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4 **Comparison of Solid and Fluid Constitutive Models of Bone Marrow During**
5 **Trabecular Bone Compression**
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Abstract

The mechanical environment and mechanobiology of bone marrow may play essential roles in bone adaptation, cancer metastasis, and immune cell regulation. However, the location of marrow within the trabecular pore space complicates experimental measurement of marrow mechanics. Computational models provide a means to assess the shear stress and pressure in the marrow during physiological loading, but they rely on accurate inputs for the marrow and the physics assumed for the interaction of bone and marrow. Elastic, viscoelastic, and fluid constitutive properties have all been reported from experimental measurements of marrow properties. It is unclear whether this ambiguity reflects the various length-scales, loading rates, and boundary conditions of the experiments, or if the material models are sufficiently similar as to be interchangeable. To address this question, we analyzed both the mean shear stress and its spatial distribution induced in marrow during compression of trabecular bone cubes when using linear elastic, neo-Hookean, viscoelastic, and power-law fluid constitutive models. Experimentally reported parameters were initially applied for all four constitutive models, resulting in poor agreement. The parameters of the soft solid models were calibrated by linear interpolation so that the volume averaged shear stress agreed with the fluid model for each, but this could only be accomplished on a specimen-by-specimen basis. Following calibration, the root-mean-squared (RMS) difference between the solid and fluid constitutive models was still greater than 26% even when the overall mean shear stress was in close agreement, indicating that the spatial distribution of stress is also sensitive to the constitutive model. As such, the choice of constitutive model should be backed by a strong rationale, and results should be interpreted with care.

62 **Keywords:** Bone marrow; Mechanobiology; Computational mechanics; Finite element
63 analysis; Fluid-structure-interaction; Constitutive model

Introduction

Bone marrow is a complex, cellular tissue residing in the pore space of trabecular bone. It is subject to cyclic motion as the bone deforms during activities of daily living. The resulting mechanical deformation and stress may cause gene and protein expression in the various bone marrow resident cell lineages, affecting immune and endocrine functions in addition to bone adaption (Mantila Roosa et al., 2011; Soves et al., 2014). Due to the complex geometry of the trabecular pores, understanding marrow mechanobiology is best approached by computational models that couple the mechanics of both bone and marrow. Such models rely on implementing appropriate constitutive models and boundary conditions.

Bone marrow has been characterized as a highly viscous fluid (Metzger et al., 2014), a viscoelastic solid (Jansen et al., 2015), or a soft elastic solid (Shin et al., 2014; Winer et al., 2009). These three constitutive models represent a range of computational complexity and expense. Modeling bone marrow as a fluid requires the time-dependent Navier-Stokes equations to be solved while coupled to the moving boundaries of the deforming solid in a fluid-structure-interaction solution. In instances with a complex solid-fluid interface, like trabecular bone, a fluid-structure interaction (FSI) simulation may be computationally prohibitive. In contrast, bone marrow can be modeled as an elastic solid, allowing the solution to be computed in a single step without time dependence. The viscoelastic assumption results in a solution with intermediate expense, as the solution is time dependent, but coupling between marrow and bone is maintained by the nodal connectivity.

It is common to apply simplified constitutive assumptions in computational models to make problems more tractable. However, it is essential to understand how these assumptions affect the model outcomes. Hence, the goal of this study was to compare the shear stress

calculated in an *in situ* model of bone marrow using fluid, solid, and viscoelastic marrow constitutive assumptions. Specifically, we compared both the mean and spatial difference in the shear stress in bone marrow modeled as 1) a power-law fluid, 2) a linear elastic solid, 3) a neo-Hookean solid, and a 4) viscoelastic solid during dynamic compression of human trabecular bone samples.

Methods

Trabecular bone images from two human femurs obtained from a national donor bank (National Disease Research Interchange) as part of a previous project were studied (Wu et al., 2013). Briefly, aligned cylindrical samples were prepared from the femoral neck (Wang et al., 2004; Wu et al., 2013). The samples were imaged by μ -CT (Scanco μ CT-80, Brüttisellen, Switzerland) at 20 μ m nominal resolution, with 500 projections at 70 kVp, 120 mA, with a 400 ms integration time. A 3 x 3 x 3 mm cubic region of interest was selected for modeling. The two samples had bone volume fractions of 0.40 and 0.09.

