AAPO RISTANIEMI

The structure–function relationships of knee joint ligaments and patellar tendon are incompletely understood. In this thesis, the mechanical and biochemical properties of bovine knee ligaments and patellar tendon were characterized and compared, and structure–function relationships were examined. A novel fibril-reinforced poroviscoelastic model was developed for the anterior cruciate ligament. The results may bring improvement for computational modeling of the knee, provide more understanding of the effect of composition on ligament and tendon function and also highlight the differences among these tissues.
STRUCTURE AND FUNCTION OF KNEE LIGAMENTS AND PATELLAR TENDON

BIOMECHANICS, BIOCHEMISTRY AND COMPUTATIONAL MODELING
Aapo Ristaniemi

STRUCTURE AND FUNCTION OF KNEE LIGAMENTS AND PATELLAR TENDON

BIOMECHANICS, BIOCHEMISTRY AND COMPUTATIONAL MODELING

Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences
No 379

University of Eastern Finland
Kuopio
2020

Academic dissertation
To be presented by permission of the Faculty of Science and Forestry for public examination at the University of Eastern Finland, Kuopio, on July 3rd, 2020, at 12 o’clock noon
Author’s address:  Aapo Ristaniemi  
University of Eastern Finland  
Department of Applied Physics  
Kuopio, Finland  
email: aapo.ristaniemi@uef.fi

Supervisors:  Professor Rami K. Korhonen  
University of Eastern Finland  
Department of Applied Physics  
Kuopio, Finland  
email: rami.korhonen@uef.fi

Mikko Finnilä, Ph.D.  
University of Oulu  
Research Unit of Medical Imaging, Physics and Technology  
Oulu, Finland  
email: mikko.finnila@oulu.fi

Lauri Stenroth, Ph.D.  
University of Eastern Finland  
Department of Applied Physics  
Kuopio, Finland  
email: lauri.stenroth@uef.fi

Reviewers:  Professor Helen L. Birch  
University College London  
Department of Orthopaedics and Musculoskeletal Science  
London, United Kingdom  
email: h.birch@ucl.ac.uk

Associate Professor Trevor J. Lujan  
Boise State University  
Department of Mechanical & Biomedical Engineering  
Boise, Idaho, USA  
email: TrevorLujan@boisestate.edu
Opponent: Professor Hazel R. C. Screen  
Queen Mary University of London  
School of Engineering and Materials Science  
London, United Kingdom  
email: h.r.c.screen@qmul.ac.uk
ABSTRACT

Knee joint ligaments are soft tissues connecting the femur to the tibia, transmitting forces, restricting movements and stabilizing the joint. The patellar tendon (PT) connects the patella to the tibia, transmitting the force generated by the quadriceps muscle to enable extension of the knee. It is not well known how the biomechanical and biochemical properties compare between these tissues and how the biochemical composition is related to the biomechanical properties. Additionally, material models of ligaments distinguishing fluid, matrix and collagen fibers, are inherently limited in their representation of viscoelastic properties. Therefore, the aims of this thesis were to comprehensively characterize and compare the biomechanical and biochemical properties of knee ligaments and PT, to elucidate their structure–function relationships, and to develop a fibril-reinforced poroviscoelastic composite material model for accurate representation of ligament tissue.

Dumbbell-shaped tensile testing samples were cut from the midsubstance of four bovine primary knee ligaments and patellar tendon (n=10 knees). The samples were subjected to a tensile stress-relaxation test, a sinusoidal loading test and an ultimate test until tissue failure. Water, hydroxyproline (collagen), uronic acid (proteoglycan) and elastin contents were biochemically determined. Multiple linear regression was used to investigate the relationship between biochemical composition and biomechanical properties. Data from 10 anterior cruciate ligament (ACL) samples were averaged and used to create a finite element model of the relaxation experiment. Existing and new poroelastic and fibril-reinforced poroviscoelastic models were implemented to replicate the experimental force-time curve.

