THE MORPHOGENESIS AND ANALYSIS OF PORCINE FEMORAL HEAD MAMMILLARY PROCESSES. A COMPUTATIONAL STUDY OF THE STRUCTURAL MECHANISMS OF BIOMECHANICAL STABILITY

Ronald Vincent Perrone Jr.

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THE MORPHOGENESIS AND ANALYSIS OF PORCINE FEMORAL HEAD MAMMILLARY PROCESSES. A COMPUTATIONAL STUDY OF THE STRUCTURAL MECHANISMS OF BIOMECHANICAL STABILITY

by

Ronald Vincent Perrone Jr.

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Major: Biomedical Engineering

The University of Memphis

Summer 2022
Dedicated to my wife and children

for their unconditional love and support

and to the memory of my father
ACKNOWLEDGEMENTS

First and foremost, I would like to thank Professor John L. Williams for allowing me the opportunity to study under his advisement. Words cannot express my gratitude for the incredible experience of having been guided by his brilliance. He has and continues to inspire me in the pursuit of science excellence and this experience has been a chapter of growth in my life that is unparalleled; for he has shown me how to see the forest for the trees and for that I am eternally grateful.

In addition, I would like to express my sincerest gratitude to my committee members, Dr. Amy Curry, Dr. Carl Herickhoff and Dr. Aaryani Sajji for their willingness to serve as members of my examination committee. I would like to thank Drs. Randy and Karyl Buddington and Dr. Ebrahim Asadi for their contributions to this work.

I would also like to thank my fellow graduate students, particularly Masumeh Kazemi and Paola James with whom I had the pleasure of working during my PhD.

Finally, I would like to thank my loving and devoted wife for her unconditional and unwavering support throughout this very long journey. I am forever grateful for you.
This dissertation consists of three manuscripts that have either been published to different peer-reviewed journals or are in a format following author guidelines for submission to a peer-reviewed journal. The manuscript in Chapter 2 entitled ‘Dimensional accuracy and repeatability of the NextEngine laser scanner for use in osteology and forensic anthropology’ was published in the Journal of Archaeological Science: Reports in April 2019. The manuscript in Chapter 3 entitled ‘The morphogenesis of porcine femoral head mammillary processes: A structural mechanism of biomechanical stability’ was published in The Anatomical Record in June 2021. The manuscript in Chapter 4 entitled ‘Computational studies of the structure-function relationship of porcine femoral head mammillary processes’, follows the author guidelines to submit to the peer-reviewed journal The Anatomical Record.
ABSTRACT

The capital femoral physis is a growth plate located between the head of the femur and the femoral neck and forms a temporary joint where growth cartilage differentiates into bone by endochondral ossification during development. A slip occurs when a shear stress across the physis overcomes the mechanical integrity of the bone-cartilage-bone interface, known as slipped capital femoral epiphysis. Though this disorder is widely studied the etiology is not completely understood. Joint morphology may play a critical role in stability, until the joint closes in early adulthood. During development morphological changes in the joint emerge and provide natural mechanical mechanisms resistant to shear: At first a large, eccentrically located epiphyseal tuberosity (tubercle), which projects into a corresponding metaphyseal fossa followed by epiphyseal cupping, which envelops the metaphysis. These features have also been observed in the domestic pig where the tubercle starts as an elongated ridge in early development, decreasing in length to a peaked structure in the caudal-lateral region as age increases. A shear component of the hip joint force acting in a plane parallel to the growth plate, through the center of the femoral head, produces a moment around the pivot point provided by the tubercle and could induce rotation of the femoral head relative to the neck unless the joint develops additional structures for stability. This work examined the development of domestic pig joint morphology from birth to adolescence through a comprehensive analysis of biomodels created from laser scans of pig femoral bones. Finite element models of idealized geometries were developed to examine the structure-function relationship of the peripheral secondary mammillary processes. The comprehensive analysis showed secondary mammillary processes in the periphery developed in a radial pattern with a degree of periodicity, well suited to resist torsional loading by interlocking the joint. Computational results showed that the radial mammillary processes
acted to limit the translations and tilting of the head but had reduced effectiveness when the
growth plate thickness was doubled. This suggests their radial pattern develops specifically to
resist torsion and enhance stability of the joint, protecting the growth plate against torsional
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**Fig. 4.8** Top Left) Epiphyseal displacements in the x, y and z directions as tracked by the reference node. The reference node (black dot) at the origin represents the initial position, where models $T_{1\text{mm}}$ and $FD_{1\text{mm}}$ have a growth plate thickness of $1\text{-mm}$ (blue and red squares respectively) and the models $T_{2\text{mm}}$ and $FD_{2\text{mm}}$ (blue and red circles respectively) have a growth plate thickness of $2\text{-mm}$. Bottom Left) The displaced growth plate of $FD_{1\text{mm}}$ (dark gray) juxtaposed with the growth plate’s initial condition (light gray), showing the metaphyseal interface. The direction of the displacement in the xy plane was caudal-medial, indicated by the black arrow. Right) contour plots for each full model (top) and the growth plate for each model (bottom). Blue indicates displacement of the epiphysis in the negative z-direction (away from the metaphysis) denoted by the blue arrow, whereas red indicates displacements in the positive z-direction (toward the metaphysis) indicated by the red arrow, as shown on the full model $FD_{2\text{mm}}$. The black dot on the complete models indicates the reference node and the origin of the global coordinate system, in Cartesian coordinates.

**Fig. B1** The top curve is the unwrapped profile. The dashed curve in the plot is the $n^{\text{th}}$ order Form polynomial and the dashed-dot curve is the $n^{\text{th}}$ order Form + Waviness polynomial used for profile deconvolution to separate the waviness and roughness profiles from the primary profile, providing the roughness profile for surface roughness analysis (bottom curve).

**Fig. B2** Waviness parameters $W_a$ and $W_z$ calculated from profile height as a function of circumferential distance. The red dashed-dot line is the centerline average (CLA) for the profile. The circumferential distance is noted at each quadrant interface, (caudal lateral, caudal medial, cranial medial, cranial lateral) from left to right. Horizontal lines indicate the maximum peak ($R_p$) and maximum valley ($R_v$) for each evaluation length. Gray shading in the first interval indicates this interval was omitted from the average calculation because there was only a peak and not a valley. The count is defined as the curve crossing the centerline twice for any interval that has not been omitted.

**Fig. B3** Roughness parameters $R_a$ and $R_z$ calculated from profile height as a function of circumferential distance. The red dashed line is the centerline average (CLA) for the profile. The circumferential distance is noted at each quadrant interface, (caudal lateral, caudal medial, cranial medial, cranial lateral) from left to right. Horizontal lines indicate the maximum peak ($R_p$) and maximum valley ($R_v$) for each evaluation length. The count is defined as the curve crossing the centerline twice for any interval that has not been omitted.
that has not been omitted. For this curve all 16 intervals were used in the roughness calculations.

Fig. B4  The averaged amplitude and wavelength ($\lambda$) for the roughness curve (solid blue) and waviness curve (red dash-dot) as a function of circumferential distance.

Fig. C1  Epiphyseal cylinders 80% the diameter of the epiphyseal rim, were created from Boolean subtractions and the perimeter of the cylinder “unwrapped” and projected to a plane. This provided cross-sectional curves of the peripheral radial mammillary processes. A total of eight parameters were measured to define an idealized cross-sectional sketch which was extruded (sweep extrusion) and patterned about the central axis of the femoral neck (axis of symmetry (Supplement 2 Table C2).

Fig. C2  A total of 22 parameters were measured to define the idealized tubercle. A&B) Cross-sections taken at the tubercle peak and length ends along with splines defined a scaffold of the tubercle). C) The average measures of these scaffolds defined an idealized base footprint of the tubercle. D) From this footprint and three cross-sectional sketches, cones were defined with average peak curvatures and angles of the tubercle. E) Splines were used as sweep guides which allowed for a smooth transition from the ridge’s peak curvature diameter to the length end ridge peak diameters, which decreased in size. F) Sweep extrusions were used to create the idealized tubercle (Supplement 2 Table C3).

Fig. C3  The location of the tubercle peak for each biomodel was determined using a custom color topography mapping algorithm, using the polar coordinate system of quadrants previously define. Each location was normalized to the diameter of the epiphyseal rim, as previously defined, and plotted onto the coordinate system. Cross hairs denote the anatomical quadrants and were projected to the surface of the tubercle and the peak.

Fig. C4  Contour plots of shear strain across the growth plate surface. Top row) The growth plate surface in contact with the epiphyseal subchondral bone plate surface (epiphyseal interface), reported in cylindrical coordinates. Bottom row) The growth plate surface in contact with the metaphyseal growth plate surface (metaphyseal interface). Solid arrows show the direction of rotation and dashed arrows denote the surface of the growth plate (gray models) the contour plot coincides with. Neighboring element values within the cartilage region only were averaged at the nodes unless
the relative difference between contributions from neighboring elements at the node was greater than 75% (as denoted in the legend).

Fig. C5 Line plots of the maximum principal strain across the growth plate surface. Top row) The growth plate surface in contact with the epiphyseal subchondral bone plate surface (epiphyseal interface), reported in cylindrical coordinates. Bottom row) The growth plate surface in contact with the metaphyseal growth plate surface (metaphyseal interface). Solid arrows show the direction of rotation and dashed arrows denote the surface of the growth plate (gray models) the contour plot coincides with. Neighboring element values within the cartilage region only were averaged at the nodes unless the relative difference between contributions from neighboring elements at the node was greater than 75% (as denoted in the legend).

Fig. D1 A cross-section of the volumetric mesh for model FD shows the minimum of two elements in each 1-mm zone of the model with higher concentrations near the tubercle and radial mammillary processes. Zone $z_1$ is shown in green, $z_2$ shown in purple, $z_3$ shown in light blue, $z_4$ shown in dark blue and $z_5$ shown in white.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BW</td>
<td>Body weight</td>
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<tr>
<td>BR</td>
<td>Epiphyseal articular bone ridge</td>
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<tr>
<td>CCD</td>
<td>Caput-collum-diaphyseal</td>
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<tr>
<td>Cdl</td>
<td>Caudal</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CLA</td>
<td>Center line average</td>
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<tr>
<td>CMM</td>
<td>Microscribe MX 3D coordinate measurement machine</td>
</tr>
<tr>
<td>Cnl</td>
<td>Cranial</td>
</tr>
<tr>
<td>CoR</td>
<td>Coefficient of repeatability</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>DOF</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>EL</td>
<td>Elongation lip</td>
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<tr>
<td>ESBP</td>
<td>Epiphyseal subchondral bone plate</td>
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<tr>
<td>FD&lt;sub&gt;1mm&lt;/sub&gt;</td>
<td>Model with tubercle, 1mm growth plate and radial mammillary processes</td>
</tr>
<tr>
<td>FD&lt;sub&gt;2mm&lt;/sub&gt;</td>
<td>Model with tubercle, 2mm growth plate and radial mammillary processes</td>
</tr>
<tr>
<td>FE</td>
<td>Finite element</td>
</tr>
<tr>
<td>HD</td>
<td>High definition</td>
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<tr>
<td>HP</td>
<td>Hewlett-Packard Pro S3 structured light scanner</td>
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<tr>
<td>ISO</td>
<td>International organization for standardization</td>
</tr>
<tr>
<td>Ltl</td>
<td>Lateral</td>
</tr>
<tr>
<td>LOA</td>
<td>Limits of agreement</td>
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<tr>
<td>Mdl</td>
<td>Medial</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MGP</td>
<td>Metaphyseal growth plate</td>
</tr>
<tr>
<td>NE</td>
<td>2010 NextEngine model 2020i laser scanner</td>
</tr>
<tr>
<td>Ra</td>
<td>Surface roughness</td>
</tr>
<tr>
<td>SCFE</td>
<td>Slipped capital femoral epiphysis</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDc</td>
<td>Corrected standard deviation</td>
</tr>
<tr>
<td>S_{dr}</td>
<td>Developed interfacial area ratios</td>
</tr>
<tr>
<td>T_{1mm}</td>
<td>Model with tubercle, 1mm growth plate and no radial mammillary processes</td>
</tr>
<tr>
<td>T_{2mm}</td>
<td>Model with tubercle, 2mm growth plate and no radial mammillary processes</td>
</tr>
<tr>
<td>VHX</td>
<td>Keyence VHX 6000 digital microscope</td>
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CHAPTER 1

INTRODUCTION

1.1 Motivation for this study and objectives

The capital femoral physis is a growth plate located between the head of the femur (epiphysis) and the femoral neck (metaphysis) and forms a temporary joint where growth cartilage differentiates into bone by endochondral ossification in young growing vertebrates. Slipped capital femoral epiphysis (SCFE) occurs when a shear stress across the growth plate overcomes the mechanical integrity of the bone-cartilage-bone interface resulting in a translational shear as well as a rotational displacement of the epiphysis relative to the metaphysis and is the most common hip disorder in adolescent children (Aronson & Tursky, 1996). Patients who suffer from a slip often present with an increased external rotation of the affected leg when the hip is flexed, with internal rotation causing mild to severe pain, which may also be restricted due to impingement (Livingstone et al., 2019). The rotational displacement has been clinically characterized as a posteromedial displacement of the epiphysis relative to the metaphysis (Hammond et al., 2016; Khaladkar et al., 2015), though cases of posterolateral displacements have been reported (Gelink et al., 2021). Though the disorder is widely studied, the etiology is not completely understood, and joint morphology may play a critical role in stabilizing the joint during development until the joint closes and the epiphysis fuses with the metaphysis.

During development an arrangement of interdigitating undulations of various sizes called mammillary processes emerge on the epiphyseal subchondral bone plate (ESBP) surface and project into corresponding concavities on the metaphyseal growth plate (MGP) surface. These mammillary processes provide natural mechanical mechanisms of resistance by interlocking the epiphysis with the metaphysis, enhancing the biomechanical stability of the joint. Scheuer et al.
(2000) described a prominent “beak-like” epiphyseal tuberosity (tubercle), which projects into a corresponding metaphyseal fossa (Novais et al., 2020). Tayton (2009) characterized the change in the ESBP surface from “one of friction to one of pegging” (Tayton, 2009). This tubercle has been suggested to be a primary stabilizer during early-stage adolescence, though it is “wide-based and fairly flat” in humans compared to their ovine, bovine and porcine counterparts (Perrone Jr & Williams, 2021; Tayton, 2007). It has been argued that the prominence and eccentric location of the tubercle provides resistance to transverse and torsional shear and an underdeveloped tubercle and enlarged metaphyseal fossa may be contributing factors to instability (Hosseinzadeh et al., 2021; Novais et al., 2020; Tayton, 2007). Tubercle height has been reported to decrease, whereas epiphyseal cupping increases as a function of age (Kiapour et al., 2019; Liu et al., 2013). These age-related changes in epiphyseal shape shift the “loading dynamics of the epiphysis in a way that the epiphyseal cupping will become the more important stabilizer” in later stages of adolescence (Kiapour et al., 2019) against transverse shear. However, a component of a distributed joint force acting in a plane parallel to the growth plate, through the center of the femoral head, could produce a moment about the pivot point provided by the eccentrically located tubercle if the growth plate is not stabilized by the development of additional structures.

The femur of the one-year-old *sus scrofa domesticus* (domestic pig) has been shown to have comparable biomechanical properties and dimensions to the adolescent human counterpart (Chung et al., 1976) and has been used for mechanical and fixation device testing (Chuinard et al., 2004; Chung, 1991; Chung et al., 1976; Jónasson et al., 2015; Snyder et al., 2006). In addition to physiological and gross anatomical similarities, the domestic pig also suffers from the hip disorder SCFE (Jubb, Kennedy, & Palmer, 1985). The torsional strength of this joint was
tested using one-year-old pig femora secured and fixed at the diaphysis, with the epiphysis secured and fixed in a custom designed torque holder. An increasing torque was applied until complete failure of the joint occurred (defined as a 30% drop in the peak torque value) (Chuinard et al., 2004). Upon examination of the growth plate surface of femurs where the epiphysis had disarticulated, a set of radially arranged peripheral mammillary processes were observed on the ESBP surface. These ridges appeared to be similar in shape, size and pattern in all the one-year-old domestic pig specimen and simply could not just be random. This led to the question of how these ridges arise with age and whether there is any degree of systematic periodicity to the pattern among pigs. Characterizing the development of these radial secondary mammillary processes with a comprehensive qualitative and quantitative analysis could provide valuable insight into these structures, which may be a key mechanical mechanism to withstand a torque induced pivot of the epiphysis about the tubercle. We hypothesized that these processes develop to form a radial pattern with some degree of periodicity beginning relatively early in development of the joint and increase in prominence with age and weight of the animal. If they are a normal feature of the joint, understanding the structure-function relationship could also provide valuable insight into the biomechanical stability of the joint. Underdevelopment could be a risk factor for SCFE. Evaluating their role in stability could prove challenging and would involve comparing the morphology of joints with slips to those without, using conventional imaging modalities such as high-resolution computed tomography and magnetic resonance imaging scans. However, with finite element methods we can evaluate the structure-function relationship with parametric models of idealized joints with and without these features. Such numerical results could provide valuable insight into the mechanical mechanisms of joint
stability and examine what variables other than underdevelopment could render them less effective.

1.2 Outline of the dissertation

This dissertation is divided into five chapters and is submitted as an article-based dissertation:

Chapter 2. Dimensional accuracy and repeatability of the NextEngine laser scanner for use in osteology and forensic anthropology. This chapter details the development of highly detailed, accurate biomodels generated from laser scans of the proximal end of porcine femurs and quantifies the dimensional accuracy of the scanner to determine the smallest reportable surface feature. We also evaluate our intra-observer scanning protocol and quantify the repeatability of the scanning process.

Chapter 3. The morphogenesis of porcine femoral head mammillary processes: A structural mechanism of biomechanical stability. This chapter provides a complete qualitative and quantitative analysis of the development femoral head mammillary processes from biomodels of the proximal end of femurs from domestic pigs ranging in age from nine-days preterm to 900-days old. We evaluated six key points of development and introduce a metric of surface roughness analysis to quantify mammillary processes and apply it to analyze the development of the observed radial pattern of peripheral mammillary processes from birth to adolescence.

Chapter 4. Computational studies of the structure-function relationship of porcine femoral head mammillary processes. This chapter contains the development of nonlinear parametric explicit finite element models using parameters derived from the comprehensive analysis of six 900-day old domestic pig femora. A set of four models were generated to evaluate
the interlocking radial mammillary processes and how increased growth plate thickness affects their role in joint stability.

Chapter 5. This chapter contains a summary of the dissertation and conclusion of the work presented, with a recommendation for future work. The motivation is to better understand the structure-function relationship of femoral head mammillary processes and define the role of the mechanical mechanisms involved with stabilizing the joint to protect the growth plate during development. Considering the anatomical similarities of the porcine joint and human counterpart, this knowledge will be useful in the development of diagnostic methods that can better detect individuals who may have a higher risk of experiencing slipped capital femoral based on mechanical risk factors associated with underdevelopment of joint morphology.
CHAPTER 2

DIMENSIONAL ACCURACY AND REPEATABILITY OF THE NEXTENGINE LASER SCANNER FOR USE IN OSTEOLOGY AND FORENSIC ANTHROPOLOGY

2.1 Introduction

The availability of low-cost, compact scanners has given researchers powerful tools for collecting 3D data that can be used for digitizing small archaeological finds such as bones and fossils for the purposes of digital archiving and geometric morphometric analysis. The NextEngine Laser Scanner model 2020i was introduced at the Solidworks (Dassault Systèmes, Waltham, MA) World 2006 event as a high-definition, lower-cost, desktop laser scanner. The scanner comes with computer software (ScanStudio) that offers a wide variety of scan control options, where “object-scans” from multiple views can be assembled and exported in a variety of standard formats. Use of this particular scanner has become increasingly popular in the field of geometric morphometric studies, forensic science and medicine, in-silico modeling and museum conservation (Fruciano et al., 2017; Guidi et al., 2007; Hammond et al., 2016; Kuzminsky and Gardiner, 2012; Sholts et al., 2010; Slizewski et al., 2010; Stephan et al., 2014; Urbanová et al., 2017; White, 2015). While this scanner and software provide an affordable means to capture high-definition 3D data of small objects, user settings and scanning protocol can introduce unnecessary error and produce results that do not take full advantage of the scanner's capabilities, as stated by the manufacturer's specifications. It is important to understand how well the virtual model (referred to here as the in-silico model) created from a scan, replicates the object being digitized. Not only should the parameters of dimensional accuracy and repeatability be considered, but also the digitizing process as a whole.
Slizewski et al. (2010) examined the performance of the NextEngine scanner and two white light scanners (Breuckmann Smartscan and Steinbichler COMET V 4M) to digitize Neanderthal specimens of teeth and bones, and compared them to 3D models derived from x-ray μCT and CT scans. They concluded, though μCT provided the best results regarding surface occlusions present in scans of teeth, the models produced by the NextEngine could easily compete with those derived by CT, for gross morphometric studies (Slizewski et al., 2010). They reported an average deviation of the NextEngine derived surfaces from those of the μCT of 0.050mm, with a maximum deviation of 0.300–0.350 mm. At an approximately 30-fold costlier purchase price μCT has some distinct advantages over surface scanning in that it allows the interior of the object to be imaged at high theoretical resolution (5–10 μm), approximately 10–30 times better than that of the NextEngine laser scanner when using the macro setting (127 μm). Their study was of a comparative nature, comparing models derived from one instrument's measurement system to models derived from another, but using representative physical specimens with otherwise unknown exact dimensions. Like any measurement system a μCT scanner is subject to its own errors including segmentation errors, partial volume effects, and artifacts introduced by noise, beam hardening, scatter, pseudo-enhancement, motion, helical, ring and metal artifacts (Boas and Fleischmann, 2012), which would need to be assessed for any particular application. Besides cost, another factor to consider is the amount of data collected and the time and computational resources to access the data for analysis which can be significant for μCT.

Kuzminsky and Gardiner (2012) in a comprehensive review of applications of 3D laser scanning publications in the field of archeology, addressed a wide variety of research opportunities that this technology brings to the fields of biological anthropology, forensic science
and museum conservation, where 3D digital databases of bones and fossils could reduce or eliminate the need for researchers and investigators to travel to where a specimen is located, saving time and expenses. They concluded that use of this technology for creating in-silico models of skeletal remains produces results suitable for archiving artifacts for use in digital extensions of museum exhibits, which could provide data-bases for researchers to share models and increase sample sizes, along with limiting the need to handle the artifacts, reducing the damage risks caused by handling. They observed that one of the limitations of using 3D laser scanners is that there is no standard protocol for creating and analyzing 3D digital models, resulting in 3D in-silico models with an accuracy that depends on the scanner, software, algorithms and expertise of the human users (Kuzminsky and Gardiner, 2012). In addition to preserving material for scientific study by remote access, creating virtual museum displays and for preserving cultural artifacts (Guidi et al., 2007) low-cost scanners are being used in a variety of scientific areas. Some examples include creating in-silico models that were used to validate anthropoid hip abduction of extinct primates (Hammond et al., 2016); creating models of marsupial skulls (Fruciano et al., 2017); studying skull fragments with evidence of perimortem fractures for virtual reassembly in forensic cases (Urbanová et al., 2017); using scans of field recovered remains of unidentified individuals from mass burials, natural disasters or wars in attempts to aid in the identification by comparison to radiographic records (Stephan et al., 2014).

The aim of our work was to characterize the performance of the lower-cost NextEngine (NE) laser scanner (NextEngine Inc., Santa Monica, CA, USA), ~$3500.00 US and mid-range cost Hewlett-Packard Pro S3 structured light scanner (HP) (Hewlett-Packard Development Company, L.P., Palo Alto, CA), ~$6000.00 US, to that of the higher-cost VHX 6000 digital microscope (VHX) (Keyence® VHX6000, Keyence Corporation of America, Itasca, IL)
We quantified repeatability, dimensional accuracy and agreement of the NE, HP and a 3D CMM (coordinate measurement machine) with that of the VHX. The CMM has been used in the past for osteology and biomechanical studies, while the digital microscope is used in profilometry to characterize surface roughness. For our applications we were particularly interested in recording small surface features on bones, such as the secondary and tertiary mammillary processes with a periodicity and amplitude of the order of 5–50 mm and 0.1–1 mm, respectively (Cohen et al., 1992), that remain at the growth plate surfaces of bones after the cartilage has been removed. In our study we use a purpose-built test block with bas-relief features as a calibration tool and compare measurements from the in-silico models of the test block to those of the physical test block, as measured by the VHX and CMM. The in-silico models were examined for geometric preservation, how well they captured the intended range of surface features and to evaluate the intra-observer scanning protocol for the NE by quantifying the variability between biomodels, created from repeated scans of eight porcine bones (capital femoral epiphyses). Here we provide a methodology for the user that establishes a set of scan protocols that we believe takes full advantage of the performance capabilities of the NE, when scanning small objects of simple and organic geometry. We believe that adoption of such a standard in the measurement protocol can provide a degree of confidence in the results for users of the models generated with such a laser scanner.

2.2 Methods

2.2.1 Method comparison

A test block with surface features of simple geometry, with varying degrees of bas-relief was made using additive manufacturing and scanned using the NextEngine laser scanner (NE) and Hewlett-Packard Pro S3 structured light scanner (HP) for offset measurement comparison to
those obtained with the VHX 6000 digital microscope (VHX) and Microscribe MX 3D coordinate measurement machine (CMM). The block was designed using computer aided design software (NX 9.0 Software, Siemens, Plano, TX), with dimensions 50 × 90 × 10 mm (width × length × thickness). Eight, 10-mm square surface features, with 2-mm thick walls, projected from the base surface of the block with offsets ranging from 3 mm down to 0.1 mm (Fig. 1A). The block was fabricated with a stereolithography 3D printer (Form2, Formlabs Inc. Somerville, MA), using opaque grey photoreactive resin (RS-F2- GPGR-04), to minimize laser light penetration into the material which could cause reflection of the laser light from material beneath the surface. The printed test block was washed twice in an isopropyl alcohol bath to remove uncured resin, and further cured with natural ultra-violet light for approximately seven days, to ensure all photo reactive resin was completely cured throughout the block. Each square offset was marked with four points centered along each wall made with a permanent ink marker pen, along with four adjacent points on the unraised base surface, to provide for a total of 32 offset measurements (Fig. 2.1A).

The 3D printer's (Form2, Formlabs Inc. Somerville, MA) printing resolution limitations include a minimum layer thickness of 0.025 mm in the z-direction and a laser spot size of 0.15 mm in the xy direction. Nevertheless, it produced an acceptably accurate test block for the purpose of serving as a comparison control. We used the VHX 6000 digital microscope, with a manufacturer's stated dimensional accuracy of ± 5 μm for the VH Z20R lens at 200×, to measure the 32 bas-relief offsets. The offset walls were each labeled using the set of points (1, 2), (3, 4), (5, 6), (7, 8), starting at the lower left wall and continuing clock-wise for each feature. Line profiles across each wall for each set of points provided the 32 offset measures (Fig. 2.1B & C).

Since the block was not a known standard, the average of two replicated measures was used to
estimate the true value of the offset's intended nominal value, which we will refer to as the VHX values.

**Fig. 2.1.** A) Test block showing 64 marks (black dots) denoting the 32 bas-relief offsets and their intended nominal values ranging from 3 mm down to 0.1 mm, along with point-to-point segments (white) used for the geometric analysis. B) The 0.1 mm offset with line profiles across the points of each offset wall, providing profiles for points (1, 2), (3, 4), (5, 6), (7, 8) for a total of 4 offset measures for each offset feature. C) Offset measure between points 1 & 2. Local variations in the area around points 1 & 2, in the base and relief segments were negligible, denoted by regional high (red H) and low (red L) spots on the base and relief.

Bas-relief measurements for each square's eight points were also acquired with the Microscribe MX 3D CMM which has a manufacturer's stated resolution of ± 0.0508 mm. The CMM was calibrated using a master 3-mm diameter ruby ball calibration tip, as prescribed by the manufacturer's calibration guide. The master tip was then replaced with a 1-mm diameter working tip, and calibrated using the provided tip calibration fixture and custom tip calibration procedure (Solution Technologies, Inc., Oella, MD), in accordance with the ASME B89.4.22 calibration performance test (Mutilba et al., 2013). The xyz coordinates for each point (64 total)
were acquired by placing the hand-held stylus on the previously marked points on the block, and the xyz coordinates recorded using Verisurf software (Verisurf Software, Inc. Anaheim CA), providing the geometry of the block to which the *in-silico* models were compared using line segments S1–S6 (Fig. 2.1A). The acquisition of the 32 offset measures was repeated, which provided a set of bas-relief replicated measures. The average of the repeated measures was used in the comparison analysis.

The test block was then scanned with the 2010 NextEngine (NE) Model 2020i laser scanner (NextEngine Inc., Santa Monica, CA, USA) using the manufacturer's stated specifications that include a capture density of at most 268,000 points/in.² and a dimensional accuracy of ± 0.127 mm in macro mode, with a field of view of 76 × 127 × 165 mm. The scanner uses a non-contact triangulation method of measurement, utilizing “twin arrays of four, Class 1M, 10 mW solid-state lasers with custom optics” and “twin 5.0 Megapixel [complimentary metal-oxide semiconductor] CMOS image sensors” (NextEngine, Inc.). This enables an operator to acquire large point cloud data sets to capture surface characteristics of an object, with a series of scans in a relatively short period of time (Guo, 2008). The test block was scanned in macro mode with the capture density set at 17,000 points/in.², scan color set to neutral and number of divisions set to ten. First, a 360° scan was performed with the block's starting position on the automated scan platform (turntable) oriented such that the length of the block was perpendicular to the turntable, with the bas-relief offsets in the scanner's z-direction, collecting a scan every 36° for a scan set of ten scans (Fig. 2.2A). The test block was then positioned with the length parallel to the turntable, with the bas-relief surface of the block upright (scanner's y-direction), and both width ends placed in the z-direction and scanned using bracket scans. The scan sets were assembled in ScanStudio (NextEngine Inc., Santa Monica, CA) and aligned using between
three and nine control points, fused, meshed and exported in STL format. The block was scanned a total of three times to provide a sufficient number of scans for statistical purposes. The average of the three scans was used in the comparison analysis. The digitizing process took approximately 2 h per object to completely scan and assemble a digital version of the object that could be exported.

The test block was then scanned with the Hewlett-Packard (HP) Pro S3 structured light scanner (Hewlett-Packard Development Company, L.P., Palo Alto, CA), with a manufacturer's stated resolution of ± 0.05 mm. The scanner uses LED light, which projects a pattern of parallel lines onto the surface of an object and uses two high-speed, high-definition cameras capable of 54 frames per second, to capture the distortions of the patterned lines on the object's surface. Triangulation is used to calculate the distance to specific points and 3D coordinates are acquired for the digital recreation of the object. The test block was placed on the turntable, oriented such that the length of the block was perpendicular to the turntable as was previously done with the NE setup, positioned between the stereo cameras and in direct line with the light projector (z-direction). The cameras and projector were positioned approximately 30 cm away from the turntable and 20 cm above the turntable at an angle of ~45° (Fig. 2.2B). The software (HP 3D Scan Pro V5.6.0) was calibrated by temporarily removing the turntable from its marked position, placing the HP 3D Calibration Pro Panels at the turntable's marked position and allowing the software to acquire the correct coordinates of the two-plane grid patterned calibration panels, using the 120 mm calibration grid. Once calibration was complete, the automated turntable was returned to the pre-scan position, and a 360° scan was acquired, collecting a scan every 36° for a set of ten scans. A series of single scans were then performed with the block in various positions to fill in gaps in the model that were not acquired in the 360°
scan. The complete model was assembled and fused in the HP Scan Pro software and exported in STL format. The block was scanned a total of two times to obtain a set of repeated scans, for which the average measures of the two scans was used in the comparison analysis. The digitizing process also took approximately 2 h per object to completely scan and assemble a digital version of the object that could be exported.

Fig. 2.2. A) Schematic of the initial scan setup and coordinate system for: A) 2010 NextEngine laser scanner model 2020i. B) Hewlett-Packard Pro S3 structured light scanner.

