MULTISCALE RELATIONSHIPS OF LIGAMENT MECHANICS

by

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ABSTRACT

Healthy knee joints require structural stability through a full range of motion. Knee stability is primarily provided by a network of ligaments that resist abnormal forces and direct smooth articulations. Ligament malfunction has deleterious effects on balance, agility, and knee laxity. Altered knee laxity redistributes the stress transmitted across articulations, which may create regional stress concentrations that are potentially damaging to articular cartilage. The high incidence of knee ligament injuries and shortfalls of related treatments are thus adverse to knee joint health. As the function of ligament is essentially mechanical, understanding the tissue-scale and molecular-scale relationships that mechanically influence ligament is critical to functional restoration. The aim of this dissertation was to strengthen the scientific knowledge of mechanical relationships that impact ligament function in the knee. Toward this objective, a tissue-scale relationship was investigated between the anterior cruciate ligament (ACL) and the medial collateral ligament (MCL). These knee ligaments are frequently injured and have inter-dependent functionality. At the molecular-scale, mechanical interactions between glycosaminoglycans (GAGs) and collagen fibrils may impact gross ligament function. Therefore, the influence of these molecular relationships on ligament material behavior was inspected.

Experimental and numerical modeling techniques (finite element analysis) were employed to fulfill these objectives. In an ACL-deficient knee, the MCL was more susceptible to damage during anterior tibial translation, as strains reached values
corresponding to the threshold of microstructural failure. The MCL midsubstance and
insertions were functionally insensitive to ACL transection during valgus loading of the
knee. Interfibrillar GAGs were found to have negligible influence on tissue-scale
mechanics during tensile loading. Therefore, interfibrillar GAGs do not directly support
ligaments’ primary function of resisting tensile loads, which contradicts the popular
theory that GAGs transmit forces between adjacent collagen fibrils. By determining the
effect of multiscale relationships on ligament function, improvements are possible in the
innovation and evaluation of ligament treatment modalities.
To my loving wife and family.
They are my joy and inspiration.
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CHAPTER 1

INTRODUCTION

Motivation

The knee is the largest synovial joint in the human body and is also the most vulnerable musculoskeletal structure [1]. In addition to transferring loads between the femur and tibia of up to 7x body weight [2], the knee must functionally permit the broad range of motion required for bipedal gait. To enable this mobility, a complex network of tendons and ligaments primarily stabilize the knee and guide smooth articulations. Through overuse, repetitive physical activity and abnormal loading, knee ligaments are prone to tear and are responsible for over 6 million hospital visits a year [1, 3]. In the short-term, knee ligament damage will restrict participation in certain sports and activities [4-6], while in the long-term, ligament damage can predispose the knee to debilitating osteoarthritis by altering gait patterns [7-9].

In order to return patients with knee ligament damage to normal activity levels and to obstruct osteoarthritic progression, clinicians and scientists have developed both surgical and nonsurgical treatment strategies. There are over 100,000 reconstructions of knee ligaments performed annually in the United States [10]. Ligament reconstruction vastly improves the gait and function of joints [11][12]. However, reconstructed knees are still beset with abnormal gait [13-15] and athletic performance is significantly hampered postsurgery [16]. Even ligaments that are adept at healing with conservative
treatment experience reductions in mechanical properties [17, 18] that can alter knee laxity [19, 20]. Moreover, ligament injury can cause physical [21, 22] and chemical alterations [23] to intact ligaments that may hamper the effectiveness of clinical treatments.

The inadequacies that exist in the present management of ligament injury can be attributed to technological limitations and deficiencies in our underlying knowledge of ligament function. It is self-evident that a ligament’s function derives from molecular interactions within its structure and is dependent on intrinsic relationships with other joint stabilizers. Therefore, a fundamental understanding of these inter- and intra-ligament relationships is absolutely necessary to develop strategies (drug therapy, functional tissue engineering, novel reconstruction procedures) that restore or enhance the native function of ligament. Since the role of ligament is mechanical by nature, mechanically based experimental and theoretical studies are appropriate to examine these tissue and molecular scale (multiscale) relationships.

Advances in experimental technologies, computational methods and biochemistry have recently enabled multiscale relationships to be investigated in detail. High resolution digital cameras have progressed to give an accurate and efficient means to track tissue deformations for mechanical analysis [24]. The astounding growth of computer power combined with improvements in medical imaging has propelled the science of numerical modeling [25-27]. Moreover, burgeoning progress in molecular biology has impelled the quantification and manipulation of tissue microstructure [28-30]. By blending these tools, the multiscale relationships that define ligament function
can be comprehensively examined and applied to the treatment and prevention of ligament injuries.

Research Goals

The overall objective of this research was to clarify multiscale biomechanical relationships in ligament. Advancement of the treatment and prevention of joint injury and disease will be facilitated by a concise understanding of the mechanical relationships within and between joint stabilizers. Experimental and theoretical biomechanics, along with biochemical techniques, were utilized to accomplish the research objective. At the tissue level, ex vivo structural experiments were employed to determine the functional interrelationship of primary knee stabilizers. Computational finite element models were then generated to examine aspects of this interaction that would otherwise be difficult or impossible to measure experimentally. At the molecular level, the mechanical relationship of collagen molecules with ground substance constituents was examined by performing a series of elastic and viscoelastic material tests before and after enzymatic treatment. Finally, the solid-fluid phase interaction of ligament was briefly examined both experimentally and computationally to investigate the mechanical relevance of fluid flow during tensile deformation.

Summary of Chapters

The mechanical relationships investigated in this dissertation start at the tissue scale and move to the molecular scale. The primary purpose of Chapter 2 was to provide sufficient background on the topics that will be addressed in the later chapters. This background information includes an overview of the structure and function of the
The microstructural composition and organization of ligament is detailed along with mathematical models and numerical methods that describe ligament material behavior.

To comprehensively study the structural interactions of ligaments, an experimental technique was developed in Chapter 3 that simultaneously measures three-dimensional joint kinematics and localized ligament strain. These coupled measurements link specific movements to functional ligament regions. A high-resolution optical tracking system was appropriate for this type of measurement, and was rigorously validated. The excellent precision and accuracy of this system make it an attractive tool for biomechanical experiments.

Chapter 4 describes the use of this validated technique to experimentally examine the relationship between the anterior cruciate ligament (ACL) and the medial collateral ligament (MCL). These two ligaments are primary knee stabilizers, have overlapping roles and account for 70% of multiligament injuries in the knee [31]. The objective of this study was to determine how ACL deficiency affects the local strain in the MCL during loading configurations applicable to these ligaments. To meet this objective, kinematic motions were applied to a prescribed force before and after ACL transection under a variety of loading conditions. The results of this study provide physiological and clinical insight into the interaction of these two primary knee stabilizers.

Certain physical phenomena associated with ligament mechanics, such as insertion and contact forces, are difficult or impossible to measure experimentally. One method to approximate these inhomogeneous forces is by means of a validated finite
element model [27]. Therefore, to further examine the relationship between the ACL and MCL, Chapter 5 focuses on using the experimental kinematics and strain measurements from Chapter 4 to generate subject-specific finite element (FE) models [26]. As a means of validation, the experimental strain values at peak load were compared to the FE predictions. This study not only improved upon past advances in subject-specific modeling, but helped clarify the specific tensile and compressive stresses projected upon the MCL after ACL transection.

In Chapters 6 and 7, the focus of this dissertation shifts to the influence of microstructure on tissue-scale mechanics. The chapter objectives are to determine whether the mechanical interactions of noncollagenous constituents play a role in the continuum material behavior of ligament. Understanding the origins of ligament material behavior is applicable to functional tissue engineering and basic science. The possible mechanical role of sulfated glycosaminoglycans (sGAGs) was investigated. SGAGs include dermatan and chondroitin sulfate, which have been widely implicated as contributors to the mechanical response of ligament. By performing experiments before and after sGAG degradation, the mechanical influence of these noncollagenous constituents was elucidated. Chapter 6 inspects the unique role of dermatan sulfate in the elastic mechanics of ligament, while Chapter 7 researches the contribution of dermatan and chondroitin sulfate on the viscoelastic material properties. Results are related to relevant prior investigations, along with basic and applied science.

The final chapter highlights the major contributions that this dissertation has made to the field of ligament biomechanics and comments on future work that is necessary to expand upon this research. The discussion on future work includes
preliminary work looking at the roles of other noncollagenous constituents and experimental factors that could be improved to better assess functionality of the ground substance. In this section, a strong emphasis was placed on a biphasic mathematical representation of ligament that describes the physical solid-fluid interaction that occurs in soft-tissue. The current state of this work was included along with the long-term perspective.

References


CHAPTER 2

BACKGROUND

Knee Joint Overview

The knee is the most complex synovial joint in the human body and is well-designed to withstand large (~7x body weight) and repetitive loads [3]. For example, the knee of an average jogger will retain 16,000 impacts per week, equivalent to 2400 tons of force [4]. The knee offers mobility in all six degrees of freedom. However, the joint primarily functions in the sagittal plane, where it has a flexion range of 100 degrees. At full extension, the knee is highly stable due to a “screw home mechanism” that locks the joint over the final 20 degrees of extension [5]. Uniform articulating contact during flexion is maintained by a smooth sliding and rolling mechanism between the tibial and femoral surfaces [6]. Although the knee is best suited to transfer compressive loads, it must also resist large torques due to forces that twist the lower extremity. At full extension, the knee is capable of resisting 90 N-m of valgus and 120 N-m of tibial axial torque [7]. The mobility and strength that is axiomatic to proper knee function is provided by the articulating surfaces, musculature and ligaments.

Articulations

Synovial, or diarthrodial, joints are classified as having a synovial filled capsule that surrounds the articulating bones. The knee has two separate articulations: the
patellofemoral joint and the tibiofemoral joint (Figure 2.1). These joints are technically condyloid joints, where convex condyles articulate with concave surfaces; however, since they mainly support motion in one planar direction (sagittal), they act more like modified hinges. The convex articulations of the patellofemoral joint are the oval medial and lateral condyles, while the trey-shaped tibial plateau provides the concave surface. Lying between the femoral condyles, the intercondyler notch forms the patellar groove where the patella slides, or tracks, upon during flexion and extension. The specific motion of articulating bones can be quantified through kinematic measurements, such as those performed in Chapter 4 and 5 of this dissertation.

The bony interfaces of these articulations are mediated by a ~2 mm layer of articular or hyaline cartilage [8]. Articular cartilage is 80% water and has qualities in-between bone and dense connective tissue. It is resilient, flexible, and in the surrounding synovial fluid has a coefficient of friction 40x lower than Teflon [9, 10]. In mature cartilage tissue, chondrocyte cells are sparsely distributed. The solid phase of

![Figure 2.1: An x-ray picture of the medial knee at 30 deg flexion.](image)
the extracellular matrix is predominantly type II collagen (~60%) and proteoglycans (~30%). The collagen interfibrillar space contains approximately 30% of the water, while the proteoglycan solution domain holds 70% of the water [11]. The cartilage surfaces are surrounded by a vascularized dense membrane called a perichondrium. The cartilage structure itself is avascular and aneural. Therefore, nutrients must diffuse from the perichondrium through the cartilage matrix to reach the cellular network of chondrocytes. The extracellular matrix is responsible for the mechanical properties of cartilage. Collagen provides tensile strength and grips aggregated proteoglycans through physical entrapment and weak electrostatic and hydrogen-bonding [12]. Through dynamic and equilibrium factors, the keratin sulfate and chondroitin sulfate glycosaminoglycans associated with the cartilage proteoglycans are able to retain water under compression. The retention of nearly incompressible water provides a solid-fluid dynamic modulus up to 50x higher than the equilibrium modulus [13-15], which is measured after water exudation.

The load bearing and shock absorption capability of the tibiofemoral articulation is augmented by the fibrocartilaginous semilunar menisci that sit on the medial and lateral concavities of the tibial plateau (Figures 2.2, 2.3). The menisci transmit 50% and 85% of the joint load at full extension and 90 deg flexion, respectively [16]. The menisci also increase the contact area of the proximal tibia by 50-70% [16] and attenuate intermittent shock waves developed during gait [17]. Structurally, the menisci are fiber reinforced composites (74% water by wet weight) that primarily insert into the anterior and posterior meniscal horns (i.e., the points of the crescent shaped menisci). The solid phase consists mostly of dense Type I collagen fibers with small amounts of
Figure 2.2: Illustration of the anterior knee joint. Adapted from [1].
Figure 2.3: Illustration of the posterior knee joint. Adapted from [1].
proteoglycans (<1% wet weight). The collagen has a circumferential orientation in the interior and a radial orientation on the surface. These orientations are adept at supporting the enormous hoop stresses that develop during joint compression [18, 19].

Muscles

The musculature provides passive (muscle tone) and dynamic support to the knee joint via forces applied to the tendenous inserts from muscular contraction. Muscle consists of a network of nerves and vessels, a muscle-specific extracellular matrix and muscle cells that contain multiple nuclei. Two major muscle groups cross the knee and enhance joint stability: the quadriceps muscle group (Figure 2.4A) and the hamstring muscle group (Figure 2.4B). Coactivation of these muscle groups during gait aids the ligaments in maintaining stability and equalizes the distribution of stress on the articular cartilage [20]. Valgus and varus stability of the knee is increased by 28-35% when the medial and lateral compartments are actively contracted [21], and Hughston et al. [22] found that medial knee stability was 25% stiffer with contraction of the semimembranosous muscle (Figure 2.4B). The hamstring group also reduces anterior tibial translation during gait [23]. The joint position sense [24] that contributes to joint stability and function is improved by stretching the muscles that surround the knee [25]. When muscle hypertrophies, the knee ligaments are at an increased risk of damage [20].

Ligaments

Ligaments are short bands of dense fibrous tissue that bind bone to bone. There are four major ligaments of the knee that play a primary role in joint stability: the anterior cruciate ligament (ACL), the posterior cruciate ligament (PCL), the medial
Figure 2.4: Illustration of the A) quadricep and B) hamstring muscle groups of the knee.
collateral ligament (MCL) or tibial collateral ligament, and the lateral collateral ligament (LCL) or fibular collateral ligament (Figures 2.2, 2.3). The joint capsule and other knee ligaments (e.g., posterior medial corner, medial and lateral retinaculum, the popliteal ligament and the meniscofemoral and meniscotibial ligament) provide additional stability. Individually, knee ligaments act as primary and/or secondary restraints to specific motions, while collectively they limit and passively guide all six degrees of freedom in the knee joint. This function is supported by an elastic modulus that is over 300 times greater than skin [26-28]. The MCL has an elastic modulus and maximum stress of 332 MPa and 38 MPa [27], respectively, while the ACL has an elastic modulus and maximum stress of 345 MPa and 36 MPa [29], respectively. These mechanical characteristics of ligament are a function of their composition and molecular organization.

**Ligament Composition and Molecular Organization**

The structural organization of ligament follows a hierarchy. In general, ligament can be described as a fiber reinforced matrix, where fibrous collagen fascicles provide structural integrity to the extrafibrillar matrix. Fibroblasts are regularly distributed in the extrafibrillar matrix and are responsible for maintaining the integrity of the collagen fascicles. The orientation of the fascicles is tissue dependent and can be parallel, oblique, and helical relative to the primary loading direction. When unloaded, collagen fibers form a sinusoidal crimp pattern that gradually straightens when strained, which forms the upwardly concave stress-strain behavior of ligament under tension [30]. Ligament is highly hydrated and 70% water by weight [31]. Of the dry weight fraction,
collagen accounts for 65-80% of the total solid phase [32]. The remaining constituents include extracellular matrix proteins (e.g., elastin) and proteoglycans [33, 34].

The insertion sites of ligament have a dramatically different composition that has adapted to reduce stress concentrations that occur between the ligament-bone interface. Two types of insertions sites exist: direct insertions and indirect insertions. Direct insertions are found at the femoral insertion in the MCL, and have fibrocartilage and mineralized fibrocartilage zones between deep ligament fibers and bone. Indirect insertions are found at the tibial insertion of the MCL, and are characterized by superficial fiber fixation directly to the bone without fibrocartilagenous transitional zones. At both the insertions and midsubstance, collagen is the molecular constituent most responsible for resisting tensile loads [35-37].

Collagen

Collagen proteins provide the “connective” ability of connective tissue and therefore, not surprisingly, constitute 25% of the total protein mass in mammals. Type I collagen represents the largest dry-weight fraction in ligament (~70%)[32]. The assembly of collagen fascicles in ligament begins with the secretion of Type I collagen molecules by fibroblast cells. A collagen molecule, or tropocollagen unit (300 nm in length and 1.5 nm in diameter), is a tightly wound triple helix of polypeptide chains, or alpha chains, that contain about 1000 amino acids each. In the extracellular matrix, tropocollagens covalently cross-link into a quarter stagger pattern to make collagen microfibrils (10-300 nm in diameter)( Figure 2.5). Structural stability of collagen is a product of the covalent crosslinks formed between alpha chains and between adjacent
tropocollagen units. Microfibrils amass to form collagen fibrils, and fibrils aggregate to form collagen fibers. Round or spindle shaped fibroblasts sparsely occupy the spaces between these fibers. Fibers group to form fiber bundles, or fascicles, which measure many micrometers in diameter and can be seen without a microscope.

Other types of collagen in ligament, such as types XII, are fibril-associated collagens. These collagens are formed similarly to type I collagen, but do not aggregate to form fibrils in the extracellular matrix. Instead, they bind periodically to the existing fibrils. It is hypothesized that these collagens mediate the interfibril interactions along with fibril-macromolecular interactions [38]. However, this subgroup of collagen has not been specifically identified with any tissue-level mechanical function.
Extrafibrillar Matrix

The extrafibrillar matrix represents the noncollagenous components of the extracellular matrix. These components include proteins such as elastin and fibronectin, along with proteoglycans and water. Elastin is a highly extensible protein that is a composite of single elastin molecules covalently cross-linked. In ligament, elastin accounts for less than 1% of ligament dry weight [39] and is found in abundance as longitudinal oriented fibers that run in parallel to collagen fibrils. Elastin mechanically contributes to the toe-region of the stress response at low strains when the collagen fibers are still partially crimped [40]. The glycoprotein fibronectin constitutes 1-2 µg/mg of dry tissue in the major knee ligaments [41]. Fibronectins function by coupling normal and growing tissue elements, and are required in vitro for collagen organization and fibroblast deposition [42].

Proteoglycans account for less than 1% of ligament dry weight and consist of a protein core covalently bonded to one or numerous glycosaminoglycans. Proteoglycans expressed in ligament include the small leucine-rich proteoglycans decorin and biglycan, and the hyalectan proteoglycans versican and aggrecan [43]. Decorin accounts for 90% of total proteoglycan content in ligament [43]. The high presence of decorin is likely due to its role in controlling the diameter and integrity of Type I collagen [43]. Decorin is secreted in the fibroblasts and “decorates” the collagen fibrils by attaching to gap spaces between longitudinally adjacent tropocollagen units [44]. The number of GAG side chains depends on the proteoglycan. Decorin has a single GAG side chain, dermatan sulfate, and biglycan has two GAG side chains that are chiefly dermatan
sulfate [45]. Versican binds 10-30 GAGs and aggrecan 100 GAGs of chondroitin and keratan sulfate [45].

Glycosaminoglycans play a significant role in the association of water with the extrafibrillar matrix and may contribute to the mechanical response of ligament [46, 47]. Biochemically, GAGs are sulfated polysaccharides, which mediate tissue hydration through electrostatic and nonelectrostatic interactions. Hydration from electrostatic interactions has been termed “charge effect,” and it occurs when the negative fixed charge density of sulfated GAGs attract cations, and thus create a concentration imbalance that swells the tissue through osmotic gradients [48]. This charge effect is reduced as the negative fixed charges become neutralized in hypertonic solution. Non-electrostatic swelling is represented by configurational entropy of the GAG chains in solution. When the solution is hypotonic, the negative charges within the GAGs repulse each other, leading to stiff GAG formations that have low entropy. In hypertonic solution (i.e., 1 M of NaCl), the charge effect is neutralized and increased flexibility of GAG chain configurations leads to higher entropy and water intake [49]. The charge and configuration effects have an inverse relationship that oppositely influences swelling depending on the tonicity of the solution [49]. These water retention mechanisms of GAGs enhance the ability of articular cartilage to bear substantial loads in compression [50, 51], and may similarly affect the mechanics of ligament. Further, GAG to GAG interactions may act as a mechanical link between discontinuous collagen fibrils [46, 52] and are a principal topic of Chapters 6 and 7.
Ligament Contribution to Knee Joint Pathology

Although the characteristic crimp pattern and molecular organization of ligament is highly adapted to control tension, irreversible damage occurs when the mechanical limits of the tissue is exceeded [53]. In 2004, there were 14.2 million visits to the hospital due to knee pain [54], and an estimated 45% of those visits were related to ligament injury [55]. The prevalence of knee ligament injuries is linked to the nonconforming articular surfaces of the knee. For example, a healthy hip is primarily stabilized from the deep “ball and socket” geometry of the femoral head and acetabulum cup, whereas the knee is minimally stabilized from the shallow “modified hinge” geometry of the tibial plateau and femoral condyles. Therefore, the connective tissues of the knee are burdened with stabilizing a highly mobile joint.

The MCL and ACL are the most commonly injured ligaments of the knee [55]. The MCL primarily restricts medial joint opening, and overstretching of the MCL typically occurs during participation in sports such as football and soccer due to a direct blow to the lateral side of the knee [56]. The MCL also restrains external rotation. Sports that rigidly-fix elongated equipment on the foot, such as alpine skiing, are exceptionally well suited to applying large external rotational torques on the knee. For this reason, the MCL is the most common injury during alpine skiing and accounts for 18% of all ski related injuries [57]. The ACL primarily restricts anterior tibial translation and internal tibial rotation. Injury to the ACL occurs in pivot sports and nearly 70% of all ACL injuries are noncontact [58]. In skiing, ACL tears account for 16% of injuries [57], and frequently occur when the skier falls back, a motion that places tremendous stress on the ACL as the tibia translates in the anterior direction. Females
have a higher incidence of ACL injuries compared to males, which has been related to measurably lower yield strength and modulus of elasticity in the female ACL [59] along with biochemical changes related to hormone fluctuations [60]. Combined MCL and ACL injuries are also common and account for 70% of all multiligament injuries [61]. The interdependent functions of the ACL and MCL relate to their frequent and associated injuries. Consequently, the relationship between these ligaments is the focus of Chapters 4 and 5.

When ligaments are torn, the failure typically occurs through the tissue substance, but can also occur by bony avulsion and in rare occurrences at the insertion junction [62, 63]. In general, bony avulsion and junction failures have better outcomes than when the tissue substance is disrupted [64]. Tissue substance failures in the MCL most frequently occur near the femoral or superior insertion [65]. The extra-articular MCL receives adequate blood supply, and after an initial inflammatory phase, matrix and cellular proliferation begins. By the time remodeling and maturation is completed after 12 months, however, the MCL has only 50-70% of its original strength [66, 67]. The two synergistic bundles of the ACL most commonly tear near the femoral insertion, although the rupture pattern of the two bundles differs in 44% of patients [68]. The intraarticular ACL receives significantly less blood than the MCL [69] and therefore, unlike the MCL, a midsubstance rupture cannot heal [70].

The dissimilar healing mechanisms of the MCL and ACL have warranted different treatment regiments. The widespread opinion with MCL injuries is that non-operative treatment is equally as effective as surgical intervention and that exercise accelerates recovery [71]. Biomechanical analysis has shown that conservative
treatment of transected canine MCLs yields better biomechanical properties than surgical treatment [67]. Avulsion injuries to the MCL, when minimally displaced, can be treated nonsurgically, but otherwise are recommended for surgical reattachment of the soft tissue to the denuded bone with sutures or screws [72].

While MCL surgery is uncommon, ACL surgery is extremely prevalent. Reconstructive ACL surgery is performed on 75,000 – 100,000 people each year in the United States [73]. Complete ACL ruptures do not heal spontaneously and can cause gross instability due to knee subluxation. The ACL reconstruction procedure typically involves removing the ruptured ACL and replacing the ligament with an autograft or allograft from the semitendinous tendon or patellar ligament by fixing the grafts in reamed femoral and tibial channels. There are also strong advocates of nonsurgical treatment, which focuses on improving joint stability through muscular conditioning [74]. However, reports have revealed that 60-80% of patients treated nonsurgically experience unsatisfactory outcomes in high-risk populations [75, 76]. Unfortunately, even ACL reconstruction often does not completely restore pre-injury gait [77] or joint stability and studies have shown a large percentage of knees with reconstructed ACLs have unsatisfactory athletic performance [78-80].