The lateral collateral ligament (LCL) had a higher phase difference compared with other ligaments and patellar tendon at 0.5 Hz loading frequency (p<0.05). Medial collateral ligament exhibited the highest Young’s modulus, strain-dependent modulus and toughness (p<0.05 compared with ACL, LCL and PT) and also the highest collagen content. Collateral ligaments had lower uronic acid contents
compared with cruciate ligaments \((p<0.05)\), and the elastin content was higher in posterior cruciate ligament as compared with ACL \((p<0.05)\). The uronic acid content significantly predicted Young’s modulus, yield stress and toughness, whereas the elastin content was related to toe region nonlinearity and Young’s modulus. Previous fibril-reinforced poroviscoelastic models were unable to completely reproduce the complex relaxation behavior \((R^2=0.815-0.976)\) while the new model with the two-relaxation-time strain-recruited viscoelastic fibrillar network developed in this thesis captured well the experimental observations \((R^2=0.997)\).

The differences in the biomechanical and biochemical properties highlight the adaptation of tissues to specific physiological functions, anatomical restrictions and loading regimes. Specifically, the LCL was the most viscous at low-frequency loads, while the MCL was the stiffest, whereas cruciate ligaments had the highest proteoglycan content. Proteoglycan and elastin contents being significant predictors of important biomechanical properties may indicate that they have crucial functional roles in ligaments and tendons, possibly influencing load transfer within the tissues. The modeling results indicate that the fast and slow viscoelastic phenomena should be incorporated into the fibrillar network formulation. The novel fibril-reinforced poroviscoelastic material model may be applied for ACL, and probably also for other ligaments, for an accurate representation of these tissues in models of the whole knee joint.

**National Library of Medicine Classification:** QT 34.5, QU 34, WE 300, WE 870

**Medical Subject Headings:** Knee Joint; Ligaments, Articular; Patellar Ligament; Biomechanical Phenomena; Elasticity; Elastic Modulus; Tensile Strength; Biochemical Phenomena; Water; Hydroxyproline; Collagen; Uronic Acids; Proteoglycans; Elastin; Models, Theoretical; Computer Simulation; Finite Element Analysis; Structure-Activity Relationship

**Yleinen suomalainen asiasanasto:** polvet; nivelet; niveletsiteet; biomekaniikka; joustavuus; kimmoisuus; vetolujuus; biokemia; vesipitoisuus; kollageenit; proteoglykaanit; mallintaminen; simulointi; elementtienetelmä
ACKNOWLEDGEMENTS

The research findings described in this thesis were made in the Department of Applied Physics at the University of Eastern Finland during the years 2016–2020. During that time, I have developed both professionally and personally, and would like to thank all the people involved.

First, I would like to thank my primary supervisor, Professor Rami Korhonen for the opportunity to conduct research in the Biophysics of Bone and Cartilage research group. I am thankful for the active guidance that pushed me to think far beyond the obvious. I warmly thank you for making it possible for me to participate in scientific conferences, which have been truly unforgettable journeys. I would like to thank my other supervisors, Mikko Finnilä and Lauri Stenroth, for bringing their perspectives and know-how to my project. Coming from different academic backgrounds, your comments have helped me to appreciate ligament biomechanics also from viewpoints other than mechanical engineering. I would like to give special thanks to Petri Tanska for his tutelage in computational modeling. I would like to thank my co-authors, Santtu Mikkonen, Tommi Paakkonen and Juha Töyräs, for improving the quality of the manuscripts.

I express my gratitude to Ewen MacDonald for the English language review. I sincerely thank the reviewers of this thesis, Helen L. Birch and Trevor J. Lujan, for your time and effort in providing the critical reviews and the feedback of my work. Special thanks go to my opponent, Hazel R. C. Screen, for accepting this task.

I am grateful to the funding sources that enabled me to work on this thesis, namely Instrumentarium Science Foundation, K. Albin Johanssons stiftelse, Kuopio University Foundation, Finnish Foundation for Technology Promotion, Academy of Finland and Sigrid Jusélius Foundation.

