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Research Paper

Rheological behavior of fresh bone marrow and the effects of storage

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ABSTRACT

The progression of several diseases, such as osteoporosis and diabetes, are associated with changes in marrow composition and physiology. As these diseases are affected by aging and activity, the biomechanical properties and mechanobiology of marrow may play a role in their progression. Bone marrow is comprised primarily of cells, and provides a niche for several mechanosensitive cell lineages. The mechanical signals imparted to the cells depend on their interaction with one another, the extracellular matrix, and the intercellular fluid. At a macroscopic scale, these interactions manifest as viscosity in marrow. Marrow viscosity has been measured in human and bovine bone. However, a large range of storage, retrieval, and measurement techniques has resulted in inconsistent data. To provide physiologically relevant data, marrow samples from young adult pigs were harvested and tested within less than 8 h of slaughter. The viscosity was over 100 Pa s at a shear rate of 1 s^{-1}, and decreased with shear rate according to a power law. However, the marrow did not exhibit a measurable yield stress as some complex fluids do. The viscosity of samples that had been frozen and thawed prior to testing was lower by an order of magnitude. The difference in properties was associated with a loss of integrity of the marrow adipocyte membranes. Previous reports of bone marrow viscosity have shown inconsistent results, which may be due to different storage and handling prior to testing. The higher viscosity compared to previous reports would impact poroelastic models of bone, and suggests that the stress on marrow cells during whole bone loading may be higher than previously believed.

1. Introduction

Bone marrow is a highly cellular soft tissue located within the central cavity of long bones, and in the pore space of trabecular bone (Tavassoli and Yoffey, 1983) (Fig. 1). It provides a niche for the mesenchymal and hematopoietic stem cell lineages, thus playing a crucial role in immune regulation and skeletal health. The various cell populations in the marrow are mechanosensitive (Riddle et al., 2006), interacting via a host of secreted and membrane bound cytokines

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that affect cell function within the niche. Progression of various degenerative diseases such as osteoporosis (Justesen et al., 2001; Verma et al., 2002; Yeung et al., 2005), marrow aplasia (Naveiras et al., 2009), and diabetes (Bethel et al., 2013) are associated with an increase in the adiposity of bone marrow, which may affect how mechanical forces are transmitted between the different cell populations. Such changes could potentially affect the mechanobiology of the resident cells, which would provide a causal link to further skeletal or immune system degeneration.

Bone marrow behaves as a fluid at body temperature, and the mechanical interactions of the components can be characterized by viscosity. The viscosity decreases with both temperature and shear rate according to a power law (Bryant et al., 1989; Gurkan and Akkus, 2008; Zhong and Akkus, 2011). The viscosity of bone marrow also depends on skeletal site (Gurkan and Akkus, 2008). Marrow from the distal end of bovine femurs is less viscous than that from the proximal end (Bryant et al., 1989), which was attributed to the higher adiposity found in the distal marrow (Bryant et al., 1989; Dietz, 1946). However, previously reported measurements were made in marrow that had been refrigerated or frozen for unknown periods (Bryant et al., 1989), which may compromise cell structure or the interactions between the cells, thereby altering the behavior.

In order to understand the effects of anatomical location, composition, and shear rates on the rheological properties of bone marrow, we measured the viscosity of bone marrow harvested from three locations in porcine femurs. Specifically, we (1) measured the rate dependence of viscosity using a rheometer; (2) repeated the experiment with bone that had been previously frozen; and (3) applied a theoretical model of viscosity of complex fluids to identify the characteristics of marrow.