In the short-term, knee instability due to ligament injury limits participation in certain sports and activities [81], but in the long-term, these injuries can lead to the more severe and debilitating pathology of osteoarthritis. Osteoarthritis is a joint disease that results in the breakdown and eventual loss of the protective layer of hyaline cartilage between the articulating bones. This condition affects 21 million people in the United States and accounts for 25% of visits to the primary care physicians [82]. The only
treatment for this painful disease is total knee replacement. In 2002, 350,000 primary total knee replacements were performed in the United States [83]. Although this operation has been extremely successful at relieving painful osteoarthritic symptoms, there is an extensive recovery period and normal joint motion and function is permanently restricted. Therefore, considerable research has focused on preventing the necessity of knee replacement. Along with developing technology to regenerate cartilage through tissue-engineering methods [84, 85], an emphasis has been placed on identifying the etiology of this disease. Osteoarthritis has been connected to age-related water loss in articular cartilage [86, 87] and recent studies have shown that ligament injury can result in early progression of this disease [88].

The link between ligament pathology and osteoarthritis resides with altered gait and biological adaptations that occur after ligament trauma. For example, the movement patterns in the ACL deficient knee will exhibit increased flexion during the swing phase and a greater hip extensor moment to reduce anterior tibial translation [77, 80]. These gait changes are identified with redistribution of stresses on the articular cartilage. A study by Andriacchi et al. [88] correlated a shift in the normal load bearing regions of the knee joint after ACL rupture with increased cartilage thinning and Maffulli et al. [89] found that 42% of ACL-deficient patients had a lesion on weight bearing regions of the articular cartilage. The mechanoreceptors that protect the knee through proprioceptive mechanisms are also damaged during ligament rupture [90]. Mechanoreceptors give afferent signals of the spacial limb position to dynamically control muscle and are most active at the limits of motion where joint supports are susceptible to injury [91]. Elimination of these receptors disrupts ligament-muscle
communication. Further, injury to one ligament in the knee can result in histological, biochemical and molecular changes to the other knee ligaments, even if they do not share functional roles [92]. One can conclude that joints behave like an integrated organ system, and it is not surprising that ligament damage and cartilage wear are related. It is therefore imperative to restore ligament mechanical function for long-term health of the knee. Proof of ligament restoration in meaningful terms of joint structural stability and ligament material properties is cardinal, and thus biomechanical experimentation is an indispensable tool for evaluating the efficacy of repair and reconstruction techniques.

**Experimental Biomechanics of Ligament**

The function of joint stabilizers is mechanical in nature and understanding the mechanical characteristics of ligament has many clinical impacts. Biomechanical studies have improved the prevention of ligament injury through intelligent design of athletic equipment [7], playing surface interfaces [93] and prophylactic braces [94]. Quantifying ligament mechanical behavior is necessary to assess surgical techniques and verify the efficacy of harvested or engineered grafts [95, 96]. The mechanisms of tissue repair and structural integrity can be elucidated to improve wound healing modalities and tissue engineering constructs [97, 98]. Finally, biomechanical studies of ligament can be used to define constitutive models that mathematically describe experimental observations. Constitutive models are essential in using computational techniques to comprehensively analyze the complex relationships of joints and provide treatment strategies [99]. Experimental mechanic studies in ligament can be grouped into structural and material tests.
Structural tests involve the physiological manipulation of whole or partial joints while kinematic and force data are collected. These experiments determine joint motion limits and the role of and interaction between joint stabilizers. The first structural experiments on cadaveric knees were performed by Brantigan in 1941 [100]. Brantigan fixed the femur and applied torques and forces to the tibia at various flexion angles. The simplification of these early experiments has evolved into modern systems that better mimic physiological loading configurations. One such system is the Oxford Rig, which permits six degrees of freedom (DOF) by simulating a flexed knee stance through tension of the quadriceps tendon [101]. Another recent system is a robotic arm that can operate in force and displacement mode in up to six DOF by finding the passive knee flexion path in joints [102]. These and other structural testing systems have been used to discern the primary and secondary ligamentous restraints in knees and the forces that ligaments transfer from bone-to-bone. Limitations of structural knee tests include problems inherent to in vitro experiments as well as misrepresentation of passive residual forces in muscle. Structural tests are also unable to characterize the behavior of a specific tissue substance; however, this can be accomplished with material tests.

Material tests are used to characterize the fundamental relationship between stress, strain and other state variables. This relationship may behave in an elastic or viscoelastic manner and may depend on material symmetries. Like all biological tissue, ligaments have elastic and viscous attributes. Elastic materials lose no energy during deformation and behave independent of time, while viscoelastic materials dissipate energy during deformation and have time dependent characteristics such as relaxation,
creep, and strain-rate sensitivity. Material symmetry classifies whether a certain tissue has any preferred directions for a given loading configuration.

To perform a material test the tissue must be isolated and inserted into a material testing system where tensile, compressive or shear loads are applied. Loads are applied along one axis, or along multiple axes to better represent physiological loading patterns. Ligaments can be isolated at their bony insertions [103] or be punched from the tissue substance [27]. Material symmetry is determined by testing a material in various cutting planes. In ligament, when tensile testing is aligned with visible collagen fascicles the material is much stiffer than when tensile testing is aligned transverse to these fibers [27]. This type of material symmetry has one preferred direction and is termed transverse isotropy. The elastic material properties can be tested by 1) loading the material to different strain-levels and allowing equilibration and 2) loading in a quasi-static manner to reduce inertial effects and time-dependent viscoelastic effects. The viscoelastic relaxation and creep properties are determined by deforming the tissue to a constant displacement or force, respectively. Strain-rate sensitivity is measured by varying loading rates. Finally, damping or energy dissipation can be measured by determining the hysteresis or energy dissipation during a loading-unloading cycle. The phase shift that occurs between sinusoidal displacements and the oscillatory force response also reflects this damping behavior [104].

**Numerical Modeling of Ligament**

**Constitutive Models**

Once the material properties of a tissue are determined, it is useful to represent the observed response mathematically for predictive modeling. The mathematical
interpretation of the material is a constitutive relationship that objectively describes the
interaction of stress, strain and other state variables such as strain rate and time. Constitutive
models contain coefficients that are fit to the experimental results through
numerical methods such as curve fitting [105], which can be supplemented with inverse
finite element methods [106]. Soft tissues experience large nonlinear deformations during
normal activity and therefore the constitutive relationship must be a function of variables
that are independent of rotation and have a nonlinear response to strain.

One constitutive model that can accommodate this tissue response is a
hyperelastic strain energy model. The strain energy $W$ represents the stored energy in
the system, and the hyperelastic model permits large deformations by being a function of
the right Cauchy-Green deformation tensor $C$, which is unaffected by rigid body
rotation. For ligament under tension, the material behavior can be described reasonably
well by a transversely isotropic strain energy [107]:

$$W = F_1(I_1) + F_2(\lambda) + \frac{K}{2} (\ln(J))^2$$  \hspace{1cm} (1)

Here $F_1$ is the matrix strain energy and $F_2$ is the collagen fiber strain energy. These two
terms represent the stored energy due to deviatoric deformation. The matrix strain
energy is a function of the first deviatoric invariant, $I_1$. The collagen fiber strain energy
is a function of the deviatoric part of the stretch ratio along the fiber direction, $\lambda$. The
dilational, or pressure response, is a function of the determinant of the deformation
gradient (i.e., volume change), $J$, and the bulk modulus, $K$. The mathematical functions
for each of these components are selected to best describe the tissue being modeled. In
ligament, a neo-Hookean model is commonly chosen to represent the matrix strain
energy [107]. With this simple model, the matrix function is only a dependent on the $I_1$
invariant. The collagen fibers are given piecewise continuous functions for stress that
have physical interpretations [108]. Under compression, the fiber strain energy equation
is zero, because collagen structures tend to buckle under small compressive forces.
During initial tension, ligament will exhibit nonlinear toe region as collagen fibers
uncrimp until the fibers are uniformly straightened at some stretch threshold, $\lambda^*$. This is
mathematically represented by including an exponential function from the start of tensile
stretch to $\lambda^*$. After the tissue is stretched past this threshold, the fiber strain energy is
linearly governed by the modulus of the straightened fibers.

The stress is derived from the strain energy (energy per unit volume):

$$ S = 2 \frac{\partial W}{\partial C}, $$

where $S$ is the 2nd Piola-Kirchhoff stress and $C$ is the right Cauchy-Green deformation
tensor. The 2nd Piola-Kirchhoff stress can easily be converted to other stress forms such
as engineering stress (1st Piola-Kirchhoff Stress) and Cauchy stress.

A limitation of this hyperelastic strain energy material is that it does not
represent the innate viscoelasticity in ligament. Y-C Fung introduced the quasilinear
viscoelastic (QLV) model in 1973 [109] to better represent the strain-history dependent
characteristics of biological tissue. In the QLV theory, the tensile stress is the product of
a reduced relaxation function (3A) and an elastic stress function (3B):

$$ T(t) = \int_{-\infty}^{t} G(t-\tau) \frac{\partial T^{(e)}}{\partial \lambda} \frac{\partial \lambda(t)}{\partial \tau} d\tau, $$

(A) (B)
where \( T \) is the Cauchy stress, \( G \) is the reduced relaxation function, \( \tau \) denotes time, \( t \) denotes time limit, and \( \lambda \) is stretch. The mechanism of this equation is that the reduced relaxation function (3A) decays for increasing values of \( t-\tau \). This decaying function introduces time-history dependence and acts to scale the elastic stress. The chain rule is used to express the instantaneous change in elastic stress (3B). Thus, these two terms scale stress at an instant in time, and the total stress \( T \) is calculated by superposing the instantaneous stresses over the entire stress history of the response.

The QLV model can accommodate any well posed elastic (e.g., hyperelasticity) and reduced relaxation function (e.g., continuous spectrum of kelvin models). Limitations of the QLV model include the nonphysical meaning of the parameter inputs and its inability to model the experimentally observed dependence of damping on strain-level [110]. Poroelasticity theory is an alternative constitutive model that has physically meaningful inputs and can possibly support strain-dependent damping in ligament.

The poroelastic constitutive model was first implemented in biological tissue by Mow et al. [111] to account for the deformation of the solid matrix and interstitial fluid flow in articular cartilage. The force interface between these two phases is a function of fluid pressure and solid/fluid velocity, therefore, a transitory response exists and the theory captures viscoelastic attributes. The poroelastic model is formulated from fundamental principles, which includes the equations of motion for the solid and fluid phase:

\[
\pi + \text{div}(T^s) = 0 \quad (4)
\]
\[
-\pi + \text{div}(T^f) = 0 \quad (5)
\]
where $T_f$ and $T_s$ are the stress tensors of the solid and fluid phase, respectively. An extra reaction force, $\pi$, is present in both equations from the phase interaction. The constitutive model specified for the solid-phase stress tensor can be any well constructed constitutive model, including equations from hyperelastic strain energy and QLV theories. The constitutive model specified for the fluid-phase can be a Newtonian viscous fluid, however, an ideal non-viscous fluid is often assumed and hence the stress tensor for the fluid phase equates to the fluid pressure. A constitutive relation is also specified for the frictional force vector, $\pi$. This drag force is typically a function of relative phase velocity, fluid pressure and permeability (constant or strain dependent).

The conservation of mass for the mixture requires:

$$\text{div}(\varphi^s \mathbf{v}^s + \varphi^f \mathbf{v}^f) = 0$$

(6)

where $\mathbf{v}^s$ and $\mathbf{v}^f$ are the respective solid-phase and fluid-phase velocities, and $\varphi^s$ and $\varphi^f$ are the respective solid-phase and fluid-phase volume fractions. The coefficient inputs to the poroelastic model include parameters (e.g., permeability) that can be experimentally measured [112]. The physical meaning of the coefficients has inherent advantage over more phenomenological based models such as the QLV theory. The poroelastic model has traditionally been used to describe compression-loaded tissues such as cartilage [113], but theoretically it can also predict material behavior under any loading configuration. The ability of the biphasic model to predict stress-relaxation and harmonic oscillation behavior in ligament is briefly explored in the final chapter.
Finite Element Models

The background of this dissertation concludes with a brief dialogue on the finite element method. The finite element method was developed to calculate how force and displacements influence the stress and strain continuum in single or multiple bodies. For 3D finite element analysis, the stress and strain continuum is solved over the volume of the object(s) being analyzed. The basic rationale is that calculations are greatly simplified for repeated geometric shapes, such as tetrahedrals and hexahedrals. Therefore, complex geometries are first discretized into a large group of simplified shapes that can be aggregated to solve for the system. After discretization, boundary conditions are applied (preprocessing) and the analysis is initiated. The vector displacements are determined at each node by minimizing the systems potential energy (internal strain energy + external forces) with a quasi-Newton method. Specified constitutive models and the associated coefficients are necessary for this calculation. Since the potential energy needs to be integrated over the volume of the system, discontinuous nodal displacements can not be used and it is necessary to evaluate differentiable shape functions. Once the total potential energy has been minimized at a load step, the process starts again for the next load step until the analysis is complete. Postprocessing is performed to visualize predictions.

Finite element methods are well established in traditional engineering disciplines and are becoming increasingly pervasive in biological sciences as their utility becomes recognized [99, 114]. To bridge the gap between clinician and engineer, however, it is critical to rigorously validate an FE model by coupling experimental and theoretical work [115]. Strong advances by Gardiner et al. [108] in validating FE models of knee
ligaments have paved the way for a clinically relevant injury model of the knee to be simulated in this dissertation. A validated FE model grants access to a full continuum of mechanical data and represents a successful synergy between experimental biomechanics, mathematical constitutive relations, and numerical methods.

References


CHAPTER 3

SIMULTANEOUS MEASUREMENT OF THREE-DIMENSIONAL
JOINT KINEMATICS AND LIGAMENT STRAINS WITH
OPTICAL METHODS

Abstract

The objective of this study was to assess the precision and accuracy of a non-
proprietary, optical three-dimensional (3D) motion analysis system for the simultaneous
measurement of soft tissue strains and joint kinematics. The system consisted of two
high-resolution digital cameras and software for calculating the 3D coordinates of
contrast markers. System precision was assessed by examining the variation in the
coordinates of static markers over time. Accuracy of 3D strain was assessed by moving
contrast markers fixed distances in the field of view and calculating the error in
predicted strain. For kinematic measurements 3D accuracy was assessed by simulating
the measurements that are required for recording joint kinematics. The field of view
(190 mm) was chosen to allow simultaneous recording of markers for soft tissue strain
measurement and knee joint kinematics. Average system precision was between ±0.004
mm and ±0.035 mm, depending on marker size and camera angle. Absolute

Lujan, Spencer P. Lake, Timothy A. Plaizier, Benjamin J. Ellis, Jeffrey A. Weiss,
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Strains with Optical Methods,” pp: 193-197, 2005, with permission from the ASME
error in strain measurement varied from a minimum of ±0.025% to a maximum of ±0.142%, depending on the angle between cameras and the direction of strain with respect to the camera axes. Kinematic accuracy for translations was between ±0.008 and ±0.034 mm, while rotational accuracy was ±0.082 to ±0.160 deg. These results demonstrate that simultaneous measurement of 3D soft tissue strain and 3D joint kinematics can be performed while achieving excellent accuracy for both sets of measurements.

Introduction

The measurement of strain is of fundamental interest in the study of soft tissue mechanics. In studies of musculoskeletal joint mechanics, the accurate measurement of three-dimensional joint kinematics is equally important. By simultaneously quantifying the strains in soft tissues such as ligaments and the joint kinematics in response to externally applied loads, it is possible to elucidate the role of these structures in guiding and restraining joint motion and to identify potential injury methods and clinical treatments [1-3]. Further, simultaneous acquisition of joint kinematics and strain fields can be used to drive and validate subject-specific models of ligament and joint mechanics [4].

The simultaneous measurement of joint kinematics and soft tissue strain is typically accomplished by using a combination of two or more different technologies. Joint kinematics are commonly quantified using video-based techniques [5,6], instrumented spatial linkages (ISLs) [7,8] or electromagnetic tracking systems [9-11]. ISL systems require the attachment of a bulky mechanical linkage across the joint, while electromagnetic tracking systems are often plagued by interference from ferrous
materials, limiting their applicability. In contrast, there are relatively few techniques that are capable of measurement of 3D soft tissue strains. Alternatives include the use of 1D measurements from contact devices such as DVRTs [12, 13]. Optical methods are currently considered to be the best option for 3D strain measurement on visible soft tissues. These methods use the direct linear transformation to calculate 3D strain measurements from two or more cameras [4,14]. Previous optical systems were primarily based on super VHS video, yielding an effective vertical resolution of 400 lines. This limited resolution requires the use of extremely small fields of view to achieve accuracies of ±0.1-0.5% error in percent strain [14]. This precludes the simultaneous tracking of markers for kinematic measurements, since a larger field of view is needed to see both the strain and kinematic markers. Currently available systems based on digital cameras typically use vendor-supplied proprietary cameras and/or framegrabbers. These systems primarily use digital cameras that have much better resolution and sensitivity than video-based systems. However, the use of proprietary vendor-supplied hardware is often costly and ties the support and upgrade of the system to a particular vendor or system integrator.

Due to ongoing improvements in the sensitivity and resolution of charge-coupled devices (CCDs), modern progressive-scan digital cameras can provide images with very high quality and resolution. The improved spatial resolution (typically at least 1024x1024) opens up the possibility of using a field of view that is large enough to track markers for both soft tissue strain and joint kinematics. The use of cameras and framegrabbers from individual vendors is especially attractive since it eliminates the need for proprietary, vendor-specific hardware and software. The objective of this study
was to develop a methodology for simultaneous measurement of 3D soft tissue strain and joint kinematics using a nonproprietary digital camera system, and to quantify the errors associated with these measurements in a test setup that mimicked the study of knee ligament biomechanics.

**Materials and Methods**

**Measurement Systems**

The measurement system consisted of two high-resolution digital cameras (Pulnix TM-1040, 1024x1024x30 frames per second (fps), Sunnyvale, CA) equipped with 50 mm 1:1.8 lenses and extension tubes, two frame grabbers (Bitflow, Woburn, MA) and Digital Motion Analysis Software (DMAS, Spica Technology Corporation, Maui, HI). The cameras were configured to record 6 fps directly to computer memory, requiring 2.1 MB of memory per frame. The cameras were focused at a target with a 190 mm diagonal field of view (FOV). The DMAS software tracked marker centroids in both camera views automatically and applied the modified direct linear transformation (DLT) to calculate the 3D centroid coordinates [15]. Preliminary tests demonstrated that black markers against a white background provided superior contrast and therefore system accuracy in comparison to markers covered with reflective tape, while two 100 W incandescent lights provided better contrast than halogen or fluorescent lighting. In the following sections, all instrument accuracy values are per the manufacturer.

A 3D calibration frame was manufactured. Twenty-seven white Delrin spherical markers (4.75 mm dia.) were arranged in three horizontal planes, with a 3x3 grid pattern on each plane and 60 mm marker spacing. The exact coordinates of each marker
centroid were determined with a coordinate measuring machine (Zeiss Eclipse 4040, accuracy ±0.0004 mm). These coordinates were used for DLT calibration.

Precision

The precision was determined by examining the variation of the 3D positions of stationary markers over time. After calibration, two different frames with twelve 4.75 and 2.38 mm dia. spherical markers were recorded for 25 seconds. The dimensions of these markers were chosen to be the exact same size as the kinematic markers (4.75 mm dia) and the strain markers (2.38 mm dia) used during actual biomechanical testing in our laboratory. The variation in marker position was determined by computing two standard deviations of the length of their position vector and the individual x, y and z coordinates over time. Experiments were repeated at camera angles of 30, 60 and 90 deg (Figure 3.1). To evaluate the precision of the system in actual test conditions, the variation of kinematic (4.75 mm dia) and strain (2.38 mm dia) marker positions were determined for four-sets of three-second passive recordings taken at a 30° camera angle during biomechanical testing of a human medial collateral ligament (MCL) (Figure 3.2). Complete details of this test configuration and marker placement can be found in our previous publications [4,14].

Accuracy of Simulated Strain Measurement

Accuracy tests were performed dynamically to determine the ability of the system to measure simulated 3D changes in strain. The effects of strain magnitude and camera angle were assessed. A 2.38 mm dia. marker was adhered to a fixed location, while a similar marker was adhered 13.5 mm apart ($L_{initial}$) to a linear actuator (Tol-O-
Figure 3.1. Plan view of the camera setup. The z-axis is directed out of the page. To assess system sensitivity to camera angle, angles of 30, 60 and 90 deg were used during testing.

Figure 3.2. Photograph of test setup for simultaneous measurement of MCL strain and knee joint kinematics. Eighteen markers (2.38 mm diameter) were adhered to the MCL for strain measurement. Femoral and tibial kinematic blocks, each with three kinematic markers (4.75 mm diameter), were affixed to the cortical bone.
Matic, Inc, Hamel, MN, accuracy ±0.0025 mm). The $L_{\text{initial}}$ was chosen to replicate the spacing between markers used to calculate 3D strains in the human MCL [4,14]. In two separate tests, the actuator was translated ($\Delta L$) along either the $z$- or $x$-axis in Figure 3.1 to simulate strains of 1, 2, 5 and 20%. Tests were performed four times for each displacement. Accuracy was calculated as the difference between the predicted displacement and the known actuator displacement. The error in simulated strain measurement was computed by determining the absolute difference between actuator strain and DMAS strain ($\Delta L/L_{\text{initial}}$).

**Accuracy of Kinematic Measurements**

When tracking joint kinematics, it is desirable to establish “embedded” coordinate systems within the bones using a convention such as the one described by Grood and Suntay [16]. The transformation matrix between embedded coordinate systems is established by tracking markers on the bones that define separate “marker” coordinate systems. The transformation between one marker coordinate system and the corresponding embedded coordinate system on a bone does not change during testing. By establishing these transformations before testing and then tracking the transformation between marker coordinate systems during testing, the transformation between embedded coordinate systems can be determined [4,14]. To assess kinematic measurement accuracy, the setup and calculations necessary to record knee joint kinematics were simulated. Two L-shaped white blocks (the “kinematic blocks,” Figure 3.2) with three 4.75 mm dia. black markers that formed a 90 deg angle were used to establish marker coordinate systems. The following tests were repeated four times for each translation or rotation, at camera angles of 30, 60 and 90 deg.
To measure accuracy of translations along the z-axis in Figure 3.1 from kinematic calculations, one kinematic block was adhered to a static fixture and a second one was attached to the linear actuator. An Inscribe 3D Digitizer (Immersion Corp, San Jose, CA accuracy ±0.085 mm) was used to determine the centroids of the markers by digitizing points on the marker surface and then fitting the coordinates to the equation of a sphere. To simulate the use of embedded coordinate systems, three points on both the static fixture and the actuator were digitized and used to establish orthonormal coordinate systems. The transformation matrices were calculated between the kinematic blocks and their respective embedded coordinate systems. The actuator was displaced 0.500, 1.000, 5.000 and 50.000 mm and an overall transformation matrix between the embedded systems was calculated by concatenation. The ratio of the calculated translations to the known translations was computed.

To measure accuracy of translation measurements along the x-axis in Figure 3.1 from kinematic calculations, a kinematic block was adhered to an x-y table and the table was moved 0.50, 1.00, 5.00, and 50.00 mm, measured with digital calipers (Mitutoyo, San Jose, accuracy ±0.02 mm). Error was calculated as the ratio of translation predicted from the motion analysis data to the known translation.

To determine accuracy of rotations about the z-axis in Figure 3.1, a rotational actuator (Tol-O-Matic, Inc, Hamel, MN, accuracy ±0.002°) was used to rotate one of the kinematic blocks through angles of 2.00° and 20.00°. The transformation matrix between the two embedded coordinate systems was calculated and the rotation between the two systems was resolved using the method of Grood and Suntay [16]. The ratio of the rotation angle from the motion analysis data to the known angle was determined.
Statistical Analysis

The effects of camera angle and marker size on 3D precision were assessed using a two-way ANOVA with repeated measures. The effects of camera angle and strain magnitudes on x-axis and z-axis strain accuracy were assessed using two separate two-way ANOVAs with repeated measures. The effects of camera angle on x-axis and z-axis translational kinematic accuracy and z-axis rotational kinematic accuracy were assessed using two separate two-way ANOVAs with repeated measures. Statistical significance was set at p<0.05 for all analyses.

Results

Precision

Results for precision were excellent for both marker sizes (Table 3.1). The larger markers exhibited significantly better precision than the smaller markers (p=0.005). There was no effect of camera angle on marker precision (p=0.089). The best results (±0.004 mm, 0.0020 % FOV) were obtained for the larger markers using a 30-deg camera angle. Precision did not vary considerably between the x, y and z coordinates of the markers. For example, at a 60 deg camera angle, the larger markers had x, y and z coordinate precisions of 0.019, 0.019 and 0.012 mm, respectively. The precisions for both marker sizes obtained with a MCL biomechanical test setup were comparable to precisions for the controlled tests (Table 3.1, 4th row).
Table 3.1. Results for measurement of 3D precision. The first three rows of data were obtained by recording stationary markers for 25 seconds with a FOV of 190 mm. The fourth row was obtained from passive recording acquired during biomechanical testing of a human MCL. Each passive recording was approximately 3 seconds long, with a camera angle of 30°, and a FOV of 190 mm. Absolute precision was calculated as two standard deviations of the position measurement, while Percent FOV was calculated as the precision divided by the FOV multiplied by 100.

