Journal of the Mechanical Behavior of Biomedical Materials 84 (2018) 168–177

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Chondron curvature mapping in growth plate cartilage under compressive loading

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ARTICLE INFO

Keywords:
Cartilage
Bone growth
Physis
Epiphyseal plate
Chondrocyte
Mechanobiology

ABSTRACT

The physis, or growth plate, is a layer of cartilage responsible for long bone growth. It is organized into reserve, proliferative and hypertrophic zones. Unlike the reserve zone where chondrocytes are randomly arranged, either singly or in pairs, the proliferative and hypertrophic chondrocytes are arranged within tubular structures called chondrons. In previous studies, the strain patterns within the compressed growth plate have been reported to be nonuniform and inhomogeneous, with an apparent random pattern in compressive strains and a localized appearance of tensile strains. In this study we measured structural deformations along the entire lengths of chondrons when the physis was subjected to physiological (20%) and hyper-physiological (30% and 40%) levels of compression. This provided a means to interpret the apparent random strain patterns seen in texture correlation maps in terms of bending deformations of chondron structures and provided a physical explanation for the inhomogeneous and nonuniform strain patterns reported in previous studies. We observed relatively large bending deformations (kinking) of the chondron structures at the interface of the reserve and proliferative zones during compression. Bending in this region may induce dividing cells to align longitudinally to maintain column formation and drive longitudinal growth.

1. Introduction

The growth plate, also known as epiphyseal plate or physis, is an undulating structure composed of hyaline cartilage and is responsible for the longitudinal bone growth of long bones. Growth plates remain in either end of the long bone between epiphysis and metaphysis until growth stops, which is typically sometime in adolescence when the bone ossifies completely (Iannotti et al., 2000). It is organized into three zones: the resting (or reserve) zone sometimes referred to as the germinal layer containing the stem cells; the proliferative zone with flattened cells that divide; and the hypertrophic zone where mineralization begins and the cells increase in size causing most of the growth. The proliferative and hypertrophic zone chondrocytes are organized in a tubular matrix called a chondron. The extracellular matrix around the chondrons consists of: a pericellular matrix immediately surrounding the cells, a territorial matrix that forms a sleeve around the column of chondrocytes, and interterritorial matrix (longitudinal septa) that lies between two chondrons and forms part of the chondron tubular wall (Eggli et al., 1985). Mechanical forces across the growth plate have been found to alter gene and protein expression and affect chondrocyte metabolism (Iannotti et al., 2000). The Hueter-Volkmann principle states that increased compression retards, whereas reduced compression accelerates the growth rate (Hueter, 1863; Villemure and Stokes, 2009). An example of this is adolescent Blount's disease. The development of bowlegs in this disease is linked to obesity. It is believed to result from asymmetric loading of the bone, which generates nonuniform mechanical forces on the growth plate (Gettys et al., 2011).

Many macro and microscale experiments have been performed to determine the mechanical properties of the growth plate cartilage zones and chondrocytes in the physis (Amini et al., 2013, 2011, 2010; Choi et al., 2007; Cohen et al., 1992; Gao et al., 2014; Sergerie et al., 2009; Sevenler et al., 2013; Villemure et al., 2007). Some have used mathematical modeling and experimental procedures to extract constants for material models by performing stress relaxation tests on compressed growth plate samples assuming transversely isotropy (Cohen et al., 1998). This assumes a structural arrangement in which the dominant direction is along the bone's long axis (also along the chondron direction) and the transverse plane is a plane of symmetry. Multiscale finite element models (Gao et al., 2015) have indicated that chondrons deform by bending when the growth plate is subjected to compression and the tissue stresses and strains in such models have been shown to agree with prevailing ideas about mechanobiology and bone growth (Gao et al., 2017).

Compression experiments have been performed to quantify

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https://doi.org/10.1016/j.jmbbm.2018.05.015
Received 21 February 2018; Received in revised form 3 May 2018; Accepted 9 May 2018

deformation at the tissue level in each zone through imaging (Amini et al., 2010), strain mapping in each zone using texture correlation (Amini et al., 2013; Villemure et al., 2007), and changes in chondrocyte dimensions (Amini et al., 2011) using confocal microscopy. It has been reported that the shear strains in the longitudinal planes induced by shearing the growth plate transverse to the growth direction by 5–15% were largest in the proliferative zone and smallest in the germinal layer and hypertrophic zone (Sevenler et al., 2013). High strains were concentrated at the boundaries between groups of chondrons suggesting that chondron clusters are bound together more tightly as a unit and less so with adjacent clusters. In fractography studies of growth plate samples tested to failure in tension it has been shown that clusters of chondrons gathered at a nutrient vessel penetrating the reserve zone (Williams et al., 2001). The clusters are pulled away at the border with the reserve zone and remain intact on the metaphyseal side, leaving behind a matrix sleeve on the epiphyseal side (Williams et al., 2001).