2. Methods

Eighteen freshly harvested porcine femurs were obtained from a local abattoir (Martin's Meats, Wakarusa, IN). Animals were 6–8 months of age at the time of slaughter. Fresh marrow specimens were harvested within 8 h post-mortem

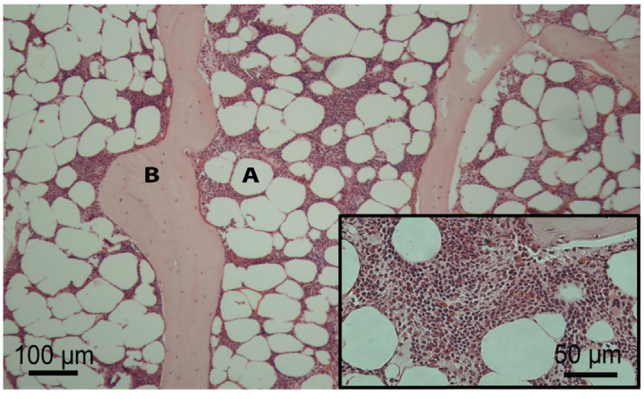

Fig. 1 – Hematoxylin and eosin staining of freshly harvested trabecular bone marrow from a porcine vertebra. Matrix is stained pink, and nuclei are blue. The marrow consists predominantly of cells. (A = adipocyte, B = bone tissue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in 12 femora. Six femora were stored at −30 °C for 5 days, and marrow samples were obtained after thawing for 3 h at 20 °C. The distance from the femoral head to the distal condyles was measured, and the femurs were sectioned and cut at 25%, 50%, and 75% of the total length using a hacksaw (Fig. 2). Marrow was retrieved from the medullary cavity at each location of the femurs with a spatula. Two to three marrow aliquots of 2 mL each could be obtained at each location. Attention was paid to ensure that no bone debris or large vasculature was included in the samples.

Marrow viscosity was measured on a parallel plate rheometer (Bohlin Gemni HR^{nano}, Malvern Instruments Ltd, Worcestershire, UK) with a gap size of 1000 μm. Approximately 2 mL of marrow was placed on the measurement plate. For strain rate dependence experiments, the temperature was held at 37 °C, while the strain rate was varied continuously from 0.1 to 10 s^{-1}. We focused on low rates because computational models suggest that *in situ* marrow deformations are small (Birmingham et al., 2013; Coughlin and Niebur, 2012).

The mechanical behavior of the marrow was quantified using two models. First, a power-law model with viscosity proportional to the strain rate raised to a negative power. Second, a Herschel–Bulkley (Coussot, 2005) model was used to characterize the behavior as a complex fluid. This model incorporates a yield stress, τ_s, below which the material behaves as a soft solid, and has previously been used to characterize cell aggregates (Iordan et al., 2008). We fit the measured shear stress to the model (Coussot, 2005):

$$\tau = \tau_s + M\dot{\gamma}^n \quad (1)$$

to determine τ_s, M, and n using the Matlab nonlinear least squares curve fitting toolbox (Matlab, Mathworks, Natick, MA), where M and n are parameters related to the shear thinning behavior.

The marrow was analyzed under microscopy before and after freezing to assess the effects on the cells. Aliquots of marrow were stained with Oil Red-O (Sigma-Aldrich, St. Louis, MO) to determine the presence of lipids in the adipocytes. To stain, 1 mL of Oil Red-O solution was added to 1.6 mL of harvested bone marrow. Marrow was agitated and broken up with a spatula and incubated for 20 min at room temperature. The marrow solution was centrifuged at 1 g for 5 min, the supernatant was withdrawn, and cells were re-suspended in 0.5 mL PBS. One hundred μL of the suspension was imaged on a bright-field light microscope at 5×. Additional aliquots of fresh and frozen marrow were stained with Trypan blue (Sigma-Aldrich, St. Louis, MO). In this case, 250 μL of Trypan blue was added to 250 μL of marrow and vortexed for 4 min at a low setting. The suspension was imaged in a bright-field microscope at 5×.

JMP software version 8.0.1 (SAS, Cary, NC) was used for all statistical analysis. Viscosity data at each location along the femoral length was pooled and non-linear regression was used to relate viscosity to shear rate. ANCOVA was used to compare power law parameters between locations and fresh vs. frozen marrow. Tukey *post hoc* analysis was conducted for pairwise comparison with a significance level of $p<0.05$. Kruskal–Wallis nonparametric test was conducted to