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Research Paper

Mechanics of intact bone marrow

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ABSTRACT

The current knowledge of bone marrow mechanics is limited to its viscous properties, neglecting the elastic contribution of the extracellular matrix. To get a more complete view of the mechanics of marrow, we characterized intact yellow porcine bone marrow using three different, but complementary techniques: rheology, indentation, and cavitation. Our analysis shows that bone marrow is elastic, and has a large amount of intra- and inter-sample heterogeneity, with an effective Young's modulus ranging from 0.25 to 24.7 kPa at physiological temperature. Each testing method was consistent across matched tissue samples, and each provided unique benefits depending on user needs. We recommend bulk rheology to capture the effects of temperature on tissue elasticity and moduli, indentation for quantifying local tissue heterogeneity, and cavitation rheology for mitigating destructive sample preparation. We anticipate the knowledge of bone marrow elastic properties for building *in vitro* models will elucidate mechanisms involved in disease progression and regenerative medicine.

1. Introduction

Bone marrow plays a significant role in body homeostasis by regulating immune and stromal cell trafficking. Researchers have characterized the matrix content and the role of local cells in bone physiology, but capturing the mechanics of bone marrow tissue has been limited in scope. The elastic modulus of engineered substrates is well known to influence cell shape, proliferation, migration, and differentiation (Marklein and Burdick, 2010; Peyton and Putnam, 2005; Peyton et al., 2008; Yang et al., 2014). While significant effort has gone into recapitulating the hematopoietic microenvironment *in vitro* for both regenerative medicine and to improve drug screening, there is no physiological measurement of the modulus of intact bone marrow (Lee et al., 2012; Mahadik et al., 2014; Nicholsa et al., 2010; Scotti et al., 2013; Torisawa et al., 2014). Though some of these model systems incorporate controlled mechanics, there is little validation for the stiffness choices, even though bone marrow stromal and progenitor cells are mechanically responsive to both engineered substrates, and the viscosity of the surrounding fluid (Engler et al., 2006; Lee et al., 2014; Sikavitsas et al., 2003; Yang et al., 2014). Knowing the modulus of *in vivo* tissue is critical for regenerative medicine as well. For example, the Blau lab found that the regenerative capacity of muscle stem cells is enhanced when

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cultured on surfaces mechanically similar to mouse muscle (Gilbert et al., 2010). This highlights the need for methods that can appropriately characterize the heterogeneous mechanics of bone marrow tissue to understand its role in driving the behaviors of the cells within.

Marrow tissue has hematopoietic-rich and adipose-rich regions, which are referred to as red and yellow marrow, respectively. Yellow marrow is enriched in the medullary cavity and red marrow in the spongy, trabecular bone (Parfitt et al., 1983; Vande Berg et al., 1998). Cell content in the marrow is a dynamic process, and yellow marrow can expand and contract as haematopoiesis occurs (Gimble et al., 1996). Unfortunately, the difficulty of harvesting red marrow has limited the ability to isolate and test its mechanics using conventional methods. Yellow marrow has been shown to be mechanically heterogeneous in studies where samples are homogenized and centrifuged to remove cell and bone debris (Bryant, 1988; Zhong and Akkus, 2011). Prepping samples in this manner removes many of the inconsistencies caused when harvesting marrow, but ignores the elastic contribution of the bone marrow extracellular matrix (ECM). The most robust study on yellow marrow mechanics measured the viscosity of the marrow from 19 human subjects and found no apparent correlation between age and marrow viscosity, though marrow has been shown to yellow with age (Justesen et al., 2001; Zhong and Akkus, 2011). Another group found proximal bovine marrow, the tissue close to the trabecular bone, to be more viscous than distal bovine marrow, and they suggest that these changes in viscosity are a function of spatial marrow composition (Bryant et al., 1988). Though both of these studies are informative, the impact of the surrounding or, potentially inclusive, trabecular bone is neglected because samples were homogenized and filtered.