The STL files of the replicated scans acquired from the NE and HP were imported into Rapidform (Rapidform Software, Geomagic Design X, Cary, NC) where the locations of points one through eight were approximated using the mesh measurement tool, and xyz coordinates recorded for the points that were marked on the test block at all eight surface features (Fig. 2.3). The repeatability of each instrument (VHX, NE, HP, CMM) was assessed and the coefficient of repeatability (CoR) reported. Bland-Altman plot analysis was used to assess agreement between the scanners and the VHX and the CMM and the VHX. Bland-Altman plot analysis is a way to evaluate a bias between the mean differences of two measurement methods, and to estimate an
agreement interval, within which 95% of the differences of one method, compared to the other one, fall (Bland and Altman, 1986; Carbone et al., 2001; Eksborg, 1981; Giavarina, 2015; Ludbrook, 2010). The dimensional accuracy of the in-silico models was compared quantitatively to the VHX values (Table 2.1).

**Table 2.1**
Offset measures for the VHX $\bar{x}$ ($n = 2$), NE $\bar{x} \pm SD$ ($n = 3$) and HP $\bar{x}$ ($n = 2$)

<table>
<thead>
<tr>
<th>Offset</th>
<th>3mm</th>
<th>2mm</th>
<th>1mm</th>
<th>0.5mm</th>
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<tbody>
<tr>
<td>VHX</td>
<td>NE</td>
<td>HP</td>
<td>VHX</td>
<td>NE</td>
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<tr>
<td>(1,2)</td>
<td>2.96</td>
<td>2.90 ± 0.04</td>
<td>3.00</td>
<td>1.98</td>
</tr>
<tr>
<td>(3,4)</td>
<td>2.98</td>
<td>2.98 ± 0.09</td>
<td>2.99</td>
<td>1.99</td>
</tr>
<tr>
<td>(5,6)</td>
<td>2.99</td>
<td>3.05 ± 0.04</td>
<td>2.99</td>
<td>1.99</td>
</tr>
<tr>
<td>(7,8)</td>
<td>3.00</td>
<td>2.97 ± 0.05</td>
<td>3.01</td>
<td>1.98</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Offset</th>
<th>0.4mm</th>
<th>0.3mm</th>
<th>0.2mm</th>
<th>0.1mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHX</td>
<td>NE</td>
<td>HP</td>
<td>VHX</td>
<td>NE</td>
</tr>
<tr>
<td>(1,2)</td>
<td>0.38</td>
<td>0.36 ± 0.01</td>
<td>0.38</td>
<td>0.30</td>
</tr>
<tr>
<td>(3,4)</td>
<td>0.39</td>
<td>0.41 ± 0.05</td>
<td>0.37</td>
<td>0.29</td>
</tr>
<tr>
<td>(5,6)</td>
<td>0.39</td>
<td>0.43 ± 0.03</td>
<td>0.39</td>
<td>0.28</td>
</tr>
<tr>
<td>(7,8)</td>
<td>0.40</td>
<td>0.38 ± 0.01</td>
<td>0.40</td>
<td>0.29</td>
</tr>
</tbody>
</table>

To evaluate how well the in-silico models from each scanner re-presented the geometry of the physical test block, we compared six point-to-point distances using the average of two sets of xyz coordinates from the digital measured points of both scanner's in-silico models, compared to the average of two sets of xyz coordinates of the CMM measured points. Points were chosen to represent a variety of vectors across the block spanning a range of segment distances from ~14 mm to ~72 mm (Fig. 2.1A). We examined the differences between the distances measured by each instrument for evidence of possible distortion or possible trends between smaller and larger distances. The point-to-point distances for the CMM and digital measures were calculated using the distance formula:

$$d(p_1, p_2) = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}$$  \(1\)
Fig. 2.3 *In-silico* models after they were imported into Rapidform, where yellow spheres show the locations where offset measures were obtained, with their location approximated using the mesh measurement tool. (Left) NextEngine *in-silico* models from scans 1 & 2. (Right) Hewlett-Packard Pro S3 *in-silico* models from scans 1 & 2.

2.2.2 Single operator variability

To quantify intra-observer variability of the intra-observer scanning protocol for the NE scanner, eight porcine capital femoral epiphyses were scanned three times each, by a single operator and surface area and volume calculated. The left and right femurs of four, 120-day-old mixed-breed pigs were harvested from a concluded animal study that was approved by the university institutional animal care and use committee. The harvested tissue was exempt from a University of Memphis IACUC protocol as it was from a concluded study where the tissue was to be otherwise discarded as non-hazardous. The femurs were sectioned at mid-diaphysis and all soft tissues completely removed by physical and chemical processes (Nawrocki, 1997). During chemical processing, the two cartilage growth plates that connect the femoral head and greater trochanter to the metaphysis, were completely digested and the bones disarticulated, leaving behind the two undulated growth plate surfaces (mammillary processes) on the metaphyseal side.
and the corresponding interlocking subchondral bone plate surfaces on epiphyseal sides of the femoral head and greater trochanter. The greater trochanter and metaphysis were omitted from the scanning process.

The eight capital femoral epiphyses (Fig. 2.4) were scanned with the NextEngine following the same protocol as the test block, for which we intentionally used a capture density (17,000 points/in.²), which excluded any detail of the micro-porous structures of the bone plate surface measuring below ~50 μm, since details of these pores were not well captured by the scans and were below the resolution of the scanner. The average area of these porous micro-structures that serve as the passageway for blood vessels penetrating epiphyseal bony plate to supply the cartilage reserve zone, was measured using the VHX 6000 digital microscope, yielding an average pore size of 20 ± 0.011 μm² (SD), with a minimum of 8 μm² and a maximum of 48 μm². The NextEngine digitizing process for the bones also took approximately 2 h per bone, to completely scan and assemble a digital version of the bone (biomodel) that could be exported. Once a bone scan set was processed and the STL file exported, the bone was removed from the turntable and the whole process started again, a total of three times for each epiphysis. The STL files were imported into Rapidform (Rapidform Soft- ware, Geomagic Design X, Cary, NC), where the total surface area and volume were calculated for each biomodel and variability quantified using the coefficient of variation (CV).
Fig. 2.4. The left and right capital femoral epiphysis of four animals were cleaned of all soft tissue and scanned a total of three times each using the NextEngine laser scanner, creating biomodels suitable for surface area and volume measures.

2.3 Results

2.3.1 Method comparison

To assess dimensional accuracy, the NE and HP *in-silico* models were first compared to each other qualitatively, for which we observed that both scanners captured the 0.1 mm surface feature of the test block. The NE scans appear less defined as compared to those produced by the HP scanner, with apparent noise showing up as a less smooth surface than what is seen in the HP models (Fig. 2.4). Though the NE models appear to display more noise than the HP models, both
have captured the smallest surface feature of the test block in each of two scans. Quantitatively the HP measures were closer to those obtained by the VHX, than the NE measures (Table 2.1).

Bland-Altman analysis was used to quantify each method's repeatability and determine if there was agreement between the measurement instruments. When assessing agreement between two measurement instruments, repeatability of each instrument should first be assessed with replicated measures and coefficient of repeatability (CoR) re-ported; poor repeatability by one or both instruments will inevitably lead to poor agreement between the two methods of measurement (Bland and Altman, 1986). The CoR is calculated as \( \sqrt{2 \times 1.96s_w} \), where \( s_w \) is the within-subject standard deviation and is estimated from the square root of the residual mean square for the replicated measures (Bartlett and Frost, 2008; Bland and Altman, 1999; Giavarina, 2015). To quantify repeatability, the differences of replicated measures were plotted against their mean for each instrument (Fig. 2.5). We assumed the mean difference between replicated measures to be zero, as we would not expect to observe differences in replicated measures from the same instrument. Plotting the differences of repeated measures against their mean, showed the data remained relatively evenly scattered about the assumed mean (zero) for the 32 difference measures obtained from the measurement instruments. This suggests repeatability did not vary in any systematic way over the range of measurements (Bland and Altman, 1986; Bland and Altman, 1996; Bland and Altman, 1999). We expect 95% of the repeated measure differences to be within two standard deviations, which is the British Standards Institution's definition of a repeatability coefficient and is what we observed (Bland and Altman, 1986; British Standards, 1979). Since the repeated measure differences for each measurement instrument were normally distributed (Shapiro-Wilks test), we also expect the difference between two re-plicated measures to vary by no more than their respective CoR for 95% of instances, which was the case for the
repeated measures for each instrument (Bartlett and Frost, 2008; Bland and Altman, 1999; Giavarina, 2015). The coefficients of repeatability were 0.010 mm, 0.137 mm, 0.068 mm and 0.193 mm, for the VHX, NE, HP and CMM, respectively.

Fig. 2.5. The difference of repeated measures plotted against the mean of the two measures, with limits of agreement (LOA) equal to two times the standard deviation (SD). All plots show agreement between repeated measures and appear randomly scattered about the mean (zero). A) VHX repeated measures. B) NE repeated measures. C) HP repeated measures. D) CMM repeated measures.

Since we had repeated measures for each instrument, the mean of each method's replicated measures was calculated and these pairs of means were used to assess the agreement between the NE, HP and CMM to that of the VHX (details in Appendix A) using Bland-Altman plots of agreement (Fig. 2.6). Like the repeatability plots (Fig. 2.5), the differences of the mean pairs were plotted against the mean of these differences and limits of agreement calculated.
2.3.1.1 3D point-to-point distance measurements 14-70 mm range

The point-to-point difference results showed no evidence of distortion in the digital block or trends between smaller and larger distances, which showed the *in-silico* models captured the geometry of block and relative distances to within $0.32 \pm 0.80$ mm ($x \pm SD$) for the CMM and NE differences and $0.41 \pm 0.98$ mm ($x \pm SD$) for the CMM and HP differences (Table 2.2).

### Table 2.2
CMM and *in-silico* point-to-point distances (see Fig. 2.1A for details)

<table>
<thead>
<tr>
<th>Segment (Points)</th>
<th>Distances</th>
<th>CMM</th>
<th>NE <em>in-silico</em></th>
<th>HP <em>in-silico</em></th>
<th>CMM - NE</th>
<th>CMM - HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (3, 2)</td>
<td>39.72</td>
<td>38.98</td>
<td>38.43</td>
<td>0.74</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>S2 (1, 4)</td>
<td>21.24</td>
<td>21.81</td>
<td>22.15</td>
<td>-0.57</td>
<td>-0.91</td>
<td></td>
</tr>
<tr>
<td>S3 (1, 8)</td>
<td>71.73</td>
<td>70.58</td>
<td>70.27</td>
<td>1.15</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>S4 (2, 1)</td>
<td>41.67</td>
<td>42.29</td>
<td>42.3</td>
<td>-0.62</td>
<td>-0.63</td>
<td></td>
</tr>
<tr>
<td>S5 (7, 5)</td>
<td>14.92</td>
<td>13.81</td>
<td>14.29</td>
<td>1.11</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>S6 (5, 4)</td>
<td>20.53</td>
<td>20.43</td>
<td>19.92</td>
<td>0.1</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>0.32</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td></td>
<td>0.8</td>
<td>0.98</td>
<td></td>
</tr>
</tbody>
</table>

2.3.2 Single operator variability in measuring bone area and volume

The coefficient of variation ($CV = \left(\frac{SD}{\overline{x}}\right) \times 100$) was calculated for each mean area and volume for the eight biomodels. The intra-observer coefficient of variation (mean CV ± SD) was $0.69 \pm 0.25\%$ for surface area and $0.77 \pm 0.26\%$ for volume (Table 2.3).
Table 2.3  
Mean area, volume and coefficient of variation (CV) of bone scans.

<table>
<thead>
<tr>
<th>Capital Femoral Epiphysis</th>
<th>Number of scans</th>
<th>Femur</th>
<th>CV [%]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>Left</td>
<td>1800.94 ± 13.95</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Right</td>
<td>1783.84 ± 02.96</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Left</td>
<td>1586.82 ± 14.80</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Right</td>
<td>1625.95 ± 13.69</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Left</td>
<td>1842.08 ± 13.98</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Right</td>
<td>1868.03 ± 16.90</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Left</td>
<td>1920.71 ± 10.96</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Right</td>
<td>1982.42 ± 11.72</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Mean 0.69  Mean 0.77  SD 0.25  SD 0.26

2.3.2 Discussion

The applications that our study provides information for on accuracy and repeatability are those where objects are small enough to fit within the scan volume of the NE scanner using macro mode (76 × 127 × 165 mm) and have small surface features with amplitudes as small as 0.1 mm above or below the surface that need to be retained in the in-silico model. Examples of these applications include bones, including those with surface marks made by animals or by man-made cuts of interest to the fields of forensics, archeology or paleontology. Capturing bone surface features associated with tendon attachments is of interest when comparing extinct species and studying how they evolved, which would be related to the inferred use of muscles attaching at those recorded sites. It could also be useful for scanning clay figurines or carvings with surface relief patterns that need to be pre- served in the model. Our own motivation for determining the repeat- ability and accuracy of the scanner in capturing and measuring bas- relief features was stimulated by our study of the formation of mammillary processes that form at the bone interfaces with the growth plate cartilage of a growing animal or human. This is vaguely similar to the knitted interfaces seen in the membrane bones of the skull, but with more of a three-dimensional topography. These structures likely help prevent sliding at the weaker
interface during growth and we are interested in measuring how they develop. Fossilized remains of younger individuals of our own or other species or animals may allow for examination of growth plate development that could contribute to our understanding of the conditions the individual was exposed to, whether there was evidence of overload, growth disturbance, malnutrition, disease or injury.

ScanStudio, provided with the NextEngine scanner, offers the user a wide variety of scan-control options, but no set protocol on scanning other than the manufacturer's recommended settings, which may or may not produce optimal scanning conditions. This leaves the scanning process largely up to the operator to determine what settings and procedures are needed for optimal scanning conditions. The protocol adopted by the operator will contribute to random error, which can be magnified if measurements are made by multiple observers working with different protocols on the same object. However, random error can be minimized by adopting a single operator protocol. The present study provides a test protocol with a macro-mode setting for scanning small objects. Single operator variability was examined for a fabricated test block with bas-relief offset features. Single operator variability was also examined for area and volume measurements of a small bone with small mammillary processes on the surface with amplitudes in the range of the bas-relief offset values of the test block. The CoR for the instruments showed each test-retest measurement of the test block features differed by no more than 0.010 mm, 0.137 mm, 0.068 mm and 0.193 mm, for the VHX, NE, HP and CMM respectively. There was agreement between all instruments and the VHX, but they all over-estimated bas-relief features on average (bias) by 0.046 ± 0.038 mm, 0.025 ± 0.033 mm and 0.026 ± 0.033 mm, for the NE, HP and CMM respectively (Fig. 2.6). For dimensional accuracy, all three instruments (NE, HP, CMM) were able to capture the 0.1 mm bas-relief from the test block. This is expected for the
HP and CMM as they have manufacturer's stated dimensional accuracies of 0.05 mm. However, the 0.1 mm feature was captured by both scanners, with no evidence of geometric distortion over the size of the test block (50 × 90 × 10 mm) in-silico models. This resolution is slightly better than the NE manufacturer's stated dimensional accuracy of 0.127 mm, which applies to the outermost region of the scan volume where results are less accurate, but the feature was not without noise. For the bones with their complex surface topography, the intra-observer coefficient of variation (mean CV ± SD) was 0.69 ± 0.25% for surface area and 0.77 ± 0.26% for volume.

**Fig. 2.6.** A, B and C) The shaded area represents the 95% confidence interval for the mean (bias) and LOA, where SDc is the corrected standard deviation (details in Appendix A). The bias represents the average an instrument overestimated the VHX 6000 measures, with all differences within the precision of the LOA, which shows there is agreement between the two measurement instruments for all three cases. D) Using the xyz data from the in-silico models for the NE and HP, distances were calculated for the six point-to-point segments seen in Fig. 2.1A, and compared to those acquired with the CMM, using the distance formula. Circles represent the CMM and NE differences and triangles represent the CMM and HP, plotted against their respective means. Both in-silico models overestimated the CMM measures on average by the amount of their respective bias values. The differences of both comparisons were all within the LOA and showed there was agreement between the compared instruments. There is also no evidence of geometric distortions as the differences of both methods appear to be evenly scattered about their respective bias, which suggests each method was capable of producing in-silico models that reproduced the geometry and overall features ranging from 0.1 mm up to ~70 mm.
2.4.1 Method comparison and single operator variability for test block with bas-relief features

In our study we compared measures from *in-silico* models of a test block obtained using a 2010 NextEngine Laser Scanner model 2020i, and the Hewlett-Packard Pro S3 structured light scanner, to those obtained from the VXH 6000 digital microscope and a Microscribe MX 3D coordinate measurement machine. We determined that the coefficients of repeatability (CoR) for the VHX, NE, HP and CMM were 0.010 mm, 0.137 mm, 0.068 mm and 0.193 mm, respectively. In other words, two replicated measures made with the same instrument should differ no more than their respective CoR. Smaller CoR are due to smaller within-subject standard deviations. The precision can be attributed to the combination of both systematic and random error. The devices that required less human interaction when collecting points showed lower CoR, which we would expect, as the VHX had the lowest CoR and the CMM had the highest. The difference between the NE and HP may be attributed to noise that is more apparent in the NE *in-silico* models and may be responsible for the larger deviations of the NE from the VHX values than the HP, seen quantitatively (Table 2.1). Points of measure acquired with the CMM, even though obtained from a single operator, were subject to human error when collected with the handheld stylus. Variation on the block's surface around points, though negligible as measured by the VHX, could add to the possible deviation from intended points of measure and be detected by the CMM, which may likely be the result of operator movements of the stylus when acquiring data. Attempts to repeatedly locate a point visually, with a handheld stylus for replicated measures can prove difficult. Points on the digital model had smaller deviation as they were located using a measurement tool, which likely contributed to better pin-pointing the location of a previously measured point. For this reason, the *in-silico* models showed better repeatability than the CMM when acquiring replicated measures for this particular use.
However, though the CoR of the methods were comparable, this did not tell us if the methods of measurement were in agreement with the VHX. It simply showed that the repeatability of the NE, HP and CMM were within less than ~0.2 mm for 95% of all repeated observations, for all methods of measurement. Yet, repeatability is a factor for agreement and if there is poor repeatability with one or both methods being compared, there will be poor agreement between the methods (Bland and Altman, 1986). A CoR simply quantifies the test-retest repeatability of a method, but it is up to the observer to determine if the variation is acceptable with a pre-determined criterion. To our knowledge there has been no publication reporting CoR for the 2010 NextEngine Laser Scanner model 2020i. Therefore we used the CoR for the CMM as the pre-defined criterion. The NE in-silico model's CoR was smaller than that of the CMM by 0.056 mm and larger than that of the HP model by 0.069 mm. We assumed this to be reasonable a repeatability for the NE scanner when used with the macro-mode setting and would not have attributed a finding of disagreement between the methods to be a result of poor repeatability, which we did not see for any instrument when compared to the VHX.

A Bland-Altman analysis showed all differences fell within the precision of the limits of agreement (LOA). All three instruments, when compared to the VHX, overestimated the VHX measures on average by 0.046 ± 0.038 mm, 0.025 ± 0.033 mm and 0.026 ± 0.033 mm, for the NE, HP and CMM respectively. In other words, measures made from the instruments were on average, higher than the VHX values by the amount of their respective bias. It is difficult to determine exactly the cause of this small systematic error, but there are several factors that could be responsible. Assembly of multiple scans in the ScanStudio and Scan Pro V5.6.0 software could also introduce error, possibly inadvertently increasing the thickness of the block, which would directly affect the measures as the reference plane for the digital measures was set to the
back of the block's surface, since that was the plane of reference for the VHX and CMM measures. If the laser from the NE was able to penetrate the material of the block, though visually opaque, it may have partly reflected from a different depth than the surface of the block, which could interfere with the received signal (Guidi et al., 2007). Nevertheless, the limits of agreement relax the assumption of zero bias between the two methods of measure being compared and provides a range for which the equipment can be found to agree, defined by the LOA (Giavarina, 2015).

2.4.1.1 Dimensional accuracy of test block with bas-relief features

The observed dimensional accuracies obtained with the NE, as compared to the VHX, were all within the limits of agreement (LOA), defined by the range of the upper LOA $0.255 \pm 0.038$ mm and lower LOA $0.162 \pm 0.038$ mm. Fig. 2.6A shows the scanner could produce results that fell well within the estimated range, at or near the resolution limits of the scanner ($0.127$ mm). It further suggests that measures made using the *in-silico* model could be anywhere below the VHX measure from the bias to the lower LOA, or above the VHX measure from the bias to the upper LOA, within the precision of both the bias and LOA as defined by the 95% CI for both. With this result, the upper range of the dimensional accuracy ($0.127$ mm) is about the best we can claim for surface features captured in the model. A previous analysis of the NextEngine scanner showed the mean dimensional accuracy of scans to be considerably worse ($\pm 0.81$ mm) than the manufacturer's stated accuracy, when scanning an object of simple geometry (Polo and Felicisimo, 2012). It is unclear why this previous analysis attained less optimal scanning conditions, but this may have been due to differences in scan settings and object dimensions, or the version of ScanStudio HD software. Earlier versions were limited when capturing in high-definition mode. We used ten divisions with a capture density of 17,000 points/in.$^2$, whereas the
previous analysis used 7 divisions with a capture density of 500 points/in.\(^2\) (Polo and Felícisimo, 2012). According to an applications engineer for the company, the stated dimensional accuracy is usually for measurements made at the boundary of the field of view or edge values, and better results are obtained when objects are in the center of the field of view at the correct focal distances (personal communication with M. Kim, NextEngine, Inc., 7/11/18). This along with our scan settings and object sizes, with the object placed well within the field of view is likely why we were able to achieve close to the manufacturer's stated dimensional accuracy of the scanner and perhaps better for some measures.

2.4.2 Single operator variability for measuring areas and volumes of small bones

Our single operator protocol remained constant for the test block scans and three consecutive scans for all eight bones, resulting in a coefficient of variation of 0.69 ± 0.25% for surface area and 0.77 ± 0.26% for volume. Our error was in line with what has been previously reported for intra-observer error on scans of organic shape, but it should be noted that our bones and protocol differed from those used in the previous study (Sholts et al., 2010). When digitizing objects a set protocol with a single operator produces the least random error (Marcy et al., 2018; Sholts et al., 2010). Sholts et al. showed an inter-observer protocol measurement error of 2% and intra-observer protocol error of 0.2% when scanning five crania three times consecutively with the NextEngine Model 2020i laser scanner. Inter-observer protocol and scan settings differed, whereas intra-observer protocols and scan settings remained constant (Sholts et al., 2010).
2.4.3 Comparison to other studies

In addition to the above-mentioned studies there have been several studies comparing the NextEngine laser and other scanners to CMM. Nouri et al. (2015) assessed the validity and reliability of a laser scanner (designed by Shahid Beheshti University, Tehran) comparing measures made using a CMM (Mora, Aschaffenburg, Germany) to those obtained from digital scans of 18 dental casts. They concluded that coordinates measured by the laser scanner were equal to coordinate measures made by the CMM (Nouri et al., 2015), which is in agreement with our findings. The assessment in their study used intraclass correlation (ICC) to determine the validity and reliability of the laser scanner as compared to the CMM, which may establish validity of the laser scanner but cannot answer the question of whether the two methods of measure can be used interchangeably (Bland and Altman, 1990). The case has been made that for method comparison studies the key is quantifying disagreement between individual measurements (Bland and Altman, 1990; Bland and Altman, 1999). Bland-Altman analysis addresses this specifically by comparing the differences of each compared measure, for a set of replicated measures, to their mean (Bland and Altman, 1986, 1999; Giavarina, 2015). For method comparison studies a Bland-Altman analysis and CoR may be considered more appropriate to report than ICC (Bland and Altman, 1990, 1999; Giavarina, 2015; Ludbrook, 2010; Vaz et al., 2013).

In addition to the comparison of the NextEngine to the structured light scanners mentioned in the Introduction (Slizewski et al., 2010), Campanelli et al. (2016) compared and tabulated the NextEngine scanner to four, higher-cost laser scanners (MMD × 50, SLP-250, ScanTrack, Metrascan 3D 70) by scanning a ceramic gage block and the distal end of a bovine femur, each scanned 10 times (Campanelli et al., 2016). For the NextEngine laser scanner, they
acquired 17,000 points/ in.², in 40° increments, although they do not specify object orientation. They also used the wide mode setting, which has a different field of view (258 × 345 × 435 mm) (x × y × z) with a manufacturer's dimensional accuracy of 0.38 mm compared to macro mode (76 × 127 × 165 mm) that we used in the present study with a dimensional accuracy of 0.127 mm. The length of the gage block was measured as the distance between two parallel planes, best fit to the short ends of the block for each of ten scans. They reported a bias of 0.36 mm with a dimensional accuracy of 0.326 mm, which is lower than the manufacturer's stated dimensional accuracy of 0.38 mm for the larger field of view setting (wide mode). We also observed measures to be well within the LOA of agreement, scattered about the bias, suggesting that dimensional accuracy obtained was at or near the resolution limits for the outer field of view for macro mode.

2.4.4 Limitations of the scanner for certain applications

Although the results indicate that laser scanning may be useful for in-silico archiving or analysis, the nature of the surfaces being scanned must also be considered. The objects used in this study had surfaces that were dull and opaque and required no surface treatment to reduce surface glare, but it is possible that the laser light penetrated the outer surfaces and partly reflected from somewhere below the surface. Objects with translucent or reflective surfaces will present difficulties for laser scanning unless the surfaces are coated with substance that does not interact chemically with the surface. The test block was fabricated due to the inability to successfully scan a standard gage block of known dimensions that had surface features at or near the resolution limits of the scanner. Specifically, we tried scanning a standard used for surface roughness analysis, but the metallic surface made it difficult to scan, reflecting the light such that the scanner could not properly determine the object's surface. This introduced artifact in the
digitized object that was observed as jagged surface anomalies that were qualitatively
determined. Surfaces could be made less reflective or translucent by lightly spraying the surface
with foot powder or flat spray paint, for example, but any effects this might have on the artifact,
or change in surface dimensions would need to be considered.

It should also be noted that there was a significant trial and error phase when developing
the protocol and scan settings for this study that led to the adaptation of the current protocol,
which produced the best results as judged qualitatively. We found that setting a capture density
one level below (10,000 points/in.$^2$) the recommended 17,000 points/in.$^2$ failed to reproduce the
smaller visible surface structures that could be observed on the bone, but not on the biomodel.
All levels above 17,000 points/in.$^2$ failed to improve observable detail, or capture any of the
porous microstructures, and appeared to add noise to the scan, which could be observed as
random jagged surface anomalies like those seen in the reflective surface of the metallic
standard. The exact cause is unclear but may be due to the effects of light scattering. We also
found for the 360° scan, setting the divisions to ten rendered better observable detail over the
manufacturer's recommended setting of seven and made the scan assembly process easier,
however, higher than ten divisions did not continue to improve the observable detail or quality of
the scan. The higher the number of divisions used, the longer the scan takes, so this becomes a
balance of determining the least number of divisions required to produce the best results and
allow for easy assembly of the scans. At the manufacturer's recommended capture density and
the number of divisions set to ten, we were able to capture surface features at or near the
resolution limits of the scanner, which can be seen in the digitized test block where the 0.1 mm
surface feature is visible.
This ~0.1 mm dimensional accuracy limit may be of importance regarding the digital archiving of small artifacts that have very small surface features, such as cut mark on bone or relief features of coins. An attempt to scan a 2018 Series D US Michigan State quarter proved rather difficult and unsuccessful. We found even with the surface reflection reduced using either white powder or flat-black spray paint, we were still unable to capture the small bas-relief features of the coin. Using the VHX we were able to measure two parts of the letter “T” on the coin, which yielded heights of 0.036 ± 0.005 mm and 0.030 ± 0.005 mm. The offset of President George Washington's nose on the face of the coin to the base of the coin measured 0.062 ± 0.005 mm. All of these measures are well below the dimensional accuracy that we were able to attain and well below the manufacturer's stated dimensional accuracy of 0.127 mm, suggesting this scanner may not be well suited for the digital archiving of items such as coins. For reference, the thickness of a human hair ranges from 0.04 to 0.12 mm.

2.4.5 Future development combining methods

Attempts to combine laser scanning with photogrammetry have shown promising results. MacDonald et al. (2017) combined laser scanning and photogrammetry to produce digital replicas of Roman coins that were digitized by combining laser scans with fine photometric detail obtained from images. However, the laser scanners (Smartscan HE and Mechscan Macro scanner) had higher acquisition resolution (0.02 mm and 0.01 mm respectively) than the NextEngine (MacDonald et al., 2017). The NextEngine just does not have the resolution capabilities that would be suited for scanning and digital archiving of coins. Lerma et al. (2010) utilized a medium-range phase based (FARO LS 880HE) terrestrial laser scanner, combined with photometric data, in order to create digital surface models of cave walls containing ancient carvings (Lerma et al., 2010). Allard et al. (2005) used the Polhemus Fastscan handheld 3D
scanner to scan a complete human skeleton, creating digital models that were suitable for 3D printing. This scanner uses a combination of laser, camera and motion tracking to create digital models that can be exported in STL format for 3D printing. Though they successfully scanned and 3D printed a replica of the skeleton, they discuss at length issues associated with the process of creating physical replicas for exhibition (Allard et al., 2005). Combining laser scans with photogrammetry appears to show relevance when scanning objects with very small surface features.

Though the NextEngine scanner may not be suited to scan metal archaeological items with reflecting surface finish, such as coins, we did find the scanner to be capable of scanning bones and capturing mammillary processes at least as small as 0.1 mm, as shown with the test block. In this study we were able to produce an in-silico model and eight biomodels with relatively short scanning periods of approximately 2 h each. The scanner proved to be well suited for scanning objects of both simple and organic geometries, capable of capturing features as small as approximately 0.1 mm, without significant distortion. These results and previous studies should help provide confidence in the use of these devices for certain applications as a low-cost, portable alternative to more costly scanning instruments. We have provided a method for performance analysis and scanning protocol that may serve as a template to the novice user and provides data relevant to the determination if use of this scanner is appropriate.

2.5 Conclusion

With appropriate scan settings, single user protocols and object placement, the NextEngine Model 2020i desktop laser scanner can capture and measure bas-relief features of varying degree offsets as small as approximately 0.1 mm. Relief offset measurements obtained from the in-silico models were in agreement with those obtained from the physical test block.
using a the VHX and CMM, demonstrating a dimensional accuracy at or near the manufacturer's stated dimensional accuracy of 0.127 mm. We quantified the repeatability of each instrument used in the study, for which the NE, HP and CMM were all below 0.2 mm, with the HP reporting the lowest CoR, then the NE, then the CMM. When scanning organic shapes, such as epiphyseal bones with mammillary processes, the intra-observer coefficient of variation (mean CV ± SD) was 0.69 ± 0.25% for surface area and 0.77 ± 0.26% for volume. This study provides a methodology for the novice NextEngine user that produced quantified results that were comparable to the manufacturer's stated dimensional accuracy and provides measures from in-silico models that are in agreement with measures acquired from a physical model measured with a high-end digital microscope and a CMM. The performance capabilities of this scanner and scanning protocol show that with a suitable protocol it is well suited for digitizing bones and capturing surface features as small as ~0.1mm, while maintaining geometric integrity.
References


CHAPTER 3

THE MORPHOGENESIS OF PORCINE FEMORAL HEAD MAMMILLARY PROCESSES: A STRUCTURAL MECHANISM OF BIOMECHANICAL STABILITY

3.1 Introduction

The capital femoral physis is a growth plate located between the head of the femur and femoral neck, which forms a temporary joint where growth plate cartilage is converted to bone by endochondral ossification during post-natal and adolescent development. The femur initially forms in the embryo as a limb bud that develops into a hyaline cartilage structure or anlage, which continues to grow by chondrogenesis. The physeal plate develops when the expanding primary center of ossification in the diaphysis meets the secondary center of ossification at the proximal end of the femur. The process of endochondral ossification continues at the metaphyseal border producing bone growth in length and circumference while the epiphyseal border forms a thickened sub-chondral bone plate before fusing in the later stages of adolescence and early adulthood. During development the interfaces between this growth cartilage and the bone on either side become increasingly undulated as epiphyseal subchondral bone plate (ESBP) interdigitations (mammillary processes) project into corresponding concavities on the metaphyseal growth interface. The matching morphology of these interfaces cannot simply be random, as the emergence and arrangement of the mammillary processes develop such that they uniquely interlock the joint, providing a mechanical mechanism resistant to shear and torsion, enhancing biomechanical stability. Their development may have significance in the prevention of the multifactorial disorder slipped capital femoral epiphysis (SCFE); a painful disorder that occurs in adolescent children where the neck of the femur slips in
relation to the femoral head (Aronsson & Karol, 1996; Crawford, 1988; Loder, Aronsson, et al., 2000). Though the disorder has been widely studied, the etiology is not completely understood. Underdevelopment of mammillary processes could be one of many risk factors for slips.