I warmly thank my roommates, Simo Ojanen, Mimmi Liukkonen, Nina Hänninen, Mohammadhossein Ebrahimi, Swetha Pala, Mikko Nissinen and Heta Orava. We have had so many scientific and non-scientific discussions. I also thank all the members of the BBC-group, it has been a wonderful working environment, as well as a social activity network. Special thanks go to Jari Torniainen and Mithilesh Prakash.

Finally, I would like to thank my family for the support during these years.

Kuopio, 28th May 2020
Aapo Ristaniemi
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL</td>
<td>Anterior Cruciate Ligament</td>
</tr>
<tr>
<td>C3D8P</td>
<td>8-node trilinear displacement and pore pressure element in Abaqus software</td>
</tr>
<tr>
<td>CaCL</td>
<td>Caudal Cruciate Ligament</td>
</tr>
<tr>
<td>CrCL</td>
<td>Cranial Cruciate Ligament</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
</tr>
<tr>
<td>LCL</td>
<td>Lateral Collateral Ligament</td>
</tr>
<tr>
<td>MCL</td>
<td>Medial Collateral Ligament</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline solution</td>
</tr>
<tr>
<td>PCL</td>
<td>Posterior Cruciate Ligament</td>
</tr>
<tr>
<td>PG</td>
<td>Proteoglycan</td>
</tr>
<tr>
<td>PT</td>
<td>Patellar Tendon</td>
</tr>
<tr>
<td>sGAG</td>
<td>Sulphated glycosaminoglycan</td>
</tr>
</tbody>
</table>
LIST OF SYMBOLS

$A$  Strain dependency coefficient (1/2 strain dependent modulus);\n    Oscillation amplitude
$A_0$  Initial cross-sectional area
$A_{1,2}$  Constant of the exponential spring
$A_{\varepsilon}$  Strain amplitude
$A_\sigma$  Stress amplitude
$B$  Initial modulus
$B_{1,2}$  Constant of the exponential spring
$C$  Coefficient
$D$  Coefficient
$e$  Void ratio; Base of the natural logarithm
$e_0$  Initial void ratio
$E$  Young’s modulus
$E_{0-6}$  Fibrillar network modulus
$E_{dyn}$  Dynamic modulus
$E_{nf}$  Young’s modulus of the non-fibrillar matrix
$f$  Frequency; Objective function
$F$  Measured force
$F$  Deformation gradient tensor
$G$  Shear modulus
$I$  Identity tensor
$J$  Determinant of the deformation gradient tensor
$k$  Hydraulic permeability
$k_0$  Initial permeability
$K$  Bulk modulus
$K_{ult}$  Toughness (energy density) at failure
$K_{yield}$  Toughness (energy density) at yield
$L$  Current length of the sample
$L_0$  Initial length of the sample
$M$  Permeability deformation dependency coefficient
$n_f$  Fluid volume fraction
$n_s$  Solid volume fraction
$p$  Fluid pressure
$q$  Fluid flow rate
$t$  Time
$z$  Value of stress of strain
$z_0$  Constant term
$\gamma$  Phase difference
$\Delta t$  Time increment
$\varepsilon$  Strain
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_f$</td>
<td>Fibril strain</td>
</tr>
<tr>
<td>$\varepsilon_{\text{linear}}$</td>
<td>Linear region length</td>
</tr>
<tr>
<td>$\varepsilon_{\text{toe}}$</td>
<td>Toe region strain</td>
</tr>
<tr>
<td>$\varepsilon_{\text{ult}}$</td>
<td>Ultimate strain</td>
</tr>
<tr>
<td>$\varepsilon_{\text{yield}}$</td>
<td>Yield strain</td>
</tr>
<tr>
<td>$\eta_{0-2}$</td>
<td>Damping coefficient of the fibrillar network</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Poisson’s ratio</td>
</tr>
<tr>
<td>$\nu_{nf}$</td>
<td>Poisson’s ratio of the non-fibrillar matrix</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Stress</td>
</tr>
<tr>
<td>$\sigma_{1,\text{old}}$</td>
<td>Maxwell element 1 stress from the previous time increment</td>
</tr>
<tr>
<td>$\sigma_{2,\text{old}}$</td>
<td>Maxwell element 2 stress from the previous time increment</td>
</tr>
<tr>
<td>$\sigma_f$</td>
<td>Fibril stress</td>
</tr>
<tr>
<td>$\sigma_{\text{toe}}$</td>
<td>Toe region stress</td>
</tr>
<tr>
<td>$\sigma_{\text{ult}}$</td>
<td>Ultimate strength</td>
</tr>
<tr>
<td>$\sigma_{\text{yield}}$</td>
<td>Yield stress</td>
</tr>
<tr>
<td>$\sigma_{\text{eff}}$</td>
<td>Effective solid stress tensor</td>
</tr>
<tr>
<td>$\sigma_f$</td>
<td>Fibrillar network stress tensor</td>
</tr>
<tr>
<td>$\sigma_{fl}$</td>
<td>Fluid stress tensor</td>
</tr>
<tr>
<td>$\sigma_{\text{neo-Hooke}}$</td>
<td>Neo-Hookean material stress tensor</td>
</tr>
<tr>
<td>$\sigma_{nf}$</td>
<td>Non-fibrillar matrix stress tensor</td>
</tr>
<tr>
<td>$\sigma_s$</td>
<td>Solid stress tensor</td>
</tr>
<tr>
<td>$\sigma_{\text{tot}}$</td>
<td>Total stress tensor</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>Phase angle</td>
</tr>
<tr>
<td>$\varphi_{\varepsilon}$</td>
<td>Phase angle of strain</td>
</tr>
<tr>
<td>$\varphi_{\sigma}$</td>
<td>Phase angle of stress</td>
</tr>
<tr>
<td>$\nabla$</td>
<td>Gradient</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I-III.