The anatomical location and surrounding cortical bone poses a unique challenge for researchers interested in mechanically studying bone marrow tissue. Many studies have looked at properties of homogenized marrow, by extracting marrow from the medullary cavity and performing bulk rheology, but these approaches are destructive and create a critical gap in our knowledge of intact marrow mechanics (Bryant, 1988; Bryant et al., 1988; Saito et al., 2002; Sobotkova et al., 1988; Zhong and Akkus, 2011). In addition, researchers have used techniques to measure intramedullary pressure (IMP) to better understand how lifestyle choices, such as loading, disuse, steroid use, and diseases such as osteoporosis and cancer change marrow content, blood flow, and bone remodeling (Bloomfield, 2010; Gurkan and Akkus, 2008; Lynch et al., 2013; Miyanishi et al., 2002; Zhang et al., 2007). It is clear that many external factors impact IMP changes, but no work has gone into characterizing the mechanics of the intact matrix, which we suggest plays a stiffness-dependent role in disease progression. Rheology is the dominant method used to characterize bone marrow tissue, with the exception of one group that used ultrasonic wave propagation (Hosokawa and Otani, 1997). However, ultrasonic wave propagation reported that the bulk modulus of bovine marrow is the same order of magnitude as what others have found for the surrounding spongy bone (~2 GPa) (Morgan et al., 2003). The stark differences between marrow and bone likely make it hard to distinguish the marrow mechanics with type of technique. Though the viscoelasticity of bone marrow makes it ideal for bulk rheological characterization, this technique lacks the ability to measure microscopic level heterogeneities and often requires destructive sample preparation. Since bone marrow tissue varies across the length of the bone, is cell-rich, and is highly vascularized, we aimed to explore two more sensitive methods in parallel with traditional rheology: indentation and cavitation rheology. This approach enabled us to explore the continuity of diverse mechanical techniques and sample preparations on the characterization of marrow mechanical properties. Together, this information will allow the field of tissue engineering to further improve the understanding of marrow mechanics and to build more accurate *in vitro* models of marrow tissue.

2. Materials and methods

2.1. *In vitro sample preparation*

Femurs from grass-fed large black Tamworth Cross pigs, 6–10 months old, were gathered from a local butcher, and mechanical testing was conducted within 2 h post-opening of the bone cavity. Indentation and rheology samples were gathered from a bone cut lengthwise down the femur, and tissue samples were biopsy punched out of the medullary cavity and stored in phosphate buffer solution (pH 7.4) for mechanical testing (Fig. 1a). The porcine bones were cut horizontally across the medullary cavity of a femur for cavitation rheology testing. All indentation and cavitation tests were performed at room temperature, which ranges from 18 to 22 °C and is annotated as 20 °C throughout the paper. A minimum of 6 porcine bones, from different pigs, were used for each mechanical test.

2.2. *Rheology*

Small amplitude oscillatory shear measurements were performed in a Kinexus Pro rheometer (Malvern Instruments, UK) using a plate–plate geometry, with a diameter of 20 mm and gap of 1 mm. Porcine bone marrow punches were placed on the lower plate, the top plate was lowered into position, and excess marrow was trimmed with a razor blade. A solvent trap was placed over the geometry and temperature was maintained at 25 °C. A 0.15% strain was selected from a strain amplitude sweep to ensure that experiments were conducted within the linear viscoelastic region (Supplementary Fig. 1). Oscillatory frequency sweeps were conducted between 0.1 and 16 Hz. To capture temperature variation, samples were heated to 35 °C, and the measurements were repeated. The effective Young's modulus, E^{Eff}, was calculated at a frequency of 0.1 Hz assuming Poisson's ratio, υ, of 0.5.

$$E^{Eff} = 2G^{*}(1+\upsilon) \tag{1}$$

2.3. *Indentation*

Indentation is a custom-built instrument that measures the forces applied to materials mounted on a stage (Chan et al., 2008). A flat, cylindrical steel probe (High-Speed M2 Tool Steel Hardened Undersized Rod, 5.2 mm diameter) was brought