During development a prominent mammillary process (physeal tubercle) emerges within the capital femoral physis along with smaller undulating peripheral mammillary processes. Observation of this prominent tubercle appeared in the English literature in 2000, when Scheuer and Black (2000) depicted a “beak-like” tuberosity located on the inferior side of the epiphysis, projecting into the metaphysis (Scheuer & Black, 2000). Tayton (2007) investigated to see if the tubercle observed in the bovine and ovine species was as prominent in the human adolescent epiphysis. Though it was significantly smaller than expected, he concluded the tubercle to be a normal feature and suggested it “would act as a significant block to any tendency for the epiphysis to slip” (Tayton, 2007). In a later study Tayton (2009) noted in younger adolescents the tubercle appeared “wide-based and fairly flat” in radiographs and magnetic resonance imaging scans, which then narrows and becomes more pointed during development and suggests the adjacent surfaces change from “one of friction to one of pegging” (Tayton, 2009).

Kandzierski, Matuszewski, et al. (2012) related morphological shape of the growth plate as a risk factor for slips, concluding there was a correlation between more spherical growth plates with less undulations, and slips (Kandzierski et al., 2012). Small undulations in the interfacial morphology can limit shear displacement across the growth plate as has been shown by finite element simulations of hypothetical growth plate shapes (Castro-Abril, Gutiérrez, et al., 2016). Liu, Armstrong, et al. (2013) expanded on Tayton's work reporting tubercle height decreased and epiphyseal morphological cupping increased as a function of age (Liu et al., 2013). Kiapour, Kiapour, et al. (2019) also observed a decrease in tubercle height and increased epiphyseal
cupping as a function of age and suggested the tubercle to be the primary stabilizer of the capital femoral epiphysis in early stages of adolescence. Age-related changes in epiphyseal shape then shift the “loading dynamics of the epiphysis in a way that the epiphyseal cupping will become a more important stabilizer” in the later stages of adolescence (Kiapour et al., 2019). These studies present evidence for the tubercle to be a primary stabilizer in the early stages of development; epiphyseal cupping then becomes the primary stabilizer as the tubercle recedes and the periphery of the epiphysis envelops the metaphysis (epiphyseal cupping), providing an alternate mechanism to withstand shear (Ipsen, Williams, et al., 2002; Kiapour et al., 2019). However, the aforementioned does not provide an alternate mechanism to withstand torsion once the tubercle recedes in both height and length, as primary stabilization against shear loading shifts from the tubercle to epiphyseal cupping.

The femur of the 1-year-old *sus scrofa domesticus* (domestic pig) has been shown to have comparable dimensions and biomechanical properties to that of the adolescent human counterpart (Chung, et al., 1976) and has served well as a model for mechanical testing and fixation device studies (Chuinard, Williams, et al., 2004; Galligan, Williams, et al., 2004; Ipsen et al., 2002; Jonasson, Ekström, et al., 2014; Jonasson, Ekström, et al., 2015; Snyder, Williams, et al., 2006). Along with gross anatomical and physiological similarities, the domestic pig is also subject to the hip disorder SCFE (Jubb, Kennedy, & Palmer, 1985). One of the coauthors of the above mechanical studies and of the current article (JLW) noted the existence of a radial pattern of bone ridges (secondary mammillary processes) on the exposed epiphyseal subchondral bone plate following failure of the cartilage. These ridges appeared to be similar in shape, size and pattern in all 1-year-old domestic pig specimens and led to the questions posed in the current study as to how these ridges arise with age and whether there is any degree of systematic
periodicity to the ridge pattern among pigs. Manual manipulation following complete failure of the growth plate cartilage and following perichondrial stripping had demonstrated that these secondary radial mammillary processes provide resistance to torsion. 3D-printed replicas of the bone models generated in the present study similarly demonstrated that the interlock provided by these processes provides an effective resistance to torsion under mild axial loading even without the presence of cartilage. We hypothesized that these processes develop to form a radial pattern with some degree of periodicity beginning relatively early in development of the joint and increase in prominence with age and weight of the animal.

Our aim in this present study was to provide a qualitative and quantitative morphometric analysis of the development of femoral head mammillary processes and epiphyseal morphogenesis in the domestic pig from birth to adolescence. The development of these peripheral mammillary processes could play an important role in the torsional stability of the joint once primary stabilization shifts from the tubercle to peripheral cupping (Kiapour et al., 2019). Our analyses focused on six key points of development: the epiphyseal tubercle, epiphyseal cupping, growth plate slope angles, expansion of the epiphyseal subchondral bone plate, epiphyseal elongation, and the emergence of smaller, radially arranged secondary mammillary processes. We introduce a metric of surface roughness analysis to quantify secondary mammillary processes and apply it to analyze the development of the observed radial pattern of peripheral mammillary processes. The radial arrangement of the peripherally located secondary mammillary processes have not previously been reported (to the best of our knowledge) and may be a key mechanism to withstand torsional loading and aid in the prevention of a slip as primary stabilization shifts from the tubercle to epiphyseal cupping.
3.2 Methods

3.2.1 Biomodels

The proximal end of 20 porcine femurs ranging in age from 9-days preterm to 900-days old were scanned with a high-definition (HD) laser scanner following a previously developed protocol (Perrone Jr & Williams, 2019). A total of 33 femurs from domestic pigs aged 9-days preterm (n = 2), 2-days old (n = 6), 20-days old (n = 4), 35-days old (n = 4), 120-days old (n = 8), 365-days old (n = 1), 540-days old (n = 2) and 900-days old, (n = 6) were obtained. The harvested tissue was exempt from a University of Memphis IACUC protocol as it was from concluded studies where the tissue was to be otherwise discarded as non-hazardous or was deemed non-hazardous after tissue removal and chemical processing. The 900, 540, 365, and 35-day old groups were all female, and the 120-day old group was all male. The 20-day old group contained one male and one female, and the bio- logical gender was unknown for the 2-day old and 9-days preterm groups. The femurs were sectioned at mid-diaphysis and all soft tissue completely removed, first by physically cutting away soft tissue and using a spatula to remove bone marrow from the medullary cavity. The bones were soaked in an ammonium hydroxide solution (NH4OH:H20) for a total of 24 hr to break down lipids and draw out remaining marrow. They were then placed in water at 100C for 1 hr, allowed to cool and soaked in a detergent solution containing the enzymes protease and amylase, until all remaining soft tissue was completely digested, which took approximately 24 hr. The bones were then rinsed with water and placed in a xylol solution of 60% denatured alcohol and 40% xylene (Nawrocki, 1997) for a period of up to 5 days, or until all lipids were completely extracted from the cortical bone. The result produced calcified remains with a clean, white, opaque surface suitable for laser scanning. During chemical processing the two cartilage growth plates that connect the femoral
head and greater trochanter to the metaphysis were completely digested and bones disarticulated, leaving behind the two undulated growth plate surfaces (mammillary processes) on the epiphyseal sides of the femoral head and greater trochanter and corresponding metaphyseal growth plate (MGP) surface. All bones had similar gross anatomy within their respective age groups, as judged qualitatively, such that one bone from each group was an adequate gross anatomical representation of the entire group, except for the 365-day old where there was only one available femur. Biomodels of one femur from each age group were generated from HD laser scans of the epiphysis (when possible) and metaphysis, capturing surface features as small as 0.1 mm (Perrone Jr & Williams, 2019). All femurs for the 120 (n = 8) and 900-day old (n = 6) groups were scanned for statistical purposes. The greater trochanters were omitted from scanning unless fused with the metaphysis (Figures 3.1 and 3.2). Statistical comparisons between age groups were limited to the 120- and 900-day old groups due to an insufficient sample size for the 365- and 540-day old groups. For ages 35 and younger, analysis showed some of the surface features were below the manufacturer's stated resolution limits of the scanner and could be considered noise, though it may be possible to achieve better resolution than the manufacturer's stated dimensional accuracy of the scanner (Perrone Jr & Williams, 2019).
FIGURE 3.1  Dorsal view of biomodels generated from the right femur of animals aged 9-days preterm to 900-days old, with the mean weight and standard deviation in kg. The epiphysis has been flipped to reveal the mammillary processes of the epiphyseal subchondral bone plate (ESBP) surface (top row) and their projections into corresponding concavities in the metaphyseal growth plate (MGP) surface (bottom row). The epiphyses for the 9-days preterm and 2-day old groups were too small to mount and scan and were omitted. The right femur for animals one of four and two of three are shown for the 120- and 900-day old groups, respectively. The greater trochanter for the 900-day old was fused to the metaphysis and included in the scan.

For regional analyses, the *in-vivo* position of the femur was approximated in three planes (Figure 3.3) using the angle of common anatomical features with respect to the horizontal and vertical (body weight) direction. The femur was oriented such that a centerline along the diaphysis was rotated 50° from the vertical in the sagittal plane. This angle was estimated from images of assembled porcine skeletal models. The femur was then aligned in the frontal plane so that the centerline along the diaphysis was parallel with both the sagittal and frontal planes. Lastly, the femur was aligned in the cranial-ventral plane (internally rotated) such that the femoral head and greater trochanter’s lateral protuberance aligned at 35° from the horizontal (Yoshioka, Siu, et al., 1987). This angle was estimated using the measured average angle of six full-length femurs by placing the femur on a level surface cranial side up, with distal end
condyles flat on the level surface. The alignment of the femoral head and greater trochanter’s lateral protuberance to a common line tangent to these anatomical features defined the measured angle from the horizontal, in the cranial-ventral plane of view (Figure 3.3). Qualitative and quantitative analysis of the biomodels focused on six key points of development: the epiphyseal tubercle, epiphyseal cupping, growth plate slope angles, expansion of the epiphyseal subchondral bone plate (ESBP), epiphyseal elongation and the emergence of smaller, radially arranged mammillary processes.

**FIGURE 3.2** Biomodels of the right and left femurs for all animals in the 120- and 900-day old groups. The femurs are numbered 1–4 (120-day old) and 1–3 (900-day old) where “R” denotes right femur and “L” denotes left femur, with weight in kg. The dorsal view (frontal plane) shows the MGP surface, with the epiphysis flipped to reveal the ESBP surface.
3.2.2 Epiphyseal tubercle

Epiphyseal tubercle height and average rate of change were evaluated as a function of age. The location, height and slope of the tubercle were determined using a custom color contour topography algorithm to map the surface of the epiphyseal subchondral bone plate (ESBP) (Rhinoceros/Grasshopper software 4.0, Robert McNeel & Associates, Seattle, WA). To establish a work plane of view for mapping the relative heights of ESBP surface relief features, a reference xy-plane was positioned tangential to the articular curvature of the femoral head, perpendicular to the central axis of the femoral neck as defined by the caput-collum-diaphysis angle or femoral neck-shaft angle. The xy-plane established a plane marking zero height [mm] in the z-direction and positioned the epiphysis such that the ESBP mammillary processes were in
the positive z-direction for the plane of view. The epiphyses were then assigned a contour color based on distance relative to the xy-plane. The tubercle peak location was identified using a polar coordinate system of quadrants (Figure 3.4) separated by intersecting sagittal and transverse planes through the central axis of the femoral neck, where $0^\circ - 90^\circ$ defined the caudal-medial quadrant, $90^\circ - 80^\circ$ defined the cranial-medial, $180^\circ - 270^\circ$ defined the cranial-lateral and $270^\circ - 0^\circ$ defined the caudal-lateral quadrant. Tubercle height was measured by locating the highest point of the tubercle and the lowest point in the local low spot (nadir) (Liu et al., 2013). The distance between the two points was measured in the coordinate system of the xy-plane and the average rate of change estimated using a secant line through the tubercle peak and lowest point (Figure 3.4).

3.2.3 Epiphyseal cupping

The radius of the metaphyseal growth plate surface curvature was an indirect approximation of epiphyseal cupping and was measured in the sagittal and transverse plane with respect to the metaphyseal fossa. Biomodels were sectioned in the sagittal and transverse positioning planes at crosshairs placed at the approximate center of the femoral head that protruded slightly above the frontal plane (NX 12.0 software, Siemens, Plano, TX; Figure 3.3). In the sagittal plane, caudal and cranial epiphyseal cupping was approximated using circles of radius $r$ defined by two control points, one placed at the metaphyseal margin and the other placed at the apex of the metaphyseal fossa. Control points approximating articular curvature of the femoral head were placed at the cranial and caudal epiphyseal margins (Figure 3.5b). In the transverse plane, lateral and medial epiphyseal cupping was estimated by placing control points the same as in the sagittal plane. Articular curvature in the transverse plane was estimated by placing control points at the medial epiphyseal margin and along the lateral articular surface.
curvature, encompassing most of the articular curvature while excluding the protruding bone lip that develops as elongation of the epiphysis occurs in older ages, judged qualitatively (Figure 3.5e).

FIGURE 3.4 Color contour topography mapping of the ESBP surface. Top left: The height of mammillary processes for a 900-day old right epiphysis, relative to the xy-plane and corresponding legend (legend values for the 900-day old only). The distance of the highest peak and the lowest point of the ESBP from the xy-plane, in the z-direction, are shown (gray ovals) along with their measured distance relative to each other (white-dashed line). Top right: A 3D rendering displaying the secant line (white arrow) that approximated the slope of the tubercle. Bottom row: The ESBP surface at different stages of development (age in days).
FIGURE 3.5  (a, d) The approximated *in-vivo* positioned 900-day old right femur in the sagittal and transverse plane, where arrows BW indicate the direction of body weight and arrows EL indicate the epiphyseal elongation lip. (b, e) The segmented femurs with circles of radius r approximating the curvature of the femoral head and curvature of epiphyseal cupping caudal (Cdl), cranial (Cnl), lateral (Ltl), and medial (Mdl) with respect to the tubercle. (c, f) Growth plate slope angle $\theta$.

3.2.4 Growth plate slope angles

Growth plate slope angles in the sagittal and transverse plane were evaluated as a function of age. In the sagittal plane, a vertical line was placed at the caudal margin of the epiphysis. A perpendicular line extended in the cranial direction with an angled line extending from the caudal epiphyseal margin to the cranial epiphyseal margin (Figure 3.5c). In the transverse plane, a vertical line was placed at the medial margin of the epiphysis. A perpendicular line extended laterally with an angled line extending from the medial epiphyseal margin to the lateral epiphyseal margin (Figure 3.5f). The angle $\theta$ was measured between the perpendicular and angled lines for both planes of view.

3.2.5 Epiphyseal expansion

Areas for the epiphyseal subchondral bone plate surface and corresponding interlocking metaphyseal growth plate surface were evaluated as a function of age and the relationship of the two surfaces assessed (Rapidform Software, Geomagic Design X, Cary, NC; Figure 12a). Expansion of the ESBP was measured in the sagittal and transverse planes with respect to the
tubercle, where expansion was the measured distance from the apex of the tubercle to the epiphyseal margins (Figure 3.6).

![Figure 3.6](image)

**FIGURE 3.6** The approximated *in-vivo* positioned right femurs, sectioned in the sagittal (top row) and transverse (bottom row) plane for 20- to 900-days old, where arrow BW indicates the direction of body weight. Black arrows indicate the measured distance of growth with respect to the apex of the tubercle.

3.2.6 Epiphyseal elongation

Epiphyseal elongation was assessed as the measured distance the epiphysis extended beyond circular symmetry of the cranial articular curvature and was evaluated as a function of age. Elongation occurs in the caudal-lateral/caudal-ventral direction (along the femoral neck isthmus) and was not accurately captured in the sagittal or transverse sectioned femurs due to the approximated *in-vivo* position. To measure elongation, the epiphysis was rotated such that the longest length (parallel with the femoral neck isthmus) was in the plane of view. Circles of radius r were defined by placing two control points along the cranial articular curvature, as judged qualitatively, to establish circular symmetry relative to cranial articular curvature. Elongation was measured as the distance the caudal region of the epiphysis extended beyond the circle of symmetry (Figure 3.7).
FIGURE 3.7 Epiphyseal elongation for ages 20- to 900-days old, where arrows EL indicate the developed elongation lip. There was no epiphyseal elongation measured for 20- and 35-days old and minimal to no elongation in the 120-day old group and 365-day old. At 540- and 900-days old the elongation lip is clearly defined with larger measures of elongation.

3.2.7 Peripheral mammillary processes

The “developed interfacial area ratios” (S_{dr}) for the central and peripheral regions of the epiphyseal subchondral bone plate surface were evaluated as a function of age. The parameter S_{dr} is a measure of the surface complexity (Blateyron, 2013) and is the ratio of the real surface area over the projected surface area as defined by ISO 25178-2 (2012). With the epiphysis positioned in the plane of view as defined for color contour topography mapping, central and peripheral regions were designated first by placing a reference circle along the outer rim of the epiphysis using two control points, each placed at the cranial-caudal interface of the epiphyseal rim. Circles of radius r, (83 ± 3% the diameter of the reference circle) were placed in the peripheral region of the epiphyses to intersect the majority of radial mammillary processes (for surface roughness analysis) and circles of radius 2/3r were placed in the interior region of the epiphyses. Surface area from the perimeter to the circle of radius 2/3r defined the peripheral region and surface area within the circle 2/3r defined the central region (Figure 3.8). Featureless plates were generated from projections of the defined regions, with surface area of the biomodels and featureless plates calculated from the triangulated meshed surfaces of the selected regions (Rapidform Software, Geomagic Design X, Cary, NC). S_{dr} was evaluated as a function of age.
The radial pattern of peripheral mammillary processes was quantified using a protocol specifically developed to provide a continuous line profile through the majority of radially arranged mammillary processes sufficient for surface roughness analysis. Boolean operations on the epiphyses generated cylinders from the circles of radius $r$ and $2/3r$, as previously defined (NX 12.0 Software, Plano, TX). The perimeters of the cylinders were “cut” at the caudal-medial/lateral interface, “unwrapped” and projected onto a plane, providing a primary profile. The form and waviness of the primary profile were extracted using best fit, least error nth order polynomials, which were subtracted from the primary profile resulting in a roughness profile suitable for surface roughness analysis (Figure 3.9; MATLAB Software, MathWorks, Natick, MA).

Surface roughness for the given profiles were quantified in terms of roughness parameter $Ra$, which were also converted to ISO N grades, as defined by ISO 4287-1997 (1997). Surface roughness in terms of $Ra$ is calculated from a given roughness profile, where $z_i = f(x)$ is the measured deviations from a reference mean line, for a profile of sampling length $L$, then the
arithmetical amplitude averages of the assessed profile, also known as the center line average (CLA), is defined by ISO 4287-1997 as:

$$Ra = \frac{1}{L} \int_{0}^{L} |zi| \, dx$$

(2)

where interval length L was determined by dividing the total roughness profile length into 16 equal intervals for all profiles for each age group, providing the CLA (ISO 4287-1997; Mitutoyo American Corporation, 2021; Bulletin No. 2229).

Roughness profiles in terms of Ra were converted to ISO N grades (Supplement 1 Table B1). To convert Ra values to N grades, Ra values are first converted from micrometers to micro-inches, where Ra (μm) × 40 1/4 Ra (μin). The starting value of the ISO grading scale is N1 1/4Ra(μin)1/41, where Nn to Nn+1 represents a range of Ra values, where increasing Nn grades indicate an increase in surface roughness.

The average amplitude, or height (H$_{avg}$) and average wavelength or periodicity (λ$_{avg}$) for assessed profiles were defined as:

$$H_{avg} = Ra = CLA$$

(3)

$$\lambda_{avg} = \frac{360^\circ}{\text{number of counts}}$$

(4)

where λ$_{avg}$ is the average wavelength of the radial mammillary processes and a count is defined as any interval of length L, for a given profile where the curve crosses the CLA twice. This established a scale for analysis as epiphyseal subchondral bone plate mammillary processes develop with a varying degree of fractal dimensioning. Intervals where a count did not occur in
the assessed profiles were omitted from the amplitude average. The amplitude $H_{\text{avg}}$ and wavelength $\lambda_{\text{avg}}$ were evaluated as a function of age.

FIGURE 3.9  Top right: The right epiphysis of a 900-day old with cylinders generated from circles of radius $r$ and $2/3r$ (concentric dash line circles shown on the epiphyseal subchondral bone plate surface). The cylinder defined by radius $r$ is shown (enlarged) with the perimeter unwrapped and projected to a plane, producing the primary profile seen in the plot (top curve). Each quadrant is represented in the plot and displays $90^\circ$ (one quadrant) of the total unwrapped profile. Numbers 1–5 demonstrate how the profile maps to the perimeter of the epiphyseal cylinder. The dashed curve in the plot is the $n$th order Form polynomial and the dashed-dot curve is the $n$th order Form + Waviness polynomial used for profile deconvolution to separate the waviness and roughness profiles from the primary profile, providing the roughness profile for surface roughness analysis.
3.2.8 Statistical analyses

Microsoft Office Excel (Excel Software, Microsoft Corporation, Redmond, WA) and MATLAB (MATLAB Software, MathWorks, Natick, MA) were used for the statistical analyses of the data. Shapiro–Wilk and d'Agostino-Pearson tests for normality were performed on data to determine the appropriate test for statistical differences, parametric or nonparametric. For normally distributed data T-tests were performed on groups with paired samples and groups with two independent samples. A Wilcoxon signed-rank test was performed on groups with paired samples where the data were not normally distributed, and a Mann–Whitney U test was performed on groups with two independent samples where the data were not normally distributed. The 95% confidence interval (CI), test statistic, p value and effect size were reported for the mean and standard deviation for parametric tests and median for nonparametric tests.

Data summaries are available in Tables in a Supplement.

3.3 Results

3.3.1 Notation and statistical note

Development as a function of age was assessed statistically between the 120- and 900-day old groups. The notation N120 Days and N900 Days in the supplement tables denote the 120- (n = 8) and 900-day old (n = 6) groups, respectively, followed by statistical results. Solid markers in plots denote the group mean with standard deviation error bars.

3.3.2 Epiphyseal tubercle

The height of the tubercle increased 44% from 5.5 ±1.1mm to 7.9 ±1.0mm as a function of age (Figure 10a) with respect to the local nadir that develops cranial-medially to the tubercle, corresponding to a domed protuberance that develops on the metaphyseal growth plate surface.
The average slope of the tubercle increased 33% from an incline of $22.4 \pm 4.4^\circ$ to $29.7 \pm 2.8^\circ$ as a function of age (Figure 3.10b; Supplement 1 Table B2).

3.3.3 Epiphyseal cupping

In the sagittal plane, the articular radius increased 37%, from $13.78 \pm 0.68$ mm to $18.87 \pm 0.67$ mm, as a function of age. The radius of epiphyseal cupping cranial with respect to the tubercle ($11.38 \pm 0.79$ mm) was 68% greater than cupping caudal ($6.75 \pm 1.93$ mm) to the tubercle for the 120-day old group. In the 900-day old group cupping cranial with respect to the tubercle ($13.11 \pm 1.65$ mm) was 26% greater than cupping caudal ($10.35 \pm 1.29$ mm) to the tubercle. Caudal cupping increased 53%, from $6.75 \pm 1.93$ mm to $10.35 \pm 1.29$ mm, as a function of age, whereas no difference could be detected for cranial cupping (Figure 3.11a). In the transverse plane, the epiphyseal articular radius increased 30%, from $13.98 \pm 0.71$ mm to $18.22 \pm 0.75$ mm, as a function of age. The radius of epiphyseal cupping medial with respect to the tubercle ($12.04 \pm 1.38$ mm) was 2.6-times greater than cupping lateral ($4.63 \pm 0.69$ mm) to the tubercle for the 120-day old group, whereas no difference could be detected for the 900-day old group. Lateral cupping increased 90%, from $4.63 \pm 0.69$ mm to $8.78 \pm 0.91$ mm, as a function of age, whereas cupping medial to the tubercle decreased 17%, from $12.04 \pm 1.38$ mm to $10.0 \pm 0.88$ mm, as a function of age (Figure 3.11b; Supplement 1 Tables B3 and B4).

3.3.4 Growth plate slope angle

Physis slope angles in the transverse plane decreased 58%, from $20.16 \pm 3.41^\circ$ to $8.47 \pm 2.26^\circ$, as a function of age, whereas no difference was detected for slope angles in the sagittal plane as a function of age (Figure 3.11c,d; Supplement 1 Table B5).
3.3.5 Epiphyseal expansion

At 120-days old the metaphyseal growth plate (MGP) surface area \((860.66 \pm 109.58 \text{ mm}^2)\) was 39\% greater than the epiphyseal subchondral bone plate (ESBP) surface area \((619.60 \pm 47.55 \text{ mm}^2)\), whereas no difference was detected between the two surface areas at 900-days old. The MGP surface area increased 61\%, from \(860.66 \pm 109.58 \text{ mm}^2\) to \(1,385.\pm 149.79 \text{ mm}^2\), as a function of age and the ESBP surface area increased 2.2-fold, from \(619.60 \pm 47.55 \text{ mm}^2\) to \(1,372.15 \pm 143.67 \text{ mm}^2\), as a function of age. Surface areas of the MGP and ESBP converged in later stages of development as the epiphysis enveloped the metaphysis (Figure 3.12a; Supplement 1 Table B6).

In the sagittal plane, expansion in the cranial direction with respect to the tubercle \((13.88 \pm 0.64 \text{ mm})\) was 54\% greater than caudal expansion \((9.0 \pm 0.53 \text{ mm})\) for the 120-day old group. In the 900-day old group expansion in the cranial direction with respect to the tubercle \((21.17 \pm 1.94 \text{ mm})\) was 55\% greater than caudal expansion \((21.17 \pm 1.03 \text{ mm})\). Cranial expansion increased 53\% from \(13.88 \pm 0.64 \text{ to } 21.17\pm 1.94 \text{ mm}\) as a function of age. Caudal expansion increased 52\%, from \(9.0 \pm 0.53 \text{ mm}\) to \(13.67 \pm 1.03 \text{ mm}\), as a function of age (Figure 3.12b). In the transverse plane, expansion in the medial direction with respect to the tubercle \((20.63 \pm 0.74 \text{ mm})\) was 53\% greater than lateral expansion \((10.01 \pm 0.56 \text{ mm})\) for the 120-day old group. In the 900-day old group expansion in the medial direction with respect to the tubercle \((33.67 \pm 1.94 \text{ mm})\) was 57\% greater than lateral expansion \((21.17 \pm 1.03 \text{ mm})\). Medial expansion increased 62\% from \(20.63 \pm 0.74 \text{ to } 33.67 \pm 1.94 \text{ mm}\) as a function of age. Lateral expansion increased 56\%, from \(10.01 \pm 0.56 \text{ mm}\) to \(15.01 \pm 0.56 \text{ mm}\), as a function of age (Figure 3.12c). In the frontal plane, expansion in the superior direction with respect to the tubercle \((18.33 \pm 0.74 \text{ mm})\) was 52\% greater than inferior expansion \((9.00 \pm 0.53 \text{ mm})\) for the 120-day old group. In the 900-day old group expansion in the superior direction with respect to the tubercle \((31.17 \pm 1.03 \text{ mm})\) was 58\% greater than inferior expansion \((21.17 \pm 1.03 \text{ mm})\). Superior expansion increased 57\% from \(18.33 \pm 0.74 \text{ to } 31.17 \pm 1.03 \text{ mm}\) as a function of age. Inferior expansion increased 55\%, from \(9.00 \pm 0.53 \text{ mm}\) to \(13.67 \pm 1.03 \text{ mm}\), as a function of age (Figure 3.12d). In the axial plane, expansion in the anterior direction with respect to the tubercle \((20.63 \pm 0.56 \text{ mm})\) was 53\% greater than posterior expansion \((10.01 \pm 0.56 \text{ mm})\) for the 120-day old group. In the 900-day old group expansion in the anterior direction with respect to the tubercle \((33.67 \pm 1.03 \text{ mm})\) was 59\% greater than posterior expansion \((21.17 \pm 1.03 \text{ mm})\). Anterior expansion increased 62\% from \(20.63 \pm 0.56 \text{ to } 33.67 \pm 1.03 \text{ mm}\) as a function of age. Posterior expansion increased 58\%, from \(10.01 \pm 0.56 \text{ mm}\) to \(15.01 \pm 0.56 \text{ mm}\), as a function of age (Figure 3.12e). In the transverse plane, expansion in the medial direction with respect to the tubercle \((20.63 \pm 0.74 \text{ mm})\) was 53\% greater than lateral expansion \((10.01 \pm 0.56 \text{ mm})\) for the 120-day old group. In the 900-day old group expansion in the medial direction with respect to the tubercle \((33.67 \pm 1.03 \text{ mm})\) was 58\% greater than lateral expansion \((21.17 \pm 1.03 \text{ mm})\). Medial expansion increased 63\% from \(20.63 \pm 0.74 \text{ to } 33.67 \pm 1.03 \text{ mm}\) as a function of age. Lateral expansion increased 59\%, from \(10.01 \pm 0.56 \text{ mm}\) to \(15.01 \pm 0.56 \text{ mm}\), as a function of age (Figure 3.12f).
mm) was 4.8-times greater than lateral expansion (4.25 ± 0.46 mm) for the 120-day old group. In the 900-day old group expansion in the medial direction with respect to the tubercle (21.0 ± 1.67 mm) was 38% greater than lateral expansion (15.17 ± 2.79 mm). Lateral expansion increased 3.5-fold, from 4.25 ± 0.46 mm to 15.17 ± 2.79 mm as a function of age, whereas no difference was detected for medial expansion (Figure 3.12c; Supplement 1 Tables B7 and B8).

3.3.6 Epiphyseal elongation

Epiphyseal elongation increased 6.4-fold, from 1.61 ± 1.52 mm to 10.32 ± 0.82 mm, as a function of age (Figure 13a). In late-stage development (540 days and older) an elongation lip developed as an epiphyseal growth front advanced along the isthmus, enveloping the femoral neck and measuring 8.9 ± 1 mm. A bone ridge also developed, encircling the caudal region on

FIGURE 3.11 (a) Estimated radius of articular curvature (AC) of the epiphysis (solid line), radius caudal to the tubercle (dashed line) and radius cranial to the tubercle (dash-dot line) in the sagittal plane, as a function of age. (b) Estimated radius of AC of the epiphysis (solid line), radius medial to the tubercle (dash-dot line) and radius lateral to the tubercle (dashed line) in the transverse plane, as a function of age. (c) The slope of the physis in the ventral direction from cnl to cdl in the sagittal plane, as a function of age. (d) The slope of the physis in the ventral direction from ltl to mdl in the transverse plane, as a function of age.
the articular side of the capital femoral epiphysis (Figure 3.13a and Video S1; Supplement 1 Table B9).

![Figure 3.12](image)

FIGURE 3.12  (a) Surface area for the metaphyseal growth plate surface (biomodel highlighted pink) and epiphyseal subchondral bone plate surface (biomodel highlighted light blue) as a function of age, with arrow EL denoting the developed elongation lip present on the 900-day old. Arrow BR denotes the epiphyseal articular bone ridge present on ages 540 days and older. At 120-days old there is a divergence in growth rates where the metaphysis expands beyond the epiphysis (black oval), but converges by 900-days old when the epiphysis envelops the metaphysis (black rectangle). (b) Growth in the caudal (dash-dot line) and cranial (solid line) direction, as a function of age. (c) Growth in the lateral (dash-dot line) and medial (solid line) direction, as a function of age.

3.3.7 Peripheral mammillary processes

Surface area in the central region of the 120-day old group (208.2 ± 28.0 mm²) was 18% greater than the same defined region for the featureless plates (177.0 ± 22.4 mm²) and surface area in the central region of the 900-day old group (359.0 ± 9.4 mm²) was 17% greater than the same defined region of the featureless plates (307.3 ± 10.6 mm²). Surface area in the peripheral region of the 120-day old group (398.2 ± 38.8 mm²) was 14% greater than the same defined region of the featureless plates (348.5 ± 31.3 mm²) and surface area in the peripheral region of
the 900-day old group (1,125.22 ± 174.7 mm$^2$) was 38% greater than the same defined region of the featureless plates (810.6 ± 114.6 mm$^2$).