The μ -CT images were segmented into bone and marrow regions using a constant threshold of 452 mg-HA/cc. Conformal tetrahedral finite element meshes were created using BioMesh3D (SCIRun, Scientific Computing and Imaging Institute, University of Utah) with an average element volume of 2000 μm^3 . In a previous study, this mesh resolution resulted in convergence of both the fluid and solid shear stress within less than 1% (Metzger et al., 2015). The bone was assigned a uniform isotropic modulus of 15 GPa and Poisson's ratio of 0.3 (Bayraktar and Keaveny, 2004; Niebur et al., 2000). A sinusoidal strain profile was applied along the inferior-superior axis of the bone by fixing the inferior nodes and uniformly displacing the nodes on the superior surface (Fig. 1) to achieve a peak compressive strain of 1000 μ -strain in $\frac{1}{2}$

s. Four models were created for each mesh with a non-Newtonian fluid, a linear elastic solid, a hyperelastic solid, and a viscoelastic solid constitutive model applied to the marrow region.

For the baseline case, the marrow was modeled as a non-Newtonian, homogenous fluid (Bryant et al., 1989; Coughlin and Niebur, 2012; Dickerson et al., 2008; Metzger et al., 2015; Ochoa et al., 1991). The bone marrow viscosity was assigned the power-law relationship $\eta = \min(\mu_0, 85.5\dot{\gamma}^{-1})$, where η is the viscosity (Pa•s), $\dot{\gamma}$ is the shear rate (s^{-1}), and μ_0 is an upper bound on the viscosity (Metzger et al., 2014). Without such a limit, the initially static fluid would have an infinite viscosity, and flow could not be initiated. The initial upper bound was 1000 Pa•s, based on experimental reports. Subsequently, an upper bound of 100 Pa•s was applied to better match the results of elastic simulations. A fluid density of 0.95 g/cm^3 was assigned (Gurkan and Akkus, 2008). A no-slip interface was applied at the bone-marrow interface, assuming that marrow cells attached to the bone matrix via integrins (Birmingham et al., 2014; Dickerson et al., 2008; Metzger et al., 2015). This was the only kinematic boundary condition assigned to the marrow, with free flow allowed at all six external surfaces. A zero pressure boundary condition was assigned on the fluid face opposite the loaded surface (Fig. 1). The resulting transient, dynamic FSI models were solved using an iterative coupling scheme in ADINA 9.0.

To investigate the response of marrow when measured as a soft solid, three constitutive models were considered: a linear elastic solid, a neo-Hookean hyperelastic solid, and a viscoelastic solid. All properties were based on literature reports (Table 1). The marrow and bone meshes were connected by equivalencing nodes at the interface. For the viscoelastic material model, a single term Prony series with an instantaneous shear modulus of 2.5 kPa, a relaxed modulus of 2 kPa, and a bulk modulus of 2.5 MPa to approach incompressibility was applied

(Jansen et al., 2015). Since the single term could not capture the reported complex modulus, decay constants of 0.1, 0.5, and 1.0 were investigated to cover a range of behavior. The shear modulus in the linear elastic and neo-Hookean models was further adjusted to minimize the error in the mean shear stress between the solid and fluid simulations.

Finite element simulations were performed using ADINA® (Watertown, MA). The elastic, hyperelastic and viscoelastic simulations were quasistatic. The solid phase of the FSI model was quasistatic and the fluid phase was fully dynamic. Results were post-processed using Paraview (Kitware, Clifton Park, NY). The data were quantified within a 2.5 x 2.5 x 2.5 mm sub-region in the center of the sample to avoid edge effects. The volumetric mean shear stress was calculated by integrating over the element volumes. To facilitate comparisons, the fluid model was taken as baseline, and the difference of each elastic marrow model was compared to it. Similarly, the spatial distribution in shear stress was compared by calculating the element by element root-mean square (RMS) difference of each elastic marrow model from the fluid model.

Results

The solution time for the FSI simulations was nearly 50 times that of the elastic models. Fluid structure interaction simulations required approximately 96 CPU hours and 15 GB of RAM. The models employing solid constitutive models for marrow required only 2 CPU hours and 2 GB of RAM. The fluid and viscoelastic simulations required multiple time steps because of the time-dependency of the material model. The elastic models were solved in a single time step, although the hyperelastic model required an iterative solution.

Compression of the bone induced shear stress in the marrow in both the solid and fluid marrow models. However, the peak volume-averaged marrow shear stress occurred at the maximum strain in the linear elastic, neo-Hookean, and viscoelastic models, while the fluid

model reached its peak stress almost immediately upon loading and decreased to a minimum at the maximum displacement. Altering the exponential parameter, α , in the viscoelastic models affected the results by less than 10%. As such, only the results for the 1 s time constant are presented.

