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UNIVERSITY OF CALIFORNIA, SAN DIEGO

ASSOCIATION OF 3-DIMENSIONAL JOINT SHAPE AND FUNCTION DURING GROWTH, REPAIR, AND IN DISEASE

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in

Bioengineering

by

Elaine Fong Ting Chan

Committee in charge:

Robert L. Sah, Chair Jeff Hasty Sanjay H. Nigam Jeffrey H. Omens Eric J. Semler Samuel R. Ward

2012

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The dissertation of Elaine Fong Ting Chan is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2012

DEDICATION

This dissertation is dedicated to my loving parents, Alan Chan and Ella Leung, who have unconditionally supported me as I pursue my dreams.

EPIGRAPH

"Shoot for the moon. Even if you miss,

you will land amongst the stars."

Jill McLemore

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ABSTRACT OF THE DISSERTATION

ASSOCIATION OF 3-DIMENSIONAL JOINT SHAPE AND FUNCTION DURING GROWTH, REPAIR, AND IN DISEASE

by

Elaine Fong Ting Chan

Doctor of Philosophy in Bioengineering University of California, San Diego, 2012 Professor Robert L. Sah, Chair

The development and maintenance of joint shape is critical for cartilage and bone biomechanics, integrity, and homeostasis. During long bone development, variations in rates of chondrocyte proliferation, hypertrophy, and matrix production within the articulo-epiphyseal cartilage complex produce the wide range of joint shapes and relative proportions of anatomical features. Difference in shape due to growth, repair, or disease, may affect or reflect joint-scale biomechanics, such as range of motion, as well as tissue-scale mechanics, such as cartilage stiffness. This dissertation aims to elucidate the relationship between joint shape and function, as well as advance the understanding of how joint shapes evolve during growth and deform in the presence of altered biomechanics during repair and in disease.

The shape of the femur was assessed using 3-D local point registration and global statistical shape modeling techniques in animal models of growth and repair, and in human pediatric hip disorders. During cartilage repair, large shape deviations at the bone-cartilage interface were associated with local articular surface recession and low cartilage stiffness, establishing the importance of joint shape in the maintenance of cartilage biomechanics. During normal development of proximal and distal femoral shape, deformations and strains at the bone-cartilage interface were found to be sitespecific and coordinated with changes at local growth plates. In humans, proximal femora underwent differential, growth-associated deformations and anisotropic areal dilations at the femoral head, femoral neck, and greater trochanter, with highest growth rates during puberty. Lastly, in the study of Legg-Calvé-Perthes disease and slipped capital femoral epiphysis, two pediatric hip disorders, proximal femora exhibited substantial disease- and site-specific deformations relative to the asymptomatic femur, with associated changes in growth plate normal vectors, suggesting biological and biomechanical mechanisms of shape deformation.

This work demonstrated correlative links between structural features of the osteochondral unit in the femur and the biomechanical properties of the articular cartilage. Delineation of regional biomechanics and morphological changes contribute to the understanding of the mechanobiology of the proximal and distal femur. In addition, metrics of displacement and strain provide tangible targets for the development of future shape modulation therapies.

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CHAPTER 1:

INTRODUCTION

The shape and biomechanics of the joint, and more specifically of the cartilage and bone, are intricately related. During prenatal development, mechanical loads and intrinsic signals are required for proper joint differentiation and limb formation [50, 92, 94, 101, 120]. During postnatal development, functional adaptation of cartilage and bone geometries occurs to provide increased joint congruence and stability [95]. Alterations to the normal joint shape due to aging, repair, or skeletal diseases may result in abnormal biomechanics including reduced range of motion at the joint-scale, and cartilage degenerative changes at the tissue-scale [10, 110, 118]. As such, joint shapes can affect or reflect growth or disease and act as snapshots in time from which *in vivo* biomechanics can be elucidated. To study changes in shape during growth, repair, and in disease, an understanding of long bone development and morphogenesis is needed.

1.1 Long bone development

Long bone development begins from the condensation of mesenchymal cells in the embryo, followed by cartilage differentiation and bone formation [110] (**Fig. 1.1**). Within the cartilage anlagen, the primary ossification center (POC) forms at the center of the shaft, or *diaphysis*, through the deposition of bone on the calcified cartilaginous core, a process known as endochondral ossification. In addition, bone is deposited directly on the cortical shell by the surrounding periosteum through intramembraneous ossification. Endochondral ossification progresses from the diaphysis peripherally towards the furthermost bone extension of the diaphysis, or *metaphysis*, and the developing cartilaginous end of the bone, or *epiphysis*. Endochondral ossification primarily contributes to the increase in bone length during development, while intramembraneous ossification contributes to shaft width and cortical bone thickness.

During endochondral ossification, cartilage undergoes interstitial and appositional growth through chondrocyte proliferation, matrix production, and hypertrophy. At a certain stage of development, the matrix surrounding the hypertrophic chondrocytes mineralizes, serving as a scaffold for new bone formation. Mesenchymal stem cells enter the tissue through vascular invasion and differentiate into osteoblasts, which synthesize bone matrix on the calcified cartilage. The process of endochondral ossification occurs both at the POC and the secondary ossification center (SOC), which develops within the epiphysis. Prior to the development of the SOC, cartilage within the epiphysis is collectively referred to as the *articulo-epiphyseal cartilage complex*. Following SOC development, cartilage between the SOC and the metaphysis is known as growth plate cartilage, while cartilage at the surface of the long bone is known as *hyaline articular cartilage*.

The shape and size of long bones are in part contributed through the coordinated development of articular and growth plate cartilages (**Fig. 1.2**). Both undergo proliferation, hypertrophy, and mineralization in distinct zones to form a highly organized structure at maturity [55, 58, 62, 63, 108, 117, 132]. In the growth plate, rates of chondrocyte proliferation, hypertrophy, and matrix production in different zones have been related to longitudinal bone growth [29, 56, 131, 132]. Differences in rates of chondrocyte proliferation, hypertrophy, and matrix production at specific bone and joint locations also give rise to differential elongation rates, such as those observed at opposite ends of long bones and between different joints [29, 56, 131, 132]. Additional variations in articular cartilage growth rates and directions by age and species produce the wide range of joint shapes and relative proportions of anatomical features (ie. lateral and medial condyle proportions in the knee).



Figure 1.1: Schematic of longitudinal bone development. Reproduced from [85] with permission. © Society for Endocrinology (2011)



Figure 1.2: Postnatal development of cartilage and bone in C57BL/6 mouse knees from (**A-E**) 12 to 120 days. (**i**) Micro-computed tomography section through the medial femoral condyle, and (**ii**) corresponding Safranin-O stained sections. High-resolution view of the (**iii**) articular cartilage and (**iv**) growth plate cartilage. White arrow in (**C-ii**) indicates the growth plate. Black arrow indicates the articular cartilage. Adapted from [18] with permission.

1.2 Shape and biomechanics

The shape plasticity in developing joints contributes to the wide range of healthy mature joint shapes [12, 135], but may also give rise to shape-related clinical disorders such as dysplasia [81], slipped capital femoral epiphysis, femoroacetabular impingement [10, 37], Legg-Calvé-Perthes disease [17, 91], and osteochondritis dissecans [25, 51]. Alterations in cartilage and subchondral bone geometry have also been associated with aging and osteoarthritis [16, 21, 52, 111, 118]. Differences in shape can affect the biomechanical function of the joint as well as the cartilage and bone composition, both of which in turn modulate joint shape. Thus, joint shape may affect or reflect growth or disease.

The development and maintenance of joint shape is critical for cartilage and bone biomechanics, integrity, and homeostasis [7]. As joint size increases, cartilage thickness and chondrocyte density decrease [28, 62, 106]. Concomitantly, articular cartilage load-bearing material properties also improve. In rabbit and bovine knee cartilage, increases during development in the tensile and compressive moduli of articular cartilage have been associated with increases in collagen content and cross-linking [64, 129, 130, 134]. In skeletally mature animals and humans, the biomechanical and biochemical characteristics of articular cartilage vary both by site (ie. patellofemoral groove and femoral condyles) and joint (i.e. knee versus ankle) [5, 65, 68, 90, 122, 129, 130], complementing the local geometry to respond to various loading demands of the body.

The macroscopic mechanisms that dictate cartilage material maturation and joint shaping remain to be determined. At the articular surface, appositional growth [4, 49] occurs with chondrocyte proliferation in the superficial zone [88, 96] and replacement of collagen fibers [57]. Articular cartilage maturation is also affected by changes at the cartilage-bone interface, with chondrocyte proliferation occurring in neonatal rabbit knee joints above the subchondral plate [88, 89]. These macroscopic tissue changes during growth may be described biomechanically by growth strains [23]. Thinning of cartilage during postnatal development is due to mineralization in the deep zone and advancement of the tidemark separating the deep and calcified cartilages. In conjunction with these axial growth processes, articular cartilage expands tangentially along with the underlying subchondral bone. Both axial and tangential growth processes may generate internal stresses [70] and affect the material quality of adult articular cartilage. Thus, a detailed understanding of the shape of the bone-cartilage interface during development would provide invaluable insight into the biomechanics of cartilage maturation and macroscopic joint size and shape.

1.3 Development of the normal femur

The femur, or thigh bone, comprises part of the knee joint on the distal epiphysis and the hip joint on the proximal epiphysis. The shape and size of the developing postnatal femur is contributed by four regional growth plates, located at the distal femur and at the proximal femoral head, femoral neck, and greater trochanter (**Fig. 1.3**). During normal growth in humans, the distal femoral growth plate contributes 70% of the total femur length, while the femoral head growth plate contributes 30% [110]. Changes in femoral geometry or growth plate kinetics may result in angular abnormalities (i.e. knock knees), limb length discrepancies, or reduced range of motion of the lower limb.

In the proximal femur, based on an antero-posterior view, the normal vector of the femoral head growth plate advances from a relatively vertical orientation during infancy to a medial orientation in adolescence, with corresponding decreases in neck-shaft angle [113]. This change is balanced by the emergence of the neck and trochanteric growth plates, with normal vectors directed supero-laterally, resulting in overall lengthening of the proximal femur along the shaft axis. In the distal femur, the growth plate maintains a relatively stable orientation with respect to the femoral shaft throughout postnatal development but significantly varies in morphology between species [18]. Compared to the relatively flat growth plate of humans, the distal femoral growth plate of mice and rabbits develop an undulating, interlocking geometry between the epiphysis and metaphysis, which may be important in distributing stresses during the highly flexed loading position of the quadruped knee [78].

While the morphology and localized growth rates of the femoral head and distal femoral growth plates have been well-studied individually, the coordinated shape developments within the femur, and growth properties of the greater trochanter and femoral neck, remain to be determined. Characterization of the 3-D shape and orientation of the normal femoral growth plates would provide better understanding of joint morphogenesis and a foundation for comparison of shape deformities after repair or in disease.


Figure 1.3: Growth of the proximal femur occurs through proliferation, matrix deposition, and cell hypertrophy at the cartilaginous regions (unshaded). The three growth plates of the proximal femur are: femoral head growth plate (LGP), greater trochanteric growth plate (TGP), and the femoral neck isthmus (FNI). Reproduced from [113] with permission.

1.4 Cartilage repair

The load-bearing function of articular cartilage, which has limited intrinsic regeneration capacity [109], can be compromised with acute injury or in chronic diseases such as osteoarthritis or osteochondritis dissecans [25, 51]. The paradigm of surgical interventions for cartilage repair depends on the size and extent of the lesion, with treatments including microfracture, osteochondral graft transplantation, autologous chondrocyte implantation, and joint resurfacing or replacement (**Fig. 1.4**). Autograft transplantation, also known as mosaicplasty for multi-graft transplantation, involves taking osteochondral grafts from non-weight bearing, donor regions of the joint to repair small (1-3 cm²) defects [2, 11, 46]. Oftentimes, the inherent mismatch between graft donor and recipient host properties [1, 9, 19, 35, 38] imposes remodeling requirements for complete structural and functional restoration.

Current Treatment Paradigms for Articular Cartilage Degeneration



Figure 1.4: Growth of the proximal femur occurs through proliferation, matrix deposition, and cell hypertrophy at the cartilaginous regions (unshaded). The three growth plates of the proximal femur are: femoral head growth plate (LGP), greater trochanteric growth plate (TGP), and the femoral neck isthmus (FNI). Reproduced from [128] with permission.

Animal models of autografts suggest that in vivo remodeling and resultant cartilage and bone properties depend on the maintenance of surface geometry at the articular surface and the bone-cartilage interface. Autografts implanted approximately flush generally display, at 3 and 6 months, articular cartilage with smooth surfaces, little integration to host cartilage, variable chondrocyte viability and clustering, and trends of slight cartilage thickening [54, 69, 76, 112]. Autografts implanted with the surface recessed in adult sheep deteriorated by 6 weeks depending on the extent of mismatch; those recessed 1mm in vivo maintained a smooth articular cartilage surface, with cartilage thickening and tidemark advancement, while grafts recessed 2mm underwent cartilage necrosis with fibrous tissue overgrowth [54]. Autografts implanted 2mm proud relative to adjacent host cartilage in adult sheep developed surface clefts after 3 months in vivo [97]. Biomechanical studies, both experimental and computational, demonstrated that the articular cartilage of proud grafts is subjected to increased peak contact pressures and compressive strains compared to the cartilage of congruent joints, while recessed grafts led to higher contact pressures in adjacent host cartilage [26, 47, 72]. Joint-scale coefficients of friction of knees with proud grafts were also elevated in vitro [75]. These studies suggest that surface geometry plays an important role in maintaining healthy cartilage, and the success of autograft repair reflects the adaptation of the graft to normal host geometry. However, few studies have quantified, particularly in 3-D, the extent of geometrical abnormalities in grafted cartilage and bone [27, 44, 59].

Structural assessment of defect repairs has traditionally focused on metrics of the central graft region and the graft-host interface, evaluated in 1-D or 2-D in one or several sites. Graft cartilage geometry has been evaluated with histology [27, 34, 44, 54, 76, 87] and magnetic resonance imaging (MRI) [24, 99, 121] using graded scales for parameters such as cartilage thickness, fill, integration, and elevation. Bone morphometry and fill have also been assessed with histology [69, 124], conventional x-ray [112], and computed tomography [61]. These 2-D methods provide valuable structural and compositional information along one section of the graft, but a limited view of remodeling within the whole joint. Knowledge of the 3-D cartilage and bone structure in and surrounding the graft, and the relationship between structure and biomechanical properties of the cartilage, would provide further insights into the role of local graft-host geometry in cartilage repair.

1.5 Diseases of the proximal femur

The importance of the articular surface and bone-cartilage interface shape for proper cartilage function and biomechanics has become increasingly apparent over the past decade, especially within the proximal femur. The conceptualization of femoroacetabular impingement (FAI), and the associated risk of labral tears and early osteoarthritis [10, 37], has led to a growing number of studies aimed at elucidating the relationship between joint shape and function or degeneration.

FAI is a morphological disorder that is manifest as a cam-type protrusion of the femoral neck, either alone or with a pincer-type over-coverage of the acetabular rim (Fig. 1.5), and can be idiopathic or a result of childhood skeletal disorders. Two common pediatric hip disorders that result in altered proximal femoral morphology, often leading to FAI, are Legg-Calvé-Perthes disease (LCPD) and slipped capital femoral epiphysis (SCFE) [33, 67, 127]. In LCPD, idiopathic osteonecrosis occurs due to disruption of blood supply to, and lateral growth arrest of, the femoral head growth plate. LPCD results in altered proximal femur morphology with a misshapen femoral head, a short and wide neck, and, in severe cases, overgrowth of the greater trochanter [110]. In SCFE, the femoral head epiphysis slips relative to the femoral neck and metaphysis, due in part to excessive mechanical shear forces. SCFE results in an increasingly displaced head and misshapen neck, depending on the acuteness and severity of slip [110]. While these diseases have been studied extensively, most analyses have been based on 2-D plain film radiographs. Thus, there is a need for detailed 3-D analyses of hip morphology in these pediatric disorders.



Figure 1.5: (A) Normal clearance of the hip. Femoroacetabular impingement (FAI) can manifest as (B) cam, (C) pincer, or (D) mixed-mode impingement. Cam impingement is a result of a bump at the femoral head-neck junction. Pincer impingement is a result of overcoverage by the acetabulum. Reproduced from [77] with permission.

For LCPD and SCFE, a variety of clinical classification schemes and surgical treatment methods [3, 40, 66, 74, 82, 93, 98, 126] have been proposed to assess proximal femoral deformities. Conventional 2-D and 3-D measures of shape abnormalities include femoral head sphericity, joint containment, degree of epiphyseal slippage, articulotrochanteric distance, and functional version and torsion, with classification based on broad ranges of parameter values (e.g., Southwick mild slip from 0-30°) or on semi-quantitative shape descriptors (e.g., Stulhberg Class IV with >1cm flattening of weight-bearing femoral head) [53, 67, 73, 98]. While the measures and classification schemes address gross deformities in proximal femoral shape, they do not capture the 3-D extent and location of shape variability which may aid in monitoring and pre-operative planning.

1.6 Modeling biological growth and deformations

The study of biological size and shape occurs in many fields and overlaps in disciplines from anatomy and anthropology to bioengineering and computer vision. The methods and endpoint measures within each field are often distinct and serve to elucidate specific aspects of growth or ontogeny.

Allometry [60, 119], or biological scaling, is the study of relative changes in body size proportions, and accounts for the mismatch in scaling and physical demands that is described by isometric scaling, where changes in size do not lead to changes in proportion. According to the square-cube law of isometric scaling, if an organism doubles in length, its surface area increases 4-fold, and its volume and mass increases 8fold. In the femur, an 8-fold increase in mass supported by a 4-fold increase in femur cross-sectional area leads to twice the amount of loads on the bone, which is energetically unfavorable and nutritionally demanding. Allometric growth is used to account for physiological factors that force the organism to deviate from isometric growth and is often described as $Y = kX^a$, where Y is the biological variable, X is a measure of body size, and **a** is the scaling component. For example, linear dimensions of an organism are 1/3 that of its surface area and 2/3 powers of its body mass, while its metabolic rate is 3/4 power of its body mass (also known as Kleiber's law in biology). Allometric studies can range from dynamic (growth of an organism) to static (variations among individuals at a given age and sex) to evolutionary (interspecies variations), and are most commonly used to elucidate relationships between function (metabolic activity or performance speed) and body mass or length.

Morphometrics is another field of study that quantifies the *form*, or size and shape, of an organism, and has been used to study fossil records, growth and mutations, as well as covariances between ecological factors or genetics and shape. The three main branches include traditional, landmark-based, and outline-based morphometrics. Traditional morphometric include previously mentioned lengths, widths, angles, ratios, and areas that are anatomically-relevant and straightforward measures of size, but are not independent measurements or representative of the 3-D shape of the joint. Landmark-based geometric morphometrics [13] contain information about the spatial distribution of shape within the organism based on locally defined landmarks at, or farthest away from, specific anatomical structures. Outline-based morphometrics uses outline tracings of an organism and techniques such as eigenshape analysis [83] and elliptical fourier analysis [30] to determine the deviations of the outline from a circle, or the minimum number of ellipses required to mimic the shape, respectively.

Morphometric techniques have been applied to study a number of different organs in the body. In the brain, voxel-based morphometric techniques have been applied to study volume of gray and white matter [20, 80] in a variety of disorders as well as the volume of structures such as the hippocampus [86]; deformation-based techniques have been used to assess morphological changes during disease across the entire brain compared to a standard brain template [39, 103, 104]. In the heart, landmark-based statistical shape modeling techniques have been used to characterize regional heart shape, wall motion, and cardiac function among and within different populations [32].

In contrast to the shape-based fields of study, models of soft tissue growth and remodeling also exist from the continuum mechanics point of view [23]. Growth deformations have been decomposed into growth and elastic parts that characterize volumetric growth via mass deposition and elastic accommodations that ensure compatibility while producing residual stresses, respectively [71, 102, 114]. Since long bones consist of "hard" tissue that experiences relatively small elastic strains, elastic accommodation deformations have traditionally been assumed to be negligible when characterizing rapid skeletal growth. Using concepts from continuum mechanics and computational modeling, morphogenesis of the finger joint and stress distributions were simulated over specific periods of growth [50]. With cell tracking techniques, *in vitro* 1-D and 2-D tissue strains have also been mapped within cartilage explants during compression and shear [41, 107, 133].

1.7 3-Dimensional shape characterization of the femur

Methods for characterizing the 3-D joint surface can be separated into descriptors of detailed surface structure or global morphology. Local curve-fitting techniques such as piecewise parametric surface patches, B-spline, or thin-plate spline representations [6] are useful for representing detailed surface structure, such as those for the assessment of osteoarthritic changes or cartilage lesions. On the other hand, parameterization techniques to obtain global morphology, such as least squares fitting [6], medial representations [123] and statistical shape models (SSM) [22], may be more appropriate for characterizing macroscopic shape patterns in development or disease. Both local and global shape representations can be further used in finite element analyses for the prediction of joint-scale mechanics [15, 31, 48, 79, 100, 116].

Whichever descriptor of joint shape is used, comparison of normal and diseased hip morphology remains challenging due to many sources of variation. The shape and size of joints vary during postnatal growth, within a population at a given age, and with disease progression. In addition, with the exception of major anatomical landmarks, corresponding locations between normal and diseased hips are difficult to discern.

SSM is a technique that quantifies complex geometries with a set of shape parameters [22, 45]. One advantage of SSM is the definition of dense matrices of corresponding surface locations that are determined across each object. Based on these point correspondences, a specific object shape is defined by the average shape and variations from the average, as calculated by the weighting parameters of specific modes. In this respect, SSM is also ideal for biomechanical analysis, as the mapping of individual locations between structures undergoing deformation is the foundation of continuum mechanics; such deformation may be due either to externally applied stress or to internally generated growth stress [36, 115].

The methods used in atlas building and SSM are similar to deformation-based morphometric analysis [103, 104]. First, an affine registration that eliminates global pose, orientation, and size differences is performed between the template shape and the sample shape. Second, a non-rigid registration using B-spline interpolation based on a method introduced by [105] is implemented to determine local deformations in shape. SSM analyses have elucidated relationships between 2-D proximal femoral shape and risk of osteoarthritis [43, 84, 125] and fractures [8, 42], as well as differences in 3-D distal femoral shape between control and incidence osteoarthritis groups [14]. However, SSM has yet to be applied in 3-D for the analysis of skeletal deformation during growth and in disease.

1.8 Dissertation objectives and overview

The overall motivation for this thesis was to contribute to understanding how joint shapes evolve during growth and deform in the presence of altered biomechanics during repair and in disease. The objectives of this dissertation were to 1) investigate the relationship between local surface shape alterations and cartilage biomechanics after cartilage repair, 2) determine the growth-associated, coordinated shape deformations of the bone-cartilage interface in the developing proximal femur, and 3) quantify abnormal shape deformations relative to the normal femoral shape in pediatric hip disorders to aid in diagnosis and intervention.

Chapter 2, which was published in *Cartilage*, describes 3-D structural and biomechanical metrics of repair tissue following defect repair with autologous osteochondral grafts. Operated and non-operated goat stifle joints were harvested at 6 and 12 months, and cartilage thickness, stiffness, and surface deviations were mapped across the joint at 63 sites. The effectiveness of autograft repair was related to the structural match between the operated and non-operated joints at the articular surface and bone-cartilage interface. This study established the importance of surface geometry in the maintenance of cartilage biomechanics.

Chapter 3, which was published in *Orthopedic Clinics of North America*, provides a qualitative comparison of the human and mouse knee shape during postnatal development as well as a detailed look at shape deformations and strains during normal development in the mouse distal femur. The shape of the bone-cartilage interface was described by statistical shape parameters and modes of variation. Deformation and strain maps illustrated how the bone-cartilage interface expands in an age- and site-specific manner during postnatal development. This study established the use of statistical shape modeling to investigate growth-associated differences in shape.

Chapter 4, which will be submitted as an original research article, investigates the abnormal deviations in proximal femoral shape during two pediatric hip disorders, Legg-Calvé-Perthes disease (LCPD) and slipped capital femoral epiphysis (SCFE). The extent and location of deformation was quantified in LCPD and SCFE femora compared to age- and size-adjusted asymptomatic proximal femora. This study identified shape patterns and 3-D metrics that are useful for clinical decision-making and treatment. In addition, strain maps of the disease femur provide insight into the abnormal biomechanics of the developing joint.

Chapter 5, which will be submitted as an original research article, further investigates the shape characteristics in asymptomatic, LCPD and SCFE hips by determining coordinated shape changes using statistical shape parameters. 3-D analyses of the gross morphological deformities in these two pediatric hip disorders provide additional understanding of disease mechanobiology, as well as insight into deformations that occur in other morphological hip disorders such as FAI or dysplasia.

Chapter 6 summarizes the major findings of these studies and discusses potential directions for future studies.

Appendix A, which was published in part in *Cartilage*, supplements the findings of Chapter 2 by providing additional results of gross morphology, collagen staining, bone morphometrics, tidemark remodeling, and vascular invasion.

Appendix B supplements the findings of Chapter 3 by determining coordinated shape changes between the proximal or distal femur bone-cartilage interface and the local growth plate surfaces. Estimates of ossification, proliferation, and hypertrophy rates were determined based on displacements of surfaces within the femur to provide a mechanistic basis of joint shape development.

Appendix C supplements the findings of Chapter 4 by providing additional validation results of the landmark correspondences and fit from statistical shape parameters.

