Finite Element Analysis and Modeling of the Reserve Zone Chondrocyte Cilium Response to Growth Plate Compression and Tension

Paola Michelle Jimenez Carrion

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FINITE ELEMENT ANALYSIS AND MODELING OF THE RESERVE ZONE
CHONDROCYTE CILIUM RESPONSE TO GROWTH PLATE COMPRESSION
AND TENSION

by
Paola Michelle Jimenez Carrion

A Thesis
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

Major: Biomedical Engineering

The University of Memphis
August 2021
To my Mom and Dad,
Who have taught me the meaning of self-sacrifice,
For your unconditional love and support
Isaiah 40:28-31
Acknowledgements

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Preface

Chapter 3, the main body of this thesis, is composed of the following journal article:

“Finite element analysis and modeling of the reserve zone chondrocyte cilium response to growth plate compression and tension”, written for submission to the *Journal of Biomechanics*. 
Abstract

Finite element models of the growth plate were created that included chondrocytes with primary cilia at different depths within the reserve zone (RZ) between the epiphyseal subchondral bone-plate (SBP) and proliferative zone (PZ). Cilia were oriented along the direction of bone growth. Loading was applied to induce 10% compression or tension across the growth plate cartilage. Under compression, axial strains in the cilium body were compressive and increased with cell depth between the SBP (-20%) to the PZ (-40%); cilium membrane radial strains were compressive (-5%) near the SBP but became tensile (10%) near the PZ. Under distraction, axial strains in the cilium body were tensile and increased from the SBP (12%) to the PZ (28%); cilium membrane radial strains were tensile (4%) at the SBP but became compressive (-12%) at the PZ. Reserve zone chondrocyte cilia perceive amplified strains that are sensitive to cell depth and direction of loading.
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### Chapter 3. Finite Element Analysis and Modeling of the Reserve Zone

**Chondrocyte Cilium Response to Growth Plate Compression and Tension**

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Chapter 1

Introduction

Motivation for study

The primary cilium of the chondrocyte is a single cytoskeleton organelle composed of microtubules. It transmits a signal from the outside to the inside of the cell and is known to be involved in maintaining homeostasis. Primary cilia have been observed in many different types of connective tissue. It is believed that as mechanotransducers, primary cilia play an essential role in the regulation of mechanisms that contribute to the creation of extracellular matrix (ECM) components (Ascenzi et al., 2007). Previous studies have demonstrated a connection of the primary cilia to the signaling of pathways such as the Indian hedgehog (Ihh). Indian hedgehog is an important pathway in chondrocyte proliferation and growth plate differentiation (Shao et al., 2012). Additionally, it is believed that the loss of primary cilia can lead to abnormalities such as cartilage reduction in articular cartilage leading to osteoarthritis (Irianto et al., 2014). Certain properties of the primary cilium such as length (Flaherty et al., 2020; Khayyeri et al., 2015; Mathieu et al., 2014), orientation (Ascenzi et al., 2007), and stiffness (Khayyeri et al., 2015) have been investigated to understand the mechanics of the primary cilium. However, no studies have been able to fully understand its mechano-sensory function.

Growth plate cartilage is described as a thin cartilaginous plate between the epiphysis and the metaphysis of an immature long bone. The growth plate is comprised of three distinct layers known as the reserve zone, proliferative zone, and hypertrophic zone. The proliferative zone and the hypertrophic zone have cells that are organized in a column-like structure surrounded by
extracellular matrix rich in collage fibers (Eggli et al., 1985). The reserve zone has fewer chondrocytes spaced further away from one another and is adjacent to the epiphyseal bone. Chondrocytes in the growth plate are surrounded by their plasma membrane, pericellular matrix (PCM,) and the extracellular matrix (ECM). Recently, studies have supported the existence of stem-like cells in the reserve zone (Mizuhashi et al., 2018). In addition, chondrocytes termed ‘neocartilage cells’ are found to exist near the subchondral bone- reserve zone tidemark (Kazemi & Williams, 2021). At the other border of the reserve zone, cells start to align and to form the columns of the ‘proliferative zone’. The relationship of the primary cilia with the reserve zone chondrocytes has only recently been described. So far, studies have reported specific cilia orientation in cells in the proliferative and hypertrophic zones, but no evidence of a preferred orientation has been found in the reserve zone (De Andrea et al., 2010). In this study we explore the primary cilium mechanics in the reserve zone. Our goal is to understand the role of the primary cilium structure when the growth plate is loaded in compression and tension. We believe that by characterizing the stresses and strains along the length of the cilium we will be able to explore the sensitivity of the cilia as a function of location within the reserve zone. This will enhance our understanding of the mechanical interactions between the primary cilium and its surrounding matrix in the reserve zone of growth plate cartilage.
References


Chapter 2

Background

Anatomy and Physiology of Cartilage

Cartilage is a fundamental part of the skeletal system. It is found in various forms as hyaline, fibrous or elastic cartilage depending on location and function. Articular cartilage is an avascular connective tissue that covers the articulating ends of the long bones. The growth and maturation of articular cartilage provide an articular surface material with low friction (Williams et al., 2008). Functions of articular cartilage include protecting bone from abrasion, transmitting loads and forces to the subchondral bone, and reducing contact stress between bones (Roschger et al., 2017). The development of this type of cartilage structure is dependent on both biological and mechanical factors tied to the effect of mechanical loading on the cells (Wong & Carter, 2003). These functions are possible due to the hydrated state of hyaline cartilage (Roschger et al., 2017). Morphologically, articular cartilage contains a small number of chondrocytes and is a tissue rich in extracellular matrix (ECM). In addition, its matrix proteoglycans and collagen network permit hydrodynamic load bearing properties for joint articulation and mechanical compression across the joint (Poole, 1997). Articular cartilage can be divided into four layers: superficial, middle, deep, and calcified cartilage. This four-zone concept is assigned depending on the characteristics between surface and depth in articular cartilage (Roschger et al., 2017). With age, there are changes in the distribution of chondrocytes in each zone. Cartilage tends to get thinner as a result of ossification front advancement. Over time, articular cartilage can lose its physical integrity causing loss of function. Loss of function can lead to injury such as osteoarthritis or, if too severe, require surgical intervention. In addition, decrease of matrix
hydration can lead to an increase in compressive stiffness and can affect the subchondral bone (Fox et al., 2009). Although articular cartilage has a unique and complex structure it has a poor capacity for repair which makes it a challenging tissue to investigate.

All long bones of a growing child consist of an epiphysis, physis (growth plate) and metaphysis at each end separated by the diaphysis. The primary center of ossification is in the cartilage anlage of the mid-diaphysis. It becomes the central shaft of a long bone and is composed of mature, lamellar bone with strong cortical exterior. The metaphyses are the flared ends of the central shaft of a long bone between the physis and the diaphysis and are composed of endosteal, spongy or trabecular bone surrounded by the exterior thin cortical bone.

![Structure of a long bone](https://www.cancer.ca/~/media/CCE/2724/83e39ac8a71b03c0d3d26704800facb6.png)

**Fig. 1.** Structure of a long bone. Image from the Canadian Cancer Society (https://www.cancer.ca/~/media/CCE/2724/83e39ac8a71b03c0d3d26704800facb6.png)

The epiphyses rest upon the physis and articulate with the adjacent bones. At birth, most long bones still contain much of the cartilage template or anlage. After the advancing primary and secondary ossification fronts meet, leaving a thin layer of growth cartilage, referred to as the
growth plate or physis, the physis remains and continues to provide bone growth until adolescence. The physis is a complex structure defined by several layers: germinal, proliferative, hypertrophic, and provisional calcification each with a designed function. The germinal zone is also called the resting or reserve zone and stores nutrients and stem cells. The proliferative zone provides for cell division, differentiation of chondrocytes oriented in columns and matrix production. In the hypertrophic zone the cells enlarge in size and some will enhance their metabolic activity though apoptosis, while other become osteoblasts. At the provisional calcification zone vascular channels invade the space of the dead columnar cells within the mineralized extracellular matrix; bone-forming osteoblasts lay down bone on the surface of the remaining calcified cartilage bars to become the primary spongiosa, which are later remodeled to form the secondary spongiosa (Peterson, 2007).

