Mechanical Stimuli in Bone Marrow During Physiologic Loading

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Disclosures:

Introduction: Skeletal adaptation is controlled by the biochemical and biomechanical signaling in bone cells and their progenitors. Bone density decreases during periods of inactivity where bones are not mechanically loaded [1], while frequent load-unload cycles increase bone mass [2]. Osteocytes are considered the primary mechanosensory cells in bone, but marrow resident cells including osteoblasts [3], mesenchymal stem cells (MSC) [4], and osteoclasts [5], also respond to mechanical cues such as pressure and shear stress, and could also play a role in skeletal health. The pressure in bone marrow increases during loading of human femurs, reaching 1 to 2 kPa during physiologic loading [6]. The pressure was higher in the head than the neck, and increased with loading rate [6]. These pressures are too low to play a mechanical role [7], and are below previously measured limits for mechanotransduction in MSCs [8]. However, pore pressure in a poroelastic solid arises from fluid interaction with the walls of the solid. As such, the pressure gradients are related to a fluid shear stress in the marrow. We hypothesized that shear stress induced in bone marrow due to poroelastic effects are sufficient to affect marrow cell fate. To address this hypothesis, we measured pressures at 4 locations in the femoral neck during stress relaxation and cyclic loading and estimated the resulting shear stress in the marrow using computational fluid dynamics models.

Methods: Nine fresh porcine femurs were obtained at the time of sacrifice, and testing was completed within 8 hours post-mortem. Four 2.3 mm diameter holes, approximately 5 mm apart and 15 mm deep, were prepared along the femoral neck, and miniature pressure transducers (Precision Measurements Inc., Ann Arbor, MI) were inserted via canulae to measure the pore pressure in situ. The femurs were loaded in compression on a servo-hydraulic load frame in a 37° C water bath. Three femurs were subjected to stress-relaxation by applying approximately 2 mm of displacement at a rate of 0.25 mm/s followed by a 900 s hold period. The remaining six femurs were subjected to cyclic loading from approximately 600 N to 1200 N with a sinusoidal wave form at 1 Hz while recording force and pressure at 100 Hz. Following testing, the femora were imaged by micro-CT (Scanco µCT-80, Brüttisellen, Switzerland) at a 20 µm resolution. A 4-mm³ cubic region of interest was selected between each pair of adjacent pressure transducers. The images were filtered and segmented with a constant threshold, and the marrow region was converted to a tetrahedral finite element mesh using VTK (VTK, Kitware, Clifton Park, NY). The marrow was modeled as a non-Newtonian fluid with density 0.95 g/cm³ and a power law viscosity law, $\mu = 77.61 \times (\text{shear rate})^{-1.13}$, determined from experimental measurements. The experimentally measured pressure differentials were prescribed to the proximal face of the bone models, a zero pressure to the distal face, and a zero flow condition to the perpendicular faces assuming infinite symmetry. A transient, dynamic solution was performed using ADINA-F (Watertown, MA). The mean shear stress in the volume was averaged over the cycle to assess the potential for mechanical stimulation.

Results: The marrow pressure increased during loading reaching as high as 5 kPa, and decreased during relaxation (Fig.1a). During cyclic loading, the pressure varied sinusoidally with the load (Fig. 1b). Pressure gradients in the range of 0.25 kPa/mm were found. Micro-CT imaging showed that location of highest porosity varied between bone specimens, but did not correlate with the measured pressure ($p>0.6$). The mean marrow shear stress, calculated from the CFD models, ranged from 2 to 24 Pa and increased with increasing pressure differential (Fig 2b). Average shear stress linearly correlated with average pressure differentials measured during cyclic loading ($p<0.05$) (Fig 2c).

Discussion: While osteocytes are the primary mechanosensor in bone, mechanical stimulation in the marrow cell population may also play a role in the response to skeletal loads. For example, MSCs exhibit increased proliferation and mRNA levels of osteogenic genes after mechanical loading [9]. As such, the goal of this study was to quantify typical mechanical stress in the marrow during activities of daily living. While the pressure was far below the reported threshold of 200 kPa for a cellular response, computational models demonstrate that the shear stress is on the order of several Pascals, which is well above the stimulatory thresholds reported [10]. As such, mechanical stimulation of the bone marrow may also play a role in bone adaptation by increasing MSC proliferation, increasing osteoblastogenesis [11] or by affecting the balance between adipogenic and osteogenic differentiation [12, 13].

Computational fluid dynamics models provide a measure of the mechanical stimuli within marrow in a continuum sense. The mechanism transmission of these shear stresses to cells would determine the potential receptors of these signals. At low shear rates, as found in the models, viscosity measurements suggest that shear stress arises primarily from cell-cell interactions or adhesion rather than flow of intercellular fluid over the cell membrane. Thus, the presence of cytoskeletal components associated with cell adhesion molecules would be an important target of this mechanical signal.

Significance: Pressure gradients arise in the pore space of trabecular bone during physiologic loading that are sufficient to...
induce shear stress in the marrow within a mechanostimulatory range for bone cells and their progenitors. Thus, the loading of bone through activities of daily living may affect bone marrow health and bone adaptation.

Acknowledgments: NSF CMMI-1100207

Figure 2: A) Micro-CT imaging and transient computational fluid dynamics (CFD) were used to model fluid flow. Measured pressure differentials were applied as boundary conditions. Marrow shear stress is represented as streamlines through trabecular architecture. B) CFD modeling shows a Gaussian distribution of the shear stress in the marrow. Dashed line represents the mean shear stress value. C) Average shear stress determined by CFD models depended on average pressure differential measured during cyclic loading (p<0.05). Solid line shows linear correlation. Dashed lines represent 95% confidence intervals. Colors represent specimens from individual bones.