When the bone marrow was modeled as a fluid, the upper bound viscosity increased the marrow shear stress almost linearly, from an average of 0.42 Pa to 4.2 Pa for the 0.09 BV/TV specimen and from 0.58 Pa to 5.6 Pa in the 0.40 BV/TV specimen. The peak velocity gradient magnitude was only 0.003 s^{-1} , leaving most of the marrow within the regime of the upper bound.

The volume-averaged shear stress decreased with decreasing shear modulus in the elastic material models (Fig. 2). The neo-Hookean model with $G = 220 \text{ Pa}$ best approximated the volume-averaged shear stress when compared to the $100 \text{ Pa}\cdot\text{s}$ -bound fluid model. The 2.5 kPa viscoelastic model best approximated the volume-averaged shear stress when compared to the $1000 \text{ Pa}\cdot\text{s}$ -bound fluid model.

The spatial distribution of shear stress varied significantly from the fluid simulations (Fig. 3). The solid models under-predicted the shear stress at the bone-bone-marrow interface when compared to the FSI simulations (Fig. 4).

The performance of the constitutive models was not consistent between the two samples studied (Fig. 5A). The relative difference with the fluid model was four times higher in the high volume fraction sample for the elastic and hyperelastic models, but had a similar magnitude for the viscoelastic model (Fig. 5A). The 220 Pa linear elastic material model had the smallest RMS difference from the fluid model when the limiting viscosity was $100 \text{ Pa}\cdot\text{s}$ in the 9% volume fraction sample (Fig. 5B), while the 2.5 kPa viscoelastic material model had the smallest difference when compared to the $1000 \text{ Pa}\cdot\text{s}$ fluid model (Fig. 5B). The RMS difference in the

linear elastic and viscoelastic models was 24% and 14% higher in the high volume fraction sample, respectively. In contrast, the RMS difference decreased by 6% between samples when the neo-Hookean model was used (Fig. 5B).

Discussion

Computational models of bone marrow mechanics are important for addressing hypotheses related to bone marrow mechanobiology. The reliability of such models depends on choosing an appropriate constitutive model. We quantified the sensitivity of the computed stress in trabecular bone marrow to the marrow constitutive model. For a single sample it was clear that the constitutive parameters could be calibrated to match the fluid simulation. However, the calibrated constitutive parameters depended on the sample, making it impractical to apply to experimental data. Even when the solid constitutive model gave similar volume-averaged shear stress within the marrow, the spatial distribution of stress exhibited substantial differences. The RMS difference was more than 25% when compared to the fluid model, and was highest at the bone surface where the highest mechanobiological signal would be expected. Finally, the elastic models could not capture rate dependency, and the viscoelastic model did not have as rapid of a decay in stress as the fluid model. As such, although marrow has been commonly modeled as a soft solid to reduce computational expense, the assumption is not consistent with a fluid marrow.

The non-Newtonian fluid constitutive model was used as the baseline for comparison. However, the true material properties of bone marrow in *in vivo* environments are not well established. Bone marrow has been characterized as a power-law fluid (Bryant et al., 1989) with properties similar to blood, a viscous power-law fluid (Metzger et al., 2014), a viscoelastic solid (Jansen et al., 2015), a gel (Winer et al., 2009), or a linear elastic soft solid (Shin et al., 2014). Although these different constitutive models could describe experimental results within

reasonable error, our results demonstrate that these constitutive models differ substantially under physiological conditions.

Bone marrow has been modeled as a linear-elastic solid (Webster et al., 2015) or a poro-viscoelastic solid (Sandino et al., 2015) in mechanobiological studies. Our results indicate that the distribution of shear stress within the marrow would differ substantially from fluid and viscoelastic constitutive models based on recent experimental measurements (Jansen et al., 2015; Metzger et al., 2014; Shin et al., 2014; Winer et al., 2009).

This study illustrates that the choice of constitutive model plays a critical role in simulations of the biomechanics of the marrow niche in trabecular bone. Although modeling bone marrow as a solid material decreases the overall computation time and memory required, elastic models could not capture the spatial distribution of shear stress that results from assuming fluid behavior. The results also differed substantially between elastic, hyperelastic, and viscoelastic constitutive models, even when using the same modulus. Hence, the constitutive model applied to marrow in computational studies should be critically evaluated based on the outcome variables of interest, and should be consistent with experimental data.

