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Effects of trabecular type and orientation on microdamage susceptibility in trabecular bone

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ABSTRACT

Trabecular architecture becomes more rod-like and anisotropic in osteoporotic and aging trabecular bone. In order to address the effects of trabecular type and orientation on trabecular bone damage mechanics, microstructural finite element modeling was used to identify the yielded tissue in ten bovine tibial trabecular bone samples compressed to 1.2% on-axis apparent strain. The yielded tissue was mapped onto individual trabeculae identified by an Individual Trabeculae Segmentation (ITS) technique, and the distribution of the predicted yielding among trabecular types and orientations was compared to the experimentally measured microdamage. Although most of the predicted yielded tissue was found in longitudinal plates ($73 \pm 11\%$), the measured microcrack density was positively correlated with the proportion of the yielded tissue in longitudinal rods ($R^2 = 0.52$, $p = 0.02$), but not in rods of other directions or plates. The overall fraction of rods and the fractions of rods along the longitudinal and transverse axes were also correlated with the measured microcrack density. In contrast, diffuse damage area did not correlate with any of these quantities. These results agree with the findings that both *in vitro* and *in vivo* microcrack densities are correlated with Structure Model Index (SMI), and are also consistent with decreased energy to failure in more rod-like trabecular bone. Together the results suggest that bending or buckling deformations of rod-like trabeculae may make trabecular structures more susceptible to microdamage formation. Moreover, while simple strain-based tissue yield criteria may account for macroscopic yielding, they may not be suitable for identifying damage.

Introduction

Trabecular tissue loss and deterioration of trabecular architecture are two factors cited in the definition of osteoporosis [1]. Trabecular tissue loss is accompanied by topological changes, such as thinning of trabeculae [2] and the conversion of trabecular plates to rods [3], such that tissue loss and architectural changes are linked. As new diagnostic and treatment procedures are developed, there is a need for an improved mechanistic understanding of bone mechanics to better delineate the effects of density and architectural changes on fracture risk [4]. Microdamage is also believed to be an important factor that increases fracture risk in osteoporotic bone [5]. Microdamage occurs *in vivo* during activities of daily living, and can be repaired through remodeling [6,7]. However, microdamage accumulation beyond the bone's capacity to repair, especially in osteoporotic or aging bone, can adversely affect the mechanical properties of bone [8,9].

Damage susceptibility in a material depends on interactions between local material properties, the presence of ultrastructural features that can enhance or inhibit crack formation and propagation, and the applied stress. In bone, matrix composition – such as mineralization and collagen chemistry [10–15] – is a major determinant of the material properties. Crack initiation and arrest sites include osteocyte lacunae and lamellar interfaces [16,17]. Trabecular tissue stress depends on external loading, and is enhanced or attenuated at different tissue locations due to the porous trabecular architecture, and, as such, several architectural parameters are associated with increased microdamage [18,19].

One architectural feature of trabecular bone that is associated with damage is the Structure Model Index (SMI). SMI is a morphometric measure of the degree to which a porous structure is composed of plate-like or rod-like elements. It takes on the value 0 for an ideal plate structure, and 3 for an ideal rod structure [20]. The density of microcracks initiated by overloading, as well as that of propagating microcracks, was positively correlated with the SMI of bovine samples [18]. *In vivo* microdamage in human vertebral trabecular bone is similarly correlated with SMI [19]. However, SMI is a single scalar metric, and does not provide details on the relative number or orientation of plates and rods. Individual Trabeculae Segmentation

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(ITS) is a technique that provides a detailed and objective characterization of the types and orientations of trabeculae [21]. Using this technique, plates have been shown to have a greater contribution to the elastic stiffness of trabecular bone than rods [21], and axially aligned trabeculae were found to provide the main resistance to axial loading [22].

Computational models provide a sample-specific means to study tissue level stress due to a given macroscopic loading for both elastic [23–25], and post-yield loading [23,26]. Notably, locations of microdamage formation were associated with higher local von Mises stress calculated by linear finite element models [27]. However, there has been no direct correlation between calculated tissue yielding from nonlinear models and experimentally measured microdamage.

The application of nonlinear microstructural Finite Element Analysis (micro-FEA), ITS, and experimental measures of microdamage may provide improved insight into the roles of trabecular architecture and mechanical loading in microdamage susceptibility. As such, the objective of this study was to determine whether microdamage susceptibility is related to the presence of highly strained tissue in specific types and orientations of trabeculae. Specifically, 1) ITS was used to decompose trabecular bone samples into individual plate and rod elements; 2) micro-FEA was used to identify trabecular tissue loaded beyond the yield point; and 3) experimentally measured microdamage was correlated with the quantity and distribution of the predicted yielded tissue.

Materials and methods

Ten cylindrical trabecular bone samples from the proximal tibia of young slaughter cattle (Martin's Meats, Wakarusa, IN) were analyzed. The orientation of the samples was controlled using micro-CT imaging to ensure that the principal trabecular orientation was aligned with the axis of the samples. Briefly, a low-resolution scan was used to create finite element models that were subsequently used to determine the principal mechanical axes of cubic bone sections [28]. The calculated axes were used to orient the cubes, and a cylindrical core was cut using a diamond edge coring tool (Starlite Industries, Rosemont, PA) [28]. The cylinder axis was aligned with the principal axis within $4.70 \pm 3.11°$ (mean ± S.D.), on average.