Appendix D supplements the findings of Chapters 4 and 5 by providing additional analyses of the shape metrics using k-means cluster analysis. The results demonstrate the potential of using statistical shape parameters and 3-D metrics as an alternative method for the classification and diagnosis of LCPD and SCFE.

Appendix E supplements the findings of Chapter 5 by providing additional schematics and metrics of atlas convergence and statistical shape modeling. In additional, correlations between all statistical shape parameters and conventional parameters are provided.

Appendix F summarizes the validation tests performed for the studies in this dissertation as related to the establishment of point correspondences and statistical shape modeling.

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CHAPTER 2

ASSOCIATION OF 3-DIMENSIONAL CARTILAGE AND BONE STRUCTURE WITH ARTICULAR CARTILAGE PROPERTIES IN AND ADJACENT TO AUTOLOGOUS OSTEOCHONDRAL GRAFTS

2.1 Abstract

Objective: The articular cartilage of autologous osteochondral grafts is typically different in structure and function from local host cartilage and thereby presents a remodeling challenge. The hypothesis of this study was that properties of the articular cartilage of trochlear autografts and adjacent femoral condyle are associated with the 3-dimensional (3-D) geometrical match between grafted and contralateral joints at 6 and 12 months after surgery. *Design:* Autografts were transferred unilaterally from the lateral trochlea (LT) to the medial femoral condyle (MFC) in adult Spanish goats. Operated and contralateral nonoperated joints were harvested at 6 and 12 months, and analyzed by indentation testing, micro-computed tomography, and histology to compare 1) histological indices of repair, 2) 3-D

structure (articular surface deviation, bone-cartilage interface deviation, cartilage thickness), 3) indentation stiffness, and 4) correlations between stiffness and 3-D structure. *Results:* Cartilage deterioration was present in grafts at 6 months and more severe at 12 months. Cartilage thickness and normalized stiffness of the operated MFC were lower than nonoperated MFC within the graft and proximal adjacent host regions. Operated MFC articular surfaces were recessed relative to the nonoperated MFC and exhibited lower cartilage stiffness with increasing recession. Sites with large bone-cartilage interface deviations, both proud and recessed, were associated with recessed articular surfaces and low cartilage stiffness. *Conclusion:* The effectiveness of cartilage repair by osteochondral grafting is associated with the match of 3-D cartilage and bone geometry to the native osteochondral structure.

2.2 Introduction

Autologous osteochondral grafts (autografts) are attractive as treatments for cartilage defects due in part to their native tissue architecture. Autografts can be taken from non-weightbearing regions of the joint and used to treat small (1-3cm²) defects [2, 8, 19]. Oftentimes, the inherent mismatch between graft donor and recipient host properties [1, 7, 10, 16, 17] imposes remodeling requirements for complete structural and functional restoration. The extent to which articular cartilage, traditionally ascribed to have limited intrinsic regenerative capacity [9], can remodel and adapt in such an autograft situation is unclear.

Animal models of autografts suggest that *in vivo* remodeling and resultant cartilage and bone properties depend on the maintenance of surface geometry. Autografts implanted approximately flush generally display, at 3 and 6 months, articular cartilage with smooth surfaces, little integration to host cartilage, variable chondrocyte viability and clustering, and trends of slight cartilage thickening [22, 28, 32, 48]. Autografts implanted with the surface recessed in adult sheep deteriorated by 6 weeks depending on the extent of mismatch; those recessed 1 mm *in vivo* maintained a smooth articular cartilage surface, with cartilage thickening and tidemark advancement, while grafts recessed 2 mm underwent cartilage necrosis with fibrous tissue overgrowth [22]. Autografts implanted 2 mm proud relative to adjacent host cartilage in adult sheep developed surface clefts after 3 months *in vivo* [44]. Biomechanical studies, both experimental and computational, demonstrated that the articular cartilage of proud grafts is subjected to increased peak contact pressures and compressive strains compared to the

cartilage of congruent joints, while recessed grafts led to higher contact pressures in adjacent host cartilage [12, 20, 29]. Joint-scale coefficients of friction of knees with proud grafts were also elevated *in vitro* [30]. These studies suggest that surface geometry plays an important role in maintaining healthy cartilage, and the success of autograft repair reflects the adaptation of the graft to normal host geometry.

Structural assessment of defect repairs has traditionally focused on metrics of the central graft region and the graft-host interface, evaluated in 1 dimension or 2 dimensions in one or several sites. Graft cartilage geometry has been evaluated with histology [13, 15, 18, 22, 32, 37] and MRI [11, 45, 53] using graded scales for parameters such as cartilage thickness, fill, integration, and elevation. Bone morphometry and fill have also been assessed with histology [28, 54], conventional x-ray [48], and computed tomography [25]. However, few studies have quantified, particularly in 3 dimensions, the extent of geometrical abnormalities in grafted cartilage and bone [13, 18, 24]. Comparisons to contralateral controls have typically involved matching relatively small tissue sections to the graft site. These 2-dimensional (2-D) methods provide valuable structural and compositional information along one section of the graft, but a limited view of remodeling within the whole joint. Three-dimensional (3-D) assessment of cartilage and bone structure in and surrounding the graft would provide further insights into the role of graft-host geometry in cartilage repair.

The reported biomechanical properties of autograft cartilage after *in vivo* remodeling vary due to measurement methodology and the underlying osteochondral structure at the test site. Single-location biomechanical measurements using indentation [28, 32, 33, 42] provide limited characterization of the state of repair within the entire

graft because repair tissues often exhibit spatially varying properties. Normalization of stiffness measurements based on cartilage thickness is useful for estimation of material properties [21, 26, 38]; when indenter dimensions are on the same order as cartilage thickness, stiffness increases as thickness decreases [34, 49]. Multiple sites of indentation and detailed analyses of the 3-D articular surface and the bone-cartilage interface could improve the characterization of repair tissue properties.

The hypothesis of this study was that properties of the articular cartilage of trochlear osteochondral autografts and of the adjacent femoral condyle are associated with the 3-D geometrical match of articular surface and bone between grafted and contralateral joints at 6 and 12 months after surgery. To address this hypothesis, the objectives of this study were to evaluate the cartilage of the implant and adjacent host region in grafted and contralateral joints for 1) histological indices of repair, 2) 3-D structure, 3) indentation stiffness, and 4) correlations between stiffness and 3-D structure.

2.3 Materials and Methods

Full-thickness grafts from the lateral trochlea (LT) were press-fit into defects of the medial femoral condyle (MFC) in one knee of adult Spanish goats, and operated and nonoperated knees were harvested at 6 and 12 months. The term "nonoperated" was chosen to describe the contralateral joints, as these joints are commonly used as longterm study controls but may not be completely "normal" or intact due to potential agingassociated changes. Metrics of repair were determined from array indentation testing at 63 test locations per joint, micro-computed tomography, and histology. Nonoperated and operated joints were compared in graft and adjacent host regions in terms of histological indices of repair, 3-D structure (articular surface deviation, bone-cartilage interface deviation, cartilage thickness, volume), and indentation stiffness (structural and thickness-normalized). Finally, correlations of stiffness with surface deviations were determined.

Methods are outlined below, and additional details and methods for analyses of other parameters (gross morphology, 3-D alignment, bone histomorphometry, and tidemark remodeling) are provided in the online supplementary material.

2.3.1 Surgical model

Adult female Spanish goats (2-3 years old) were UCSD Institutional Animal Care and Use Committee approval. In the operated knee of each goat, a full-thickness osteochondral graft (diameter $[\emptyset] = 3.5$ mm, height [h] = 6 mm) was harvested from the LT using a trephine (Smith and Nephew, Andover, MA) and press-fit into recipient osteochondral defects ($\emptyset = 3.5$ mm) drilled in the weight-bearing surface of the MFC

(see supplementary material). Care was taken to ensure that the graft articular surfaces were approximately flush with host articular surfaces. At 6 and 12 months (n = 4 each), animals were euthanized, and both operated and contralateral nonoperated knees were harvested for analysis (**Fig. 2.1**).

2.3.2 Indentation mechanical testing

Cartilage load-bearing function was mapped at 63 sites per knee surrounding the defect region. At each site, rapid indentation of cartilage was performed for 1 second to a depth of 100 μ m using a porous, plane-ended indenter ($\emptyset = 0.4$ mm) attached to a Mach-1 V500cs (BioSyntech, Quebec, Canada) to allow measurement of load and determination of structural stiffness (force per indentation depth) (see **Appendix A**) [4]. Testing was performed in 0.5 mm intervals along a 10 mm proximal-to-distal path through the central axis of the defect, as well as paths 1.1 mm lateral and medial to the central axis (**Fig. 2.1B**). Scalpel marks were created 1.5 mm proximal and distal to the beginning and end of the central path for registration with other measurements. Following indentation, condyles were fixed in 10% neutral buffered formalin.



Figure 2.1: (A) In operated knee joints, osteochondral autografts 3.5 mm in diameter were obtained from the lateral trochlea (LT) and transplanted into the medial femoral condyle (MFC). (B) Both operated and contralateral nonoperated joints were analyzed at and adjacent to the graft region by indentation testing in an array of 63 positions along central (C), medial (M), and lateral (L) paths oriented proximal-distal. Positions were classified as graft, proximal adjacent host (PAHC), or distal adjacent host (DAHC) regions. (C) Operated and nonoperated joint MFC and LT regions (boxed) were then analyzed with micro-computer tomography, histology, and immunohistochemistry.

2.3.3 Micro-computed tomography (μCT)

 μ CT imaging was performed to visualize the cartilage and bone relative to the indentation test sites. Radio-opaque pins ($\emptyset = 0.25$ mm, h = 3 mm) were inserted into the scalpel marks of each sample as markers to register μ CT data with other metrics. Imaging was at 45 μ m³ resolution (GE eXplore Locus, GE Healthcare, London, Canada). X-ray scattering from pins was negligible in areas of analysis (see **Appendix A**). Data export and 3-D visualization were performed with Microview v2.1.2 (GE Healthcare).

2.3.4 Histology

Samples were processed for histological sections at the central, medial, and lateral test paths, and analyzed by histochemistry (hematoxylin and eosin [H&E]; Safranin-O) and immunohistochemistry (types I and II collagen [COL-I and COL-II]). Safranin-O sections were scored independently by two users using the modified O'Driscoll scale [15, 43] (maximum total score = 28), including a category for "degeneration in graff" (maximum score = 4), and the International Cartilage Repair Society Visual Assessment Scale (ICRS I) [37] (each category, maximum score = 3). Maximum scores represent normal cartilage (see **Appendix A**).

2.3.5 Data analysis

 μ CT data from pairs of operated and nonoperated joints were analyzed individually and together to allow determination and comparison of cartilage and bone properties at anatomically site-matched locations. The articular cartilage surface and bone-cartilage interface were segmented from μ CT scans by thresholding in Mimics (Materialise, Leuven, Belgium). Then, positions of registration pins were identified,
from which sites of indentation testing on the μ CT-segmented surface were determined (**Fig. A.1A**). Contralateral nonoperated joints were mirror-imaged and matched to operated joints using a 3-D registration technique (STL registration algorithm, Mimics, Materialise) applied to the bone-cartilage interface. This allowed comparison of site-matched properties within and between nonoperated and operated joints (**Fig. A.1B**). 3-D operated and nonoperated bone interfaces were well-aligned, with a root mean square error of 0.07 ± 0.01 mm.

In both operated and nonoperated knees, data points were categorized as graft region, or proximal/distal adjacent host cartilage (PAHC/DAHC) region, based on a set distance from the graft center (**Fig. 2.1B**). Graft centers in μ CT data sets were determined for operated joints as the midpoint between the proximal and distal edges of the graft subchondral bone along the central path. Corresponding graft centers in the nonoperated knee were defined in the same anatomical location based on the registered surfaces. All references to "graft region" in the subsequent text refers to tissue at the implant location. Thus, "operated graft" includes tissue originating from the grafted and/or adjacent host tissue, and "nonoperated graft" includes tissue at a corresponding anatomical location of the nonoperated contralateral MFC.

Geometrical deviations of operated surfaces from nonoperated contralateral controls were calculated at the articular surface and bone-cartilage interface. Deviations from each point of the nonoperated surfaces were determined as the shortest distance to the operated surface along the local surface normal vector. Proud surfaces were denoted by positive deviations and recessed surfaces by negative deviations.

Cartilage thickness was determined from μ CT scans at each indentation site as the height from the cartilage surface to the bone-cartilage interface. These measures were similar to those from histology (see supplementary material) but could be determined semiautomatically.

Tissue volume in the graft was calculated as the volume between the articular cartilage surface and bone-cartilage interface within a cylinder, 1.75 mm radius around the graft center, aligned parallel to the local surface normal vector. The difference in volume between the operated graft and contralateral nonoperated graft regions was also computed.

Normalized cartilage stiffness was determined from indentation structural stiffness and thickness to allow comparisons of material properties. The normalization factor was determined from a function that curve fit structural stiffness versus thickness data from healthy goat cartilage for both MFC and LT samples (**Fig. A.2**). Normalized stiffness (SS_{NORM}) at each test site, *i*, was calculated as $SS_{NORM,i} = SS_i / SS_F(th_i)$, where SS_i was the measured structural stiffness at location *i*, *th_i* was the cartilage thickness taken as the average of 3 immediately adjacent locations (*i*-1, *i*, and *i*+1), and SS_F was the curve-fit value for the stiffness of healthy cartilage with thickness *th_i* (see supplementary material). Thus, normalized nonoperated values should be approximately 1. Both structural and normalized stiffness are reported.

Variability across the joint was determined to assess how uniform the repair tissue was compared to nonoperated and adjacent host. Two indices of variability were determined: host-implant variability, representing the average variability across an equal region of host and graft tissue, and incremental variability, representing the average of differences between immediately adjacent sites (see supplementary material).

The relationships between normalized stiffness and articular surface deviation, and between normalized stiffness and bone-cartilage interface deviation, were determined by binning together data in 0.15 mm increments of deviation. Adjacent bins were grouped when the number of points was low (< 20) to obtain estimates with a confidence interval of \pm 10%.

2.3.6 Statistical analysis

Data are reported as mean \pm standard error of the mean (SEM) and compared as follows. To address Objectives 1 and 2, parametric data (thickness, structural and normalized stiffness) that varied substantially (>2-fold) with standard deviations proportional to the mean were log-transformed [50]. Nonparametric data (ICRS I, O'Driscoll scores) were transformed to ranks to allow for subsequent 2-way analysis of variance (ANOVA); this is analogous to the Kruskal-Wallis 1-way ANOVA for nonparametric data [47, 50]. After respective transformations, data were analyzed by 2way repeated-measures ANOVA to assess effects with a fixed factor of remodeling time (6, 12 months) and a repeated factor of surgical operation (operated, nonoperated). Student *t* tests were used to compare operated MFC to nonoperated MFC and LT thickness and stiffness at individual indentation sites.

To address Objective 3, a 1-way ANOVA with a *post-hoc* Dunnett's test was used to compare normalized stiffness of operated joints at each deviation level to nonoperated average stiffness at 6 and 12 months. Comparisons between deviation levels were performed with a *post-hoc* Tukey test.

2.4 Results

2.4.1 Histology

Modified O'Driscoll and ICRS I scores of Safranin-O-stained sections were significantly lower in operated than nonoperated joints in all categories (**Figs. 2.2** and **2.3**). Total modified O'Driscoll scores for nonoperated joints were 27.6 ± 0.4 and 27.9 ± 0.2 at 6 and 12 months, respectively, and were 39% and 48% lower in operated joints, respectively (p < 0.005) (**Fig. 2.3A**). Operated graft cartilage showed minimal integration with adjacent host cartilage at both times (**Fig. 2.2C(i)**, **J(i)**), with undulating surfaces that did not appear to match the convexity of the natural joint contour. The largest difference in scores from nonoperated controls were associated with degenerative changes (O'Driscoll score: -67% at 6 months, -81% at 12 months) (**Fig. 2.3B**) within the graft.

At 6 months, Safranin-O sections showed chondrocyte clustering in the deep zone and loss of cellularity and proteoglycan staining in the superficial zone of graft cartilage (**Fig. 2.2C(iii**)). Adjacent host had superficial proteoglycan loss and normal deep zone staining (**Fig. 2.2C(ii**)). At 12 months, 3 of 4 grafts had significant loss of chondrocytes and were devoid of Safranin-O staining throughout the depth of the cartilage (**Fig. 2.2J(iii**)). Adjacent host cartilage exhibited flow into the repair region, with chondrocyte clustering in the deep zone (**Fig. 2.2J(iii**)) and fragmented tissue at the graft-host junction (**Fig. 2.2J(i)**). Graft-host subchondral bone junctions were well-integrated in all operated knees. At both times, 2 of 4 knees contained fibrotic cysts.



Figure 2.2: Representative (A-C, G-J) histology sections and (D-F, K-M) microcomputed tomography (μ CT) planes, all of which were taken through the center of the graft, approximately along the central proximal-to-distal indentation test path, at postoperative time of (A-F) 6 and (G-M) 12 months, demonstrating corresponding bone trabecular structure in (A, D, G, K) nonoperated lateral trochlea (LT), (B, E, H, L) nonoperated medial femoral condyle (MFC), and (C, F, J, M) operated MFC regions. Higher magnification to visualize the (i) graft-host interface, and (ii, iii) cellular organization in host and graft cartilage shows a lack of integration between transplanted and host cartilage, cell clustering, and diminished Safranin-O (proteoglycan) staining in the graft. (D-F, K-M) In μ CT images at a plane approximately corresponding to Safranin-O histology sections, the articular surface (yellow arrow) and bone-cartilage interface (blue arrow) were localized by image processing. A cylindrical volume of interest (dotted circle) was thresholded for morphometric analyses of bone (bone in purple and marrow space in green).



Figure 2.2 (continued): Representative histology sections and micro-computed tomography (μ CT) planes, all of which were taken through the center of the graft, approximately along the central proximal-to-distal indentation test path, at post-operative time of 6 and 12 months.



Figure 2.3: (A) Modified O'Driscoll and (B) International Cartilage Repair Society (ICRS) I histology scores. Maximum scores per modified O'Driscoll category are the following: nature of tissue, 7; structural characteristics, 9; degeneration in graft, 4; degeneration in AHC, 3; subchondral bone, 3; inflammation, 2. The maximum score per ICRS I category is 3. Significant effects of treatment (operated vs. nonoperated joints) and postoperative time (6 and 12 months) are indicated as *p<0.05, **p<0.01, ***p<0.005. Data are shown as mean \pm SEM.

2.4.2 Surface deviations

The articular surfaces across all operated adjacent host and graft regions were recessed relative to contralateral nonoperated regions (**Fig. 2.4**). The average graft region was recessed by 0.24 mm at 6 months and 0.37 mm at 12 months (p < 0.005). The bone-cartilage interface of the grafts tended to be proud relative to nonoperated, with a ring of recessed host bone immediately surrounding the graft (**Fig. 2.4E,F**). 3-D reconstructions of graft cartilage and bone showed variability of the bone-cartilage interface (**Fig. 2.5**).

2.4.3 Cartilage thickness and volume

Cartilage thickness increased from proximal to distal across nonoperated joints and varied across operated grafts (**Figs. 2.6A**,**B** and **2.7A**-**F**). In the nonoperated joint, cartilage in the MFC recipient region (0.97mm) (**Fig. 2.2B**,**H**) was twice as thick as the LT donor (0.49mm) (**Figs. 2.2A**,**G** and **2.7A**,**B**), demonstrating inherent structural differences between graft and host cartilage. Compared to site-matched locations in the nonoperated MFC, operated graft thicknesses were lower at the graft center (p < 0.005; -25% at 6 months, -43% at 12 months), and tended to be lower in PAHC (**Figs. 2.6A** and **2.7A**,**B**). Host-implant and incremental variability, 2 measures of "roughness" of properties across the joint, were both higher than nonoperated at 6 and 12 months (p <0.005) (**Fig. 2.6B**). Thickness maps across the operated surface showed low values in the graft regions (**Fig. 2.7C-F**).

Concomitantly, cartilage volume in the 3.5 mm diameter graft regions varied between operated and nonoperated joints (p < 0.05). Operated graft volumes at 6 and 12 months were 7.06 ± 1.48 and 7.52 ± 0.60 mm³, respectively, in contrast to nonoperated

cartilage volumes in a site-matched region $(8.89 \pm 0.48 \text{ mm}^3 \text{ at } 6 \text{ months}, 11.04 \pm 0.80 \text{ mm}^3 \text{ at } 12 \text{ months})$. The difference in cartilage volume between operated and nonoperated graft regions (being lower in the operated region in all samples) was $1.83 \pm 1.33 \text{ mm}^3$ at 6 months and higher at 12 months $(3.52 \pm 1.18 \text{ mm}^3, \text{p} < 0.05)$.

2.4.4 Normalized stiffness

Cartilage stiffness varied across the joint in the proximal to distal direction and was lower in operated graft regions (**Figs. 2.6C-F** and **2.7G-M**). Operated graft structural stiffness at 6 and 12 months were 1.22 N/mm and 0.62 N/mm, respectively, compared to 1.69 N/mm and 0.67 N/mm in nonoperated MFC, and 11.4 N/mm and 4.8 N/mm in nonoperated LT graft regions (**Fig. 2.6C**). Normalized cartilage stiffness of the operated graft was lower than nonoperated MFC (p < 0.01) and decreased with time (p <0.005) (**Figs. 2.6E** and **2.7G,H**). In the graft region, normalized stiffness in operated MFC was 0.28 and 0.19 for 6 and 12 months, respectively, compared to 0.92 and 0.62 for nonoperated MFC, and 1.85 and 0.90 for nonoperated LT. Both host-implant and incremental variability of normalized stiffness were higher in operated compared to nonoperated joints (**Fig. 2.6F**). Stiffness maps across the operated surface showed low values extending into the PAHC and DAHC regions (**Fig. 2.7J-M**).



Figure 2.4: Surface deviations in graft and proximal and distal adjacent host (PAHC, DAHC) regions at 6 and 12 months. Surface height deviation (**A**, **B**) profiles along the central test path and (**C-F**) spatial maps of the operated articular surface (AS) and bone-cartilage interface (BCI) with respect to matching contralateral nonoperated joints, depicting proud or recessed surfaces. Horizontal dashed lines on surface maps indicate location of central test path. Vertical dashed lines indicate approximate positions of interfaces between PAHC, graft, and DAHC. Data are shown as mean \pm SEM.



Figure 2.5: Three-dimensional bone reconstructions showing variably proud bonecartilage interface with respect to the adjacent host (A) with and (B) without a transparent cartilage layer overlaid on top of the bone.



Figure 2.6: (A-C) Regional averages of cartilage thickness, structural stiffness, and normalized stiffness, with (D-F) corresponding host-implant and incremental variability measures for operated and nonoperated medial femoral condyle (MFC). Significant effects of treatment (operated vs. nonoperated joints) and post-operative time (6 and 12 months) are indicated as p<0.05, p<0.01, p<0.05. Data are shown as mean \pm SEM.



Figure 2.7: (A-F) Cartilage thickness and **(G-M)** normalized stiffness in graft and surrounding proximal and distal adjacent host (PAHC, DAHC) regions at 6 and 12 months. The operated medial femoral condyle (MFC), nonoperated MFC, and nonoperated lateral trochlea (LT) **(A,B)** cartilage thickness and **(G,H)** normalized stiffness profiles along the central test path, and **(C-F, J-M)** corresponding spatial maps for the operated and nonoperated MFC. Horizontal dashed lines on spatial maps indicate location of central test path. Vertical dashed lines indicate approximate positions of the interfaces between the PAHC, graft, and DAHC. Data are shown as mean \pm SEM. Comparisons of the operated MFC to the nonoperated MFC and LT are shown as *p<0.05, #p<0.01.

2.4.5 Correlation between normalized stiffness and 3-D structure

Cartilage stiffness at 6 and 12 months was associated with deviations in the articular surface (**Fig. 2.8A,C**). At 6 months, normalized cartilage stiffness of operated knees was lower than nonoperated at all articular surface deviations (p < 0.005), with lower stiffness for increasing articular recession (-17% stiffness for 0- to +0.30 mm deviation vs. -54% stiffness for -0.75 mm to -0.30 mm deviation). Similarly, at 12 months, sites with articular surfaces recessed >-0.15 mm had substantially lower normalized stiffness (>-60%) than nonoperated (p < 0.005), whereas sites near 0 mm deviation had stiffness within 15% of nonoperated values.

Cartilage stiffness was also associated with bone-cartilage interface deviation (**Figs. 2.8B,D**). At 6 and 12 months, normalized cartilage stiffness of operated knees with bone-cartilage interface deviations <-0.15 mm and >+0.15 mm was lower (>-50%) than nonoperated values (p < 0.005), and also lower than sites with smaller deviations (between -0.15 mm and +0.15 mm, p < 0.05).

Deviations at the bone-cartilage interface were associated with deviations at the articular surface (**Fig. 2.9**). With substantial bone-cartilage interface deviation (<-0.15 mm or >+0.15 mm) (**Fig. 2.9C,D**) the articular surface was recessed (>-0.20 mm) at 6 and 12 months. In contrast, with little bone-cartilage interface deviations (between -0.15 mm and +0.15 mm), articular surface deviations were not detectable at 6 months and small (-0.10 mm) at 12 months. Conversely, deviations of the articular surface were not substantially associated with deviations of the bone-cartilage interface (**Fig. 2.9A,B**), consistent with the relationships of both proud and recessed bone-cartilage interfaces (**Fig. 2.9C,D**).