<table>
<thead>
<tr>
<th>Camera Angle (θ)</th>
<th>4.75 mm dia. Markers</th>
<th>2.38 mm dia. Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision (mm)</td>
<td>Percent FOV (%)</td>
</tr>
<tr>
<td>30°</td>
<td>0.004</td>
<td>0.0020%</td>
</tr>
<tr>
<td>60°</td>
<td>0.011</td>
<td>0.0049%</td>
</tr>
<tr>
<td>90°</td>
<td>0.006</td>
<td>0.0026%</td>
</tr>
<tr>
<td>MCL Study (30°)</td>
<td>.009</td>
<td>0.0044%</td>
</tr>
</tbody>
</table>
Accuracy of Simulated Strain Measurement

The optical system delivered excellent results for strain error (Table 3.2). There was a significant effect of camera angle on accuracy for z- and x-axis strain accuracy (p=0.004 and p<0.001, respectively, Figure 3.3). The most accurate camera angle for strains along the z-axis was 30 deg, having an average accuracy of ±0.005 mm with a strain error of ±0.035%. Conversely, the most accurate camera angle when strains were measured along the x-axis was 90 deg, having average accuracies of ±0.003 resulting in a strain error of ±0.025%. There was a significant effect of strain magnitude on accuracy for z- and x-axis strain accuracy (p=0.008 and p<0.001, respectively). The condition conferring the least accuracy occurred for both the z-axis and x-axis cases at 90 deg and 30 deg, respectively, when a 20% strain was applied.

Accuracy of Kinematic Measurements

The optical system delivered very good results for kinematic accuracy (Table 3.3). Data for z-axis kinematic accuracy are shown as an example (Figure 3.4). The average x- and z-axis translational accuracies across all three camera angles and all four actuator displacements were ±0.025 and ±0.016 mm, respectively. Average accuracy for rotation was ±0.124 deg (Table 3.3). There was a significant effect of camera angle on accuracy of kinematic measurements of translation along the z- and x-axes (p=0.029 and p<0.001, respectively), but there was no effect of camera angle on kinematic rotational accuracy (p=0.378). The effect of camera angle on the kinematic translational accuracies was similar to that for the strain accuracies. There was a significant effect of the magnitude of translation/rotation on accuracy of kinematic measurements of
Table 3.2. Accuracy of 3D simulated strain measurement along the z- and x-axes for all four strain levels. Accuracy (mm) was calculated as the difference between the actuator-based value and the value calculated from the optical system data. Strain error (%) is the accuracy divided by the gauge length (13.5 mm) multiplied by 100.

<table>
<thead>
<tr>
<th>Camera Angle (θ)</th>
<th>1.0% Strain</th>
<th>2.0% Strain</th>
<th>5.0% Strain</th>
<th>20.0% Strain</th>
<th>Averages across all Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>z-axis 30 deg</td>
<td>Accuracy (mm) 0.004 ± 0.007</td>
<td>0.011 ± 0.003</td>
<td>0.003 ± 0.004</td>
<td>0.001 ± 0.007</td>
<td><strong>0.005 ± 0.005</strong></td>
</tr>
<tr>
<td></td>
<td>Strain Error (%) 0.028 ± 0.052</td>
<td>0.084 ± 0.018</td>
<td>0.019 ± 0.030</td>
<td>0.010 ± 0.052</td>
<td><strong>0.035 ± 0.033</strong></td>
</tr>
<tr>
<td>60 deg</td>
<td>Accuracy (mm) 0.002 ± 0.001</td>
<td>0.013 ± 0.005</td>
<td>0.005 ± 0.005</td>
<td>0.009 ± 0.012</td>
<td><strong>0.007 ± 0.006</strong></td>
</tr>
<tr>
<td></td>
<td>Strain Error (%) 0.014 ± 0.009</td>
<td>0.099 ± 0.037</td>
<td>0.040 ± 0.035</td>
<td>0.067 ± 0.091</td>
<td><strong>0.055 ± 0.037</strong></td>
</tr>
<tr>
<td>90 deg</td>
<td>Accuracy (mm) 0.009 ± 0.001</td>
<td>0.016 ± 0.003</td>
<td>0.011 ± 0.004</td>
<td>0.024 ± 0.005</td>
<td><strong>0.015 ± 0.003</strong></td>
</tr>
<tr>
<td></td>
<td>Strain Error (%) 0.065 ± 0.006</td>
<td>0.115 ± 0.025</td>
<td>0.081 ± 0.027</td>
<td>0.175 ± 0.034</td>
<td>0.109 ± 0.049</td>
</tr>
<tr>
<td>x-axis 30 deg</td>
<td>Accuracy (mm) 0.011 ± 0.002</td>
<td>0.011 ± 0.002</td>
<td>0.019 ± 0.002</td>
<td>0.036 ± 0.003</td>
<td><strong>0.019 ± 0.012</strong></td>
</tr>
<tr>
<td></td>
<td>Strain Error (%) 0.081 ± 0.018</td>
<td>0.082 ± 0.014</td>
<td>0.138 ± 0.017</td>
<td>0.267 ± 0.024</td>
<td><strong>0.142 ± 0.088</strong></td>
</tr>
<tr>
<td>60 deg</td>
<td>Accuracy (mm) 0.002 ± 0.002</td>
<td>0.018 ± 0.002</td>
<td>0.006 ± 0.011</td>
<td>0.003 ± 0.002</td>
<td><strong>0.007 ± 0.007</strong></td>
</tr>
<tr>
<td></td>
<td>Strain Error (%) 0.015 ± 0.015</td>
<td>0.130 ± 0.017</td>
<td>0.045 ± 0.082</td>
<td>0.021 ± 0.011</td>
<td><strong>0.053 ± 0.053</strong></td>
</tr>
<tr>
<td>90 deg</td>
<td>Accuracy (mm) 0.002 ± 0.001</td>
<td>0.006 ± 0.001</td>
<td>0.003 ± 0.007</td>
<td>0.003 ± 0.001</td>
<td><strong>0.003 ± 0.002</strong></td>
</tr>
<tr>
<td></td>
<td>Strain Error (%) 0.017 ± 0.008</td>
<td>0.045 ± 0.007</td>
<td>0.022 ± 0.049</td>
<td>0.018 ± 0.006</td>
<td>0.025 ± 0.013</td>
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Figure 3.3. A - Simulated 3D strain error along the z-axis. B – Simulated 3D strain error along the x-axes. Strain Error was computed as the difference between the actuator-based strain and the strain calculated by the motion analysis system, divided by the gauge length.
Table 3.3. Accuracy of 3D kinematic measurements. Accuracy is the difference between the actuator translation/rotation and the value calculated from the optical system data. Accuracy in terms of Percent FOV is the accuracy divided by FOV multiplied by 100. Results are the average across all translations/rotations described in the Methods section.

<table>
<thead>
<tr>
<th>Camera Angle (θ)</th>
<th>Translation (x-axis)</th>
<th>Translation (z-axis)</th>
<th>Rotation (z-axis)</th>
</tr>
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<td></td>
<td>Accuracy (mm)</td>
<td>% FOV</td>
<td>Accuracy (mm)</td>
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<tr>
<td>30 deg</td>
<td>0.034 ± 0.031</td>
<td>0.012 ± 0.004</td>
<td>0.008 ± 0.011</td>
</tr>
<tr>
<td>60 deg</td>
<td>0.018 ± 0.005</td>
<td>0.009 ± 0.006</td>
<td>0.019 ± 0.023</td>
</tr>
<tr>
<td>90 deg</td>
<td>0.021 ± 0.002</td>
<td>0.009 ± 0.008</td>
<td>0.020 ± 0.017</td>
</tr>
</tbody>
</table>

Figure 3.4. Results for the measurement of 3D kinematics along the z-axis direction. Accuracy was measured as the difference between the actuator based translation and the value calculated by the motion analysis system, divided by the actuator translation.
translation along the z- and x-axes and rotation about the z-axis (p<0.001, p<0.001 and p=0.033, respectively). Larger translations/rotations reduced kinematic accuracy.

**Discussion**

This study demonstrated that the 3D system can accurately measure simulated strain and kinematics using physical and optical conditions that accommodate simultaneous tracking of markers for both measurements. A reduced camera angle significantly improved accuracy for frontal plane (z-axis) displacements, while an increased camera angle significantly improved accuracies of displacements along the intersection of the sagittal and transverse planes (x-axis). Moreover, in comparison to similar systems using proprietary vendor-specific hardware, this system is a small fraction of the cost.

Accuracy and precision of the system were determined using a testing environment that was specifically designed to mimic the physical and optical conditions for experiments on the human MCL in intact knees. For actual testing of the human MCL, only precision was determined. It is not possible to determine strain accuracy during an actual biomechanical test since a gold standard for strain measurements is difficult if not impossible to establish. However, by setting all testing variables (i.e., FOV, marker size, marker spacing, lighting) appropriately, the controlled tests faithfully reproduced the physical and optical conditions of actual biomechanical tests for the MCL. Results for precision from the controlled tests and from actual measurements on the MCL were similar (Table 3.1), supporting the notion that the controlled tests provided a good surrogate for the physical and optical conditions that are encountered during actual tests on the MCL.
System precision (Table 3.1) was calculated using the length of the position vectors of the markers, and thus these measurements should be considered average errors that take into account the precision in all three spatial directions. For the simulated strain measurements, the major spatial directions were accounted for by testing along the x- and z-axes. By positioning the cameras at an angle that permitted the most marker motion perpendicular to the cameras, therefore reducing the incremental distances that each pixel in the video system represents, the strain error was significantly decreased. For z-axis strains this occurred at a 30 deg camera angle, and for x-axis strains this occurred at a 90 deg camera angle. Based upon the accuracy results (Figure 3.3), it is recommended that these camera angles be optimized accordingly, especially if large strains are predicted. The measurements of strain accuracy should be considered a best case when considering general measurements in 3D using comparable camera angles. Although a similar argument applies to the kinematic measurements, the “gold standard” for these measurements was based on a combination of digitizer, actuator encoder, or digital caliper measurements. Because of differences in the accuracy of these measurement techniques and the propagation of errors in the kinematic measurements, the results for translational and rotational kinematic accuracies likely represent worst cases. This error propagation is likely responsible for the reduction of accuracy with increased axial translation (Figure 3.4).

As with any system based on video or digital cameras, the most important determinants of precision and accuracy are the resolution of the CCD and the FOV used for measurements. This assumes that an accurate DLT calibration has been performed and that this calibration has taken into account errors associated with lens distortion. In
this study, the FOV was chosen to allow simultaneous tracking of markers for strain and kinematic measurements in the context of studying the human MCL [4,14]. Limitations on the rate of data transfer from the camera to the framegrabber cards and then to computer memory, primarily imposed by the bandwidth of the computer system’s bus, result in a tradeoff between the frame rate and spatial resolution of the CCD. Cameras with higher resolution CCDs typically have slower frame rates. This limitation will likely be eliminated with improvements in computer architecture. Marker contrast is also very important, with improved contrast yielding better system precision and thus accuracy. During actual biomechanical testing, contrast may become reduced by extraneous objects in the foreground and background. Draping the testing backdrop and fixtures with white material, and applying white gauze to any uninvolved tissue that may darken the captured image will alleviate this problem. Extreme specimen discoloration could similarly reduce marker contrast. Affixing the strain markers to the tissue with adhesives may cause local strain abnormalities; therefore a minimal amount of adhesive should be applied. Finally, the physical size of a CCD affects the sensitivity through its responsivity and dynamic range, with larger CCDs yielding better sensitivity and thus better image quality (see, e.g., [17]). The cameras used in this study had 1” CCDs, the largest size that was available.

In summary, the 3D measurement system provided excellent accuracy for simulated strain measurement and very good accuracy for kinematic measurements. The absolute and percent errors are considered to be more than acceptable for simultaneous 3D measurements of ligament strain and joint kinematics.
References


CHAPTER 4

EFFECT OF ACL DEFICIENCY ON MCL STRAINS
AND JOINT KINEMATICS

Abstract

The knee joint is partially stabilized by the interaction of multiple ligament structures. This study tested the interdependent functions of the anterior cruciate ligament (ACL) and the medial collateral ligament (MCL) by evaluating the effects of ACL deficiency on local MCL strain while simultaneously measuring joint kinematics under specific loading scenarios. A structural testing machine applied anterior translation and valgus rotation (limits 100 N and 10 N m, respectively) to the tibia of 10 human cadaveric knees with the ACL intact or severed. A three-dimensional motion analysis system measured joint kinematics and MCL tissue strain in 18 regions of the superficial MCL. ACL deficiency significantly increased MCL strains by 1.8% ($p<0.05$) during anterior translation, bringing ligament fibers to strain levels characteristic of microtrauma. In contrast, ACL transection had no effect on MCL strains during valgus rotation (increase of only 0.1%). Therefore, isolated valgus

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rotation in the ACL-deficient knee was nondetrimental to the MCL. The ACL was also found to promote internal tibial rotation during anterior translation, which in turn decreased strains near the femoral insertion of the MCL. These data advance the basic structure-function understanding of the MCL, and may benefit the treatment of ACL injuries by improving the knowledge of ACL function and clarifying motions that are potentially harmful to secondary stabilizers.

Introduction

The mechanical functions of knee ligaments are interrelated, with multiple soft tissue structures contributing to joint stability under externally applied loading conditions [1, 2]. The overlapping function of the anterior cruciate ligament (ACL) and medial collateral ligament (MCL) is a prime example of this concept, as these ligaments share responsibility in stabilizing anterior translation of the tibia and valgus joint opening [3]. Injuries to the ACL and MCL account for 26% of knee trauma [4], with combined ACL/MCL injuries comprising 70% of all multiligament knee injuries [5]. Isolated MCL injuries often adequately heal without surgical intervention. However, conservatively treated ACL injuries have a high incidence of unsatisfactory outcomes [6, 7]. Even ACL reconstructed knees exhibit abnormal kinematics [8-10] that may lead to cartilage degeneration [11]. Due to the relationship between the ACL and MCL, treatment of combined or isolated ACL injuries may be improved by an understanding of the mechanical effects of ACL deficiency on MCL function.

The current knowledge of ligament function in the knee joint is largely based on ligament cutting studies that measured changes in laxity after dissecting a specific structure. Experimental studies in cadaveric knees have demonstrated that the
superficial MCL is the primary restraint to valgus rotation, and a secondary restraint to anterior translation [3, 12-16], while the ACL is the primary restraint to anterior translation, and a secondary restraint to valgus rotation [3, 13, 14, 17-19]. In addition, the MCL and ACL both resist internal tibial rotation [20-22], with the MCL also resisting external tibial rotation [22, 23]. Recent experiments have investigated local tissue strains and overall force in the ligament during applied loading conditions. Local MCL strains have been measured for single or combined loading conditions, and with the exception of studies by Fischer et al. [24] and Yasuda et al. [25], all MCL strain studies have focused on intact knees [26-31]. Fischer utilized strain measurement techniques to determine if function of the superficial MCL was affected when the posterior aspect of the longitudinal parallel fibers was severed. Significant changes in strain were only seen in an ACL deficient knee, prompting future research to look into the interaction between the superficial MCL and the ACL. Yasuda found that the ACL has minimal effect on the dynamic strain behavior of the MCL when a lateral impact load is applied to the knee, and kinematic studies determined that when the MCL is intact, the ACL has only a small influence on valgus laxity near full knee extension [12, 22]. Nevertheless, force measurement studies found that when the MCL is intact, ACL tension significantly increases with the application of a valgus load over a range of flexion angles [20, 32]. These results leave the role of the ACL in resisting valgus rotation in an MCL-intact knee unclear; moreover, it is unknown how ACL deficiency quantitatively affects regional MCL strain under specific loading conditions.

Interpretation of these interactions may be aided by investigating how ACL deficiency alters localized MCL strains and joint kinematics. Local measurement of
ligament strain provides insight into regional function and the values of strain directly relate to the propensity of the tissue to damage, tear or rupture [33]. Further, local strain measurements on heterogeneous tissue structures are necessary to understand how externally applied kinematic motions are resisted by specific regions [26, 34]. This information would provide a broad visualization of MCL structural behavior and would identify the loading configurations that the MCL resists actively. Finally, studying MCL strain patterns in normal and ACL-deficient knees can afford a physiological baseline to compare the in vitro efficacy of ACL reconstruction techniques. The objective of this research was to quantify regional MCL strains and joint kinematics in the normal and ACL deficient knee during anterior translation and valgus rotation at varying flexion angles and tibial axial constraint. Two hypotheses were tested: (1) Strains in the MCL increase following ACL transection during application of anterior translation, and (2) strains in the MCL increase following ACL transection during application of valgus rotation.

**Materials and Methods**

**Experimental Design**

Kinematic tests were performed on human knees before and after ACL transection. Briefly, the tibia of each knee was subjected to cyclic anterior-posterior (A-P) translation and varus-valgus (V-V) rotation at flexion angles of 0, 30, 60, and 90 deg with tibial axial rotation constrained or unconstrained. MCL tissue strains and joint kinematics were recorded during the entire application of anterior translation and valgus rotation to the tibia. Following testing, the MCL was dissected free from the joint to
measure the stress-free strain pattern of the MCL. All tissues were kept moist with 0.9% saline solution throughout dissection and testing.

Specimen Preparation

Ten cadaveric right knees were acquired fresh-frozen from male donors (donor age = 56±7 y, range 18-65). Each knee was from midtibia to midfemur and was allowed to thaw for 16 h prior to dissection. All skin, fascia, muscle, and other periarticular soft tissue surrounding the knee joint was removed, including the patella and patellar tendon. One knee was eliminated from testing due to the absence of a medial meniscus. Otherwise all knees showed no sign of arthritis or previous soft tissue injury. The fibula was secured to the tibia with a stainless steel screw to ensure an anatomical position was maintained. The femur and tibia were potted in mounting tubes using catalyzed polymer resin (Bondo, Mar-Hyde, Atlanta, GA). Two L-shaped white blocks (the “kinematic blocks”) with three black acrylic markers (4.75 mm dia.) were fastened to the anterior femoral condyle and the posterior aspect of the tibia using nylon screws. Kinematic blocks were used to record the three-dimensional kinematic motions of the tibia and femur during testing.

A 3 x 7 grid of markers (2.3 mm dia.) was adhered to the MCL using cyanoacrylate (Figure 4.1). These markers formed 18 gauge lengths for strain measurement, with each gauge length spanning approximately 15 mm along the collagen fiber direction. The markers were teased with tweezers after adhesion to verify that they were attached to the superficial MCL fibers and not to the fascia. The markers in the first and second rows were arranged along the anterior and posterior longitudinal parallel fibers of the superficial MCL, respectively (Figure 4.1). Distal to the joint line, the
Figure 4.1. Location of fiducial markers. Twenty-one markers defined 18 regions for strain measurement. The markers in row 1 and row 2 were affixed to the anterior and posterior longitudinal fibers of the superficial MCL. Markers in row 3 inferior to the joint line were considered affixed to the distal oblique fibers of the superficial MCL. Markers in row 3 superior to the joint line were considered affixed to the anterior posteromedial corner.

Markers in the third row were affixed to the distal oblique fibers of the superficial MCL. Proximal to the joint line, the markers in the third row were affixed to the anterior portion of the posteromedial corner. These naming conventions are consistent with Robinson et al. [35] and Warren and Marshall [36].

Testing Procedure

Each knee was mounted in fixtures on a custom testing machine. The machine and fixtures allowed up to four degrees of freedom (DOF) through a combination of linear and rotary bearings and actuators (Figure 4.2). Flexion was fixed, and either A-P displacement during V-V rotation or V-V rotation during A-P displacement was fixed. The tibial fixture permitted tibial axial rotation to be either constrained or unconstrained.
Figure 4.2. Schematic of the loading apparatus, depicting a medial view of the knee at 0 deg flexion. Kinematic blocks are rigidly attached to the tibia and femur for 3-D motion measurement. A – applied anterior-posterior tibial translation. B - applied varus-valgus rotation. C – adjustable flexion angle. D - constrained or unconstrained tibial axial rotation. E - unconstrained medial-lateral translation and joint distraction. F – load/torque cell.

Thirty-two tests were performed on each knee. A-P displacements were applied to a set force limit and V-V rotations were applied to a set torque limit (limits of ±100 N and ±10 N m, respectively [22, 26, 37]). Both A-P and V-V tests were performed at four flexion angles (0, 30, 60, and 90 deg), with tibial rotation either unconstrained or constrained, and the ACL either intact or deficient. Ten cycles were run for each test to precondition the soft tissue structures of the knee. Data were analyzed at the tenth cycle during anterior translation and valgus rotation. Linear and angular velocities (1.5 mm/s and 1 deg/s, respectively) were selected to achieve quasi-static test conditions, thus minimizing tissue viscoelastic and inertial effects. A bus cable (RTSI, Plano, TX) was integrated with LabView software to enable real-time capture of both the loading data from the multiaxial load cell (Futek T5105, Irvine, CA, accuracy ±2.2 N and ±0.056 N
MCL strains and joint kinematics were measured simultaneously using a 3D motion analysis system that tracked the centroids of the markers attached to the MCL and kinematic blocks (Figure 4.2) [34]. The associated software used the modified direct linear transformation method to calculate the 3D spatial coordinates of the markers [34]. The 3D motion analysis system consisted of two high-resolution digital cameras (Pulnix TM-1040, 1024x1024x30 fps, Sunnyvale, CA) equipped with 50 mm 1:1.8 lenses and extension tubes, two frame grabbers (Bitflow, Woburn, MA) and digital motion analysis software (DMAS, Spica Technology Corp, Maui, HI). The extracapsular location of the MCL and its planar geometry facilitated the use of this motion analysis system for strain measurement. Unconstrained tibial axial rotation of the knee was calculated using the established kinematic conventions of Grood and Suntay [38].

Prior to testing, a mechanical digitizer (Immersion Corp, San Jose, CA accuracy ±0.085 mm) was used to create “embedded” coordinate systems based on anatomical landmarks [39, 40]. The centroids of the markers on the kinematic blocks were determined by averaging four digitized points around the circumference of each marker. These centroids were used to create marker coordinate systems. The transformation matrix between the femur and tibia could then be calculated by using the transformation matrices formed between the embedded and marker coordinate systems and the video-tracked kinematic block systems [34].

A testing methodology was developed to initiate ACL-deficient tests from the ACL-intact neutral position. This neutral position was defined for each flexion angle by
finding the inflection point of the force response resulting from small cyclic A-P and V-V displacements, with tibial axial rotation unconstrained. Actuator translation and rotation positions were logged so that the original neutral positions could be restored after ACL transection. To mimic ACL deficiency, the ACL was transected through its midsubstance without removing the knee from the fixture. Care was taken to avoid damage to the PCL. To verify that the ACL-intact testing position was reproduced for the ACL-deficient knee, kinematic block positions were measured in relation to each other and the multiaxial test frame for each flexion angle. After ACL transection, positional information was compared at each flexion angle and adjustments were made if necessary.

Establishment of Reference Configuration for Strain Measurement

Following testing, the MCL was dissected from its femoral and tibial attachments to measure the stress-free reference length \( l_0 \) between all 18 marker pairs. Using validated procedures [26, 37], the motion analysis system measured the stress-free configuration after the isolated ligament relaxed for 10 min on a saline covered glass plate. This was an important step for the calculation of absolute strain, as force exists in the ligament when it is attached to its insertion sites. Material properties of ligament, including ultimate and substructural failure limits, have been quantified in the literature using stress-free configurations [33, 41]. Accurate interpretation of strain data therefore required the use of stress-free reference lengths. In this study, it was found that basing strain results on \textit{in situ} gauge lengths measured at 0 and 30 deg passive knee flexion, on average significantly underpredicted strain by 2.7±0.1\% (\( p<0.001 \)) and 1.1±0.1\% (\( p<0.001 \)), respectively.
Data and Statistical Analysis

The lengths between marker pairs were measured in the previously described stress-free reference state \((l_0)\) and during kinematic tests \((l)\) at peak valgus rotation, peak anterior translation, and in the neutral position. Tensile strain along the fiber direction was calculated as \(\varepsilon = (l - l_0) / l_0\). Repeated measures ANOVA analysis with three within-subject factors (ACL state, knee flexion angle, tibial axial constraint) was used in conjunction with Bonferroni adjusted pair-wise comparisons to measure significance of factors, factor interactions and between factor levels. If significance was found \((p \leq 0.05)\), adjusted paired \(t\)-tests were used for case by case comparisons. A similar analysis was performed for the kinematic data. A power analysis demonstrated that a sample size of 10 was sufficient to obtain a power of 0.8 when detecting a 1.0\% change in the strain, a 1.0 deg kinematic rotation, and 1.5 mm kinematic displacement. Data are reported as mean±standard error, unless otherwise stated.