The physis is composed of noncellular components, the matrix, through which the cell columns transverse. The physis, like articular cartilage, is avascular; thus, oxygen and nutrients are provided by the epiphyseal vessels that pass through the reserve zone and end at the base of the columnar zone. The fibrous perichondrium surrounds the outer surface of the physis. The perichondrium is composed of structures such as the Zone of Ranvier which contributes cells to the reserve zone and to peripheral growth and Lacroix’s perichondrial ring which provides mechanical support to the physis (Peterson, 2007).
Hormones that affect Growth Plate Development

Bone is composed of a tough organic matrix that is strengthened by deposits of crystalline calcium and phosphate salts. The relative ratio of calcium to phosphate varies according to nutritional conditions. Precipitation and absorption of these salts are important in maintaining an equilibrium with the extracellular fluid as well as in the role of bone deposition and remodeling. Regulation of vitamin D, parathyroid hormone (PTH) and calcitonin play an important role in the formation of bone (Guyton and Hall, 2016). Vitamin D is integral to the mineralization process of cartilage prior to the formation of bone. Vitamin D is a biologically inactive steroid made in the skin by the action of sunlight. It is converted to 25-hydroxycholecalciferol in the liver and then to 1,25-dihydroxycholecalciferol in the kidneys. 1,25-dihydroxycholecalciferol is the active form of Vitamin D which increases the absorption of calcium and phosphate in the extracellular
fluid and contributes to the feedback regulation of these substances (Guyton and Hall, 2016) which allows for the deposition of calcium into the matrices in normal quantities. Recently, vitamin D has been found to also play an important role in growth plate cartilage development as receptors for 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol have been found in the growth plate cartilage (Shapiro, 2016).

On the other hand, the thyroid hormones also exert their effects on the growth plate through mediation of insulin-like growth factors (IGF). The insulin-like growth factors are regulatory molecules that stimulate longitudinal skeletal growth during the fetal period and throughout postnatal growth until skeletal maturation. Thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are both secreted by the thyroid gland and profoundly increase the metabolic rate of the body as well as increase the transcription of large numbers of genes (Guyton and Hall, 2016). When affecting cartilage, T3 and T4 are mediated through IGF which also mediates the action of the growth hormone in the growth plate. Specifically, in the growth plate, thyroid hormones act on the chondrocytes of the proliferative and upper hypertrophic zone by increasing DNA synthesis in cells and increasing cell maturation, proteoglycan and collagen synthesis, and alkaline phosphatase activity (Shapiro, 2016).

The Parathyroid hormone (PTH) and its receptor protein (PTHrP) have also been identified in the role of growth plate chondrocytes as stimulating the proliferation of chondrocytes and preventing their early hypertrophy in the growth plate (Shapiro, 2016). The parathyroid hormone is important in the control of the extracellular calcium and phosphate concentration by regulating intestinal reabsorption, renal excretion, and exchange between the extracellular fluid and bone of these ions. In addition, PTH also determines the functional effects of vitamin D in the body (Guyton and Hall, 2016).
Bone growth theories

It is believed that under physiological loading the growth plate is primarily subjected to a compressive state of stress (Cohen et al., 1994). The observed relationship between mechanical loading of the growth plate and the longitudinal growth of bones is described by the Hueter-Volkmann principle: bone growth in skeletal immaturity is retarded by incrementing mechanical compression on the growth plate and is accelerated by reducing compression loading (Hueter, 1863; Mehlman et al., 1997; Villemure & Stokes, 2009; Volkmann, 1862). Similarly, Wolf’s law indicates that bone remodeling is a response to the stress in the surrounding environments. When studying dynamic versus static loads in bone growth, Frost concluded that dynamic compression, when in the physiological range, results in bone growth acceleration (Frost, 1990). In fact, without movement and weight bearing, growth will not occur (Golding, 1994). However excessive compression can lead to altered histological features and metabolic functions (Wong & Carter, 2003). Pauwels has developed a tissue differentiation theory which attempts to explain factors that accelerate bone formation and/or maintain cartilage and has been used as a guide for many computational models (Pauwels, 1960).

Previous Studies on Cartilage

Growth plate tested in compression along the growth direction can be considered as a viscoelastic material (Villemure & Stokes, 2009). However, the biomechanics of growth plate cartilage is yet to be fully understood. On the other hand, there has been a significant amount of research done on articular cartilage that might contribute to the understanding of growth plate cartilage mechanics. Articular cartilage is considered a biphasic material composed of 1) a solid matrix phase (proteoglycan macromolecules and collagen fibers) and 2) a fluid phase (interstitial fluid, predominantly water). The deformation of cartilage not only depends on the mechanical
properties of the constituents of the solid matrix but also on its interstitial water (Mow et al., 1982). Researchers have attempted to explain the role of the interstitial fluid in articular cartilage by performing creep and relaxation tests. From the stress relaxation test, a relaxation equilibrium state is obtained where no further fluid exudation occurs and the internal fluid is redistributed and ceases to move through the matrix (Armstrong et al., 1984) enabling equilibrium modulus values to be obtained at various applied stress levels. In the biphasic model of cartilage, the interstitial fluid flow and solid deformation are coupled through drag forces related to the permeability of the tissue. The mechanical behavior of growth plate is comparable to articular cartilage as they have similarities in their composition and structure (Villemure & Stokes, 2009). A handful of studies have attempted to explain the behavior of growth plate cartilage in compression. Various models based on the biphasic theory have been developed, including linear biphasic (Mow et al., 1980), nonlinear biphasic (Cohen et al., 1994), and transversely isotropic biphasic models (Cohen et al., 1998).

To study the mechanics of hydrated soft tissue, cartilage was modeled by Mow’s linear biphasic theory (Mow et al., 1980) as having two phases composed of: 1) an inviscid fluid and 2) an elastic isotropic solid. The material properties of growth plate cartilage can be obtained through experiments such as confined and unconfined tests (Cohen et al., 1998; Cohen et al., 1994; Mow et al., 1980; Sergerie et al., 2009; Wosu et al., 2012). A transversely isotropic biphasic model (TIBM) was developed (Cohen et al., 1998) to see if a better curve fit could be obtained than was obtained earlier using an isotropic model (Armstrong et al., 1984). In the TIBM model two regions of the growth plate were tested under confined and unconfined tests: the chondroepiphysis/reserve zone region and the proliferative/hypertrophic zones region. Results showed that the proliferative/hypertrophic zone was half as stiff and twice as permeable
as the chondroepiphysis zone and that the elastic modulus in the transverse plane was about ten times greater in tension than in compression. It was also concluded that a transversely isotropic model provides a better fit to the experimental data than the isotropic model (Cohen et al., 1998). Transverse isotropy was also used for each of the growth plate zones and showed that the reserve zone is twice as stiff as the proliferative and hypertrophic zones along the compression axis and three times stiffer in the transverse plane (Sergerie et al., 2009). The permeability of the proliferative zone and hypertrophic zone were similar in order of magnitude to those presented by Cohen et al.; however, the permeability of the reserve zone was 2.3% lower than what Cohen et al. reported as the permeability for the chondroepiphysis/reserve zone in four-month-old bovine growth plate. Good agreement was obtained between the experimental and theoretical values, although peak stresses were generally higher in the experimental curves. In another study (Wosu et al., 2012) two methods were used to obtain mechanical properties of the growth plate at four different ages: newborn, 4, 8, and 18 weeks. The TIBM approach was also used in combination with a differential evolution global optimization algorithm (Price et al., 2005) to extract material properties in each method. Results showed that the transverse and out-of-plane Young’s modulus decreased, and the permeability increased as the age increased. Overall, it was concluded that the growth plate becomes more flexible and more permeable with age.