Figure 2.8: Normalized stiffness versus surface deviation of graft and adjacent host test sites of operated joints, binned according to (A, C) articular surface deviation and (B, D) bone-cartilage interface deviation, for samples at (A, B) 6 and (C, D) 12 months. Data are shown as mean \pm SEM. Horizontal lines indicate normalized stiffness of nonoperated medial femoral condyle (MFC); dashed lines indicate mean, and solid lines indicate ± 1 SEM. Difference in stiffness between operated and nonoperated MFC are shown as *p<0.05, **p<0.01, ***p<0.005.



Figure 2.9: (A, B) Effect of articular surface deviation on bone-cartilage interface deviation, and (C, D) vice versa at (A, C) 6 and (B, D) 12 months. Data were binned according to (A, B) articular surface deviation or (C, D) bone-cartilage interface deviation in the same way as data were binned for analysis in Figure 2.8.

2.5 Discussion

This study examined the properties of the articular cartilage within and around an osteochondral autograft after 6 and 12 months *in vivo*, and related the biomechanical quality of the cartilage to the 3-D structure of the repair region. Features of matrix and cellular deterioration were present in graft and adjacent host regions of operated MFC, with time-dependent recession of the operated articular surface and volume loss with respect to nonoperated structures (**Figs. 2.2-2.4**). Cartilage thickness and stiffness were lower and more variable in graft as well as proximal adjacent host regions of operated compared to nonoperated joints (**Figs. 2.6** and **2.7**). Recession of the articular surface was associated with lowering of normalized cartilage stiffness, and regions with substantial deviations at the bone-cartilage interface (proud and recessed) were associated with low normalized stiffness and recessed articular surfaces (**Figs. 2.8** and **2.9**). Together, these results indicate that the health (vs. deterioration) of operated knee cartilage, both in and surrounding the autograft, is maintained (vs. altered) in association with the geometry of the articular surface and bone-cartilage interface.

A number of issues involving the graft and animal model were taken into consideration in this study. The sample size of 8 animals over 2 time points was adequate to detect significant differences between operated and nonoperated joints. However, the assessment of time-dependent effects was limited by having only 4 animals per time point. The small ($\emptyset = 3.5$ mm, h = 6 mm) graft size was chosen in order to harvest a relatively flat graft from the Spanish goat lateral trochlea and avoid the groove curvature. The approach of this study was to investigate grafts placed

approximately flush and assess graft properties at 6 and 12 months. Matched contralateral nonoperated joints from each animal were analyzed for direct comparison and provided indices of initial graft properties. While the treated joints were not analyzed pre-operatively, with 3-D registration techniques and additional structural and biomechanical measures, it was possible to estimate location-matched geometrical and stiffness properties of the donor (LT) graft based on the contralateral nonoperated joint. The interpretation of the differences in operated joints assumes negligible changes in the contralateral joint during the study. In support of this, the animals were skeletally mature (as defined by the cartilage zonal architecture and continuous calcified cartilage layer) [23] at the time of surgery and had similar thigh circumferences at harvest. However, general age-related changes may have occurred during the post-operative period [39, 51]. These factors should be considered in comparing conclusions from this study to those from other animal models or extrapolating results to clinical scenarios.

In the present study, 3-D articular surface deviation maps highlighted regions of cartilage recession that correlated with lower mechanical stiffness. Recession of the articular surface was time-dependent within the graft (0.24 mm at 6 months, 0.37 mm at 12 months) and was also evident in the adjacent host cartilage, in agreement with histological observations (**Figs. 2.2C,J** and **2.4C-F**). The trends for lower cartilage stiffness with recession of the articular surface in operated knees, and for cartilage stiffness values close to nonoperated with small articular surface deviations (**Fig. 2.8A,C**), suggest that local surface deviations may influence cartilage remodeling and homeostasis. Evidence of graft subsidence is consistent with previous studies where 2-D preoperative and postoperative measurements of autograft contours indicated 0.32 mm

recession in sheep MFC [24]. Articular surface recession may lead to altered mechanics, different from those needed to maintain normal cartilage viability and mechanical properties [40, 52]. The time course and location of altered cartilage surface geometry remain to be elucidated.

The variability in bone-cartilage interface structure, both within and between grafts, may also have contributed to the variations in cartilage homeostasis and remodeling. While grafts were initially implanted such that the articular surface was flush with adjacent host, the bone-cartilage interface was variably matched to the host. The initial implant geometry, and subsequent remodeling, may have resulted in bone-cartilage interfaces being oriented variably from flat to angled (**Fig. 2.5**). The association between bone-cartilage interface location and cartilage stiffness suggests that regions of large deviations (proud or recessed) at the bone-cartilage interface may also have contributed to articular surface subsidence and lower normalized cartilage stiffness (**Figs. 2.8** and **2.9**). These results support the idea that certain geometrical features of an osteochondral graft may adversely affect repair, leading to cartilage tissue with suboptimal biomechanical properties.

The multi-site array measurements [4, 31] of cartilage stiffness allowed characterization of stiffness properties and their variability across the joint, as well as differences between operated and nonoperated graft and adjacent host cartilage regions. The indentation technique has been well-characterized [3, 5, 14, 27, 35, 36, 41, 46] and is sensitive to local cartilage degeneration [6] and the integrity of the graft-host interface [4, 49]. However, it has rarely [31] been used it to systematically assessed stiffness variability within and around a cartilage repair site. In this study, normalization of

structural stiffness accounted for variable tissue thickness to reduce the variability relative to that of raw measurements; this enabled sensitive detection of graft treatment effects (**Fig. 2.6**). The large number of test sites within the graft led to a precise estimate of overall tissue properties, while individual sites allowed for characterization of local variability. Host-implant and incremental variability, 2 variables computed to describe the "roughness" of parameters (i.e. thickness, stiffness) across the joint, were both higher in the operated graft compared to nonoperated, demonstrating the inhomogeneity of graft cartilage compared to contralateral healthy cartilage. The multiple-site indentation scheme used in this study was essential to characterize the consequences of grafting on repair tissue properties due to intra-site variability.

Differences in cartilage thickness and other properties between operated MFC, nonoperated LT, and nonoperated MFC graft regions may be due to a number of factors. In the operated graft, histological indices of deterioration (GAG depletion, chondrocyte clustering) (**Fig. 2.2**) and cartilage thickening were consistent with features of early OA, while cartilage thinning and low stiffness may be related to late OA-like degeneration (**Fig. 2.6**). In the nonoperated MFC, aging-related changes may have occurred during the 6- or 12-month postoperative period, with softening of the collagen network leading to increases in water content and cartilage thickness and decreases in indentation stiffness. Innate differences in thickness between contralateral joints are likely to have been minimal, as cartilage thickness in nonoperated distal regions of left and right MFCs were well-matched. Cartilage thickening may also be associated with tidemark remodeling within the graft. While no correlation was observed between proud bone and vascular invasion, operated grafts had significantly more blood vessels crossing the

tidemark closest to the articular surface compared to nonoperated donor LT and recipient MFC sites (see **Appendix A**), indicative of vigorous and possibly OA-like remodeling.

Thus, cartilage structure and quality within the graft likely reflect a number of factors and remodeling responses. Deleterious indices, such as chondrocyte clustering, hypocellularity, and progressive loss of proteoglycan staining with time (**Figs. 2.2** and **2.3**), may reflect locally excessive or insufficient mechanical regulatory stimuli. Future investigations to match articular surface and bone-cartilage interface geometry and to promote remodeling to achieve native cartilage structure may lead to an increased longevity of osteochondral grafts.

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CHAPTER 3:

STRUCTURAL AND FUNCTIONAL MATURATION OF DISTAL FEMORAL CARTILAGE AND BONE DURING POSTNATAL DEVELOPMENT AND GROWTH IN HUMANS AND MICE

3.1 Abstract

The size and shape of joints markedly affect their biomechanical properties, but the macroscopic 3-dimensional (3-D) mechanism and extent of cartilage and joint maturation during normal growth are largely unknown. This study qualitatively illustrates the development of the bone-cartilage interface in the knee during postnatal growth in humans and C57BL/6 wild-type mice, quantitatively defines the 3-D shape using statistical shape modeling, and assesses growth strain rates in the mouse distal femur. Accurate quantification of the cartilage-bone interface geometry is imperative for furthering the understanding of the macroscopic mechanisms of cartilage maturation and overall joint development.

3.2 Introduction

The load-bearing and compositional properties of articular cartilage vary with both the size and shape of the diarthrodial joint. In skeletally mature animals and humans, the biomechanical and biochemical characteristics of articular cartilage vary by site (eg, patellofemoral groove vs femoral condyles) and joint (eg, knee versus ankle) [2, 27, 30, 37, 53, 58, 59], complementing the local geometry to respond to various loading demands of the body. During postnatal development (the processes of differentiation and growth), joint size increases, and cartilage thickness and chondrocyte density decrease [9, 24, 42]. Concomitantly, articular cartilage loadbearing material properties improve. In animal knees, increases during development in the tensile and compressive moduli of articular cartilage have been associated with increases in collagen content and cross-linking [26, 58, 59, 62].

The macroscopic mechanisms that dictate cartilage material maturation and joint shaping remain to be determined. At the articular surface, appositional growth [1, 18] occurs with chondrocyte proliferation in the superficial zone [35, 40] and deposition of collagen fibers [22]. Articular cartilage maturation is also affected by changes at the bone-cartilage interface, with chondrocyte proliferation occurring in neonatal rabbit knee joints above the subchondral plate [35, 36]. These macroscopic tissue changes during growth may be described biomechanically by growth deformations and strains [8]. Thinning of cartilage during postnatal development is due to mineralization in the deep zone and advancement of the tidemark, separating the deep and calcified cartilages. In conjunction with these axial growth processes, articular cartilage expands tangentially along with the underlying subchondral bone. Both axial and tangential growth processes may generate internal stresses [31] and affect the material quality of adult articular cartilage. Thus, a detailed understanding of the shape of the bone-cartilage interface during development would provide insight into the biomechanics of cartilage maturation and macroscopic joint size and shape.

The length and shape of long bones during *in vivo* development is in part contributed through the differential growth and remodeling of articular and growth plate cartilages. Both begin as a homogenous condensation of chondrocytes that undergo proliferation, hypertrophy, and mineralization in distinct zones to form a highly organized structure at maturity [20, 23-25, 43, 49, 61]. In the growth plate, rates of chondrocyte proliferation, hypertrophy, and matrix production in different zones have been related to longitudinal bone growth [10, 21, 60, 61]. Differences in these rates at specific locations give rise to differential elongation rates, such as those observed at opposite ends of long bones and between different joints. Additional variations in cartilage growth rates and directions with age and species produce the wide range of joint shapes and relative proportions of anatomical features (eg, lateral and medial condyle proportions in the knee).

Current knowledge of the macroscopic joint shape is based on measurements of cartilage and bone geometries from gross specimens, plain radiographs, magnetic resonance imaging (MRI), and computed tomography (CT). However, the complexities of joint shape are challenging to represent by 2-D or best-fit 3-D measurements [19, 52]. While the distal femur has been characterized by measurements such as anterior-posterior length, medial-lateral width, and intercondylar height [32, 34], these parameters are confounded by the size of the knee, especially in growing knees, making it difficult to distinguish shape changes during joint maturation. Statistical shape modeling (SSM) [7, 17] is one technique that can concisely quantify complex shapes in a limited number of independent parameters based on a sample population. This method can automatically identify corresponding anatomical regions between samples with high reproducibility [57], making it ideal for studying shape changes during growth and development. Both 2-D and 3-D SSMs have been used to analyze joint shape and bone density as risk factors for the development of osteoarthritis [5, 14, 33, 46, 55-57] and osteoporotic fractures [3, 13, 39], but have yet to be applied to the growth of healthy joints.

The hypothesis of this study was that shape changes of the distal femur at the cartilage-bone interface vary differentially with age and anatomical region during postnatal growth and development. The aims were to (1) illustrate and qualitatively compare the shape changes of the bone-cartilage interface during normal development in humans and C57BL/6 wild-type mice, (2) establish a statistical shape model and determine shape parameters for the growth of the mouse distal femur from postnatal day 12 to 120, and (3) determine growth deformation and strain rates of the bone-cartilage interface. Quantification of the shape plasticity throughout growth and development provides the foundation for investigating *in vivo* developmental biomechanics of the knee. In addition, quantitative models of the developing knee are useful as design targets for tissue engineering that extends to the joint scale, as well as and developing new technologies for clinical diagnosis and treatment of skeletal disorders.

3.3 Materials and Methods

3.3.1 Sample preparation and imaging

With Institution Review Board approval, clinical CT scans were obtained from 6 patients (range: 3.9-11.9 years; mean: 8.2 years) with tibial torsion abnormalities but morphologically normal distal femora at 0.4-0.6mm in-plane resolution and 0.63mm slice thickness (GE Lightspeed VCT, GE Healthcare, USA).

The structure of mouse knee joints was assessed by micro-computed tomography (μ CT) and histology. With IACUC approval, both hindlimbs of twenty-one C57BL/6 male mice, n=3 pairs each at 12, 16, 20, 24, 30, 60, and 120 days postnatal, were harvested, fixed in 10% neutral buffered formalin, and scanned intact by μ CT at (9 μ m)³ isotropic voxel resolution (SkyScan 1076, SkyScan, Belgium; 70kVp, 140 μ A, 1750ms exposure). Following μ CT, one femur at each age point was randomly selected for histological processing. Proximal and distal femora were decalcified in 10% formic acid and paraffin-embedded. 5 μ m thick sagittal sections were obtained approximately through the center of the medial femoral condyle of the distal femur. Sections were stained with Safranin-O and digitized at 20X magnification (0.5 μ m resolution) with Aperio ScanScope (Aperio Technologies, Vista, CA).

3.3.2 Gross morphology

Human and mouse CT images were qualitatively analyzed for morphological changes at the bone-cartilage interface during developmental growth in 2-D coronal and sagittal planes through the center of the load-bearing region of the condyle and in 3-D reconstructions. Distal femora were assessed for contour of the condyles,

posterior condyle prominence, medial versus lateral femoral condyle (MFC/LFC) size, and trochlear ridge development, as well as growth plate location and morphology. Contour of the opposing tibial plateau, as well as joint space width between the distal femoral and proximal tibial secondary ossification centers (SOCs), were also noted.

3.3.3 Histology

Safranin-O sections were compared to matching μ CT sections of mouse femora to interpret the bone-cartilage interface of the SOC relative to the overlying articular-epiphyseal cartilage. In addition, histology sections were assessed for chondrocyte hypertrophy and organization.

3.3.4 Image processing

All CT and μ CT scans were imported into Mimics (Materialise, Belgium) for surface segmentation and 3-D reconstruction. Left femora were flipped in orientation to match right femora. Bone-cartilage interfaces were identified by thresholding for bone, segmented, and exported as point clouds.

3.3.5 Width and thickness measurements

Trans-epicondylar widths were determined in human and mouse distal femora as a measure of growth. In 3-D reconstructed models, trans-epicondylar width was measured as the nearest distance from the edge of the lateral epicondyle to the edge of the medial epicondyle. In the mouse, the overall length of the femur was also determined from the most proximal point on the femoral head to the most distal point on the femoral condyles.

Thicknesses of the articular-epiphyseal and growth plate cartilage were calculated from 2-D histology images. Articular-epiphyseal cartilage was defined from

the articular surface to the calcified cartilage tidemark, or to the distal edge of large hypertrophic cells for young joints with no tidemark. Growth plate cartilage was defined from the epiphyseal side of reserve zone chondrocytes to the metaphyseal side of terminal hypertrophic zone chondrocytes. Because articular-epiphyseal and growth plate cartilage contours were highly curved in the mouse distal femur, making it difficult to estimate thickness directly, cartilage thickness was calculated as the area of the cartilage divided by the average width of the cartilage.

3.3.6 Statistical shape modeling (SSM)

Both femora of fifteen mice, n = 3 pairs each at days 12, 24, 30, 60, 120, were used as the initial training samples for SSM. Training samples were rigidly registered and isotropically scaled [38] to the largest femur (day 120 sample). Point-to-point correspondences between coordinates of each femur were defined automatically, following previously established methods [11] by first constructing a normalized average atlas shape and then extrapolating points (landmark coordinates) in corresponding locations to each femur. Each distal femur was described by 412 landmarks, located from the femoral condyles to the proximal edge of the trochlea.

The statistical shape model was built from the landmarks of the training samples using principal component analysis [7, 11]. A mean shape was calculated from training sample landmarks, and deviations of each shape from the mean were determined. Singular value decomposition of the covariance matrix was performed to obtain the eigenvectors and corresponding eigenvalues (in descending order). The eigenvectors represent the *modes of variation* within the training set, analogous to the principal axes of an ellipse. The eigenvalues represent the *variance* explained by each

mode, or the amount of contribution of each mode to overall joint shape variation. Using the modes of variation from the model, the *shape parameters*, **b**, of each sample was calculated by $\mathbf{x} = \overline{\mathbf{x}} + \mathbf{P}\mathbf{b}$, where $\overline{\mathbf{x}}$ is the mean shape, $\mathbf{P} = (p_1 | p_2 | ... | p_t)$ are the first *t* modes of variation that explain >90% of the total shape variance, and **x** is the sample shape. Shape parameters are analogous to principal radii of an ellipse. Parameters were normalized to one standard deviation of the mode, calculated as the square root of the corresponding eigenvalue.

Additional samples at days 16 and 20 were analyzed by applying the SSM. Samples were segmented in Mimics, rigidly aligned to the same reference femur from the training set, and non-rigidly aligned to the model atlas shape to extrapolate landmarks. Shape parameters were then determined as described above.

3.3.7 Growth maps: deformation rates, strain rates, and strain directions

Deformation and principal surface strain rates between ages were calculated from corresponding landmark coordinates of the average shape at each age. Here, landmark coordinates represented non-scaled shapes. Samples were aligned at the centroid of the growth plate to determine deformations during growth. Magnitudes of deformation were calculated as the distance between corresponding landmarks, and deformations in the same direction as the outward surface normal vector were defined as positive. The two-dimensional components of Green's strain [12] E_{ij} were calculated from $ds^2 - ds_0^2 = 2E_{ij}dX_idX_j$ for i,j = 1:2, where $ds_0^2 = dX_idX_j$ is the squared segment length of a pair of landmarks at the first age point, and ds^2 is the squared length at the second age point. Maximum principal strain rates and directions were calculated from the Green's strain components [12]. Deformation and strain rates were mapped in 3-D onto the triangulated surfaces of the average shape at each age point. Surface patches intersecting the transverse plane and sagittal plane through the medial condyle were determined, and the 3-D principal strain directions of those patches were projected onto each plane to illustrate strain directions along the 2-D surface contour.

3.3.8 Statistics

Differences in mode parameters between age points were assessed by a oneway ANOVA with post-hoc Tukey test. All data are expressed as mean±SD.
3.4 Results

3.4.1 Gross morphology of the developing distal femur: human and mouse

The overall size and shape of both human (Figs. 3.1A and 3.2A) and mouse (Figs 3.1B and 3.2B) distal femoral bone-cartilage interface changed markedly over the evaluated growth period, as visualized by μ CT in coronal (Fig. 3.1(a)) and sagittal (Fig. 3.1(b)) planes, and in 3-D reconstructions (Fig. 3.2).

In the human, the femoral growth plate was situated just proximal to the posterior edge of the condyles and was relatively flat in the transverse plane (**Figs. 3.1A(b)** and **3.2A(b,c)**). At age 4, femoral condyle contours were round in both coronal and sagittal planes (**Fig. 3.1A(i)**). At age 7 years, the posterior condyle was prominent, and the sagittal contour became elliptical. The MFC appeared slightly larger than the LFC. By age 12 years, the load-bearing region of the femoral condyles flattened in the coronal plane, while the sagittal profile remained elliptical (**Fig. 3.1A(iii)**). A prominent intercondylar notch was observed in the coronal view. Extension of the lateral trochlear ridge was evident at 7 years and prominent by 12 years (**Fig. 3.2A(a)**). As the lateral trochlea developed, the lateral side of the growth plate became larger than the medial side (**Fig. 3.2A(b)**). Joint space width between femoral and tibial epiphyseal bone decreased noticeably with age (**Fig 3.1A(a)**). At the opposing joint surface, proximal tibia SOC was round at age 4, developed flat plateaus by age 7, and had concave plateaus by age 12 (**Fig. 3.1A(a)**).

In the mouse, growth plates were also situated proximal to the posterior edge of the condyle but had an undulating shape in the transverse plane, with four regions that extended convex towards the articular surface (Fig. 3.2B(b,c)). At postnatal day 12 (Fig. 3.1B(i-b)), the femoral SOC had a rounded distal contour and an undulating proximal contour matching the growth plate geometry. Between days 16 and 24, SOCs expanded radially as femoral condyles extended outward, and the geometry of the epiphysis interlocked with the metaphysis (Fig. 3.1B(ii)). At day 30, the posterior condyle became prominent, and the load-bearing regions of the condyles were flattened in the coronal plane (Fig. 3.1B(iii)). The MFC also extended more medially compared to the LFC. By day 60, the shape of the knee stabilized. Prominence of the lateral trochlear ridge, an evolutionary feature of bipedalism [51], was not observed in mice. Similar to humans, joint space width between femoral and tibial epiphyseal bone decreased with age (Fig. 3.1B(a)). In contrast to humans, mouse growth plates remained approximately symmetrical in the medial-lateral direction throughout development (Fig. 3.2B(b)). Proximal tibial SOCs were rounded at day 12, flattened with two plateaus at day 20, concave towards the distal femur at day 30, and fully developed by day 60 (Fig. 3.1B(a)).



Figure 3.1: (A) Clinical CT scans of the asymptomatic human knee from (i-iii) ages 4 to 12 years, and (B) μ CT scans and Safranin-O sections of C57BL/6 wild-type mouse knees from (i-v) postnatal days 12-120, in (a) coronal and (b,c) sagittal views. L=lateral, M=medial, A=anterior, P=posterior.



Figure 3.2: 3-D reconstructions of the developing right distal femur and growth plate (A) in the human from ages 4 to 12 years, and (B) in the mouse from postnatal days 12-120. Transverse views of the (i) distal femur and (ii) growth plate, and (iii) sagittal views of the growth plate overlaid on the distal femur are shown. L=lateral, M=medial, A=anterior, P=posterior.

Rapid joint-scale growth occurred from ages 4 to 12 in the human and during the first 30 days in mice. Trans-epicondylar width in the human distal femur (**Fig. 3.3A**) increased linearly from age 4 to 12 (R^2 =0.95; 4 y.o., 45.3mm; 12 y.o., 78.9mm), while trans-epicondylar width in the mouse femur (**Fig. 3.3B**) increased up to day 30, after which it plateaued (12 day, 1.9mm; 30 day, 2.7mm; 120 day, 2.8mm). Overall femur length in the mouse increased at a slower pace and did not plateau until day 60 (**Fig. 3.3C**). Femur length at day 12 was approximately 50% that of day 120. The final length and volume of the mouse femur at day 120 were 15.2±0.2mm and 47.0±4.3 mm³, respectively.

3.4.2 Histology

In C57BL/6 mice, both articular-epiphyseal and growth plate cartilage stained intensely with Safranin-O at all ages, with zonal and age-associated variations in cellular organization (Figs. 3.1B(c) and 3.4).

At day 12, articular-epiphyseal cartilage regions contained randomly distributed chondrocytes, with large hypertrophic chondrocytes at the SOC (**Fig. 3.4A(i)**). By day 30, the zonal architecture of articular cartilage was apparent with underlying bone formation, and by day 60, the articular cartilage was fully formed with a continuous subchondral bone plate.

Growth plate cartilage exhibited a distinct architecture with reserve, proliferative, and hypertrophic zones that decreased in height with age (**Fig. 3.4(ii)**). At day 12, hypertrophic chondrocytes in the articular-epiphyseal complex that stained with Safranin-O were not visible in μ CT (**Figs. 3.1B(b,c)** and **3.4A**). At day 20, bony regions in histology sections corresponded well with mineralized regions observed in μ CT, and proliferative zone chondrocyte nuclei stained prominently (**Fig. 3.4B**). By day 60, hypertrophic chondrocytes were essentially absent from the growth plate (**Fig. 3.4D**).

Articular-epiphyseal and growth plate cartilage of the opposing proximal tibia showed similar age-dependent patterns of cellular organization and matrix staining (Fig. 3.4(iii)).

3.4.3 Cartilage thickness

Articular-epiphyseal cartilage thickness in the mouse decreased with age and plateaued after day 60 (**Fig. 3.5**). Thickness measurements from histology at days 12 and 120 were 0.17mm and 0.03mm, respectively. Growth plate cartilage thickness also decreased with age and did not plateau by day 120. Growth plate thickness at days 12 and 120 were 0.56mm and 0.10mm, respectively. Both articular-epiphyseal and growth plate cartilage thickness were inversely correlated to overall femur length (articular-epiphyseal cartilage, R^2 =0.93; growth plate cartilage, R^2 =0.94; data not shown).



Figure 3.3: Transepicondylar width of (A) human and (B) mouse distal femur, and (C) mouse femur length, measured in 3-D reconstructions of individual samples.