To represent MCL strains graphically, mean values of regional fiber strain were applied to a finite element mesh of a MCL constructed from one of the specimens [37]. This mesh was input to TOPAZ3D (LLNL, Livermore, CA) to perform a least squares interpolation of fiber strain values between discrete measurement locations, which yielded a continuous spatial representation of the results.

Results

Effect of ACL Transection

ACL deficiency significantly increased anterior translation by an average 10.0 ± 1.1 mm \((p<0.001, \text{Figure 4.3A})\), and MCL strains were significantly greater for ACL-deficient cases at peak anterior translation \((\text{Figure 4.3B})\). ACL deficiency did not
Figure 4.3. Experimental results from anterior-posterior translation. A) Anterior tibial translation at all flexion angles, with unconstrained tibial axial rotation, before and after ACL transection. B) Average MCL strains at ATT as a function of knee flexion angle, with unconstrained tibial axial rotation, before and after ACL transection. ACL transection had no significant effect on valgus laxity or MCL strains. Error bars = SD.
significantly affect valgus rotation ($p=0.12$, Figure 4.4A), and MCL strains were not significantly affected by ACL deficiency at peak valgus rotation (Figure 4.4B). ACL transection increased MCL strains by an average $1.8 \pm 0.5\%$ at peak anterior translation. In contrast, ACL transection increased MCL strains by only $0.1 \pm 0.1\%$ at peak valgus rotation (Figure 4.5). The significant strain increases at peak anterior translation ($p<0.05$) occurred along every region of the superficial longitudinal MCL and the region representing the anterior fibers of the posteromedial corner.

ACL transection caused the largest increase in MCL strain during anterior translation at 30 deg of knee flexion ($2.0 \pm 1.5\%$), corresponding with the greatest increase in anterior laxity ($12.4 \pm 1.3$ mm). During anterior translation, the lowest aggregate strain increases due to ACL transection occurred at 0 deg of knee flexion ($1.4 \pm 0.7\%$); however, even with these lower strain increases, 0 deg flexion had the greatest absolute strain in both ACL intact and ACL-deficient cases. For all anterior translation cases, the region with the largest overall strain increase due to ACL transection was near the femoral insertion ($3.8 \pm 1.1\%$), while the region with the least overall increase was along the distal oblique fibers of the superficial MCL ($0.3 \pm 0.4\%$) (Figure 4.5).

Effect of Knee Flexion Angle

Knee flexion angle had a significant effect on both anterior translation and valgus rotation ($p<0.001$ and $p=0.01$, respectively). Flexing or extending the knee to 30 deg from all other flexion angles significantly increased anterior translation (average of $3.1 \pm 0.5$ mm for all cases, Figure 4.3A). Extending the knee to 0 deg from all other angles significantly decreased valgus rotation (average of $1.5 \pm 0.3$ deg for all cases,
Figure 4.4. Experimental results from varus-valgus rotation. A) Valgus rotation at all flexion angles, with unconstrained tibial axial rotation, before and after ACL transection.  B) Average MCL strains at peak valgus rotation as a function of knee flexion angle, with unconstrained tibial axial rotation, before and after ACL transection.  ACL transection had no significant effect on valgus laxity or MCL strains.  Error bars = SD.
Figure 4.5. MCL strain changes due to ACL transection at peak anterior translation and valgus rotation, averaged over all cases. Transection significantly increased MCL strains during anterior translation, but had no effect on MCL strains during valgus rotation. * p < 0.05 (within a region).
Medial collateral ligament strains in most measurement regions were also significantly affected by flexion angle for all test cases. Interestingly, MCL strain patterns were changed in a nearly uniform manner with each successive 30 deg flexion, for both loading configurations. This uniform change in strain followed a pattern of small yet significant strain increases along the most anterior row distal to the joint line (0.3 ± 0.2%), coupled with larger and significant decreases in change around the posteromedial corner (-3.5 ± 0.6%). Both ACL-intact knees and ACL-deficient knees exhibited this trend in MCL strain behavior.

Effect of Tibial Axial Constraint

Unconstraining tibial axial rotation significantly increased anterior translation by an average of 0.6 ± 0.1 mm and valgus rotation by an average of 0.7 ± 0.2 deg (p<0.001 and p=0.001, respectively), under all test conditions. Overall increases in laxity corresponded with overall decreases in MCL strains of 0.45 ± 0.24% during anterior translation and 0.10 ± 0.17% during valgus rotation. These strain decreases were significant across the majority of longitudinal parallel fibers during anterior translation and near the femoral insertion during valgus rotation.

When tibial axial rotation was unconstrained for the ACL-intact cases, an average internal tibial rotation (ITR) of 9.3 ± 3.8 deg occurred during anterior translation. Transecting the ACL significantly reduced ITR during anterior translation at 30, 60 and 90 deg flexion by an average 6.9 ± 3.8 deg (Figure 4.6A). When tibial rotation was unconstrained, the larger ITR in knees with an intact ACL resulted in significantly lower MCL strains in the longitudinal fibers near the femoral insertion (2.5
± 0.4%, Figure 4.6B). In contrast, for ACL deficient knees, ITR was reduced and the decreases in strain near the femoral insertion were insignificant (0.6 ± 0.2%, Figure 4.6B). This illustrates that decreased ITR after ACL transection results in increased MCL strains in the longitudinal fibers near the femoral insertion. Statistical analysis further supported this observation, as there was a significant interaction between tibial axial constraint and ACL transection along strain regions near the femoral insertion at peak anterior translation ($p<0.05$).

**Discussion**

Understanding the interdependent functions of the ACL and MCL can clarify the structure-function relationship of both ligaments. This study found that ACL deficiency significantly increased MCL strains during anterior translation, but had no effect on MCL strains during valgus rotation. Joint kinematics measured simultaneously with MCL strains were consistent with comparable studies [19, 22, 26]. The results support our hypothesis that ACL deficiency increases MCL strain during anterior translation, which is logical considering the respective primary and secondary roles of the ACL and MCL in restraining anterior translation. Conversely, our hypothesis that ACL deficiency would increase MCL strain during valgus rotation was rejected. This means that application of a valgus rotation to 10 N m in the ACL-deficient knee was non-detrimental to the MCL.

The finding that strains in the superficial MCL are insensitive to ACL transection during valgus rotation was surprising considering that the ACL has been shown to be an active stabilizer to valgus rotation when the MCL is healthy [20, 32]. Studies by Fukuda
Figure 4.6. Interdependence of the ACL and tibial axial rotation. A) Internal tibial axial rotation from neutral to peak anterior translation, 30 deg knee flexion, before and after ACL transection. B) Average MCL strains at peak anterior translation, 30 deg knee flexion, with fixed and unconstrained tibial axial rotation, before and after ACL transection. In the ACL-intact knee, unconstraining tibia axial rotation significantly reduced strain along the anterior MCL. After ACL transection, internal tibial rotation was significantly decreased and MCL strain was unaffected when tibial axial rotation was unconstrained. Thus, in the intact knee, the ACL promoted internal tibial rotation during anterior translation, which relieved strain in the MCL. This also occurred at 60 and 90 deg flexion. * p < 0.05
et al. [32] and Miyasaka et al. [20] used force superimposition techniques and strain gauges, respectively, determining that the ACL resists valgus rotation from full extension to 90 deg flexion. Our results showed that ACL transection produced small, insignificant increases in valgus laxity, yet this increased valgus rotation minimally impacted MCL strains at all flexion angles (average increase was 0.1%, average \( p=0.64 \)). A few explanations on this discrepancy are offered. First, it is possible that the reported ACL force contributions during valgus rotation in an intact knee are easily accommodated by the MCL after ACL transection. Therefore, MCL strain changes are imperceptible and the integrity of the MCL is unaltered. Another possibility is that other secondary stabilizers might increase their contribution to resisting valgus rotation after ACL transection, allowing the MCL to continue to function normally. Yet, the most likely explanation involves differences in degrees of freedom between testing systems. The testing machine and fixtures in this study permitted up to 4 DOF, while experiments by Fukuda et al. [32] used a 5 DOF system. The 5 DOF system permitted A-P translation during V-V rotation, and demonstrated that coupled A-P translation during V-V rotation increases after ACL transection. Therefore, in an intact knee, the function of the ACL during valgus rotation may be to resist coupled anterior translation, and the ACL only resists pure valgus rotation after the MCL is compromised.

To make clinical interpretations, it was necessary to identify loading conditions that generate damaging strains, which was feasible since a stress-free reference was used for strain calculation. A stress-free reference allows direct comparison with material properties reported in the literature. Ligament rupture typically occurs at \(~18\%\) strain [41], and the onset of microtrauma or substructural failure in ligament occurs at 5.2%
strain [33]. During valgus rotation, maximum absolute strains in the midlongitudinal MCL fibers remained around 4.4% in both the intact and ACL-deficient knee, below the microtrauma threshold. During anterior translation, ACL transection significantly increased maximum absolute strains along the mid-longitudinal fibers from 2.9% to 5.7%, a strain level that could induce microtrauma. These results show evidence that longitudinal MCL fibers in ACL-deficient knees are initially predisposed to damage from anterior translation. This finding is useful in interpreting results from a study by Tashman et al. [42] who measured kinematic gait changes over two years in ACL-deficient and ACL-intact canines. Consistent with our results and the literature [19, 22, 43], ACL transection immediately caused large translational increases during anterior translation and small rotational increases during valgus rotation. In the ACL-deficient knee, anterior translation significantly escalated with time. Our data suggest that the MCL initially assisted in stabilizing anterior translation; however, the MCL became strained over time leading to increased anterior tibial displacements. This potential increase in MCL laxity may be one factor in the unsatisfactory outcomes characteristic of conservatively treated ACL injuries [44].

Interestingly, the strains of around 10% in the anterior posteromedial corner during both loading conditions greatly exceed the reported substructure failure threshold. However, these results are deceiving. The material tests that defined damaging strains [33, 41] were tested along the midlongitudinal MCL fibers and therefore are not directly comparable with regions of the posteromedial corner. Considering that the posteromedial corner has been shown to play a limited role in resisting valgus rotation
[22], this tissue is likely less stiff with greater failure strain thresholds than the adjoining longitudinal fibers.

Relating joint kinematics and local strains has enabled a better understanding of functional MCL regions and is applicable to clinical diagnosis. Following ACL transection, increased anterior laxity was resisted by fibers near the femoral insertion and along the midsubstance of the parallel longitudinal fibers. The greatest average increase in MCL strain following ACL transection occurred at 30 deg flexion, consistent with the largest increase in anterior translation. Yet, 0 deg flexion held the distinguished position of having the greatest absolute strains both before and after ACL transection. Increasing the flexion angle, for both loading conditions, slightly stretched the fibers distal to the joint line along the anterior superficial MCL. Meanwhile, the posterior regions of the superficial MCL and anterior posteromedial corner uniformly slackened with this increased flexion. This behavioral pattern and the corresponding magnitude of the strain changes were unaffected by ACL transection, and were insensitive to the discrepancies between anterior translation and valgus subluxation patterns observed at different flexion angles. Therefore, regardless of directional loading or ACL condition, progressive flexing of the knee will reduce overall MCL strain. These findings support using a knee flexion angle of 15-30 deg when administering the Lachman test rather than performing the test at full extension [45]. At a slightly flexed angle, the MCL will not be overstressed, and deviations in joint laxity with the contralateral knee are maximized.

The relationship between tibial axial rotation and the MCL and ACL was further developed in this study. When tibial axial rotation was constrained, knee laxity
decreased under both loading conditions. This was at least partially due to increased resistance along the longitudinal fibers of the MCL, which experienced significantly higher strains, particularly during anterior translation. Unconstraining tibial axial rotation permitted internal tibial rotation, which in turn decreased MCL strains. Internal tibial rotation during anterior translation was reduced after the ACL was transected. Thus, the ACL encourages internal rotation, perhaps by “unwinding” during anterior translation [46]. In summary, in an intact knee, the ACL promotes internal tibial rotation, which in turn reduces MCL strains along the longitudinal parallel fibers of the superficial MCL near the femoral insertion.

The specific limitations of the methods used in this study deserve discussion. Joint kinematics may have been altered due to the dissection necessary for strain measurement or because joint compressive forces and stabilizing muscle activity were not represented. Muscle activity has been shown to reduce knee laxity [47]. Therefore, to reproduce the magnitudes of strains from this study in vivo, greater force and torque limits would likely be required. Removing the patella may have also influenced MCL strain patterns and joint kinematics. Tests were performed in a controlled environment, and did not undergo high speed motions or combined loading configurations that would have been more analogous to injury causing mechanisms. Strain measurement was based on changes to gauge length between marker pairs. This assumed that strain was homogeneous over the length of these discrete regions. For graphical representation, MCL strain values were interpolated between marker rows, which may not accurately account for inhomogeneities orthogonal to the fiber direction. Finally, strains in the deep MCL were not measured, although previous studies have shown that the deep MCL
is half as stiff as the superficial MCL [48] and has a minimal contribution to valgus restraint when the superficial MCL is intact [49].

Through measurement of tissue strain and joint kinematics, this research has improved the understanding of how the ACL and MCL interact. Additionally, results from this study can be used to validate finite element models and improve the governing constitutive equations. The present results and methods can also serve as a baseline to verify that a specific ACL reconstruction technique not only returns the knee to regular joint kinematics, but is capable of returning normal functionality to the intact MCL.

References


CHAPTER 5

MCL INSERTION SITE AND CONTACT FORCES
IN THE ACL-DEFICIENT KNEE

Abstract

The objectives of this research were to determine the effects of ACL deficiency on MCL insertion site and contact forces during anterior tibial loading and valgus loading using a combined experimental-finite element (FE) approach. Our hypothesis was that ACL deficiency would increase MCL insertion site forces at its attachment to the tibia and femur and increase contact forces between the MCL and these bones. Six male knees were subjected to varus-valgus and anterior-posterior loading at flexion angles of 0 and 30 deg. Three-dimensional joint kinematics and MCL strains were recorded during kinematic testing. Following testing, the MCL of each knee was removed to establish a stress-free reference configuration. A FE model of the femur-MCL-tibia complex was constructed for each knee to simulate valgus rotation and anterior translation at 0 and 30 deg, using subject-specific bone and ligament geometry and joint kinematics. A transversely isotropic hyperelastic material model with average material coefficients taken from a previous study was used to represent the MCL.
Subject-specific MCL in situ strain distributions were used in each model. Insertion site and contact forces were determined from the FE analyses. FE predictions were validated by comparing MCL fiber strains to experimental measurements. The subject-specific FE predictions of MCL fiber stretch correlated well with the experimentally measured values ($R^2 = 0.953$). ACL deficiency caused a significant increase in MCL insertion site and contact forces in response to anterior tibial loading. In contrast, ACL deficiency did not significantly increase MCL insertion site and contact forces in response to valgus loading, demonstrating that the ACL is not a restraint to valgus rotation in knees that have an intact MCL. When evaluating valgus laxity in the ACL-deficient knee, increased valgus laxity indicates a compromised MCL.

**Introduction**

The effect of anterior cruciate ligament (ACL) deficiency on the mechanical function of other knee ligaments remains unclear, although it is known that even knees with reconstructed ACLs often exhibit abnormal knee kinematics [1]. The ACL is a primary restraint to anterior tibial translation and a secondary restraint to valgus rotation [2-12], while the medial collateral ligament (MCL) is a primary restraint to valgus rotation [3-14] and a secondary restraint to anterior tibial translation [2, 4-6, 11, 14-18]. The MCL is involved in approximately 40% of all severe knee injuries [19], while approximately 50% of partial MCL tears and 80% of complete MCL tears occur in conjunction with injury to other knee ligaments [20]. In alpine skiing, the most common ligament that is injured in conjunction with the MCL is the ACL [21].

Animal studies have shown that MCL healing is substantially poorer in the case of a combined MCL/ACL injury than for an isolated MCL injury [2, 5-7, 11, 12].
12 weeks of healing, MCLs from knees with combined MCL/ACL injuries had a tensile strength of only 10% of control values [12]. It has been proposed that the healing MCL in the ACL-deficient knee is subjected to increased strains and forces as a result of ACL deficiency [6]. An ACL graft acts as a stabilizer initially, but as it heals, forces are transferred to the MCL that hinder healing and result in a hypertrophy of the MCL with tissue of lower quality. As long as two years after injury, healing MCLs still had “significantly different biological composition, biomechanical properties, and matrix organization” [11].

Although animal studies have shown that the MCL may be at risk for injury in an ACL deficient knee, conclusions in the literature as to the exact contributions of the MCL and ACL to valgus stability vary within and between studies of ligament healing in animal models and joint kinematics in cadaver models. In animal models, the variation in results is confounded by the variation in the type of injury model used. Results from a rabbit healing study showed that valgus rotation does not increase over time in response to healing of the ACL graft after an O'Donoghue triad injury (rupture of the medial collateral ligament with removal of the anterior cruciate ligament and part of the medial meniscus) although anterior translation did significantly increase over the same healing period [2]. The conclusions of this animal study that created an O'Donoghue triad injury are in contrast to other animal studies that have shown that there are higher ACL forces and increased valgus laxity in response to a valgus load in a MCL deficient knee [3-6, 9, 10]. Two previous cadaver studies concluded that valgus laxity is relatively unaffected by ACL deficiency [13, 14] and Mazzocca et al. concluded that, “the ACL can be compromised in isolated grade III MCL injuries” caused by a
valgus load [10]. The actual insertion site and contact forces in the MCL in response to a valgus torque in the intact and ACL-deficient knee, which arguably are the most relevant data for interpretation of ligament contribution to joint function, are unknown.

The aim of this study was to examine the effects of ACL deficiency on MCL insertion site and contact forces when the knee is subjected to anterior tibial loading and valgus torque. It was hypothesized that ACL deficiency would cause an increase in MCL insertion site and contact forces in response to both loading conditions.

**Materials and Methods**

**Overview**

This study combined experimental and computational methods to determine the effect of ACL injury on MCL insertion site and contact forces during anterior tibial loading and valgus loading. Computed tomography (CT) images were used to obtain the subject-specific geometry of the femur, tibia and MCL in a series of six cadaveric knees. Each knee was tested with the ACL intact and the ACL completely severed. For each injury state the knee was subjected to anterior-posterior (A-P) translation and varus-valgus (V-V) rotation at two flexion angles (0 and 30 deg) with tibial rotation constrained and unconstrained while knee kinematics and MCL strains were recorded. Polygonal surfaces were extracted from the CT data and were used to generate subject-specific FE models of each knee. The FE models were analyzed under the experimentally measured kinematics to determine MCL strains, contact forces, and insertion site forces. FE predicted fiber stretches were compared to experimental values as a means of validation and the effect of injury state, flexion angle, and tibial constraint on MCL insertion site and contact forces was determined.
Specimen Preparation and CT Scan

Six intact human male cadaver knees were used (donor age 60 ± 8.3 years). Preparation for testing followed the same protocol as Gardiner et al. [22], with the exception that additional contrast markers were used to define gauge lengths for measurement of MCL fiber strain. Markers were distributed along the visible fiber direction of the MCL in a 3x7 grid pattern, forming 18 gauge lengths (Figure 4.1). Each gauge length was approximately 15 mm long. The position of the markers was chosen based on anatomical landmarks. The boundaries of the MCL insertion sites on the femur and tibia were marked with copper wires to aid with identification of their geometry in the volumetric CT images. Nylon kinematic blocks were fastened to the distal femur and proximal tibia (shown in Figure 3.2), while positioning blocks with three beveled cavities (not shown in figure), forming a right angle, were fastened to the proximal femur and distal tibia [23]. After dissection, a volumetric CT scan was obtained for each knee at 0 deg flexion (slice thickness = 1.3 mm with 1.0 mm overlap, 142-168 mm field of view, 512 × 512 acquisition matrix).

Kinematic Testing

Following the CT scan, each knee was mounted in fixtures on a custom materials testing machine, which allowed both A-P translation and V-V rotation to be applied at fixed flexion angles with constrained or unconstrained tibial axial rotation and unconstrained medial-lateral translation and joint distraction (Figure 4.2). During testing, 10 cycles of A-P translation (load limits of ±100 N at 1.5 mm/sec) and 10 cycles of V-V rotation (torque limits of ±10 N-m at 1 deg/sec) were independently applied to the tibia. The A-P load and V-V torque limits were established so that they were large
enough to achieve the terminal stiffness of the ligament without inflicting injury to the tissue and thereby allowing multiple tests with the same specimen [24]. A-P and V-V loading were conducted at 0° and 30° flexion. The tests were repeated with tibial axial rotation constrained and unconstrained at each flexion angle. The load and torque were measured with a multi-axis load cell (Futek T5105, Irvine, CA, accuracy ±2.2 N and ±0.056 N-m). Following the eight ACL intact tests, the ACL was transected through the midsubstance without damage to the PCL or removal of the knee from the fixture and all the tests were repeated. Finally, following ACL transection, the attachment of the medial meniscus to the MCL was transected. This test was performed to verify that the attachment did not influence joint kinematics and MCL strains under A-P and V-V loading; a similar conclusion was reached for the effect of the meniscus attachment on MCL strains in the intact knee in our previous study [22]. To minimize hysteresis effects, data from the 10th cycle of loading were analyzed for all tests.

Care was taken to ensure that the relative kinematic positions of the bones were duplicated for a given flexion angle and tibial axial rotation constraint for both injury states. When testing the intact knee, a neutral A-P and V-V position was determined at each flexion angle with tibial axial rotation unconstrained. The neutral A-P and V-V positions were determined by iteratively adjusting the starting position and running A-P and V-V motion cycles until the given load (±100 N) and torque (±10 N-m) limits produced equal anterior and posterior translation and equal varus and valgus rotations, respectively. Once these reference positions were established, actuator translation and rotation positions were logged so the positions could be restored after ACL transection. The three-dimensional kinematic position of the femur relative to the tibia was verified
through the use of a Microscribe digitizer (Immersion Corp, San Jose, CA, accuracy ±0.085 mm) in combination with the positioning blocks. The digitizer and positioning blocks were used to precisely determine the relative three-dimensional kinematics of the femur and tibia [25]. In this manner, positional repeatability between the different injury states was insured.

Measurement of Joint Kinematics and Ligament Strains

A digital motion analysis system consisting of two high-resolution digital cameras (Pulnix TM-1040, 1024x1024x30 fps, Sunnyvale, CA) and Digital Motion Analysis Software (DMAS, Spica Technology Corporation, Maui, HI) was used to record MCL strain in the 18 measurement regions and joint kinematics simultaneously (strain measurement accuracy: ±0.035 percent; joint kinematic translational accuracy: ±0.025 mm; joint kinematic rotational accuracy: ±0.124 deg) [23].

In Situ Strain

At the conclusion of testing, the MCL was dissected from the bones and placed in a buffered saline bath for 10 minutes to allow the ligament to achieve a stress-free reference configuration. The 3D coordinates of the fiducial markers on the MCL were determined using the digital motion analysis system. This provided reference (zero-load) lengths for each strain region, \( l_0 \) [12, 22, 26]. These values were combined with length measurements taken during the kinematic testing to calculate in situ fiber strain between marker pairs. These data were used as input to the subject-specific FE models [22, 27].
CT Scan, Surface Reconstruction and FE Mesh Generation

Using the copper insertion site wires and MCL strain contrast markers as guides, cross-sectional contours of the MCL, femur, and tibia were extracted from the CT dataset (SurfDriver, Kailua, Hawaii). Polygonal surfaces were generated by stacking and lacing together the contours [28] and smoothing was applied [29]. The polygons composing the surfaces of the femur and tibia were converted directly to shell elements and used to represent the bones as rigid bodies [30]. The MCL surface was imported into FE preprocessing software (TrueGrid, XYZ Scientific, Livermore, CA) and a hexahedral mesh was created.

