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The effects of tensile-compressive loading mode and microarchitecture on microdamage in human vertebral cancellous bone

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ABSTRACT

The amount of microdamage in bone tissue impairs mechanical performance and may act as a stimulus for bone remodeling. Here we determine how loading mode (tension vs. compression) and microstructure (trabecular microarchitecture, local trabecular thickness, and presence of resorption cavities) influence the number and volume of microdamage sites generated in cancellous bone following a single overload. Twenty paired cylindrical specimens of human vertebral cancellous bone from 10 donors (47–78 years) were mechanically loaded to apparent yield in either compression or tension, and imaged in three dimensions for microarchitecture and microdamage (voxel size $0.7 \times 0.7 \times 5.0\ \mu m^3$). We found that the overall proportion of damaged tissue was greater ($p=0.01$) for apparent tension loading ($3.9 \pm 2.4\%$, mean $\pm$ SD) than for apparent compression loading ($1.9 \pm 1.3\%$). Individual microdamage sites generated in tension were larger in volume ($p < 0.001$) but not more numerous ($p=0.64$) than sites in compression. For both loading modes, the proportion of damaged tissue varied more across donors than with bone volume fraction, traditional measures of microarchitecture (trabecular thickness, trabecular separation, etc.), apparent Young's modulus, or strength. Microdamage tended to occur in regions of greater trabecular thickness but not near observable resorption cavities. Taken together, these findings indicate that, regardless of loading mode, accumulation of microdamage in cancellous bone after monotonic loading to yield is influenced by donor characteristics other than traditional measures of microarchitecture, suggesting a possible role for tissue material properties.

1. Introduction

Microscopic tissue damage in cancellous bone, referred here as microdamage, impairs stiffness, strength and fatigue life (Hernandez and Keaveny, 2006; Hernandez et al., 2014; Lambers et al., 2013; Wachtel and Keaveny, 1997a) and is also thought to stimulate bone resorption and remodeling (Herman et al., 2010; Mori and Burr, 1993). The amount and morphology of microdamage formed in bone is likely influenced by apparent loading mode, microarchitecture and tissue material properties.

In cancellous bone, the microstructure allows for load redistribution as localized regions are damaged, such that increases in microdamage may be due to the initiation of new locations of microdamage or to the extension of already existing microdamage. Extension of existing microdamage sites is more likely to lead to trabecular microfracture, which has a disproportionate negative effect on mechanical performance of cancellous bone (Silva and Gibson, 1997; Yeh and Keaveny, 2001). The degree to which a microdamage site extends can be influenced by loading mode (Wang et al., 2005; Wu et al., 2013). Microdamage generated in cancellous bone is commonly identified with a bulk stain and evaluated in two-dimensional sections (Burr et al., 1997; Moore and Gibson, 2002, 2003; Vashishth et al., 2000). Two-dimensional assessment of microdamage is useful for characterizing whole specimen amounts of damage, but cannot provide accurate measures of the number or size of damage sites. Three-dimensional assessment of microdamage in cancellous bone has recently been demonstrated (Bigley et al., 2008; Slyfield et al., 2012; Tang and Vashishth, 2007, 2010) and could measure the

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number, size and shape of individual microdamage sites, but has so far only been applied to study microdamage generated by apparent compression.

Theoretical and experimental models suggest that more tissue will yield under apparent tension than under apparent compression as a result of difference in local tissue failure modes (failure due to excessive tensile principal strains vs. compressive principal strains) (Bayraktar and Keaveny, 2004), suggesting that apparent tension will result in more microdamage than apparent compression. In addition to apparent loading mode, trabecular microarchitecture, and local geometry such as the presence of resorption cavities or thickness of individual trabeculae, have also been implicated as factors that may influence the generation of microdamage (Green et al., 2011; Slyfield et al., 2012). To our knowledge, differences in microdamage generation between tensile and compressive loading have not been assessed using three-dimensional analysis and it is not known how the number and size of microdamage sites vary among the apparent loading modes. Additionally, the effect of whole specimen microarchitecture and local geometry (including the presence of resorption cavities and thickness of trabeculae) on the generation of microdamage in these two apparent loading modes is not known.

The long-term goal of this research is to understand the generation of microdamage in cancellous bone and its effect on apparent mechanical properties. Specifically, in this study we consider a single uniaxial load and determine (1) the differences in the number and volume of microdamage sites in cancellous bone subjected to apparent tension or compression; (2) the effect of the cancellous microarchitecture and local microstructure on the number and size of microdamage sites; and (3) the degree to which microdamage formed under the two loading modes is spatially related to resorption cavities.

2. Material and methods

2.1. Overview

Two specimens of vertebral cancellous bone were collected from each donor then loaded in either compression or tension. The resulting amount of microdamage was determined using three-dimensional imaging and the number and size of individual microdamage sites was determined along with traditional measures of microarchitecture and local trabecular thickness. The spatial association between microdamage and resorption cavities was also evaluated.

