Distance from Growth Plate Influences Compressive Properties of Developing Tissue in the Pediatric Knee Joint

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INTRODUCTION: Regenerative tissue engineering in orthopaedics often focuses on the evolution of biological tissues as they form into skeletally mature tissue. However, little is known about the actual mechanical properties of human pediatric tissues due to the scarcity of cadaveric donors. With respect to trabecular bone and cartilage, the relationships between mechanical properties, proximity to the ossification center, and age remain unclear. Our group has the privilege to be included in a study that receives pediatric cadaveric tissues from anonymous donors. We selected a small cohort of proximal tibiae and distal femora to quantify the compressive mechanical properties of developing tissue in the knee. We hypothesized that increased distance from the growth plate in the metaphyseal direction, as well as increased age, would results in increased material properties.

METHODS: *Specimen Information*: Six human cadaveric donor specimens (3 femora, 3 tibiae) from 6 donors (3 males, 1 female, 2 unknown sex, age range 1-18 months) were used in this study. All specimens were deidentified, so the use of an IRB protocol was not required. All specimens were frozen at -20ºC until dissection. *Dissection and sample preparation:* Specimens were defrosted overnight at room temperature and kept in 1% PBS solution to prevent dehydration. Samples were finely dissected to remove all muscle, fat, and connective tissue, leaving the bone, ossification center, and cartilage intact. Specimens were potted in polymethyl methacrylate (PMMA) in polycarbonate tubes (**Fig. 1A**). Potted samples were cut into 2mm-thick transverse slices using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL) (**Fig. 1B**), and the relative distance of each slice from the growth plate was recorded. To provide a standardized shape for mechanical testing, discs were cut from slices using a 6mm-diameter biopsy punch (**Fig. 1C**). In total, 164 discs were collected, and the thickness and cross-sectional area of each disc were measured with Vernier calipers. *Mechanical Testing:* Discs were tested in unconfined compression to determine material properties using established protocols [1-3]. Testing was performed on a mechanical test frame (Instron 5542) using a 500N load cell and custom aluminum platens (**Fig. 1D**). The upper platen was connected to a universal joint to ensure normal application of forces. Discs were compressed to 10% strain under displacement control, and force-displacement data was saved for analysis. *Data Analysis:* Material properties were calculated using custom MATLAB scripts (MATLAB R2022a). We made measures of compressive modulus (MPa, slope of elastic region of stress-strain curve) and Peak Stress (MPa) at 10% strain. Results were batched into three age ranges (< 3months, 8 months, and 18 months) and were evaluated using mixed-effect analysis (GraphPad Prism) to determine the effects growth plate distance and donor age on material properties.

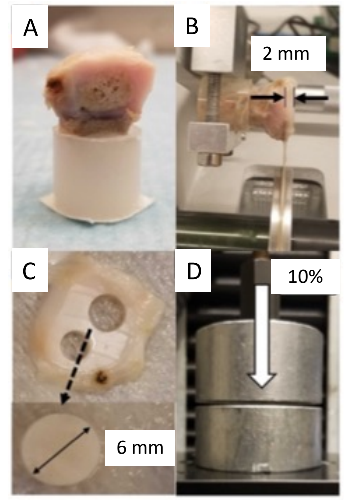

Fig. 1: *Photographic summary of the experimental protocol. Samples were (A) potted in PMMA, (B) cut into 2 mm slices, (C) cored into cylinders, and (D) subjected to unconfined compression to 10% strain.*

RESULTS: Pooled data from human samples indicates that the moduli for discs harvested from the metaphyseal region (< 3mo: 48.46 MPa; 8mo: 51.11 MPa; 18mo: 55.63 MPa) had higher moduli than those from the growth plate (< 3mo: 5.76 MPa, p < .0001; 8mo: 28.49 MPa, p = .052; 18mo: 11.21 MPa, p < .0001) and epiphyseal region (< 3mo: 3.47 MPa, p < .0001; 8mo: 12.70 MPa, p < .0001; 18mo: 7.27 MPa, p < .0001) (**Fig. 2**). Similarly, metaphyseal samples resulted in significantly higher Peak Stress values (< 3mo: 2.90 MPa; 8mo: 3.51 MPa; 18mo: 3.98 MPa) than the growth plate (< 3mo: 0.25 MPa, p = .0002; 8mo: 1.86 MPa, p = .0063; 18mo: 0.59 MPa, p < .0001) and epiphyseal region (< 3mo: 0.17 MPa, p < .0001; 8mo: 0.87 MPa, p < .0001; 18mo: 0.37 MPa, p < .0001). There was only one age-related difference in mechanical properties: Peak Stress in the 18mo metaphysis was significantly higher than in the < 3mo metaphysis (p = .0004).

DISCUSSION: To the best of our knowledge, this study is the first to characterize mechanical properties of developing knee tissue from human pediatric donors. Results from this preliminary study confirmed the first part of our hypothesis, that material properties would increase in the metaphyseal direction. Interestingly, the second half of our hypothesis, that material properties would consistently increase due to age, was rejected. This study includes a small range of ages (1-18 months), and more data is required to fully test this hypothesis. The moduli in this study are substantially lower than skeletally mature samples of trabecular bone, which range up to approximately 2 GPa [4]. This study had several limitations. Specifically, the age range of donors is small and the sample size of the data set is limited. The use of 6 mm diameter cylindrical specimens was helpful to standardize mechanical testing, but the discs limited the number of samples that could be used for testing and samples were not always homogenous in nature. In particular, samples sometimes had a mix of bone and cartilage tissue, but only the hardest tissue provided resistance to the mechanical loads during testing. Future work will include the analysis of stress relaxation and use of a nano indenter to quantify isolated regions of tissue more accurately.

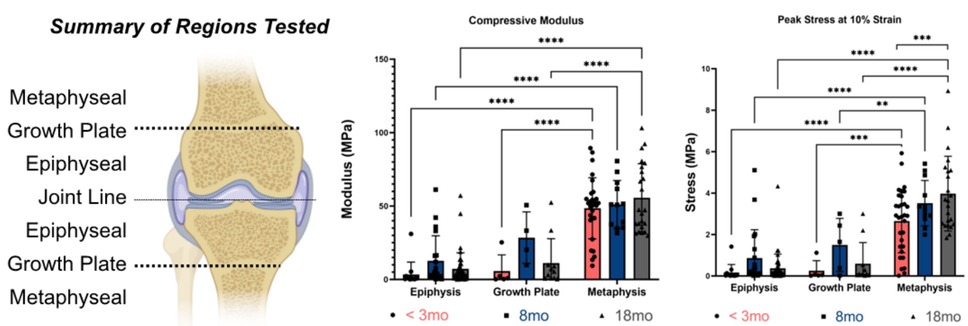

Figure 2: (A) Diagram showing anatomical locations of samples tested in the study. (B) Modulus and (C) Peak Stress results from compression testing (average +/- 1 standard deviation). Modulus and Peak Stress both increased in the metaphyseal direction of the growth plate, but not with age

SIGNIFICANCE/CLINICAL RELEVANCE: Results from this study provide meaningful data in the regenerative orthopaedic tissue engineering research space. Modulus and peak stress values from this study can be used as inputs into computational models, as well as provide guidelines for the development of biomaterials for bone regeneration.

REFERENCES: 1) Julkunen, et al, J Biomech 2008. 2) Korhonen, et al, J Biomech 2002. 3) Delaine-Smith, et al, J Mech Behav Biomed Mater 2016. 4) Morgan+ J Biomech, 2003.

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