Surface complexity was quantified with the developed interfacial area ratios ($S_{dr}$) and showed a 27% increase in $S_{dr}$ in the peripheral region, whereas no difference was detected in the central region, as a function of age (Figure 3.13b; Supplement 1 Table B10).

The average amplitude ($H_{avg}$) of peripheral region mammillary processes for the 120-day old group (0.16 ± 0.06 mm) was 60% greater than the central region mammillary processes (0.10 ± 0.02 mm). The amplitude $H_{avg}$ of peripheral region mammillary processes for the 900-day old group (0.44 ± 0.03 mm) was 2.6-times greater than the central region mammillary processes (0.17 ± 0.06 mm). As a function of age, the central region $H_{avg}$ increased 70%, from (0.10 ± 0.02 mm) to (0.17 ± 0.06 mm), whereas the peripheral region increased 2.8-fold, from 0.16 ± 0.06 mm to 0.44 ± 0.03 mm (Figure 3.13c).

The average angle between the radial arrangement of peripheral region mammillary processes, or periodicity ($\lambda_{avg}$) for the 120-day old group was 22.2 ± 4.4˚ and 20.4 ± 5.9˚ for the 900-day old group. The central region $\lambda_{avg}$ for the 120-day old group was 35.2 ± 9.2˚ and 32.0 ± 11.9˚ for the 900-day old group (Figure 13d; Supplement 1 Table B11).

Surface roughness (Ra) ISO N grades were converted from the amplitude $H_{avg}$ and increased from 0.10 ± 0.02 mm (grade N$1_3$) to 0.17 ± 0.06 mm (grade N$1_4$) for the central region as a function of age and increased in the peripheral region from 0.16 ± 0.06 mm (grade N$1_4$) to 0.44 ± 0.03 mm (grade N$1_6$) (Supplement 1 Table B12).
3.4 Discussion

3.4.1 A mechanism to withstand torsion

Slipped capital femoral epiphysis (SCFE) in humans is characterized clinically as an epiphyseal posteromedial displacement relative to the metaphysis (Khaladkar, Sherawat, et al., 2015; Tayton, 2007). In a small fraction of cases there is a posterolateral displacement of the epiphysis relative to the metaphysis (Gelink, Cúneo, et al., 2021). Torsion has been reported as an etiologic factor in initial slips (Aronson & Tursky, 1996). Treatment of acute slips may require medial or internal rotation (Amara, Leroux, et al., 2014; Broughton, Todd, et al., 1988; Casey, Hamilton, et al., 1972; Fairbank, 1969), suggesting a torsional displacement in the initial slip. It has been argued that the eccentric docking mechanism of the epiphyseal tubercle into the metaphyseal fossa is important for stability against both transverse and torsional shear and that an underdeveloped tubercle and large fossa may contribute to instability (Hosseinzadeh, Novais,
et al., 2021; Novais, Maranho, et al., 2020; Tayton, 2007). While the eccentric location of the tubercle provides resistance to transverse shear, it can also act as a pivot point for torque (Figure 3.14) unless the growth plate is stabilized by additional structures to counter the torque. We argue that this torque is countered during growth by the developmental shift to epiphyseal cupping combined with the resistance provided by the evolving secondary mammillary processes. Also, it has been suggested that increasing the height of the growth plate unlocks the interdigitating secondary mammillary processes (Speer & Braun, 1985) to reduce resistance to shear. Radiologists have reported widening of the growth plate accompanying slips, which may be subtle or as much as a five-fold increase in thickness (Khaladkar et al., 2015; Leblanc, Bellemore, et al., 2017; Tresoldi, Modesti, et al., 2017), although it is unclear if this widening is a contributing factor to the slip or a consequence thereof. The thickness or height of the growth plate varies with age, rate of growth and endocrine function. This would suggest that thickening of the plate may be a contributing factor to the slip and warrants further investigation.

In the present study, six key points of development (the epiphyseal tubercle, epiphyseal cupping, growth plate slope angles, expansion of the epiphyseal sub-chondral bone plate, epiphyseal elongation and the emergence of smaller, and radially arranged secondary mammillary processes) were investigated for evidence of mechanical mechanisms to withstand torsional loading once primary stabilization shifts from the epiphyseal tubercle to peripheral cupping (Kiapour et al., 2019). The results provide evidence of mechanical mechanisms of torsional stability provided by secondary mammillary processes that appear early in development and persist through adolescence. As epiphyseal cupping increased, a distinct radial pattern of smaller mammillary processes emerged in both the central and peripheral region with a degree of periodicity. The peripheral region mammillary processes were more prominent than the central
region and are well suited to resist torsional forces. For the domestic pig aged approximately 120-days old, an observer could reasonably expect to observe 11 ± 3 mammillary processes (ridges) in the central region with a height of 0.10 ± 0.02 mm, radially arranged with a ridge every 35.2 ± 9.2°. In the periphery an observer could reasonably expect to observe 17 ± 3 ridges with a height of 0.16 ± 0.06 mm, radially arranged with a ridge every 22.2 ± 4.4°. For the domestic pig aged approximately 900-days old, an observer could reasonably expect to observe 13 ± 5 ridges in the central region with a height of 0.17 ± 0.06 mm, radially arranged with a ridge every 32.0 ± 11.9°. In the periphery an observer could reasonably expect to observe 19 ± 5 ridges with a height of 0.44 ± 0.03 mm, radially arranged with a ridge every 20.4 ± 5.9°. The development of these radially arranged peripheral mammillary processes were quantified using surface roughness analysis, which has been used to analyze biological surfaces and surfaces of materials interacting with biological surfaces (Costa-Rodrigues, Fernandes, et al., 2012; Nosonovsky & Bhushan, 2008; Persson & Gorb, 2003). There are several parameters of surface roughness and choosing the appropriate parameter for analysis could be preference- or application-dependent. Previous studies have used roughness parameter Ra (root mean square method) to determine roughness for the articular cartilage surfaces of various equine synovial joints to assess fractal dimensioning of surface features during drying (Smyth, Rifkin, et al., 2014; Smyth, Rifkin, et al., 2012). The mammillary processes observed in the present study had a degree of fractal dimensioning, and the scale for analysis was determined by scale of interest (macro-scale) and instrument resolution (Perrone Jr & Williams, 2019).
FIGURE 3.14  Diagram to illustrate how an interlocking epiphyseal tubercle (T) and metaphyseal fossa could function as a pivot. Ideally joint forces would act normal to the physis. A joint force acting at an incline will have a shear component tending to tilt the epiphysis about the pivot point. Left: Sagittal view of a 365-day old right femur in the approximate in-vivo position during stance, where arrows indicate joint forces as a distributed load over the femoral head. Middle: The epiphysis with the metaphysis and physis removed to show the location of the tubercle (T), at a distance r from an axis passing along the center of the femoral neck to the center of the physis (C). Right: Two diagrams of the epiphysis oriented such that the epiphyseal subchondral bone plate is in the plane of view. Top-Right: The shear component of the distributed joint forces acting in a plane through C, parallel to the physis. Bottom-Right: The shear component (FC) of the resultant joint force, which acts through C and causes a moment (torque) about the pivot point provided by the tubercle (T).

The radially arranged secondary mammillary processes were consistent between animals within their respective groups (Figure 3.2 and Video S1: https://players.brightcove.net/656326989001/default_default/index.html?videoId=626299132900), which suggests they are not simply random but develop to enhance the biomechanical stability of the joint specifically to withstand torsional loading. The emergence of similar, radially arranged mammillary processes also appear on the cranial and caudal surfaces of the
vertebral body of the domestic pig, despite being separated by large cartilaginous vertebral discs. In addition, interlocking undulations were also observed on the metaphyseal growth plate and epiphyseal subchondral bone plate surface of the humoral head and greater tuberosity. Stamos and Weaver (2020) observed similar undulations at the distal metaphyseal end of the femur for various hominoids, concluding them to be relatively simple during the fetal and infant periods relative to later development, where surface morphology increased in developed interfacial area ratio. However, they concluded the human surfaces of the joint were relatively flat throughout ontogeny by comparison (Stamos & Weaver, 2020). The morphogenesis and development of femoral head mammillary processes characterized in the present study were also observed in the right and left femur of a 690-day old miniature pig species, which suggests they are a normal feature that develop as a natural defense against forces that could damage the growth plate and disrupt the process of endochondral ossification prior to closure.

3.4.2 Joint morphogenesis and mechanical stability

Joint morphogenesis was assessed qualitatively and quantitatively in relation to the direction of bodyweight with the femur in the approximate in-vivo position while in stance. The porcine tubercle at 20 and 35-days old was “wide-based” and “fairly flat”, similar to observations made on the early adolescent human capital femoral epiphysis (Tayton, 2009). At 120-days old (human equivalent of 6 years) the tubercle had developed into a peaked ridge. By 900-days old (human equivalent of 18 years) the ridge-ends had receded and the tubercle was a prominent “beak-like” peak (Scheuer & Black, 2000), which has also been reported for the ovine and bovine species (Tayton, 2007). However, tubercle height measures were slightly smaller in the human counterpart, which could be the result of differences between quadruped and bipedal development. Tayton (2007) reported a tubercle height of 4.1 ± 1.1 mm for adolescent children.
11–15 years old (n = 11, xSD) and Liu et al. (2013) reported a tubercle height of 4.4 ± 1.1 mm for adolescent children 3–17 years old (n = 22, x SD). The 120-day old domestic pig had a tubercle height of 5.5 ± 1.1 mm, whereas the 900-day old had a tubercle height of 7.9 ± 1.0 mm (x SD). It is unclear if the tubercle's late stage position is the result of isometric or allometric scaling (Stern, Aviram, et al., 2015). However, symmetric expansion in the sagittal plane suggests growth expands relative to the tubercle and implies isometric scaling (where the tubercle emerges in its relative final position and maintains that position throughout development), which occurs for many anatomical landmarks of the femur (Stern et al., 2015; Suzuki, Matsubayashi, et al., 2019). In the sagittal plane the tubercle remained pronounced throughout development, whereas a decrease was observed in the transverse plane (Figure 6) due to the height of the tubercle ridge-ends receding. Transverse plane lateral expansion increased 3.5-fold, from 4.25 ± 0.46 mm to 15.17 ± 2.79 mm as a function of age, whereas no expansion was detected medially. This is associated with a growth front along the isthmus of the femoral neck and suggests allometric scaling. Isometric versus allometric scaling of landmark superstructures in bone development is of interest, as bone structures can- not simply be relocated by cell migration or proliferation (Stern et al., 2015). Understanding the locational development of these structures may provide insight into developmental changes that occur in response to the external mechanical environment and warrant further investigation.

Mechanical mechanisms of stability in the transverse plane differed from that of the sagittal plane. In the transverse plane the tubercle receded as epiphyseal cupping increased as a function of age, demonstrating the shift of primary shear stabilization from the tubercle to epiphyseal cupping (Kiapour et al., 2019), while the growth plate slope angle decreased by half. Growth plate angle decreases also occur similarly with normal growth in adolescent children in
normal weight ranges when measuring Southwick's anteroposterior angle (Damaceno, Santili, et al., 2007; Hesper, Zilkens, et al., 2017). It has been argued using a finite element model of an 11-year-old proximal femur, that body mass, type of physical activity and presence of the perichondrial ring are greater factors for slipped capital femoral epiphysis than the physeal-diaphysis angle and growth plate thickness (Castro-Abril et al., 2016). However, this model did not include the hypothetical irregular growth plate shapes included in a later model (Castro-Abril et al., 2016). It is difficult to determine from the present study if there is a correlation in the reduction of growth plate slope angle in the transverse plane of the domestic pig and Southwick's anteroposterior angle.

In the sagittal plane epiphyseal cupping relative to the tubercle was less pronounced with radius increases better characterizing flattening of the growth plate surface rather than curving, as seen in the transverse plane. The slope of the growth plate was retained throughout development. A distinct domed protuberance on the metaphyseal growth plate surface cranial to the tubercle emerged in early development and increased as a function of age, providing a mechanism of stability as loading shifts from the tubercle in the stance phase of the gait cycle to the cranial domed protuberance in the “toe-off” phase. This is possible due to the sigmoid shaped growth plate and retained slope angle, which provides mechanisms of stability throughout the gait cycle (see Video S2:


Femoral head radii were slightly smaller in the domestic pig as compared to their human counterpart and elongated with age. The radius of the femoral head with respect to the cranial articular curvature in the sagittal and transverse planes were nearly identical within and between
the samples in each of their respective groups, measuring 14.0 ± 0.7 mm, 17.7 mm, 20.2 mm, and 18.9 ± 0.9 mm for the 120, 365, 540, and 900-day old groups, respectively. A previous study of femoral head radii measured models generated from DICOM files to establish a set of normal values for adolescent children 14–16 years-of-age (n = 50), reported average femoral head radii of 24.5 ± 1.7 mm for males and 21.9 ± 1.4 mm for females (Jamali, Mak, et al., 2013).

Elongation was a combination of the spherical shape of the femoral head elongating and the development of an elongation lip on the articular side of the capital femoral epiphysis in the caudal region that enveloped the isthmus. This growth front may also contribute as an additional alternate mechanism to withstand torsion as the age and weight of the animal increased.

Elongation of the epiphyses (as the measured distance outside of circular symmetry) for 540-days and older were approximately nine times greater than the 120-day old group and 365-day old. This growth front not only increases the surface contact area but it also increases the polar moment of inertia, which increases the resistance to torsion and enhances stability (Hibbeler, 2013). The elongation lip developed near an articular bone-ridge that also developed and encircled the caudal region of the articular side of the epiphysis. The purpose of the bone ridge (Figure 12a) that developed on ages 540-days and older is not clear, but it could function as the attachment site of the perichondrial ring of LaCroix; a thick fibrous tissue that spans the circumference of the epiphyseal margin and reinforces stability of the joint (Schneider, Ipsen, et al., 2003).

It is unclear what drives the development of femoral head mammillary processes, but it has been shown that variation in mechanical loading influences chondrogenesis during joint formation (Congdon, Hammond, et al., 2012; Moncayo-Donoso, Guevara, et al., 2019). It is also unclear as to what extent if at all, radially arranged peripheral mammillary processes develop in
the human capital femoral epiphysis. The development of radially arranged mammillary processes have not previously been reported, to the best of our knowledge and underdevelopment may be a risk factor that increases the likelihood of a slip in the multifactorial disorder slipped capital femoral epiphysis (Aronson & Tursky, 1996).

3.4.3 Limitations

In this study, we investigated the development of different animals at various ages rather than following animals throughout the period of developmental interest. Though some of our groups had sufficient sample sizes for statistical purposes, a larger population would better characterize a baseline for the porcine model. This study also assessed developmental findings without consideration of the role of soft tissue. The growth plate can be 4–6 mm thick in early adolescent stages (20-day old) and as thin as 1 mm in later stages (365-day and older). The offset created by growth cartilage could reduce the mechanical effectiveness of the interlocking bone-cartilage-bone interface. Another significant component in the prevention of interface failure is the perichondrium. This fibrous band of tissue wraps around femoral head at the joint seam of the epiphyseal and metaphyseal interface and in a 1-year-old pig accounts for approximately 20% of the joint's strength in both transverse shear (Ipsen et al., 2002) and torsion. Interestingly, when the transverse shear test was repeated following the first test in which the growth plate cartilage failed completely but left the perichondrium intact, the shear load to failure during the second test was 65% of the contralateral control femora. This paradoxical result was explained by noting that after the cartilage had failed, the perichondrial ring acted to tether the femoral head to the metaphysis, causing the interdigitating mammillary processes to interlock. Further posterior displacement of the epiphysis required elevating the epiphysis over the physeal tubercle
and overcoming the tensile forces in the perichondrium anteriorly, which could only occur by ultimately rupturing (Schneider et al., 2003).

The metaphyseal growth plate surface was also subject to chemical processes during tissue removal that may have inadvertently removed surface features in the zone of provisional calcification and primary spongiosa, as in-vivo growth plate differentiates into calcified cartilage before being replaced with trabecular bone. It is difficult to interpret 3D topography of the trabeculated metaphyseal surface. The topography of the epiphyseal subchondral bone plate (ESBP) can be easily evaluated as it is a thin layer of cortical bone with much smaller pore sizes. For this reason, mammillary processes were evaluated using the ESBP surface.

It should also be noted that the two animals in the 35-day old group were part of a methotrexate study. Methotrexate has been shown to arrest bone growth by suppressing chondrocyte proliferation and cause osteoporosis in young rats given five once-daily doses of 0.75 mg/kg, with “damage” most obvious 9 days after final treatment, but bone growth returning to normal by day 14 (Xian, Cool, et al., 2007). The 35-day old group in this study received a one-time dose of methotrexate at 2 g/kg and were sacrificed 6 days later. Though they were sacrificed prior to the time damage was reported in the rat model, there is a chance that the high dose could cause immediate bone growth arrest at the time of infusion. For this reason, the 35-day old group could be more representative of animals 6 days younger, assuming arrest occurred immediately. At the gross level, the bones showed no obvious signs of deformity or osteochondrosis when compared to the adjacent age groups, as judged qualitatively.

Finally, the average heights of the peripheral mammillary processes of animals younger than 120 days fell below the scanner manufacturer's stated resolution limit of 0.1 mm. It is possible to obtain higher resolution with the NextEngine HD laser scanner, but for this study we
can only claim the results of average height and periodicity of mammillary processes for animals that were 120-days and older in age. Measurements of <0.1 mm could be the result of noise in the scanning process (Perrone Jr & Williams, 2019).
References


CHAPTER 4

COMPUTATION STUDIES OF THE STRUCTURE-FUNCTION RELATIONSHIP OF PORCINE FEMORAL HEAD MAMMILLARY PROCESSES

4.1 Introduction

Slipped capital femoral epiphysis (SCFE) occurs when the neck of the femur slips in relation to the femoral head and is the most common hip disorder in adolescent children between 11 and 16 years of age (Aronson & Tursky, 1996). The capital femoral physis is a growth plate located between the head of the femur and the femoral neck and forms a temporary joint where growth cartilage differentiates into bone by endochondral ossification during adolescent development. A slip occurs when a shear force across the capital femoral physis overcomes the mechanical integrity of the bone-cartilage-bone interface resulting in an epiphyseal posteromedial displacement relative to the metaphysis involving a rotation of the femoral head relative to the neck (Hammond et al., 2016; Khaladkar et al., 2015; Tayton, 2007), though a small number of posterolateral displacements occur (Gelink et al., 2021). Patients who suffer a slip will often present with increased external rotation of the affected leg when the hip is flexed. Internal rotation of the femur following a slip may cause mild to severe pain and may be restricted due to impingement (Livingstone et al., 2019). Though the disorder is widely studied the etiology is not completely understood and is likely multifactorial. However, joint morphology may play a critical role in the stability of the bone-cartilage-bone interface throughout development until the joint closes in early adulthood, when the epiphysis fuses with the metaphysis.
During development morphological changes in the joint emerge and provide natural mechanical mechanisms resistant to shear and torsion, enhancing biomechanical stability of the joint. Scheuer et al. (2000) described the emergence of a prominent, “beak-like” eccentrically located epiphyseal tuberosity (tubercle), which projects into a corresponding metaphyseal fossa (Novais et al., 2020; Scheuer et al., 2000). Tayton (2009) characterized the tubercle’s development as a change of the epiphyseal subchondral bone plate surface from “one of friction to one of pegging” (Tayton, 2009). Though the tubercle is “wide-based and fairly flat” in humans compared to their ovine, bovine and porcine counterparts (Perrone Jr & Williams, 2021; Tayton, 2007), the tubercle has been suggested to be a primary stabilizer during early stage adolescence (Kiapour et al., 2019). It has been argued that the prominence and eccentric location of the tubercle provides stability against transverse and torsional shear and that an underdeveloped tubercle and enlarged metaphyseal fossa may be contributing factors to instability (Hosseinzadeh et al., 2021; Novais et al., 2020; Tayton, 2007). In later stage development the tubercle recedes in both height and length as epiphyseal cupping increases (Kiapour et al., 2019; R. W. Liu et al., 2013; Perrone Jr & Williams, 2021) and primary stabilization shifts from the tubercle to epiphyseal cupping (Kiapour et al., 2019). In the *sus scrofa domesticus* (domestic pig) the tubercle begins as an elongated ridge spanning the diameter of the epiphysis in early development, decreasing in length to a peaked structure in the caudal-lateral (analogous to posterolateral) region (Perrone Jr & Williams, 2021) bearing resemblance to the eccentric “beak-like” tuberosity Sheuer et al. (2000) described. The change from an elongated ridge to an eccentric peaked structure can act in concert with epiphyseal cupping to provide shear resistance but can also act as a pivot point for a torque if the growth plate is not stabilized with the
development of additional structures, as primary stabilization shifts from the tubercle to
epiphysial cupping.

In the domestic pig a set of smaller, radially arranged secondary mammillary processes in
the periphery of the epiphysis develop with a degree of periodicity and project into
the corresponding concavities in the metaphyseal growth plate surface, interlocking the epiphysis
with the metaphysis. The emergence and pattern of these structures begin early in development
and increase in prominence as the weight and age of the animal increase. It has been argued that
these structures increase biomechanical stability and provide resistance to increasing torque
throughout development that could otherwise cause the epiphysis to pivot about the eccentrically
located tubercle (Perrone Jr & Williams, 2021). Small undulations in the interfacial morphology
have been shown in finite element simulations of hypothetical growth plate shapes to limit shear
(Castro-Abril et al., 2016). However, radiologists have reported widening of the growth plate
ranging from mild up to a 5-fold increase accompanying cases of SCFE (Khaladkar et al., 2015;
Leblanc et al., 2017) but it is unclear if the observed widening of the growth plate contributes to
the slip or is a consequence thereof. It has been suggested that increasing the height of the
growth plate unlocks the interdigitating mammillary processes reducing resistance to shear
(Speer & Braun, 1985) but it is unclear if this also decreases torsional resistance.

Our aim in this present study was to develop a finite element modeling approach to
examine the structure-function relationship of the radially arranged secondary mammillary
processes with respect to torsional resistance and evaluate how an increased growth plate
thickness affects their role in joint stability. We sought to answer the following: (1) Do the
radially arranged, peripheral mammillary processes reduce pivoting about the tubercle when a
torque is applied?
(2) Do these peripheral mammillary processes reduce stress distributions on the tubercle when a torque is applied? (3) What affect does an increased growth plate thickness have on the structure-function relationship of the interdigitating mammillary processes?

4.2 Methods

4.2.1 Analog model construction

A set of parameters used to define the geometry of an idealized femoral head joint were approximated using the averaged measures obtained from biomodels created from laser scans of the proximal end of six 900-day old domestic pig femurs with all soft tissue removed (Perrone Jr & Williams, 2019, 2021). All measures and model geometry were developed using computer aided design software (CAD) (NX 2020 software, Siemens, Plano, TX). The approximate in-vivo position during stance for each biomodel was previously defined for model orientation in 3D space (Perrone Jr & Williams, 2021). A total of 42 parameters using the averaged measures of the six biomodels defined the idealized femoral head joint and are detailed in supplement figures 1-3 and supplement tables 1-5. To define the primary shape of the epiphysis, an axisymmetric sketch was derived from sagittal and transverse cross-sections through the central axis of the femoral neck, as defined by the caput-collum-diaphyseal angle, for each epiphysis. A set of five parameters were measured from the cross-sections and used to derive the axisymmetric sketch (Figure 4.1A; Supplement 2 Table C1). To define the idealized shape of the radial mammillary processes, cylinders were created from the epiphyses using Boolean subtractions and cylinder perimeters “unwrapped” and projected to a plane to create profiles of the radial mammillary processes (Perrone Jr & Williams, 2021). A set of eight parameters were measured for each cross-section in the curve and defined the idealized shape of a single, radial mammillary process (Figure, 4.1A; Supplement 2 Figure C1 & Table C2). To define the idealized shape of the
tubercle, a set of three sketches were developed from cross-sections taken at the peak of the
tubercle and one at each length end, linked together with splines placed on the tubercle surface to
create a scaffold of the tubercle. A set of 22 parameters were measured from the three cross-
sections and splines, which defined the idealized shape of the tubercle (Figure 1A; Supplement 2
Figure C2 & Table C3). The scaffold of the tubercle was positioned using the average peak
location and angle of the cranial-medial length end, as measured from centerlines defining the
four anatomical quadrants: caudal-medial, caudal-lateral, cranial-medial and cranial-lateral
(Figure 4.1B & 4.2; Supplement 2 Figure C3). The sketch of the idealized, single mammillary
process was positioned and patterned about the central axis of the femoral neck (axis of
symmetry) for a total of 19 radial mammillary processes spaced every 20.4 deg (Perrone Jr &
Williams, 2021)(Figure 4.1B; Supplement 2 Figure C1). Solids of each component were created
using revolve and sweep extrusions and the three parts merged, creating the idealized epiphysis
(Figure 4.1B & C).

To define the idealized shape of the metaphysis, an axisymmetric sketch was developed
from cross-sections as previously defined. A set of seven parameters were measured and defined
the primary shape of the metaphysis and a revolve extrusion about the axis of symmetry was
used to create the solid metaphysis. The epiphyseal subchondral bone plate surface of the
idealized epiphysis was positioned onto the metaphysis such that opposing contact surfaces were
shared. A Boolean subtraction was performed on the metaphysis using the epiphysis as the tool,
which created the corresponding concavities for the epiphyseal tubercle and secondary
mammillary processes to reside (Figure 4.1D & E). The central region between the epiphysis and
metaphysis was partitioned into five, 1-mm thick zones that maintained the geometry of the
mammillary processes throughout, such that each layer interlocked with the adjacent layer. This
allowed for variation of material properties through the model to better represent the transitions of the bone-cartilage-bone interface at the macroscale, which includes subchondral bone, growth plate cartilage, calcified cartilage and trabecular bone (Gao et al., 2015; Gao et al., 2014; Kazemi & Williams, 2019) (Figure 1F). Finally, two versions of the model’s geometry were developed, one with the large epiphyseal tubercle and radially arranged peripheral mammillary processes, which idealized full development (model FD) and one with just the tubercle and no peripheral mammillary processes (model T) which idealized possible underdevelopment of peripheral structures.

**FIGURE 4.1**  A) An axisymmetric sketch (blue half-moon shaped curve) defined the primary shape of the epiphysis (Supplement 2 Table C1). A circle 80% the diameter of the epiphyseal rim defined the cylinders for Boolean subtractions. (Supplement 2 Table C2 & Supplement Fig. C1). Cross-sections Through the tubercle and splines created the tubercle scaffold. (Supplement 2 Table C3 & Supplement 2 Fig. C2). B) Sweep and revolve extrusions created the primary shape of the epiphysis, tubercle and one single, radial mammillary process that was patterned about the axis of symmetry. C) The idealized epiphysis. D & E) An axisymmetric sketch (blue curve) defined by parameters measured from cross-sections of the femoral head and idealized epiphysis was revolved about the central axis of the femoral neck to create the metaphysis (Supplement Table 4). F) The idealized model, with the epiphyseal cap (E), 1 mm zones z1-z5 and the metaphysis (M) defining the regions of the model.
The model was orientated to match the approximate *in-vivo* position of the pig femur during stance, as previously defined in a polar coordinate system of anatomical quadrants (Perrone Jr & Williams, 2021). The origin of this global coordinate system was at the apex of the articular side of the epiphysis with the z-axis oriented along the central axis of the femoral neck. A plane along the y-axis divided the medial and lateral region and a plane along the x-axis divided the caudal and cranial regions, which are analogous to posterior and anterior respectively for the biped (Figure 4.2).

**FIGURE 4.2** Anatomical orientation for the parametric model with black arrows denoting the direction of a prescribed torque “T” about the central axis of the femoral neck (reference node axis), at the reference node attached to the anatomical surface at the origin of the global coordinate system. Left) a polar coordinate system of quadrants about the reference node axis (z-axis) define the anatomical orientation of the model’s geometry. Right) The caudal and cranial regions for a quadruped are somewhat analagous to the posterior and anterior regions respectively for a biped. Model parts E, z1 and z2 (red) make up the epiphysis. The remaining partitions make up the transitional zones of the bone-cartilage-bone interface of the femoral head.
4.2.2 Volumetric Mesh Model

The seven individual CAD parts of each model were imported into a geometry and mesh generation toolkit software (Cubit 2021.11 software, Coreform, Orem, UT) where they were assembled and converted to volumetric meshes. The assembled models were “imprinted” such that the mesh between shared (contact) surfaces of the individual parts were identical and a 10-node quadratic tetrahedral solid element (C3D10) was assigned. The mesh density propagated toward the central region to generate a minimum two layers of elements through each 1-mm thick zone, which generated a highly dense mesh through zones z₁-z₅, resulting in volumetric meshes containing over 600,000 elements for model T and over 1 million for model FD. Zone z₃ represents the 1 mm thick growth plate. When zone z₃ and z₄ are combined they represent a 2 mm thick growth plate. For model T, z₃ contains 88,553 elements and when z₃ and z₄ are combined they contain a total of 175,673. For model FD, z₃ contains 184,672 elements and when z₃ and z₄ are combined they contain a total of 368,634 elements.

4.2.3 Finite Element Model

Volumetric mesh models were imported into ABAQUS/Explicit finite element (FE) software (ABAQUS/Explicit 2021, SIMULI, Providence, RI) to generate FE models for analysis. The models were assembled and native meshes merged, keeping each zone partitioned. Material properties were defined using data from previous studies and assigned to each zone to produce four variations of the model based on geometry and material property assignment, which defined a growth plate thickness of either 1-mm (T₁mm and FD₁mm) or 2-mm (T₂mm and FD₂mm) (Table 4.1). Materials were modeled as isotropic and linear elastic except for growth plate cartilage, which was modeled as isotropic and hyper-elastic. Each linear elastic material was assigned a Young’s modulus \( E \), Poisson’s ratio \( \nu \) and mass density \( \rho \) as follows: \( E = 550 \text{ MPa} \) (Lee &
Jasiuk, 2014), $\nu = 0.3$ (R. Liu et al.) and $\rho = 0.001 \text{ g/mm}^3$ (Martin et al., 1998) for epiphyseal trabecular bone; $E = 350 \text{ MPa}$ (Bravo et al., 2019), $\nu = 0.3$ (R. Liu et al.) and $\rho = 0.001 \text{ g/mm}^3$ (Martin et al., 1998) for metaphyseal trabecular bone; $E = 1100 \text{ MPa}$, $\nu = 0.3$ (Wei et al., 2005) and $\rho = 2.0 \text{ g/mm}^3$ (Martin et al., 1998) for subcondral bone; $E = 100 \text{ MPa}$ (Mente & Lewis, 1994), $\nu = 0.2$ (Carter et al., 1998) and $\rho = 0.001 \text{ g/mm}^3$ (Martin et al., 1998) for calcified cartilage.