The cylindrical samples were scanned at 20 μm isotropic resolution in a micro-CT scanner (μCT-80, Scanco Medical AG, Brüttisellen, Switzerland) and the architecture was quantified using the standard software (μCT Evaluation Program V4.3, Scanco Medical AG, Brüttisellen, Switzerland, Table 1).

A cuboid region, approximately $5 \times 5 \times 6$ mm^3 in size, was taken from the center of each cylindrical sample image, and the images were region-averaged to 40 μm isotropic resolution to reduce computational expenses while still maintaining sufficient fidelity for numerical convergence [29–32]. ITS was then used to identify individual plates and rods within each sample [22]. This method applies Digital Topological Analysis (DTA) to transform the thresholded micro-CT images into a collection of individual trabecular plates and rods. Individual trabeculae (plates and rods) were classified as longitudinal (0–30°), oblique (30–60°), and transverse (60–90°) with respect to the cylinder axis. The amount of bone tissue in each trabecular type, and within each of the three orientation categories was calculated.

Table 1
Architectural parameters of the samples ($n = 10$).

Parameters[a]	Mean	S.D.	Range
VF[b]	0.243	0.044	0.190–0.320
ρ_{app} (g/cm^3)	0.462	0.085	0.363–0.610
Tb.Th. (mm)	0.184	0.034	0.133–0.235
Tb.Sp. (mm)	0.606	0.080	0.467–0.700
SMI (−)	0.568	0.391	−0.131–1.078
Conn.D. (1/mm^3)	5.410	2.674	2.611–11.449
DA (−)	1.828	0.195	1.603–2.265

[a] VF = volume fraction; ρ_{app} = apparent density; Tb.Th. = trabecular thickness; Tb.Sp. = trabecular spacing; SMI = Structure Model Index; Conn.D. = connectivity density; and DA = degree of anisotropy.
[b] Measured by Archimedes' Principle.

Micro-FEA models were created from the 40 μm resolution images for each sample by directly converting the bone voxels into eight-node finite elements [33]. The trabecular tissue was modeled as a homogenous isotropic material with a specimen-specific back-calculated tissue modulus [34] and a Poisson's ratio of 0.3. A bilinear elastic model with an asymmetric principal strain tissue yield criterion [23] was applied with compressive and tensile yield strains of 0.83% and 0.41%, respectively [35]. We verified that the yielded regions did not undergo unloading (although the surrounding elastically loaded tissue did), so that the bilinear constitutive model behaves similarly to a plasticity model [23]. However, the absence of tissue level softening does not allow the model to be used for apparent strains beyond the ultimate strain [25].

Each sample was analyzed with boundary conditions corresponding to 1.2% apparent compressive strain along the principal trabecular orientation. Geometric nonlinearity was not included, but the effects would be small for the dense bone samples and low apparent strain used here [31]. The nonlinear models were solved using a custom finite element code implementing an implicit incremental solution method.

The tissue level strains were analyzed to determine which trabeculae contained yielded tissue. Due to the porous architecture of trabecular bone, bone tissue can yield due to either compressive strain or tensile strain under apparent compressive loading. Tissue that had a principal strain exceeding either the compressive or tensile elastic strain limit (0.83% strain in compression and 0.41% strain in tension) was detected and segmented at 1.2% apparent strain. The locations of the yielded tissue were mapped onto individual trabeculae, and the distribution of the yielded tissue within each trabecular type and orientation combination was calculated. A predominance of compressive or tensile yielding suggested uniaxial compression or extension of trabeculae, while combined compressive and tensile yielding was taken as an indication of bending failure.

The amount and location of yielded tissue were compared to measurements of microdamage from a previous study in our laboratory on the same samples [36]. Briefly, *in vivo* microdamage was stained with alizarin. Subsequently, microdamage induced by a 2% apparent on-axis compressive overload was stained by xylenol orange, thereby allowing the two types of damage to be quantified separately [37]. The microcrack density (Cr.Dn.) and diffuse damage area (Dx.Ar.) were quantified by direct counting under 100× magnification on an epifluorescent microscope. The two-dimensional nature of the measurement did not allow damage in rods and plates to be differentiated.

The damage due to the overload was compared to the rod fraction – defined as the total number of rods divided by the total number of trabeculae (Rd.N./Tr.N.) – and the rod fractions in the longitudinal, oblique, and transverse directions — defined as the number of rods in each direction divided by the total number of trabeculae in the corresponding directions (L.Rd.N./L.Tr.N., O.Rd.N./O.Tr.N., and T.Rd.N./T.Tr.N.).

Statistical analyses were performed using Student's *t*-test in Microsoft Excel 2003 and linear regression in JMP 7.0 (SAS Institute Inc., Cary, NC) with a significance level of $p = 0.05$. The normality of the parameters and of the regression residuals was verified using JMP.

Results

The bone samples were primarily composed of longitudinally oriented plates. When measured by tissue volume, $80 \pm 10\%$ (mean ± S.D.) of the tissue was plates, and over 70% of the plates were oriented