Constitutive Model

The MCL was represented as transversely isotropic hyperelastic, with the strain energy \( W \) [22]:

\[
W = F_1(\tilde{I}_1) + F_2(\tilde{\lambda}) + \frac{K}{2}(\ln(J))^2. \tag{1}
\]

Here, \( \tilde{I}_1 \) is the first deviatoric invariant, \( \tilde{\lambda} \) is the deviatoric part of the stretch ratio along the local fiber direction, and \( J \) is the determinant of the deformation gradient, \( \mathbf{F} \). The matrix strain energy \( F_1(\tilde{I}_1) \) was chosen so that \( \partial F_1/\partial \tilde{I}_1 = C_1 \), yielding the neo-Hookean constitutive model. The derivatives of the fiber strain energy function \( F_2(\tilde{\lambda}) \) were defined as a function of the fiber stretch:

\[
\tilde{\lambda} \frac{\partial F_2}{\partial \tilde{\lambda}} = 0, \quad \tilde{\lambda} \leq 1;
\]
\[ \lambda \frac{\partial F_2}{\partial \lambda} = C_3 \left[ \exp \left( C_4 \left( \lambda - 1 \right) \right) - 1 \right], \quad 1 < \lambda < \lambda^* ; \]

\[ \lambda \frac{\partial F_2}{\partial \lambda} = C_5 \lambda + C_6, \quad \lambda \geq \lambda^* . \]

\( C_3 \) scales the exponential stress, \( C_4 \) specifies the rate of collagen uncrimping, \( C_5 \) is the modulus of straightened collagen fibers, and \( \lambda^* \) is the stretch at which the collagen is straightened. The third term in Eq (1) represents the bulk (volumetric) response, with the bulk modulus \( K \) controlling the entire volumetric response of the material. The population-average material coefficients from Gardiner et al. were used [22]:

\[ C_1 = 1.44 \text{ MPa}, \quad \lambda^* = 1.062 \text{ (no units)}, \quad C_5 = 0.57 \text{ MPa}, \quad C_4 = 48.0 \text{ (no units)}, \quad C_5 = 467.1 \text{ MPa} . \]

Population average material coefficients were used based on the finding that using average coefficients versus subject specific coefficients yielded no significant difference in the accuracy of FE strain predictions [31]. Due to a lack of experimental data describing ligament bulk behavior, the bulk modulus was specified to be two orders of magnitude greater than \( C_1 \), yielding nearly incompressible material behavior [22].

**Boundary Conditions**

The experimentally measured kinematic dataset was used to prescribe the motion of the tibia relative to the femur in the FE analyses [22]. The coordinates of the kinematic blocks in both the CT and kinematic datasets allowed for correlation of the two datasets. The entire FE model was transformed so that the global coordinate system was aligned with the coordinate system of the femur kinematic block. Motion of the tibia was described using incremental translations and rotations referenced to the femur.
kinematic block [30, 32]. The MCL mesh was attached to the bones by defining node sets, based on the area within the copper wires, at the proximal and distal ends of the MCL as the same rigid material as the femur and tibia, respectively. Contact was enforced using the penalty method.

Finite Element Analysis

The implicitly integrated FE code NIKE3D was used for all analyses [32]. An automatic time stepping strategy was employed, with iterations based on a quasi-Newton method. Each analysis was performed in three parts. In the first part, the knee was moved from the position in which it was placed at the time of the CT scan to the initial testing position (either 0 or 30 deg of flexion). During the second part, the experimentally measured in situ strains for a given flexion angle and injury state were applied to the MCL. During the third part the experimental kinematic motion was applied (either anterior translation or valgus rotation). FE results were analyzed with GRIZ [33].

Regional Strains, Insertion Site and Contact Forces

FE predicted fiber stretches for nodes within each measurement region were averaged. Average FE predicted fiber stretches were compared to the experimentally measured values. The magnitude of ligament forces at the insertion sites and the magnitude of the resultant forces due to MCL-bone contact were obtained from the NIKE3D output.
Statistical Analysis

Regression analyses were used to evaluate the ability of the FE models to predict experimentally measured values of MCL fiber stretch. FE predictions of regional fiber stretch were determined as a function of location along the length of the MCL. The predicted stretches were calculated and tabulated for all six knees according to test case and compared to experimental results. Coefficients of determination ($R^2$), regression lines, and p-values were determined.

The effect of tibial axial rotation constraint on insertion site and contact forces was assessed with a paired t-test using all the force data (insertion site and contact forces for both injury states at both angles and both loading conditions). The effects of within-subject treatment (injury state and flexion angle) in response to anterior and valgus loading on insertion site and contact forces were assessed using 2-way repeated measures ANOVAs. The results of the paired t-test showed no significant effect of tibial constraint on insertion site and contact forces (see results section below), so only the force data for the tests with constrained tibial axial rotation were used in the 2-way ANOVAs. In cases when significance was found (p<0.05), multiple comparisons were performed using the Tukey procedure.

Results

Experimental Kinematics

Before ACL resection, the average anterior displacements at 0 and 30 deg knee flexion in response to a 100 N anterior tibial load were $6.8 \pm 2.6$ mm and $6.7 \pm 2.2$ mm, respectively. ACL transection significantly increased anterior displacement in response to a 100 N anterior tibial load ($16.5 \pm 6.1$ mm and $20.8 \pm 4.4$ mm at 0 and 30 deg,
respectively) (p<0.001 for both flexion angles). Before ACL transection, the average valgus rotation at 0 and 30 deg knee flexion in response to a 10 N-m valgus torque were 3.6 ± 1.8 deg and 5.1 ± 2.0 deg, respectively. ACL deficiency did not significantly change valgus rotation in response to a 10 N-m valgus torque (4.3 ± 1.9 deg and 5.3 ± 1.7 deg at 0 and 30 deg, respectively). Subsequent separation of the medial meniscus attachment had no significant effect on knee joint kinematics for both A-P and V-V loading (data not shown). Because there was no change in joint kinematics following separation of the medial meniscus from the MCL in the ACL-deficient knee, these data were not subsequently analyzed via FE analysis.

FE Predictions of Regional Fiber Stretch

The FE values for fiber stretch were excellent predictors of experimental fiber stretch. Regression analysis of FE predicted fiber stretch versus experimentally measured fiber stretch for all regions, knees and test cases of intact and ACL-deficient knees yielded a coefficient of determination of $R^2 = 0.953$ (p=0.001) (Figure 5.1). Fringe plots of the FE fiber strain illustrate the MCL strain patterns and increases in strain caused by ACL-deficiency in response to anterior and valgus loading (Figure 5.2). In response to both types of load and regardless of injury state, the highest MCL strains were found in the posterior-proximal region [34]. MCL strain increased significantly in response to anterior loading when the ACL was injured, but the increase was not significantly different in response to a valgus load. Higher MCL strains tended to be more distributed in response to valgus loading than anterior loading regardless of injury.
Figure 5.1. FE predicted vs. experimental fiber stretch for all knees, test conditions, and measurement regions (N=1632).
Figure 5.2. Representative fringe plots of FE predicted fiber strain for a 100 N anterior load (top row) and a 10 N-m valgus load (bottom row) for the uninjured knee and the ACL-deficient knee. MCL strains increased in response to anterior tibial loading when the ACL was injured. MCL strains also increased locally in the ACL-deficient knee in response to a valgus torque, but these local increases did not result in significant changes in insertion site or contact forces.
state. A comprehensive set of MCL strain data as well as the kinematic data from this testing can be found in Lujan et al. [34].

Tibial Axial Rotation Constraint

A paired t-test using all insertion site and contact forces for both flexion angles and loading conditions showed that there was no effect of tibial axial rotation constraint on the predicted forces (p = 0.154).

Insertion Site Force

ACL deficiency caused significant increases in MCL insertion site forces at both the femur and tibia during anterior tibial translation. The forces at both insertion sites were significantly higher at 0 than at 30 deg in ACL-deficient knees in response to the 100 N anterior tibial load. The MCL femoral insertion site forces corresponding to the in situ strains in the intact knee (before application of the experimental kinematics) at 0 and 30 deg were 39.7 ± 38.1 N and 6.0 ± 5.4 N, respectively. The MCL tibial insertion site forces due to in situ strain in the intact knee at 0 and 30 deg were 42.9 ± 43.1 N and 6.0 ± 5.5 N, respectively. Before ACL resection, the MCL femoral insertion site forces during anterior translation at 0 and 30 deg were 55.9 ± 38.2 N and 8.3 ± 5.4 N, respectively. Before ACL resection, the MCL tibial insertion site force during anterior tibial translation at 0 and 30 deg were 58.7 ± 42.0 N and 8.3 ± 5.5 N, respectively (Figure 5.3, left panel). ACL-deficiency significantly increased MCL insertion site forces at the femur (126.6 ± 84.8 N and 74.1 ± 57.8 N at 0 and 30 deg, respectively) and tibia (133.9 ± 88.5 N and 80.9 ± 62.4 N at 0 and 30 deg, respectively) during anterior
tibial translation (p<0.05 for all cases). Insertion site forces were significantly higher at 0 than at 30 deg during anterior tibial translation (p=0.012 for both insertions).

In contrast to the anterior loading results, ACL deficiency did not significantly affect insertion site forces during application of valgus torque (Figure 5.3, right panel). This was true at both the femoral and tibial insertion sites and for both flexion angles. Although the increases caused by ACL deficiency were not statistically significant in response to a valgus load, the results followed the same trend as the anterior loading results, with higher forces and increases in forces at the tibial insertion and at 0 deg flexion. Before ACL resection, the MCL femoral insertion site forces during valgus rotation at 0 and 30 deg were 72.8 ± 49.9 N and 46.0 ± 34.4 N, respectively. Before ACL resection, the MCL tibial insertion site forces during valgus rotation at 0 and 30 deg were 77.8 ± 55.6 N and 47.9 ± 37.9 N, respectively. After the ACL was transected,

Figure 5.3. FE predictions of insertion site forces for femoral and tibial insertion sites as a function of flexion angle and ACL state. Left panel - anterior tibial translation. Right panel – valgus rotation. Asterisks indicate statistically significant comparisons. There was a significant increase in MCL insertion site forces at the femur and tibia during anterior tibial translation after ACL injury at both 0 and 30 deg. In contrast, there was no significant effect of ACL deficiency on MCL insertion site forces in response to valgus loading. Both tibial and femoral insertion site forces were significantly higher at 0 deg in the ACL-deficient knee during anterior tibial translation. (mean ± stdev).
the MCL femoral insertion site forces during valgus rotation at 0 and 30 deg were 92.0 ± 64.0 N and 57.5 ± 45.0 N, respectively. After the ACL was transected, the MCL tibial insertion site forces during valgus rotation at 0 and 30 deg were 99.0 ± 72.2 N and 60.7 ± 49.3 N, respectively.

Contact Forces

ACL deficiency resulted in significantly increased MCL contact forces on the tibia during anterior tibial translation at both flexion angles, and MCL contact forces on the tibia were significantly higher at 0 than at 30 deg in the ACL-deficient knee. Before ACL resection, the MCL contact forces on the tibia during anterior translation at 0 and 30 deg were 11.6 ± 11.9 N and 0.7 ± 0.9 N, respectively (Figure 5.4, left panel). ACL deficiency significantly increased MCL contact forces on the tibia (28.4 ± 18.9 N and 21.1 ± 15.8 N at 0 and 30 deg, respectively) during anterior translation (p=0.001 at both angles). MCL contact forces on the tibia in the ACL-deficient knee were significantly higher at 0 than at 30 deg in response to anterior tibial loading (p=0.044). ACL deficiency did not significantly affect contact forces during application of valgus torque (Figure 5.4, right panel).

Discussion

The hypothesis of this research was that ACL deficiency would increase MCL insertion site forces at the femur and tibia and increase contact forces between the MCL and the bones in response to both anterior and valgus loading. This hypothesis was partially disproved. In the ACL-deficient knee, the MCL is indeed subjected to higher insertion site and contact forces in response to an anterior load. However, MCL forces
Figure 5.4. FE predictions of contact forces between the MCL and femur and between the MCL and tibia as a function of flexion angle and ACL injury state. Left - anterior tibial translation. Right – valgus rotation. Asterisks indicate statistically significant comparisons. ACL deficiency significantly increased tibial contact forces at both flexion angles during anterior tibial translation. Further, tibial contact forces in the ACL-deficient knee at 0 deg were significantly higher than at 30 deg during anterior loading. In contrast, there was no significant effect of ACL deficiency on MCL contact forces during valgus loading (mean ± standard deviation).

due to a valgus torque are not significantly increased in the ACL-deficient knee. It follows that the MCL resists anterior tibial translation in knees with intact ACLs, but the ACL is not a restraint to valgus rotation when a healthy MCL is present.

ACL deficiency caused a significant increase in MCL insertion site and contact forces in response to anterior tibial loading. This result is supported by an FE study that examined MCL insertion site and contact forces in the ACL-deficient knee [35] and by cadaver studies that have utilized a robotic/universal force-moment sensor system to calculate MCL insertion site forces in the ACL-deficient knee [16, 17]. Moglo et al. created a single FE model of the knee including the MCL, ACL, posterior cruciate ligament, lateral collateral ligament, menisci, and cartilage [35]. A 100 N posterior load
was applied to the femur at a range of flexion angles from 0 to 90 deg to study the forces in the remaining structures after removing the ACL. At full extension, forces in collateral ligaments increased in the ACL-deficient knee. Better support for our findings can be found in two studies that used a robotic/universal force-moment sensor system to calculate MCL insertion site forces in the intact and ACL-deficient knee [16, 17]. In each study, anterior loads were applied to intact and ACL-deficient cadaver knees and the resulting MCL insertion site forces were measured. Both studies found a significant increase in MCL insertion site forces during anterior tibial loading in the ACL-deficient knee.

In vivo studies of MCL healing have demonstrated that MCL healing is inferior when injured in conjunction with the ACL [2, 5, 6, 12]. Each of these studies found increased knee laxity and decreased MCL material properties when the MCL is injured in conjunction with the ACL as compared to MCL injury with intact ACL, but only one of these studies measured MCL forces. Using a goat model, the insertion site forces in healing MCLs in response to an anterior tibial load in knees with reconstructed ACLs were measured by Abramowitch et al. using a robotic/universal force-moment sensor system [6]. It was their conclusion that “the healing MCL may have been required to take on excessive loads and was unable to heal sufficiently as compared to an isolated MCL injury.” Although these conclusions were reached based on healing studies in animal models, application of the results of the present study suggest that differences in MCL healing between knees with intact ACLs and those with transected ACLs could be due to either anterior or valgus loading. The insertion site forces in response to a valgus torque were generally of the same magnitude for a given flexion angle as the insertion
site forces in response to an anterior tibial load, although they did not significantly increase with ACL deficiency, and the types and magnitudes of loads that hinder MCL healing are unknown.

The ACL is not a restraint to valgus rotation if the MCL is intact (Figures 5.4 and 5.5, right panels). At first this may seem contradictory to the widely held notion that the ACL is a secondary restraint to valgus rotation [3-8, 11]. However, upon closer examination, these studies reached this conclusion based on the results of MCL transection. Specifically, when the MCL was injured or transected, the ACL experienced increased loading during application of a valgus torque. Although our conclusion has not been reported previously in the literature, the results of other studies support the conclusions indirectly. Engle et al. examined the effect of ACL repair and graft restructuring on MCL healing after an O'Donoghue triad injury [2]. At 0, 6, and 12 weeks postoperatively, the anterior translation and valgus rotation of the knees were tested. From time point zero to 12 weeks anterior laxity significantly increased, but valgus laxity did not. Markolf et al. found that valgus knee laxity was relatively unaffected by sectioning of the cruciate ligaments [14]. This idea is further supported by a study looking at medial and lateral laxity in intact cadaver knees [13]. Using a six degree of freedom linkage attached to six different knees with applied varus-valgus loading, Grood et al. found that the ACL and PCL combined accounted for only 14.8% of the medial restraining moment at five degrees knee flexion and only 13.4% of the restraining moment at 25 degrees knee flexion. Thus, when evaluating valgus laxity in the ACL-injured knee, any increase in valgus laxity indicates a compromised MCL.
Applying the in situ strain to the MCL during the second part of the FE analysis creates insertion site forces. These forces represent the contribution of the MCL to knee stability when there is little or no muscle activation or external loading. The insertion site forces caused by the in situ strain were smaller than the insertion site forces present after anterior or valgus loading in the intact knee, although not always significantly smaller. This result is reflective of the relatively low load limits used in this study. In the ACL-deficient knee, insertion site forces significantly increased in response to an anterior tibial load, but not a valgus load, from the insertion site forces caused by the in situ strain. This followed the trend of the results comparing the insertion site forces in the ACL-deficient knee to the intact knee.

Changes in MCL contact forces followed the trend of MCL insertion site forces in that there was a significant increase in contact forces between the MCL and tibia following ACL transection in response to an anterior tibial load, but not a valgus torque. Contact forces were generated between the MCL and tibia during anterior tibial translation as the MCL slid over the convex surface of the tibia. These forces were relatively small in knees with intact ACL. Contact forces increased when the ACL was transected, and on average anterior tibial translation was more than doubled, forcing the MCL to slide over parts of the bone that have increased curvature. To our knowledge this is the first study to examine ligament contact forces using subject-specific FE modeling.

The attachment of the medial meniscus to the MCL was not represented in the FE analyses. This approach was justified by the results of our previous study, which demonstrated that transection of the attachment had no effect on knee kinematics under
valgus loading in the intact knee [22]. In the present study, it was confirmed that separation of the attachment of the medial meniscus to the MCL had no significant effect on joint kinematics for both A-P and V-V loading in the ACL-deficient knee. Of course, it is possible that other soft tissue structures that were dissected away from the knees may contribute to knee stability under A-P and V-V loading, and thus as with any cadaveric study, caution should be taken when extrapolating results to other situations.

Improvements in the experimental methods that were used in the present study resulted in substantially better agreement between FE predictions and experimental measurements of fiber stretch than was obtained in our previous study [22]. Improvements included the use of a more accurate digital motion analysis system, placement of wires around the MCL insertion sites to aid in identifying their locations in the CT images, and the placement of additional strain markers along and across the MCL [23]. The excellent correlation between experimental and FE predicted fiber strains ($R^2 = 0.953$) provides confidence in the fidelity of the subject-specific FE model predictions. Data such as insertion site forces and contact forces, which elucidate other injury mechanisms and risks, can be evaluated using subject-specific FE methods. Further, the combination of results available through a combined experimental and computational protocol can be used to determine the likely location of injury and to what extent it may occur.

Several assumptions were made in the constitutive model used for the MCL to decrease both the experimental and computational time it took to conduct the study. Average MCL material coefficients from a previous study [22] were used. In the previous study, results from FE simulations using subject-specific material properties
were compared to those using average material properties and no statistical differences were found. The MCL was assumed to have homogenous material properties. This assumption was used in our previous study [22] that yielded good correlations between experimental and FE strain results so the assumption was used again for this study which yielded an even better correlation.

It should be noted that the A-P and V-V mechanical testing performed for this research simulated an ideal clinical exam for knee laxity and no attempt was made to simulate weight bearing or muscle forces. Caution should be use when extrapolating the results reported here to a knee under muscle activation forces and/or ground contact forces. The anterior load and valgus torque limits for this research were specifically chosen to allow multiple tests on a single knee. Future research examining other loading conditions including muscle and body weight forces during regular daily activities is still needed.

In summary, ACL deficiency significantly increases MCL insertion site and contact forces in response to an anterior tibial load, and the largest increases occur at full extension. In contrast, ACL deficiency does not significantly increase MCL insertion site and contact forces in response to a valgus torque. Since it was demonstrated that the ACL is not a restraint to valgus rotation if the MCL is intact, increased valgus laxity in the ACL-deficient knee indicates a compromised MCL.

References


CHAPTER 6

EFFECT OF DERMATAN SULFATE GLYCOSAMINOGLYCNANS ON THE QUASI-STATIC MATERIAL PROPERTIES OF THE HUMAN MEDIAL COLLATERAL LIGAMENT

Abstract

The glycosaminoglycan of decorin, dermatan sulfate (DS), has been suggested to contribute to the mechanical properties of soft connective tissues such as ligaments and tendons. This study investigated the mechanical function of DS in human medial collateral ligaments (MCL) using nondestructive shear and tensile material tests performed before and after targeted removal of DS with Chondroitinase B (ChB). The quasi-static elastic material properties of human MCL were unchanged after DS removal. At peak deformation, tensile and shear stresses in ChB treated tissue were within 0.5% (p>0.70) and 2.0% (p>0.30) of pretreatment values, respectively. From pre- to post-ChB treatment under tensile loading, the tensile tangent modulus went from 242 ± 64 to 233 ± 57 MPa (p=0.44), and tissue strain went from 4.3 ± 0.3% to 4.4 ± 0.3%.

0.3% (p=0.54). Tissue hysteresis was unaffected by DS removal for both tensile and shear loading. Biochemical analysis confirmed that 90% of DS was removed by ChB treatment when compared to control samples, and TEM imaging further verified the degradation of DS by showing an 88% reduction (p<.001) of sulfated glycosaminoglycans in ChB treated tissue. These results demonstrate that DS in mature knee MCL tissue does not resist tensile or shear deformation under quasi-static loading conditions, challenging the theory that decorin proteoglycans contribute to the material behavior of ligament.

Introduction

The mechanical characteristics of ligament and tendon are a function of their composition and molecular organization. The major solid phase constituent of ligament and tendon is type I collagen, which represents over 70% of the total solid phase [1, 2]. The remaining constituents include other collagens (III,V,VI,XI,XIV), extracellular matrix proteins (e.g., elastin) and proteoglycans [3, 4]. The hierarchical organization of type I collagen in ligament has been extensively studied; type I collagen fibrils form parallel arrays of fibers that are the main contributors to ligament material properties [5]. However, there are very limited data on the mechanical influence of the noncollagenous components of ligament. Understanding the contributions of these components to connective tissue mechanics can clarify their roles and aid efforts to engineer replacement tissues.

The primary proteoglycan in ligament [6, 7], decorin, and its single sulfated glycosaminoglycan (GAG) have been the subject of numerous studies due to their respective proven and theoretical roles in tissue-level organization and mechanics at the
molecular level. Dermatan sulfate (DS) is the GAG that associates with the decorin core protein in nearly all mature tissues [8, 9](Figure 6.1, A). The concave face of decorin has been modeled to straddle the D-period binding site of a single collagen triple helix [10, 11] (Figure 6.1, B). Decorin appears to be excluded from the tightly tropocollagen packed fibril interior [12], localizing to the surface of the collagen fibrils (Figure 6.1, C). The highly charged DS GAG projects away from the decorin core protein [13], generally orthogonally aligned to the fibril direction [8].

Scanning electron and atomic force microscopy of connective tissues demonstrate that the majority of DS GAGs span the space between neighboring fibrils [12, 15]. Several studies report that collagen fibrils in various species are short and discontinuous [16-18], suggesting that a secondary microstructure could be involved in transferring axial force between contiguous fibrils. This finding led to an appealing hypothesis that decorin based DS GAGs on adjacent fibril surfaces interact, creating functional “cross-links” that transfer forces between discontinuous fibrils [8, 15, 17, 19-24] (Figure 6.1, C). Supporting this hypothesis, molecular dynamics predict that the summation of GAG interaction forces in collagen-decorin networks are capable of transferring inter-fibril stress [15, 25], and experiments confirm that DS does indeed self-associate [26]. The proposed fibril-fibril link would prevent fibril sliding and potentially contribute to quasi-static mechanical properties under both shear and tensile loading.

Using knockout mice and competitive peptides, previous studies have investigated the possible mechanical role of decorin indirectly. Knockout studies have
Figure 6.1. Illustration of interfibrillar GAG interaction. (A) Magnified schematic of decorin proteoglycan. The boxed domains “L” represent the Leucine-rich repeats of the protein core. “C” represents the cysteine rich domain. The dermatan sulfate GAG is attached to the protein core near the amino (NH$_2$) terminus through a serine linked oligosaccharide (“LO”). On the macroscopic level, dermatan sulfate is made up of iduronic acid-containing regions “I” and glucuronic acid-containing regions “G”. Chondroitinase B will degrade the iduronic acid regions mainly into disaccharides. (B) Frontal view of collagen fibril assembly. Five quarter staggered tropocollagen units construct a microfibril. Decorin core proteins likely bind to tropocollagen triple helix units at the D-period gap region of the microfibril. Although decorin binds to collagen, the exact mechanism of this attachment is debated [14]. (C) Cross-sectional view of collagen fibril assembly. A fibril consists of a variable number of microfibrils, however decorin is only able to bind to tropocollagen units on the outer surface of the fibril. The proposed interaction between collagen fibrils involves the association of at least two dermatan sulfate GAG side-chains bonded to decorin core proteins on adjacent collagen fibrils.
reported that decorin deficiency decreases the tensile strength in the dermis [27], does not affect tensile strength or modulus in tail tendon fascicles [28, 29], and increases modulus in the patellar tendon [28, 29]. NKISK, a pentapeptide that inhibits the ability of decorin to bind to collagen, caused fibril to fibril disassociation [18], and ultimately greater tissue laxity without reduction in material strength [30]. The mechanical behavior of connective tissues from knockout mice may be influenced by changes in tissue development due to the absence of decorin, such as irregular collagen structure [27] and regulatory activity that increases biglycan production as a compensatory mechanism [31-33]. Lack of biochemical analyses in the NKISK studies has left the molecular alterations in question. If NKISK specifically inhibits decorin binding, observed changes may be due to the absence of the decorin core protein and not necessarily the associated GAG. Altogether these studies have left the mechanical role of DS under elastic tensile deformation unclear and controversial.