Figure 3.4: Safranin-O sections of mouse distal femoral (i) articular-epiphyseal cartilage, (ii) growth plate cartilage, and proximal tibial (iii) growth plate cartilage showing distinct patterns of cellular organization at postnatal days (A) 12, (B) 20, (C) 30, (D) 60, and (E) 120. Images are oriented such that the articular surface is at the top.



Figure 3.5: Articulo-epiphyseal and growth plate cartilage thickness in C57BL/6 wild-type mice with age, determined in 2-D histology sections.

3.4.4 Statistical shape model: mouse distal femoral bone-cartilage interface

In size-normalized data, 11 modes of variation accounted for >90% of the total shape variation during developmental growth of the distal femur. The first 5 modes of variation accounted for 83% of total shape variation and described mid-condyle outward extension, posterior condyle upward extension, the relative size and medial extension of the MFC, varus/valgus rotation, and trochlea protrusion and intercondylar notch width, respectively (**Fig. 3.6**). Modes 6 to 11 accounted for 7% of the total shape variation and described minor shape changes of the condyles and trochlear groove.

Shape parameters for Modes 1, 2, 3, and 5 were significantly different between age points in the distal femur (**Fig. 3.7** and **Table 3.1**, p<0.01). From day 12 to 60, the femoral condyles underwent mid-condyle extension (decrease in Mode 1 parameter from 1.42 to -0.93), after which Mode 1 parameters plateaued. The posterior condyle region extended outward from day 12 to day 30 (decrease in Mode 2 parameter from 1.25 to -0.76), and then retracted relative to the rest of the distal femur up to day 120. The relative size of the MFC (Mode 3 parameter) peaked at day 20, and slowly decreased up to day 120. Intercondylar notch width (Mode 5 parameter) widened from day 12 to 24, then became narrow up until day 60, and widened again at day 120.

Mode 1 parameters, describing mid-condyle extension, were linearly correlated with femur length, articular-epiphyseal cartilage thickness, and growth plate cartilage thickness (R^2 =0.96, 0.83, and 0.94, respectively; data not shown).



Figure 3.6: First 5 modes of variation of the distal femur of CB57BL/6 mice. Colored solid/dashed lines indicate +/-3 standard deviations from the mean. Black lines depict major changes in shape.



Figure 3.7: Normalized parameters for Modes of Variation 1, 2, 3, and 5 as a function of age. Data are shown as mean±SD.

	Age	Mode of Variation				
_	(Days)	1	2	3	4	5
	12	1.42 ± 0.13	1.25±0.31	-0.42±0.59	-0.02 ± 0.70	0.21±1.45
	16	1.14 ± 0.07	-0.21±0.17	1.08±0.09	-0.27±0.46	0.06±0.13
	20	0.77 ± 0.08	-0.71±0.09	1.55±0.18	0.74±0.35	-0.26±0.15
	24	0.53 ± 0.05	-0.73±0.14	0.88±0.31	0.18±0.52	-0.73±0.60
	30	0.15 ± 0.07	-0.76 ± 0.04	0.90±0.17	-0.18±0.78	0.13±0.41
	60	-0.93±0.11	0.09±0.26	-0.14±0.53	-0.65±1.42	0.95±0.86
	120	-1.23 ± 0.10	0.56±0.13	-0.45 ± 0.24	0.67±1.16	-0.62 ± 0.56
_	% Variance Explained	56.49	15.71	6.45	2.29	2.10

 Table 3.1: Distal Femur Mode Parameters at Different Age Points (mean±SD)

3.4.5 Growth maps: deformation rates, strain rates, and strain directions

Deformation and surface growth strains per day in the distal femur were highest at the condyles at day 12 and decreased with time (**Figs. 3.8** and **3.9**). Deformation rates were 0.14 mm per day between days 12 and 16, and decreased to <0.01 mm per day between days 60 and 120. Between days 16 and 20, strain rates were higher in the MFC than LFC, with maximum strains of 0.12 per day. Between days 20 and 24, strain rates were highest on the medial side of the LFC and the intercondylar notch, and between days 24 and 30, on the lateral side of the MFC. After day 30, strain rates were small (<0.03 per day). Regions of maximum strain corresponded well with regions of high deformation.

In general, directions of maximum strain were similar between all ages in the transverse and sagittal planes (**Fig. 3.10**). Strain directions in the transverse plane between days 12 and 16 were most variable, with strains in both directions along the surface contour. Along both sides of the epicondyles, directions of maximum strain primarily pointed up toward the trochlea (**Fig. 3.10A**). At the load-bearing surface of the medial condyle, principal strains were directed medially. Strain directions in the lateral condyle were more variable, with direction vectors pointing medial and lateral between days 12 and 30, and lateral after day 30. In the sagittal plane (**Fig. 3.10B**), a distinct transition point was visible near the center of the load-bearing surface of the MFC (and LFC, not shown) at all ages, where strains posterior to this location pointed toward the trochlea. Surface strains of the trochlea pointed proximally, away from the condyles.



Figure 3.8: Distal femoral shape at days 12, 16, 20, 24, 30, 60, and 120. Color maps on the younger shape indicate deformation rates (mm per day), calculated between the age intervals indicated at the top. Distal femoral shape at the older age point is overlaid (outline) for comparison.



Figure 3.9: Distal femoral shape at days 12, 16, 20, 24, 30, and 60. Color maps indicate maximum principal strain rates (per day), calculated between the age intervals indicated at the top and mapped onto the shape of the younger age point. L=lateral, M=medial, A=anterior, P=posterior.



Figure 3.10: Directions of maximum strain (corresponding to strain rates shown in **Figure 3.8**) across the right distal femur (i) transverse plane and (ii) sagittal plane through the medial condyle. Direction vectors were calculated between age intervals indicated at the top and mapped onto the shape of the younger age point. Orientation and color of arrowhead indicate direction of the vector with respect to the horizontal axis. Gray dots are points of reference at corresponding locations. L=lateral, M=medial, A=anterior, P=posterior.

3.5 Discussion

Development of the distal femoral bone-cartilage interface was generally similar between humans and mice, with subtle differences in condyle and trochlea morphology between the two species (Figs. 3.1-3.3). Distal femur size increased linearly up to age 12 in humans and day 30 in mice, with trans-epicondylar widths of 79 mm and 2.7 mm, respectively. In both species, the distal femoral SOC began with a rounded contour, followed by protrusion of the condyles and trochlear ridges and the appearance of the intercondylar notch. Using statistical shape modeling, it was possible to represent these intricate and complex 3-D size and shape changes with 11 modes of variation and corresponding shape parameters (Figs. 3.6 and 3.7). From day 12, mouse mid-condyles extended outward up to day 60, associated with a decrease in Mode 1 parameter from 1.42 to -0.93, as posterior condyle regions extended up to day 30 and then partially retracted (Mode 2, Fig. 3.7). Concomitantly, the relative size and medial extension of the MFC increased and peaked at day 24 (Mode 3), with related trends in intercondylar notch width (Mode 5). Mode 1 shape parameters (mid-condyle extension) were highly correlated with overall femur length and the thickness of articular-epiphyseal and growth plate cartilages (Fig. 3.5). In addition, growth deformations and strains decreased with age and were consistent with shape parameters (Fig. 3.8-3.10). Principal strain directions demonstrated that trochlear and shaft strains pointed proximally, while a sharp transition point in strain directions existed at the center of the MFC load-bearing region. Together, these results

quantitatively illustrate how the bone-cartilage interface takes form and expands during postnatal development and growth.

As with all animal and modeling studies, a number of limitations exist in the study design and interpretation of results. Quantitative assessment of shape changes during development was performed with wild-type C57BL/6 mice, a commonly used laboratory strain. The ages chosen for this study cover the range of mouse development starting from the appearance of the SOC as detectable by μ CT, to puberty (\sim 30 days), skeletal maturity (\sim 60 days), and up to early adulthood [29, 44, 63]. Human development was observed up to puberty (age 12) for comparison. While inherent differences in anatomy exist between humans and mice, trends in the model are applicable to human development since both species undergo the same general sequence of development that leads to functional adaptation and skeletal maturity. In the growth model, statistical shape analysis was used to describe shape-related variations of the mouse distal femur, normalized for joint size. As such, shape parameters from the model quantified relative, and not absolute, changes in proportions of anatomical features. In addition, surface strain rates between ages were calculated by assuming an exact anatomical correspondence between landmark coordinates of samples extrapolated during statistical shape modeling (extrapolation precision was within 0.03mm).

The growth and attainment of shape in the distal femur was similar in humans and mice, even with substantial differences in growth plate morphology. Transepicondylar width increased in humans from age 4 up to puberty, comparable to that of mice between days 12 to 30 (**Fig. 3.3**), and as observed to plateau similarly after puberty [15]. In both species, femoral condyles developed from a rounded contour in sagittal and coronal planes to elliptical and flattened contours (**Fig. 3.1** and **3.2**). As condyle shape and relative proportions of the MFC to LFC changed, growth plate morphology did not vary noticeably, supporting the theory that growth plates primarily contribute to longitudinal bone growth while radial expansion of the SOC affects joint shape, with final shape modulated by biomechanical loading [16, 22, 48, 61]. However, lateral extension of the human growth plate occurred in conjunction with prominence of the lateral trochlear ridge, suggesting that the distal femoral growth plate may have a role in dictating trochlear morphology. The observed similarities and differences between human and mouse bone-cartilage interfaces serve as a basis for the interpretation and generalization of statistical shape modeling results.

This is the first study to quantitatively describe shape variations, normalized for size, throughout postnatal development in MFC/LFC proportion, extension of the condyles, and intercondylar notch width. The plateau of shape parameters at ~ 60 days in mice corresponded well with the cessation of femur growth, finalization of zonal organization in articulo-epiphyseal and growth plate cartilage, and stabilization of distal femur geometry (**Figs. 3.1-3.4** and **3.7**). However, during the rapid lengthening phase, the distal femur underwent variations in shape that were not directly related to femur length and chondrocyte organization. Extension of the posterior condyle peaked around day 20 and decreased afterward, similar to the relative size of the MFC. In contrast, intercondylar notch width widened from day 12 to 20, narrowed up to day 60, and became wider again at day 120. It is unclear how functional adaptation or pre-programmed differential growth played a role in defining these transient shapes. One

of the most striking changes during pediatric skeletal development in humans is the reorientation of the tibio-femoral angle from >15° varus at birth to 10° valgus around 3 years of age, and finally decreasing to ~6° valgus by 6-7 years of age, with associated growth of the MFC [41, 45]. Similar angular remodeling changes in the mouse may be related to the observed variations in MFC size and shape described above. Analysis of these transient developmental shapes may also provide insight into questions such as why certain intercondylar notch shapes predispose the joint to osteoarthritis in adults [47], but not in adolescents.

This study also is the first to provide a direct estimate of growth deformation and strain vectors. Growth maps highlighted the spatially-distinct shape changes throughout development and corresponded well with shape parameters. As deformation and strain rates were higher in the MFC than LFC between days 16-20, Mode 3 parameters for relative MFC size peaked (large MFC:LFC size), and as rates were high on the medial side of the LFC between days 20-24, Mode 5 parameters for notch width decreased (narrow width). The alternating patterns of higher growth rates on the MFC and LFC surfaces are reminiscent of adaptive shaping of the femoral condyles as the body responds to internal and external factors. Temporal and jointspecific variations during growth and development have previously been observed in the dynamics of collagen remodeling [4, 28, 54] as well as biomechanics of articular cartilage [6, 26, 58, 59]. However, little is known about the spatial distribution of matrix components during growth in the knee, especially between the MFC and LFC. It would be interesting in future studies to investigate the relationship between developmental shape changes within the MFC and LFC, and the properties of articular cartilage in those regions of highest change.

Principal strain directions in the transverse and sagittal planes illustrated directional patterns of growth that have previously only been assumed or qualitatively described [50]. The general pattern of principal directions remained fairly consistent between ages, even as strain rates decreased with age. One interesting finding from this study was the distinct transition point in principal directions located within the load-bearing region of the condyle in the sagittal view (Fig. 3.10B). This location may be the central point of mid-condyle growth and extension, as surface strains around it were directed in opposite directions. Strains on the sides of the epicondyles in the transverse view were directed up towards the trochlea (Fig. 3.10A), which may be related to the emergence of trochlear ridges. Along the load-bearing regions of the MFC in the transverse view, strains were directed medially, matching the medial extension and growth of the MFC as described by the shape parameters (Figs. 3.7 and **3.9A**). These unique strain direction patterns of the developing distal femur serve as snapshots in time through which in vivo joint development and the mechanisms behind cartilage structural maturation can be better understood.

In conclusion, this study characterized the macroscopic shape and *in vivo* growth strains of the distal femur at the bone-cartilage interface throughout postnatal growth. Comparisons between human and mice provided a qualitative assessment of the morphological differences between species and insight into the evolutionary mechanisms of joint development. Statistical shape modeling and resultant parameters quantitatively defined initial, final, and transient geometries of the mouse distal femur

during growth. The first five modes of variation accounted for the shapes of major anatomical features such as the condyles and intercondylar notch, while latter modes were associated with fine-tuning of positions and curvatures within the joint. Growth deformation and strain maps showed highest rates in the femoral condyles and strain direction vectors that corresponded with the emergence of anatomical features. Accurate quantification of the bone-cartilage interface geometry is imperative for furthering the understanding of macroscopic mechanisms of cartilage maturation and overall joint development, as well as for developing new technologies for the diagnosis and treatment of joint disorders.

3.6 Acknowledgments

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CHAPTER 4:

3-DIMENSIONAL METRICS OF PROXIMAL FEMORAL SHAPE DEFORMITIES IN LEGG-CALVÉ-PERTHES DISEASE AND SLIPPED CAPITAL FEMORAL EPIPHYSIS

4.1 Abstract

Legg-Calve-Perthes disease (LCPD) and slipped capital femoral epiphysis (SCFE) are two common pediatric hip disorders that affect the 3-dimensional shape and function of the proximal femur. However, the current understanding and diagnosis of these disorders is based on 2-dimensional radiographic measures, primarily involving the femoral head, that only partially describe complex skeletal morphology and growth biomechanics. This study aimed to improve the 3-dimensional understanding of LCPD and SCFE morphology and provide metrics for treatment through the quantification of global proximal femoral shape using statistical shape modeling. Proximal femur deformations, determined from clinical CT scans of 32 asymptomatic, LCPD, and SCFE patients, were described with displacement, strain, and growth plate angle metrics. Asymptomatic femora underwent coordinated, growth-associated displacements and

anisotropic area dilation that were site-specific and highest at the greater trochanter. After size and age-based shape adjustment, diseased proximal femora exhibited distinct deformation and dilation patterns relative to asymptomatic femora, with corresponding differences in growth plate vector angles. LCPD femora had large displacements and surface dilations in the superior femoral head, and displacement of the lesser trochanter. SCFE femora had large displacements but minimal dilations in the femoral head, and increased greater trochanteric dilation with severity of slip. 3-D quantitative comparisons of size and age-adjusted femora are useful for preoperative clinical decision-making in the choice and execution of treatment, as well as the development of therapeutic or preventive interventions. In addition, results of this study provide insight into the mechanobiology of disease through regional and coordinated tissue deformations.

4.2 Introduction

The importance of the shape of the proximal femur for proper hip joint function has become increasingly apparent over the past decade with the recognition of femoroacetabular impingement (FAI) and the associated risk of early osteoarthritis [4, 16]. Two common pediatric hip disorders that result in altered proximal femoral morphology, often leading to FAI, are Legg-Calvé-Perthes disease (LCPD) and slipped capital femoral epiphysis (SCFE) [11, 25, 44], with an incidence of 5.5 and 10.8 cases, respectively, per 100,000 children in North America [29, 35]. In LCPD, idiopathic osteonecrosis occurs due to disruption of blood supply to, and lateral growth arrest of, the femoral head growth plate. LPCD results in altered proximal femur morphology with a misshapen femoral head, a short and wide neck, and, in severe cases, overgrowth of the greater trochanter [38]. In SCFE, the femoral head epiphysis slips relative to the femoral neck and metaphysis, due in part to excessive mechanical shear forces. SCFE results in an increasingly displaced head and misshapen neck, depending on the acuteness and severity of slip [38]. While LCPD and SCFE have been studied extensively, most analyses have been based on 2-D plain film radiographs with metrics primarily of the femoral head. Thus, there is a need for detailed 3-D analyses of global hip morphology in these pediatric disorders.

One approach to describe 3-D global morphology is statistical shape modeling (SSM) [10]. SSM provides a small number of parameters that characterize macroscopic shape patterns and may serve as practical metrics for development or disease. SSM is also ideal for biomechanical analysis, as the mapping of individual locations between

structures undergoing deformation is the foundation of continuum mechanics [15]; such deformation may be due either to externally applied stress or to internally generated growth stress [40]. SSM analyses have elucidated relationships between proximal femoral shape and risk of osteoarthritis [6, 19, 30, 42] and fractures [3, 18]. However, SSM has yet to be applied in 3-D for the analysis of skeletal deformation during growth.

The shape and size of the growing proximal femur is in part determined by the coordinated development of the three regional growth plates located at the femoral head, femoral neck, and greater trochanter. Femur lengthening at the proximal epiphysis along the shaft axis is achieved through growth of the femoral head growth plate in the superomedial direction, balanced by growth of the greater trochanteric growth plate and femoral neck isthmus in the supero-lateral direction [39]. While the femoral head growth plate has been well-studied, growth properties of the greater trochanter and femoral neck, and the coordinated shape developments within the proximal femur, remain to be determined. Characterization of the shape and orientation of the normal proximal femur growth plates would provide a foundation for comparison of developmental deformities.

For LCPD and SCFE, a variety of clinical classification schemes and surgical treatment methods [2, 17, 23, 27, 28, 32, 35, 43] have been proposed to assess proximal femoral deformities. Conventional 2-D and 3-D measures of shape abnormalities include femoral head sphericity, joint containment, degree of epiphyseal slippage, articulotrochanteric distance, and functional version and torsion, with classification based on broad ranges of parameter values (e.g., Southwick mild slip from 0-30°) or on semi-quantitative shape descriptors (e.g., Stulhberg Class IV with >1cm flattening of

weight-bearing femoral head) [22, 25, 26, 35]. While the measures and classification schemes address gross deformities in proximal femoral shape, they do not capture the 3-D extent and location of shape variability which may aid in monitoring disease and pre-operative planning. Surgical treatment includes *in situ* pinning for stabilization or osteochondroplasty for re-establishing normal contours of the femoral head, or osteotomy for the correction of chronic angular deformity, [25, 35]. Current dilemmas involve the treatment of choice and whether residual shape deformities are benign or will progress to disease including FAI and arthritis [20, 21, 28].

The hypothesis of this study was that site-specific deformations of the diseased (SCFE and LCPD) hip from the normal hip shape can be distinguished and elucidated by 3-D metrics of displacements and strains of the proximal femoral bone surface as well as angles of the femoral head growth plate. The aims of the study were to 1) advance the understanding of abnormal deformations of LCPD and SCFE through 3-D shape and growth plate metrics, and 2) provide targets for treatment by quantifying the amount and location of shape deformations relative to the asymptomatic joint. The results of this study may be useful for understanding the pathogenesis and extent of proximal femoral shape deformities, planning pre-operatively for treatment, developing preventive interventions in high-risk populations.

4.3 Results

4.3.1 The proximal femoral surface underwent spatially varying and anisotropic expansion during normal postnatal growth

In order to visualize target regions for surgical correction, normal baseline growth characteristics were established first, and compared subsequently, to abnormal growth deformations occurring with disease. Asymptomatic proximal femora in 4 age groups (n=3 each) were chosen to represent normal growth between 6 to 18 years, corresponding approximately to late childhood (6-9 y.o.), juvenile (9-12 y.o.), puberty growth spurt (12-15 y.o.), and late adolescent (15-18 y.o.) stages of human development [5].

To illustrate the normal biomechanical growth characteristics of the proximal femur, the deformations of the femoral head and the greater trochanteric epiphyses were mapped between the age groups (with each age group referred here by the average age) of the 27 asymptomatic hips (**Fig. 4.1**). First, regional growth deformations were determined, using femoral head or greater trochanter growth plates as local reference positions, registering all proximal femora at the centroid of the epiphyseal side of the growth plate, and determining displacements rates (**Fig. 4.2A-F**,**N**). Displacement rates of the femoral head and greater trochanter were both 0.8mm per year from 6 to 18 years, with a trend towards higher rates from 10.5 to 13.5 years (femoral head, 1.1mm per year; greater trochanter, 1.5mm per year; **Fig. 4.2N**). Displacement rates were relatively uniform across the femoral head, and highest at the apex of the greater trochanter. The orientations (normal vectors) of the femoral head and greater trochanter is growth plates.
were approximately constant. Theta (θ) and phi (ϕ) angles, measured from the x- and zaxes, respectively (**Fig. 4.3A,B**), were $\theta = -8.1\pm2.4^{\circ}$ and $\phi = 22.5\pm0.7^{\circ}$ for the femoral head growth plate, and $\theta = 4.3\pm1.5^{\circ}$ and $\phi = 41.2\pm0.9^{\circ}$ for the greater trochanteric growth plate (**Fig. 4.2A-F** and **Table 4.1**).

The expansion of the proximal femoral bone surface during growth was subsequently determined and illustrated by maps of regional strain (Fig. 4.2G-M,O). Principal strains and directions were depicted by ellipses indicating anisotropic growth strains or by circles indicating uniform growth strain, with the extent of expansion (the percent dilation in surface area between age groups) depicted by color of the ellipse or circle. From a biomechanical perspective, these deformations represent growth, since load-induced elastic deformations of bone are minimal (see Appendix C). Area dilation rates varied over the proximal femoral surface (Fig. 4.2G-M) with age-related trends (Fig. 4.20). Overall area dilation rates from 6 to 18 years for the femoral head, neck, and greater trochanter were 16.1% per year, 13.5% per year, and 20.6% per year, respectively, the latter two being significantly different (p=0.01). Dilation rates tended to be higher between 10.5 to 13.5 years (femoral head, 22.2% per year; femoral neck, 20.1% per year; greater trochanter, 23.9% per year; Fig. 4.2H,L) than those for the overall age range. Principal strain directions varied with age. Between 7.5 to 10.5 years, the intertrochanteric surfaces were stretched with maximum principal strains in the greater-lesser trochanter direction (Fig. 4.2G). Between 13.5 to 16.5 years, the trochanters dilated uniformly (Fig. 4.2J). Femoral neck strains were maximal in the medio-lateral direction between 7.5 to 13.5 years (Fig. 4.2G,H), and aligned along the

neck-shaft axis between 13.5 to 16.5 years (**Fig. 4.2J**). Femoral head surfaces dilated relatively isotropically in both principal directions at all ages. The corresponding volumetric scaling factor of the asymptomatic proximal femur increased linearly with age at a rate of 5.3% per year (R^2 =0.75, p<0.001; **Table 4.1**).

4.3.2 Femoral head and trochanters of LCPD and SCFE proximal femora were displaced and deformed relative to the asymptomatic femur

To quantify and localize abnormal deformation and dilatation in LCPD and SCFE, proximal femora were reconstructed using age-adjusted statistical shape parameters, and aligned to and compared with the average asymptomatic femur at the shaft and trochanter regions. Scans of disease hips included LCPD femora subdivided into cam or cam+pincer groups (n=3 each) based on alpha angle [34] and acetabular coverage parameters [45] to assess effects of impingement on femoral shape, and SCFE femora subdivided into mild, moderate and severe slips based on Southwick epiphyseal slip angles (n=3 each) [41].

In LCPD, deformation of the femoral head was substantial (**Fig. 4.3C,D,H,J**), being higher in the cam group (19.0±3.3mm) than the cam+pincer group (13.9±5.2mm). Displacements of the femoral neck and greater trochanter were similar between cam and cam+pincer groups (neck, 8.0mm; greater trochanter, 4.0mm). Femoral head growth plate normal vectors were oriented more medially in LCPD groups compared to asymptomatic (asymptomatic, $\phi = 22.5\pm0.7^{\circ}$; cam, $\phi = 42.0\pm2.9^{\circ}$; cam+pincer, $\phi = 37.9\pm6.0^{\circ}$; each, p<0.05). In SCFE hips, deformation of the femoral head, neck, and greater trochanter generally increased with severity of slip (**Fig. 4.3E-G,K-M**). Femoral head, neck, and trochanter displacements were 5.5 ± 0.8 mm, 2.9 ± 0.3 mm, and 1.8 ± 0.3 mm, respectively, for mild slips, and 21.2 ± 3.6 mm, 7.5 ± 1.2 mm, and 4.4 ± 1.1 mm, respectively, for severe slips. Femoral head growth plate vector directions became increasingly posterior and inferior with severity of slip, with moderate and severe slips significantly different from asymptomatic (asymptomatic, $\theta = -8.1\pm2.4^{\circ}$; moderate, $\theta = -53.9\pm5.0^{\circ}$; severe, $\theta = -75.4\pm15.0^{\circ}$; asymptomatic, $\theta = 22.5\pm0.7^{\circ}$; moderate, $\theta = 47.5\pm5.8^{\circ}$; severe, $\theta = 64.7\pm6.1^{\circ}$; each, p<0.001). Both growth plate angles were correlated with Southwick angles (θ : R²=0.42, p<0.05; ϕ : R²=0.79, p<0.001).