Growth plate cartilage was modeled as hyper-elastic due to the expected finite rotations and strains resulting from applied torques applied to the epiphysis for comparison to experimental data, which far exceeded physiological loading conditions. All strains were reported as logarithmic strains. A mass density of $\rho = 0.001 \text{ g/mm}^3$ was assigned as a reasonable density due to the high-water content in growth cartilage. Early trials of a simplified model showed high mesh deformation of the two stack elements in this 1-mm thick zone when assigned linear elastic material properties, which produced unreliable results. Hyper-elastic material properties sustained mesh integrity and produced results equivalent to hand calculations. Therefore, cartilage was assigned the two parameter hyper-elastic Mooney-Rivlin type with two deviatoric and one volumetric coefficient, using the coefficients proposed for articular cartilage $C_{01} = 4.1 \text{ MPa}$ and $C_{10} = 0.41 \text{ MPa}$ (Li et al., 2007; Marqués Gómez et al., 2022). In ABAQUS the default compressibility constant $D_1$ (when left blank), assumes an initial bulk modulus to be 20 times that of the initial shear modulus, given by:

$$\frac{K_0}{\mu_0} = 20$$  \hspace{1cm} (5)

where the initial shear modulus $\mu_0 = 20 \text{ MPa}$, bulk modulus $K_0 = 2 \text{ MPa}/D_1$ where $D_1$ is the compressibility constant and when left blank will correspond to a Poisson’s ratio of $\nu = 0.475$. 
Table 4.1. Material Property Assignment For Each Model (E = Epiphysis M = Metaphysis)

<table>
<thead>
<tr>
<th>Zones</th>
<th>T_{1mm}</th>
<th>T_{2mm}</th>
<th>FD_{1mm}</th>
<th>FD_{2mm}</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Trabecular Bone (E)</td>
<td>Trabecular Bone (E)</td>
<td>Trabecular Bone (E)</td>
<td>Trabecular Bone (E)</td>
</tr>
<tr>
<td>Z_1</td>
<td>Trabecular Bone (E)</td>
<td>Trabecular Bone (E)</td>
<td>Trabecular Bone (E)</td>
<td>Trabecular Bone (E)</td>
</tr>
<tr>
<td>Z_2</td>
<td>Subchondral Bone</td>
<td>Subchondral Bone</td>
<td>Subchondral Bone</td>
<td>Subchondral Bone</td>
</tr>
<tr>
<td>Z_3</td>
<td>Cartilage</td>
<td>Cartilage</td>
<td>Cartilage</td>
<td>Cartilage</td>
</tr>
<tr>
<td>Z_4</td>
<td>Calcified Cartilage</td>
<td>Cartilage</td>
<td>Calcified Cartilage</td>
<td>Cartilage</td>
</tr>
<tr>
<td>Z_5</td>
<td>Trabecular Bone (M)</td>
<td>Calcified Cartilage</td>
<td>Trabecular Bone (M)</td>
<td>Calcified Cartilage</td>
</tr>
<tr>
<td>M</td>
<td>Trabecular Bone (M)</td>
<td>Trabecular Bone (M)</td>
<td>Trabecular Bone (M)</td>
<td>Trabecular Bone (M)</td>
</tr>
</tbody>
</table>

4.2.4 Experimental Loading Conditions

Chuinard et al. (2004) evaluated the contribution of the perichondrial ring with respect to torsional loads applied to the femoral capital epiphysis. To measure this, eight pairs of one-year old femora were obtained and the perichondrial ring sharply excised from half of the set and left in-tact for the other. The diaphysis of the femur during testing was securely mounted and fixed, with the epiphysis mounted and fixed in a specially designed cylindrical torque holder (see schematic in Figure 4.3). All degrees of freedom (DOF) were constrained except the rotation resulting from the prescribed torque that was applied to the epiphysis about an axis perpendicular to the growth plate. An increasing torque was applied at a rate of 0.11 degrees/sec, simulating external rotation of the femur with respect to the epiphysis, until failure, defined as a 30% drop in torque from the peak value resulting in major destruction of the growth plate. The average peak value for the femurs with the perichondrial ring excised was 19.6 ± 3.0 Nm. Results were evaluated using torque-rotation plots and showed the femora with intact perichondrial rings were able to support larger torques, averaging 23.4 ± 3.6 Nm. Energy at peak torque for the femurs with the excised perichondrial ring at peak torque and failure were 1.5 ± 0.4 and 1.6 ± 0.5 Joule rad respectively. With the perichondrial ring intact, energy at peak torque and failure was 2.1 ± 0.5 and 4.5 ± 0.5 Joule rad respectively. (Chuinard et al., 2004). The current FE models did not include a perichondrial ring and comparisons were made to the experimental data produced by the femora with the perichondrial ring excised.
4.2.5 Model Loading and Boundary Conditions

The loading and boundary conditions for the FE models were set up to simulate the experiment. The surface nodes from zones E, z₁ and z₂ were coupled to the reference node at the origin, representing the epiphysis (Figure 4.2). The boundary condition for the nodes at the cross-sectional area of the neck were fixed in all DOF (encastré). The reference node was free to rotate about the z-axis but fixed in the remaining five DOF, simulating the constrained femora from the experiment. A 19.6 Nm torque was prescribed at the reference node about the central axis of the femoral neck (z-axis) simulating external rotation of the model’s metaphysis with respect to the epiphysis at the ultimate experimental failure torque. The simulation was run in ABAQUS/Explicit as quasi-static and completed the full torque in three seconds distributed over 20 evenly timed increments, for which the kinetic energy was negligible (nearly zero) as determined by monitoring the internal and kinetic energy. Material and geometric non-linearities were addressed by setting the NLgeom parameter in ABAQUS to yes (default for Explicit). To analyze how well the model simulated the experiment and if the solutions were reasonable, the rotational stiffness was calculated and evaluated from torque-rotation plots for the experimental femora and models T₁mm and FD₁mm.

The structure function relationship of the peripheral mammillary processes were evaluated along with the effects of doubling the growth plate thickness from 1-mm to 2 mm, for all four models (T₁mm, T₂mm, FD₁mm, and FD₂mm). Unlike the in vitro experiments of torque to failure, in the case of a slipped capital femoral epiphysis (SCFE) the epiphysis is free to translate and tilt relative to the femoral neck in all six DOF with only the integrity of the growth plate cartilage and tension in the perichondrium/periosteum, and compression do to body weight to restrain the motion. To simulate conditions more relevant to the in-vivo state the four models
were prescribed the same torque values as used to simulate the in vitro experiment, while allowing movement in all six DOF. No loads other than the torque were prescribed and the perichondrium was not included.

To analyze the structure-function relationship of the radial mammillary processes and the effect of growth plate thickness, we first calculated and evaluated rotational stiffness from torque-rotation plots. To determine if the peripheral mammillary processes reduced torsional induced stress distributions on the tubercle and assess the effect of increased growth plate thickness, we evaluated stress distributions on the tubercle and across the growth plate four all four models. Since the stress tensor can be decomposed into the sum of the hydrostatic and deviatoric stresses, we created contour plots of the hydrostatic stress (volumetric or dilatational stress) and equivalent stress (von Mises), defined by Eqs. (2) and (3) respectively.

\[
\text{Hydrostatic stress, } \sigma_H = \frac{1}{3}(\sigma_{11} + \sigma_{22} + \sigma_{33}) \quad (6)
\]

where \(\sigma_H\) is the average of the principal stresses \(\sigma_{11}, \sigma_{22}\) and \(\sigma_{33}\). Hydrostatic stresses are negative in compression and positive in tension.

\[
\text{Equivalent stress (von Mises), } \sigma_{VM} = \left\{ \frac{1}{2} \left[ (\sigma_{11} - \sigma_{22})^2 + (\sigma_{22} - \sigma_{33})^2 + (\sigma_{33} - \sigma_{11})^2 \right] \right\}^{1/2} \quad (7)
\]

The equivalent or von Mises stresses in 3D stress states are often used to compare with the tensile stresses associated with yielding in experimental uniaxial tension tests in which only one of the principal stresses is nonzero. Cartilage being a nearly incompressible material under short duration loading is susceptible to damage due to large distortional or deviatoric stresses and to tensile hydrostatic stresses that are resisted by collagen fibers. High compressive hydrostatic
stresses may cause vascular occlusion or collapse of the zone of provisional calcification and primary spongiosa. This separation of stresses into dilatational deviatoric components has been used in bone growth theories to model endochondral bone growth (Wong & Carter, 1990). Regions in the cartilage anlage that are subjected to relatively large hydrostatic stresses in comparison with the von Mises stresses are more likely to remain as cartilage, whereas increases in von Mises stresses in relation to hydrostatic values will drive the process toward bone formation. Contour plots of both stress components may help with understanding failure mechanism and of the development of the mammillary processes.

4.3 Results

4.3.1 Comparison to Experiment (epiphysis constrained in 5 DOF)

The average reported rotational stiffness and epiphyseal rotation of the experimental femoral growth plates was $2.6 \pm 1.4$ Nm/deg and $7.8 \pm 1.3$ deg ($\mu \pm SD$), respectively (Chuinard et al., 2004). The rotational stiffness value of our constrained model $FD_{1mm}$ (3.8 Nm/deg) simulating the experiment was 46% higher than the average experimental value ($2.6 \pm 1.4$ Nm/deg), resulting in 35% less rotation than the average experimental value ($7.8 \pm 1.3$ deg) (5.1 deg). The stiffness of the constrained model $T_{1mm}$ (3.8 Nm/deg) was one and a half times higher than the average experimental stiffness, resulting in 56% less rotation (Figure 4.3). However, the eight experimental femurs had a wide range of rotations to failure (defined as a 30% drop in torque from the peak value), ranging between 5.3 and 9.3 deg and model $FD_{1mm}$ approximated the experimental test results for the stiffest specimen.
4.3.2 Unconstrained models

Models $T_{1\text{mm}}$ and $FD_{1\text{mm}}$ (unconstrained) rotated 3.4 and 3.9 deg, respectively, about the z-axis as a result of the torque. Rotational stiffness decreased for both models by 12%, from 5.7 Nm/deg to 5.0 Nm/deg and 5.1 Nm/deg to 4.5 Nm/deg, respectively, when the growth plate thickness doubled (modeled by $T_{2\text{mm}}$ and $FD_{2\text{mm}}$). This resulted in a 15% increase in rotation about the z-axis for model T, from 3.4 to 3.9 deg, and a 13% increase for model FD, from 3.9 to 4.3 deg. Model FD rotated about a half a degree more than model T, for both 1-mm and 2-mm growth plate thicknesses. Rotation about the x-axis increased 2.5-fold, from 0.006 deg to 0.015 deg for model T and increased 8-fold, from 0.001 deg to 0.008 deg for model FD when the growth plate thickness doubled. Rotation about the y-axis increased 2.3-fold for both models, from 0.019 deg to 0.043 deg for model T and 0.018 deg to 0.043 deg for model FD when the growth plate thickness doubled (Figure 4.4).
FIGURE 4.4  Left) Torque rotation plot for models T\textsubscript{1mm}, FD\textsubscript{1mm} (blue and red squares respectively), T\textsubscript{2mm} and FD\textsubscript{2mm} (blue and red circles respectively) with a black arrow showing direction of rotation about the z-axis. Rotations about the x and y axes [deg] are shown in the legend under the corresponding model. Right) Each of the 7 partitions were assigned a material property (previously defined), with growth plate (GP) assigned to establish a 1-mm thick z\textsubscript{3} or 2mm thick (z\textsubscript{3} + z\textsubscript{4}) for both models.

Pivoting about the tubercle was higher in the models with no radial mammillary processes compared to models with radial mammillary processes (Figure 4.5; Supplement 2 Figure C4).

The average circumferential shear strain at the outer most edge of the growth plate on the epiphyseal interface, was 25% but the epiphysis translated posteromedially and tilted as well as rotating about the femoral neck, causing variations in the circumferential shear strains around the periphery of the growth plate.
FIGURE 4.5  Contour plots of shear strain across the growth plate surface that is in contact with the subchondral bone plate surface (epiphyseal interface), reported in cylindrical coordinates. All four models show the metaphyseal fossa where the tubercle resides, and models FD1&2 show the radial concavities where the interlocking radial mammillary processes reside. The growth plates in the top row are oriented to show more of the cranial (anterior) region, whereas the growth plates in the bottom row are oriented to show more of the caudal (posterior) region surface. Curved arrows on model T1mm denote the direction of rotation. Neighboring element values within the cartilage region only were averaged at the nodes unless the relative difference between contributions from neighboring elements at the node was greater than 75% (as denoted in the legend).

Contour plots of von Mises stress distribution across the growth plate surfaces were generally higher in the periphery (Figure 4.6), which correlated with maximum principal strains (Supplement 2 Figure C5). Stresses for model T1mm were as high as 4.8 MPa in the peripheral region in the cranial-medial quadrant on the surface in contact with the metaphyseal growth plate surface (metaphyseal interface). Stresses as high as 4.8 MPa also occurred on the epiphyseal interface but were more uniformly distributed circumferentially. When the thickness of the growth plate doubled (T2mm), stresses increased on the leading contact ridge of the corresponding tubercle for both interfaces, reaching up to 5.4 MPa but were more uniformly distributed circumferentially.
Von Mises stress for model FD\textsubscript{1mm} were also higher in the peripheral region in the cranial-medial direction reaching values as high as 6.1 MPa, but were confined to the peripheral ends of the radial mammillary processes versus the outer most periphery on the metaphyseal interface. Stress concentrations on the epiphyseal interface were mostly confined to the grooves of the radial mammillary processes with a very thin line in the circumference of the outermost cranial region. This resulted in lower, more evenly distributed stress in the outermost peripheral region for both interfaces. When the thickness of the growth plate doubled (FD\textsubscript{2mm}), stresses in the metaphyseal interface were higher in the outer most peripheral region in the cranial-medial direction, reaching values up to 6 MPa but with only a trace amount seen at the peripheral ends of the mammillary processes in the cranial-lateral region. On the epiphyseal interface von Mises stress was concentrated at the leading contact edge of the tubercle, within the grooves of the radial mammillary processes and at the outermost periphery up to 6 MPa but was more uniformly distributed circumferentially.
FIGURE 4.6  Contour plots of von Mises stress distribution across the growth plate surface. Top row) The growth plate surface in contact with the epiphyseal subchondral bone plate surface (epiphyseal interface). Bottom row) The growth plate surface in contact with the metaphyseal growth plate surface (metaphyseal interface). Solid arrows show the direction of rotation and dashed arrows denote the surface of the growth plate (gray models) the contour plot coincides with. Neighboring element values within the cartilage region were averaged at the nodes unless the relative difference between contributions from neighboring cartilage elements at the node was greater than 75% (as denoted in the legend).

Contour plots of the hydrostatic stress showed stress distribution across the tubercle and through the growth plate were lowest in model FD_{1mm}, containing the radially arranged peripheral mammillary processes and a growth plate thickness of 1-mm. Hydrostatic compressive stresses as high as 5.9 MPa occurred at the leading contact ridge of the tubercle and were more prominent in the epiphyseal interface, with tensile forces as high as 1.8 MPa on the trailing contact ridge. For model T_{1mm}, with no radial mammillary processes, compressive stresses as high as 6.7 MPa occurred over a larger area of the tubercle’s leading contact edge and surface, whereas hydrostatic tensile stresses up to 1.8 MPa did not increase over a larger area on the trailing contact ridge. Hydrostatic stresses were concentrated in the grooves of the radial
mammillary processes for model FD\textsubscript{1mm} on the epiphyseal interface also reaching compressive values as high as 5.9 MPa, whereas tensile values were as high as 6.3 MPa (Figure 4.7).

When the thickness of the growth plate doubled from 1-mm to 2-mm, hydrostatic stresses on the leading contact ridge of the tubercle reached compressive values as high as 10.4 MPa and tensile values as high as 2.3 MPa on the Trailing contact ridge for model T\textsubscript{2mm} (no radial mammillary processes). Stresses on the leading contact ridge of the tubercle for the model FD\textsubscript{2mm} (with radial mammillary processes) were lower, reaching compressive values up to 5.6 MPa, with tensile values up to 2.3 MPa on the trailing contact ridge. Stresses in the grooves of the radial mammillary processes on the epiphyseal interface reached compressive and tensile values of 5.6 MPa and 2.8 MPa, respectively (Figure 4.7). Stresses on the leading contact surface of the tubercle were distributed through the growth plate from the epiphyseal interface to the metaphyseal interface the least for model FD\textsubscript{1mm}. 
4.4 Discussion

4.4.1 Comparison to experiment

The analog model $FD_{1mm}$ under constrained conditions was comparable to the femurs in the experiment but on the higher end of the rotational stiffness range (5.3 to 9.3 deg), rotating 5.1 deg. The zones of the model were assigned material properties at the macroscale (Gao et al., 2017) with the growth plate cartilage assigned hyper-elastic material properties with coefficients proposed for articular cartilage in the acetabulum (Li et al., 2007). However, there are substantial biochemical and structural composition differences between articular cartilage and growth plate cartilage, which may act to stabilize articular cartilage against shearing and compressive forces (Gregory et al., 2001). Articular cartilage may be more representative of the growth plate’s
reserve zone, with a higher resistance to shear strain resulting in higher rotational stiffness, but when comparing model FD_{1mm} to the experimental results the solutions were reasonable. Depending on the rate of growth and age, the reserve zone may occupy a variable fraction of the growth plate height ranging from 20 to 80% (Shah et al., 2003). Constitutive properties of growth plate cartilage derived experimentally from shear tests of the proliferative and hypertrophic zones have not been published to the best of our knowledge and would require the cartilage to be separated from the mammillary processes. Shear tests of beams cut across the ‘cartilage bridge’ 2.3 mm thick bi-polar growth plate between the tibial tuberosity and epiphysis of yearling calves generated average ultimate engineering shear strains of 48% (39% logarithmic strain) and average ultimate shear stresses of 2 MPa (Datta et al.) which can be compared with the average peripheral circumferential strains in the model of 25%. The rotational stiffness was higher in the model with no mammillary processes (T_{1mm}) than model FD_{1mm} causing it to rotate less and the results from this study provide insight as to why this was the case in the following discussion.

4.4.2 Unconstrained models

The majority cases of slipped capital femoral epiphysis (SCFE) have been characterized clinically as an epiphyseal posteromedial displacement relative to the metaphysis (Khaladkar et al., 2015; Tayton, 2007). Treatment of acute slips may require medial or internal rotation reduction (Amara et al., 2014; Casey et al., 1972; Fairbank, 1969) as torsion has been reported as an etiologic factor in the initial slip (Aronson & Tursky, 1996). There are several factors that could contribute to torsional loading across the growth plate. Synovial fluid in osteoarthritic joints contains less of the major component hyaluronic acid which is key for lubrication of synovial joints to reduce start-up friction (Foy et al., 1999; Higaki et al., 1998; Hills & Butler,
Joint friction also increases when joint load duration increases causing morphological changes in the conformity of sliding surfaces and the initial fluid film that exists during short periods of load duration by the squeeze-film mechanism, may become less effective (Mabuchi et al., 1998). In addition to high contact forces, friction moments have been associated with implant failures by loosening the fixation of acetabular cups in total hip replacement implants. Torque values as small as 2.2 Nm have been reported with cup failures (Tabata et al., 2015). Friction torques as high as approximately 1.75 Nm have been reported from in-vivo hip implants equipped with six strain gauges that provide telemetry data for 3D forces and moments incurred at the center of the ball of the implant during walking (Bergmann et al., 2016). These are relatively small torque values, but a shear component of the distributed joint forces acting in a plane parallel to the growth plate, through the center of the femoral head, could produce a moment about the pivot point provided by the eccentrically located tubercle. It has been argued that this torque is countered during growth by the resistance provided by the emergence of the radially arranged peripheral mammillary processes (Perrone Jr & Williams, 2021).

Rotation was a half degree higher in the model with radial mammillary processes (FD) versus that of the model without T, for both growth plate thicknesses (1- & 2-mm). Viewing displacement of the reference point at the apex of the epiphysis, demonstrates how the epiphysis is moving in relation to the metaphysis under the prescribed torque about the femoral neck axis (z-axis). During rotation, the leading edge of the tubercle begins to ride up the contact wall of the metaphyseal fossa, displacing the epiphysis from the metaphysis in the negative z-direction (separating the head from the neck). While this is occurring, the original location of the epiphysis, as tracked by the reference node, displaces in the caudal-medial direction (analogous
to posteromedial), for all four models, with model FD$_{2\text{mm}}$ having the highest caudal-medial displacement. This would be equivalent to a posteromedial displacement for the biped (Fig. 8). The resulting strain energies were 589, 674, 661 and 741 Nmm for models T$_{1\text{mm}}$, T$_{2\text{mm}}$, FD$_{1\text{mm}}$ and FD$_{2\text{mm}}$, respectively.

**FIGURE 4.8** Top Left) Epiphyseal displacements in the x, y and z directions as tracked by the reference node. The reference node (black dot) at the origin represents the initial position, where models T$_{1\text{mm}}$ and FD$_{1\text{mm}}$ have a growth plate thickness of 1-mm (blue and red squares respectively) and the models T$_{2\text{mm}}$ and FD$_{2\text{mm}}$ (blue and red circles respectively) have a growth plate thickness of 2-mm. Bottom Left) The displaced growth plate of FD$_{1\text{mm}}$ (dark gray) juxtaposed with the growth plate’s initial condition (light gray), showing the metaphyseal interface. The direction of the displacement in the xy plane was caudal-medial, indicated by the black arrow. Right) contour plots for each full model (top) and the growth plate for each model (bottom). Blue indicates displacement of the epiphysis in the negative z-direction (away from the metaphysis) denoted by the blue arrow, whereas red indicates displacements in the positive z-direction (toward the metaphysis) indicated by the red arrow, as shown on the full model FD$_{2\text{mm}}$. The black dot on the complete models indicates the reference node and the origin of the global coordinate system, in Cartesian coordinates.
4.4.3 The role of radial mammillary processes and joint stability

In this study we examined the structure-function relationship of the radially arranged peripheral mammillary processes using a finite element method. Our models showed that the idealized geometry did reduce pivoting of the epiphysis in the cranial region of the growth plate for the model with the radial mammillary processes and 1-mm thick growth plate, which reduced strains in the growth plate’s outermost region and reduced stresses on the tubercle. When the growth plate thickness doubled, the peripheral interlocking interdigitations were less effective, resulting in higher stress concentrations on the tubercle and increased pivoting of the epiphysis, resulting in higher shear strain in the growth plate. Widening of the growth plate has been reported with cases of SCFE (Khaladkar et al., 2015; Leblanc et al., 2017) and it has been suggested such increases in growth plate thickness could unlock the interdigitating mammillary processes reducing resistance to shear (Speer & Braun, 1985). Our model supports this hypothesis and suggests increased growth plate thickness also reduces the effectiveness of the interlocking interdigitations to resist torsion.

4.4.4 Limitations

The models used in this study were idealized versions of the 900-day old domestic pig femur. Elongation of the epiphysis has been reported for this animal as a growth front in the caudal region of the epiphysis in the form of an elongation lip develops and converges with a growth front from the greater trochanter, along the femoral neck. Epiphyseal elongation observed in the pig model would increase the polar moment of inertia, which in turn would increase the resistance to torsion resulting in less rotation and increased biomechanical stability (Hibbeler, 2013). However, the femora used in the experimental study were that of the one-year-old domestic pig, which had not yet developed epiphyseal elongation though the tubercle and
radially arranged mammillary processes were well developed (Perrone Jr & Williams, 2021). Therefore, elongation was omitted from the model. Another key structure in the biomechanical stability of the joint is the perichondrial ring. The models in this study excluded this feature, comparing to the experimental femurs with this tissue excised, but it should be noted that this thick, fibrous band of tissue encompasses the circumference of the physis margin, acting to tether the epiphysis to the metaphysis. This aids the interlocking mammillary processes keeping them held tightly in place. This structure accounts for approximately 20% of the joint’s strength in both transvers shear (Ipsen et al., 2002) and torsion. However, even after complete failure of the growth plate in the femora initially tested, where the perichondrial ring was left intact, a second test failed at 65% of the contralateral control femora. This is explained by the tethering provided by the perichondrial ring keeping the epiphysis interlocked with the metaphysis even though the growth plate interface was completely destroyed, further exemplifying the structure-function relationship of the interdigitating mammillary processes (Schneider et al., 2003). Finally, the loads used in the experiment were not intended to simulate physiologic loads that would occur in-vivo. The prescribed torque that came from the experiment was just the average torque recorded at the time of failure, as previously defined. We used these data to gauge how reasonable our model’s solutions were when loaded and constrained to simulate the experiment. By keeping the loading conditions consistent, only changing the constrained degrees of freedom to unconstrained, we were able to continue to track the reasonableness of the model’s solution compared to the experimental data without introducing new variables, such as changing the prescribed torque. Though the loading conditions far exceed physiological conditions, the experiment and simulations from the models were adequate to examine the structure-function relationship of the radially arranged peripheral mammillary processes. The tubercle has been
widely studied in the human epiphysis (Kiapour et al., 2019; R. W. Liu et al., 2013; Scheuer et al., 2000; Tayton, 2009) but it is unclear if these radially arranged peripheral mammillary processes occur in the human epiphysis and warrants further study.
References


CHAPTER 5

SUMMARY AND FUTURE WORK

5.1 Summary and conclusion

This study focused on the development of femoral head mammillary processes and their contribution to joint stability. A comprehensive analysis of the joint’s development revealed evidence that the emergence and patterned arrangement of the interdigitating mammillary processes that interlock the epiphysis with the metaphysis were not simply random, but a normal feature of the joint. A computational investigation into the structure-function relationship revealed evidence to suggest they serve to specifically resist torsional loading, enhancing the biomechanical stability of the joint.

A protocol developed to generate highly accurate, high-definition digital biomodels of bones from laser scans, capturing surface features as small as 0.1 mm was employed to create a set of biomodels ranging in age from nine-days preterm to 900-days old. A comprehensive qualitative and quantitative analysis of the joint revealed the emergence of secondary mammillary processes develop to form a radial pattern beginning relatively early in development and increase in prominence with age and weight of the animal. A metric of surface roughness analysis quantitatively characterized the mammillary processes, which revealed they were not simply random but developed radially with some degree of periodicity. The results of the comprehensive analysis study suggest they are a normal feature of the joint and work in concert with the eccentrically located tubercle and epiphyseal cupping, to serve as a defense mechanism against joint forces that could damage the growth plate and disrupt the process of endochondral ossification.
The computational results from the nonlinear parametric explicit finite element models revealed the idealized geometry did reduce pivoting of the epiphysis relative to the metaphysis about the tubercle’s pivot point for the model with a 1-mm thick growth plate. This reduced strains in the growth plate’s outermost region of the cranial quadrants and reduced stresses on the tubercle when compared to the model without these interlocking surface features. When the growth plate thickness was doubled the interlocking interdigitations were disengaged and rendered less effective, resulting in higher stress concentrations on the tubercle and increased pivoting of the epiphysis causing higher shear strain in the growth plate. The results of this study suggest secondary mammillary processes develop specifically as a natural mechanical mechanism to withstand torsion to protect the growth plate and enhance joint stability.

In summary, the main objectives of this work were to provide insight into the development of mammillary processes and evaluate their role in joint stability. The information from the qualitative and quantitative analysis provides new insight about secondary mammillary processes and introduces their emergence and arrangement as a normal feature of the joint’s development. The information from the computational analysis provides new insight regarding their role as a natural mechanical mechanism providing resistance to torsion, protecting the growth plate from shear strains, enhancing biomechanical stability of the joint throughout development.

5.2 Recommendation for future work

In this study we developed a set of nonlinear parametric explicit finite element models to evaluate the role of the radially arranged peripheral mammillary processes in joint stability and how increased growth plate thickness affected their resistance to torsion. However, the increased thickness of the growth plate was doubled for this study, but could be much thicker under certain
circumstances, which could further reduce not just the effectiveness of the peripheral mammillary processes to resist torsion but also that of the tubercle to resist transverse shearing.

These models were compared to experimental data from femurs with the perichondrial ring excised and our models subsequently omitted this feature. However, the perichondrial ring is a thick band of fibrous tissue that encircles the circumference of the epiphyseal margin. This band of tissue transitions seamlessly from articular cartilage into periosteum and acts as a tether to keep the epiphysis tightly seated with the metaphysis. This is a key structure in the resistance of both shear and torsion.

The torque applied to the models far exceeded that of physiological loading and was intended for comparison purposes to experimental data evaluating the torsional strength of the joint. The torque was also applied about the central axis of the femoral neck, which would only be one component of a joint force. Now that the mechanical role of the radial, peripheral mammillary processes has been defined it would be desirable to incorporate the perichondrial in the model and evaluate the effects of growth plate thickness under physiological loading conditions.
REFERENCES


APPENDIX A

The assessment of agreement between the VHX 6000 digital microscope (Keyence® VHX6000, Keyence Corporation of America, Itasca, IL) and the 2010 NextEngine (NE) model 2020I laser scanner (NextEngine Inc., Santa Monica, CA, USA), Hewlett-Packard (HP) Pros S3 white light scanner (Hewlett-Packard Development Company, L.P., Palo Alto, CA) and the Microscribe MX 3D coordinate measurement machine (CMM) (Solution Technologies, Inc., Oella, MD) was made using the mean of the repeated measurements of each instrument.

We calculated limits of agreement (LOA) for which 95% of differences should fall within for there to be considered agreement between the two methods of measurement. The LOA were calculated as the bias ± 1.96 SD (SD = standard deviation). Since we used the mean of repeated measures, the standard deviation was underestimated due to some of the repeated measurement error being removed when averaging the replicated measures. We corrected for this by first calculating the SD for the differences between repeated measures for each method (s₁ and s₂), then the SD for the differences between the means for each method (S_D). The corrected standard deviation (SDc) was calculated as (Bland and Altman, 1986).

\[ SDc = \sqrt{s_D^2 + \left(\frac{1}{4}\right)s_1^2 + \left(\frac{1}{4}\right)s_2^2} = 0.068 \text{ mm} \]

Therefore, the bias ± 1.96 SDc determined the LOA for which 95% of the differences should fall (Bland and Altman, 1986). The bias and LOA however, are just an estimate so a 95% confidence interval (CI) was calculated to determine the precision of the bias and LOA (Fig. 6C shaded area). For the bias, the 95% CI was calculated as

\[ x \pm t \left(\frac{SDc}{n}\right)(\text{bias}\pm0.025 \text{ mm}) \]
For the limits of agreement (LOA) the 95% CI was calculated as

\[ x \pm t(3 \times \text{SDc}/ n)(\text{LOA} \pm 0.021 \text{ mm}), \]

where \( t \) was the t-statistic from the t-table (Giavarina, 2015). We determined there to be agreement between the compared instruments with more than 95% of the differences within the precision of the limits of agreement (LOA).
APPENDIX B

B1.1 Supplement 1 Tables

Table B1 converts roughness profiles Ra into ISO $N$ grades, first to micro-inches, then to $N$ grades.

Table B1: Surface Roughness $N$ Grade Conversions.

<table>
<thead>
<tr>
<th>Ra [µm]</th>
<th>Ra [µin]</th>
<th>ISO N Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>1</td>
<td>$N_1$</td>
</tr>
<tr>
<td>0.05</td>
<td>2</td>
<td>$N_2$</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>$N_3$</td>
</tr>
<tr>
<td>0.2</td>
<td>8</td>
<td>$N_4$</td>
</tr>
<tr>
<td>0.4</td>
<td>16</td>
<td>$N_5$</td>
</tr>
<tr>
<td>0.8</td>
<td>32</td>
<td>$N_6$</td>
</tr>
<tr>
<td>1.6</td>
<td>63</td>
<td>$N_7$</td>
</tr>
<tr>
<td>3.1</td>
<td>125</td>
<td>$N_8$</td>
</tr>
<tr>
<td>6.3</td>
<td>250</td>
<td>$N_9$</td>
</tr>
<tr>
<td>12.5</td>
<td>500</td>
<td>$N_{10}$</td>
</tr>
<tr>
<td>25</td>
<td>1,000</td>
<td>$N_{11}$</td>
</tr>
<tr>
<td>50</td>
<td>2,000</td>
<td>$N_{12}$</td>
</tr>
<tr>
<td>100</td>
<td>4,000</td>
<td>$N_{13}$</td>
</tr>
<tr>
<td>200</td>
<td>8,000</td>
<td>$N_{14}$</td>
</tr>
<tr>
<td>400</td>
<td>16,000</td>
<td>$N_{15}$</td>
</tr>
<tr>
<td>800</td>
<td>32,000</td>
<td>$N_{16}$</td>
</tr>
</tbody>
</table>

Development as a function of age was assessed statistically between the 120- and 900-day old groups. The notation $N_{120 \text{ Days}}$ and $N_{900 \text{ Days}}$ in the supplement tables B2-B11 denote the 120- ($n = 8$) and 900-day old ($n = 6$) groups, respectively, followed by statistical results.
Table B2: Tubercle Height and Average Rate of Change.

<table>
<thead>
<tr>
<th>N120 Days [mm]</th>
<th>N900 Days [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5 ± 1.1</td>
<td>7.9 ± 1.0</td>
<td>2.4</td>
<td>[1.2, 3.6]</td>
<td>t(11.18) = 4.30</td>
<td>P = .001</td>
<td>d = 2.31</td>
</tr>
</tbody>
</table>

Change in the slope of the tubercle from N120 Days to N900 Days (T-test: Two independent samples) (x̅±SD)

<table>
<thead>
<tr>
<th>N120 Days [Deg]</th>
<th>N900 Days [Deg]</th>
<th>Diff [Deg]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.4 ± 4.4</td>
<td>29.7 ± 2.8</td>
<td>7.3</td>
<td>[3.0, 11.6]</td>
<td>t(11.92) = 3.69</td>
<td>P = .003</td>
<td>d = 1.88</td>
</tr>
</tbody>
</table>

Table B3: Epiphyseal cupping in the sagittal plane.