The behavior of growth plate under compression in both the peripheral sites and interior sites of the intact growth plate cartilage were analyzed by a nonlinear biphasic theory (Cohen et al., 1994) by means of three models: permeable at epiphyseal and metaphyseal sides, permeable at metaphyseal side and impermeable at epiphyseal side, and impermeable at epiphyseal and metaphyseal interfaces. In this study, agreement was obtained between the curve-fit finite element model and the experimental data from the compression stress-relaxation tests. In
addition, it was concluded that the intrinsic permeability for articular cartilage was much lower than that of the growth plate. Recently, a poroelastic biphasic model of growth plate cartilage was developed (Kazemi and Williams, 2021) that showed chondrocytes in the reserve zone experience strains that are highly dependent on location and strain rate. It was also concluded that shear stresses caused by fluid flow may be sufficient to stimulate the reserve zone chondrocytes located near the subchondral bone border, and that this may be important in understanding the role of the reserve zone cells in modeling and remodeling the subchondral bone-plate. It was also shown that at high strain rates the behavior of the growth plate may be analyzed using a purely elastic model, as the fluid phase has too little time to move through the matrix to cause any time-dependent effects during short-duration loading events such as gait. Thus, many biphasic models have been used to describe the behavior of growth plate cartilage under loading to aid in the understanding of cartilage mechanics, but short duration events with time scales far shorter than those of the time for stress relaxation to occur, may be analyzed using a purely elastic model.

**Previous Studies on Primary Cilium in Cartilage**

The primary cilium is a 0.2 µm, singular, non-motile organelle protruding from the cytoskeleton that plays a key role in signal transmission, mechanosensation, and the cell cycle (Khayyeri et al., 2015). The cilium has been observed in many different types of tissues, including cartilage (McGlashan et al., 2008), tendons (Donnelly et al., 2010b) and bone (Malone et al., 2007). It is believed that by sensing the mechanical environment the cilium plays an essential role in homeostasis, contributes to the creation of extracellular (ECM) components (Ascenzi et al., 2007), and regulates matrix secretion (Tao et al., 2020). Prior models of kidney and vascular cells have proposed that the primary cilium responds to fluid flow by amplifying
tensile strains of the plasma membrane (Moo et al., 2012). Tensile strain results in the opening of stretch-activated calcium channels near the base of the cilium (Mathieu et al., 2014; Rydholm et al., 2010). Other studies demonstrated that the signaling of events in the Indian hedgehog (Ihh) pathway are depleted when the primary cilium is not present. The Ihh pathway plays an important part in chondrocyte proliferation and differentiation in growth plate cartilage (Shao et al., 2012). In addition, it is believed that the loss of primary cilia causes the deeper zones of articular cartilage to have reduced instantaneous and equilibrium moduli which could lead to thickening of cartilage and abnormal joint formation (Irianto et al., 2014).

Cilia have variable characteristics such as length, orientation, and stiffness that can help the cell control mechanosensation. Previous studies investigating cilium length reported this parameter to be important for several reasons. In articular cartilage, there is an increase in chondrocytic cilium length that results from stress, strain, and fluid flow experienced as a function of cell location from superficial to deep zones (McGlashan et al., 2008). In addition, longer cilia are also observed with a decrease or removal of mechanical stimulation under cyclic compression (McGlashan et al., 2010). In a cell modeled under fluid flow conditions, modeling a longer cilium resulted in higher strains in the nucleus, cytoplasm and the cilium itself (Khayyeri et al., 2015). In growth plate cartilage, the cilium is shortest in the proliferative zone when compared to the reserve and hypertrophic zones (de Andrea et al., 2010) suggesting length is associated with cell cycle. On the other hand, a finite element study of a cell embedded in a collagen gel subjected to tensile loading concluded that cilium length had little influence on the strain in the ciliary membrane (Mathieu et al., 2014). Thus, other properties of the cilium must be considered besides cilium length.
Several studies have investigated cilium orientation in connective tissue. When observed in tendon, primary cilia were found to be highly oriented with respect to the collagen fibers in the ECM (Donnelly et al., 2010a). In articular cartilage, the cilium was found on the inferior side of the cell in the superficial zone oriented away from the articular surface, while in the middle to deep zones the orientation remained unclear (McGlashan et al., 2008). In a load-bearing area, primary cilia were oriented towards the subchondral bone (Farnum & Wilsman, 2011). However, the cilia in deeper zones were oriented either toward the articular surface or toward the subchondral bone, while in non-load bearing areas this pattern disappeared. In growth plate cartilage, the cilia did not acquire a clear orientation in the reserve zone. In the proliferative and hypertrophic zones the cilia formed what is called a “virtual axis”, where they pointed either towards the epiphyseal or metaphysical side (Ascenzi et al., 2007, 2011; de Andrea et al., 2010). This pattern is similar to what was reported in the deep zone of articular primary cilia chondrocytes.

In studies using transmission electron microscopy (TEM) it has been observed that the cilium can remain partially intracellular as a result of the flagellar pocket. This pocket is formed by the plasma membrane and is located near the base of the cilium before joining the ciliary membrane (Molla-Herman et al., 2010). Primary cilia orientation also seems to influence membrane stress and strain. Previously studies reported stress concentration found in the membrane near the base of the cilium which could lead to the location of stretch activated calcium channels that are crucial for signaling (Rydholm et al., 2010). In a finite element study where tensile strain was applied perpendicularly and parallel to the cilium, results showed greater tensile strain amplification in the ciliary membrane when the strain is applied parallel rather than perpendicular to the cilium (Mathieu et al., 2014).
In addition to the cell membrane, the flexural rigidity of the primary cilium is also a factor that plays a role in the transduction of external signals (Downs et al., 2014; Young et al., 2012). Khayyeri et al. (2015) explored the stiffness of the cilium by comparing two Young’s modulus values derived from calculations of flexural rigidity in the cilium axoneme (Herzog, 2010; Rydholm et al., 2010; Young et al., 2012). Their simulations conclude that different flexural rigidity properties of the cilium do not lead to major differences in the strains within the nucleus, cytoplasm, or cortex; however, a softer cilium develops greater strains under loading. These results led them to conclude that the regulation of sensory channels located in the cilium, is modulated by the cilium itself (Khayyeri et al., 2015).

Lastly, it is known that the nucleus also plays a key role in mechanotransduction. Studies that have compressed cartilage by 15% report a significant decrease in chondrocyte and nucleus height and volume (Guilak, 1995). Compression influences gene transcription, chromatin conformation (Leipzig & Athanasiou, 2008) and reduces aggrecan synthesis (Buschmann et al., 1996). It has been suggested that excessive forces transmitted to the nucleus could lead to structural damage in chromatin and cause damage in DNA (Wang et al., 2018). Studies reporting mechanical properties of the nucleus state that the nucleus is three to four times stiffer than the cell cytoplasm (Chen et al., 2012; Guilak et al., 2000). However, in situ experimental studies suggest a one-to-one correlation in cellular and nuclear strains (Leipzig & Athanasiou, 2008) and a finite-element model found the best fit of cytoplasm to nuclear stiffness ratio to be 1.4. This disagreement may be explained if there is a difference in the mechanical behavior of chondrocytes when isolated in vitro than in when studied in situ (Ofek et al., 2009). Thus, we need to understand whether the cilium is sensing mechanical loading from other structures and/or transmitting mechanical loading to other structures and if this affects its function.
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Chapter 3

Finite Element Analysis and Modeling of the Reserve Zone Chondrocyte Cilium Response to Growth Plate Compression and Tension

Introduction

Growth plate chondrocytes moderate bone growth in response to mechanical loads by transducing strains and stresses into intracellular biochemical signals through a variety of mechanisms under active investigation. Efforts to understand the role of mechanics through computational models of proliferative (PZ), hypertrophic zone (HZ) chondrocytes (Gao et al., 2015; 2017) and reserve zone (RZ) chondrocytes (Kazemi & Williams, 2020; 2021) in their surrounding 3D matrix structures have revealed depth- and zone-dependent variations in cellular stress and strain. Computational modeling has also shown the reserve zone chondrocyte pericellular matrix (PCM) to be an essential component of the cell’s mechanosensory mechanism (Kazemi & Williams, 2020). Not included in these models is the primary cilium located in the PCM which has been shown to be an important mechanism for signal and force transduction in cells (Ruhlen & Marberry, 2014) and cartilage development (Tao et al., 2020).