AUTHOR’S CONTRIBUTION

I) The author was the main contributor: performed the experiments, analyzed the data and was the main writer. A co-author was responsible for the statistical model.

II) The author was the main contributor: analyzed the data and was the main writer. Co-authors were responsible for measuring biochemical contents and multiple linear regression analysis.

III) The author was the main contributor: derived and implemented the new fibrillar network formulations, performed material parameter optimizations and was the main writer.
CONTENTS

1 Introduction .................................................................................................................. 19
2 Knee ligaments and patellar tendon .............................................................................. 21
  2.1 Anatomy and functional role .................................................................................. 21
  2.2 Biochemical composition ...................................................................................... 24
  2.3 Structural organization ......................................................................................... 26
3 Biomechanics and modeling ......................................................................................... 27
  3.1 Quasi-static properties ......................................................................................... 27
  3.2 Viscoelastic properties .......................................................................................... 27
  3.3 Fibril-reinforced poroviscoelastic modeling .......................................................... 29
    3.3.1 Poroelastic theory .......................................................................................... 29
    3.3.2 Neo-Hookean poroelastic material model ....................................................... 30
    3.3.3 Fibril-reinforced poroviscoelastic material model ......................................... 30
    3.3.4 Fibrillar network formulations ..................................................................... 31
4 Clinical relevance ......................................................................................................... 35
5 Aims ............................................................................................................................... 36
6 Materials and methods ................................................................................................. 37
  6.1 Sample preparation ............................................................................................... 37
  6.2 Tensile testing ....................................................................................................... 39
  6.3 Biochemical analyses ............................................................................................ 39
  6.4 Data analysis .......................................................................................................... 40
  6.5 Fibril-reinforced poroviscoelastic modeling .......................................................... 41
  6.6 Statistical methods ................................................................................................ 44
7 Results ........................................................................................................................... 45
  7.1 Mechanical properties .......................................................................................... 45
  7.2 Biochemical analyses ........................................................................................... 49
  7.3 Structure–function relationships ............................................................................ 50
  7.4 Poroelastic and fibril-reinforced poroviscoelastic material models ...................... 54
8 Discussion ....................................................................................................................... 57
  8.1 Mechanical properties .......................................................................................... 57
  8.2 Biochemical contents ........................................................................................... 59
  8.3 Structure–function relationships .......................................................................... 61
  8.4 Poroelastic and fibril-reinforced poroviscoelastic material models ...................... 62
  8.5 Limitations ............................................................................................................ 64
  8.6 Future work ............................................................................................................ 66
9 Summary and conclusions ............................................................................................ 67
10 Bibliography ................................................................................................................ 68
Appendices ....................................................................................................................... 83
  Appendix A: Equations of fibril stress for fibrillar network formulations ................. 83
  Appendix B: Effect of initial permeability, permeability deformation dependency
    coefficient, water content and Poisson’s ratio of the non-fibrillar matrix on the
    stress-relaxation response ......................................................................................... 87
1 INTRODUCTION