Articular radius from N120 Days to N900 Days (Mann-Whitney U test: Two independent samples) (Median)

<table>
<thead>
<tr>
<th>N120 Days [mm]</th>
<th>N900 Days [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>19.2</td>
<td>5.2</td>
<td>[4.0, 6.3]</td>
<td>U = 0, z = 3.10</td>
<td>P &lt; .001</td>
<td>r = .82</td>
</tr>
</tbody>
</table>

Radius of cranial cupping vs caudal at N120 Days (Wilcoxon singed-rank test: Paired samples) (Median)

<table>
<thead>
<tr>
<th>Cranial [mm]</th>
<th>Caudal [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.5</td>
<td>6.2</td>
<td>5.3</td>
<td>[2.3, 6.6]</td>
<td>T = 0, z = 2.52</td>
<td>P = .008</td>
<td>r = .63</td>
</tr>
</tbody>
</table>

Radius of cranial cupping vs caudal at N900 Days (T-test: Paired samples) (x̅±SD)

<table>
<thead>
<tr>
<th>Cranial [mm]</th>
<th>Caudal [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.1 ± 1.7</td>
<td>10.4 ± 1.3</td>
<td>2.8 ± 1.6</td>
<td>[1.1, 4.4]</td>
<td>t(5) = 4.27</td>
<td>P = .008</td>
<td>d = 1.74</td>
</tr>
</tbody>
</table>

Radius of cranial cupping from N120 Days to N900 Days (Mann-Whitney U test: Two independent samples) (Median)

<table>
<thead>
<tr>
<th>N120 Days [mm]</th>
<th>N900 Days [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>10.4</td>
<td>4.2</td>
<td>[1.1, 5.6]</td>
<td>U = 4, z = 2.58</td>
<td>P = .008</td>
<td>r = .70</td>
</tr>
</tbody>
</table>

Radius of cranial cupping from N120 Days to N900 Days (T-test: Two independent samples) (x̅±SD)

<table>
<thead>
<tr>
<th>N120 Days [mm]</th>
<th>N900 Days [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.4 ± 0.8</td>
<td>13.1 ± 1.7</td>
<td>1.7</td>
<td>[-0.01, 3.5]</td>
<td>t(6.74) = 2.37</td>
<td>P = .05</td>
<td>d = 1.41</td>
</tr>
</tbody>
</table>
### Table B4: Epiphysial cupping in the transverse plane.

| Articular radius from $\text{N}_{120\text{ Days}}$ to $\text{N}_{900\text{ Days}}$ (T-test: Two independent samples) ($\bar{x} \pm \text{SD}$) |  |
|---|---|---|---|---|---|---|
| $\text{N}_{120\text{ Days}}$ [mm] | $\text{N}_{900\text{ Days}}$ [mm] | Diff [mm] | 95%CI | Test Statistic | $P$-value | Effect Size |
| 14.0 ± 0.7 | 18.2 ± 0.8 | 4.2 | [3.4, 5.1] | $t(10.55) = 10.75$ | $P < .001$ | $d = 5.86$ |

Radius of medial cupping vs lateral at $\text{N}_{120\text{ Days}}$ (T-test: Paired samples) ($\bar{x} \pm \text{SD}$)

<table>
<thead>
<tr>
<th>Medial [mm]</th>
<th>Lateral [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>$P$-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0 ± 1.4</td>
<td>4.6 ± 0.7</td>
<td>7.4 ± 1.6</td>
<td>[6.1, 8.7]</td>
<td>$t(7) = 12.97$</td>
<td>$P &lt; .001$</td>
<td>$d = 4.58$</td>
</tr>
</tbody>
</table>

Radius of medial cupping vs lateral at $\text{N}_{900\text{ Days}}$ (T-test: Paired samples) ($\bar{x} \pm \text{SD}$)

<table>
<thead>
<tr>
<th>Medial [mm]</th>
<th>Lateral [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>$P$-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0 ± 0.9</td>
<td>8.8 ± 0.9</td>
<td>1.2 ± 1.1</td>
<td>[0.04, 2.4]</td>
<td>$t(5) = 2.66$</td>
<td>$P = .045$</td>
<td>$d = 1.09$</td>
</tr>
</tbody>
</table>

Radius of medial cupping from $\text{N}_{120\text{ Days}}$ to $\text{N}_{900\text{ Days}}$ (T-test: Two independent samples) ($\bar{x} \pm \text{SD}$)

<table>
<thead>
<tr>
<th>$\text{N}_{120\text{ Days}}$ [mm]</th>
<th>$\text{N}_{900\text{ Days}}$ [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>$P$-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0 ± 1.4</td>
<td>10.0 ± 0.9</td>
<td>2</td>
<td>[0.7, 3.4]</td>
<td>$t(11.79) = 3.37$</td>
<td>$P = .006$</td>
<td>$d = 1.70$</td>
</tr>
</tbody>
</table>

Radius of lateral cupping from $\text{N}_{120\text{ Days}}$ to $\text{N}_{900\text{ Days}}$ (T-test: Two independent samples) ($\bar{x} \pm \text{SD}$)

<table>
<thead>
<tr>
<th>$\text{N}_{120\text{ Days}}$ [mm]</th>
<th>$\text{N}_{900\text{ Days}}$ [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>$P$-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.63 ± 0.1</td>
<td>8.8 ± 0.9</td>
<td>4.1</td>
<td>[3.1, 5.2]</td>
<td>$t(9.03) = 9.33$</td>
<td>$P &lt; .001$</td>
<td>$d = 5.26$</td>
</tr>
</tbody>
</table>

### Table B5: Physial slope angles.

| Transverse plane growth plate slope angles from $\text{N}_{120\text{ Days}}$ to $\text{N}_{900\text{ Days}}$ (T-test: Two Independent Samples) ($\bar{x} \pm \text{SD}$) |  |
|---|---|---|---|---|---|---|
| $\text{N}_{120\text{ Days}}$ [Deg] | $\text{N}_{900\text{ Days}}$ [Deg] | Diff [Deg] | 95%CI | Test Statistic | $P$-value | Effect Size |
| 20.2 ± 3.4 | 8.5 ± 2.3 | 11.7 | [8.4, 15.0] | $t(11.89) = 7.70$ | $P < .001$ | $d = 3.91$ |

| Sagittal plane growth plate slope angles from $\text{N}_{120\text{ Days}}$ to $\text{N}_{900\text{ Days}}$ (T-test: Two Independent Samples) ($\bar{x} \pm \text{SD}$) |  |
|---|---|---|---|---|---|---|
| $\text{N}_{120\text{ Days}}$ [Deg] | $\text{N}_{900\text{ Days}}$ [Deg] | Diff [Deg] | 95%CI | Test Statistic | $P$-value | Effect Size |
| 27.4 ± 2.1 | 21.7 ± 5.5 | 5.7 | [-0.1, 11.5] | $t(6.10) = 2.38$ | $P = .05$ | $d = 1.44$ |

### Table B6: Metaphysial growth plate and epiphysial subchondral bone plate surface area.

| Surface area of the metaphysis vs epiphysis at $\text{N}_{120\text{ Days}}$ (Wilcoxon signed-rank test: Paired samples) (Median) |  |
|---|---|---|---|---|---|---|
| Metaphysis [mm$^2$] | Epiphysis [mm$^2$] | Diff [mm$^2$] | 95%CI | Test Statistic | $P$-value | Effect Size |
| 865.5 | 622.4 | 243.1 | [166.9, 331.8] | $T = 0$, $z = 2.52$ | $P = .008$ | $r = .63$ |

| Surface area of the metaphysis vs epiphysis at $\text{N}_{900\text{ Days}}$ (T-test: Paired samples) ($\bar{x} \pm \text{SD}$) |  |
|---|---|---|---|---|---|
| $\text{N}_{120\text{ Days}}$ [mm$^2$] | $\text{N}_{900\text{ Days}}$ [mm$^2$] | Diff [mm$^2$] | 95%CI | Test Statistic | $P$-value | Effect Size |
| 1385.4 ± 149.8 | 1372.2 ± 143.7 | 13.3 ± 20.2 | [-7.9, 34.5] | $t(5) = 1.61$ | $P = .17$ | $d = 68$ |

| Surface area of the epiphysis from $\text{N}_{120\text{ Days}}$ to $\text{N}_{900\text{ Days}}$ (T-test: Two independent samples) ($\bar{x} \pm \text{SD}$) |  |
|---|---|---|---|---|---|
| $\text{N}_{120\text{ Days}}$ [mm$^2$] | $\text{N}_{900\text{ Days}}$ [mm$^2$] | Diff [mm$^2$] | 95%CI | Test Statistic | $P$-value | Effect Size |
| 619.6 ± 47.6 | 619.6 ± 47.6 | 752.6 | [602.2, 902.9] | $t(5.82) = 12.33$ | $P < .001$ | $d = 7.56$ |

| Surface area of the metaphysis from $\text{N}_{120\text{ Days}}$ to $\text{N}_{900\text{ Days}}$ (T-test: Two independent samples) ($\bar{x} \pm \text{SD}$) |  |
|---|---|---|---|---|---|
| $\text{N}_{120\text{ Days}}$ [mm$^2$] | $\text{N}_{900\text{ Days}}$ [mm$^2$] | Diff [mm$^2$] | 95%CI | Test Statistic | $P$-value | Effect Size |
| 860.7 ± 109.6 | 860.7 ± 109.6 | 524.8 | [360.4, 689.2] | $t(8.81) = 7.25$ | $P < .001$ | $d = 4.10$ |
Table B7: Expansion in the sagittal plane.

<table>
<thead>
<tr>
<th>Cranial vs caudal epiphyseal expansion at N&lt;sub&gt;120 Days&lt;/sub&gt; (Wilcoxon singed-rank test: Paired samples) (Median)</th>
<th>Cranial [mm]</th>
<th>Caudal [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>9</td>
<td>5</td>
<td>[4.0, 5.5]</td>
<td>T = 0, z = 2.52</td>
<td>P = .001</td>
<td>r = .64</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cranial vs caudal epiphyseal expansion at N&lt;sub&gt;900 Days&lt;/sub&gt; (T-test: Paired samples) (x±SD)</th>
<th>Cranial [mm]</th>
<th>Caudal [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.2 ± 1.9</td>
<td>13.7 ± 1.0</td>
<td>7.5 ± 1.0</td>
<td>[6.4, 8.6]</td>
<td>t(5) = 17.52</td>
<td>P &lt; .001</td>
<td>d = 7.15</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cranial expansion from N&lt;sub&gt;120 Days&lt;/sub&gt; to N&lt;sub&gt;900 Days&lt;/sub&gt; (Mann-Whitney U test: Two independent samples) (Median)</th>
<th>N&lt;sub&gt;120 Days&lt;/sub&gt; [mm]</th>
<th>N&lt;sub&gt;900 Days&lt;/sub&gt; [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>20.5</td>
<td>5.0</td>
<td>[5.0, 10.0]</td>
<td>U = 0, z = 3.18</td>
<td>P &lt; .001</td>
<td>r = .84</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medial vs lateral epiphyseal expansion at N&lt;sub&gt;120 Days&lt;/sub&gt; (Wilcoxon singed-rank test: Paired samples) (Median)</th>
<th>Medial [mm]</th>
<th>Lateral [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.5</td>
<td>4</td>
<td>16.5</td>
<td>[16.0, 17.0]</td>
<td>T = 0, z = 2.63</td>
<td>P = .008</td>
<td>r = .66</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medial vs lateral epiphyseal expansion at N&lt;sub&gt;900 Days&lt;/sub&gt; (T-test: Paired samples) (x±SD)</th>
<th>Medial [mm]</th>
<th>Lateral [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.0 ± 1.7</td>
<td>15.2 ± 2.8</td>
<td>5.8 ± 4.3</td>
<td>[1.3, 10.4]</td>
<td>t(5) = 3.31</td>
<td>P &lt; .001</td>
<td>d = 1.35</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medial expansion from N&lt;sub&gt;120 Days&lt;/sub&gt; to N&lt;sub&gt;900 Days&lt;/sub&gt; (T-test: Two independent samples) (x±SD)</th>
<th>N&lt;sub&gt;120 Days&lt;/sub&gt; [mm]</th>
<th>N&lt;sub&gt;900 Days&lt;/sub&gt; [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.6 ± 0.7</td>
<td>21.0 ± 1.7</td>
<td>0.4</td>
<td>[-1.5, 2.1]</td>
<td>t(6.49) = 0.51</td>
<td>P = .63</td>
<td>d = .31</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lateral expansion from N&lt;sub&gt;120 Days&lt;/sub&gt; to N&lt;sub&gt;900 Days&lt;/sub&gt; (Mann-Whitney U test: Two independent samples) (Median)</th>
<th>N&lt;sub&gt;120 Days&lt;/sub&gt; [mm]</th>
<th>N&lt;sub&gt;900 Days&lt;/sub&gt; [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>15</td>
<td>11</td>
<td>[1.0, 15.0]</td>
<td>U = 0, z = 3.23</td>
<td>P = .001</td>
<td>r = .86</td>
<td></td>
</tr>
</tbody>
</table>

Table B8: Expansion in the transverse plane.

<table>
<thead>
<tr>
<th>Medial vs lateral epiphyseal expansion at N&lt;sub&gt;120 Days&lt;/sub&gt; (Wilcoxon singed-rank test: Paired samples) (Median)</th>
<th>Medial [mm]</th>
<th>Lateral [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.5</td>
<td>4</td>
<td>16.5</td>
<td>[16.0, 17.0]</td>
<td>T = 0, z = 2.63</td>
<td>P = .008</td>
<td>r = .66</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medial vs lateral epiphyseal expansion at N&lt;sub&gt;900 Days&lt;/sub&gt; (T-test: Paired samples) (x±SD)</th>
<th>Medial [mm]</th>
<th>Lateral [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.0 ± 1.7</td>
<td>15.2 ± 2.8</td>
<td>5.8 ± 4.3</td>
<td>[1.3, 10.4]</td>
<td>t(5) = 3.31</td>
<td>P &lt; .001</td>
<td>d = 1.35</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medial expansion from N&lt;sub&gt;120 Days&lt;/sub&gt; to N&lt;sub&gt;900 Days&lt;/sub&gt; (T-test: Two independent samples) (x±SD)</th>
<th>N&lt;sub&gt;120 Days&lt;/sub&gt; [mm]</th>
<th>N&lt;sub&gt;900 Days&lt;/sub&gt; [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.6 ± 0.7</td>
<td>21.0 ± 1.7</td>
<td>0.4</td>
<td>[-1.5, 2.1]</td>
<td>t(6.49) = 0.51</td>
<td>P = .63</td>
<td>d = .31</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lateral expansion from N&lt;sub&gt;120 Days&lt;/sub&gt; to N&lt;sub&gt;900 Days&lt;/sub&gt; (Mann-Whitney U test: Two independent samples) (Median)</th>
<th>N&lt;sub&gt;120 Days&lt;/sub&gt; [mm]</th>
<th>N&lt;sub&gt;900 Days&lt;/sub&gt; [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>15</td>
<td>11</td>
<td>[1.0, 15.0]</td>
<td>U = 0, z = 3.23</td>
<td>P = .001</td>
<td>r = .86</td>
<td></td>
</tr>
</tbody>
</table>

Table B9: Elongation of the capital femoral epiphysis.

<table>
<thead>
<tr>
<th>Epiphyseal elongation from N&lt;sub&gt;120 Days&lt;/sub&gt; to N&lt;sub&gt;900 Days&lt;/sub&gt; (Mann-Whitney U test: Two independent samples) (Median)</th>
<th>N&lt;sub&gt;120 Days&lt;/sub&gt; [mm]</th>
<th>N&lt;sub&gt;900 Days&lt;/sub&gt; [mm]</th>
<th>Diff [mm]</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.5</td>
<td>9.5</td>
<td>[5.5, 10.5]</td>
<td>U = 0, z = 3.10</td>
<td>P &lt; .001</td>
<td>r = .83</td>
<td></td>
</tr>
<tr>
<td>Table B10: Developed interfacial area ratios ($S_{dr}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Real vs projected surface area for the central region at N$_{120}$ Days (T-test: Paired samples) (x±SD)

<table>
<thead>
<tr>
<th></th>
<th>Projected</th>
<th>Diff</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real</td>
<td>208.2 ± 28.0</td>
<td>177.0 ± 22.4</td>
<td>31.2 ± 5.9</td>
<td>[26.3, 36.2]</td>
<td>t(7) = 14.94</td>
<td>P &lt; .001</td>
</tr>
</tbody>
</table>

### Real vs projected surface area for the central region at N$_{900}$ Days (T-test: Paired samples) (x±SD)

<table>
<thead>
<tr>
<th></th>
<th>Projected</th>
<th>Diff</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real</td>
<td>359.0 ± 9.4</td>
<td>307.3 ± 10.6</td>
<td>51.7 ± 3.8</td>
<td>[47.7, 55.7]</td>
<td>t(5) = 33.11</td>
<td>P &lt; .001</td>
</tr>
</tbody>
</table>

### Central region $S_{dr}$ from N$_{120}$ Days to N$_{900}$ Days (Mann-Whitney U test: Two independent samples) (Median)

<table>
<thead>
<tr>
<th></th>
<th>Projected</th>
<th>Diff</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_{120}$ Days</td>
<td>0.4</td>
<td>0.4</td>
<td>0</td>
<td>[-0.03, 0.01]</td>
<td>U = 19, z = 0.65</td>
<td>P = .57</td>
</tr>
</tbody>
</table>

### Real vs projected surface area for the peripheral region at N$_{120}$ Days (T-test: Paired samples) (x±SD)

<table>
<thead>
<tr>
<th></th>
<th>Projected</th>
<th>Diff</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real</td>
<td>398.2 ± 38.8</td>
<td>348.5 ± 31.3</td>
<td>49.7 ± 11.2</td>
<td>[40.4, 59.0]</td>
<td>t(7) = 12.57</td>
<td>P &lt; .001</td>
</tr>
</tbody>
</table>

### Real vs projected surface area peripheral region at N$_{120}$ Days (Wilcoxon signed-rank test: Paired samples) (Median)

<table>
<thead>
<tr>
<th></th>
<th>Projected</th>
<th>Diff</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real</td>
<td>1088.8</td>
<td>804.1</td>
<td>284.7</td>
<td>[259.8, 384.7]</td>
<td>T = 0, z = 2.20</td>
<td>P = .03</td>
</tr>
</tbody>
</table>

### Peripheral region $S_{dr}$ from N$_{120}$ Days to N$_{900}$ Days (T-test: Two independent samples) (x±SD)

<table>
<thead>
<tr>
<th></th>
<th>Projected</th>
<th>Diff</th>
<th>95%CI</th>
<th>Test Statistic</th>
<th>P-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_{120}$ Days</td>
<td>1.1 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>0.3</td>
<td>[0.2, 0.3]</td>
<td>t(7.31) = 11.55</td>
<td>P &lt; .001</td>
</tr>
</tbody>
</table>
Table B11: Average amplitude and periodicity of central and peripheral mammillary processes.

<table>
<thead>
<tr>
<th></th>
<th>Average height for regions defined by ( r ) vs regions defined by ( 2/3r ) at ( N_{120} ) Days (T-test: Paired samples) (( \bar{x} \pm SD ))</th>
<th></th>
<th>Average height for regions defined by ( r ) vs regions defined by ( 2/3r ) at ( N_{900} ) Days (T-test: Paired samples) (( \bar{x} \pm SD ))</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( r ) [mm]</td>
<td>0.16 ± 0.06</td>
<td>0.44 ± 0.03</td>
<td>( 2/3r ) [mm]</td>
<td>0.10 ± 0.06</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>Diff [mm]</td>
<td>0.06 ± 0.06</td>
<td>0.27 ± 0.06</td>
<td>95%CI</td>
<td>[0.01, 0.11]</td>
<td>[0.20, 0.33]</td>
</tr>
<tr>
<td>Test Statistic</td>
<td>( t(7) = 2.61 )</td>
<td>( t(5) = 10.89 )</td>
<td>P-value</td>
<td>( P = .03 )</td>
<td>( P &lt; .001 )</td>
</tr>
<tr>
<td>Effect Size</td>
<td>( d = .92 )</td>
<td>( d = 4.45 )</td>
<td>Effect Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diff [mm]</td>
<td>0.06 ± 0.06</td>
<td>0.27 ± 0.06</td>
<td>95%CI</td>
<td>[0.01, 0.11]</td>
<td>[0.20, 0.33]</td>
</tr>
<tr>
<td>Test Statistic</td>
<td>( t(7) = 2.61 )</td>
<td>( t(5) = 10.89 )</td>
<td>P-value</td>
<td>( P = .03 )</td>
<td>( P &lt; .001 )</td>
</tr>
<tr>
<td>Effect Size</td>
<td>( d = .92 )</td>
<td>( d = 4.45 )</td>
<td>Effect Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age [Days]</td>
<td>95%CI</td>
<td>Test Statistic</td>
<td>P-value</td>
<td>Effect Size</td>
<td></td>
</tr>
<tr>
<td>( t(7) = 2.61 )</td>
<td>( P = .03 )</td>
<td>( d = .92 )</td>
<td>Effect Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t(5) = 10.89 )</td>
<td>( P &lt; .001 )</td>
<td>( d = 4.45 )</td>
<td>Effect Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodicity of regions defined by ( r ) vs ( 2/3r ) at ( N_{120} ) Days (T-test: Paired samples) (( \bar{x} \pm SD ))</td>
<td></td>
<td></td>
<td>Periodicity of regions defined by ( r ) vs ( 2/3r ) at ( N_{900} ) Days (T-test: Paired Samples) (( \bar{x} \pm SD ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r ) [Deg]</td>
<td>22.2 ± 4.4</td>
<td>35.2 ± 9.2</td>
<td>Diff [Deg]</td>
<td>13.0 ± 10.2</td>
<td>1.36 ± 10.2</td>
</tr>
<tr>
<td>Diff [Deg]</td>
<td>13.0 ± 10.2</td>
<td>1.36 ± 10.2</td>
<td>95%CI</td>
<td>[4.5, 21.6]</td>
<td>[4.5, 21.6]</td>
</tr>
<tr>
<td>Test Statistic</td>
<td>( t(7) = 3.61 )</td>
<td>( t(5) = 2.56 )</td>
<td>P-value</td>
<td>( P = .009 )</td>
<td>( P = .048 )</td>
</tr>
<tr>
<td>Effect Size</td>
<td>( d = 1.28 )</td>
<td>( d = 0.35 )</td>
<td>Effect Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodicity of regions defined by ( r ) vs ( 2/3r ) at ( N_{120} ) Days (T-test: Paired Samples) (( \bar{x} \pm SD ))</td>
<td></td>
<td></td>
<td>Periodicity of the central region (( 2/3r )) from ( N_{120} ) Days to ( N_{900} ) Days (T-test: Two Independent Samples) (( \bar{x} \pm SD ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r ) [Deg]</td>
<td>35.2 ± 9.2</td>
<td>32.0 ± 11.9</td>
<td>Diff [Deg]</td>
<td>11.7 ± 10.9</td>
<td>3.16 ± 10.9</td>
</tr>
<tr>
<td>Diff [Deg]</td>
<td>3.16 ± 10.9</td>
<td>11.7 ± 10.9</td>
<td>95%CI</td>
<td>[0.13, 23.2]</td>
<td>[0.13, 23.2]</td>
</tr>
<tr>
<td>Test Statistic</td>
<td>( t(7.3) = 0.54 )</td>
<td>( t(5) = 2.60 )</td>
<td>P-value</td>
<td>( P = .60 )</td>
<td>( P = .048 )</td>
</tr>
<tr>
<td>Effect Size</td>
<td>( d = 0.30 )</td>
<td>( d = 0.35 )</td>
<td>Effect Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodicity of the peripheral region (( r )) from ( N_{120} ) Days to ( N_{900} ) Days (T-test: Two Independent Samples) (( \bar{x} \pm SD ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r ) [Deg]</td>
<td>22.2 ± 4.4</td>
<td>20.4 ± 5.9</td>
<td>Diff [Deg]</td>
<td>1.8 ± 8.3</td>
<td>1.8 ± 8.3</td>
</tr>
<tr>
<td>Diff [Deg]</td>
<td>1.8 ± 8.3</td>
<td>1.8 ± 8.3</td>
<td>95%CI</td>
<td>[4.7, 8.3]</td>
<td>[4.7, 8.3]</td>
</tr>
<tr>
<td>Test Statistic</td>
<td>( t(8.98) = 0.62 )</td>
<td>( t(5) = 2.60 )</td>
<td>P-value</td>
<td>( P = .55 )</td>
<td>( P = .048 )</td>
</tr>
<tr>
<td>Effect Size</td>
<td>( d = 0.35 )</td>
<td>( d = 0.35 )</td>
<td>Effect Size</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table B12 shows surface roughness conversions and the \( N \) grade that applied to the central and peripheral regions for ages 20 to 900-days old.

Table B12: Surface roughness converted to \( N \) grades (\( \bar{x} \pm SD \)).

<table>
<thead>
<tr>
<th>Age [Days]</th>
<th>Central Region Ra [mm]</th>
<th>ISO N Grade</th>
<th>Central Region Ra [mm]</th>
<th>ISO N Grade</th>
<th>Peripheral Region Ra [mm]</th>
<th>ISO N Grade</th>
<th>Peripheral Region Ra [mm]</th>
<th>ISO N Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.05</td>
<td>11</td>
<td>0.07</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>0.05</td>
<td>11</td>
<td>0.08</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120_{avg}</td>
<td>0.10 ± 0.02</td>
<td>13</td>
<td>0.16 ± 0.06</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>365</td>
<td>0.18</td>
<td>13</td>
<td>0.35</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>540</td>
<td>0.15</td>
<td>13</td>
<td>0.34</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>900_{avg}</td>
<td>0.17 ± 0.06</td>
<td>14</td>
<td>0.44 ± 0.03</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
B1.2 Supplement 1 MATLAB script

The following MATLAB code calculated surface roughness parameters $Ra$ and $Rz$, surface waviness parameters $Wa$ and $Wz$, average amplitude, and periodicity from the unwrapped profiles (900-day old left epiphysis of animal 1 shown) as described in chapter 3 and 4. However, surface waviness and roughness parameter and $Rz$ were not reported. Black and purple lettering in the script indicates operators and green lettering is annotation. A percent symbol is used to either turn off parts of the code that are not in use, turning the operators into annotation or used after an operator to begin annotation.

A Quantitative Qualitative Analysis of Femoral Head Mammillary Processes

Ron Perrone

Boolean Operations

Boolean operations were performed on the epiphysis of porcine femoral heads, which created cylinders. The perimeters of the cylinders were "unwrapped" and projected onto a plane, which gave a descriptive characteristic of the topography in particular regions of the epiphysis. These curves were analyzed with this algorithm to determine the number of peaks and their locations by degrees of rotation from a central axis. Each curve was an array of 2000 data points over a 360 degree perimeter of a cylinder $r$ and $2/3r$.

Algorithm Purpose

To analyze the profile curves using surface texture techniques, namely $Ra$, $Rz$, $Wa$, $Wz$ values, which are commonly used in metrology analysis.

The Profile (Surface Texture)

Surface Roughness.

$% Ra = CLA = (A1 + A2 + ... + An)/L$
Ra is the arithmetic mean of the absolute ordinate values z(x) within a sampling length

\[ \frac{1}{L} \int_{0}^{L} |z(x)| \, dx \]

\[ R_z = \frac{[(P_1 + P_2 + \ldots + P_n) + (V_1 + V_2 + \ldots + V_n)]}{nS} \]

Rz is the summation of the highest peak in a sample length plus the absolute lowest valley in the sample length, divided by the number of sample lengths. \([P_1 + P_2 + \ldots + P_n] + [V_1 + V_2 + \ldots + V_n] \)/nS where nS is the number of sampling lengths.

Start

Profiles

Profiles are considered a summation of sine and cosine waves.

A best fit polynomial of nth order was used to determine up to three levels of underlying wave forms that make up the profile.

Primary - nth order best fit polynomial based on observed topography.

Secondary - nth order best fit polynomial with least error.

Tertiary - Primary wave with secondary waveform removed.

    clear \% Clears workspace.

    clc \% Clears command window.

Data

Imports the array data (x and y) to the workspace by loading Excel data that has been imported and converted to *.mat format.

    load('UM1_272L_Curve_r') \% y data.

    load('degrees'); \% x axis data = 0:0.1802:360.2198.
Starts at 0 and goes to 360.2198 degrees in steps of 0.1802.

\[ x = \text{degrees}; \% \text{Quadrants in degrees.} \]

\[ y = \text{UM1}_272L\_\text{Curve}_r; \% \text{Cylinder profile data.} \]

\[ y = \text{flipud}(y); \% \text{Flips the array reversing the order of the array.} \]

===================================================================

**Remove Linear & Nonlinear Trends**

% y = detrend(y); % Remove linear trend.

Remove nonlinear trends.

% p = polyfit(x,y,n) \%returns the coefficients for a polynomial p(x) of degree n that is a best fit (in a least-squares sense) for the data in y.

The coefficients in p are in descending powers, and the length of p is:

% n+1 p(x)=p_1x^n+p_2x^{n-1}+...+p_nx+p_{n+1}.

% [p,S,mu] = polyfit(x,y,n) \% Returns mu, which is a two-element vector with centering and scaling values. mu(1) is mean(x), and mu(2) is std(x). Using these values, polyfit centers x at zero and scales it to have unit.

% standard deviation x = (x-xbar)/?

This centering and scaling transformation improves the numerical properties of both the polynomial and the fitting algorithm. A best-fit polynomial is fit to the data and subtracted from the data to remove non-linear trends. For the purposes of this data, nonlinear trends would include trends that would be categorized as primary or secondary, which are the two levels above the level of interest which is surface roughness (Ra and Rz) and waviness (Wa and Wz)

====================================================================
"Form" Polynomial

Sets a polynomial of nth order $opol = n$ to the profile, which is then subtracted from the profile.

$opol1 = 6$; % Sets order of the polynomial.

$[p,s,mu] = \text{polyfit}(x,y,opol1)$; % Polyfit function.

$f_y1 = \text{polyval}(p,x,[],mu)$; % Returns values of the best fit polynomial.

To see how good the fit is, evaluate the polynomial at the data points and generate a table showing the data, fit, and error.

$T1 = \text{table}(x,y,f_y1,y-f_y1,'\text{VariableNames}',\{'X','Y','Fit','FitError'\})$;

$y1 = y - f_y1$; % Subtracts the best fit polynomial from the data.

Returns the average fit error from table $T$ for the fit polynomial of order "opol".

$\text{average\_fit\_error1} = \text{mean}(y1)$

"Waviness" Polynomial

Sets a polynomial of nth order $opol = n$ to the profile, which is then subtracted from the profile.

$opol2 = 15$; % Sets order of the polynomial.

$[p,s,mu] = \text{polyfit}(x,y,opol2)$; % Polyfit function.

$f_y2 = \text{polyval}(p,x,[],mu)$; % Returns values of the best fit polynomial.

To see how good the fit is, evaluate the polynomial at the data points and generate a table showing the data, fit, and error.

$T2 = \text{table}(x,y,f_y2,y-f_y2,'\text{VariableNames}',\{'X','Y','Fit','FitError'\})$;

$y2 = y - f_y2$; % Subtracts the best fit polynomial from the data.

Returns the average fit error for the fit polynomial of order "opol".

$\text{average\_fit\_error2} = \text{mean}(y2)$
**Tertiary (Roughness)**

\[ yt = y_2; \]  
% Is the profile, less the secondary polynomial. The secondary polynomial is a best fit polynomial to the profile.

**Secondary (Waviness)**

Primary curve less the secondary polynomial and tertiary curve.

\[ ys = y - y_2; \]  
% Profile less the tertiary curve.

\[ ys = ys - f_y1; \]  
% ys less primary polynomial.

**Plot Curves**

Plot profile, Form polynomial, Waviness polynomial Secondary & tertiary curves.

Profile = Starting perimeter of cylinder unwrapped/projected on plane.

Form polynomial = Best fit polynomial for overall form.

Waviness polynomial = Best fit polynomial for form and waviness.

Secondary = waviness extracted from profile.

Tertiary = roughness extracted from profile.