The relevance of cilia in cartilage was demonstrated in a study in which depletion of cilia in articular cartilage chondrocytes resulted in an upregulation of osteoarthritic antigens and increase in cartilage thickness (Irianto et al., 2014). Besides the presence or absence of cilia, their lengths also appear to be relevant to the cell’s mechanosensory mechanism as shown by the association of increased cilia lengths in osteoblast cells of patients with idiopathic scoliosis (Oliazadeh et al., 2017). In addition to cilium length, cilium orientation is important to establish the axis of bone growth. The primary cilia in the PZ and HZ are aligned parallel to the longitudinal axis of the bone, implying that polarization gradients exist inside the growth plate that direct the cells to their specific positions and orientations (de Andrea et al., 2010). This
polarity was shown to be disturbed but partly retained in growth plate sub-populations of small columns of osteochondroma cells (De Andrea et al., 2010). Cilia do not appear to be organized in the RZ and it has been suggested that the RZ generates and maintains the polarization gradients by secreting morphogens (De Andrea et al., 2010) that control the alignment of clones in preparation for the transition into the proliferative zone in the PZ (Abad et al., 2002). Despite the apparent importance of the RZ as a regulator of the physis (Mizuhashi et al., 2018) and of primary cilia as mechanotransducers, we have found no computational studies on the mechanical signals in and around cilia in the RZ related to their function as signal transducers in growth plate cartilage.

In this study we used computational modeling to explore the following questions: (1) do cilia perceive strains differently depending on cell depth in the RZ region between the subchondral epiphyseal bone plate and PZ in ways that could be associated with cell recruitment into the PZ? (2) do cilia perceive strains differently when the growth plate is subjected to compression versus tension in ways that could explain modulation of bone growth by the Heuter-Volkmann principle? (3) Is the PCM shear stress environment indicative of cilium orientation and could it be a factor in chondrocyte kinesis and rotation near the PZ?

Methods

A 2D axisymmetric, static finite element model of a central region of bone-growth plate-bone volume was created using ABAQUS/CAE 2019 in which circular chondrocytes were embedded in three locations within the RZ. Each cell in the model is surrounded by a pericellular matrix (PCM), cell membrane, cilium, and ciliary membrane, and is composed of a cytosol, and nucleus. A uniform displacement of 0.08 mm was prescribed on the surface of the test sample along the long axis of the bone (Y-direction) which equals 10% of the 0.8 growth plate cartilage
thickness. This displacement was applied in the negative Y-direction for compressive loading and in the positive Y-direction for tensile loading (Fig. 1).

Fig. 3. 2D plane axisymmetric model of a central cylindrical volume of bone-growth plate-bone. Nodes along the axis of symmetry (Y-axis) are free to move in the axial or Y-direction and are fixed in the radial or X-direction. Nodes along the bottom are fixed in the Y-direction and free in the X-direction. Cells are included at three locations in the reserve zone.

Axisymmetric 4-node bilinear, hybrid linear pressure elements (CA4XH) were used to avoid volumetric locking due to the nearly incompressible material properties assigned to some of the regions (Table 1). Mesh convergence studies showed the model converged when the Von Misses stresses in the nucleus and cilium of the model varied less by than 5% between consecutive refinements. Non-linear geometric effects and large strains were accounted for by
turning on NLGEOM. The growth plate was partitioned into two sections to represent the reserve zone (RZ) and the proliferative/hypertrophic zone (PZ/HZ). To examine the influence of cell depth within the RZ, cells were embedded in three locations (Fig. 4): Cell 1 close to the epiphyseal subchondral bone-plate border at the upper end of the RZ, Cell 2 in the middle and Cell 3 at the lowest level near the PZ border.

Fig. 4. Reserve zone (RZ) region of the idealized axisymmetric model consisting of bone-cartilage-bone components with three cells located in the RZ. Each cell is embedded in the RZ and consists of a pericellular matrix (PCM), a primary cilium with a ciliary membrane, a cell membrane, a cytoplasm, and a nucleus, as shown for Cell 2.

Isotropic linear elastic material properties and dimensions for the various regions in the model were obtained from the literature (Table 1). The primary cilium had a radius of 0.1 µm and length of 2.5 µm (0.5 µm inside the cell and 2 µm in the PCM). All regions of the cilium body were assigned a Young’s modulus (YM) of 178 kPa, Poisson’s ratio (PR) of 0.33. The cilium was surrounded by a membrane of 10 nm thickness (Rydholm et al., 2010). The analyses were static, representing short time, fast occurring loading where the fluid was assumed to not flow; thus, treating the cartilage as a single-phase material.

Table 1. Linear elastic material properties of the model
<table>
<thead>
<tr>
<th>Component</th>
<th>Young’s Modulus (MPa)</th>
<th>Poisson’s ratio</th>
<th>Radius (mm)</th>
<th>Height/Thickness (mm)</th>
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<tr>
<td>Epiphysis/ Metaphysis</td>
<td>1000/1000</td>
<td>0.2/0.2</td>
<td>1</td>
<td>0.5/0.5</td>
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<tr>
<td>Subchondral Bone (SB)</td>
<td>2000 (Moo et al., 2014)</td>
<td>0.2 (Moo et al., 2014)</td>
<td>1</td>
<td>0.6</td>
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<tr>
<td>Calcified Cartilage (CC)</td>
<td>300 (Kazemi &amp; Williams, 2021)</td>
<td>0.2 (Fan et al., 2008)</td>
<td>1</td>
<td>0.06</td>
</tr>
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<td>Cytoplasm</td>
<td>0.0035 (Ofek et al., 2009)</td>
<td>0.4999 (Ofek et al., 2009)</td>
<td>0.005 (Chen et al., 2012)</td>
<td>-</td>
</tr>
<tr>
<td>Nucleus</td>
<td>0.005 (Ofek et al., 2009)</td>
<td>0.4999 (Ofek et al., 2009)</td>
<td>0.0025 (Ofek et al., 2009)</td>
<td>-</td>
</tr>
<tr>
<td>Chondrocyte &amp; Cilium Membranes</td>
<td>0.040 (Moo et al., 2012)</td>
<td>0.47</td>
<td>-</td>
<td>0.00001 (Rydholm et al., 2010)</td>
</tr>
<tr>
<td>Primary Cilium</td>
<td>0.178 (Rydholm et al., 2010)</td>
<td>0.33 (Rydholm et al., 2010)</td>
<td>0.0001 (Rydholm et al., 2010)</td>
<td>-</td>
</tr>
<tr>
<td>PCM</td>
<td>0.265 (Allen &amp; Mao, 2004)</td>
<td>0.45 (Gao et al., 2015)</td>
<td>0.0075 (Korhonen et al., 2008)</td>
<td>0.0025</td>
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<td>Reserve Zone (ECM)</td>
<td>0.98 (Gao et al., 2015)</td>
<td>0.47 (Gao et al., 2015)</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Proliferative Zone</td>
<td>0.49 (Kazemi &amp; Williams, 2020)</td>
<td>0.47 (Kazemi &amp; Williams, 2020)</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Provisional Calcification</td>
<td>100 (Kazemi &amp; Williams, 2021)</td>
<td>0.2 (Kazemi &amp; Williams, 2021)</td>
<td>1</td>
<td>0.16 (Kazemi &amp; Williams, 2020)</td>
</tr>
</tbody>
</table>