In LCPD, surface dilation was substantial (**Fig. 4.3N,O,S,T**). In cam and cam+pincer hips, dilation of the femoral head were $17.9\pm14.4\%$ and $22.7\pm7.5\%$ (p<0.001), respectively, with the majority of dilation occurring in the antero-superior region. Surfaces in those regions were stretched primarily in the medial direction. Femoral neck dilation of the cam+pincer group ($10.5\pm0.9\%$) was higher than that of the cam group ($4.6\pm1.5\%$), with maximum strain directions in both groups oriented circumferentially around the neck. In the cam group, a localized region of high dilation was present at the greater trochanter and superior neck junction that was not apparent from displacement maps. Dilations at the greater trochanter were small in both groups (<3%).

In SCFE hips, surface dilation was less marked and localized. At the femoral head, dilation was minimal (**Fig. 4.3P-R,U-W**). However, in the greater trochanter, area

dilations and anisotropic stretch increased with the severity of slip (Fig. 4.3P-R). Dilations in mild, moderate, and severe groups were $2.7\pm2.0\%$, $4.4\pm3.9\%$, and $11.5\pm6.7\%$, respectively. At the postero-superior ridge of the greater trochanter, surfaces were increasingly stretched in the medio-lateral direction. Within the femoral neck, surfaces were contracted in mild and moderate slips, and dilated in severe slips (mild, – $6.5\pm1.3\%$; moderate, $-2.7\pm1.8\%$; severe, $5.6\pm6.9\%$). Large dilations and anisotropic strains were evident at the posterior head-neck junction in severe SCFE hips (Fig. 4.3R,W).

	#	Femora	ll Head	Greater 1	trochanter
Age Group	Scaling Factor	(_o) θ	(_o) Φ	θ -180 (°)	(°) q
6-9 y.o.	1.16 ± 0.10	-14±5.4	23±1.3	-3.6±2.6	40.8±1.1
9-12 y.o.	1.32 ± 0.10	-4.6±4.8	23±1.0	1.5±2.5	38.5±2.1
12-15 y.o.	1.42 ± 0.10	-2.7±2.4	23±1.5	5.5±0.7	44±1.3
15-18 y.o.	1.58 ± 0.1	-13±5.6	22 ± 2.0	12±2.3	41.9±1.5

Table 4.1: Scaling factor and growth plate angles with respect to

		LC	D		SCFE	
Metric	Asymptomatic	cam	cam+pincer	mild	moderate	severe
		Femoral	Head			
Displacement (mm)	N/A	19.0±3.3	13.8±5.2	5.5±0.8	13.7±1.7	21.2±3.6
Area dilation (%)	N/A	17.9±14.4	22.7±7.5	-2.5±1.7	0.3 ± 2.2	0.1 ± 3.0
(_o) (-8.1±2.4	15.9±11.4	20.8±5.9	-28.7±7.8	-53.9±5.0	-75.4±15.0
(°) q	22.5±0.7	42.0±2.9	37.9±6.0	27.1±2.0	47.5±5.8	64.7±6.1
		Greater tro	ochanter			
Displacement (mm)	N/A	4.0 ± 0.4	4.0 ± 0.2	1.8 ± 0.3	3.3±0.7	$4.4{\pm}1.1$
Area dilation (%)	N/A	1.2±7.7	2.2±9.0	2.7±2.1	4.4±3.9	11.5±6.7
9 -180 (°)	4.3 ± 1.5	8.1±6.9	-7.7±4.8	13.2±3.6	7.4±3.6	10.0±2.6
(°) q	41.2 ± 0.9	40.6 ± 1.3	41.2±1.8	40.7±1.4	41.6±1.4	39.1±1.0
		Femora	l neck			
Displacement (mm)	N/A	8.3±1.3	8.0±2.2	2.9 ± 0.3	4.7±0.7	7.5±1.2
Area dilation (%)	N/A	4.6 ± 1.5	10.5 ± 0.9	-6.5±1.3	-2.7±1.8	5.6±6.9



 $dx_i'dx_i'-dx_idx_i = 2E_{ij}dx_idx_j$

Figure 4.1: Schematic of deformation and surface strain calculations in 2-D and 3-D.



Figure 4.2: (A-F) Displacement rates (colormap) of the femoral head and greater trochanter epiphyses during proximal femur development. Shapes and ages indicated at the top represent the mean within each age group (for example, age group 6-9 years is represented by the mean shape of 7.5 years). Colors are mapped onto the shape of the younger age, with the mesh shape of the older age overlaid on top. Growth plate normal vectors for the femoral head and greater trochanter are indicated as solid magenta lines for the younger age, and black lines for the older age. (G-M) Corresponding area dilation rates (colormap) and principal strains and directions (ellipse shape and orientation) for each surface patch. Dilations were determined as the percentage difference in area with respect to the younger age point over the indicated age interval. Deformations of ellipses are proportional to principal strains. The solid line within each ellipse indicates the direction of maximum strain.



Figure 4.2 (continued): (**N**) Regional displacement and (**O**) area dilation with age at the femoral head, neck, and greater trochanter. Solid symbols represent the regional values for each sample. Open symbols represent averages within each age group.



Figure 4.3: Displacement and area dilation rates of age- and size-adjusted LCPD and SCFE femora relative to the asymptomatic femur. (A) Sagittal and (B) transverse views of the reference femur shape (age-adjusted, average asymptomatic), from which displacements and area dilations were calculated. Red meshes indicate representative normal epiphyseal surfaces of the femoral head and greater trochanteric growth plates, with corresponding black lines indicating the growth plate normal vector. Growth plate vector angles were determined with respect to the x-axis (θ) in the x-y plane, and the z-axis (ϕ) in the x-z plane. (C-M) Displacements (colormap) of the femoral head and greater trochanter epiphyses in each disease subgroup. Colors are mapped onto the disease shape, with the reference shape overlaid on top. Growth plate normal vectors for the disease hip are indicated as solid magenta lines. (N-W) Corresponding area dilation (colormap) and principal strains and directions (ellipse shape and orientation) for each surface patch. Deformations of ellipses are proportional to principal strains. The solid line within each ellipse indicates the direction of maximum strain.

4.4 Discussion

This study illustrates the applicability of statistical shape modeling to define data-driven shape and deformation parameters during normal growth and in the pediatric hip disorders LCPD and SCFE. Development of the normal proximal femur was characterized by site-specific growth metrics (volumetric scaling, displacement, and dilation rates) and corresponding growth plate normal vectors. In addition, LCPD and SCFE femora exhibited site-specific deformations and anisotropic dilations relative to size- and age-adjusted asymptomatic femora, with associated differences in growth plate vectors, providing insight into the mechanobiology of disease. The metrics of the developing adolescent proximal femur elucidate regional and coordinated tissue deformation that is useful for preoperative clinical decision-making and the development of therapeutic interventions.

The interpretation of deformation maps in the context of continuum mechanics allowed determination and illustration of both displacement and dilation, complementary indices for both the understanding and treatment of disease. The progression of LCPD and SCFE shape deformities has been extensively described in 2-D from longitudinal radiographic studies [1, 7, 8, 31, 39]. However, quantification of 3-D deformation relative to the normal femur at landmark locations is essential for delineating normal and aberrant growth biomechanics. Displacement maps in the current study depicted the difference in position between the surface of a proximal femur sample and that of a reference bone; they thereby provide tangible local distance measures, useful for reshaping the proximal femur to that of a normal freely-moving

hip. In contrast, area dilation maps quantified the difference in surface area and also the directions of dilatation, reflecting the site-specific mechanobiology of hip disease. In LCPD, femoral heads exhibited relatively large displacements and also large dilations, consistent with local aberrations in growth. However, in SCFE, femoral heads displayed relatively large displacement but little dilation, indicative of rotation about the femoral epiphysis. SSM-biomechanics analysis also revealed localized dilations of the bone surface, not always manifest as substantial displacements, as evident in LCPD hips at the greater trochanter and superior neck junction (**Fig. 4.3C,H,N,S**), and in SCFE hips at the greater trochanteric ridge (**Fig. 4.3G,M,R,W**). Together, the two metrics or deformation and dilation help elucidate the *in vivo* biomechanics of health and disease.

The analyses of surface displacements and dilations were dependent on the anatomical correspondence between landmark coordinates of samples defined during SSM building. In this study, the average error between manual and automatic extrapolation methods (~2 voxels) was comparable to intra- and inter-observer variability of the manual method and suggests the automatic method as a reasonable alternative to creating dense sets of landmark coordinates. In addition, average root mean square error between reconstructed shapes using statistical shape parameters and the original shape were 0.79 mm (<1 pixel, **Fig. C.1**), demonstrating the accuracy of shape parameters in representing the 3-D proximal femur.

In LCPD, metrics at the femoral head and lesser trochanter provided insight into the mechanobiology of shape deformation. Within the antero-superior femoral head region of both groups, surface areas were dilated >25%, with maximum strains in the antero-lateral to postero-medial directions, supporting the idea of viscous flow of material within LCPD femoral heads towards regions of lower pressure [24, 33]. Significant differences from asymptomatic hips were observed even with the relatively small sample size of LCPD proximal femora in this study.

In SCFE, morphological differences at the femoral head, trochanter, and neck regions, suggested site-specific biomechanics with disease progression. With increasing severity of slip, the greater trochanter was dilated and stretched in the medio-lateral direction, and the intertrochanteric ridge was also stretched. As the greater and lesser trochanters are sites of attachment for a number of muscles, displacements of these regions result in differences in the muscle lever arm length and resultant forces on the hip joint. The femoral neck of severe slips was dilated, with compressive strains in the posterior neck along the neck axis and tensile strains in the circumferential direction (**Fig. 4.3R**), possibly due to posterior bending of the femoral neck. Together, these results suggest that overgrowth of the greater trochanter in SCFE is not simply due to growth arrest of the femoral head, but likely also involves compensatory growth of the greater trochanteric epiphysis driven by local biomechanical forces.

In conclusion, this study quantified 3-D deformations relative to the asymptomatic femur in two common pediatric hip disorders, LCPD and SCFE. Measures of displacement and dilation contribute to the understanding of the mechanobiology of the femoral head, neck and trochanters, and are useful during preoperative decision- in the choice and execution of treatment. While clinical CT scans were used in this study, the methodology is applicable to other 3-D imaging techniques, such as MRI, at appropriate image resolutions. Future directions include analyzing the articular cartilage together with bone for a better understanding of the changes occurring

in the articular-epiphyseal cartilage complex during development, using shape models and metrics for analysis of growth mechanobiology in health and disease including FAI, and evaluating the utility of shape metrics for classification or monitoring of disease progression. Delineation of regional biomechanics and resultant morphological changes within the hip joint provide tangible shape targets for surgical correction as well as therapeutic or preventive shape modulation therapies.

4.5 Materials and Methods

4.5.1 Patients

With Institutional Review Board approval, clinical computed tomography (CT) scans of 32 patients (range: 7.0-18.2 years; mean: 13.0 years) with a range of hip diagnoses were obtained. Informed consent from patients was obtained after the nature and possible consequences of the studies were explained. The number of patients was targeted to obtain a minimum of 3 hips per age or disease group (described below) to build the SSM. CT scans were obtained at a voxel size of (0.5-0.9mm)² in-plane and 0.63mm slice thickness (GE Lightspeed VCT, GE Healthcare, USA). Left and right hips were classified into asymptomatic, LCPD, or SCFE disease subgroups (**Table C.1**) by a pediatric orthopedic surgeon (H.S.H.) based on history, clinical presentation, CT assessment, and antero-posterior (A-P), frog-leg, and/or cross-table lateral radiograph assessment.

Asymptomatic (n=27) hips were comprised of morphologically normal, contralateral hips of LCPD patients, or hips of patients with tibial torsion, and analyzed in 4 age groups (6-9 years, 9-12 years, 12-15 years, and 15-18 years; n=3 each) corresponding to distinct stages during human postnatal growth. LCPD hips were classified by intra-articular impingement sequelae [43] into cam and cam+pincer subgroups. Cam impingement (n=3) was identified as an aspherical femoral head with impingement of the superior head-neck junction and confirmed using alpha angle measurements [34]. Pincer impingement (n=3) was identified as reduced range of motion due to acetabular over-coverage, assessed using Hip2Norm software [45]. SCFE

severity was classified by the Southwick angle [41], with <30° as mild (n=9), 30-50° as moderate (n=7), and >50° as severe (n=4) slip. Average Southwick angles for mild, moderate, and severe slip groups were $14\pm4^{\circ}$, $40\pm6^{\circ}$, and $62\pm4^{\circ}$, respectively. Hips with other pathologies (extra-articular impingement, developmental dysplasia, etc., n=11) were not analyzed further.

4.5.2 CT image processing

CT scans were imported into Mimics (Materialise, Belgium) for surface segmentation and 3-D reconstruction. Left femora were flipped in orientation to match right femora. Cartilage-bone interfaces were identified by thresholding for bone, segmented, cropped at the base of the lesser trochanter, and exported as point clouds for statistical shape modeling.

A coordinate system for the asymptomatic proximal femur was defined similar to previous methods [26, 37]. Femoral neck and femoral shaft axes were determined as the axes of the least squares best-fit cylinders to the respective regions. The z-axis (supero-inferior) was defined as the femoral shaft axis, the y-axis (antero-posterior) as the cross product of the shaft and neck axes, and the x-axis (medio-lateral) as the cross product of the y- and z-axes. Thus, the x-z plane contained both the femoral shaft and femoral neck axes.

4.5.3 Growth plate analyses

The epiphyseal surfaces of the growth plates of the femoral head and greater trochanter were segmented from CT scans and exported as point clouds. For each growth plate, position and direction were determined as the centroid of the point cloud and the normal vectors to a least-squares best-fit plane, respectively. Growth plate angles were defined in spherical coordinates, with $+\theta$ indicating the angle with respect to the x-axis in the x-y plane in the anterior direction (**Fig. 4.3A**), and $+\phi$ indicating the angle from the positive z axis in the inferior direction (**Fig. 4.3B**).

4.5.4 **Point-to-point Correspondences**

Point-to-point correspondences between coordinates of each femur were defined during statistical shape modeling (SSM) following previously established methods [10, 12] and described elsewhere [9]. Briefly, point-to-point correspondences were defined automatically by iteratively constructing an atlas shape [12, 13, 36] and then extrapolating points, termed *landmark coordinates*, in corresponding locations to each proximal femur. The human proximal femur SSM contained 1000 landmarks, which are the vertices of each patch in the 3-D images (**Figs. 4.2 and 4.3**). This corresponds to roughly 1 landmark for every 4.3 mm (~5 pixels) across the oldest asymptomatic hip. Point correspondences defined during atlas construction were validated by comparisons of selected landmarks to manually-defined anatomical locations (see **Appendix C, Fig. C.2** and **Table C.2**).

For comparisons between the average asymptomatic and disease groups, statistical shape parameters from SSM were used to adjust for age-related shape differences. For comparisons between the average asymptomatic and disease groups, landmark coordinates for all femora were age-adjusted to account for differences in age ranges between groups. First, statistical shape parameters from SSM that varied with age in the asymptomatic samples were identified by linear regression, and parameters subsequently adjusted to that of age 12 (mean age of asymptomatic samples). Ageadjusted landmark coordinates were then reconstructed based on the SSM mean shape and mode of variations [9].

4.5.5 **Displacement and Dilation Maps**

Displacement and surface area dilation maps were created by analyzing the corresponding landmark coordinates and then displaying these metrics in 3-D on the local surface patches of the average shape for each age or disease group.

Magnitudes of displacement were calculated as the distance between corresponding landmarks. For age growth maps, landmark coordinates represented nonscaled shapes, and all displacements were calculated with respect to the youngest femoral shape. Samples were aligned at the centroid of the growth plate (separately for the femoral head and greater trochanter) to determine displacements during growth. Only displacements for landmarks on the epiphyseal side of the growth plate were determined between age groups. For comparisons between asymptomatic and disease hips, landmark coordinates represented scaled and age-adjusted shapes, and displacements were calculated with respect to the average age-adjusted asymptomatic femoral shape by aligning to the shaft and trochanters.

Surface area dilations were plotted with directions of principal strains to illustrate local strains on the bone surface. Area dilation was calculated as the percent difference in area of each surface patch, with respect to the younger age point for growth maps, and with respect to the asymptomatic shape for disease maps. The two-dimensional components of Green's strain [14] E_{ij} were calculated from $ds^2 - ds_0^2 = 2E_{ij}dX_idX_j$ for i,j = 1:2, where $ds_0^2 = dX_idX_j$ is the squared segment length of a pair of landmarks at the first age point, and ds^2 is the squared length at the second age point.

Principal strains and directions were calculated from the Green's strain components. A unit circle was generated for each surface patch and deformed in the principal directions by the principal strains E_1 and E_2 to illustrate strain patterns.

4.5.6 Statistics

Differences in conventional and statistical shape parameters between age groups of asymptomatic patients were assessed by a one-way ANOVA with post-hoc Tukey test. Differences in parameters between disease groups were assessed by a one-way ANCOVA (fixed factor of disease group, covariate of age) with post-hoc Tukey test. In addition, disease groups were compared to the average asymptomatic group with a post-hoc Dunnett test. Correlations between conventional and statistical shape parameters, displacement and age, and area dilation and age were determined by linear regression. Displacements and dilations for each disease group were compared to a value of 0 by a 1-sample Student's t test. All data are expressed as mean \pm standard error of the mean (SEM) except where indicated.

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CHAPTER 5:

STATISTICAL SHAPE MODELING OF PROXIMAL FEMORAL SHAPE DEFORMITIES IN LEGG-CALVÉ-PERTHES DISEASE AND SLIPPED CAPITAL FEMORAL EPIPHYSIS

5.1 Abstract

The current understanding of morphological deformities of the hip such as femoroacetabular impingement (FAI), Legg-Calve-Perthes disease (LCPD), and slipped capital femoral epiphysis (SCFE) is based on 2-dimensional metrics, primarily involving the femoral head, that only partially describe the complex skeletal morphology. This study aimed to improve the 3-dimensional understanding of shape variations during normal growth, and in LCPD and SCFE, through statistical shape modeling. Proximal femur shape, determined from clinical CT scans of 32 asymptomatic, LCPD, and SCFE patients, was described by 8 modes of variation and corresponding shape parameters. Statistical shape parameters were distinct with age and revealed coordinated, growthassociated differences in neck length-to-width ratio, femoral head medicalization, and trochanter protrusion. After size and age-based shape adjustment, diseased proximal femora were characterized by shape parameters distinct from asymptomatic hips, which described region-specific differences in morphology. Statistical shape parameters were correlated with certain conventional parameters of shape, including neck-shaft angle, head diameter, and neck diameter. 3-D quantitative analyses of proximal femoral bone shape during growth and in disease are useful for furthering the understanding of normal and abnormal shape deviations which affect cartilage biomechanics and risk of developing osteoarthritis.

5.2 Introduction

The importance of the shape of the proximal femur for proper hip joint function and maintenance of cartilage biomechanics has become increasingly apparent over the past decade with the conceptualization of femoroacetabular impingement (FAI) and the associated risk of early osteoarthritis in adults [3, 7, 11]. FAI is manifest as a cam-type protrusion of the femoral neck, either alone or with a pincer-type over-coverage of the acetabular rim, and can be idiopathic or a result of childhood skeletal disorders. While the etiology of FAI is unknown, two common pediatric hip disorders that result in altered proximal femoral morphology often leading to FAI are Legg-Calvé-Perthes disease (LCPD) and slipped capital femoral epiphysis (SCFE) [5, 12, 27].

The complex and evolving femoral morphologies of LCPD and SCFE are often challenging to quantify, manage, and treat clinically [8, 12, 14, 19]. In LCPD, idiopathic osteonecrosis occurs due to disruption of blood supply to, and growth arrest of, the femoral head growth plate. LPCD results in altered proximal femur morphology with a misshapen femoral head, a short and wide neck, and, in severe cases, overgrowth of the greater trochanter [21]. In SCFE, the femoral head epiphysis slips relative to the femoral neck and metaphysis, due in part to excessive mechanical shear forces. SCFE results in an increasingly displaced head and misshapen neck, depending on the acuteness and severity of slip [21]. While these diseases have been studied extensively, most quantitative analyses have been based on 2-D plain film radiographs. Classification of these shape abnormalities are often based on broad ranges of parameter values (e.g., Southwick mild slip from 0-30°) or on semi-quantitative shape descriptors (e.g.,

Stulhberg Class IV with >1cm flattening of weight-bearing femoral head) [9, 12, 13, 18]. 3-D analyses of the gross morphological deformities in these two pediatric hip disorders would provide additional understanding of disease mechanobiology, as well as insight into deformations that occur in other morphological hip disorders such as FAI.

The 3-D shape of joints can be described by fits to local surface structure or global morphology. Local fitting of surface positions with techniques involving large numbers of parameters such as piecewise parametric surface patches, B-spline, or thinplate spline representations [2] are useful for representing detailed surface structure. Such approaches are particularly suitable for the delineating the substantial variations with age-associated osteoarthritic erosion or injury-associated cartilage lesions. On the other hand, fitting of surface positions with relatively few parameters that define global morphology, such as medial representations [25] and statistical shape models (SSM), may provide practical metrics for characteristic macroscopic shape patterns in development or disease.

The hypothesis of this study was that coordinated, regional shape deformations of the proximal femoral bone surface occur in pediatric hip disorders LCPD and SCFE, and these shapes can be quantitatively described in 3-D using statistical shape modeling. The aims of the study were to advance the understanding of normal development of the proximal femur and abnormal deformations of LCPD and SCFE through 3-D statistical shape parameters and correlations with conventional parameters.

5.3 Material and Methods

5.3.1 Patients

With Institutional Review Board approval, clinical computed tomography (CT) scans of 32 patients (range: 7.0-18.2 years; mean: 13.0 years) with a range of hip diagnoses were obtained. Informed consent from patients was obtained after the nature and possible consequences of the studies were explained. The number of patients was targeted to obtain a minimum of 3 hips per age or disease group (described below) to build the SSM. CT scans were obtained at a voxel size of (0.5-0.9mm)² in-plane and 0.63mm slice thickness (GE Lightspeed VCT, GE Healthcare, USA). Left and right hips were classified into asymptomatic, LCPD, or SCFE disease subgroups (**Fig. 5.1** and **Table E.1**) by a pediatric orthopedic surgeon (H.S.H.) based on history, clinical presentation, CT assessment, and antero-posterior (A-P), frog-leg, and/or cross-table lateral radiograph assessment.

Asymptomatic (n=27) hips were comprised of morphologically normal, contralateral hips of LCPD patients, or hips of patients with tibial torsion, and analyzed in 4 age groups (6-9 years, 9-12 years, 12-15 years, and 15-18 years; n=3 each) corresponding to distinct stages during human postnatal growth. LCPD hips were classified by intra-articular impingement sequelae [26] into cam and cam+pincer subgroups. Cam impingement (n=3) was identified as an aspherical femoral head with impingement of the superior head-neck junction and confirmed using alpha angle measurements [17]. Pincer impingement (n=3) was identified as reduced range of motion due to acetabular over-coverage, assessed using Hip2Norm software [29]. SCFE

severity was classified by the Southwick angle [22], with <30° as mild (n=9), 30-50° as moderate (n=7), and >50° as severe (n=4) slip. Average Southwick angles for mild, moderate, and severe slip groups were $14\pm4^{\circ}$, $40\pm6^{\circ}$, and $62\pm4^{\circ}$, respectively. Hips with other pathologies (extra-articular impingement, developmental dysplasia, etc., n=11) were not analyzed further.

5.3.2 CT image processing

CT scans were imported into Mimics (Materialise, Belgium) for surface segmentation and 3-D reconstruction. Left femora were flipped in orientation to match right femora. Cartilage-bone interfaces were identified by thresholding for bone, segmented, cropped at the base of the lesser trochanter, and exported as point clouds for statistical shape modeling.

A coordinate system for the asymptomatic proximal femur was defined similar to previous methods [13, 20]. Femoral neck and femoral shaft axes were determined as the axes of the least squares best-fit cylinders to the respective regions. The z-axis (supero-inferior) was defined as the femoral shaft axis, the y-axis (antero-posterior) as the cross product of the shaft and neck axes, and the x-axis (medio-lateral) as the cross product of the y- and z-axes. Thus, the x-z plane contained both the femoral shaft and femoral neck axes.

5.3.3 Conventional shape parameters

After rotation of all femurs to the coordinate system, points of the femoral head were best-fit with an ellipsoid to determine the three principal radii and the location of the center of the femoral head. In addition, the apices of the greater and lesser trochanters were identified. The following six conventional shape parameters were then determined in the 3-D datasets:

- 1. *Femoral neck-shaft angle*: the angle between the femoral neck and shaft axes.
- 2. *Intertrochanteric distance*: the shortest distance between the apices of the greater and lesser trochanters.
- 3. *Femoral head diameter*: twice the maximum radius of a least-squares best-fit ellipsoid to the femoral head.
- 4. *Femoral neck diameter*: the diameter of the best-fit cylinder of the neck.
- 5. *Femoral head eccentricity*: $(1 (b^2/a^2))^{1/2}$, where *a* is the maximum radius, and *b* the minimum radius, of the best-fit ellipsoid of the femoral head. Eccentricity ranges from 0 for a perfect sphere to a maximum approaching 1, with flattened heads having a value of ~0.7.
- 6. *Femoral head medial offset*: the medio-lateral distance from the center of the femoral head to the shaft axis in the proximal femur coordinate system.

For comparisons between the average asymptomatic and disease groups, conventional shape parameters were age-adjusted to account for differences in age ranges between groups. Parameters that varied with age in the asymptomatic samples were identified by linear regression, and parameters subsequently adjusted to that of age 12 (mean age of asymptomatic samples).