**Figure(1)**

Set/control figure 1 for plot.

\[
\text{figure('units','normalized','position',[.5 .5 .5 .5]);}
\]

Plot profile, polynomials, secondary and tertiary curves.

\[
\text{plot(x,y+23,'k', } \ x,f_y1+16,'--k', \ x,f_y2+10,'-.k', \ x,ys+7,'k'; ...}
\]
\[
\text{x,yt,'k','LineWidth',1.2);}
\]

\[
\text{legend('Profile (top)','Form Poly.','Wav. Poly.', 'Secondary',...}
\]
\[
'\text{Tertiary','Location','southoutside','Orientation','horizontal');}
\]
set(0,'DefaultLegendAutoUpdate','off') % This keeps the legend from adding data to it later in the script.

text(364, y(363)+16, ['n_{p}=' num2str(opol1)],'fontsize', 15);
text(364, y(363)+10, ['n_{p}=' num2str(opol2)],'fontsize', 15);

**Plot Characteristics**

axis('auto') % Makes the graph fill the figure.

grid on % Turns the grid on (major not minor).

box on % Tuns on the box that outlines the plot.

Sets up the (fig) for uniform changes in font, font size, color etc...

    fig = gca;

    set(fig,'fontname','Bodoni 72','fontsize',20,'LineWidth',1.5);

Set y limits to auto. Set x limits and x tick marks.

    ylim([-5, 37]); % Sets y axis to fit the curve between min and max value.

    xlim([0, 360]); % Sets the x axis limits from 0 to 360.

    set (fig, 'XTick', [0, 45, 90, 135, 180, 225, 270, 315, 360])

Set fig for presentation appearance.

    x_plot = xlabel(...

    '$Location\hspace{1mm}On\hspace{1mm}Cylinder\hspace{1mm}Perimiter\hspace{1mm}{[\text{degrees}]}$'); % x label.

    y_plot = ylabel(...

    '$Height\hspace{1mm}{{[\text{mm}]}}$'); % y label.

    t_plot = title(...
'\$900\hspace{1mm}\text{Day}\hspace{1mm}(1L)\hspace{1mm}\text{Cylinder}\hspace{1mm}\text{Profile}\hspace{1mm}\text{Deconvolution}\$'); % Title.

Interpretation of text characters for presentation.

    hold on

    set(t_plot,'Interpreter','Latex');

    set(x_plot,'Interpreter','Latex');

    set(y_plot,'Interpreter','Latex');

Define Quadrant Boundaries

    x1 = 90;
    x2 = 180;
    x3 = 270;
    x4 = 360;

    y1 = get(gca,'ylim');

    plot([x1 x1],y1,'k','LineWidth',1) % Caudal Lateral quadrant(0:90).

    plot([x2 x2],y1,'k','LineWidth',1) % Caudal Medial quadrant(90:180).

    plot([x3 x3],y1,'k','LineWidth',1) % Cranial Medial quadrant(180:270).

    plot([x4 x4],y1,'k','LineWidth',1) % Cranial Lateral quadrant(270:360).

Color Intervals With Negligible Peak/Valleys

% Blue     [0.7,0.8,1.0]
% Yellow   [1.0,1.0,0.7]
% Purple   [0.9,0.7,1.0]
% Green    [0.7,1.0,0.7]
Interval 15 (x >= 0) & (x <= 90) colored light green.

\[ y_p = \text{get(gca,'YLim')}; \]
\[ \text{patch('XData',[0 0 90 90],'YData',[y_p fliplr(y_p)],...} \]
\[ 'FaceColor',[0.7,1.0,0.7],'FaceAlpha',0.3) \]

Interval 13 (x >= 90) & (x <= 180) colored light purple.

\[ y_p = \text{get(gca,'YLim')}; \]
\[ \text{patch('XData',[90 90 180 180],'YData',[y_p fliplr(y_p)],...} \]
\[ 'FaceColor',[0.9,0.7,1.0],'FaceAlpha',0.3) \]

Interval 15 (x >= 180) & (x <= 270) colored light yellow.

\[ y_p = \text{get(gca,'YLim')}; \]
\[ \text{patch('XData',[180 180 270 270],'YData',[y_p fliplr(y_p)],...} \]
\[ 'FaceColor',[1.0,1.0,0.7],'FaceAlpha',0.3) \]

Interval 13 (x >= 270) & (x <= 360) colored light blue.

\[ y_p = \text{get(gca,'YLim')}; \]
\[ \text{patch('XData',[270 270 360 360],'YData',[y_p fliplr(y_p)],...} \]
\[ 'FaceColor',[0.7,0.8,1.0],'FaceAlpha',0.3) \]

Secondary (Wa) Analysis.

Figure (2)

Set/control figure 2 for plot.

\[ \text{figure('units','normalized','position',[.5 .5 .5 .5]);} \]
Convert Degrees to Distance [mm]

% $r = \frac{(103.4971/\pi)}{2}$; % Radius of cylinder $r$ [mm]. Use if "r" is not known

$r = 15; 

circ = (2*\pi*r);$ % Circumference of cylinder $r$ [mm].

quad = circ/4; % Length of 1 quadrant.

x = 0:circ/1999:circ; % x vector [mm].

Normalize & Shift profile

max_x = max(ys); % Find maximum in array.

$y = \frac{ys}{max_x};$ % Normalize height to max.

min_x = min(ys); % Find minimum in array.

$y = ys - min_x;$ % Shift profile (lowest value in array = 0).

Plot Characteristics

axis('auto') % Makes the graph fill the figure.

% grid on % Turns the grid on (major not minor).

box on % Tuns on the box that outlines the plot.

Sets up the (fig) for uniform changes in font, font size, color etc...

fig3 = gca;

set(fig3,'fontname','Bodoni 72','fontsize',20,'LineWidth',1.5); % Set y limits to auto. Set x limits and x tick marks.

ylim([-2,2]); % Sets the y axis to fit the curve between the min and max value.

xlim([0, circ]); % Sets the x axis limits from 0 to circumference.
set(fig3,'XTick',[0,quad,2*quad,3*quad,circ]) \% Sets x axis tick marks at the specified numbers.

Set fig for presentation appearance.

x_plot = xlabel(...
'\$Circumference\hspace{1mm}of\hspace{1mm}Cylinder\hspace{1mm}{{[mm]}}$');

y_plot = ylabel(...
'\$Height\hspace{1mm}{{[mm]}}$');

t_plot = title(...
'\$900\hspace{1mm}Day\hspace{1mm}(1L)\hspace{1mm}Cylinder\hspace{1mm}r\hspace{1mm}Waviness$');

Interpretation of text characters for presentation.

set(t_plot,'Interpreter','Latex');

set(x_plot,'Interpreter','Latex');

set(y_plot,'Interpreter','Latex');

hold on

Find Center Line Average

CL = 1.028696; \% Dividing line such that area above = area below.

level = CL;\% Set the centerline as the level for area shading.

Highlight Region of Interest\% Index section.

indx = (x >= 0) & (x <= circ); \% Index section.

plot(x(indx),y(indx)-CL,'k','linewidth',2)

Shade Area Under Curve Above & Below Center Line.

Shade the area of the curve in the indexed section grey [.7 .7 .7] above the level CL.
Shifts all curves to set CL as the zero

area(x(indx), max(y(indx)-CL, level-CL),level-CL,...

'EdgeColor', 'none', 'FaceColor',[.7 .7 .7],'showbaseline', 'on');

Shade the area of the curve in the indexed section dark grey below the level CL.

Shifts all curves to set CL as the zero.

area(x(indx), min(y(indx)-CL, level-CL),level-CL,...

'EdgeColor', 'none', 'FaceColor',[.9 .9 .9],'showbaseline', 'on');

Define Quadrant Boundaries

x1 = quad;
x2 = 2*quad;
x3 = 3*quad;
x4 = circ;
y1 = get(gca, 'ylim');

plot ([x1 x1],y1,'k','LineWidth',1) % Caudal Lateral quadrant(0:90).
plot ([x2 x2],y1,'k','LineWidth',1) % Caudal Medial quadrant(90:180).
plot ([x3 x3],y1,'k','LineWidth',1) % Cranial Medial quadrant(180:270).
plot ([x4 x4],y1,'k','LineWidth',1) % Cranial Lateral quadrant(270:360).

Plot Center Line.

hold on
x1 = 0;
x2 = 4*quad;
y1 = CL-CL;
plot ([x1 x2],[y1 y1],'-r','linewidth',1.5);
Calculate Area

Area under the curve above and below centerline (shaded regions).

As\_above = -\text{trapz}(x(\text{indx}), \max(y(\text{indx}), \text{level}) - \text{level})

As\_below = -\text{trapz}(x(\text{indx}), \min(y(\text{indx}), \text{level}) - \text{level})

Calculate Ra Surface Roughness Value

As = |As\_above| + |As\_below|

CLA = As/(\text{circ})

Wa = CLA

Count Zero Crossings

1 count = the interval where the curve crosses the zero line in the positive direction and returns through the zero line in the negative direction.

threshold = y - CL > 0; % Set threshold to y data with CL set to zero

y is the data and number of crossing (NOC) is the number of counts that occur in the curve.

[y, NOC] = \text{bwlabel}(threshold);

The curves are cylindrical; therefore, the first "count" and the last "count" are two halves of one count.

counts\_Wa = NOC-1 % The count to the far right excluded;

Reset y data back to yt (tertiary curve) at the level prior to the shift of CL to be placed as the zero line.

y = ys + CL;

====================================================================
Secondary (Wz) Analysis

Sampling length [mm]

sl = quad;
x1 = 0;
x2 = quad;
x3 = quad + sl;
x4 = quad + 2*sl;
y1 = get(gca, 'ylim');
plot ([x1 x1],y1,':','Color',[0,0.5,0],'linewidth',1.8);
plot ([x2 x2],y1,':','Color',[0,0.5,0],'linewidth',1.8);
plot ([x3 x3],y1,':','Color',[0,0.5,0],'linewidth',1.8);
plot ([x4 x4],y1,':','Color',[0,0.5,0],'linewidth',1.8);

Sampling Lengths. (To be used when it applies to the curve in the interval)

% plot(x(indx),y(indx),'k','linewidth',.05)
% max_x = max(y(indx)); % Find max for indexed section.
% min_x = min(y(indx)); % Find min for indexed section.

For when the peak is not the highest value in the data.

% plot(x(indx),y(indx),'k','linewidth',.05)
% [pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
% max_x = max(pks); % Find max peak for indexed section.
% min_x = min(y(indx)); % Find min for indexed section.
For when the valley is not the lowest value in the data.

```matlab
%     plot(x(indx),y(indx),'k','linewidth',.05)
%     max_x = max(y(indx)); % Find max for indexed section.
%     [vlys,min_locs] = findpeaks(-y(indx));% Find peaks indexed section.
%     min_x = -max(vlys); % Find max valley for indexed section.
```

**Highlight Sampling Length 1**

Sampling length 1 (Each length is 24.8186 mm).

```matlab
indx = (x >= 0) & (x <= quad); % Index section.

y(indx) = y(indx) - CL; % Shift curve so CL is centered at zero.
```

Plot Rmin.

```matlab
s1 = 0;
```

**Color Intervals Without Peak and Valleys**

Interval 15 (x >= sl*14) & (x <= sl*15) colored light blue.

If there is not a peak and valley in the same interval it is omitted.

```matlab
y_p = get(gca,'YLim');

patch('XData',[0 0 sl sl],'YData',[y_p fliplr(y_p)],...
      'FaceColor',[.8,.8,.8],'FaceAlpha',0.3)
```

**Highlight Sampling Length 2**

```matlab
indx = (x >= quad) & (x <= quad + sl); % Index section.
```

Shift curve so CL is centered at zero.

```matlab
y(indx) = y(indx) - CL;
```

```matlab
plot(x(indx),y(indx),'k','linewidth',1.5)
```
For when the peak is not the highest value in the data.

```matlab
plot(x(indx),y(indx),'k','linewidth',1.5)
[pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
max_x = max(pks); % Find max peak for indexed section.
min_x = min(y(indx)); % Find min for indexed section.
```

For when the valley is not the lowest value in the data.

```matlab
plot(x(indx),y(indx),'k','linewidth',1.5)
max_x = max(y(indx)); % Find max for indexed section.
[vlys,min_locs] = findpeaks(-y(indx)); % Find peaks indexed section.
min_x = -max(vlys); % Find max valley for indexed section
```

Plot Rmax.

```matlab
hold on
x1 = quad;
x2 = quad + sl;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
```

Plot Rmin.

```matlab
hold on
x1 = quad;
x2 = quad + sl;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
s2 = max_x - min_x;
```
Highlight Sampling Length 3

\[ \text{indx} = (x \geq \text{quad} + \text{sl}) \& (x \leq \text{quad} + \text{sl}\times2); \% \text{ Index section.} \]

Shift curve so CL is centered at zero.

\[ \text{y(indx)} = \text{y(indx)} - \text{CL}; \]
\[ \text{plot(x(indx),y(indx),'k','linewidth',1.5)} \]
\[ \text{max}_x = \text{max(y(indx))}; \% \text{ Find max for indexed section.} \]
\[ \text{min}_x = \text{min(y(indx))}; \% \text{ Find min for indexed section} \]

Plot Rmax.

\[ \text{hold on} \]
\[ \text{x1} = \text{quad} + \text{sl}; \]
\[ \text{x2} = \text{quad} + \text{sl}\times2; \]
\[ \text{y1} = \text{max}_x; \]
\[ \text{plot ([x1 x2],[y1 y1],'k','linewidth',0.5)} \]

Plot Rmin.

\[ \text{hold on} \]
\[ \text{x1} = \text{quad} + \text{sl}; \]
\[ \text{x2} = \text{quad} + \text{sl}\times2; \]
\[ \text{y1} = \text{min}_x; \]
\[ \text{plot ([x1 x2],[y1 y1],'k','linewidth',0.5)} \]
\[ s3 = \text{max}_x - \text{min}_x; \]

Highlight Sampling Length 4

\[ \text{indx} = (x \geq \text{quad} + 2\times\text{sl}) \& (x \leq \text{quad} + \text{sl}\times4); \% \text{ Index section.} \]
Shift curve so CL is centered at zero.

\[ y(\text{indx}) = y(\text{indx}) - \text{CL}; \]

plot(x(\text{indx}),y(\text{indx}),'k','linewidth',1.5)

For when the peak is not the highest value in the data.

plot(x(\text{indx}),y(\text{indx}),'k','linewidth',1.5)

[pks,locs] = findpeaks(y(\text{indx})); % Find peaks for indexed section.

max_x = max(pks); % Find max peak for indexed section.

min_x = min(y(\text{indx})); % Find min for indexed section.

Plot Rmax.

hold on

x1 = quad + 2*sl;

x2 = quad + sl*4;

y1 = max_x;

plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

Plot Rmin.

hold on

x1 = quad + 2*sl;

x2 = quad + sl*4;

y1 = min_x;

plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

s4 = max_x - min_x;
**Calculate Wz**

Wz is the average peak to valley height

\[
Wz = \frac{(s1+s2+s3+s4)}{3};
\]

**Peaks.**

\[
\text{indx} = (x \geq 0) \& (x \leq \text{circ}); \quad \% \text{ Index section.}
\]

Returns peaks [pks] and their location [loc] indices along the 1000 point array (y), which is the projected cylinder curve. A peak is defined as any point on the curve that has a lower value in both adjacent indices.

\[
[pks, \text{locs}] = \text{findpeaks}(y(\text{indx}));
\]

\[
y_{\text{inv}} = \max(y) - y; \quad \% \text{ Invert y data to enable find peaks to find valleys.}
\]

**Valleys.**

Returns valleys [vlys] and their location [min_loc] indices along the 1000 point array (y), which is the projected cylinder curve. A valley is defined as any point on the curve that has a higher value in both adjacent indices.

\[
[vlys, \text{min_locs}] = \text{findpeaks}(y_{\text{inv}});
\]

\[
vlys = \max(vlys) - vlys; \quad \% \text{ Invert data so markers will be placed in correct location.}
\]

**Plot markers over peaks**

\[
\% \quad \text{plot}(x(\text{locs}),pks+0.07,'v','\text{markersize}',6,...
\]

\[
\% \quad '\text{markerfacecolor}',k','\text{linewidth}',1);
\]

**Plot Rmax (highest peak).**

\[
\text{Rmax} = \max(pks);
\]

\[
p = \text{find}(y == \text{Rmax});
\]

\[
xRmax = x(p);
\]
Plot Rv_{max} (lowest valley).

[vlys, min_locs] = findpeaks(-y(indx)); % Find peaks for indexed section.

Rmin = -max(vlys); % Find max valley for indexed section.

v = find(y == Rmin);

xRmin = x(v);

txt2 = ['Rv_{max} = ', num2str(Rmin, '%.2f')];

text(xRmin-3.0, Rmin-0.09, txt2, 'HorizontalAlignment', 'left', 'fontname', ...
' Bodoni 72', 'fontsize', 15, 'LineWidth', 1.5);

Averaged Undulations (Secondary)

x = 0:0.1:circ; % Distance evaluated.

Wave equation y = A*cos((2*pi)/lambda*x) or y = A*sin((2*pi)/lambda*x)
% A = amplitude.

% lambda = one wavelength cycle per distance.

\[ A_{Wa} = Wz/2; \] % Rz is the peak to valley distance average. Rz/2 = amplitude.

\[ \text{lambda}_{Wa} = (\text{circ})/\text{counts}_{Wa}; \] % Determine lambda (wavelength).

\[ y_{Wa} = A_{Wa}\cos((2\pi/\text{lambda}_{Wa})x); \] % Equation.

_Tertiary (Ra) Analysis_

**Figure(3)**

Set/control figure 5 for plot.

\[
\text{figure('units','normalized','position',[.5 .5 .5 .5]);}
\]

**Convert Degrees to Distance [mm]**

\[ r = (70.3277/pi)/2; \] % Radius of cylinder r [mm]. Use if r is unknown.

\[ r = 15; \]

\[ \text{circ} = (2\pi*r); \] % Circumference of cylinder r [mm].

\[ \text{quad} = \text{circ}/4; \] % Length of 1 quadrant.

\[ x = 0:\text{circ}/1999:\text{circ}; \] % x vector [mm].

Normalize & Shift profile

\[ \text{max}_x = \text{max}(yt); \] % Find maximum in array.

\[ y = yt/\text{max}_x; \] % Normalize height to max.

\[ \text{min}_x = \text{min}(ys); \] % Find minimum in array.

\[ y = yt - \text{min}_x; \] % Shift profile (lowest value in array = 0).
**Plot Characteristics**

- `axis('auto')` % Makes the graph fill the figure.
- `grid on` % Turns the grid on (major not minor).
- `box on` % Tuns on the box that outlines the plot

Sets up the (fig) for uniform changes in font, font size, color etc...

```
fig5 = gca;
set(fig5,'fontname','Bodoni 72','fontsize',20,'LineWidth',1.5);
```

Set y limits to auto. Set x limits and x tick marks.

```
ylim([-2, 2]); % Sets the y axis to fit the curve between the min and max value.
xlim([0, circ]); % Sets the x axis limits from 0 to circumference.
```

Sets x axis tick marks at the specified numbers.

```
set (fig5, 'XTick', [0, quad, 2*quad, 3*quad, circ])
```

Set fig for presentation appearance.

```
x_plot = xlabel(...
'$Circumference$\hspace{1mm}of\hspace{1mm}Cylinder\hspace{1mm}{{[mm]}}$);
```

```
y_plot = ylabel(...
'$Height$\hspace{1mm}{{[mm]}}$);
```

```
t_plot = title(...
'$900$\hspace{1mm}Day\hspace{1mm}(1L)\hspace{1mm}Cylinder\hspace{1mm}r\hspace{1mm}{{[mm]}}$\hspace{1mm}Roughness$');
```

Interpretation of text characters for presentation.

```
set(t_plot,'Interpreter','Latex');
```

```
set(x_plot,'Interpreter','Latex');
```
set(y_plot,'Interpreter','Latex');

hold on

**Find Center Line Average**

CL = 1.028884; % Dividing line such that area above = area below.

level = CL; % set the centerline as the level for area shading.

**Highlight Region of Interest**

indx = (x >= 0) & (x <= circ); % Index section.

plot(x(indx),y(indx) - CL,'k','linewidth',2)

**Shade Area Under Curve Above & Below Center Line**

Shade the area of the curve in the indexed section grey [.7 .7 .7] above the level CL.

Shifts all curves to set CL as the zero.

area(x(indx), max(y(indx) - CL, level - CL), level - CL,...

    'EdgeColor', 'none', 'FaceColor', [.7 .7 .7],'showbaseline', 'on');

Shade the area of the curve in the indexed section dark grey below the level CL.

Shifts all curves to set CL as the zero.

area(x(indx), min(y(indx) - CL, level - CL), level - CL,...

    'EdgeColor', 'none', 'FaceColor', [.9 .9 .9],'showbaseline', 'on');

**Define Quadrant Boundaries**

x1 = quad;

x2 = 2*quad;

x3 = 3*quad;

x4 = circ;

y1 = get(gca, 'ylim');
plot ([x1 x1],y1,'k','LineWidth',1) %Caudal Lateral quadrant(0:90).
plot ([x2 x2],y1,'k','LineWidth',1) %Caudal Medial quadrant(90:180).
plot ([x3 x3],y1,'k','LineWidth',1) %Cranial Medial quadrant(180:270).
plot ([x4 x4],y1,'k','LineWidth',1) %Cranial Lateral quadrant(270:360).

Plot Center Line.

hold on
x1 = 0;
x2 = 4*quad;
y1 = CL-CL;
plot ([x1 x2],[y1 y1],'-r','linewidth',1.5);

Calculate Area

Area under the curve above and below centerline (shaded regions).

\[ \text{At}_{\text{above}} = -\text{trapz}(x(\text{indx}), \max(y(\text{indx}), \text{level}) - \text{level}) \]

\[ \text{At}_{\text{below}} = -\text{trapz}(x(\text{indx}), \min(y(\text{indx}), \text{level}) - \text{level}) \]

Calculate Ra Surface Roughness Value

\[ \text{At} = \text{abs}(\text{At}_{\text{above}}) + \text{abs}(\text{At}_{\text{below}}) \]

\[ \text{CLA} = \frac{\text{At}}{\text{circ}} \]

\[ \text{Ra} = \text{CLA} \]

Count Zero Crossings

1 count = the interval where the curve crosses the zero line in the positive direction and returns through the zero line in the negative direction.
Set threshold to y data with CL set to zero

    threshold = y - CL > 0;

y is the data and number of crossing (NOC) is the number of counts that occur in the curve.

    [y, NOC] = bwlabel(threshold);

The curves are cylindrical; therefore, the first "count" and the last "count" are two halves of one count.

    counts = NOC - 1; % Counts on far left and right excluded.

Reset y data back to yt (tertiary curve) at the level prior to the shift of CL to be placed as the zero line.

    y = yt + CL;

=================================================================================================

Tertiary (Rz)

Sampling length is 6.2046 mm

    sl = (circ)/16; % Sampling length (ISO recommendation for Rz).

    x1 = 0;
    x2 = sl;
    x3 = sl*2;
    x4 = sl*3;
    x5 = sl*4;
    x6 = sl*5;
    x7 = sl*6;
    x8 = sl*7;
    x9 = sl*8;
x10 = sl*9;
x11 = sl*10;
x12 = sl*11;
x13 = sl*12;
x14 = sl*13;
x15 = sl*14;
x16 = sl*15;
y1 = get(gca, 'ylim');
plot ([x1 x1], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x2 x2], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x3 x3], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x4 x4], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x5 x5], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x6 x6], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x7 x7], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x8 x8], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x9 x9], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x10 x10], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x11 x11], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x12 x12], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x13 x13], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x14 x14], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
plot ([x15 x15], y1, ':', 'Color', [0, 0.5, 0], 'linewidth', 1.8);
Sampling Lengths

%     plot(x(indx),y(indx),'k','linewidth',1.5).
%     max_x = max(y(indx)); % Find max for indexed section.
%     min_x = min(y(indx)); % Find min for indexed section.
For when the peak is not the highest value in the data.
%     plot(x(indx),y(indx),'k','linewidth',1.5)
%     [pks,locs] = findpeaks(y(indx));% Find peaks for indexed section.
%     max_x = max(pks); % Find max peak for indexed section.
%     min_x = min(pks); % Find min for indexed section.
For when the valley is not the lowest value in the data.
%     plot(x(indx),y(indx),'k','linewidth',1.5)
%     max_x = max(y(indx)); % Find max for indexed section.
%     [vlys,min_locs] = findpeaks(-y(indx));% Find peaks indexed section.
%     min_x = -max(vlys); % Find max valley for indexed section.

Highlight Sampling Length 1

indx = (x >= 0) & (x <= sl); % Index section.
y(indx) = y(indx)-CL; % Shift curve so CL is centered at zero.
plot(x(indx),y(indx),'k','linewidth',1.5)
max_x = max(y(indx)); % Find max for indexed section.
For when the valley is not the lowest value in the data.
plot(x(indx),y(indx),'k','linewidth',1.5)
max_x = max(y(indx)); % Find max for indexed section.
[vlys,min_locs] = findpeaks(-y(indx)); % Find peaks indexed section.
min_x = -max(vlys); % Find max valley for indexed section.

Plot Rmax.

hold on
x1 = 0;
x2 = sl;
y1 = max_x;
plot ([x1 x2],[y1 y1], 'k', 'linewidth', 0.5);

Plot Rmin.

hold on
x1 = 0;
x2 = sl;
y1 = min_x;
plot ([x1 x2],[y1 y1], 'k', 'linewidth', 0.5);
s1 = max_x - min_x;

**Highlight Sampling Length 2**

indx = (x >= sl) & (x <= sl*2); % Indexed section.
y(indx) = y(indx)-CL; % Shift curve so CL is centered at zero
plot(x(indx),y(indx),'k','linewidth',1.5)
min_x = min(y(indx)); % Find min for indexed section.

For when the peak is not the highest value in the data.
plot(x(indx),y(indx), 'k', 'linewidth', 1.5)
[pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
max_x = max(pks); % Find max peak for indexed section

Plot Rmax.

hold on
x1 = sl;
x2 = sl*2;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

Plot Rmin.

hold on
x1 = sl;
x2 = sl*2;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5)
s2 = max_x - min_x;

Highlight Sampling Length 3

Sampling length 1 (each length is 6.2046[mm])
indx = (x >= sl*2) & (x <= sl*3); % Index section.
y indx) = y(indx) - CL; % Shift curve so CL is centered at zero
plot(x(indx),y(indx),'k','linewidth',1.5)
max_x = max(y(indx)); % Find max for indexed section.
min_x = min(y(indx)); % Find min for indexed section.
Plot Rmax.

    hold on
    x1 = sl*2;
    x2 = sl*3;
    y1 = max_x;
    plot ([x1 x2],[y1 y1],’k’,’linewidth’,0.5);

Plot Rmin.

    hold on
    x1 = sl*2;
    x2 = sl*3;
    y1 = min_x;
    plot ([x1 x2],[y1 y1],’k’,’linewidth’,0.5);
    s3 = max_x - min_x;

Highlight Sampling Length 4

    indx = (x >= sl*3) & (x <= sl*4); % Index section.
    y(indx) = y(indx)-CL; % Shift curve so CL is centered at zero.

For when the peak is not the highest value in the data.

    plot(x(indx),y(indx),’k’,’linewidth’,1.5)
    [pks,locs] = findpeaks(y(indx)) ;% Find peaks for indexed section.
    max_x = max(pks); % Find max peak for indexed section.
    [vlys,min_locs] = findpeaks(-y(indx)); % Find peaks indexed section.
    min_x = -max(vlys); % Find max valley for indexed section.
Plot Rmax.

hold on
x1 = sl*3;

x2 = sl*4;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5)

Plot Rmin.

hold on
x1 = sl*3;

x2 = sl*4;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
s4 = max_x - min_x;

Highlight Sampling Length 5

indx = (x >= sl*4) & (x <= sl*5);% Index section.
y(indx) = y(indx)-CL; % Shift curve so CL is centered at zero.
min_x = min(y(indx)); % Find min for indexed section.

For when the peak is not the highest value in the data.

plot(x(indx),y(indx),'k','linewidth',1.5)

[pks,locs] = findpeaks(y(indx));% Find peaks for indexed section.
max_x = max(pks); % Find max peak for indexed section.
Plot Rmax.

```matlab
hold on
x1 = sl*4;
x2 = sl*5;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
```

Plot Rmin.

```matlab
hold on
x1 = sl*4;
x2 = sl*5;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
s5 = max_x - min_x;
```

Highlight Sampling Length 6

```matlab
indx = (x >= sl*5) & (x <= sl*6); % Index section.
y(indx) = y(indx)-CL; % Shift curve so CL is centered at zero.
plot(x(indx),y(indx),'k','linewidth',1.5)
min_x = min(y(indx)); % Find min for indexed section.
```

For when the peak is not the highest value in the data.

```matlab
plot(x(indx),y(indx),'k','linewidth',1.5)
[pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
max_x = max(pks); % Find max peak for indexed section.
```
Plot Rmax.

hold on
x1 = sl*5;
x2 = sl*6;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

Plot Rmin.

hold on
x1 = sl*5;
x2 = sl*6;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
s6 = max_x - min_x;

Highlight Sampling Length 7
indx = (x >= sl*6) & (x <= sl*7); % Index section.
y(indx) = y(indx)-CL; % Shift curve so CL is centered at zero.
plot(x(indx),y(indx),'k','linewidth',1.5)
max_x = max(y(indx)); % Find max for indexed section.
min_x = min(y(indx)); % Find min for indexed section.

For when the peak is not the highest value in the data.
plot(x(indx),y(indx),'k','linewidth',1.5)
[pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
max_x = max(pks); % Find max peak for indexed section.
[vlys, min_locs] = findpeaks(-y(indx)); % Find peaks indexed section.

min_x = -max(vlys); % Find max valley for indexed section.

Plot Rmax.

hold on

x1 = sl*6;
x2 = sl*7;
y1 = max_x;
plot ([x1 x2], [y1 y1], 'k', 'linewidth', 0.5);

Plot Rmin.

hold on

x1 = sl*6;
x2 = sl*7;
y1 = min_x;
plot ([x1 x2],[y1 y1], 'k', 'linewidth', 0.5);
s7 = max_x - min_x;

Highlight Sampling Length 8

indx = (x >= sl*7) & (x <= sl*8); % Index section.
y(indx) = y(indx) - CL; % Shift curve so CL is centered at zero.
plot(x(indx),y(indx), 'k', 'linewidth', 1.5)

max_x = max(y(indx)); % Find max for indexed section.
min_x = min(y(indx)); % Find min for indexed section.
Plot \( R_{\text{max}} \).

\begin{verbatim}
hold on
x1 = sl*7;
x2 = sl*8;
y1 = max_x;
plot ([x1 x2],[y1 y1], 'k', 'linewidth', 0.5);
\end{verbatim}

Plot \( R_{\text{min}} \).

\begin{verbatim}
hold on
x1 = sl*7;
x2 = sl*8;
y1 = min_x;
plot ([x1 x2],[y1 y1], 'k', 'linewidth', 0.5);
s8 = max_x - min_x;
\end{verbatim}

Highlight Sampling Length 9

\begin{verbatim}
indx = (x >= sl*8) & (x <= sl*9); % Index section.
y(indx) = y(indx) - CL; % Shift curve so CL is centered at zero.
plot(x(indx),y(indx), 'k', 'linewidth', 1.5)
\end{verbatim}

For when the peak is not the highest value in the data.

\begin{verbatim}
plot(x(indx),y(indx), 'k', 'linewidth', 1.5)
[pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
max_x = max(pks); % Find max peak for indexed section.
\end{verbatim}
For when the valley is not the lowest value in the data.

\[
\text{max}_x = \max(y(\text{indx})); \quad \% \text{Find max for indexed section.}
\]

\[
[vlys,\text{min}_x] = \text{findpeaks}(-y(\text{indx})); \quad \% \text{Find peaks indexed section.}
\]

\[
\text{min}_x = -\max(vlys); \quad \% \text{Find max valley for indexed section.}
\]

Plot Rmax.

\[
\text{hold on}
\]

\[
x1 = \text{sl}*8;
\]

\[
x2 = \text{sl}*9;
\]

\[
y1 = \max_x;
\]

\[
\text{plot } ([x1 \ x2],[y1 \ y1],'k','\text{linewidth}',0.5);
\]

Plot Rmin.

\[
\text{hold on}
\]

\[
x1 = \text{sl}*8;
\]

\[
x2 = \text{sl}*9;
\]

\[
y1 = \min_x;
\]

\[
\text{plot } ([x1 \ x2],[y1 \ y1],'k','\text{linewidth}',0.5);
\]

\[
s9 = \max_x - \min_x;
\]

Highlight Sampling Length 10

\[
\text{indx} = (x \geq \text{sl}*9) \& (x \leq \text{sl}*10); \quad \% \text{Index section.}
\]

\[
y(\text{indx}) = y(\text{indx})-\text{CL}; \quad \% \text{Shift curve so CL is centered at zero.}
\]

\[
\text{plot}(x(\text{indx}),y(\text{indx}),'k','\text{linewidth}',1.5)
\]

\[
\text{max}_x = \max(y(\text{indx})); \quad \% \text{Find max for indexed section.}
\]

\[
\text{min}_x = \min(y(\text{indx})); \quad \% \text{Find min for indexed section.}
\]
Plot Rmax.

    hold on
    x1 = sl*9;
    x2 = sl*10;
    y1 = max_x;
    plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

Plot Rmin.

    hold on
    x1 = sl*9;
    x2 = sl*10;
    y1 = min_x;
    plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
    s10 = max_x - min_x;

Highlight Sampling Length 11

    indx = (x >= sl*10) & (x <= sl*11+0.04); % Index section.
    y(indx) = y(indx) - CL; % Shift curve so CL is centered at zero.