Two orientations of the cilium were considered, both aligned with the long axis of the bone in the major direction of bone growth: oriented towards the epiphysis or the metaphysis. To describe the results the cilium was segmented into several regions: basal body, transition zone, proximal axoneme, middle axoneme and distal axoneme. The basal body extended 0.5 μm inside the cell beneath the cell membrane, the transition zone was at the level of the cell membrane, and the proximal, middle, and distal axoneme regions projected 2 μm into the PCM with the distal
end of the cilium located 0.5 µm from the outer radius of the PCM. The ciliary membrane covered the portion of the cilium extending into the PCM and contained three regions: proximal, middle, and distal ciliary membrane. The centroidal value for the elements in each cilium and cilium membrane region were used to obtain axial (Y-) and radial (X-) strains. Cell height and cell width strains were reported as engineering strains from the change in horizontal radius and vertical diameter before and after compression.

Axisymmetry prevents consideration of other orientations in which bending would play a role in deforming the cilium. To investigate stress conditions within the PCM at other possible cilium orientations, in-plane shear stresses and maximum shear stress contour plots were obtained within the PCM. Shear stress values at 0, 90 and 180 degrees were plotted at the location where the distal end of the cilium would be in the PCM (2 µm out from the cell membrane and 0.5 µm from the PCM/ECM interface).

To investigate the sensitivity of the results to the assumed material property values for the cytoplasm we increased the cytoplasm’s Young’s modulus ten-fold from 3.5 kPa to 35 kPa. This increase may also represent various states of cytoskeletal activity in response to growth plate loading. Cell height- and width-strains were calculated and reported as engineering strains. The cell height strain was calculated from the ratio of the change in the vertical diameter of the cell before and after compression to the undeformed diameter. The width strain was similarly obtained from the horizontal radius of the cell. Nucleus height- and width- strains were obtained in a similar fashion. All other strains were reported as logarithmic strains. Likewise, to investigate the sensitivity of the results to the assumed material properties of the nucleus we increased the Young’s modulus of the nucleus 10-fold from 5 kPa to 50 kPa.
Results

Axial (Y-) strains within the cilium body were compressive and remained so along the cilium length for all cell locations, increasing in magnitude with cell depth, and were amplified 2X in Cell 1 and nearly 4X in Cell 3 where cells prepare to enter the proliferative zone (Fig. 5b).

Radial strains within the cilium remained compressive along the length for Cell 1, decreasing in magnitude and leveling off distally. This pattern was similar for Cell 2 with lower strain magnitudes. In Cell 3 a switch is observed from compressive in the basal body to tensile strains between the basal body and the transition zone where the tensile strains leveled off (Fig 5c). The results for axial and radial strains were identical whether the cilium was placed at 180 or 0 degrees to the +Y-axis, demonstrating no apparent up or down preference in terms of strain sensitivity for the cilium in the RZ when aligned with the axis of bone growth.

Fig. 5. Radial and axial strains of the primary cilium in three cell locations within the RZ: Near subchondral bone plate (Cell 1), middle of the RZ (Cell 2), near the PZ (Cell 3) for 10% compression of growth plate cartilage. a) Representation of cilium regions; b) Radial (X-) strain; c) Axial (Y-) strain
During 10% compression of the growth plate, axial strains in the cillum membrane were amplified as much as two-fold over the applied 10% strain and were compressive along the cillum body (Fig. 6). They also increased in magnitude with cell depth from maximum strains in the proximal portion of the cillum membrane equal to the applied strain for Cell 1 near the epiphyseal bone plate to double the applied strain for Cell 3 near the PZ.

Radial strains in the cillum membrane were compressive and about half the applied strain in Cell 1 but became tensile as cell depth increased for Cells 2 and 3 for which a maximum radial strain was equal in magnitude to the applied compressive strain of 10% in the proximal region of the membrane.

Fig. 6. Radial and axial strains of cillum membrane in three cell locations within the RZ: near subchondral bone plate (Cell 1), middle of RZ (Cell 2), near PZ (Cell 3) for 10% compression of growth plate cartilage. a) Representation of cillum membrane regions; b) Radial (X-) strain; c) Axial (Y-) strain.
Simulating a distraction of 10% tensile strain applied across the growth plate generated an inverted pattern of radial (X-axis) and axial (Y-axis) strains along the cilium compared with 10% compression, with a sign inversion and slightly lower peak magnitudes (Appendix A). This sign inversion is best observed by direct comparison for Cell 3 (Fig. 7). Axial strains were compressive when the growth plate was compressed and tensile when distracted. Axial strains were amplified almost 4X in compression and 3X in tension predominantly in the transition zone of the cilium interior. Cilium membrane strains were tensile when the growth plate was compressed and compressive when it was distracted by 10%. Radial strains across the cilium membrane in the proximal region were equal in magnitude to the applied axial strain across the growth plate and decreased to half distally.

Fig. 7. Cilium and cilium membrane radial and axial strains in Cell 3 (cell near the PZ) for 10% growth plate subjected to either a 10% tensile strain and or a 10% compressive strain across the growth plate.
The in-plane shear stresses increased with cell depth within the RZ. In-plane shear stresses in the PCM varied with location around the perimeter of the cell as well as with radial distance from the cell surface. In-plane shear stresses at a radius (7 µm) corresponding to the distal end of the cilium ranged in magnitude from 0.05 to 0.25 kPa at 0 and 180 degrees, and from 0.35 to 0.4 kPa at 90 degrees, increasing in magnitude with the depth of the cell within the RZ. In-plane shear stresses were concentrated at 45 and 135 degrees from the positive Y-axis near the cell membrane surface (contour plots in Fig. 8a) at all three cell locations increasing from 8 kPa in Cell 1 to 13 kPa in Cell 3. In-plane shear stresses at the distal end of the cilium relate to stresses associated with bending of the cilium for cilium orientations deviating from the axis of bone growth.

Maximum 3D shear stresses at a radius corresponding to the distal end of the cilium (7 µm) ranged in magnitude from 15 to 40 kPa at 0 and 180 degrees, and from 14 to 46 kPa at 90 degrees, decreasing in magnitude with the depth of the cell within the RZ. Maximum 3D shear stress values were concentrated at the cell membrane surface at 0- and 180-degree locations ranging from 31 kPa for Cell 1 to 69 kPa for Cell 3 near the PZ and along the cell surface at 90 degrees for Cells 2 and 3 (45 to 55 kPa) (Fig. 8).
Fig. 8. Line graphs and contour plots of a) in-plane shear stress and b) 3D maximum (Tresca) shear stress within the PCM of cells in three locations in the RZ when subjected to 10% compression of growth plate cartilage. Line graphs are based on three locations: 0, 90 and 180 degrees (contour plots are in MPa). Cell 1 is located near the subchondral bone plate. Cell 2 is in the middle of reserve zone and Cell 3 is near the proliferative zone. The outer radius of the PCM is 7.5 µm and the inner radius of the PCM is 5 µm. In-Plane shear stresses and maximum shear stresses in the line plots were extracted at a radius of 7 µm corresponding to the distal tip if the cilium.
The results were relatively insensitive to the assumed material properties of the cytoplasm (Fig. 9). A ten-fold increase in Young’s modulus of the cytoplasm resulted in negligible changes (0.04% to 0.4%) in chondrocyte height and width strains under 10% growth plate compression (Fig. 9). Relatively small changes were seen for the nucleus strains and were more pronounced at greater cell depths in the RZ. For Cell 3, near the PZ, increasing the cytoplasm modulus caused the nucleus width strain to increase from 9% to 13%, and the height strain to increase from -17% to -22%. Likewise, a ten-fold increase in Young’s modulus of the nucleus had a negligible influence on cell height and width strains, however, the nucleus height and width strains decreased as much as 3-fold in Cell 3.