In this study, a new experimental model was developed to quantify the effects of DS GAGs on the elastic material behavior of mature ligament tissue. The objective of this study was to assess the role of DS in resisting quasi-static tensile and shear deformation along the fiber direction in human ligament by targeted removal of DS crosslinks with enzymatic degradation. Understanding the elastic contribution of DS will clarify the structure-function relationships in ligament and address the validity of the decorin cross-linking theory.
Materials and Methods

Experimental Design

The medial collateral ligament (MCL) of the human knee was chosen for study due to the extensive prior mechanical test data available [34-37] and our well-developed experimental protocols for mechanical characterization [34, 36, 38]. Knees were acquired fresh-frozen and were allowed 16 h to thaw prior to dissection. The freeze-thaw cycle does not influence the quasi-static material properties of ligament [39, 40]. Knees with surgical scars, ligament injury or cartilage degeneration characteristic of osteoarthritis were eliminated. To account for material symmetry characteristics of ligament, two types of mechanical tests were performed – uniaxial tensile testing [34] and shear testing [36]. For tensile testing, 16 specimens were harvested from 4 unpaired human MCLs (donor age = 57±5 yrs). For shear testing, 16 specimens were harvested from 5 unpaired human MCLs (donor age = 55±8 yrs). The specimens were divided between two treatment groups: a control treatment group and a Chondroitinase B (ChB) treatment group. ChB specifically degrades DS side chains of proteoglycans [41]. Mechanical testing was performed pre- and posttreatment on the same specimen.

Uniaxial Tensile Testing

Four unpaired human MCLs were used for tensile testing. A hardened steel punch [34] was used to extract four samples from different locations in each superficial MCL between the tibial and femoral insertions, for a total of 16 tested samples. The punch shape included beveled ends for gripping and was oriented so that its long axis was aligned with visible fiber bundles. Sample geometry met ASTM requirements for fiber reinforced composite materials [42]. Samples were randomly divided between the
control and ChB treatment groups and loaded in a clamp assembly. A 0.1 N preload was applied to establish a consistent reference length. Vertical position of the actuator (Tol-O-Matic, Hamel, MN, accuracy ±1.0 µm) and high-resolution micrometer measurements (Newport, Irvine, CA, accuracy ±0.5 µm) of the x-y table were logged so that the reference configuration could be reproduced after treatment. Sample position was further measured by tracking 4.75 mm dia acrylic white spheres adhered to each clamp and to a fixed reference with a digital camera (Pulnix TM-1040, 1024x1024x30 fps, Sunnyvale, CA) and digital motion analysis software (DMAS, Spica Technology Corp, Maui, HI, accuracy ± 0.005 mm) [43]. This protocol verified that posttreatment clamp positions were within 0.04 ± 0.01 mm of pretreatment clamp positions.

Specimen dimensions were measured using digital calipers (Mitutoyo, San Jose, accuracy ±0.02 mm) by taking an average of three measurements (Table 6.1). Following five minutes of relaxation, a triangular displacement profile was applied for 10 cycles at a strain rate of 1.0%/sec with a clamp-to-clamp strain amplitude of 8% of the specimen length. Clamp-to-clamp strain was based on substructural failure limits of ligament, reported to occur after 5% tissue strain [44]. Tissue tissue strain is approximately half the clamp-to-clamp strain [37], so an 8% clamp-to-clamp strain was chosen to avoid structural damage. The strain rate was selected to minimize viscoelastic and inertial effects [34]. Tissue strain was measured by tracking 1 mm diameter contrast

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Mean clamp-to-clamp length ± SD</th>
<th>Mean width ± SD</th>
<th>Mean thickness ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile</td>
<td>15.06 ± 1.84</td>
<td>1.81 ± 0.38</td>
<td>1.48 ± 0.58</td>
</tr>
<tr>
<td>Shear</td>
<td>6.93 ± 0.91</td>
<td>9.81 ± 0.93</td>
<td>1.84 ± 0.42</td>
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markers on the specimen midsubstance using the DMAS system. Force and displacement were monitored continuously, with peak stress calculations based on the 10th cycle. Tangent modulus was calculated as the slope of the linear region of the stress-strain curve using local tissue strain, and was standardized for each case by using linear regression to fit the stress-strain data for the final 1% strain region. Hysteresis was determined from the area enclosed by the loading and unloading stress-strain curves from the last loading cycle.

Shear Testing

Five unpaired human MCLs were used. A rectangular punch (10 x 25 mm) was used to extract up to four samples from each superficial, for a total of 16 tested samples. Samples were randomly divided between the control and ChB treatment groups and loaded in a clamp assembly so that visible fibers were along the direction of displacement [36]. Load cells (Sensotec Inc, Columbus, OH, 1000 g, accuracy ±0.1 g) monitored both transverse and longitudinal clamp reaction forces. After the specimen was loaded and aligned in an unstrained state, a 1 g preload was applied in the transverse direction. The vertical displacement was then set at a neutral position, defined as the inflection point of the force response resulting from small cyclic up-down clamp displacements. Clamp position and physical dimensions were recorded (Table 6.1) using methods identical to the tensile mechanical protocol. Following a five-minute equilibration period, a triangular displacement profile was applied for 10 cycles at a strain rate of 6.5%/sec, with peak displacement based on \( \tan(\theta) = \frac{\text{peak displacement}}{\text{clamp-to-clamp length}} = 0.4 \) [36]. Strain rate was chosen to minimize viscoelastic and inertial effects and peak displacement was selected to maximize loading without
damaging the tissue. Transverse force, longitudinal force and vertical displacement were monitored continuously, with peak stress calculations based on the 10th cycle. Shear modulus was defined as the slope of the stress-strain curve over the final 0.1 shear strain region. Hysteresis effect was measured using the previously mentioned methods.

Chondroitinase B Treatment Protocol

Following initial shear and tensile testing, each specimen was removed from the test machine while still mounted in the clamps. The entire specimen and clamp assembly was bathed for 1 h at room temperature in 15 ml of buffer solution (15 ml of 20 mM Tris pH 7.5, 150 mM NaCl, 5 mM CaCl2) with protease inhibitors (1 tablet of mini-complete per 10 ml of buffer). Samples were then bathed in 15 ml of either the control buffer solution or the ChB (ChB, 1.0 IU/mL) for 6 h at room temperature with gentle agitation using an orbital shaker. Preliminary tests confirmed that all DS was completely degraded using 0.25 IU/mL for 6 h (data not shown). Immediately after treatment, the clamp assembly was reattached to the test machine and returned to the original testing position. The sample was allowed to equilibrate for five minutes. Shear or tensile posttreatment testing was then performed with the same test parameters. For the tensile test specimens, failure tests were performed subsequently using our published protocol [34]. The sample was then removed from the clamps and placed in a stop buffer (20 mM Tris, pH 7.5, 150 mM Sodium Chloride, and 10 mM EDTA) to inhibit any further ChB activity. Samples were kept continuously moist by applying 0.9% saline solution through a nozzle.
Specificity of Chondroitinase B

A large quantity of ChB was required to treat all ligament specimens. ChB was cloned into a prokaryotic expression vector as previously described [45]. ChB degrades DS into disaccharides by cleaving regions that contain iduronic acid (Figure 6.1, A). One international unit (IU) of ChB activity corresponds to the amount of enzyme required to liberate 1 μM of unsaturated uronic acid from DS per minute at 30°C (pH 7.5, [DS] = 2 mg/mL).

The specificity of ChB was determined by incubating ChB with various sulfated GAGs and then determining the GAG concentration remaining after digestion using the dimethylmethylene blue (DMB) assay [46]. Individual reactions (30 μl, n=6 for each condition) were set up containing 1.0 IU/mL ChB and 500 μg/mL of sulfated GAG (DS, chondroitin sulfate A and C, heparan sulfate, or keratan sulfate). Control reactions contained GAGs and buffer only: 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 5 mM CaCl₂. The reaction was allowed to proceed for 6 h at RT. Five μl of each reaction (diluted 2 fold) was transferred to a 96 well plate in duplicate, along with GAG standards. Two hundred μl of dimethylmethylene blue reagent were added to each well, and the plate was immediately read in a plate reader at 530 and 590 nm. GAG concentrations were expressed as a percentage of control reactions.

Verification of ChB Activity and Removal of DS

Following mechanical testing, the ligament tissue between the clamps was isolated with a scalpel and cut into three pieces for GAG quantification, decorin Western blot analysis and transmission electron microscopy (TEM). GAG quantification followed established guidelines [47], which involved taking DMB assays from digested
papain extracts. These guidelines permitted DS content in control and ChB treated samples to be calculated by subtracting the amount of GAG present in the papain extract treated with additional ChB from the papain extract that was treated with additional buffer only.

Western blot analysis examined the glycosylation state of decorin from extracted proteoglycans. Wet weights were obtained and samples were then frozen in liquid nitrogen and pulverized. Proteoglycans were extracted twice over 24 h at 4°C in a total of 25 volumes of 4 M GuHCl, 50 mM acetate buffer pH 6.0 plus protease inhibitors. Two-hundred microliters of extract were precipitated overnight by the addition of 1 ml of 100% ethanol. Following centrifugation at 14,100 g, the protein pellet was washed with 70% ethanol and resuspended in 20 μl of 8 M urea, followed by an additional 140 μl of 10 mM Tris pH 7.4. Twenty-five microliters of this extract were treated with and without additional ChB, as described above. Fifteen microliters of each reaction was resolved by size on 4-16% gradient SDS-polyacrylamide gels. Proteins were electrophoretically transferred to nitrocellulose membranes and blocked in 3% BSA in 1X TBST (Tris-buffered saline plus 0.1% Tween 20) for 1 hr at RT. Blots were incubated with 0.2 μg/ml biotinylated anti-human decorin antibody (BAF140, R&D Systems, Minneapolis, MN) for 3 h at RT on a nutator. Blots were washed and incubated with 0.8 μg/ml with Neutravidin-HRP (Pierce, Rockford, IL) for 1 h at RT. Blots were developed using a chemiluminescent substrate (Bio-Rad, Hercules, CA) and imaged with a gel documentation system with an integrated 12 bit camera.

TEM was used to investigate the presence of sulfated GAGs in the five control and ChB treated MCL specimens that were tested in shear. Ligament sections were
Samples were sectioned to 20 µm (cryostat, Leica CM3050S, Exton PA), mounted on slides, and stained with Cupromeronic Blue [8, 48]. Ultrathin sections, approximately 70 nm, were obtained via ultramicrotome (Leica Ultracut UCT, Exton, PA) and viewed using a Hitachi H7100 TEM with a LAB6 filament. Digital images of a minimum of four fields of view were obtained from each examined MCL. An image processing program was used to determine the number of stained GAGs per image. All images from a sample were averaged to produce a representative number of detected objects for each sample.

Statistical Analysis

The effects of control and ChB treatment on tensile and shear peak stresses, tensile and shear tangent moduli and peak tissue strain were assessed using paired t-tests to measure significance between pre- and posttreatment. Control versus ChB treatment effect upon hysteresis was assessed using ANCOVA, controlling for pretreatment results, with Bonferroni adjustment for multiple comparisons. TEM and biochemistry results were assessed using independent t-tests to measure control versus ChB treatment effect. A power analysis demonstrated that a sample size of 8 was sufficient to detect a 10% change with 80% confidence for all measured quasi-static mechanical results (Power = 0.80). Unless otherwise noted, all results are reported with standard error.

Results

ChB and control treatments had no significant effect on tensile (Figure 6.2) or shear (Figure 6.3) stress-strain response. At peak deformation, there were no significant differences in the tensile peak stress due to control or ChB treatments (percent decreases
Figure 6.2. Stress-Strain curves for tensile tests of control (n=8) and chondroitinase B (n=8) treated ligament samples. (A) Tensile mechanical response immediately after dissection (pretreatment) and after 6 h of control treatment (posttreatment). (B) Tensile mechanical response immediately after dissection (pretreatment) and after 6 h of chondroitinase B treatment (posttreatment). Bars = standard error.

Figure 6.3. Stress-Strain curves for shear tests of control (n=8) and chondroitinase B (n=8) treated ligament samples. (A) Shear mechanical response immediately after dissection (pretreatment) and after 6 h of control treatment (posttreatment). (B) Shear mechanical response immediately after dissection (pretreatment) and after 6 h of chondroitinase B treatment (posttreatment). Bars = standard error.
of 0.4 ± 1.3% (p=0.75) and 0.4 ± 0.9% (p=0.718) due to control and ChB treatments, respectively. Similarly, there were no significant differences in the shear peak stress due to control or ChB treatments (percent increases of 5.6 ± 3.3% (p=0.13) and 1.7 ± 1.6% (p=0.32) due to control and ChB treatments, respectively).

Tensile tangent modulus, shear modulus and tissue strain were unaffected by ChB and control treatments. For tensile tests, an 8.0% clamp strain equated to 5.1 ± 0.4% tissue strain. Between time stages, tensile control specimens went from 5.8 ± 0.5% to 5.7 ± 0.6% (p=0.58) tissue strain, while tensile ChB treated specimens went from 4.3 ± 0.3% to 4.4 ± 0.3% (p=0.54) tissue strain. The pre- and posttreatment tensile moduli from the 8% cyclic tests were, respectively, 160 ± 56 and 159 ± 55 MPa for the control (p=0.79), 242 ± 64 and 233 ± 57 MPa for the ChB treated (p=0.37). The tensile tangent modulus data calculated from the 8% cyclic strain tests (198 ± 28 MPa) had a high correlation with the tensile tangent modulus data calculated from the failure tests (202 ± 37 MPa) (r²=0.8). The pre- and posttreatment shear moduli were, respectively, 133 ± 11 and 142 ± 14 KPa for the control (p=0.08), 146 ± 39 and 151 ± 41 KPa for the ChB treated (p=0.08).

There were no significant differences in percent hysteresis between control and ChB treatments for tensile (p=0.16) and shear (p=0.79) material tests. The pre- and posttreatment percent hysteresis for tensile specimens were, respectively, 18.8 ± 0.9% and 20.4 ± 0.9% for control treated, and 20.0 ± 0.7% and 20.8 ± 0.9% for ChB treated. The pre- and posttreatment percent hysteresis for shear specimens were, respectively, 21.2 ± 1.8% and 27.5 ± 1.5% for control, and 21.2 ± 1.1% and 28.0 ± 1.9% for ChB treated.
ChB reduced the concentration of the DS standard by 78% (Figure 6.4). Concentrations of Chondroitin sulfates A and C, heparan sulfate and keratan sulfate were unaffected by ChB treatment. A likely reason that the specificity test did not show a complete degradation of DS is due to the presence of DMB detectable glucuronic acid-containing regions in DS, which are not digested by ChB [49, 50]. Biochemical analysis of the mechanically tested samples found that over 90% of the DS in ChB treated ligament samples (0.1 ± 0.2 μg DS / mg dry tissue) were eliminated when compared to control specimens (1.5 ± 0.5 μg DS / mg dry tissue) (p < 0.001). DS accounted for 44 ± 4% of all sulfated GAGs (3.6 ± 0.6 μg sulfated GAGs / mg dry tissue) in the mechanically tested controls. Western blot results for three control and three treated specimens are shown in Figure 6.5, but all shear samples and random tensile samples were tested and yielded similar results. Control specimens (lanes 1, 3, and 5) had two predominant species, a band at ~43 kDa and a smear from 50-100 kDa. When extracts

Figure 6.4. Specificity of Chondroitinase B. Chondroitinase B (1 U/ml) was incubated with glycosaminoglycans (500 μg/ml) for 6 h. GAG concentration after 6 h was determined using the DMB assay. Concentrations were normalized to control reactions which did not contain chondroitinase B. DS = dermatan sulfate, CS = equal mixture of chondroitin sulfates A and C, HS = heparan sulfate, KS = keratan sulfate. N = 6. Error bars = SD.
Figure 6.5. Decorin western blot of proteins extracted from 3 control and 3 ChB treated ligament samples. Each lane represents protein extracted from approximately 375 µg of wet tissue. Two tests were performed on each sample. They were either given an additional control buffer treatment (-) or given an additional treatment of ChB (+). Decorin core protein migrated as a doublet at ~45 kDa. Decorin containing its dermatan sulfate side-chain migrated as a smear from ~55 to 120 kDa.

were treated with ChB (lanes 2, 4, and 6) the smear was eliminated and there were two major bands at 45 and 90 kDa. The species are consistent with published results in the literature - the 45 kDa band, which appears as a doublet with shorter exposure times, represents decorin that has lost its glycosaminoglycan side chain, and the 90 kDa band is a dimeric form of decorin [51, 52]. Protein extracts obtained from ChB treated ligament samples (lanes 7, 9, and 11) did not show the smear pattern between 50 and 100 kDa, suggesting that decorin obtained from these samples had already lost its DS side chain. Additional ChB treatment did not cause any further changes (lanes 8, 10, and 12).
TEM images further verified degradation of sulfated GAGs. ChB treated samples showed a significant reduction in the number of sulfated GAGs when compared to controls (88% reduction, p<0.001). The quantity of stained GAGs was reasonably uniform within groups having 47 ± 13 and 350 ± 40 stained GAGs per field for ChB treated and control images, respectively. Stained GAGs remaining after ChB treatment were preferentially aligned with the local collagen fibrils and generally larger than the GAGs that were removed (Figure 6.6).

Discussion

The DS GAG of decorin has been implicated as a contributor to the material behavior of connective tissue [27-29]. Our present findings do not support the theory that sulfated GAG crosslinks influence continuum-level mechanical behavior during

Figure 6.6. Representative TEM images of tissue stained with Cupromeroneic Blue after mechanical testing. Large arrows indicate collagen fibril direction, small arrows indicate darkly stained sulfated GAGs. (A) Control treated specimen. (B) CnB treated specimen with 88% reduction in sulfated GAGs. Note the decrease in the overall number of fibril spanning GAGs and the preferred alignment of remaining sulfated GAGs along the collagen fibril direction. (Bar = 200 nm).
quasi-static tensile loading [8, 15, 17, 19-23]. Shear testing was conducted to determine whether DS contributes to the mechanical behavior of mature human MCL when fibrils are sheared relative to each other [29, 53]. This hypothesis was similarly rejected. Therefore, DS in mature human medial collateral ligament does not resist quasi-static tensile or shear loads.

Mathematical studies have hypothesized that GAG crosslinks contribute to the resistance of loads along the fiber direction by transferring force between discontinuous fibrils. For instance, Redaelli et al. [22] used a molecular dynamics model to explore the ability of decorin GAG crosslinks to prevent sliding between discontinuous 100 µm fibrils. Results suggested that interfibrillar GAGs could transfer force between adjacent fibrils, with maximum load transfer and GAG strain occurring at 5% tissue strain. The current study demonstrated that even near 5% tissue strain, interfibrillar GAG crosslinks did not contribute to the tensile material response of mature human MCL. This result does not negate the theory that DS may resist fibril motion, but it does confirm that any interfibrillar resistance from DS interaction does not affect quasi-static mechanics at the macro scale. Past studies support this conclusion. Cribb and Scott [8] demonstrated that the orientation of sulfated GAGs does not change under tensile stretch along the direction of the fibrils, indicating that there was no relative sliding of the fibrils. Further, the mathematical model used by Radaelli [22] assumed fibril length to be discontinuous in 100 µm segments. Fibril discontinuity is debated in the literature, with many investigators reporting that mature fibrils are continuous structures [53-56], with tapered ends only present on embryonic and in vitro fibrils [21, 57, 58]. It should be noted that non-DS molecules, including other sulfated GAGs, may transfer forces
between shorter discontinuous fibrils or contribute to the mechanical properties of ligaments in other test configurations.

Our findings aid the interpretation of results from studies using knockout mice and competitive peptide techniques. Results of this study suggest that material alterations reported for tendons from decorin knockouts [27] are likely due to genetic compensation or developmental abnormalities inherent in decorin deficiency. In another study, pentapeptide NKISK treatment [30] disrupted the decorin-collagen interaction in mouse tendon, resulting in increased strain behavior. Since the current study found that DS degradation did not influence strain behavior, another factor may have influenced tissue mechanics in the NKISK study. Biochemical analyses are needed to understand how NKISK alters molecular composition.

Since negatively charged GAGs have an affinity for water molecules, we investigated hysteresis during both shear and tensile tests. There were no significant changes in hysteresis following the control and ChB treatments. However, DS may still contribute to the viscoelastic response when other loading rates or protocols are used. In studies of decorin-deficient knockout mice, decorin content correlated with strain-rate sensitivity [28] and stress-relaxation behavior [59]. As stated previously, knockout models are unable to directly measure DS contribution. Thus to completely characterize the possible role of DS in ligament mechanical behavior, viscoelastic testing in both tensile and compressive configurations is necessary.

To have confidence in our results, the experiments needed to be performed in a reproducible manner that provided data consistent with the literature. Ligament mechanical behavior can vary between donors, and this can be especially problematic in
studies using cadaveric tissues. Measured mechanical response can also be influenced by tissue heterogeneity, clamping, and specimen alignment in the material testing system. These variables were controlled with the repeated measures design of the experiments. Measured mechanical properties were also in good agreement with past research. Peak shear stress for this experiment was 22 KPa, while published peak shear stress using similar shear strains and loading configurations was 25 KPa [36]. Past studies measured an average tangent modulus of 332 MPa in human MCL specimens taken along the anterior portion of the superficial MCL between the tibial insertion and the meniscal attachment [34]. Samples taken from this same region in the current study had a similar average tangent modulus of 330 MPa.

Biochemical and TEM analysis verified selective degradation of DS and elimination of interfibrillar crosslinks. Samples treated with ChB had 90% less DS than control samples and an absence of the DS GAG side chain commonly associated with decorin. TEM results demonstrated that ChB treatment removed the vast majority of GAGs. Since the sulfated GAGs that remained after ChB treatment were oriented along the fibrils, DS represented the majority of fibril-spanning sulfated GAGs in the mature human MCL.

Since ChB degrades all DS chains, it likely degraded DS side chains on biglycan, a small leucine rich proteoglycan that accounts for less than 10% of all proteoglycans in ligament [7]. Biglycan has two associated GAG side chains and a core protein that binds to collagen [60] and is highly homologous to decorin. Biglycan is another proteoglycan that could contribute to the mechanical properties of tendon [29]. However, since biglycan side chains are predominantly DS, and DS was eliminated via
ChB treatment, biglycan proteoglycans similarly did not contribute to quasi-static mechanical behavior of the human MCL.

Several limitations of this research must be mentioned. A quasi-static loading rate was used and thus DS depletion could have an effect if mechanical testing was performed using faster loading rates. Further, DS may contribute to ligament mechanical properties in other test configurations such as compressive loading. Results from this study are based on in vitro tests and long-term effects of DS depletion were not considered. The human MCL tissue used in this study came from relatively old donors with several different causes of death. The exact time from death to postmortem freezing was unknown but less than 24 h. Although the experimental design utilized pre-and posttreatment testing of the same sample (repeated measures design), which controls for the effect of initial differences between samples, the postmortem conditions and differences in cause of death may have affected the starting concentration of DS in the tissues. Similar to previous studies that used a repeated measures design to assess treatment [59, 61], multiple samples were taken from each donor. However, it should be noted that the measured quasi-static mechanical parameters and GAG content had as much variability between samples from a specific donor as between donors. Had mechanical test data from each donor been averaged (n=4) to focus on variability between donors, an acceptable change of 20% would be detected with 80% confidence for all measured quasi-static mechanical results.

In summary, mechanical tests on the same mature human MCL specimens before and after targeted DS removal demonstrated that DS does not contribute to the quasi-static tensile or shear material properties of mature human MCL. Future studies should
examine how DS contributes to viscoelasticity, and if other GAGs influence tissue mechanics. By continuing to examine microstructural influence on mechanical properties of tissue, the origins of tissue material response can be elucidated and applied to the improvement of tissue engineering and wound healing methods. Studies seeking to engineer replacement tissues can use the results of this study and others like it to understand the contribution of specific molecules to the mechanics of the tissue that is being replaced.

References


Abstract

The viscoelastic properties of human ligament potentially guard against structural failure, yet the microstructural origins of these transient behaviors are unknown. Glycosaminoglycans (GAGs) are widely suspected to affect ligament viscoelasticity by forming molecular bridges between neighboring collagen fibrils. This study investigated whether GAGs directly affect viscoelastic material behavior in human medial collateral ligament (MCL) by using nondestructive tensile tests before and after degradation of glycosaminoglycans with Chondroitinase ABC (ChABC). Control and ChABC treatment (83% GAG removal) produced similar alterations to ligament viscoelasticity. This finding was consistent at different levels of collagen fiber stretch and tissue hydration. On average, stress relaxation increased after incubation by 2.2% (control) and 2.1% (ChABC), dynamic modulus increased after incubation by 3.6% (control) and 3.8% (ChABC), and phase shift increased after incubation by 8.5% (control) and 8.4% (ChABC). The changes in viscoelastic behavior after treatment were significantly more pronounced at lower clamp-to-clamp strain levels. A 10% difference in the water content of tested specimens had minor influence on ligament viscoelastic properties. The major finding of this study was that mechanical interactions between collagen fibrils and GAGs
do not affect tissue-level viscoelastic mechanics in mature human MCL. These findings narrow the number of extracellular matrix proteins that have a direct contribution to ligament viscoelasticity.