For when the peak is not the highest value in the data.

    plot(x(indx),y(indx),'k','linewidth',1.5)
    [pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
    max_x = max(pks); % Find max peak for indexed section.

For when the valley is not the lowest value in the data.

    [vlys,min_locs] = findpeaks(-y(indx)); % Find peaks indexed section.
    min_x = -max(vlys); % Find max valley for indexed section.
Plot Rmax.

```matlab
hold on
x1 = sl*10;
x2 = sl*11;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
```

Plot Rmin.

```matlab
hold on
x1 = sl*10;
x2 = sl*11;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
s11 = max_x - min_x;
```

**Highlight Sampling Length 12**

```matlab
indx = (x >= sl*11+.04) & (x <= sl*12); % Index section.
y(indx) = y(indx) - CL; % Shift curve so CL is centered at zero.
```

For when the peak is not the highest value in the data.

```matlab
plot(x(indx),y(indx),'k','linewidth',1.5)
[pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
max_x = max(pks); % Find max peak for indexed section.
```

For when the valley is not the lowest value in the data.

```matlab
[vlys,min_locs] = findpeaks(-y(indx)); % Find peaks indexed section.
min_x = -max(vlys); % Find max valley for indexed section.
```
Plot $R_{\text{max}}$.

```
    hold on
    x1 = sl*11;
    x2 = sl*12;
    y1 = max_x;
    plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
```

Plot $R_{\text{min}}$.

```
    hold on
    x1 = sl*11;
    x2 = sl*12;
    y1 = min_x;
    plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
    s12 = max_x - min_x;
```

**Highlight Sampling Length 13**

```
    indx = (x >= sl*12) & (x <= sl*13); % Index section.
    y(indx) = y(indx) - CL; % Shift curve so CL is centered at zero.
```

For when the peak is not the highest value in the data.

```
    plot(x(indx),y(indx),'k','linewidth',1.5)
    [pks,locs] = findpeaks(y(indx)); % Find peaks for indexed section.
    max_x = max(pks); % Find max peak for indexed section.
```

For when the valley is not the lowest value in the data.

```
    [vlys,min_locs] = findpeaks(-y(indx)); % Find peaks indexed section.
    min_x = -max(vlys); % Find max valley for indexed section.
```
Plot Rmax.

hold on
x1 = sl*12;
x2 = sl*13;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

Plot Rmin.

hold on
x1 = sl*12;
x2 = sl*13;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
s13 = max_x - min_x;

Highlight Sampling Length 14

indx = (x >= sl*13) & (x <= sl*14); % Index section.
y(indx) = y(indx)-CL; % Shift curve so CL is centered at zero.
plot(x(indx),y(indx),'k','linewidth',1.5)
max_x = max(y(indx)); % Find max for indexed section.
min_x = min(y(indx)); % Find min for indexed section.

Plot Rmax.

hold on
x1 = sl*13;
x2 = sl*14;
\[ y_1 = \text{max}_x; \]
\[
\text{plot } ([x_1 \ x_2],[y_1 \ y_1],'k','linewidth',0.5); \]

Plot Rmin.

\[
\text{hold on} \]
\[
x_1 = sl*13; \]
\[
x_2 = sl*14; \]
\[
y_1 = \text{min}_x; \]
\[
\text{plot } ([x_1 \ x_2],[y_1 \ y_1],'k','linewidth',0.5); \]
\[
s_{14} = \text{max}_x - \text{min}_x; \]

Highlight Sampling Length 15

\[
\text{indx} = (x >= sl*14) \& (x <= sl*15); \quad \% \text{Index section.} \]
\[
y(\text{indx}) = y(\text{indx}) - \text{CL}; \quad \% \text{Shift curve so CL is centered at zero.} \]
\[
\text{plot}(x(\text{indx}),y(\text{indx}),'k','linewidth',1.5) \]
\[
\text{max}_x = \max(y(\text{indx})); \quad \% \text{Find max for indexed section.} \]

For when the valley is not the lowest value in the data.

\[
\text{max}_x = \max(y(\text{indx})); \quad \% \text{Find max for indexed section.} \]
\[
[vlys,\text{min}\_\text{locs}] = \text{findpeaks}(-y(\text{indx})); \quad \% \text{Find peaks indexed section.} \]
\[
\text{min}_x = -\max(vlys); \quad \% \text{Find max valley for indexed section.} \]

Plot Rmax.

\[
\text{hold on} \]
\[
x_1 = sl*14; \]
\[
x_2 = sl*15; \]
\[
y_1 = \text{max}_x; \]
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

Plot Rmin.

hold on
x1 = sl*14;
x2 = sl*15;
y1 = min_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);
s15 = max_x - min_x;

Highlight Sampling Length 16

indx = (x >= sl*15) & (x <= sl*16);% Index section.
y(indx) = y(indx) - CL;% Shift curve so CL is centered at zero.

For when the peak is not the highest value in the data.

plot(x(indx),y(indx),'k','linewidth',1.5)

[pks,locs] = findpeaks(y(indx));% Find peaks for indexed section.
max_x = max(pks);% Find max peak for indexed section.

For when the valley is not the lowest value in the data.

[vlys,min_locs] = findpeaks(-y(indx));% Find peaks indexed section.
min_x = -max(vlys);% Find max valley for indexed section.

Plot Rmax.

hold on
x1 = sl*15;
x2 = sl*16;
y1 = max_x;
plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

Plot Rmin.

hold on

x1 = sl*15;

x2 = sl*16;

y1 = min_x;

plot ([x1 x2],[y1 y1],'k','linewidth',0.5);

s16 = max_x - min_x;

Calculate Rz

Rz is the average peak to valley height.

Rz = (s1+s2+s3+s4+s5+s6+s7+s8+...

s9+s10+s11+s12+s13+s14+s15+s16)/16;

Count Peaks

A = Rz/2; % Rz is the peak to valley distance average. Rz/2 = amplitude.

indx = (x >= 0) & (x <= circ); % Index section.

Returns peaks [pks] and their location [loc] indices along the 1000 point array (y), which is the projected cylinder curve. A peak is defined as any point on the curve that has a lower value in both adjacent indices.

[pks, locs] = findpeaks(yt); %,'MinPeakProminence', A/2.

y_inv = max(y)-y; % Invert y data to enable find peaks to find valleys.
Returns valleys [vlys] and their location [min_loc] indices along the 1000 point array (y), which is the projected cylinder curve. A valley is defined as any point on the curve that has a higher value in both adjacent indices.

[vlys, min_locs] = findpeaks(y_inv);

Plot Rp1max (highest peak).

Rmax = max(pks);
p = find(y == Rmax);
xRmax = x(p);
txt1 = ['Rp_{max} = ',num2str(Rmax,'%.2f')];
text(Rmax+4,Rmax-0.2,txt1,'HorizontalAlignment','left','fontname','Bodoni 72','fontsize',15,'LineWidth',1.5);

Plot Rv1min (lowest valley).

[vlys,min_locs] = findpeaks(-y(indx)); % Find peaks for indexed section.
Rmin = -max(vlys); % Find max valley for indexed section.
v = find(y == Rmin); % Find max valley for indexed section.
xRmin = x(v);
txt2 = ['Rv_{max} = ',num2str(Rmin,'%.2f')];
text(xRmin-1,Rmin-0.09,txt2,'HorizontalAlignment','left','fontname','Bodoni 72','fontsize',15,'LineWidth',1.5);

Annotation Box

Add Ra value to the plot.

[0 0 1 1];[x_begin y_begin length height].

    annotation('textbox',[0.8 0.2 0.6 0.7],'String',...
Averaged Undulations (Tertiary)

Distance evaluated

divisor = numel(y_Wa)-1;

x = 0:(circ/divisor):circ;

Wave equation \( y = A \cos\left(\frac{2\pi}{\lambda}x\right) \) or \( y = A \sin\left(\frac{2\pi}{\lambda}x\right) \).

% A = amplitude.

% \( \lambda \) = one wavelength cycle per distance.

\( A = \frac{Rz}{2}; \) % Rz is the peak to valley distance average. \( \frac{Rz}{2} \) = amplitude.

\( \lambda = \frac{\text{circ}}{\text{counts}}; \) % Determine \( \lambda \) (wavelesnght).

\( y = A \cos\left(\frac{2\pi}{\lambda}x\right) \); % Equation.

Plot Characteristics

Figure(4)

Set/control figure 4 for plot.

figure('units','normalized','position',[.5 .5 .5 .5]);

Plot the averaged undulations (ideal waveform).

plot(x,y,'color',[0,0,0.8],'LineWidth',1)

hold on
plot(x, y_Wa, '-', 'color', [0.8, 0.0, 0.0], 'LineWidth', 1)

hold on

axis('auto') % Makes the graph fill the figure.

grid on % Turns the grid on (major not minor).

box on % Tuns on the box that outlines the plot.

Sets up the figure (fig) for uniform changes in font size, color etc...

fig4 = gca;

set(fig4, 'fontname', 'Bodoni 72', 'fontsize', 20, 'LineWidth', 1.5);

Set y limits to auto. Set x limits and x tick marks.

ylim([-2, 2]); % Sets the y axis to fit the curve between the min and max value.

xlim([0, circ]); % Sets the x axis limits from 0 to circumference.

Sets x axis tick marks at the specified numbers.

set (fig4, 'XTick', [0, quad, 2*quad, 3*quad, circ]);

Set fig for presentation appearance.

x_plot = xlabel('...$Circumference \hspace{1mm} of \hspace{1mm} Cylinder \hspace{1mm} \{[mm]\}$');

y_plot = ylabel('...$Height \hspace{1mm} \{[mm]\}$');

t_plot = title('...$900 \hspace{1mm} Day \hspace{1mm} (1L) \hspace{1mm} Cylinder \hspace{1mm} \{[mm]\} \hspace{1mm} m \hspace{1mm} Averaged \hspace{1mm} \{[mm]\} \hspace{1mm} Undulations$');

Interpretation of text characters for presentation.

set(t_plot, 'Interpreter', 'Latex');
set(x_plot,'Interpreter','Latex');
set(y_plot,'Interpreter','Latex');

**Define Quadrant Boundaries**

\[
\begin{align*}
x_1 &= \text{quad}; \\
x_2 &= 2*\text{quad}; \\
x_3 &= 3*\text{quad}; \\
x_4 &= \text{circ}; \\
y_1 &= \text{get(gca, 'ylim')};
\end{align*}
\]

\[
\text{plot ([x1 x1],y1,'k','LineWidth',1)%Caudal Lateral quadrant(0:90).}
\]

\[
\text{plot ([x2 x2],y1,'k','LineWidth',1)%Caudal Medial quadrant(90:180).}
\]

\[
\text{plot ([x3 x3],y1,'k','LineWidth',1)%Cranial Medial quadrant(180:270).}
\]

\[
\text{plot ([x4 x4],y1,'k','LineWidth',1)%Cranial Lateral quadrant(270:360).}
\]

**Annotation Box**

Add wavelength lambda to graph.

\[
[0 0 1 1];[x\_begin y\_begin length height].
\]

\[
\text{annotation('textbox',[0.3 0.2 0.6 0.715],'}color',[0,0,0.8],'}String',... \\
\text{[']['Roughness'],... \\
\text{['\lambda = ' num2str(lambda,'%.2f'), ' [mm]'],... \\
\text{['Amplitude = ' num2str(A,'%.2f'), ' [mm]']],'FitBoxToText','on','BackgroundColor',[1. 1. 1.],'fontname','Bodoni 72','fontsize',15,'LineWidth',1.5);}
\]

\[
[0 0 1 1];[x\_begin y\_begin length height].
\]

\[
\text{annotation('textbox',[0.55 0.2 0.2 0.715],'}color',[0.8,0,0],'}String',... \\
\text{[']['Waviness'],...}
\]
The MATLAB code returns the following plots (Fig B1, B2 & B3) where the waviness, roughness, average amplitude, and periodicity are calculated.

**FIGURE B1**  The top curve is the unwrapped profile. The dashed curve in the plot is the n<sup>th</sup> order Form polynomial and the dashed-dot curve is the nth order Form + Waviness polynomial used for profile deconvolution to separate the waviness and roughness profiles from the primary profile, providing the roughness profile for surface roughness analysis (bottom curve).

The following two figures (Fig. B2 & B3) are an example of the results returned from the roughness analysis that was performed on each epiphysis, as detailed in chapter 3.
FIGURE B2  Waviness parameters $Wa$ and $Wz$ calculated from profile height as a function of circumferential distance. The red dashed-dot line is the centerline average (CLA) for the profile. The circumferential distance is noted at each quadrant interface, (caudal lateral, caudal medial, cranial medial, cranial lateral) from left to right. Horizontal lines indicate the maximum peak ($R_p$) and maximum valley ($R_v$) for each evaluation length. Gray shading in the first interval indicates this interval was omitted from the average calculation because there was only a peak and not a valley. The count is defined as the curve crossing the centerline twice for any interval that has not been omitted.
FIGURE B3  Roughness parameters Ra and Rz calculated from profile height as a function of circumferential distance. The red dashed line is the centerline average (CLA) for the profile. The circumferential distance is noted at each quadrant interface, (caudal lateral, caudal medial, cranial medial, cranial lateral) from left to right. Horizontal lines indicate the maximum peak (Rp) and maximum valley (Rv) for each evaluation length. The count is defined as the curve crossing the centerline twice for any interval that has not been omitted. For this curve all 16 intervals were used in the roughness calculations.
The averaged amplitude and wavelength ($\lambda$) for the roughness curve (solid blue) and waviness curve (red dash-dot) as a function of circumferential distance.

**FIGURE B4** The averaged amplitude and wavelength ($\lambda$) for the roughness curve (solid blue) and waviness curve (red dash-dot) as a function of circumferential distance.
APPENDIX C

C1.1 Supplement 2 Tables

The following set of tables provide the parameters used to define the idealized models, with parameter description, notation, averaged measures and figure the tables correspond with. Table C1 shows the parameters for the epiphysis; C2 shows the parameters for the radial mammillary processes; C3 shows the parameters for tubercle and C4 shows the parameters for the metaphysis.

Table C1: Epiphysis (averaged measures)

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>Notation</th>
<th>Value</th>
<th>Units</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphyseal depth (thickness)</td>
<td>d_E</td>
<td>15.5</td>
<td>mm</td>
<td>Fig. 1A</td>
</tr>
<tr>
<td>Depth of epiphyseal cupping</td>
<td>d_c</td>
<td>6.7</td>
<td>mm</td>
<td>Fig. 1A</td>
</tr>
<tr>
<td>Radius of epiphysis</td>
<td>r_E</td>
<td>18.8</td>
<td>mm</td>
<td>Fig. 1A</td>
</tr>
<tr>
<td>Diameter of articular curvature</td>
<td>ø_AS</td>
<td>37.6</td>
<td>mm</td>
<td>Fig. 1A</td>
</tr>
<tr>
<td>Diameter of epiphyseal cupping</td>
<td>ø_C</td>
<td>30</td>
<td>mm</td>
<td>Fig. 1A</td>
</tr>
</tbody>
</table>

Table C2: Radial Mammillary Processes (averaged measures)

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>Notation</th>
<th>Value</th>
<th>Units</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder diameter percent of rim diameter</td>
<td>ø_Cyl</td>
<td>80</td>
<td>%</td>
<td>Sup. Fig. 1</td>
</tr>
<tr>
<td>Peak curvature diameter</td>
<td>P_o</td>
<td>1.6</td>
<td>mm</td>
<td>Sup. Fig. 1</td>
</tr>
<tr>
<td>Length</td>
<td>L</td>
<td>7.7</td>
<td>mm</td>
<td>Sup. Fig. 1</td>
</tr>
<tr>
<td>Base width</td>
<td>b_w</td>
<td>1</td>
<td>mm</td>
<td>Sup. Fig. 1</td>
</tr>
<tr>
<td>Height</td>
<td>h</td>
<td>0.44</td>
<td>mm</td>
<td>Sup. Fig. 1</td>
</tr>
<tr>
<td>Peripheral end distance from rim</td>
<td>d_r</td>
<td>2</td>
<td>mm</td>
<td>Sup. Fig. 1</td>
</tr>
<tr>
<td>Number of mammillary processes</td>
<td>M(x19)</td>
<td>19</td>
<td>num</td>
<td>Sup. Fig. 1</td>
</tr>
<tr>
<td>Periodicity</td>
<td>θ</td>
<td>20.4</td>
<td>deg</td>
<td>Sup. Fig. 1</td>
</tr>
</tbody>
</table>
### Table C3: Tubercle (averaged measures)

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>Notation</th>
<th>Value</th>
<th>Units</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length end 1 peak diameter</td>
<td>$\varphi_1$</td>
<td>1.4</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Tubercle peak diameter</td>
<td>$\varphi_2$</td>
<td>1.6</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Length end 2 peak diameter</td>
<td>$\varphi_3$</td>
<td>1.4</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Length end 1 cone base diameter</td>
<td>$\varphi_4$</td>
<td>6.8</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Tubercle cone base diameter</td>
<td>$\varphi_5$</td>
<td>10</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Length end 2 cone base diameter</td>
<td>$\varphi_6$</td>
<td>6.8</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Angle of length end 1 cone</td>
<td>$\theta_1$</td>
<td>26.7</td>
<td>deg</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Angle of tubercle cone</td>
<td>$\theta_2$</td>
<td>30</td>
<td>deg</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Angle of length end 2 cone</td>
<td>$\theta_3$</td>
<td>26.7</td>
<td>deg</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Length end 1 peak height</td>
<td>$h_{LE1}$</td>
<td>1.6</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Tubercle peak height</td>
<td>$h_T$</td>
<td>7.9</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Length end 2 peak height</td>
<td>$h_{LE2}$</td>
<td>1.6</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Radius of base central region</td>
<td>$r_{bc}$</td>
<td>5</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Radius of base peripheral region</td>
<td>$r_{bp}$</td>
<td>10.8</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Radius of ridge curvature</td>
<td>$r_r$</td>
<td>9.4</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Cranial-medial length</td>
<td>$L_1$</td>
<td>7.5</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Cranial-medial length end</td>
<td>$L_{1E}$</td>
<td>3</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Lateral length</td>
<td>$L_2$</td>
<td>7.5</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Lateral length end</td>
<td>$L_{2E}$</td>
<td>3</td>
<td>mm</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Angle between length $L_1$ and $L_2$</td>
<td>$\theta$</td>
<td>125.7</td>
<td>deg</td>
<td>Sup. 2 Fig. 2</td>
</tr>
<tr>
<td>Peak location order pair</td>
<td>$(x,y)_{avg}$</td>
<td>(1.5,6.1)</td>
<td>mm</td>
<td>Sup. 2 Fig. 3</td>
</tr>
<tr>
<td>Angle of cranial-medial length end</td>
<td>$\theta_T$</td>
<td>35.7</td>
<td>deg</td>
<td>Sup. 2 Fig. 3</td>
</tr>
</tbody>
</table>

### Table C4: Metaphysis (averaged measures)

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>Notation</th>
<th>Value</th>
<th>Units</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of epiphyseal cupping</td>
<td>$\varphi_C$</td>
<td>30</td>
<td>mm</td>
<td>Fig. 1D&amp;E</td>
</tr>
<tr>
<td>Diameter of articular curvature</td>
<td>$\varphi_{AS}$</td>
<td>37.6</td>
<td>mm</td>
<td>Fig. 1D&amp;E</td>
</tr>
<tr>
<td>Radius of epiphysis</td>
<td>$r_E$</td>
<td>18.8</td>
<td>mm</td>
<td>Fig. 1D&amp;E</td>
</tr>
<tr>
<td>Radius of femoral neck</td>
<td>$r_N$</td>
<td>10</td>
<td>mm</td>
<td>Fig. 1D&amp;E</td>
</tr>
<tr>
<td>Diameter of neck</td>
<td>$\varphi_{NC}$</td>
<td>10</td>
<td>mm</td>
<td>Fig. 1D&amp;E</td>
</tr>
<tr>
<td>Neck base to articular curve distance</td>
<td>$d_{M1}$</td>
<td>7.6</td>
<td>mm</td>
<td>Fig. 1D&amp;E</td>
</tr>
<tr>
<td>Articular curve to epiphyseal rim distance</td>
<td>$d_{M2}$</td>
<td>10</td>
<td>mm</td>
<td>Fig. 1D&amp;E</td>
</tr>
</tbody>
</table>
C1.2 Supplement 2 Figures

Boolean subtractions created cylinders for which the perimeter was unwrapped and projected to a plane, where a set of parameters were measured from the profile of each mammillary process, defined in table C2 and seen in figure C1. The average of these measures from each mammillary process from each epiphysis defined the idealized mammillary process.

FIGURE C1 Epiphyseal cylinders 80% the diameter of the epiphyseal rim, were created from Boolean subtractions and the perimeter of the cylinder “unwrapped” and projected to a plane. This provided cross-sectional curves of the peripheral radial mammillary processes. A total of eight parameters were measured to define an idealized cross-sectional sketch which was extruded (sweep extrusion) and patterned about the central axis of the femoral neck (axis of symmetry (Supplement Table 2).

Scaffolds of each tubercle were defined from cross-sectional sketches connected with splines. Sketches of the tubercle’s profile were taken at the tubercle peak and length ends 3 mm from where the curvature of the tubercle ends converged with the cupping curvature of the subchondral bone plate surface. Splines laid upon the surface of the tubercle connected the sketches and created a scaffold for which the set of parameters detailed in table C3 and seen in
figure C2, were measured. The average of these measures defined the idealized scaffold and sweep extrusion resulted in the idealized tubercle.

**FIGURE C2** A total of 22 parameters were measured to define the idealized tubercle. A&B) Cross-sections taken at the tubercle peak and length ends along with splines defined a scaffold of the tubercle. C) The average measures of these scaffolds defined an idealized base footprint of the tubercle. D) From this footprint and three cross-sectional sketches, cones were defined with average peak curvatures and angles of the tubercle. E) Splines were used as sweep guides which allowed for a smooth transition from the ridge’s peak curvature diameter to the length end ridge peak diameters, which decreased in size. F) Sweep extrusions were used to create the idealized tubercle (Supplement Table 3).

The tubercle was positioned onto the epiphysis using average tubercle peak location and the angle of the cranial-medial length end (L₁) from a cross-sectional centerline dividing the medial and lateral halves.
FIGURE C3  The location of the tubercle peak for each biomodel was determined using a custom color
topography mapping algorithm, using the polar coordinate system of quadrants previously define. Each
location was normalized to the diameter of the epiphyseal rim, as previously defined, and plotted onto the
coordinate system. Cross hairs denote the anatomical quadrants and were projected to the surface of the
tubercle and the peak.

Contour plots of shear strain presented in chapter 4 were rotated and flipped to show the
shear strains across the metaphyseal interface (Fig. C4) for comparison with the epiphyseal
interface plots of Fig.4.5 in Chapter 4. Shear strains in the radial-circumferential (r-θ) plane
increased radially to average peripheral values of 23% for model FD$_{1\text{mm}}$ and 26% for FD$_{2\text{mm}}$,
with peak local strains of 33% and 32% in the grooves of the radial mammillary processes for
model FD$_{1\text{mm}}$ and model FD$_{2\text{mm}}$, respectively, indicating a slight increase in peripheral strain for
the FD$_{2\text{mm}}$ model where the mammillary processes were less effective in restraining the
epiphyseal rotation due to increased growth plate thickness. This view of the growth plate
surface interfaces shows how the strains are distributed across both surface interfaces, that are
not well seen in figure 4.5 (Chapter 4).
Figure C4  Contour plots of shear strain across the growth plate surface. Top row) The growth plate surface in contact with the epiphyseal subchondral bone plate surface (epiphyseal interface), reported in cylindrical coordinates. Bottom row) The growth plate surface in contact with the metaphyseal growth plate surface (metaphyseal interface). Solid arrows show the direction of rotation and dashed arrows denote the surface of the growth plate (gray models) the contour plot coincides with. Neighboring element values within the cartilage region only were averaged at the nodes unless the relative difference between contributions from neighboring elements at the node was greater than 75% (as denoted in the legend).

Maximum principal strains (Fig. C5) for all four models correlated with the stress distribution seen in the von Mises contour plots, detailed in Chapter 4. Maximum principal strains increased radially up to 19% and slightly higher (19.7%) for FD1mm.
FIGURE C5  Line plots of the maximum principal strain across the growth plate surface. Top row) The growth plate surface in contact with the epiphyseal subchondral bone plate surface (epiphyseal interface), reported in cylindrical coordinates. Bottom row) The growth plate surface in contact with the metaphyseal growth plate surface (metaphyseal interface). Solid arrows show the direction of rotation and dashed arrows denote the surface of the growth plate (gray models) the contour plot coincides with. Neighboring element values within the cartilage region only were averaged at the nodes unless the relative difference between contributions from neighboring elements at the node was greater than 75% (as denoted in the legend).
APPENDIX D

D1.1 Volumetric mesh

Figure D1 shows a cross-section of the volumetric mesh (Cubit 2021.11 software, Coreform, Orem, UT) for the model that had the tubercle and radial mammillary processes and shows how the geometry of the 1-mm thick zones $z_1$-$z_5$ continued through each layer modeling how the geometry of the subchondral bone plate mammillary processes interlock the bone-cartilage-bone interface.

![Cross-section of the volumetric mesh](image)

**FIGURE D1.** A cross-section of the volumetric mesh for model FD shows the minimum of two elements in each 1-mm zone of the model with higher concentrations near the tubercle and radial mammillary processes. Zone $z_1$ is shown in green, $z_2$ shown in purple, $z_3$ shown in light blue, $z_4$ shown in dark blue and $z_5$ shown in white.

D1.2 ABAQUS input file

The following is part of the ABAQUS input file for the nonlinear parametric explicit finite element model $\text{FD}_{1\text{mm}}$. Due to the large number of elements and nodes, the element and node coordinates were omitted from the input file. This model was subjected to a torque of 19.6
Nm of the unconstrained epiphysis and run as a quasi-static analysis. Material properties are defined in chapter 4.

*Heading
** Job name: FD_1mm_1s Model name: FD 1mm 1 sec run
** Generated by: Abaqus/CAE 2021
*Preprint, echo=NO, model=NO, history=NO, contact=NO
**
** PARTS
**
*Part, name=Part-1
*Node
%%% This model has 1,492,124 nodes. Node coordinates are not included in this input file.
*Element, type=C3D10
%%% This model has 1,110,210 elements. Element coordinates are not included in this input file.
*Surface, type=ELEMENT, name=ES_ES_S1, S1
** Section: TB
*Solid Section, elset=EE, material=TB

,** Section: TB
*Solid Section, elset=ME, material=TB

,** Section: TB
*Solid Section, elset=Z1E, material=TB

,** Section: SB
*Solid Section, elset=Z2E, material=SB

,** Section: GP Hyper-elastic
*Solid Section, elset=Z3E, material="GP Hyperelastic"

,** Section: CC
*Solid Section, elset=Z4E, material=CC

,** Section: TB
*Solid Section, elset=Z5E, material=TB

,*End Part
**
**
** ASSEMBLY
**
*Assembly, name=Assembly
**
*Instance, name=Part-1-1, part=Part-1
*End Instance
**

*Node
  1, 1.65145696e-15, -4.82947016e-15, -4.26325608e-14
*Nset, nset=RP-1
  1,
*Nset, nset=RP-1BC
  1,
*Nset, nset=RP-1L
  1,
*Nset, nset=Set-3, instance=Part-1-1
  12751, 12924, 13271, 13441, 13724, 16062, 16191, 16377, 16444, 16512, 16589, 16824, 17154, 19371, 19455, 19540
  19607, 19682, 19755, 19825, 19899, 20170, 22460, 22560, 22712, 22853, 22941, 23039, 23144, 23242, 23335, 23680
  25306, 25457, 25601, 25684, 25833, 26023, 26216, 26354, 26503, 26620, 28983, 29059, 29137, 29216, 29283, 29367
  29502, 29697, 29972, 30303, 32363, 32516, 32587, 32784, 32947, 33123, 33233, 33353, 35327, 35402, 35471, 35541
  35623, 35702, 35780, 36096, 36238, 36343, 36447, 38600, 38728, 38884, 38957, 39040, 39041, 39114, 39211, 39387
  39686, 39789, 41831, 41908, 42052, 42205, 42289, 42419, 42591, 42720, 44598, 44884, 44974, 45049, 45131, 45210
  45290, 45541, 45679, 48103, 48337, 48667, 48891, 49092
*Nset, nset= _T-Cylindrical, internal
  RP-1L,
*Transform, nset= _T-Cylindrical, type=C
    0., 0., 0., 0., 0., 1.
*Orientation, name=Cylindrical, system=CYLINDRICAL
    0., 0., 0., 0., 0., 1.
  1, 0.
** Constraint: Constraint-1
*Coupling, constraint name=Constraint-1, ref node=RP-1, surface=Part-1-1.ES, orientation=Cylindrical
*Distributing, weighting method=UNIFORM
*End Assembly
*Amplitude, name=Amp-1, definition=SMOOTH STEP
    0., 0., 3., 1.
**
** MATERIALS
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*Material, name=CC
*Density
  0.001,
*Elastic
100., 0.2
*Material, name=GP
*Density
 0.001,
*Elastic
 1., 0.45
*Material, name="GP Hyperelastic"
*Density
 0.001,
*Hyperelastic, mooney-rivlin
 4.1, 0.41, 0.
*Material, name=HB
*Density
 0.002,
*Elastic
6500., 0.3
*Material, name=SB
*Density
 0.002,
*Elastic
1100., 0.3
*Material, name=TB
*Density
 0.001,
*Elastic
350., 0.3
*Material, name=TB-E
*Density
 0.002,
*Elastic
550., 0.3
**  
** -- .............................................................................
**  
** STEP: Step-1  
**  
*Step, name=Step-1, ngeom=YES
*Dynamic, Explicit
 , 3.
*Bulk Viscosity
 0.06, 1.2
**  
** BOUNDARY CONDITIONS  
**  
** Name: BC-1 Type: Symmetry/Antisymmetry/Encastre
*Boundary
Set-3, ENCASTRE

**
** LOADS
**
** Name: Load-1  Type: Moment
*Cload, amplitude=Amp-1
RP-1L, 6, -19580.
**
** OUTPUT REQUESTS
**
*Restart, write, number interval=1, time marks=NO
**
** FIELD OUTPUT: F-Output-1
**
*Output, field, variable=PRESELECT
**
** HISTORY OUTPUT: H-Output-1
**
*Output, history
*Energy Output
ALLAE, ALLCD, ALLCW, ALLDMD, ALLFD, ALLIE, ALLKE, ALLMW, ALLPD,
ALLPW, ALLSE, ALLVD, ALLWK, ETOTAL
**
** HISTORY OUTPUT: H-Output-2
**
*Node Output, nset=RP-1
CF1, CF2, CF3, CM1, CM2, CM3, RF1, RF2
RF3, RM, RM1, RM2, RM3
*End Step