![Fig. 9. Comparison of height and width strains for the chondrocyte and the nucleus in three cell locations in the RZ when subjected to 10% compression of growth plate cartilage: Near the subchondral bone plate (Cell 1), in the middle of RZ (Cell 2), near PZ (Cell 3). a) Chondrocyte height and width strain at each cell location. b) Nucleus height and width strain for each cell location. The influence of the cytoplasm modulus is more pronounced as the cell location approaches the PZ. The curves for both models with and without cilium overlap.](image)
Discussion

We developed a model to investigate the role of the primary cilium in reserve zone (RZ) chondrocytes. We explored the sensitivity of ciliary strains to chondrocyte location within the growth plate RZ relative to the subchondral bone plate and proliferative zone (PZ) borders; this information is relevant to the role of cells near and within the tidemark region adjacent to the subchondral bone plate and of cells at the border of the PZ that enter the proliferative phase. We examined the sensitivity of ciliary strains to changes in loading direction. Understanding this may provide insight into how loading modulates growth rate. Lastly, we examined the relationship of pericellular matrix shear stresses to the observed ciliary orientations in the reserve zone noting that studies have shown no preferred ciliary orientation in reserve zone chondrocytes until after the cells enter the columnar zone.

The model represents a 2-mm diameter cylindrical volume of tissue from an interior region of a growth plate in which the outer circumferential surface of the model is far enough from the axis where the cells were located, for boundary conditions to not affect the local stress field around the cells, as was determined by comparing various diameter models. The thickness values of each growth plate zone were chosen based on histology sections of the proximal femoral epiphysis of 480-day-old pigs (Kazemi and Williams, 2021). The RZ thickness was half that of the previous study while the other zones had similar values. This range in values is representative of what is seen in the histological sections. The model is limited to high strain rates during short time intervals justifying the assumption of elastic behavior (Cohen et al., 1998; Kazemi & Williams, 2021). The use of 2D axisymmetry assumes the structure and loading do not vary in the hoop direction. This limited cilium orientations to coincide with the axis of axisymmetry.
Material properties were assumed to be isotropic and homogeneous within each region. The simple model of the cilium did not include fibers that form a bridge between the transition zone microtubules and the ciliary membrane (Young et al., 2012) or the nine pair-ring structure of the axoneme which is composed of microtubules (Molla-Herman et al., 2010) which are likely to be important to understanding the mechanobiology of the cilium.

For a compression of the growth plate by 10%, cell strains increased with cell depth within the 0.4 mm thick RZ reaching maximum values of -20% height strain and 11% width strain for the chondrocyte at the columnar zone border. This trend is the same as shown in a previous study on the RZ (Kazemi & Williams, 2020a) in which the RZ thickness was 0.95 mm and the cell strain values at 0.4 mm depth were -25% for height strain and 15% for width strain when the growth plate was compressed by 15%. In the present model cell strains were insensitive to a 10-fold increase in Young’s modulus for the cytoplasm, while nucleus strains showed some sensitivity suggesting that the cytoskeleton could play a role in transmitting strains to intracellular organelles perhaps in connection with deflection of the primary cilium (Khayyeri et al., 2015). The model would need to include intracellular details to investigate this further.

Compression of the growth plate by 10% produced the highest compressive strains (40%) in the transition zone of the cilium in the cell closest to the PZ. High strains in this region have also been reported for other tissues subjected to other loading environments (Mathieu et al., 2014; Young et al., 2012). These high strain levels in the cilium transition zone hint at the mechanosensory role of the cilium not only through transmembrane channels present in base of the cilium, but also, bridging proteins such as integrins that help the cilium bind with the extracellular matrix or cytoskeleton. The transition zone of the cilium is known for accumulating and filtering the entry of multiple proteins including transport motor proteins that run along the
long axis of the axoneme (Garcia et al., 2018; Moore & Jacobs, 2016). Thus, the high concentration of molecules and proteins in the proximal axoneme might be related to the stress built up in the proximal membrane.

Strains in the radial direction inside the cilium and ciliary membrane became tensile and equal in magnitude to the applied compressive strain in RZ cells at the border of the PZ, which has half the elastic modulus of the RZ, but remained compressive near the calcified cartilage of the subchondral bone-plate, which has a modulus 300 times greater than the RZ. At the PZ border these tensile radial membrane strains are likely transmitted from the PCM to the cilium through integrins attaching the cell and cilium to the PCM. RZ chondrocytes at the PZ transition organize themselves to enter PZ columns through cell division and rotation (Aszodi et al. 2003; De Andrea et al., 2010). Previous studies (Chang et al., 2012; Yuan et al., 2015) have linked the primary cilia to cell differentiation and signaling factors which stimulate the proliferation, differentiation and rotation of the chondrocyte as it enters the PZ. In addition, the transition zone of the cilium, also known also as the ‘check-point’, is the location where most of the proteins are being regulated and sorted (Garcia et al., 2018; Reiter et al., 2012). Thus, the tensile radial strains across the membrane, that are maximum at its transition zone, may stimulate the organization of cells to prepare for entry into the proliferative zone tubular columns.

Both compression and tension produced a notable depth-dependent strain response in chondrocyte cilium strains suggesting that chondrocytes sense external loading differently depending on their position in relation to the subchondral bone-plate and the PZ. Tension across the growth plate produced a similar pattern of depth-dependence in axial cilium and membrane strains as was seen for compression, except that the strains were reversed in sign. Under applied tension the axial cilium membrane strains in the cell near the PZ were tensile and radial strains
compressive, whereas under applied compression the axial cilium membrane strains were compressive and the membrane radial strains tensile. In both tension and compression, the primary cilium strains for cells close to the PZ were larger than for cells near the subchondral bone-plate. In both cases the largest axial strains occurred in the transition zone of the cilium and in the proximal cilium membrane; thus, applying compression or tension to the growth plate causes the most changes when the cells are transitioning to the proliferative zone.

As to whether cells can distinguish external compression from tension by means of the cilium, it may be noted that both the axial and radial strains in the cilium basal body of the cell near the PZ follow the applied loading sign, i.e., growth plate compression produced compressive radial and axial strains, while tension produced tensile radial and axial strains in the basal body. These results suggest one way by which cilia could distinguish compression from tension. The basal body plays a role in motility and cell cycle progression as well as morphogenesis. It is possible that tension and compression of the basal body could initiate different biological responses. Another possibility is that the cilium membrane acts as a sensor. Tension across the growth plate stretches the proximal cilium membrane thereby opening stretch activated ion channels, while compressive loading results in tensile strains in the radial direction which act across the membrane/PCM interface and stretch integrin receptors (McGlashan et al., 2006). Each of these events may lead to different cellular responses that modulate bone growth.

The orientation of the cilium has been noted to be disrupted in cartilage diseases such as osteoarthritis, osteochondroma, an chondrosarcoma (Barsch et al., 2020; De Andrea et al., 2010, 2015; McGlashan et al., 2008). In the proliferative and hypertrophic zones of growth plate cartilage, primary cilia are present at the top or bottom of the cell and oriented towards the subchondral bone or towards the metaphysis. In the reserve zone, however, no clear pattern for
orientation has been determined (De Andrea et al., 2010). We found no differences in cilium strains between the 0- and 180-degree orientations of cilia in the present study. It has been proposed that cilia are oriented in a particular direction partly for protection of the cilium itself as well as preserving intracellular structures (Barsch et al., 2020; McGlashan et al., 2008; Rich & Clark, 2012).