**Introduction**

The intrinsic viscoelastic behavior of ligament originates from molecular realignments that are neither infinitely slow (elastic) nor instantaneous (viscous). This transient response may guard against structural failure. In particular, ligament stiffness and damping properties increase at greater rates of strain [1, 2], and ligament stress reduces with time during cyclic loading [3, 4]. These mechanisms may brake excessive joint loads and protect against ligament fatigue failure [5]. Although such functionally significant behaviors in ligament are well-defined, the microstructural genesis of these transient behaviors is unknown. Determination of microstructural influence on tissue-scale viscoelasticity could improve the innovation and evaluation of ligament treatment modalities (e.g. tissue engineering, drug therapy).

The origins of viscoelastic material behavior in ligament are certainly related to its organization and composition. Ligament can be described as a hydrated (~70% water) fiber-reinforced matrix, where collagen fibers provide structural integrity to the extrafibrillar matrix [6, 7]. Collagen fibers are formed by arrays of fibrils that in turn are composed of aggregating tropocollagen monomers. These tropocollagen units are tightly wound Type I collagen helices, which represent 70% of the solid phase weight [8]. The remaining constituents include other collagens (III, V, X, XII, and XIV), extracellular matrix proteins and proteoglycans. Theories on the viscoelastic origin in ligament and tendon include inherent viscoelasticity of the collagen fibers [6, 9], interaction of collagen
fibers with the extracellular constituents [10, 11] and fluid movement through the solid phase [10, 11]. One theory that has received considerable attention concerns the interaction between collagen fibrils and sulfated glycosaminoglycans [6, 12, 13].

Sulfated glycosaminoglycans (sGAGs) are negatively-charged polysaccharide macromolecules that covalently bind to proteoglycan core proteins. The sGAGs in ligament include dermatan sulfate, chondroitin sulfate (A & C), and keratin sulfate. Dermatan and chondroitin sulfate make up over 95% of all sGAGs in ligament [14], and are the exclusive sGAG chains of the interfibrillar proteoglycans biglycan and decorin. Biglycan attaches to collagen fibrils through its sGAG chains [15], while decorin, which represents ~90% of all ligament proteoglycans [16], attaches to collagen fibrils through the regular binding of its core protein on D-period sites [17]. The sGAG chains of biglycan and decorin span fibrils [17, 18] and are preferentially distributed orthogonal to the fibril direction [19]. Since sGAGs have been shown to self-associate [20], interactions between sGAGs on neighboring fibrils have the capacity to influence the fibril sliding that is attributed with tensile stretch [6]. Although it has been shown that interfibrillar sGAGs do not appear to influence quasi-static ligaments mechanics [21], sGAGs may still resist transient tensile loads [13, 22]. Further, sGAGs are highly negatively charged and through collagen fibril interactions create a fixed charge density that influences tissue hydration [23], and potentially viscoelastic behavior [24, 25].

Experimental and theoretical studies have investigated the viscoelastic mechanical influence of sulfated GAGs during tensile deformation. Research by Robinson et al. [26] and Elliot et al. [7] found that tendon tail fascicles in decorin-deficient mice were less strain-rate sensitive and experienced faster stress relaxation than those of wild type mice,
while tendon tail fascicles in young wild type mice with elevated levels of decorin were more strain-rate sensitive and experienced slower stress relaxation than those of wild type mice. These results indicate that decorin deficiency is strongly associated with altered tensile viscoelastic properties in tendon. Further, degradation of sulfated GAGs significantly altered tensile viscoelastic properties in lung parenchymal strips [27] and in bovine cartilage [13, 22]. No studies have directly measured the influence of sGAGs in ligament, but theoretical studies did successfully predict viscoelastic behavior of structurally similar tendon with models that account for sGAG-fibril interactions [6, 13].

Collectively, these previous studies have identified sGAGs as a macromolecule likely connected to viscoelastic tensile properties in ligament. Yet, the mechanical alterations observed in decorin-deficient mice [7, 28] were not specific to sGAGs and experimental results in parenchymal lung and cartilage tissue may not translate to ligament, where sGAGs are more sparsely distributed [8, 29]. Therefore, the aim of this study was to specifically determine whether interfibrilar sGAGs influence the viscoelastic material behavior of ligament under tension. To meet this objective, it was necessary to control for tissue hydration and collagen fiber stretch, which have been demonstrated to independently impact ligament viscoelasticity [2, 24, 25].

**Materials and Methods**

**Experimental Design**

The medial collateral ligament (MCL) of the human knee was chosen as a model ligament due to a relatively high distribution of sulfated GAGs [8, 14, 30] and our well established experimental protocol for mechanical characterization [1, 31]. Samples extracted from MCLs were subjected to tensile loading along the fiber direction on a
material testing system. This configuration best represents the principal loading orientation in ligament [32]. After tensile testing was completed, samples were removed from the material testing system and incubated in either a control or chondroitinase ABC (ChABC) treatment. ChABC degrades chondroitin sulfate (A+C) and dermatan sulfate without affecting other proteoglycan macromolecules [33, 34] and has been reported to remove 98% of interfibrillar sGAGs [14]. Mechanical testing was then repeated on each specimen to determine the treatment effect. To measure whether this treatment effect was affected by testing parameters, strain level and buffer solution were varied to make eight groups, with n=5 samples in each group, for a total of 40 samples.

Viscoelastic Testing

Eleven unpaired human MCLs were used for this experiment (donor age = 54±10 yrs). Six MCLs were used for viscoelastic testing at a 4% strain level and five MCLs were used for viscoelastic testing at a 6% strain level. Knees were acquired fresh-frozen and were allowed 16 h to thaw prior to dissection. Knees with ligament lacerations, surgical scars, or cartilage wear characteristic of osteoarthritis were eliminated. During dissection, the tissue was kept moist by applying a physiological 0.9% saline buffer solution through a nozzle. A hardened steel punch was used to extract four tensile samples from different locations in each superficial MCL between the tibial and femoral insertions (Figure 7.1, A) [31]. Two strips of tissue immediately adjacent to the punched tensile sample were retained to monitor the tissue hydration in an unloaded state (Figure 7.1, B). The punch shape included beveled ends for gripping and was oriented so that its long axis was aligned with visible fiber bundles. Black optical markers were adhered to the narrow central third of the tensile sample using cyanoacrylate. Samples
Figure 7.1: Specimen acquisition. A) Up to four specimen sets were extracted from each MCL, with three samples to each set. Each set included one punched sample for viscoelastic tensile testing (dark grey) and two adjacent strips to monitor tissue hydration (light grey). B) Photograph of the specimen set.

were loaded in the clamping assembly and width and thickness dimensions were determined by taking an average of three measurements with digital calipers (Mitutoyo, San Jose, CA; accuracy ±0.02 mm). Mechanical testing was performed in a custom built testing chamber that interfaced with a heating recirculator (PolyScience, Niles, IL; accuracy ± 0.2°C) to maintain a solution temperature of 37°C [3, 35].

The testing protocol involved a series of preconditioning loads followed by a period of recovery and then viscoelastic testing (Figure 7.2). This testing protocol allowed the measurement of multiple viscoelastic characteristics while not permanently altering the elastic tissue properties. To establish a consistent reference position for all samples, a 0.1 N preload was applied (Sensotec Inc, Columbus, OH; 1,000 g, max range 2,900 grams, accuracy 0.3g). At this reference position, clamp-to-clamp specimen length was determined. This was calculated by averaging three measurements (Mitutoyo, San Jose, accuracy ±0.02 mm) of an inside calipers prong-to-prong distance after insertion between the testing clamps. To precondition the samples, the tissue was first ramped at 1.0%/sec to 8% of the reference length, and held for 5 minutes. This was immediately followed by a triangular displacement profile applied to 8% of the reference length for
10 cycles at a strain rate of 1%/sec. The 8% strain amplitude was selected to exceed the highest strain level experienced during the testing protocol [36], while not elastically damaging the tissue [21]. To verify that no elastic damage occurred between pre- and posttreatment, the peak stress of the final preconditioning cycle was recorded (Figure 7.2).

After preconditioning, the sample was allowed to recover for 10 minutes at its reference length. The specimen was then ramped at 1%/sec to a prescribed strain level of 4% or 6% clamp-to-clamp strain and allowed to stress-relax for 10 minutes (Figure 7.2). At a 4% strain level, the stress-strain response is still in the toe region [21] and the collagen fibers are not completely uncrimped [37]. At a 6% strain, the stress-strain response is in the linear region and the collagen fibers are collectively straightened [37, 38]. Testing these two strain levels permitted the assessment of sulfated GAGs at different states of collagen and extrafibrillar matrix contribution. Following stress relaxation, sinusoidal displacement waves (0.1, 1, 5, 10, and 15 Hz) were applied to a 0.125% strain amplitude (Figure 7.2). The 15 HZ sinusoidal oscillation corresponds to a
max strain-rate of ~12%/s. Since the optical tracking system recorded at a maximum of 30 HZ, the local tissue strain during 0.1 HZ oscillations was used to predict the local strain for all sinusoidal frequencies [1]. There was an average drop of 0.6% ± 0.4% (SD) in the equilibrium stress level between the start and completion of the entire set of oscillations. This small drop verified that 10 minutes of stress relaxation was sufficient to allow equilibrium to be reached.

After the test protocol was completed, each sample was removed from the material testing system while still mounted in the clamps. The entire specimen and clamp assembly was bathed for 1 h at room temperature in the buffer solution (detailed in the next section) with protease inhibitors (one tablet of minicomplete per 10 ml of buffer). This was followed by 6 h of incubation in the buffer solution with or without ChABC treatment (1.0 IU/mL, Sigma-Aldrich, Buchs, Switzerland). Following incubation, the clamp assembly was reattached to the material testing system and returned to the original testing position. Mechanical tests were then repeated using parameters identical to the pretreatment mechanical tests. Treatment and repeatability protocols are described in greater detail in our previous publications [14, 21](Chapter. 6).

Tissue Swelling

To control tissue swelling, two testing solutions were used throughout the experiment. One solution consisted of a physiological buffer mixture (15 ml of 20 mM Tris pH 7.5, 150 mM NaCl, 5 mM CaCl₂), and was designated the standard buffer. A second solution was this buffer plus 7.5% polyethylene glycol (10 kDa; Sigma-Aldrich, Buchs, Switzerland), and was designated the PEG buffer. Polyethylene glycol has been previously used to control swelling in biological tissue [39, 40] and this concentration has
been shown to maintain wet weight in ligament during prolonged immersion [41]. Samples were equilibrated in their respective buffer solutions for at least 2 h prior to pretreatment mechanical tests. All mechanical tests and incubations were performed in their respective buffer solutions.

Multiple techniques were employed to quantify tissue swelling during the course of the experiment. Adjacent to each tensile sample, two strips were harvested to monitor tissue hydration (Figure 7.1, B). One strip was weighed and immediately frozen to serve as the time-zero control for water content. The second strip shadowed the testing protocol of the mechanically tested specimen in an unloaded state. For example, this sample was immersed in solution from the testing chamber during mechanical tests and was included in the same incubation compartment as the mechanically tested sample. It was therefore possible to assess whether the wet weight of the tissue had been altered during mechanical testing and incubation. After the posttreatment mechanical tests, tensile samples were excised from the clamps. Tensile samples and their adjacent strips were then weighed, rinsed, frozen and lyophilized. Water content was calculated from the ratio of dry weight to final wet weight (APX-60, Denver Instruments, Denver; readability ±0.1 mg).

Verification of Sulfated GAG Removal

The efficacy of the ChABC treatment was determined by using dimethylmethylene blue assays to first measure the concentration of sulfated GAGs in papain digested samples before and after retreatment with ChABC [21, 27]. The percentage of nondegraded sGAGs in ChABC incubated samples was estimated by subtracting the amount of sGAGs (μg of sGAG / mg of dry weight) in the papain digested
sample from the sGAGs in the same sample after additional ChABC treatment. This value, which represents the amount of sGAGs unsuccessfully digested during incubation, was divided by the amount of sGAGs native to the sample. Native sGAG estimates were calculated by averaging the amount of sGAGs in all the time-zero control specimens. Since ChABC does not degrade keratin or heparin sulfate, this native sGAG estimate was first reduced by the amount of sGAGs in the time-zero control after ChABC treatment. An approximation of the efficacy of ChABC could thus be attained, along with the percentage of sGAGs that could not be degraded by ChABC.

Data Reduction and Statistical Analysis

To generate stress-strain curves, the first Piola-Kirchhoff stress was determined with tissue engineering strains approximated from the digital camera data \(((l-l_0) / l_0\), where \(l\) is the current length and \(l_0\) is the reference length). The stress–time curves from the stress relaxation tests were normalized by the peak stress to obtain reduced relaxation curves [42]. The cyclic strain–time and stress–time data from the final four cycles of each frequency were fit to a four-parameter sine function in Matlab:

\[
y = y_0 + A \sin \left( \frac{2\pi t}{b} + \phi \right)
\]

Here, \(y\) and \(t\) represent the strain (or stress) and time data, respectively, \(y_0\) represents the equilibrium strain (or stress) level and \(A\) denotes the amplitude of the sine wave. \(\phi\) represents the phase and \(f\) denotes the frequency (Hz). The final four cycles of each frequency were chosen as input to give a sufficient number of preconditioning cycles, while maintaining an excellent fit (average \(r^2 = 0.998 \pm 0.002\)). The fit parameters were not significantly altered when the final two cycles were substituted as input (data not
shown). Dynamic modulus $M$ (MPa) and phase shift $\phi$ (radians) were calculated for all frequencies:

$$M = \frac{A_\sigma}{A_\varepsilon}; \quad \phi = \phi_\sigma - \phi_\varepsilon$$

(2)

$A_\sigma$ and $A_\varepsilon$ denote amplitudes of the cyclic stress–time and strain–time data, respectively, while $\phi_\sigma$ and $\phi_\varepsilon$ denote the corresponding phase angles [43].

Statistical analysis was performed in four parts. First, a repeated measures design was used to determine the effect of incubation and frequency on material properties for each of the eight test groups. The effect of incubation on peak stress, stress relaxation, average dynamic modulus, and average phase shift was assessed using paired t-tests with pre- and posttreatment data. The effect of frequency on dynamic modulus and phase shift, along with factor interaction between frequency and time, was assessed using repeated measures ANOVA with two within-subject factors (frequency, time), with Bonferroni adjusted pair-wise comparisons to measure significance between levels. Second, the effect of treatment, buffer solution and strain-level on mechanical changes due to incubation was assessed using ANCOVA, by controlling for pretreatment results, with Bonferroni adjustment for multiple comparisons. Third, to determine whether the pretreatment material properties were different between the eight test groups, ANOVA analysis was used with factors of treatment, buffer solution and strain-level. Lastly, biochemistry results were assessed using independent t-tests to measure treatment effect on sGAG content. A power analysis demonstrated that a sample size of 5 was sufficient to minimally detect a 20% change in all measured mechanical properties with 80%
confidence (Power = 0.80). Significance was set to $p \leq 0.05$, and all results are reported with standard error unless otherwise noted.

Results

Reduced Relaxation Data

The reduced relaxation curves were unaffected by incubation for all combinations of treatments, buffer solutions and strain-levels (Table 7.1, Fig. 7.3). The percent relaxation for all tests was $30.0 \pm 1.0\%$ before incubation and $30.6 \pm 1.0\%$ after incubation. The reduced relaxation of all samples tested at the lower strain-level, $31.8 \pm 1.0\%$, was significantly greater than at the higher strain-level, $28.1 \pm 1.0\%$ ($p=0.01$). The

![Table 7.1: Mechanical properties for all eight test groups (n=5) before and after treatment. SEM – “Standard error of the mean” for paired differences. Bolded SEM values represent statistically significant differences between related pre- and posttreatment mechanical properties (p<0.05).](image-url)
Figure 7.3: Reduced relaxation as a function of oscillatory frequency for control treated in either standard buffer (top, right), and ChABC treated in either standard buffer (bottom, left) or PEG buffer (bottom, right). Legends indicate applied clamp-to-clamp strain for pre- and posttreatment results. Mean ± standard error.
type of treatment and buffer solution did not affect the change in relaxation after incubation (p=0.69 and p=0.41, respectively). However, the strain-level did indeed influence the change in relaxation after incubation (p=0.02). The percent increase in percent relaxation after incubation varied by 0.2% due to treatment, 3.2% due to buffer solution (greater increase with PEG buffer), and 4.1% due to strain-level (greater increase at a 4% strain level).

Dynamic Modulus

The average dynamic modulus (averaged over all frequencies) was significantly affected by incubation in only two of the eight test groups (Fig. 7.4, Table 7.1). These groups were both at a 6% clamp-to-clamp strain and had an average increase in dynamic modulus of 4.0 ± 1.1% after treatment. The average dynamic modulus for all specimens at 0.1 Hz was 424 ± 48 MPa before incubation and 434 ± 50 after incubation, while average dynamic modulus at 15 Hz was 501 ± 55 before incubation and 522 ± 59 after incubation. The samples tested at the higher strain level had a dynamic modulus 226% greater than the lower strain level (p<0.001). Frequency only had a significant effect on dynamic modulus for the two test groups control treated in standard buffer. The frequency effect on dynamic modulus was not influenced by incubation. The type of treatment, buffer solution, and strain-level did not affect the change in dynamic modulus between pre- and posttreatment (p=0.86, p=0.20 and p=0.96, respectively). The percent increase in dynamic modulus after incubation only varied by 0.3% due to treatment, 0.1% due to buffer solution, and 4.2% due to strain-level (greater increase at the 6% strain-level).
Figure 7.4: Dynamic modulus as a function of oscillatory frequency for control treated in either standard buffer (top, left) or PEG buffer (top, right), and ChABC treated in either standard buffer (bottom, left) or PEG buffer (bottom, right). Legends indicate applied clamp-to-clamp strain for pre- and posttreatment results. Mean ± standard error.
Phase Shift

The average phase shift (averaged over all frequencies) was significantly affected by incubation in four of the eight test groups (Fig. 7.5, Table 7.1). Groups significantly affected had an increase in phase shift of 9.4 ± 2.6%, while groups not significantly affected had an increase in phase shift of 7.5 ± 0.8%. The average phase shift for all specimens at 0.1 Hz was 0.062 ± 0.004 radians before incubation and 0.069 ± 0.004 radians after incubation, while at 15 Hz was 0.155 ± 0.004 radians before incubation and 0.169 ± 0.005 radians after incubation. The average phase shift at the 4% strain-level, 0.114 ± 0.003 radians, was significantly greater than at the 6% strain-level, 0.095 ± 0.003 radians (p<0.001). Additionally, the average phase shift in PEG buffer, 0.111 ± 0.003, was significantly greater than in standard buffer, 0.098 ± 0.003 radians (p=0.004). Frequency had a significant effect on phase shift for all eight test groups. The frequency effect on phase shift for all eight groups was not influenced by incubation. The type of treatment and buffer solution did not affect the change in phase shift after incubation (p=0.98 and p=0.32, respectively). The strain-level, however, did significantly influence the change in phase shift after incubation (p=0.02). The percent increase in phase shift after incubation varied by 0.1% due to treatment, 5.2% due to buffer solution (greater increase in standard buffer), and 5.3% due to strain-level (greater increase at a 4% strain-level).

Tissue Swelling

The wet weights of the unloaded samples were normalized to their initial weight and graphed with respect to discrete measurement points during testing (Figure 7.6). On average, the wet weight of unloaded samples increased by 18 ± 2% when tested in
Figure 7.5: Phase shift as a function of oscillatory frequency for control treated in either standard buffer (top, left) or PEG buffer (top, right), and ChABC treated in either standard buffer (bottom, left) or PEG buffer (bottom, right). Legends indicate applied clamp-to-clamp strain for pre- and posttreatment results. Mean ± standard error.
standard buffer and decreased by 11 ± 2% when tested in PEG buffer. The unloaded samples tested in standard buffer had a significantly greater wet weight than those tested in PEG buffer (p<0.001). There was no effect of treatment on the overall normalized wet weight (p=0.68). During the incubation period, the average wet weight in the unloaded samples decreased by 1.2 ± 1.7% (p=0.48) in the standard buffer and increased by 3.2 ± 1.4% (p=0.08) in the PEG buffers. The greatest change during the incubation period was a 4.5 ± 2.8% decrease in wet weight in samples treated with ChABC in standard buffer (p=0.15).

The native water content was consistent between treatment and buffer solution groups, with an average range from 71.6% to 73.1% (Figure 7.7). All tested samples had significantly different water content than the native samples, with the exception of the mechanically loaded samples tested in standard buffer (average water content = 72.3, p=0.24). Compared to the native samples, unloaded samples incubated in the PEG buffer had a significantly decreased water content, and unloaded samples incubated in standard buffer had a significantly increased water content (p<0.001 and p<0.001, respectively). There was no effect of treatment or strain-level on water content in incubated samples (p=0.29 and p=0.52, respectively), although in samples incubated in standard buffer, the ChABC treated samples had 2.2% less water content than control treated samples. The mechanically loaded samples had 2.5% less water content than the unloaded samples (p=0.002).
Figure 7.6: Time history of the unloaded samples wet weight through the course of the experiment. Wet weight was normalized to the initial weight of the unloaded sample immediately after dissection. All samples equilibrated in buffer solution for at least 2 h before pretreatment test. The 4% and 6% strain-level groups were combined.

Figure 7.7: Water content of the time-zero reference samples (native), the incubated samples that were not mechanically tested (unloaded), and the incubated samples that were mechanically tested (loaded). Data from 4% and 6% strain-levels were combined.
Experimental Repeatability and Efficacy

Repeatability of the pretreatment mechanical tests was confirmed by optically measuring posttreatment clamp positions to be within $0.03 \pm 0.01$ mm of the pretreatment clamp positions. To verify that the mechanical testing protocol did not elastically damage the specimens, a comparison was made between pretreatment and posttreatment peak stress during the last preconditioning cycle (Table 7.1). For all treatment, buffer solution and strain level combinations, incubation affected the average peak stress by less than 5%. All changes to the peak stress after incubation were not significant, with the exception of the group treated with ChABC in PEG buffer solution at a 6% strain-level, which had a significant increase of only $2.7 \pm 0.8\%$ (Table 7.1)(p=0.03). A qualitative comparison validated that the 4% and 6% clamp-to-clamp strains were indeed in the toe and linear stress-strain regions, respectively (data not shown). This comparison was made by superimposing the 4% and 6% clamp-to-clamp strains on the final cycle of their respective 8% clamp-to-clamp preconditioning loads.

The efficiency of ChABC digestion of sGAGs was dependent on the buffer solution. ChABC treatment of the time-zero reference samples removed $90 \pm 6\%$ (SD) of sulfated GAGs. The time-zero reference samples had $4.2 \pm 0.4$ and $4.4 \pm 0.5$ μg sulfated gag / mg dry tissue in standard and PEG buffer groups, respectively. Samples that were mechanically tested in the standard buffer and treated with ChABC had an average of $0.7$ μg sulfated gag / mg dry tissue at the end of testing, a 91.8% reduction from the native reference. Samples that were mechanically tested in the PEG buffer and treated with ChABC had an average of $1.6$ μg sulfated gag / mg dry tissue at the end of testing, a 68.4% reduction from the native reference. The sulfated GAG reduction in mechanically
tested samples treated with ChABC was significantly less in the PEG buffer compared to the standard buffer (p=0.03). The native samples had on average 20% more sulfated GAGs than the unloaded samples that endured the lengthy testing protocol (p=0.23).

Discussion

The aim of this research was to determine the specific influence of interfibrillar sGAGs on ligament viscoelasticity. Viscoelastic properties were measured before and after incubation in either control or ChABC treatments. Control and ChABC treatments had no effect on stress relaxation and strain-rate dependent behavior, and moderately increased dynamic modulus and phase shift. Testing in either control or ChABC treatment did not influence any of the observed viscoelastic changes after incubation. This was consistent at different levels of collagen fiber stretch and tissue hydration. Therefore, this study concludes that mechanical interactions between collagen fibrils and interfibrillar sGAGs do not affect tissue-level viscoelastic mechanics in human MCL.

The mechanical property most impacted by incubation was phase shift. Phase shift, which quantifies energy dissipation, increased by 8.4% in control treated samples and 8.5% in ChABC samples. Although this increase was only significant in half the tested groups (Table 7.1), it was observed in 35 of the 40 tested samples. A similar time-dependent effect occurs in articular cartilage. Cartilage tested after control-treated incubation exhibited greater creep [22] and phase shift [44] than cartilage tested immediately upon dissection. This effect may tie into the observed relationship between strain-level and changes in viscoelastic properties after incubation. Samples tested at a 4% strain level were more apt to dissipate energy after incubation than samples tested at the 6% strain level. This would indicate a time-dependent degradation of structures or
networks that provide elastic function at low-strain rates, such as elastin [45]. Interestingly, in our final preliminary studies with the same test procedure (Fig. 7.2), the phase shift increased by only $1.4 \pm 3.3\%$ after incubation ($n=4$). The only deviation in the preliminary protocol was that tests were conducted on specimens that had been thawed and refrozen multiple times. Therefore, this ex vivo loss in energy storage appears to equilibrate in time.

