 microstrain (3.0%) locally, which are up to 15 times greater than the continuum level strains measured *in vivo*. Microdamage alters the local strain field thus increasing the magnitude of strain available to nearby osteocytes. These results indicate tissue strains sensed by osteocytes can be much greater than macroscopic strains measured using strain gauges.

In vitro cell deformation due to fluid flow

The cell strains resulting from steady fluid flow-induced shear stress of 16 dynes/cm^2 (1,6 Pa) were quantified at 16, 32, 40, 48, and 64 seconds. Average osteocyte strains increase from 7,972 microstrains after 16 seconds of flow to 22,856 microstrains after 64 seconds of flow. Maximum strains in the cell populations studied reached 23,300 microstrains at 16 seconds to 174,400 microstrains at 64 seconds. In contrast, maximum strains measured *in vivo* are approximately 1,800 microstrain in humans and up to 3,000 microstrain in other species. These data may help to explain why bone cells are more sensitive to fluid flow than substrate strain; fluid forces result in cell deformations much higher than those considered to be “physiological”.

Conclusions

1. Local osteocyte level strains in undamaged cortical bone can be over five times greater than measured continuum level strains.
2. When microdamage is present, local osteocyte level strains can be up to 3.0% close to the microcrack.
3. Osteocyte deformations resulting from fluid flow generated shear stress *in vitro* varies significantly among cells and can be as high as 3.0%.

References

1. Wronski TJ, Morey ER. Inhibition of cortical and trabecular bone formation in the long bones of immobilized monkeys. Clin Orthop 1983; (181)269-276.
2. Jee WSS, Wronski TJ, Morey ER, Kimmel DB. Effects of space flight on trabecular bone in rats. Am J Physiol 1983; 244:R310-R314.
3. Rubin CT, Lanyon LE. Regulation of bone formation by applied dynamic loads. J Bone Joint Surg Am 1984; 66:397-402.

4. Rubin CT, Lanyon LE. Regulation of bone mass by mechanical strain magnitude. Calcif Tissue Int 1985; 37:411-417.
5. Lanyon LE. Osteocytes, strain detection, bone modeling and remodeling. Calcif Tissue Int 1993; 53:Suppl 1:S102-S106.
6. Mullender MG, Huiskes R. Osteocytes and bone lining cells: which are the best candidates for mechano-sensors in cancellous bone? Bone 1997; 20:527-532.
7. Owan I, Burr DB, Turner CH, Qiu J, Tu Y, Onyia JE, Duncan RL. Mechanotransduction in bone: osteoblasts are more responsive to fluid forces than mechanical strain. Am J Physiol 1997; 273:C810-C815.
8. Smalt R, Mitchell FT, Howard RL, Chambers TJ. Induction of NO and prostaglandin E2 in osteoblasts by wall-shear stress but not mechanical strain. Am J Physiol 1997;273:E751-758.
9. Smalt R, Mitchell FT, Howard RL, Chambers TJ. Mechanotransduction in bone cells: induction of nitric oxide and prostaglandin synthesis by fluid shear stress, but not by mechanical strain. Adv Exp Med Biol 1997; 433:311-314.
10. Nicolella DP, Nicholls AE, Lankford J, Davy DT. Machine Vision Photogrammetry: a technique for measurement of microstructural strain in cortical bone. J Biomech 2001; 34:135-139.
11. Williams DR, Davidson, DL, Lankford J. Fatigue-crack-tip plastic strains by the stereoimaging technique. Exper Mech 1980; 20:134-139.