5.3.4 Statistical shape modeling (SSM)

To build the SSM (**Fig. E.1**), 27 proximal femora (n=3 per group) were used as initial training samples to capture the full range of shape variations in the study population. Training samples were rigidly registered and isotropically scaled by the coherent point drift algorithm [16] to the largest-volume sample. Point-to-point correspondences between coordinates of each femur were defined automatically, following previously established methods [6] by first iteratively constructing a normalized, average atlas shape and then extrapolating points, termed *landmark coordinates*, in corresponding locations to each proximal femur. The atlas converged after 4 iterations, as determined by the kappa statistic [6] (**Fig. E.2A**). The human proximal femur SSM contained 1000 landmarks, equivalent to 1 landmark every 4.3mm across the surface.

SSMs were built from landmarks of the respective training samples using principal component analysis [4, 6]. A mean shape was calculated from training sample landmarks, and deviations of each shape from the mean were determined. Singular value decomposition of the covariance matrix was performed to obtain the eigenvectors and corresponding eigenvalues (in descending order). The eigenvectors represent the *modes of variation* within the training set, analogous to the principal axes of an ellipse. The eigenvalues represent the *variance* explained by each mode, or the amount of contribution of each mode to overall joint shape variation. Using the modes of variation from the model, the *shape parameters*, **b**, of each sample was calculated from $\mathbf{x} = \overline{\mathbf{x}}+\mathbf{Pb}$, where $\overline{\mathbf{x}}$ is the mean shape, $\mathbf{P} = (\mathbf{p}_1 \mid \mathbf{p}_2 \mid ... \mid \mathbf{p}_i)$ are the first *t* modes of variation that explain >90% of the total shape variance, and **x** is the sample shape as represented

by the location of the landmark coordinates. Shape parameters represent the distance weighting factor for each mode of variation.

Statistical shape parameters were then determined for all samples by applying the SSM. Each sample's CT data set was segmented in Mimics, rigidly aligned to the reference femur from the training set, non-rigidly aligned to the model atlas shape to extrapolate landmarks, and then fit to the SSM to determine the shape parameters. All shape parameters were normalized to one standard deviation of the mode, calculated as the square root of the corresponding eigenvalue, to elucidate the relative variation of each mode [4]. For comparisons between the average asymptomatic and disease groups, parameters were also age-adjusted to account for differences in age ranges between groups, in the same manner as for the conventional shape parameters.

5.3.5 Statistics

Differences in conventional and statistical shape parameters between age groups of asymptomatic patients were assessed by a one-way ANOVA with post-hoc Tukey test. Differences in parameters between disease groups were assessed by a one-way ANCOVA (fixed factor of disease group, covariate of age) with post-hoc Tukey test. In addition, disease groups were compared to the average asymptomatic group with a post-hoc Dunnett test. Correlations between conventional and statistical shape parameters, displacement and age, and area dilation and age were determined by linear regression. All data are expressed as mean \pm standard error of the mean (SEM) except where indicated.

5.4 Results

5.4.1 Abnormalities in proximal femur shape were described by 8 modes of variation

The first 8 modes of variation in the SSM accounted for >90% of overall shape variation between asymptomatic and disease states, with the modes numbered in descending order based on contribution to overall shape (**Fig. 5.2**). The modes of variation described shape variations within the population, normalized for size. Mode 1 affected the overall sphericity of the femoral head and femoral neck length and width. Modes 2-4 accounted for the femoral head antero-posterior epiphyseal position and greater trochanter medial-lateral width, supero-inferior epiphyseal position and trochanter protrusion, and neck shape, respectively. Modes 5 to 8 defined femoral head shape, femoral head posterior protrusion and lesser trochanter location, and trochanteric fossa shape.

5.4.2 Statistical shape parameters were distinct between asymptomatic, LCPD, and SCFE groups

The normal growth-associated differences in shape of the asymptomatic proximal femora were quantified first. Femora at each age differed in shape in a number of ways (**Fig. 5.3A-E** and **Table 5.1**). The femoral neck length-to-width ratio decreased with age, corresponding to an increase in Mode 1 (p<0.001) (**Fig. 5.3A**). Greater and lesser trochanters became increasingly protuberant with age as the femoral epiphysis

moved medially (decrease in Mode 3, p<0.001), with small variations in the head-neck transition region, and head protrusion and sphericity (Modes 4-7, p=0.05-0.25).

To facilitate comparisons between disease and asymptomatic samples, shape parameters for modes of variation that were age-dependent (Modes 1 and 3, p<0.005), were age-adjusted to 12 years (**Table 5.2**).

In LCPD, the effects of impingement on femoral shape were evaluated overall and also for hips subdivided into cam or cam+pincer groups. Both LCPD cam and cam+pincer groups had short and wide femoral necks and flattened heads compared to asymptomatic hips (Mode 1: asymptomatic, -0.71 ± 0.06 ; cam, $+1.33\pm0.43$; cam+pincer, $+0.98\pm0.40$; p<0.01, **Fig. 5.3F**). However, the two LCPD groups were distinguished by the posterior extension of the femoral head in the cam+pincer group and the posterolateral position of the lesser trochanter in the cam group (Mode 7: asymptomatic: $+0.25\pm0.10$; cam, $+1.06\pm0.23$; cam+pincer, -1.62 ± 0.54 ; p<0.01, **Fig. 5.3J**).

In SCFE, the effects of the degree of epiphyseal slip (mild, moderate, and severe) on morphological changes were assessed. Differences in statistical shape parameters between SCFE and asymptomatic proximal femora generally increased with the severity of epiphyseal slip. With increasing slip, the femoral neck shortened (Mode 1: asymptomatic, -0.70 ± 0.06 ; severe, $+0.51\pm0.18$; p<0.05, **Fig. 5.3F**), femoral head epiphyses displaced posteriorly (Mode 2: asymptomatic: $+0.03\pm0.14$; severe, -1.63 ± 0.31 ; p<0.05, **Fig. 5.3G**), and the superior head-neck transition became indistinguishable (Mode 4: asymptomatic, -0.20 ± 0.15 ; severe, $+0.95\pm0.40$; p<0.05, **Fig. 5.3I**). Mild and severe slip groups were also distinguishable based on femoral neck shortening (Mode 1) and definition of the head-neck transition region (Mode 4).

5.4.3 Statistical shape parameters were correlated with conventional shape parameters

The statistical shape parameters (**Fig. 5.3A-J**), while describing 3-D variations in morphology often not limited to a single anatomical region, did correlate with a number of conventional indices of shape and size (**Fig. 5.3K-T**). Mode 1, describing femoral neck length and width as well as femoral head sphericity, correlated strongly with neck-shaft angle as well as neck diameter (**Fig. 5.3U,X**). Mode 3, describing femoral head supero-inferior epiphyseal position and trochanter protrusion, correlated strongly with medial offset of the femoral head and detectably with intertrochanteric distance (**Fig. 5.3V,Y**). Mode 5, affecting medial head protrusion, correlated with femoral head diameter (**Fig. 5.3W**), and Mode 6, affecting superior femoral head shape, correlated with head eccentricity (**Fig. 5.3Z**). Detailed statistics are available in **Appendix E, Tables E.2** and **E.3**.


Figure 5.1: Representative sagittal and transverse CT cross-sections of (A-H) asymptomatic left hips at 7, 10, 13, and 16 years, and affected hips with LCPD (J, O) cam impingement, (K, P) cam+pincer impingement, and SCFE (L, Q) mild, (M, R) moderate, and (N, S) severe slip.



Figure 5.2: Schematic and description of the eight modes of variation that account for 92% of the total shape variation in the human proximal femur (normalized to size). Solid/dashed lines indicate ± 3 standard deviations from the mean shape. Percentages indicate the amount of total shape variance explained by a particular mode. A = anterior, I = inferior, L = lateral, M = medial, P = posterior S = superior.



Figure 5.3: (A-J) Statistical and (K-T) conventional shape parameters of Modes 1-4 and 7, determined from 3-D datasets of proximal femora. (A-E, K-O) Parameters were directly compared between age groups of asymptomatic hips. (F-J, P-T) Parameters were age-adjusted to 12 years for comparisons between disease groups and the average asymptomatic proximal femur to account for age-associated shape differences.



0

-2

0.3

= 0.30

p = 0.003

0.5

Head eccentricity

·υ

2

1

0

-1

-2

2

1

0

-2

15

1 Mode 1 1-1

Х

0.6

35

-2

20

p = 0.04

40

Intertrochanteric

distance [mm]

60

p < 0.001

25

Neck diameter [mm]

Mode 1

Normalized statistical

shape parameter

Figure 5.3 (continued): (U-Z) Correlations between statistical and conventional shape parameters in asymptomatic proximal femora from 6 to 18 years of age. Data are shown as mean±SE. *p<0.05 compared to the average asymptomatic proximal femur. — p<0.05, — p<0.01, — p<0.005 between groups.

		Table 5	.1: Statistical	l shape pai	rameters	s for asympton	matic age groi	1ps. ^{a,b,c,d} p<0.(05	
anon J	1					Mode of V	ariation			
duore	=	1	2	3		4	5	6	7	8
6-9 years	5 -1	.13±0.08ª	0.14±0.33ab	0.69±0).23a (0.54±0.21ª	0.26±0.12ª	-0.21±0.42ª	0.29±0.25ª	-0.03±0.05ª
9-12 years	8 -0	.83±0.05ac	-0.43±0.18ª	0.65±0	.13ª -(0.41±0.26ª -	-0.06±0.11ª ^b	0.27±0.17ª	-0.07±0.10ª	-0.07±0.23ª
12-15 years	8 0-	.39±0.11 ^{bc}	0.52 ± 0.14^{b}	0-09±0).14 ^{bc} -(0.24±0.26ª -	-0.53±0.24 ^b	0.71±0.33ª	0.58±0.19ª	0.07±0.19ª
15-19 years	9-0	.51±0.15°	-0.07±0.31 ^{ab}	-0.24 ±0).15° -(0.50±0.29ª -	-0.15±0.11 ^{ab}	0.73±0.35ª	0.20±0.21ª	0.15±0.30ª
					:					
	Ial	01e 5.2: Stat	usucal snape	parameter	S IOT GIS	sease groups (age-adjusted t	0 12 years). 4,4	c0.0>d	
anon J	\$					Mode of	Variation			
duore	=	1	2		3	4	5	6	7	8
Asymptomatic	27	-0.71±0.0	6ªc 0.04±0.	14 ^{ac} 0.28	3±0.08ª	-0.20±0.15ª	-0.16±0.10ª	0.41±0.17ª	0.25±0.10ª	0.03±0.11ª
LCPD										
cam	3	1.33 ± 0.4	3 ^b 0.58±0.	13ª 0.50)±0.22ª	-0.86±0.39ªb	0.74±1.12ª	0.05±0.56ª	1.06±0.23 ^{ab}	-0.89±0.76ª
cam+pincer	3	0.98±0.4	0 ^b 1.12±0.	08ª 1.02	2±0.13ab	0.21±0.56ª	-0.22±0.47ª	-0.29±0.73ª	-1.62±0.54 ^{bc}	0.48±0.63ª
SCFE										
mild	10	- 0.66±0.1	1 ^{ac} -0.89±0.	14 ^b 0.24	t±0.09ª	-0.50±0.17 ^{ab}	0.07±0.14ª	0.54±0.21ª	-0.15±0.15 ^{ac}	-0.11±0.23ª
moderate	٢	-0.09±0.2	2 ^{cd} -0.88±0.	36 ^{bc} -0.58	3±0.55 ^b	0.26±0.20ª	0.06±0.23ª	-0.02±0.27ª	-0.68±0.40°	-0.16±0.37ª
severe	4	0.51 ± 0.1	8 ^{bd} -1.63±0.	31 ^b 0.38	3±0.23ª	0.96 ± 0.40^{b}	-0.51±0.43ª	-0.46±0.26ª	-0.04±0.54ac	0.44±0.32ª

5.5 Discussion

The complex 3-D shapes of the proximal femora in asymptomatic, LCPD, and SCFE hips were described with SSM in 8 modes of variation that accounted for >90% of total shape variation. The 8 shape parameters were distinct with age and disease group and correlated with conventional parameter such as neck-shaft angle, head and neck diameter, intertrochanteric distance, and head eccentricity (Fig. 5.3). Asymptomatic proximal femur development with age was characterized by extension of the femoral neck and greater trochanter, definition of the head-neck transition, and protrusion and increased sphericity of the medial and superior femoral head. After adjustment for age-related shape variations, LCPD cam and cam+pincer groups were characterized by flattened femoral heads, short and wide femoral necks, and posteriorly positioned lesser trochanters compared to asymptomatic femora (Modes 1 and 7), with distinct differences in A-P head morphology between the two groups. SCFE proximal femora exhibited short femoral necks, posterior displacement of the femoral head epiphyses, and lack of distinct head-neck transition with increasing slip severity (Modes 1, 2, and 4). Shape parameters were distinct with age and disease, described coordinated shape differences between asymptomatic and diseased femora, and provided insight into the morphological development of proximal femoral deformities.

Some limitations exist in the study design and interpretation of results. The current study only assessed morphological changes within the proximal femur and did not analyze torsional angles with respect to the femoral condyles or coverage from the acetabulum. In addition, the analysis was performed on ossified bone morphology, which may be affected by the stage of disease progression. Due to the limited number of LCPD cases each year that necessitated clinical CT scans, only 6 LCPD proximal femora were included in the study, compared to 27 asymptomatic and 21 SCFE femora. As the SSM was built with 3 training samples per age and disease subgroup, the small sample size in LCPD did not affect the model and shape parameters, but may limit the generalizability of results. Also, the asymptomatic group included contralateral joints from unilateral LCPD patients. While these hips were radiographically normal, there is a potential for pre-radiographic abnormalities in the joint [1, 10].

Statistical shape parameters provide information that is complementary to conventional parameters. Substantial deviations were evident for certain modes of variation (**Fig. 5.2**), and shape parameters delineate details that have been traditionally difficult to characterize, such as the head-neck transition and the trochanteric fossa shape. Statistical shape parameters were able to capture the traditionally observed anatomical-based shape changes (correlations with conventional parameters, **Fig. 5.3**) as well as complex interaction patterns of shape changes in different regions that occurred simultaneously. It is interesting to note that Mode 2 parameters (**Fig. 5.3G**), which account for posterior slip of the epiphysis, were large and negative in all three subgroups of SCFE, which may indicate the presence of a very distinct morphology present even during mild SCFE that is not necessarily captured with the conventional Southwick angle. Differential regulation of growth plate and articulo-epiphyseal cartilage kinetics during development may play a role in these coordinated shape changes during normal growth and in disease [24, 28]. In larger population studies, as well as with serial

analysis of individuals, statistical shape parameters may also be useful for the classification of disease and identification of patterns of disease progression.

During normal growth, asymptomatic femora were distinct in morphology and showed age-associated trends in shape parameters. The progression of Mode 1 (neck length and head sphericity) and Mode 3 (trochanter protrusion and superior-inferior head epiphyseal position) parameters with age may represent a normal pattern of proximal femoral shape changes that are required to develop a healthy hip joint, with deviations at a specific age point leading to morphological deformities. Decrease in neck-shaft angle from 155° to 130° from birth to adult observed previously [23] match well with the increased Mode 1 and decreased Mode 3 parameters with age, which move the femoral head epiphysis medially and relatively closer to the trochanters. Variations within the parameters capture the extent of shape plasticity during normal femur development.

Age-adjusted parameters describing the proximal femoral shape of LCPD and SCFE provided insight into *in vivo* biomechanics. In LCPD, intertrochanteric distances were similar between LCPD subgroups and asymptomatic hips; however, the position of the lesser trochanter was postero-lateral in the cam group relative to cam+pincer (Mode 7, **Fig. 5.3J**). Lateral transfer of the lesser trochanter theoretically enhances the effect of attached muscles by increasing the lever arm distance [15], but has not been noted previously. This morphological difference between cam and cam+pincer hips may also contribute to differences in femoral head shape and degree of posterior extrusion, as the muscle forces act to contain the head within the acetabulum. In SCFE, the greater trochanter medial-lateral width was high (Mode 2, **Fig. 5.3G**). As the greater trochanter

also serves as the insertion site for a number of muscles, the SCFE hip may be morphologically adapting to increased muscles forces as patients attempt to achieve the full range of motion while suffering from epiphyseal slip and consequent impingement.

In conclusion, this study characterized the 3-D proximal femoral shape during normal growth and in two pediatric hip disorders, LCPD and SCFE. Statistical shape parameters described coordinated, regional shape deformations that were distinct with age and disease and correlated with certain conventional parameters of shape. Quantification of 3-D shape during growth and with respect to the age-adjusted normal shape in disease is important for understanding normal and abnormal deformations that are also relevant to morphological disorders including FAI and dysplasia, as well as differences in morphology due to race or gender, which may increase the risk of developing osteoarthritis.

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CHAPTER 6:

CONCLUSION

The studies presented in the preceding chapters were conducted to 1) investigate the relationship between local surface shape alterations and cartilage biomechanics after cartilage repair, 2) determine the growth-associated, coordinated shape deformations of the bone-cartilage interface in the developing proximal femur, and 3) quantify abnormal shape deformations relative to the normal femoral shape in pediatric hip disorders to aid in diagnosis and intervention. The primary findings of these studies are summarized below and then discussed with suggestions for future research directions.

6.1 Summary of Findings

- 1. The effectiveness of autograft repair is associated with the structural match between the operated and non-operated joints at the articular surface and bone-cartilage interface (**Chapter 2**).
 - a. Cartilage thickness, stiffness, and histological metrics of repair were lower in the graft and adjacent host compared to the nonoperated joint.
 - b. Articular surfaces of the graft and adjacent host after 6 and 12 months *in vivo* were recessed relative to the nonoperated joint, while bone-cartilage interface deviations were variable across the joint.

- c. Cartilage stiffness was lower with increased recession of the articular surface and large deviations (both recession and protrusion) of the bone-cartilage interface.
- d. In addition, large deviations at the bone-cartilage interface were associated with articular surface recession. A normally-located bone-cartilage interface was associated with a normally-located articular surface.
- 2. Distal femoral shape at the bone-cartilage interface varied differentially with age and anatomical region during postnatal development (**Chapter 3**).
 - a. The attainment of bone-cartilage interface shape of the distal femur and proximal tibia were qualitatively similar in humans and mice, with marked differences in growth plate morphology.
 - b. Mouse distal femoral shape was described by 11 independent parameters that accounted for >90% of total shape variation during growth. Each shape parameter described changes in specific anatomical regions of the distal femur and varied with age.
 - c. Displacements and strains in the medial and lateral condyles, as well as intercondylar notch, were site-specific and varied greatly between postnatal days 12 to 120.
 - d. Principal strains corresponded well with the appearance of anatomical landmarks within the distal femur and suggest directional growth of the trochlea and condyles.

- 3. Proximal femoral shape underwent site-specific deformations at the bone-cartilage interface during normal growth and in LCPD and SCFE diseases, with corresponding changes in growth plate orientation (**Chapter 4**).
 - a. Normal, asymptomatic proximal femora underwent differential, growthassociated deformations and anisotropic areal dilations at the femoral head, femoral neck, and greater trochanter, with highest growth rates during puberty.
 - LCPD femora exhibited substantial displacements and dilations at the femoral neck and superior femoral head regions, with associated medial orientation of the growth plate.
 - c. SCFE femora exhibited displacement and dilations at the femoral head and posterior greater trochanter that increased with severity of disease, with altered orientation of the femoral head growth plate in the inferior and posterior directions.
- 4. Proximal femoral shape varied with age and anatomical region during normal postnatal growth and in LCPD and SCFE diseases (**Chapter 5**).
 - a. Human proximal femoral shape was described by 8 independent statistical shape parameters that accounted for >90% of total shape variation and described coordinated, global shape changes during growth and in disease.
 - Normal, asymptomatic femora exhibited coordinated, growth-associated differences in neck length-to-width ratio, femoral head medicalization, and trochanter protrusion with age.

- c. After size and age-based shape adjustment, diseased proximal femora were characterized by shape parameters distinct from asymptomatic hips, which described region-specific differences in morphology
- d. Statistical shape parameters were correlated to certain conventional parameters describing local anatomical features.

6.2 Discussion

The main contribution of this thesis is encompassed in the 3-D deformation and area dilation maps during normal joint development and with disease, which offer insight into *in vivo* joint deformations and strains that affect shape development. The results presented here also demonstrate correlative links between structural features of the osteochondral unit of the synovial joint and the biomechanical properties of the articular cartilage that are important for understanding the role of joint shape in cartilage maturation. Comparisons between normal and abnormal surface geometries were possible with the definition of dense matrices of corresponding locations from point registration techniques and statistical shape modeling. Results of this work provide tangible 3-D metrics that may be useful for future shape modulation therapies and offer a platform for future analyses of longitudinal shape changes as well as effects of treatment on joint shape.

In the formulation of this dissertation, consideration was given to the various animal models and diseases that could be studied. Growth and cartilage repair studies were conducted in murine and caprine animal models to determine the effects of unperturbed and altered biomechanics, respectively, on the shape of cartilage and bone. Advantages of using these animal models include the ability to perform histological and biomechanical analyses, as well as future opportunities to correlate the results of this work with the extensive database of molecular and genetic studies. While inherent differences in anatomy exist between humans and animal models, trends in the shape development and cartilage remodeling are applicable to humans, since both undergo the same general sequence of development that leads to functional adaptation and skeletal maturity. The hip disorders, LCPD and SCFE, were chosen as targets for shape analysis because the disease morphology originates from the proximal femur, as opposed to disorders such as hip dysplasia, in which the femoral morphology may be a result of acetabular undercoverage. These diseases were studied in the pediatric population to apply the 3-D joint shape analysis in a translational setting and provide useful metrics for treatment and intervention.

As with all animal and modeling studies, a number of limitations exist in the interpretation of statistical shape modeling results. Surface deformation and area dilations were determined by assuming an exact anatomical correspondence between landmark coordinates of samples extrapolated during statistical shape modeling (extrapolation precision was within 0.03mm). These landmark coordinates, determined during atlas building, are dependent on the choice of affine and non-rigid registration techniques, and different correspondence definitions of these landmarks may ultimately result in different displacement and strain values. The algorithms applied in this study have been previously tested and validated in statistical shape modeling studies of different organs [5, 6, 9, 10]. In addition, numerous validation tests were also performed by the author of this dissertation to verify correspondence mappings and landmark

extrapolations (see Appendix F). However, as the true correspondences between femurs during growth or after deformation cannot be known without using biomarkers, there is an innate amount of error in surface shape modeling. Another point to note is that statistical shape analysis was used to describe shape-related variations, normalized for joint size. As such, shape parameters from the model quantified relative, and not absolute, changes in proportions of anatomical features.

Chapter 2 was one of the first studies to compare the 3-D structure of osteochondral grafts with cartilage biomechanical properties across a large region of the repaired joint. The trends for lower cartilage stiffness with recession of the articular surface in operated knees, and for cartilage stiffness values close to nonoperated with small deviations, suggest that local surface deviations may influence cartilage remodeling and homeostasis. Articular surface recession may lead to altered mechanics, different from those needed to maintain normal cartilage viability and mechanical properties [8, 16]. In addition, the association between bone-cartilage interface location and cartilage stiffness suggests that regions of large deviations (proud or recessed) at the bone-cartilage interface may have contributed to articular surface subsidence and lower normalized cartilage stiffness.

Cartilage structure and quality within the graft likely reflect a number of factors and remodeling responses. Histological indices of deterioration (GAG depletion, chondrocyte clustering) and cartilage thickening were consistent with features of early OA, while cartilage thinning and low stiffness may be related to late OA-like degeneration. Cartilage changes may also be associated with tidemark remodeling within the graft. While no correlation was observed between proud bone and vascular invasion, operated grafts had significantly more blood vessels crossing the tidemark closest to the articular surface compared to nonoperated donor LT and recipient MFC sites, indicative of vigorous and possibly OA-like remodeling. Together, these findings support the idea that aberrant local geometry of the articular surface and bone-cartilage interface may adversely affect cartilage biomechanical properties.

With the establishment of the importance of joint shape to the maintenance of cartilage biomechanics, the normal deformations in shape of the femoral bone-cartilage interface were quantitatively defined in **Chapters 3** and **Appendix B**. Normal femur development has previously been described qualitatively and quantitatively by regional shape measures such as femoral head diameter. Using statistical shape modeling, it was possible to represent the coordinated, global size and shape changes of the mouse distal femur with 11 modes of variation and corresponding shape parameters. In addition, growth deformation and strain maps illustrated spatially-distinct, directional patterns of growth that have previously only been assumed or qualitatively described [15]. These strain patterns of the developing distal femur serve as snapshots in time through which *in vivo* joint development and the mechanisms behind cartilage structural maturation can be better understood.

During the rapid growth phase, the distal femur underwent site-specific variations in shape at the condyles and intercondylar notch that were not directly related to femur length and chondrocyte organization. It remains to be established how functional adaptation or pre-programmed differential growth played a role in defining these transient shapes. One of the most striking changes during pediatric skeletal development in humans is the reorientation of the tibio-femoral angle from >15° varus

at birth to 10° valgus around 3 years of age, and finally decreasing to \sim 6° valgus by 6-7 years of age, with associated growth of the MFC [11, 12]. Similar angular remodeling changes in the mouse may be related to the observed variations in MFC size and shape. Analysis of these transient developmental shapes may also provide insight into questions such as why certain intercondylar notch shapes predispose the joint to osteoarthritis in adults [13], but not in adolescents.