From the aforementioned experiments, we know that the tubular structure or chondron containing the proliferative through hypertrophic cells play an important role in protecting and distributing stresses when the growth plate is compressed. The fiber orientation and arrangement in and around the chondrons must play an important role in maintaining chondrons in cluster formation while being subjected to relatively large compressive deformations. However, little research exists which would elucidate the microscale chondron level mechanical behavior of the growth plate.

In previous compression studies (Tutorino et al., 2001), no evidence of physical damage could be detected even after subjecting the growth plate to nominal compressive strains approaching 80–90%. However, there were repeatable patterns of periodic oscillations in the stress-strain curves when the growth plate was repeatedly loaded and unloaded to increasing levels of strain, which was hypothesized to be a result of local chondron level deformation (Tutorino et al., 2001). To explore this concept further, the present study aimed to characterize the chondron level deformation in the growth plate under compression by observing and measuring the change in shape of the chondrons under increasing compression levels of physiologic (20%) and hyper-physiologic (30% and 40%) compressive strains. This was achieved by means of confocal microscopy to obtain 3D views of the chondron structures within the growth plate sample before and after stress relaxation tests at different strains.

2. Methods and materials

2.1. Preparation of bone-growth plate cartilage samples

The distal end of an ulnar bone (Fig. 1) was obtained from a twenty-day mongrel-breed pig. The specimens were obtained from animals sacrificed as part of another study that was approved by the University of Memphis animal care and use committee. The samples were washed with phosphate buffered saline (PBS, American Bio Innovations, pH 7.4) and wrapped in gauze and stored at −20 °C until use. After thawing in PBS the distal end of the ulna was cut into three 4-mm thick slices parallel to the longitudinal axis of the bone (Fig. 1) using a diamond wafering blade and an Isomet 1000 bone saw (Buehler, Lake Bluff, USA). Three compression test samples (8 × 4 × 4 mm) were cut from the central region of each slice, stored in PBS at 40 °C until ready for use, or frozen for a later time.

2.2. Growth plate thickness measurements

Each test sample was imaged with a stereomicroscope (Olympus SZX16, Tokyo, Japan) and calibration slide at 5× magnification. Average growth plate thickness measurements (< 0.01 mm resolution) were obtained by measuring the longitudinal distance between epiphyseal and metaphyseal borders at twenty locations on each test sample using Image J (NIH, Maryland, USA). Displacements required to achieve average compressive strains of 20%, 30% and 40% across the growth plate cartilage were calculated for these thickness measurements.

2.3. Staining

The chondrocyte nuclei were stained with PBS: Hoechst 33342 (Invitrogen, Waltham MA) at 1:10,000 dilution for 30 min. The sample remained in a PBS wash for 10 min before being transferred to the compression stage on top of a glass slide.

2.4. Compression device

To compress the samples we used a purpose-built device that incorporated a mechanical micrometer (Pittsburgh 0–1 in. micrometer, Camarillo, CA) with a displacement resolution of 0.0001 in. A custom compression jig was attached to the anvil of a micrometer (Fig. 2a). A custom stage was designed to hold the compression device which could be attached to the confocal microscope stage during imaging and reduce translation of the field of view during imaging. To achieve nominal strains of 20%, 30% and 40% across the growth plate cartilage the micrometer spindle was advanced a distance calculated using the average growth plate thickness for each specimen.

2.5. Confocal microscopy and stress relaxation compression tests

The growth plate was imaged with the sample mounted in the compression setup using a laser scanning confocal fluorescence inverted microscope (Nikon A1, Nikon Instruments, Inc. Melville, NY) to obtain a z-stack of image slices at 10× magnification in planes parallel to the long axis of the bone. The x-y grid size was set to 1024 × 1024 pixels with a 3 μm step size in the z direction and a pinhole size of 55.89 μm. After imaging without compression the sample was compressed to achieve an estimated average strain of 20% across the growth plate, which was maintained for 15 min before imaging again to allow for stress relaxation. These steps were repeated to attain subsequent

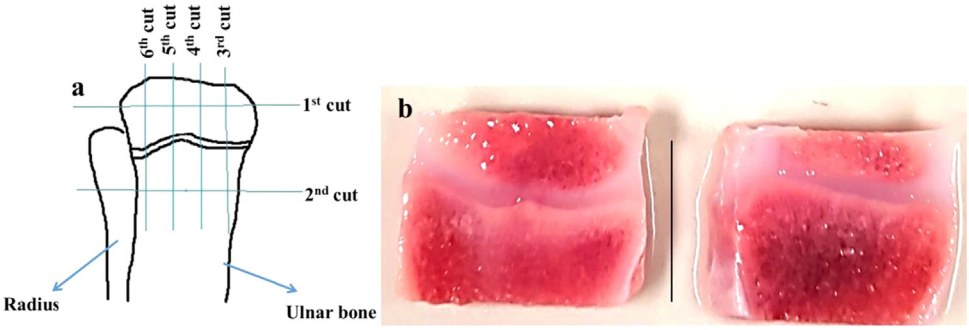

Fig. 1. a) Illustration of the locations for sectioning of the ulnar bone, and b) two sectioned 4-mm thick slices. Scale bar in (b) is 8 mm.