In this study, the pericellular matrix shear stresses were concentrated in three locations along the cell surface and were greatest at the 0-, 90- and 180-degrees locations indicating that a primary cilium located there will experience more shear stress at the cell membrane junction than at other locations. The maximum matrix tissue shear stresses of 31 kPa to 69 kPa in the present study are comparable to the 55 kPa value reported for articular cartilage under 10% compression (Athesian et al., 2007). These maximum shear stresses increased in magnitude with cell depth and peak values were seen for the cell near at the PZ, where cells are positioned to join a column or divide to form daughter cells and orient their cilia vertically in the proliferative zones. It has been shown that cilia in columnar chondrocytes cluster at 10- and 80- degrees (Aszenzi et al., 2011), coinciding with regions where we found peak maximum shear stresses. The shear stresses are of interest because they could cause stretching of integrins anchoring the cell and cilium to the PCM and stimulate cell kinesis and rotation, which are likely to be involved as RZ cells transition from RZ to PZ.
References


Gao, J., Roan, E., & Williams, J. L. (2015). Regional variations in growth plate chondrocyte


Chapter 4

Conclusions

In this study our goals were to (1) examine the sensitivity of ciliary strains to changes in loading direction by comparing compression and tension loading, (2) characterize the stresses and strains along the length of the cilium to explore the sensitivity of the cilia as a function of location within the reserve zone, and (3) explore the relationship of pericellular matrix shear stresses to ciliary orientations in reserve zone chondrocytes.

Our results indicate that in tension and compression, the largest axial strains occurred in the transition zone of the cilium and in the proximal cilium membrane. In addition, the largest changes were observed when the cells were transitioning from the reserve zone to the proliferative zone. Both compression and tension produced a notable depth-dependent strain response in chondrocyte cilium strains, suggesting that chondrocytes sense external loading differently depending on their position in relation to the subchondral bone-plate and the proliferative zone. We found that maximum shear stresses increased in magnitude with cell depth and peak values were seen for the cell near at the proliferative zone, where cells are positioned to join a column or divide to form daughter cells and orient their cilia vertically in the proliferative zones.

In summary, the information obtained from this finite element model provides new insight into the mechanics of reserve zone chondrocyte cilia and sheds light on the possible mechanosensory function of the primary cilium role at different depths in the reserve zone of growth plate cartilage.
APPENDICES

Appendix A. Light microscopy images of tibia cartilage bridge

Figure 1A is a ‘stitched’ image of the proximal tibia growth plate a 4-to-5-month calf sample at 2x magnification. Figures A2-A6 show images of the growth plate cartilage at 10x magnification. Figures A7-A10 show images of the growth plate cartilage at 20x magnification. All images were corrected using ImageJ software (see appendix E).

Figure 1. 2x view of a 4–5-month calf growth plate in the proximal tibia. H&E stain.

Figure 2. 10x Growth plate reserve zone in the center. H&E stain.
Figure 3. 10x Growth plate cartilage showing reserve zone, proliferative zone, and hypertrophic zone. H&E stain.
Figure 4. 10X. Growth plate cartilage showing reserve zone, and short proliferative and hypertrophic zones. A layer of calcified cartilage can be seen in the lower portion of the image. H&E stain.

Figure 5. 10x Growth plate cartilage showing only cells in the reserve zone.
Figure 6. 10x View of tibia cartilage bridge showing the reserve zone and cells forming short columns in the proliferative and hypertrophic zones. Calcified cartilage bars can be seen in the upper right corner. H&E stain.

Figure 7. 20x Growth plate cartilage showing chondrocyte clusters. A layer of calcified cartilage is seen in the lower part of the image. H&E stain.
Fig. 8. 20x Growth plate cartilage showing clusters of hypertrophic chondrocytes. H&E stain.

Fig. 9. 20x Growth plate cartilage showing cells in the reserve zone. H&E stain.
Fig. 10. 20x Growth plate cartilage showing cells in the hypertrophic zone. H&E stain.
Appendix B. Primary Cilium and Ciliary Membrane Results when subjected to 10% tension

A 2D axisymmetric, static finite element model of a central region of bone-growth plate-bone volume was created using ABAQUS/CAE 2019 in which circular chondrocytes were embedded in three locations within the RZ. Each cell in the model is surrounded by a pericellular matrix (PCM), cell membrane, cilium, and ciliary membrane, and is composed of a cytosol, and nucleus. A uniform displacement of 0.08 mm was prescribed on the surface of the test sample along the long axis of the bone (Y-direction) which equals 10% of the 0.8 growth plate cartilage thickness. This displacement was applied in the positive Y-direction for tensile loading (Fig.1).

Normal logarithmic strains in the radial (X-axis) and axial (Y-axis) directions were obtained in the cilium and in the membrane similar to results obtained in compression. The strains in the radial and axial directions are non-uniform as the axoneme transitions from proximal to distal Fig 1. In the radial direction in the cilium, the strains become compressive up to about 7% when in the proximal axoneme and as the cell depth increases. The opposite occurs in the axial direction, the strains become tensile up to about 30% in the transition zone of the cilium and as the cell depth increases.
Fig 1. Radial and axial strains of primary cilium in three cell locations within reserve zone - near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3) when subjected to 10% tension of growth plate cartilage. Graphs show cilium regions for each cilium. a) Representation of cilium regions; b) Radial Strains; highest compressive radial strain is about 7% in the proximal axoneme in Cell 3; c) Axial Strains; highest tensile strain is about 30% in the transition zone of the cilium in Cell 3.

Additionally, results for three ciliary membrane regions from three cell locations were extracted and plotted (Fig. 2): proximal, middle, and distal membrane. In the radial direction, the strains become compressive up to about 13% when in the proximal membrane and as the cell depth increases. The opposite occurs in the axial direction, the strains become tensile up to about 20% in the proximal ciliary membrane and in the cell that is closes to the proliferative zone.
Fig 2. Radial and axial strains of ciliary membrane in three cell locations within reserve zone—near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3) when subjected to 10% tension of growth plate cartilage. Graphs show membrane regions around each cilium. a) Representation of membrane regions; b) Radial Strains; highest compressive radial strain is about 12% in the proximal membrane in Cell 3; c) Axial Strains; highest tensile strain is about 20% in the proximal membrane in the cilium of the cell nearest to the proliferative zone.
Appendix C. Primary Cilium and Ciliary Membrane Results when compared to a published study

To relate the model to cartilage and bone regeneration studies in 3D cultures of chondrocytes embedded in a collagen gel (Mathieu et al., 2014) a separate model was created in which the primary cilia (YM = 178 kPa) and primary cilium membrane properties (YM = 1 kPa) matched those in a prior study (Mathieu et al., 2014) and all regions in the mesh surrounding the chondrocyte, including bone, calcified cartilage, PCM, and ECM were assigned material properties of a collagen gel (YM = 17 kPa). In the model by Mathieu et al (2014) the dimensions of each region were: primary cilium radius = 95 nm, ciliary membrane thickness = 5 nm and collagen gel = 5 µm³. The model was then subjected to a uniform displacement at the top edge of 0.26 mm, corresponding to an end-to-end nominal strain of 10% corresponding to the loads in a published study (Mathieu et al., 2014).