Although the specific contribution of sGAGs on viscoelastic properties has not been tested previously in ligament or tendon, it has been examined in other connective tissues. Enzymatic degradation with ChABC has affirmed that sGAGS moderately control the kinetics of the viscoelastic response in cartilage through sGAG-collagen interactions [22, 44, 46, 47]. Based on the negligible effect of ChABC treatment on ligament viscoelasticity, it can be concluded that sGAGs do not physically provide the functional integrity in ligament that they do in cartilage. This may be due to the low concentrations of sGAGs in ligament (<1% dry weight) relative to cartilage (15-30% dry weight), and the differences in anisotropy and collagen organization. It is important to note that although sGAGs did affect viscoelasticity in cartilage under tension and compression, collagen is a more significant determinant of cartilage viscoelastic properties than sGAG concentration [46]. Al Jamal et al. [27] used a repeated measures design with ChABC on parenchymal lung tissue to show that dermatan and chondroitin sulfate removal resulted in increased hysteresis. Compositional and structural differences between ligament and lung tissue may again account for the conflicting results. Additionally, the parenchymal lung study was unable to reproduce material properties with the control group, which complicates their data interpretations.
The primary function of ligament is to resist tensile loads. It is becoming clear that interfibrillar sGAGs do not directly support this function, although they likely have an indirect effect by modulating collagen fibrillogenesis [48, 49]. This inference is supported by our previous study, which showed that sGAGs have no effect on ligament quasi-static material behavior [14] and in other studies that examined decorin-deficiency. Mice knockout studies identified decorin as a proteoglycan that influences elastic and viscoelastic tensile behavior in tendon [7, 28]. Based on the results of the present study, it appears that these mechanical alterations were due to compensatory or developmental abnormalities intrinsic to decorin deficient mice. For instance, histological studies have shown decorin-deficiency to cause irregularly sized and spaced fibrils, which is likely caused by the absence of interfibrillar GAGs that normally inhibit lateral fibril fusion [50]. Although tendon and ligament have unique histological and biochemical characteristics, they also exhibit gross compositional similarities and ligaments have higher GAG concentrations than tendons [8]. Therefore, it is appropriate to extend the results of the present study to tendon. Finally, the results of the present study are only applicable to tensile loads, and it is still possible that sGAGs physically resist compression in mature human ligament. Sulfated GAG-fibril networks have been shown to resist compressive stresses by retarding fluid exudation [51], and the sGAG concentrations in ligament are highest near the insertion sites, which experience the highest compressive stresses [14, 30, 52].

The experimental protocol provided reproducible results and material properties consistent with the literature. The protocol incorporated a repeated measures design to reduce the inter- and intraspecimen variability that has been observed mechanically and
chemically in ligament [21]. To test the reproducibility of the protocol, a quantitative comparison of peak stress values was made to confirm that pretreatment mechanical tests did not damage the sample and cause a reduction in posttreatment mechanical behaviors. Pretreatment and posttreatment peak stress values were indeed verified to be within 5% for all test groups. Several protocol revisions were required to achieve this consistency.

The viscoelastic results are consistent with previous research that defined the viscoelastic properties of human MCL [1]. Variations in phase shift between these studies may be due to different testing environments (fluid chamber versus humidity chamber [1]). Since specimens were extracted from four distinct regions in the MCL, the material properties and conclusions of this study are representative of the longitudinal (proximal and distal) and oblique fiber zones of the superficial MCL.

Incubation in ChABC successfully removed the interfibrillar sGAGs, chondroitin and dermatan sulfate. In standard buffer, ChABC treated samples had a 92% reduction of dermatan and chondroitin sulfates, while in PEG buffer, ChABC treated samples had a 69% reduction in chondroitin and dermatan sulfates. These degradation values are higher or similar to those reported in other studies that used ChABC in connective tissue [27, 53, 54]. Our previous studies in human ligament used TEM imaging with cupromeronic blue staining to determine that 80% removal of dermatan and chondroitin sulfate resulted in a 98% reduction in interfibrillar sGAGs [14]. Therefore, we are confident that the majority of interfibrillar sGAGs were degraded. The PEG buffer significantly lessened the effectiveness of ChABC, which is likely due to the increased viscosity of the buffer solution and the inverse relationship between viscosity and diffusion (Stokes-Einstein relation). ChABC will also degrade hyaluronic acid, a macromolecule that forms non-
covalent bonds with aggrecan and is chemically similar to chondroitin sulfate. Based on the effectiveness of ChABC in degrading hyaluronic acid [14], this acid does not appear to influence viscoelasticity in human MCL. Biochemical assays showed that chondroitin and dermatan sulfate represent 90% of total sGAGs in the MCL samples. The remaining 10% of sGAGs likely includes keratin sulfate and heparin sulfate. These sGAGs may contribute to ligament viscoelasticity, but this is unlikely based on their low concentrations and distribution near fibroblasts [55].

Tissue hydration was controlled by immersing samples in two types of treatment buffer solutions throughout the entire testing protocol. One of the buffers included 7.5% PEG to create a hypertonic solution that reduced tissue swelling. The philosophy behind testing at varying states of hydration was to ensure that our results were not dependent on a particular tissue hydration state or masked by tissue swelling. Previous research indicated that concentrations of 0.9% saline and 7.5% PEG maintained initial wet weight in ligament, with a 5% decrease in water content [41]. This study found that tissue dehydration was more pronounced with these same concentrations, likely from the addition of 5mM calcium chloride or surrounding temperature variations.

The minor effect of hydration on mechanical properties has implications for other theoretical explanations on the origins of viscoelasticity in ligaments and tendons. Fluid flow can contribute to viscoelasticity through solid-fluid interactions that results in frictional drag [56, 57]. These biphasic interactions are dependent on permeability, which is a function of sGAG concentration and water content [58]. Since sGAG extraction and water content fluctuations had no effect on viscoelastic characteristics, fluid flow appears to not contribute to viscoelastic effects in organized structures such as
ligament that have low concentrations of sGAGs. Other studies have reached similar conclusions [27, 59]. The present study, however, is limited in supporting this statement, for two reasons. First, sulfate groups may have remained in the tissue after being cleaved and continued to influence fluid permeability. Secondly, fluid flow mechanisms in ligament may be insensitive to 10% fluctuations in gross water content. Future studies that specifically examine fluid flow mechanisms in ligament through experimental or theoretical approaches could better address this postulation.

A principal limitation of this research is that biochemical composition within the ligaments may have been altered postmortem. This concern is justified by research that found freezing rabbit ligament caused significant changes in hysteresis during tensile tests [3, 60], yet, the measured change was small and its relevance has been questioned [3]. It is unlikely that the in vivo conditions of the MCL were perfectly replicated in this experiment, although every effort was made to provide a physiological testing environment (e.g., temperature, hydration, protease inhibitors). Lastly, the prolonged effect of sGAG deprivation was not investigated in this study.

In conclusion, this study demonstrated that mechanical interactions between sGAGs and collagen fibrils insignificantly contribute to tissue-level viscoelastic behavior in mature ligament. Although the origins of viscoelasticity in human ligament remains unresolved, our findings have helped narrow the possible contributions of extracellular matrix proteins. Future studies need to assess the inherent viscoelasticity of collagen by determining the influence of inter- and intramolecular collagen bonds and fibril-associated collagens (i.e. Type XII) on transient ligament behavior. The findings of this
study have progressed the structure-function knowledge of ligament, and are applicable to research seeking to engineer and evaluate replacement tissues.

References


CHAPTER 8

DISCUSSION

Summary

The objective of this dissertation was to strengthen the scientific knowledge of mechanical relationships that impact ligament function in the human knee. Two mechanical relationships were studied in this dissertation: The first between the medial collateral ligament (MCL) and the anterior cruciate ligament (ACL), the second between sulfated glycosaminoglycans and collagen fibrils. This discussion will focus on conclusions from these studies. The principal conclusions are first summarized:

- The MCL was more susceptible to damage after ACL transection. This finding favors reconstruction of the ACL to minimize damage to interrelated ligaments.
- The ACL does not directly resist valgus torque. However, the ACL indirectly resists valgus torques by limiting coupled anterior tibial translation during valgus rotation.
- The ACL promotes internal tibial rotation during anterior translation. This should be considered for reconstructive surgery techniques that aim to restore ACL function.
- Sulfated glycosaminoglycans had negligible influence on tissue level mechanics of ligament during tensile loading, and therefore do not directly support ligaments’ primary function of resisting tensile loads.
- Ligament viscoelasticity was insensitive to tissue hydration, which questions the theory that interstitial fluid flow controls the tensile time-dependent mechanics.
The anterior cruciate ligament and medial collateral ligament have overlapping functionality that results in a high incidence of multiligament injuries [1]. An experimental and theoretical approach was used to study the interdependence between these two primary knee stabilizers. Cadaveric joint kinematics and local strain distributions upon the MCL were measured with the ACL intact and deficient for specific loading configurations. Finite element models of the experimental tests were generated from subject-specific geometries and kinematic measurements. Ligament strain and joint kinematics were measured with a newly validated method of optical tracking. For a field of view typical of cadaveric knee experiments (~190 mm), this optical system had an accuracy of 0.04% for measuring strain and an accuracy better than 0.07% for measuring three-dimensional joint kinematics. This optical technique demonstrated superior accuracy and flexibility to previous methods [2, 3], and was likely responsible for this study's improved correlation of experimental-theoretical strain measurements ($r^2 = 0.95$) compared to previous subject-specific studies of human knee ligaments ($r^2=0.75$)[4]. This optical measurement system has now been implemented in other national labs for biomechanical experiments [5-7].

This research clarified the specific and interdependent function of the MCL. As hypothesized, the MCL actively resists anterior tibial translation (ATT) and this resistance increases in the ACL-deficient knee. ACL transection significantly increased peak strain in the MCL's midlongitudinal fibers from 2.9% to 5.7%. The 5.7% strain is above the 5.2% threshold where microtrauma initiates [8]. This would indicate that the MCL is sensitive to overuse in an ACL deficient knee. The FE model predicted peak insertion site forces in the tibia and femur to increase after ACL transection by 54% and
56%, respectively. The associated force values at the insertions (max=140 N) are difficult to measure experimentally and provide parameters to evaluate fatigue propensity in MCL reattachment surgeries and engineered replacement tissues. The FE model also helped resolve a query on the source of strain heterogeneity in the MCL [9]. High contact gradients coincided with strain heterogeneity, while regions with consistent bone contact had uniform strain fields. For example, the MCL substance between the medial condyle of the tibia (high contact) and distal to the femoral insertion (low contact) experienced elevated strain heterogeneity. Regions of uniform contact proximal to the tibial insertion had homogenous localized strain.

The observations of this research also explicitly defined ACL function. In an intact knee, the ACL was found to promote internal tibial rotation during anterior translation. This “unwinding” [10] in turn reduced MCL strains along the longitudinal parallel fibers of the superficial MCL near the femoral insertion, which is the region that experiences the highest strains and is most prone to rupture [11]. Additionally, it is well known that the ACL is the primary restraint to ATT, but controversy exists as to whether the ACL is an active secondary restraint to valgus rotation when the MCL is intact [12-15]. This study found that ACL-deficiency was nondetrimental to the MCL’s ability to restrain pure valgus rotation. The discrepancy between our results and previous reports, in that the ACL actively resists valgus rotation [14, 15], can be logically explained. The ACL and posterior cruciate ligament (PCL) counteract each other during valgus rotation through respective application of posterior and anterior force on the tibia [16]. After ACL transection, this force balance is disrupted and the tibia will slide anteriorly during valgus rotation [15]. Since the tibia was constrained from anterior-posterior translation,
this kinematic alteration manifested as a $121 \pm 49\%$ increase in posterior force during peak valgus load at full knee extension. Coupled ATT thus likely explains the previously measured ACL force response during valgus torque [15, 17]. Removing the anterior-posterior constraint may have resulted in increased valgus rotation and MCL strains in our study due to coupled ATT. However, based on the negligible increases in MCL strains after ACL transection, it appears that the ACL does not directly resist valgus torque, but serves its primary function of resisting ATT during valgus rotation. These functional roles of the ACL should be considered when evaluating surgical reconstructions, and may be helpful in diagnosing whether an ACL deficient patient has a combined MCL injury.

The ability of the MCL and ACL to perform their functions of joint stabilization is directly related to the molecular relationships that provide structural integrity. A molecular relationship that has been widely implicated in contributing to tissue level mechanics is between proteoglycans and collagen fibrils. This relationship has been theoretically and experimentally shown to affect elastic and viscoelastic mechanical behavior in ligament [18-22]. However, the sulfated glycosaminoglycans (GAG) that would mediate this mechanical interaction have not been specifically studied. Therefore, two experimental protocols were performed to test whether elastic and viscoelastic material properties of ligament where affected by GAG degradation via enzymatic treatments. A repeated measures design was utilized to afford a powerful means of measuring treatment effect. Successful execution of these experiments required the design and fabrication of a new material test system that utilized detailed protocols to control experimental repeatability.
The principal conclusion of these extensive mechanical tests was that sulfated glycosaminoglycans have negligible influence on tissue level mechanics during tensile loading. The degradation of interfibrillar GAGs had less than a 5% effect on almost all mechanical properties. In comparison, removal of intermolecular collagen crosslinks in tendon fascicles reduced peak stress by 90% [23], and removal of elastin in tendon and aponeuroses significantly increased hysteresis [24]. Relative to these molecular structures, it becomes quite clear that interfibrillar GAGs do not directly support ligaments primary function of resisting tensile loads. Nonetheless, GAGs likely have an indirect affect on tissue-level mechanics [19, 21] by modulating collagen fibrillogenesis through inhibition of lateral fibril fusion [25].

The findings of this research are relevant to past studies. First, the results contradict the popular theory that interfibrillar GAG interactions are responsible for transmitting forces along discontinuous collagen fibrils [22, 26, 27], and support studies that have predicted fibrils to be continuous [28, 29]. Additionally, the observed insensitivity of viscoelastic material behavior to tissue hydration is inconsistent with the theory that interstitial fluid flow [30] controls the time-dependent mechanical response of ligament in tension. The removal of GAGs should have theoretically increased permeability and therefore affected pressure gradients and time-dependent drag interactions between the fluid and solid phases. This inference can be confirmed through future experiments that measure the affect of GAG extraction on permeability [31]. Lastly, the role of GAGs in ligament and cartilage is drastically different. In cartilage, GAGs retard fluid flow through the porous solid phase to partially control elastic and viscoelastic material properties in both tension and compression [32-34].
The different mechanical roles of GAGs in ligament and cartilage may be due to the low concentrations of GAGs in ligament (<1% dry weight) relative to cartilage (15-30% dry weight), and the differences in anisotropy and collagen organization (Type I vs. Type II). This body of work has expanded the structure-function knowledge of ligament and the findings are applicable to research seeking to engineer and evaluate replacement tissues.

**Limitations and Future Work**

An important limitation to the numerical analysis covered in Chapter 5 is the inability of the material model to represent time-dependent effects. To negotiate this limitation, it was necessary to apply quasi-static loads to the associated experimental study, thus limiting the viscoelastic effects and permitting the finite element model to make accurate predictions. These slow strain-rates are justified in the context of simulating diagnostic exams. However, the applied strain-rates (1%/s) are not appropriate for modeling ligament injury mechanisms, which occur at strain-rates in excess of 28%/s [35]. To broaden the application of these subject-specific finite element models, it is necessary to implement and validate a material that represents the intrinsic viscoelastic behavior of ligament. The functionality of such a viscoelastic material model would be enhanced if it was physical, as opposed to phenomenological. A physical model would not only permit further investigation into the structural mechanisms contributing to mechanical integrity, but it would permit the study of compositional inhomogeneities and pathologies (e.g., Ehlers-Danlos syndrome) that alter the physical structure of ligament [36]. The concurrent validation of functional
physical models with experiments examining structure-function relationships is a natural
expansion of the work covered in this dissertation.

Future work should immediately test the ability of a poroelastic material to
represent the time-dependent effects experimentally observed in Chapter 7. Poroelastic
constitutive equations are derived from first principles and physically represent water
transport through a porous solid medium [37]. Analytically, pressures at continuum
points are solved in addition to displacements. The pressure is a function of fluid flux
and tissue permeability. Through conservation of mass, fluid flux relates to the velocity
of the solid-fluid mixture. These relations provide a time-dependent material response.
Experimental studies support the supposition that water freely moves through the solid
phase of ligament. Hannafin and Arnoczky [38] determined that 6% of water in a canine
flexor tendon, which is compositionally similar to ligament [39], is exuded shortly after
application of small static loads. Additionally, MRI studies measured an increase in free
water molecules after tendon was exposed to axial stress, which suggests that previously
bound molecules were released [40]. In these studies, fluid transport was indicated by
higher concentrations of water molecules along the tissue periphery. The ability of
poroelastic theory to describe experimental behavior will allow conclusions to be drawn
about the solid-fluid mechanical relationship in ligament.

Preliminary studies in our lab have demonstrated that the poroelastic model is
able to accurately predict stress relaxation behavior using experimental permeability
coefficients [31] and validated bulk modulus values [4]. The poroelastic model,
however, was found to incorrectly predict how variations to strain-level and frequency
influence aspects of the mechanical response. For example, stress relaxation increased
with strain-level, which conflicts with experimental results (Figure 7.3). Furthermore, the poroelastic FE model incorrectly predicted a decrease in phase shift as oscillation frequency increased, while experimentally the phase shift significantly increased progressively at greater frequencies (Figure 7.5). This poroelastic behavior is in agreement with a study by Ateshian et al. [41]. Ateshian et al. demonstrated that with instantaneous loads the interstitial fluid pressure within a poroelastic model is nearly identical to the hydrostatic pressure of an incompressible elastic material at every point except near the fluid boundary. Hence, the poroelastic material will behave elastically. It is important to note that the loading rate at which a poroelastic material begins to behave elastically is dependent on permeability and bulk modulus. Alterations to these parameters may result in model predictions that better represent experimental behavior. This raises the question of whether the parameters used in this preliminary study accurately represent ligament.

Experimental research supports a low bulk modulus by demonstrating that the Poisson’s ratio (longitudinal vs. transverse) in ligament is greater than 1.5 [42]. This high poisons ratio is indicative of significant volumetric deformation during tension, which would require a value for bulk modulus lower than values commonly used in the literature [4]. Furthermore, a Poissons ratio above 1.5 would indicate that fluid is flowing out of the tissue during tension. Therefore, the model used in this preliminary study, along with numerous studies in the literature [4, 43, 44], have been inaccurately modeling the volumetric deformation of ligament during tensile loading. Although the incorrect orientation of fluid flow will still model damping effects, it is not
physiological. In the future, hyperelastic constitutive models should be emulated that utilize strain energy functions permitting Poisson’s ratios greater than 0.5 [45].

Other inconsistencies exist between physical ligament mechanics and the implemented poroelastic material. The permeability used in the current model is isotropic and strain-independent. Experimentation has in fact shown that permeability is anisotropic [30] and strain-dependent [31]. The isotropic permeability can be replaced with an anisotropic tensor [50] that physiologically reduces permeability along the collagen fiber direction [51]. This anisotropy should allow more realistic boundary conditions to be applied at off-axis surfaces and will direct lateral fluid flow. The permeability tensor can also be readily updated to be strain-dependent [52]. Due to the high Poisson’s ratio for ligament, the permeability should decrease with tensile stress, which could describe the experimental reduction in stress relaxation at higher strain levels. Furthermore, based on the insensitivity of ligament viscoelastic behavior to extraction of sulfated glycosaminoglycan macromolecules (Chapter 7), it would appear that collagen networks mediate fluid flow and not constituents in the extracellular matrix. Overall, poroelastic theory may be an effective tool to describe ligament material behavior and future investigations offer significant challenges and rewards.

Another principal limitation to this body of work is the in vitro model used for study. The structural cadaveric test employed for the MCL-ACL experiments have inherent error associated with muscle excision, tissue swelling, chemical decay, and motion constraints (i.e., limited degrees of freedom). For example, the removal of joint stabilizing muscles would result in an over-estimation of the translations and rotations associated with particular load limits. However, regardless of this over-estimation,
ligament strains are still valid for the measured kinematics. All limitations inherent in
cadaveric models could be eliminated by using image registration to noninvasively
measure strain distribution in vivo [49, 50]. For example, ligament strains and joint
kinematics in different population groups (e.g., normal, ACL-deficient) could be
measured with open MRI technology, which facilitates anatomical positions and joint
loads. The highly accurate data provided in this research would serve as an excellent
source for future studies that use such noninvasive methods.

In all experiments, ligament biochemical composition may have been altered
postmortem. Although efforts were made to provide physiological testing environments,
it is unlikely that the in vivo conditions of the MCL were perfectly replicated. To
remove this limitation, future studies of GAG interactions could be performed on
appropriate animal models [51, 52]. The observation made in Chapter 7 that viscoelastic
energy dissipation is dependent on exposure time could be controlled with this type of
study. An animal model could also be used to examine the effect of GAG degradation
over long time periods. It is feasible that interfibrillar space reductions from GAG
extraction [25] could eventually cause fibril damage due to interfibrillar friction. Lastly,
gene alterations could be used to propagate interfibrillar GAGs to determine if a certain
concentration of GAGs influenced tissue mechanics.

Supplementary experiments were performed to test certain aspects of
biochemical alterations. One valid apprehension of the GAG studies was that
mechanical interaction of the GAGs is dependent on intermediary ions (e.g., calcium,
sodium) that neutralize the repulsion of negatively charged GAGs. To ensure that
calcium exudation was not masking the mechanical contributions from GAGs,
supplementary experiments were performed. In these pilot studies, ligament tissue was rinsed during dissection and mechanical testing with a solution that included high concentrations of calcium (0.9% NaCl + 2mM Ca). Upon completion of testing, these samples were soaked in normal saline (1 hr, 0.9% NaCl), retested, soaked again in the calcium solution (1 hr) and retested. This sequential treatment minimally affected peak stress in tensile (~2%) and shear (~4%) loading configurations. Even though these experiments did not implicate calcium exudation as a cause of mechanical defect, physiological calcium concentrations were always included in the testing buffer (5 mM CaCl₂). Supplementary studies were also conducted with elastase to test the sensitivity of the testing system. Elastin was expected to contribute to the tensile response at low strains [53]. Compared to the control group elastase treatment doubled the phase shift and decreased the peak stress at a 4% clamp-to-clamp strain level by 35%. It is logical that elastin provides mechanical support when fibers are crimped and loss of elastin would reduce energy storage mechanisms. Considering the small sample size (n=2) and lack of chemical verification of elastase efficacy and specificity, these results should be interpreted with caution. Nonetheless, this pilot study confirmed that the testing apparatus was sensitive to molecular alterations and it is recommended that elastin be further investigated to quantify its contribution to the mechanical function of ligament.

Although the origins of elasticity and viscoelasticity in human ligament are incomplete, future work can begin where this dissertation ends. First, to completely characterize the mechanical effect of GAGs in ligament, compression and permeability tests need to be performed. Theoretically, GAGs should contribute to compressive stiffness [54] near insertions and articulations where compressive forces are innate. This
theory is supported by regionally high GAG concentrations in these zones [55]. Experiments can be synchronized with an investigation of GAG influence on permeability transverse to the fiber direction. By identifying mechanisms responsible for resisting fluid exudation, these findings would be applicable to the poroelastic model. Next, mounting evidence from this study and others [28, 40] suggests that the intrinsic viscoelasticity in collagen and the fluid exudation from collagen controls the viscoelastic response when ligament is tensioned. The effect of intercollagen bonds on viscoelasticity can be examined in a rat model with β-APN [23] and the effect of intracollagen bonds on viscoelasticity may be tested with molecular dynamics models [56]. One postulation is that intercollagen bonds primarily control viscoelasticity in ligament through molecular realignments that originate from fluid interaction and tropocollagen sliding [23].

In conclusion, continued investigations in the structure-function relationships of ligament will drive the long-term goal of representing material behavior with physical models and be applicable to treatment strategies. Implementing physical constitutive relationships into finite element models may improve adaptability of the model to anatomically distinct regions [21, 57] and facilitate representation of viscoelastic mechanisms that guard against structural failure. Connective tissue is composed of similar constituents that have diverse concentrations and organizations. Understanding the akin and dissimilar functions of these constituents in specific tissues will aid interpretations of mechanical alterations that occur from injury repair mechanisms and disease states, as well as spur innovations in clinical treatments.
References