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Conflict of Interest Statement

The authors have no conflicts of interest related to this publication.

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Tables

Table 1. Three solid constitutive models were investigated: linear elastic, neo-Hookean, and viscoelastic. Material values were taken from the literature. Intermediate values were interpolated based on the error in the mean shear stress from one of the fluid models. The viscoelastic properties were based on the highest temperature tested in order to simulate physiological behavior, and a bulk modulus of 2500 kPa with a 10 Pa decay was applied to approximate near incompressibility, and viscous behavior constrained to the deviatoric stress.

Material Model						
Linear Elastic						
$S_{ij} = \frac{EU}{(1 + \nu)(1 - 2\nu)} e_{kk} d'_{ij} + \frac{E}{1 + \nu} e_{ij}$		G (Pa)	ν			
Isaksson <i>et al.</i> (2009)		700000	0.16			
Linear interpolation		318	0.48			
Winer <i>et al.</i> (2009)		220	0.48			
Shin <i>et al.</i> (2014)		107	0.48			
neo-Hookean						
$S_{ij} = \frac{G}{J^{2/3}} \left(B_{ij} - \frac{1}{3} B_{kk} \right) + k (J - 1) d'_{ij}$		G (Pa)	κ			
$e_{ij} = \frac{1}{2} \left(d'_{ij} - B_{ij}^{-1} \right), J = \sqrt{\det(B)}$						
Linear interpolation		320	160000			
Winer et al., (2009)		220	110000			
Viscoelastic						
$S_{ij} = 2 \left(G_{\infty} + G_1 \right) e_{ij}(t) - 2 \partial G_1 \int\limits_{0+}^t e_{ij}(t - t') e^{-\partial t'} dt'$						
$+ d'_{ij} \left[\left(k_{\infty} + k_1 \right) e_{kk}(t) - \partial k_1 \int\limits_{0+}^t e_{kk}(t - t') e^{-\partial t'} dt' \right]$						
where $e_{ij} = e_{ij} - \frac{1}{3} d'_{ij} e_{kk}$		G_{∞} (Pa)	G_1 (Pa)	κ_{∞} (Pa)	κ_1 (Pa)	α (1/s)
Jansen <i>et al.</i> (2015)		2000	500	2500000	10	0.1
		2000	500	2500000	10	0.5
		2000	500	2500000	10	1.0

Figure Captions

Figure 1: A) Trabecular bone and marrow models were created from u-CT reconstructions and meshed with BioMesh3D (SCIRun, University of Utah). B) Compressive strain was applied to the superior surface of the bone models. C) For the FSI models the pressure was set to zero on the inferior surface of the marrow. The only kinematic constraint on the marrow was a no-slip boundary condition at the interface with the mineralized bone tissue.

Figure 2: The mean shear stress in the linear elastic and neo-Hookean models decreased nonlinearly with decreasing shear modulus. The constitutive parameters were adjusted by linear interpolation to reduce the average error. Elastic models with lower moduli were compared to the fluid model with an upper bound viscosity of 100 Pa•s (A) while the elastic models with higher moduli were compared to the 1000 Pa•s bound power-law fluid model (B) in order to obtain the best possible agreement.

Figure 3: The solid constitutive models resulted in higher stresses than the fluid models. The locations of high stress and stress distributions differed between the elastic and fluid constitutive models, as well as between the various elastic constitutive models.

Figure 4: The absolute value of the difference in shear stress between elastic and fluid constitutive models exceeded 100% in many locations. A) Linear elastic, and B) neo-Hookean compared to fluid with $\eta_{\max} = 100$ Pa•s. C) Viscoelastic material compared to fluid with $\eta_{\max} = 1000$ Pa•s. Note that the regions of highest error differ between all models, indicating that the

three elastic constitutive models differ from one another as well as from the fluid constitutive model.

Figure 5: A) When the same constitutive parameters were applied to samples of differing volume fraction, the error in the mean shear stress differed sharply. As such, calibration of the material properties is unlikely to be successful. B) The RMS difference from the fluid result was also dependent on the sample analyzed, further indicating the differences in the constitutive models. In both A) and B) the linear elastic and neo-Hookean materials were compared to the 100 Pa•s upper bound power-law model, while the viscoelastic model was compared to the 1000 Pa•s upper bound power-law model.

A

B

C

A

B

A