Finally, **Chapters 4** and **5**, and **Appendices C-E**, illustrated the applicability of statistical shape modeling to define data-driven shape and deformation parameters during normal growth and in the pediatric hip disorders LCPD and SCFE. The progression of LCPD and SCFE shape deformities has been extensively described in 2-D from longitudinal radiographic studies [1-3, 7, 14]. However, quantification of 3-D deformation relative to the normal femur at landmark locations is essential for delineating normal and aberrant growth biomechanics. Displacement maps in the study depicted the difference in position between the surface of a proximal femur sample and that of a reference bone; they thereby provide tangible local distance measures, useful for reshaping the proximal femur to that of a normal freely-moving hip. In contrast, area dilation maps quantified the difference in surface area and also the directions of dilation, reflecting the site-specific mechanobiology of hip disease.

Together, the two metrics or deformation and dilation help elucidate the *in vivo* biomechanics of health and disease. In LCPD, displacement of the lesser trochanter between cam and cam+pincer hips may contribute to differences in femoral head shape and degree of posterior extrusion, as the lever arm of muscles that attach to the lesser trochanter are altered. In SCFE, dilation and anisotropic stretch near the greater

trochanter, which serves as the insertion site for a number of muscles including the piriformis and obturators, suggests morphological adaptation due to increased muscles forces as patients attempt to achieve the full range of motion with femoral deformities. The metrics of the developing adolescent proximal femur elucidate regional and coordinated tissue deformation that may aid in clinical decision-making and the development of intervention therapies.

6.3 **Future Directions**

The work presented in this dissertation can be expanded in a number of ways. Some of the major directions include investigating additional tissue structures or joints with the femoral bone-cartilage interface to elucidate the coordinate shape changes that occur during joint morphogenesis, using the 3-D metrics for finite element and other analyses to enhance the understanding of mechanobiology during postnatal development, and applying the 3-D metrics in the clinical setting to aid in the diagnosis, intervention, and prevention of skeletal disorders.

The shape analyses performed in this work were based on clinical CT and μ CT scans of the femoral bone, and can be extended to different tissues and joints. The methodology presented in this dissertation is applicable to other 3-D imaging techniques, such as MRI, at appropriate image resolutions, which may provide additional visualization of soft tissue structures such as articular cartilage and synovium. Immediate future applications of this work include analyzing the articular cartilage together with bone for a better understanding of the changes occurring in the articulo-epiphyseal cartilage complex during development and in disease, as well as analyzing

opposing surfaces of the joint together to investigate coordinated shape changes and dynamic joint diseases such as femoroacetabular impingement. 3-D shape modeling can also be applied to study the effects of gene knock-outs or biochemical and biomechanical modulation on shape development in animal models, and can be extended to different joints and organ systems in the body.

3-D shape metrics can be used in a number of different applications to further the understanding of mechanobiology during development and in disease. The statistical shape model describes shape changes that are limited to the variations present within the sample population used to build the model [4]. As such, different 3-D joint shape scenarios within the population variance can be reconstructed by altering the statistical shape parameters. These shape scenarios may be useful in finite element analyses to determine stress distributions with progressive alterations in shape, or for tissue engineering approaches to create joint-scale grafts with variable, but populationrelevant, geometries. Site-specific displacement and strain metrics can also be correlated to local extracellular matrix distribution or cellular organization to elucidate the molecular basis of shape changes during growth or in repair.

Finally, as suggested in **Chapter 4** and **Appendix D**, shape metrics may be applied to the clinical setting in the future to aid in diagnosis and treatment of skeletal disorders. With the development of a robust statistical shape model based on a large population of training samples, 3-D shape analyses can be automatically performed on CT or MRI scans to evaluate the extent and location of shape deformations relative to the normal joint. These analyses would provide clinicians with visualization of the regions of joint deformation along with tangible metrics for recontouring the joint surface. Furthermore, cluster analysis of statistical shape parameters may be an alternative method for disease diagnosis or classification, and may provide additional sensitivity to early manifestations of skeletal disorders. With the additional insight into various joint morphologies provided by 3-D shape metrics, clinicians and scientists will be able to develop novel surgical and non-surgical therapies for shape modulation of joints during growth, repair, and in disease.

6.4 References

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APPENDIX A:

SUPPLEMENTARY MATERIAL FOR CHAPTER 2

A.1 Introduction

Autologous osteochondral grafts (autografts) are attractive as treatments for small cartilage defects due to their native tissue architecture. However, the extent to which autografts can repair and integrate with the host tissue remains unclear. The hypothesis of this study was that properties of the articular cartilage of trochlear osteochondral autografts and of the adjacent femoral condyle are associated with the 3-dimensional geometrical match of articular surface and bone between grafted and contralateral joints at 6 and 12 months after surgery.

A.2 Methods

A.2.1 Surgical Model

Unilateral graft transfers were performed using the Mosaicplasty Complete Instrumentation kit (Smith and Nephew, Andover, MA). The graft was harvested with a trephine from the lateral trochlea (LT) to obtain an osteochondral core with outer diameter 3.5 mm and height 6 mm. The recipient site was prepared using the provided drill guide, drill, and dilator to create a recipient socket of inner diameter 3.5 mm and a depth of 6 mm. The graft was removed from the coring tool with a tamp and gently inserted to the desired depth of 6 mm using the delivery tamp and adjustable plunger.

Animals were cast in a modified Thomas splint for 7 days postoperatively and had free range of motion after cast removal. LT defects were allowed to spontaneously heal, and contralateral knees served as non-operated controls. Thigh circumferences for the operated leg at euthanasia were 31 ± 1.7 and 30 ± 0.8 cm for 6 and 12 month groups. In the 12 month group, one animal died 3 weeks early due to endotoxemia from unknown causes, and joints were immediately harvested and analyzed.

Joints were photographed upon harvest and grossly examined for osteophytes, cartilage surface quality (smooth, rough, or eroded), discoloration, and integration with the adjacent host cartilage.

A.2.2 Indentation Mechanical Testing

At each indentation site, the cartilage surface was identified using a contact tare force of 9.8×10^{-4} N (equivalent to 7.80 kPa), followed by a constant displacement rate (100 µm/second), single indentation to a depth of 100 µm for 1 second. Samples were aligned such that the articular surface was perpendicular to the indentation testing axis. The mechanical testing sampling pattern was chosen to minimize testing time while capturing significant variations across the joint, as cartilage properties varied more in the proximal-distal than medial-lateral directions. Samples were kept moist during testing with drops of phosphate-buffered saline (no calcium or magnesium) supplemented with proteinase inhibitors (2 mM Na₂-EDTA, 1 mM PMSF, 5 mM Benz-HCL, 10 mM NEM). Care was taken not to affect the graft or interface regions during removal of condyles for additional analyses. Indentation testing took approximately 45 minutes for 63 sites per joint.

A.2.3 Micro-Computed Tomography (µCT)

Samples were secured inside a closed container containing a wet Kimwipe to preserve moisture, with air as contrast. Samples were scanned at 45 μ m³ resolution, 80 kV, and 450 μ A (GE eXplore Locus system, GE Healthcare, London, Canada) and reconstructed with GE Reconstruction software.

Radio-opaque pins (diameter $[\emptyset] = 0.25$ mm, height [h] = 3 mm, stainless steel; Fine Science Tools, Foster City, USA), used to register indentation measurements to μ CT images, were located >1.5 mm away from all surface and thickness measurements. X-ray scatter from the pins was analyzed using profile gradients across the backgroundcartilage-bone interfaces and determined to have a negligible effect on thickness measurements >1.0 mm away from the pins.

A.2.4 Histology

Tissue blocks were decalcified in 10% formic acid, paraffin-embedded, and bisected along the central axis based on the registration marks. 5µm thick sections were obtained along the indentation test paths. Sections were stained for hematoxylin and eosin (H&E), collagen I (COL-I), and collagen II (COL-II), and digitized at 20X magnification (ScanScope, Aperio Technologies, Vista, USA). Sections were evaluated with the modified O'Driscoll and International Cartilage Repair Society (ICRS) I scores, with scores of 0 describing osteoarthritic samples and maximum scores representing normal cartilage. The modified O'Driscoll score evaluated sections for the nature of the

predominant tissue (score 0-7, cellular morphology, Safranin-O staining), structural characteristics (score 0-9, surface regularity, structural integrity, thickness, bonding to the adjacent cartilage), freedom from degenerative changes in the graft (score 0-4, hypocellularity, chondrocyte clustering), freedom from degenerative changes in the adjacent host (score 0-3), subchondral bone reconstitution (score 0-3), and inflammatory response in the subchondral bone (score 0-2). Data are presented as the summation of scores from each main category. With the ICRS I Visual Assessment Scale, tissue was scored for surface appearance (smooth/irregular), matrix structure (hyaline/fibrous), cell distribution (columnar/disorganized), cell population viability, subchondral bone (normal/necrosis), and cartilage mineralization (normal/abnormal), with scores for each category ranging from 0-3.

A.2.5 Data Analysis

Articular cartilage surfaces and bone-cartilage interfaces were segmented and reconstructed from μ CT scans in Mimics using a combination of thresholding, region growing, and morphologic operations. 3-D registration of joints to determine anatomical site-matched cartilage properties was performed by 1) locating sites of indentation on the reconstructed articular surface of both operated and nonoperated condyles, and 2) aligning operated and contralateral nonoperated surfaces together (**Fig. A.1**). In the first step, the articular surface was aligned such that the graft surface was parallel with the transverse plane. The centers of the registration pins were located, and sites of indentation were identified based on set distances from the pins (**Fig. A.1A**). The second step involved rigidly aligning the nonoperated bone-cartilage interface to the operated interface using the Mimics built-in STL registration algorithm, which iteratively

minimizes the distance between two surfaces (**Fig. A.1B**). The transformation matrix of the alignment was calculated using custom MATLAB code and applied to similarly transform the cartilage surface. Cartilage stiffness and thickness measurements were compared, and surface shape deviations determined, at anatomical site-matched locations based on nonoperated to operated condyle registrations. Accuracy of the registration method was determined by calculating the root mean square error between non-operated distal adjacent host cartilage of operated and nonoperated joints.

Test sites within a 1.25 mm radius of the graft center were defined as graft region. Test sites beyond a 1.75 mm radius from the graft center were defined as either proximal or distal adjacent host cartilage (PAHC, DAHC) region. A 0.5 mm ring of points along the graft-host interface was omitted from regional averages to avoid edge effects.

Cartilage thickness was calculated from μ CT images and compared to measurements made from histology. μ CT scans were oriented using the registration markers such that the indentation testing axis was parallel to the condyle sagittal plane, and the graft articular surface was parallel to the transverse plane. Corresponding histology and μ CT sections were found by manually matching trabecular bone structure to determine the accuracy of histology section locations relative to indentation sites and thickness measurements defined in the μ CT scans. Accuracy of cartilage thickness measurements from μ CT were assessed by comparison to manual measurements from histology for central, medial, and lateral indentation test axes at 504 test sites in the MFC. In histology sections, cartilage thickness was measured at the indentation sites as the vertical distance between the articular surface and bone-cartilage interface. Structural stiffness (N/mm) was determined as the peak force at each testing site divided by the applied 100 μ m indentation depth. The custom-fit function to healthy goat cartilage thickness and structural stiffness (*n* = 8 joints, separate study, **Fig. A.2**) was:

$$SS_{F}(th) = 0.2013 + [5.59e^{-2.091(th - 0.1667)}] / [th - 0.1667]^{0.5218}$$

where SS_F is the structural stiffness (N/mm) calculated from the fit, and *th* is the cartilage thickness (mm).

Host-implant variability was calculated as the standard deviation of a data set containing an equal number of graft and adjacent host cartilage locations along the path. Incremental variability was determined by averaging the change in value between locations *i* and i+1 for the same set of data.

Trabecular bone morphometric parameters in graft and adjacent host regions (**Fig. 2.2E,F,L,M**) were calculated from cylindrical ($\emptyset = 3 \text{ mm}$, h = 4 mm) volumes of interest (VOI), centered 4.5 mm below the bone-cartilage interface. The cylinder axis was placed parallel to the coronal plane in graft and AHC regions, with the VOI center at the sagittal slice through the center of the graft. Parameters of percent bone volume (BV/TV), trabecular thickness (Tb.Th), number (Tb.N), and separation (Tb.Sp) were determined within the VOIs using CTAn software (Skyscan, Belgium).

Tidemark remodeling and vascularization of the calcified cartilage region were analyzed using sagittal histology sections along the center of the graft. The number of tidemarks and the number of blood vessels (and surrounding lamellar bone) crossing the tidemark closest to the articular surface were calculated in a 2 x 1 mm (WxH) region at the center of the graft. The relationship between normalized stiffness and the difference in thickness between operated MFC and nonoperated LT samples (i.e., the difference between cartilage thickness at test sites within the operated graft region and the mean thickness of contralateral nonoperated LT donor regions) were determined to estimate cartilage thickening in the graft.

A.2.6 Statistical Analysis

Data are reported as mean ± standard error of the mean (SEM) and compared as follows. Bone morphometric parameters were log-transformed because the data varied substantially (>2-fold), and standard deviations were proportional to the mean [22]. Cartilage volume measurements were not transformed. Data were subsequently analyzed by two-way repeated-measures ANOVA to assess effects with a fixed factor of remodeling time (6, 12 months) and a repeated factor of surgical operation (operated, nonoperated). Correlations between normalized stiffness and difference in thickness from nonoperated LT were determined by linear regression.



Figure A.1: Schematic illustrating registration method to determine site-specific cartilage and bone properties. **(A)** Biomechanical indentation sites were mapped onto micro-computed tomography (μ CT) and histology image data for operatedd and contralateral nonoperated articular surfaces using the registration pins (μ CT) or vertical lacerations (histology) as landmarks. Surfaces were oriented so that the graft region was parallel to the transverse plane, and indentation sites were identified based on distances from pins, as set during biomechanical testing. **(B)** Sites on nonoperated joints were mapped to those of operated joints for each animal by matching sites on the joint surface. From the overall joint contour, articular surfaces of nonoperated and operated joints were aligned using an algorithm that minimizes the distance between the two surfaces. Then, the series of sites on nonoperated joints was matched to those on operated joints, typically with a shift of 0 or 1 site (proximal or distal).



Figure A.2: Indentation stiffness measurements were normalized to cartilage thickness according to a curve (red) that was fit to the indentation stiffness of 413 sites from healthy goat knees of 8 animals.

A.3 Results

A.3.1 Gross Morphology

Overall shapes of both operated and contralateral nonoperated were normal at harvest, with local alterations in structure around the graft. No osteophytes were observed in all joints. At 6 months, one graft contained macroscopically smooth cartilage, two grafts were rough and fragmented, and one graft was eroded. In all grafts, cartilage coloration matched adjacent host cartilage. A gap was present at the graft-host boundary in two of four joints. At 12 months, one graft was smooth at the surface, two grafts were rough, and one graft contained a focal lesion in the center. An orange discoloration and gap was visible at all graft edges. In all joints, host cartilage immediately adjacent to the graft was mildly roughened, while distal posterior condyle cartilage was smooth. No degenerative changes were observed in contralateral nonoperated knees or the lateral femoral condyle of operated knees. Defects in the LT donor sites were filled with fibrous tissue.

A.3.2 Histology

H&E sections showed loss of cell viability and chondrocyte clustering in the deep zone of graft cartilage (**Fig. A.3**). An abundance of osteoblasts, osteocytes, and fibrotic tissue was present in the marrow space directly underneath graft cartilage, while adjacent lamellar bone appeared normal. Typical bulk tissue staining of COL-II and slight surface staining of COL-I was present in both graft and host regions of operated and nonoperated joints (**Figs. A.4** and **A.5**).


Figure A.3: Hematoxylin and eosin (H&E) sections along the central test path at (A-C) 6 and (D-F) 12 months in nonoperated lateral trochlea, nonoperated medial femoral condyle (MFC), and operated MFC regions. Higher magnifications of the (i) graft-host interface, (ii) host cartilage, and (iii) graft cartilage are shown.



Figure A.4: Collagen I sections along the central test path at (A-C) 6 and (D-F) 12 months in nonoperated lateral trochlea, nonoperated medial femoral condyle (MFC), and operated MFC regions. Higher magnifications of the (i) graft-host interface, (ii) host cartilage, and (iii) graft cartilage are shown.



Figure A.5: Collagen II sections along the central test path at (A-C) 6 and (D-F) 12 months in nonoperated lateral trochlea, nonoperated medial femoral condyle (MFC), and operated MFC regions. Higher magnifications of the (i) graft-host interface, (ii) host cartilage, and (iii) graft cartilage are shown.

A.3.3 Cartilage Thickness: Comparisons of Histology and µCT

 μ CT images along the central testing axis matched well with cartilage geometry and bone structure from histology sections. Histology sections were within ±0.25 mm from the testing paths defined from registration markers in the μ CT scans. Thickness measurements between μ CT and histology sections were highly correlated (p < 0.001), with a 95% confidence interval of 0.96 to 0.99 for the slope (**Fig. A.6**).

A.3.4 3-D Whole-Joint Alignment

Cartilage surfaces were clearly distinguishable from air and bone and were successfully segmented from μ CT datasets. 3-D operated and nonoperated bone interfaces were well-aligned, with a root mean square error of 0.07 ± 0.01 mm. In all animals, operated thickness of DAHC matched that of nonoperated (6 month, 1.27 ± 0.10 mm; 12 month, 1.25 ± 0.08 mm).

A.3.5 Bone Morphometry

Bone structural differences were observed between μ CT scans of operated and contralateral nonoperated joints at 6 and 12 months (**Fig. 2.3E,F,L,M**) and quantified by morphometric analysis (**Fig. A.7**). Trabecular thickness in operated graft regions (6 month, 0.29 ± 0.03 mm; 12 month, 0.30 ± 0.03 mm) was higher than nonoperated (6 month, 0.21 ± 0.01 mm; 12 month, 0.21 ± 0.00 mm). Trabecular separation was also higher in operated compared to nonoperated graft regions. Compared to graft regions, adjacent host tended to have higher trabecular number and ratio of bone to total volume.



Figure A.6: Linear correlation between thickness measurements from histology and micro-computed tomography at 507 test sites.



Figure A.7: (A) Bone volume to total volume, (B) trabecular thickness, (C) trabecular number, and (D) trabecular separation measurements for 6 and 12 month operated and nonoperated graft and adjacent host regions. Significant effects of treatment (operated versus nonoperated joints) and postoperative time (6 and 12 months) indicated as p<0.05, p<0.01, p<0.01, p<0.05. Data are shown as mean \pm SEM.

A.3.6 Tidemark Remodeling and Vascular Invasion in the Graft

The number of tidemarks within the graft was higher than corresponding recipient sites in nonoperated MFC (p < 0.01) but similar to donor site nonoperated LT at 6 and 12 months (operated graft, 2.6 ± 0.3 ; nonoperated MFC, 5.0 ± 0.2 ; nonoperated LT, 3.0 ± 0.3). The number of vessels crossing the tidemark closest to the articular surface in the operated graft was higher than both nonoperated MFC and nonoperated LT (operated graft, 2.1 ± 0.3 ; nonoperated MFC, 0.0 ± 0.0 ; nonoperated LT, 0.6 ± 0.1 ; p < 0.05).

A.3.7 Correlation between Normalized Stiffness and 3-D Structure

Structural changes due to remodeling were apparent, based on the difference between operated graft and nonoperated LT donor cartilage properties. The difference in cartilage thickness between operated graft and nonoperated LT donor regions was positively but weakly correlated with normalized stiffness of the graft at both 6 and 12 months, and 6 and 12 months combined ($R^2 = 0.07-0.11$, p < 0.001) (Fig. A.8).

A.3.8 Sample Summaries

Summaries of histological, structural, and mechanical results of each individual operated and nonoperated sample are shown in **Figs. A.9-A.16** for 6 months, and **Figs. A.17-A.24** for 12 months.



Figure A.8: Correlation between stiffness and recovery of cartilage thickness, measured as the difference in thickness from nonoperated lateral trochlea cartilage, at 6 and 12 months of operated medial femoral condyles.



Figure A.9: Summary of 6 month nonoperated sample, 3106L.



Figure A.10: Summary of 6 month nonoperated sample, 3127L.



Figure A.11: Summary of 6 month nonoperated sample, 3131L.



Figure A.12: Summary of 6 month nonoperated sample, 3149L.



Figure A.13: Summary of 6 month operated sample, 3106R.



Figure A.14: Summary of 6 month operated sample, 3127R.



Figure A.15: Summary of 6 month operated sample, 3131R.



Figure A.16: Summary of 6 month operated sample, 3149R.



Figure A.17: Summary of 12 month nonoperated sample, 3107L.



Figure A.18: Summary of 12 month nonoperated sample, 3110L.



Figure A.19: Summary of 12 month nonoperated sample, 3134L.



Figure A.20: Summary of 12 month nonoperated sample, 3148L.



Figure A.21: Summary of 12 month operated sample, 3107R.



Figure A.22: Summary of 12 month operated sample, 3110R.



Figure A.23: Summary of 12 month operated sample, 3134R.



Figure A.24: Summary of 12 month operated sample, 3148R.

A.4 Discussion

3-D registration of operated and contralateral nonoperated joints offered advantages of whole-joint matched comparisons of cartilage and bone surface contours as well as location-dependent properties. The scalpel mark and pin system successfully registered different analyses of repair in this study. 2-D histology sections taken along each test path corresponded well with path locations from µCT registration markers. In addition, the high correlations between thickness measurements made from histology and μ CT demonstrate the success of registration. Alignment and registration can be performed in a variety of ways [3, 11, 21], and appeared effective in the present study with excellent agreement for the surfaces of the posterior condyle (non-operated region) between the left and right knees of each animal. Comparisons of properties such as cartilage thickness at matched locations have been done by defining regions [5, 24] or anatomical coordinate systems on the femur [4]. Locations for mechanical tests are often defined based on the graft edge or center and visually matched on the contralateral joint, or by measuring a specific distance from anatomical landmarks. Analyses based on 3-D joint registration reduce the effect of user variability associated with visually estimating sites or defining anatomical coordinate systems on the condyle surface. In addition, this method is applicable for a variety of metrics and may be further extended to quantify whole-joint shape changes due to surgical operation or shape disparities between contralateral joints.

Subchondral bone remodeling was present but variable between grafts, which may have contributed to the variations in cartilage remodeling responses. Deviation maps of the bone-cartilage interface confirmed incongruities in subchondral bone height at the graft-host interface observed in 2-D histology and μ CT images (**Figs. 2.2C,F,J,M** and **2.4E,F**). The presence of cysts with dense surrounding bone walls is consistent with observed changes in bony architecture of autografts in sheep after 3 and 6 months,[15] and may account for the higher trabecular separation and trabecular thickness, and trend in lower trabecular number, within the 3-D VOI of operated grafts compared to nonoperated. Currently, strategies to control bone orientation, cyst formation, and tidemark remodeling within the graft remain elusive [20, 23].

The adjacent host cartilage, which has been less well-characterized in the literature, [6, 13, 23] also showed mild surface fibrillation and had lower O'Driscoll scores compared to the contralateral nonoperated cartilage (Fig. 2.3). "Cartilage flow" of the adjacent host, where peripheral cartilage begins to curve and push into the graft region (Fig. 2.2J, boxed), was present at 6 months and more so at 12 months, as evidenced by slanted columns of deep zone chondrocytes. A ring of recessed bone surrounding the graft corresponded to regions of adjacent host cartilage flow and may have been a result of surgical trauma or subchondral cysts near the graft-host interface, as excessive disruption of the subchondral plate has been suggested to result in bone resorption and cyst formation [10, 19]. This phenomenon has previously been observed in osteochondral grafts [12, 13] as well as spontaneous defect repairs after the collapse of the defect bone walls [14]. In the operated knee, a combination of poor graft nutrition [2, 8] and subchondral bone resorption, commonly observed with osteochondral grafting procedures [7, 23], may have resulted in a small-scale collapse of the bone near the graft edge, leading to cartilage flow and surface fibrillation. Profiles of cartilage thickness and

stiffness across the operated joint showed decreasing values approaching the graft-host interface (**Fig. 2.7**) and support macroscopic observations of degeneration and lack of cartilage integration. These results indicate that the presence of an AOCG alters normal joint geometry and biomechanics in a way that can also significantly affect the adjacent host cartilage.

Stiffness measurements were affected substantially by cartilage thickness, which is relatively thin in the normal Spanish goat cartilage and varies markedly across the repair tissue. Theoretically, indentation stiffness measurements are independent from thickness for tissue >2 mm thick [18]. However, Spanish goat cartilage is <1.5 mm in the MFC and <1 mm in the LT, and previous methods [9] that modeled cartilage as a homogeneous, elastic, incompressible solid, with a Poisson's ratio of 0.5 and a perfectly rigid underlying bone, did not produce a good fit for healthy goat cartilage. In addition, repair tissue was thin and contained non-uniform underlying bone, which would result in high apparent stiffness values that were not representative of cartilage properties, but rather reflect the underlying bone. A custom curve fit was determined for normal goat cartilage properties with the conditions that stiffness reaches a finite value as cartilage becomes infinitely thick and approaches infinity at a non-zero thickness threshold to account for very thin cartilage or underlying bone effects. Results from this study were consistent with stiffness measurements of normal goat cartilage from past studies. Raw stiffnesses of adult nonoperated goat MFCs were 1.6 ± 1.0 N/mm[1] and 0.79 ± 0.15 N/mm [17], compared to 1.69 ± 0.49 N/mm from this study. Graft stiffness was $7.7 \pm$ 7.9 N/mm in a 6 month goat study [16], compared to 1.22 ± 0.80 N/mm and 0.62 ± 0.16 N/mm at 6 and 12 months from this study. Slight disparities in nonoperated values may be due to the location of testing along the femoral condyle. Differences in graft stiffness could be due to a number of factors including graft size, cartilage thickness, and healing response of the animals, emphasizing the advantage of using a normalized metric of stiffness.