Three cilia were located in the collagen gel at the same distances from each other as in the reserve zone models (0.16 mm); Cell 1 (near subchondral bone), Cell 2 (middle of the reserve zone) and Cell 3 (near proliferative zone). The results showed the maximum principal strains are not very sensitive to the small differences in depth of the cells located in the central region of the collagen gel. The maximum principal tensile strains along the cilium body peaked at 7.5% in the proximal axoneme and decreased in the distal region of the axoneme to about 4.5%.

Maximum principal strains for the ciliary membrane of cells at three depths within the collagen gel. The maximum principal tensile strain increases along the length of the cilium as the ciliary membrane reaches the distal axoneme of the cilium and peaks at 50% in the distal region of the ciliary membrane. The least amount of maximum tensile strain (10%) occurs in the proximal membrane.
Fig 1. Maximum Principal Tensile Strain in a) cilium regions and b) ciliary membrane regions in three cells within the reserve zone-near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3) when 10% tension is applied to the growth plate. The maximum principal tensile strain is 7.5% when in the proximal axoneme. The least amount of maximum tensile strain occurs in the distal region of the axoneme at about 4.5%. Maximum principal tensile strain is highest at 50% in the distal ciliary membrane. There is an overlap in the principal strain curves of the three cells due the minimal to no difference obtained within location.
Appendix D. Height and Width Strains of the cell and nucleus under 10% Compression

To investigate the sensitivity of the results to the assumed material properties of the nucleus we increased the Young’s modulus of the nucleus 10-fold from 5 kPa to 50 kPa.

Figure 1. The Young’s Modulus of the nucleus region in the model was increased 10 times the original Young’s Modulus. No modulus-dependent difference is observed in the cell strains. In the nucleus the width and height strains decrease by a 3-fold at the location of Cell 3.
Appendix E. Primary Cilium and Ciliary Membrane Results when subjected to 5% compression

A 2D axisymmetric, static finite element model of a central region of bone-growth plate-bone volume was created using ABAQUS/CAE 2019 in which circular chondrocytes were embedded in three locations within the RZ. Each cell in the model is surrounded by a pericellular matrix (PCM), cell membrane, cilium, and ciliary membrane, and is composed of a cytosol, and nucleus. A uniform displacement of 0.04 mm was prescribed on the surface of the test sample along the long axis of the bone (Y-direction) which equals 5% of the 0.8 growth plate cartilage thickness. This displacement was applied in the negative Y-direction for compressive loading (Fig.1).
Figure 1. Radial (X-) and axial (Y-) strains of a primary cilium in three cell locations within the reserve zone - near the subchondral bone plate (Cell 1), in the middle of reserve zone (Cell 2), and near the proliferative zone (Cell 3) - when subjected to 5% compression of growth plate cartilage. Graphs show cilium regions for each cilium. a) Representation of cilium regions; b) Radial Strains; highest tensile radial strain is ~3% in the proximal axoneme in Cell 3; c) Axial Strains; highest compressive strain is ~18% in the transition zone of the cilium in Cell 3.
Figure 2. Radial (X-) and axial (Y-) strains of ciliary membrane in three cell locations within reserve zone—near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3)—when subjected to 5% compression of growth plate cartilage. The horizontal axis indicates the membrane regions around each cilium. a) Representation of membrane regions; b) Radial Strains; highest tensile radial strain is about 5% in the proximal membrane in cell 3; c) Axial Strains; highest compressive strain is ~11% in the proximal membrane in the cilium of the cell nearest to the proliferative zone.
Fig 3. Comparison of height and width strains for the chondrocyte and the nucleus in three cell locations within reserve zone- near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3). Results for two models are shown: one with cilium properties, one without cilium properties. The results are identical for both models. a) Chondrocyte height and width strain for each cell location b) Nucleus height and width strain for each cell location. Cell depth in both a) and b) in left to right order from subchondral bone to the proliferative zone.
Appendix F. Comparison of primary cilia for two orientations: 0-and-180-degrees under 5% compressive strain

To investigate if there were differences in the axial and radial strains when the primary cilium was placed at 0 and 180 degrees in the cell, and differences when a cilium is present vs no cilium present, strains in the normal directions were obtained. The radial strain (LE11) is the normal strain in the X-direction and the axial strain (LE22) is the normal strain in the Y-direction (from metaphysis to epiphysis). Two models were created by using the same mesh but assigning different material properties. In the first model, the elements representing the primary cilium were assigned the same properties as the PCM (named ‘no cilium’), whereas in the second they were assigned the properties of the primary cilium (named ‘cilium’).

Fig. 1. Cell indicating possible cilium positions at 0 and 180 degrees.
Figure 2. Comparison of radial (LE11) and axial (LE22) strains in the distal axoneme region of the primary cilium, in three cell locations within reserve zone: near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3).
Figure 3. Comparison of radial (LE11) and axial (LE22) in the middle axoneme region of the primary cilium, in three cell locations within reserve zone - near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3).
Figure 4. Comparison of radial (LE11) and axial (LE22) strains in the proximal axoneme region of the primary cilium, in three cell locations within reserve zone—near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3).
Figure 5. Comparison of radial (LE11) and axial (LE22) strains in the transition zone of the primary cilium, in three cell locations within reserve zone—near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3).
Figure 6. Comparison of radial (LE11) and axial (LE22) strains in the basal body of the primary cilium in three cell locations within reserve zone—near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3).

Radial and axial strains of ciliary membrane regions were also obtained when the model was subjected to 5% compression. Two models were created by using the same mesh but assigning different material properties. In the first model, the elements representing the ciliary membrane were assigned the same properties as the PCM (labeled ‘no cilium’ in the plots), whereas in the second they were assigned the properties of the ciliary membrane (labeled ‘cilium’).
Figure 7. Comparison of radial (LE11) and axial (LE22) strains in the proximal, middle, and distal regions of the ciliary membrane in three cell locations within reserve zone - near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3).

Appendix G. Maximum principal strains for the growth plate at 10% tensile strain
Maximum principal strains were obtained when the growth plate was subjected to 10% tension to characterize the strains in each region of the cilium and ciliary membrane.

![Graph 1](image1.png)

![Graph 2](image2.png)

Fig 1. Maximum principal strains of the primary cilium regions and ciliary membrane regions in three cell locations within reserve zone- near subchondral bone (Cell 1), middle of reserve zone (Cell 2), near proliferative zone (Cell 3)- when subjected to 10% tension of growth plate cartilage. Graphs show cilium regions and membrane regions for each cilium.
Appendix H. ImageJ correction

A macro code was used in ImageJ to correct the background illumination for the histology obtained from light microscopy.

I. For each magnification, focus the sample, set exposure time, correct brightness with condenser.

II. Block the light path in microscope and take a picture. Call it ‘Dark Field’.

III. Allow light to penetrate through the sample and move the eye piece of the microscope to a place in the slide where there is no tissue. Take a picture and call it ‘Bright Field’.

IV. Capture your region of interest in the slide. This will be your ‘sample’.

V. Open ImageJ and import the three pictures taken: Dark Field, Bright Field, and sample.

VI. Run the macro code to correct the background illumination.

The macro code is as follows:

```
imageCalculator ("Subtract create", "BrightField.tif","DarkField.tif");
selectWindow ("Result of BrightField.tif");
rename ("Divisor");
imageCalculator ("Subtract create", "Sample.tif","DarkField.tif");
selectWindow ("Result of Sample.tif");
rename ("Numerator");
run ("Calculator Plus", "i1=Numerator i2=Divisor operation=[Divide: i2 = (i1/i2) x k1 + k2]
k1=255 k2=0 create");
selectWindow ("Result");
```
Appendix I. Abaqus input file

The following script is part of the model input file used in Abaqus for a static, elastic model of growth plate cartilage, including three cells in the reserve zone. A uniform displacement of 0.08 mm was prescribed on the surface of the test sample along the long axis of the bone (Y-direction) which equals 10% of the 0.8 mm growth plate cartilage thickness.

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