2.2. Specimen preparation and mechanical testing (additional details in supplementary materials)

The fourth lumbar vertebral bodies of 10 donors (8 male, aged 47–78 years, 70 ± 10, mean ± SD, 2 female, aged 72, 80, tissue source National Disease Research Interchange, Philadelphia, PA, USA) with no medical history of metabolic bone disease or cancer and no obvious vertebral deformities were included in the study. Cylindrical cores of cancellous bone (nominal diameter 8 mm, nominal height 25–30 mm, nominal effective gage length 15 mm) aligned in the superior–inferior direction were collected using a diamond tipped coring tool. Two specimens were collected from each vertebra resulting in a total of 20 specimens. Due to errors in image acquisition, two specimens were excluded, resulting in 18 specimens (8 pairs and 2 single samples).

Specimens were stained with xylenol orange (0.5 mM in PBS, 2 h) to label pre-existing microdamage. Specimens were then potted into brass fixtures using bone cement in preparation for mechanical loading (Bevill et al., 2009). Microscopic tissue damage was induced by applying a single load to 0.8% strain (~apparent yield) in compression ($n=9$) or tension ($n=9$) at a rate of 0.5%/s using a materials testing device. Apparent level yield stress, yield strain, Young's modulus (E), residual strain, inelastic strain and applied energy were determined from the stress–strain curve. After loading, specimens were stained with calcein (0.5 mM in PBS, 2 h) to label microdamage caused by the applied load. The central 5 mm in length of each specimen was then cut away, and embedded undecalcified in methyl-methacrylate in preparation for serial milling imaging.

2.3. Image acquisition, processing and analysis

Three-dimensional images of bone and fluorescent labels of microdamage were collected at a voxel size of $0.7 \times 0.7 \times 5.0\ \mu m^3$ using serial milling (Slyfield et al., 2009, 2012; Tkachenko et al., 2009). As the image acquisition and pre-processing methodology has been well described previously (see Supplementary materials for a summary), we concentrate here on image thresholding and analysis. To avoid damage caused during specimen preparation and, only the central region of each specimen was analyzed, resulting in a region of interest corresponding to 5.4 mm in diameter and 4 mm in height. Bone was segmented from each image using a manually determined global threshold. The bone surface was smoothed by closing with a spherical structuring element with a radius of 14 μm.

Images of microdamage were resampled to $2.8 \times 2.8 \times 2.5\ \mu m^3$ to achieve more isotropic voxels (enabling morphological processing) and thresholds were determined manually. Regions stained with xylenol orange represented microdamage

Table 1
Measures of microdamage, microarchitecture, and apparent mechanical properties are shown. Results are shown as mean (95% confidence interval). p Value indicates significant difference between tension and compression. Tests assessing damage volume per microdamage site include all microdamage sites (not a specimen average).

	Tension ($n=9$)	Compression ($n=9$)	p-Value
Microdamage			
Damage volume fraction (DV/BV, %)	3.93 (2.10, 5.77)	1.89 (0.86, 2.92)	0.01
Number of damage sites (#)	511 (294, 727)	478 (261, 695)	0.72
Number of damage sites per bone volume (#/mm^3)	74 (51, 97)	77 (48, 106)	0.64
Damage volume per microdamage site (10^5 μm^3)	5.2 (4.0, 6.4)	2.1 (1.6, 2.6)	< 0.001
Microarchitecture			
Bone volume fraction (BV/TV,%)	7.34 (5.73, 8.94)	7.02 (5.03, 9.00)	0.80
Bone volume (BV, mm^3)	6.72 (5.25, 8.19)	6.42 (4.60, 8.24)	0.80
Bone surface (BS, mm^2)	168 (141, 196)	160 (124, 196)	0.58
Trabecular thickness (Tb.Th, μm)	112 (102, 121)	107 (95, 118)	0.73
Trabecular separation (Tb.Sp, mm)	1.00 (0.90, 1.10)	1.06 (0.90, 1.22)	0.42
Trabecular number (Tb.N, 1/mm)	1.06 (0.96, 1.16)	1.01 (0.86, 1.17)	0.46
Structure model index (SMI)	1.60 (1.21, 1.99)	1.46 (1.10, 1.82)	0.41
Degree of anisotropy (DA)	1.40 (1.28, 1.53)	1.54 (1.32, 1.75)	0.24
Connectivity density (Conn.D, 1/mm^3)	3.75 (2.83, 4.66)	3.22 (1.93, 4.52)	0.37
Apparent mechanical properties			
Young's modulus (N/mm^2)	276 (217, 336)	231 (202, 260)	0.15
Yield strain (%)	0.73 (0.70, 0.76)	0.75 (0.71, 0.78)	0.41
Yield stress (N/mm^2)	1.48 (1.20, 1.76)	1.19 (0.99, 1.39)	0.07
Applied strain (%)	0.81 (0.76, 0.86)	0.84 (0.77, 0.91)	0.43
Residual strain (%)	0.095 (0.085, 0.105)	0.140 (0.165, 0.115)	< 0.01
Inelastic strain (%)	0.25 (0.22, 0.28)	0.25 (0.23, 0.28)	0.90
Energy applied (mJ/mm^3)	0.0072 (0.0058, 0.0085)	0.0066 (0.0053, 0.0079)	0.52