The number and location of indentation sites represent a trade-off between spatial resolution and total test duration. Selection of an appropriate number of sites to capture repair cartilage variations is an important consideration of indentation testing, but few studies have determined typical means and standard deviations across normal and repair cartilage. In this study, intra-sample variability of structural stiffnesses were higher than inter-sample variability in operated (0.46 vs. 0.35 N/mm, respectively), and lower in nonoperated (0.24 vs. 0.44 N/mm) joints. The number of test sites necessary to obtain a $\pm 10\%$ confidence interval around the mean at a 95% confidence level was calculated using the nonoperated mean, as an evaluation of cartilage repair success relative to normal tissue, and the operated mean, to provide a precise index of repair tissue mechanics. Based on structural stiffness data, 107 test sites across the operated joint would be required to obtain a $\pm 10\%$ confidence interval around the nonoperated typical structural stiffness of 0.87 N/mm, based on the average of 6 and 12 month data, while 136 measurements would be required for a $\pm 10\%$ confidence interval around the operated mean of 0.77 N/mm. Intra-sample variability of normalized stiffnesses was higher than inter-sample variability in both operated (0.20 vs. 0.11) and nonoperated (0.23 vs. 0.13) joints. 32 test sites across the operated joint are necessary to obtain a $\pm 10\%$ confidence interval around the nonoperated typical normalized stiffness of 0.71, based on the average of 6 and 12 month data, while 67 measurements are necessary for a $\pm 10\%$ confidence interval around the operated mean of 0.49. The 63-site indentation array used in this study was thus appropriate for delineating normalized stiffness variations in repaired cartilage.

This study provides a quantitative 3-D assessment of graft repair success and insight into the process of *in vivo* remodeling for grafts with different structural and biomechanical properties from the adjacent host. While long-term graft cartilage properties were inferior to the host and contralateral nonoperated controls, the results of this study suggest that graft cartilage and bone have the capability to remodel, and maintenance of cartilage properties may be dependent on the ability to adapt. Strategies that can promote cartilage thickening and bone contour remodeling while preventing cyst formation may enhance the long-term success of grafts. The 3-D analyses and array indentation techniques presented in this study provide novel methods to quantitatively assess osteochondral repair success and are applicable for the characterization of both native and engineered tissues. Future work to further reduce the number of sampling sites and optimize the efficiency of data acquisition will allow more widespread applicability of these methods.

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APPENDIX B:

SUPPLEMENTARY MATERIAL FOR CHAPTER 3 -COORDINATED DEVELOPMENT OF THE PHYSIS AND EPIPHYSIS

B.1 Hypothesis and Aims

The hypothesis of this study was that regional ossification and tissue expansion rates (including proliferation, matrix deposition, and hypertrophy) in the articulo-epiphyseal cartilage complex occur in association with shape changes at the bone-cartilage interface of the femoral secondary ossification centers. The aims of this study were to 1) estimate articulo-epiphyseal cartilage kinetics (proliferation, matrix deposition, hypertrophy, and ossification rates) using high resolution micro-computed tomography scans of the mouse femur, and 2) determine the association between cartilage kinetics and shape formation in the proximal and distal femoral epiphyses.



Figure B.1: Safranin-O stained histology sections of the (A,B) proximal and (C,D) distal femur at (A,C) 12 and (B,D) 30 days in C57BL/6 mice. Magnified images of the (i) articulo-epiphyseal or and articular cartilage, and (ii,iii) growth plate regions. Magnified images of the distal femur are flipped vertically to maintain a constant orientation of the epiphyseal surface at the top and metaphyseal surface at the bottom. White arrows indicate direction of chondrocyte columns in the growth plate.



Figure B.2: 2-D schematic of cartilage and bone surfaces within the articuloepiphyseal complex at the proximal and distal ends of the femur. Micro-CT sagittal and transverse images of C57BL/6 mouse femora at days 16 and 60 are shown for comparison. Growth rates (growth plate ossification and expansion, SOC ossification) were determined based on displacement of these surfaces relative to a fixed location (the nutrient foramen). C = articular cartilage surface, SOC = secondary ossification center bone surface, eGP = epiphyseal surface of growth plate cartilage, mGP = metaphyseal surface of growth plate cartilage, NF = nutrient foramen.


Figure B.3: Macroscopic growth characteristics of the femur. (A) Displacements of the different surfaces described in **Fig. B.2** relative to the nutrient foramen (at z=0) in C57BL/6 mouse femurs from day 12 to 120. SOC = secondary ossification center bone surface, eGP = epiphyseal surface of growth plate cartilage, mGP = metaphyseal surface of growth plate cartilage, NF = nutrient foramen.



Figure B.3 (continued): (B) Femur length and lengthening rate with age. (C) Percentage of femur growth accounted for by the proximal femoral head and distal femoral growth plates with age.



Figure B.4: Growth rates of the proximal and distal femur. Rates of growth plate (GP) ossification and expansion, and secondary ossification center (SOC) ossification in the (A) proximal femoral head and (B) distal femur. (C) Correlation between rate of expansion versus rate of ossification in the proximal femoral head and distal femoral growth plates.



Figure B.5: Displacement rates of the femoral epiphyses after rigid alignment to the epiphyseal side of the growth plate. (**A**,**B**) Displacement rates (color) of the proximal femoral head secondary ossification center bone surfaces, calculate between (**i**-**iv**) age intervals indicated at top and mapped onto the shape of the younger age point. Meshes of the older age are overlaid on top for comparison. Med = medial, Inf = inferior, Ant = anterior.



Figure B.6: Area dilation rates and principal strain directions of the femoral epiphyses. Area dilation rates (color) of the proximal (**A**,**B**) femoral head secondary ossification center (SOC) surfaces, and (**C**,**D**) epiphyseal/metaphyseal growth plate (eGP/mGP) surfaces. Black lines on the surface indicate maximum principal directions of strain on the surface (plotted in gray over dark regions). Med = medial, Inf = inferior, Ant = anterior.



Figure B.6 (continued): Area dilation rates (color) of the distal (E,F) SOC surfaces, and (G,H) eGP/mGP surfaces.



Figure B.7: Schematic of the contours of the distal femur secondary ossification center (SOC) after rigid alignment to the epiphyseal side of the growth plate (eGP).



Figure B.8: Micro-computed tomography and corresponding Safranin-O stained histology sections of the proximal femur.



Figure B.9: Area dilations of the (A-C) proximal and (D-F) distal femoral secondary ossification center (SOC) surface and epiphyseal/metaphyseal growth plate (eGP/mGP) surfaces. Dilations of the proximal region were determined from the femoral head. Dilations of the distal femur were divided into four regions corresponding to the distal growth plate mammillary processes. LFC/MFC = lateral/medial femoral condyle, LT/MT = lateral/medial trochlea, PL = posterolateral, PM = posteromedial, AL = anterolateral, AM = anteromedial.



GP area dilation [%]

Figure B.10: Correlations between dilations of the secondary ossification center (SOC) surface and dilations of the epiphyseal/metaphyseal growth plate surfaces (eGP/mGP). (**A**,**C**) Proximal and (**B**,**D**) distal femur SOC dilations versus (**A**,**B**) eGP and (**C**,**D**) mGP dilations. Dilations of the distal femur were divided into four regions corresponding to the distal growth plate mammillary processes. LFC/MFC = lateral/medial femoral condyle, LT/MT = lateral/medial trochlea, PL = posterolateral, PM = posteromedial, AL = anterolateral, AM = anteromedial.

B.2 Ackowledgments

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APPENDIX C:

SUPPLEMENTARY MATERIAL FOR CHAPTER 4

C.1 Methods

All automatically defined landmark correspondences within the femoral head, neck, and greater and lesser trochanters were visually verified to be in the correct anatomical region. Discrepancy error between the automatic landmark extrapolation technique presented in this study and traditional manual landmarking of individual training shapes were determined for the model. Manual landmarks were defined by two independent observers at 4 anatomical locations on the proximal femur in each of the 53 samples during two separate sessions. *Automatic* landmark extrapolation was simulated using a semi-automatic technique in which the model atlases were manually landmarked by the observers at the defined anatomical locations, and landmarks were automatically extrapolated to the samples by backcalculation of the non-rigid registrations to the atlas shape. Landmark errors were calculated as the Euclidean distance between corresponding landmarks.

Intra-observer error was determined as the error between sessions of each observer averaged over the training set and two observers (Fig. C.2). Inter-observer

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error was calculated as the error between the session averages of the two observers. Error between manual and semi-automatic extrapolation was determined between the average of the two sets of training set landmarks over the sessions and observers and then averaged over the training set.

C.2 Results

To define corresponding points between proximal femora of different patients, a shape atlas was created for segmented bone. The atlas was constructed iteratively, with convergence after 4 iterations and final kappa agreement of 0.99.

The validity of automatic extrapolation of corresponding landmark coordinates in each sample was tested by comparison to manual definitions at 4 anatomical locations in asymptomatic femurs (**Table C.2**). The same locations were also defined on the averaged atlas shape, from which semi-automatic landmarks were extrapolated based on the atlas. Intra-observer variability tended to be higher in the manual versus semiautomatic method (p=0.09; average manual variability, 2.16mm; average semiautomatic variability, 1.33mm). Inter-observer variability was similar between the two methods (3.55mm and 3.48mm for manual and semi-automatic, respectively). The average Euclidean distance between manual and semi-automatic landmarks was 1.82mm, or approximately 2 voxels.

Condition		А	.ge (years	5)	S	ex
Condition	n -	Mean	Min.	Max.	Μ	F
		Asympto	matic			
6-9 years	5	8.18	6.97	8.98	3	2
9-12 years	8	11.05	10.17	11.94	5	3
12-15 years	8	13.14	12.19	14.55	4	4
15-19 years	6	16.28	15.16	18.16	5	1
Overall	27	12.30	6.97	18.16	17	10
		LCPI	D			
cam	3	13.79	12.41	18.16	1	2
cam+pincer	3	11.68	8.98	15.70	3	0
		SCFI	E			
mild	9	13.74	11.01	16.38	5	4
moderate	7	13.97	11.05	16.38	5	2
severe	4	14.96	11.01	16.54	3	1

Table C.1: Patient population statistics

Table C.2: Manual versus semi-automatic

 landmarking observer variabilities (mm)

	0			/	
Landmank	Mai	nual	Semi	-Auto	Manual vs
Lanumark	Intra	Inter	Intra	Inter	Semi-Auto
Greater trochanter apex	1.48	2.55	0.00	1.89	1.71
Lesser trochanter apex	1.69	4.67	1.74	6.80	2.89
Superior head-neck transition	3.04	4.13	1.90	3.56	1.41
Inferior head-neck transition	2.44	2.84	1.67	1.67	1.27
Average	2.16	3.55	1.33	3.48	1.82



Figure C.1: Representative samples depicting the accuracy of fit of the SSM reconstructed shape to the original shape of the joint directly segmented from CT scans at 0.5-0.9mm in-plane voxel resolution and 0.63mm slice thickness.



Figure C.2: Discrepancy error calculations between manual and automatic methods for determining landmark correspondences,

C.3 Discussion

In previous studies [1, 3, 4], growth deformations have been decomposed into growth and elastic parts that characterize volumetric growth via mass deposition and elastic accommodations that ensure compatibility while producing residual stresses, respectively. Since long bones consist of "hard" tissue that experiences relatively small elastic strains, elastic accommodation deformations may be neglected when characterizing rapid developmental growth; thus, in this study measured deformations are interpreted to represent growth deformations only.

This is the first study to quantitatively describe in 3-D the isotropic expansion of the femoral head epiphysis and anisotropic expansion of the greater trochanteric epiphysis during normal development of the proximal femur. Alignment of the joints based on the epiphyseal surface of the growth plate allowed for calculations of displacement rates within the femoral head and greater trochanteric regions. Highest displacement and surface dilation rates were observed between the ages of 11 and 13 years, approximately the time of the growth spurt during puberty. Femoral head displacement rates and changes in neck-shaft angle in the current study were similar to previous measures of epiphyseal height growth rates of 0.8mm/year between 5 and 15 years [2], and change in neck-shaft angle from 155° to 130° from birth to adult [5]. While displacement rates of the femoral neck isthmus could not be determined due to complexities in aligning the femoral neck axis and dilations were greatest during the period of highest growth, suggesting thickening of the femoral neck with age.

C.4 Acknowledgments

Appendix C, in part, will be submitted for publication along with Chapter 4. The dissertation author was the primary author and thanks co-authors, Christine L. Farnsworth, Stephen M. Klisch, Harish S. Hosalkar, and Robert L. Sah for their contribution. The dissertation author also gratefully acknowledges the assistance of Ricky Harjanto, Karen Samy, and J.D. Bomar. This work was supported by grants from the National Institutes of Health (NIH R01 AR044058) and the National Science Foundation. Additional individual support was received through a NSF Graduate Research Fellowship (to E.F.C.).

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APPENDIX D:

TRANSLATIONAL APPROACH: STATISTICAL SHAPE PARAMETERS FOR DISEASE CLASSIFICATION AND DIAGNOSIS

D.1 Introduction

The aim of this study was to introduce, through cluster analysis of the SSM shape parameters presented in **Chapters 4** and **5**, a potential method of classifying disease and monitoring progression.

D.2 Methods

To demonstrate the potential of using statistical shape parameters for disease classification, k-means cluster analysis was performed on the 8 statistical shape parameters of all samples [2]. The number of clusters was determined by comparing mean silhouette values, a measure of how close each sample in one cluster is to samples in neighboring clusters [1]. After cluster analysis, conventional and statistical shape parameters were determined for each cluster mean shape. In addition, the largest two clusters were analyzed further by division into subclusters using similar considerations.

In addition, k-means cluster analysis was performed on displacement, θ , and φ metrics for asymptomatic, mild, moderate, and severe SCFE hips.

D.3 Results

To describe patterns of proximal femoral shape within the study population and identify potential pathways of shape deformation, statistical shape parameters of all samples were grouped using k-means cluster analysis. Cluster analysis resulted in 6 distinct clusters of which 2 had >10 samples and were further subdivided to analyze minor patterns (**Figs. D.1** and **D.2**). The largest two clusters revealed distinct patterns of proximal femoral shape. Cluster A was divided into 4 subclusters, A1 through A4, which depicted varying degrees of femoral head epiphyseal slip, lesser trochanter medial positioning, and greater trochanter protrusion, characteristic of SCFE progression (**Fig. D.1**). Further subdivision of the most normal subcluster, A1, revealed slight differences in the head, neck, and lesser trochanter regions. Cluster B was subdivided into two clusters that demonstrated femoral head enlargement and lateral positioning of the lesser trochanter, with little change in intertrochanteric distance, characteristic of LCPD femora during disease progression. The remaining clusters were composed of severe cases of LCPD and SCFE that were distinct in shape.

Cluster analysis of asymptomatic and SCFE hips using displacement and growth plate angles resulted in 4 clusters of various disease progression (**Fig. D.3**).



Figure D.1: Possible progression patterns of proximal femoral shape determined from k-means cluster analysis. Colormaps depict displacements of the average cluster shape from the asymptomatic proximal femoral shape.



Figure D.2: Conventional and statistical shape parameters (adjusted to age 12) corresponding to Fig. D.1 for each cluster and subcluster determined from k-means cluster analysis.



Figure D.3: Cluster analysis of displacement and growth plate angle (θ, ϕ) metrics in asymptomatic and SCFE femora. Colored patches indicate 4 clusters determined by k-means cluster analysis. Colored points indicate asymptomatic and SCFE categories as determined by the Southwick angle [3].

D.4 Discussion

Cluster analysis revealed overarching differences in proximal femoral morphology that may lead to specific manifestations of shape deformities with time. The two main clusters matched well with qualitative descriptions of SCFE and LCPD progression, respectively, with epiphyseal slip in the former and femoral head enlargement in the latter (**Fig. D.2**). Between the relatively asymptomatic clusters A and B, differences existed in epiphyseal position, neck transition shapes, and femoral head protrusion. These shapes may arise from variations within the normal population or may be precursors to certain disease morphologies. As there were a limited number of LCPD hips with highly variable healed morphologies, those proximal femora were divided into individual clusters with few samples. Further analysis of a larger sample population may provide new methods for classifying proximal femoral diseases and earlier detection and treatment for LCPD and SCFE.

D.5 Acknowledgments

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APPENDIX E:

SUPPLEMENTARY MATERIAL FOR CHAPTER 5

E.1 List of supplementary materials

Fig. E.1. Schematic of the process of statistical shape model.

Fig. E.2. Statistical shape model (A) atlas convergence and (B) cumulative variance explained.

Table E.1. P-values for linear correlations between conventional and statistical shape parameters in asymptomatic proximal femora

Table E.2. R^2 values for linear correlations between conventional and statistical shape parameters in asymptomatic proximal femora



Statistical Shape Modeling

- 1. Segmentation
- Rigid registration to largest shape
 Transformation to
- binary volume
- 4. Iterative shape blending
- Rigid registration to atlas
 Non-rigid registration to atlas
 Atlas landmarking
 Landmark extrapolation

- 9. PCA

Figure E.1: Schematic of the process of statistical shape modeling.



Figure E.2: Statistical shape model (A) atlas convergence and (B) cumulative variance explained with each additional mode.

Condition		А	.ge (years	5)	S	ex
Condition	n	Mean	Min.	Max.	Μ	F
		Asympto	matic			
6-9 years	5	8.18	6.97	8.98	3	2
9-12 years	8	11.05	10.17	11.94	5	3
12-15 years	8	13.14	12.19	14.55	4	4
15-19 years	6	16.28	15.16	18.16	5	1
Overall	27	12.30	6.97	18.16	17	10
		LCPI	D			
cam	3	13.79	12.41	18.16	1	2
cam+pincer	3	11.68	8.98	15.70	3	0
		SCFI	E			
mild	9	13.74	11.01	16.38	5	4
moderate	7	13.97	11.05	16.38	5	2
severe	4	14.96	11.01	16.54	3	1

 Table E.1: Patient population statistics

Table E.2: P-values and statistical shape	E.2: P-value tistical shape		s for linear correlation parameters in asymp	as between of tornatic prov	conventional simal femora	
Neck-						
Shaft Medial Int Angle Offset	Medial Int Offset	Int	ertrochanteric Distance	Head Diameter	Neck Diameter	Eccentricity
0.000 0.000	0.000		0.001	0.000	0.000	0.095
0.001 0.191	0.191		060.0	0.503	0.238	0.189
0.000 0.000	0.000		0.043	0.009	0.028	0.738
0.723 0.113	0.113		0.000	0.412	0.718	0.240
0.000 0.004	0.004		0.158	0.006	0.000	0.045
0.185 0.161	0.161		0.404	0.010	0.110	0.003
0.005 0.083	0.083		0.996	0.162	0.008	0.527
0.529 0.954	0.954		0.184	0.188	0.990	0.163

	Table 1	E.3: R ² value	es for linear correlatio	ins between	conventional	_
	and stat	tistical shape	e parameters in asymp	tomatic prov	kimal femora	_
	Neck-	Medial	Intertrochanteric	Head	Neck	
Mode	Shaft Angle	Offset	Distance	Diameter	Diameter	Eccentricity
1	0.58	0.62	0.34	0.49	0.67	0.11
6	0.38	0.07	0.11	0.02	0.06	0.07
3	0.49	09.0	0.15	0.24	0.18	0.00
4	0.01	0.10	0.41	0.03	0.01	0.05
S	0.45	0.28	0.08	0.27	0.47	0.15
9	0.07	0.08	0.03	0.24	0.10	0.30
L	0.27	0.12	0.00	0.08	0.25	0.02
8	0.02	0.00	0.07	0.07	0.00	0.08

E.2 Acknowledgments

Appendix E, in part, will be submitted for publication with Chapter 5. The dissertation author was the primary author and thanks co-authors, Christine L. Farnsworth, Harish S. Hosalkar, and Robert L. Sah for their contribution. The dissertation author also gratefully acknowledges the assistance of Ricky Harjanto and J.D. Bomar. This work was supported by grants from the National Institutes of Health (NIH R01 AR044058) and the National Science Foundation. Additional individual support was received through a NSF Graduate Research Fellowship (to E.F.C.).

APPENDIX F:

SUMMARY OF VALIDATION OF POINT CORRESPONDENCES AND STATISTICAL SHAPE MODELING TECHNIQUES

F.1 Introduction

As the results of this dissertation are dependent on the proper definition of corresponding landmark coordinates between samples, and representation of the original shape by the landmark coordinates, the methods for point correspondences and statistical shape modeling were assessed and validated at a number of different stages, and summarized below.

F.2 Atlas convergence

An atlas shape was constructed based on the Sparse Active Shape Modeling (SPASM) algorithm [1] in order to extract corresponding landmark coordinates. The atlas was iteratively constructed by averaging the signed distance transforms of the rigidly registered, binarized training shapes, effectively blending the binary volumes together. After each iteration, training shapes were rigidly aligned to the new atlas by minimizing the normalized squared difference between the two volumes, equivalent to

the proportion of non-overlapping voxels. Agreement between successive iterations was recorded using the Cohen's kappa coefficient [2], K = (Po - Pe)/(1-Pe), where *Po* is the proportion of voxels overlapping and *Pe* is the proportion of voxels expected to overlap by chance. The atlas was defined as converged at the ith iteration when kappa values for the i+1 iteration started to decrease. In general, atlases converged after 4-5 iterations. Refer to **Figure C.3** and **Figure F.1** for human and mouse atlas kappa agreements, respectively.

F.3 Manual versus Semi-Automatic Landmarking

Discrepancy error between the automatic landmark extrapolation technique presented in this study and traditional manual landmarking of individual training shapes were determined for both proximal and distal femur models. Two independent observers *manually* landmarked four anatomical locations on the proximal femur and three locations on the distal femur in each of the 30 training shapes during two separate sessions. *Automatic* landmark extrapolation was simulated using a semi-automatic technique in which the model atlases were manually landmarked by the observers at the defined anatomical locations, and landmarks were automatically extrapolated to the 30 training shapes by backcalculation of the non-rigid registrations to the atlas shape. Landmark errors were calculated as the Euclidean distance between corresponding landmarks. Refer to **Figure C.2** for a schematic of error calculations.

Intra-observer error was determined as the error between sessions of each observer averaged over the training set and two observers. Inter-observer error was calculated as the error between the session averages of the two observers. Error between
manual and semi-automatic extrapolation was determined between the average of the two sets of training set landmarks over the sessions and observers and then averaged over the training set.

In the mouse studies, error between manual and semi-automatic landmarking was 0.037 mm (~4 pixels) in the proximal femur and 0.028 mm (~3 pixels) in the distal femur (**Table F.1**). No statistical differences were found in intra-observer and inter-observer variabilities between manual and automatic methods. For the human hip study, CT scans were processed at full resolution, and discrepancy error between manual and automatic landmarking was ~2 pixels (refer to **Table C.2**).

F.4 Landmark distribution across joint surface

The exact number of landmarks in each model was based on remeshing of the atlas. This resulted in a landmark distribution of 1 landmark at roughly every 0.08 mm (or ~9 pixels) across the oldest mouse femur subchondral bone and growth plate surfaces, and 1 landmark roughly every 4.3 mm (~5 pixels) across the oldest asymptomatic human hip.

F.5 Leave-one-out Experiments

Leave-one-out tests [1, 2] were performed to characterize how well the SSMs could be generalized to samples outside of the training set. Each training shape was left out of the model building process in turn, and the resulting new model was used to reconstruct the left out shape. Reconstruction error was calculated as the mean Euclidean distance between landmarks of the actual and segmented training shape.

In the mouse femur model, reconstruction error decreased as the number of modes included in the model increased (**Figure F.2A**). Errors with all modes included for the proximal and distal femora were both 0.04mm (4.9 pixels).

F.6 Landmark precision

To determine the precision of landmark coordinates defined using the atlas shape, a day 16 mouse femur was scanned using μ CT at (9 μ m)³ isotropic resolution in two different sessions, and corresponding landmark coordinates were extrapolated. Precision of landmark extrapolation was assessed by calculating the root mean square error (RMSE) between corresponding landmarks of the two scans.

RMSE between the extrapolated landmarks of the same sample was 0.024 mm (2.7 pixels), compared to 0.16 mm (\sim 17.7 pixels) between samples of the same age group.

Model	Manual		Semi-automatic		Manual vs.
	Intra	Inter	Intra	Inter	Semi-auto
Prox. Femur	0.21	0.23	0.25	0.32	0.31
Dist. Femur	0.29	0.35	0.31	0.22	0.23

 Table F.1: Manual versus semi-automatic landmarking observer variabilities [mm] in mice



Figure F.1: Kappa agreement and squared difference during mouse femur atlas construction.



Figure F.2: Reconstruction errors from leave-one-out experiments as a function of included modes.

F.7 Acknowledgments

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