Ligaments and tendons are soft connective tissues in the musculoskeletal system. Anatomically, ligaments connect bone to bone, whereas tendons connect muscle to bone. They are fundamental connective links that enable locomotion and function of the musculoskeletal system. In the knee joint, the four primary ligaments transmit forces, guide and restrict joint movement, stabilize the joint, and serve as mechanical dampers [1–4]. The patellar tendon connects the patella to the tibia, transmitting force from the quadriceps muscle and thus enabling the extension of the knee.

During normal locomotion, the connective tissues of the knee joint, ligaments, tendons, menisci, cartilages, and bones, work in concert [5,6]. The mechanical properties of ligaments and tendons highly affect the loading of the other tissues and are thus important for the joint’s physiological functioning [2,7]. The mechanical properties of individual ligaments and tendons are well adapted to their particular function and exhibit complex anisotropic nonlinear viscoelastic behavior. A detailed understanding of the mechanical properties is thus needed, both for understanding the normal function of the tissues and in examinations of diseases. However, a limited number of studies have examined the mechanical properties of knee ligaments and patellar tendon from the same set of knees [8–10]. Moreover, a comparison of the viscoelastic properties for these tissues has never been performed under sinusoidal loading, which mimics the dynamic physiological loading during walking.

Mechanical properties originate from biochemical composition and structural organization. Therefore, composition and structure probably differ between the tissues. Nonetheless, it is not well known how biochemical composition compares between knee ligaments and the patellar tendon, as the literature is limited and conflicting [8,11–16]. For example, no previous data were available on the elastin content of lateral collateral ligament.

Relating the biochemical composition with the mechanical properties, i.e., determining structure–function relationships, provides a fundamental understanding of the tissue function and is important when evaluating the effect of degradation or the adaptation of tissue constituents on the mechanical properties, when developing new material models of tissues, and when developing imaging methods for diagnostics [17–22]. Commonly structure–function relationships have been examined by considering the effect of a single biochemical constituent on the mechanical response [23–26]. For example, the collagen content was found to correlate with Young’s modulus and tensile strength of medial collateral ligament [23]. However, according to the author’s knowledge, structure–function relationships have never been investigated in the primary knee ligaments and patellar tendon obtained from the same set of knees, with all the main constituents, water, collagen, elastin, and proteoglycans, assessed at the same time.
Knee joint mechanical function may be effectively evaluated by using computational models of the whole joint. The outcome of those models, however, depends on the material model and material properties that are chosen for ligaments [21,22,27]. It is thus important that the tissue-level behavior of ligaments should be represented as accurately as possible. Compositionally inspired material models offer a useful option for accurate modeling, and they may also be used to investigate the effects of different constituents on the mechanical response. The current material models of ligaments and tendons that distinguish fibers, matrix and fluid are, however, limited in their viscoelastic representation and are unable to capture the complex stress-relaxation behavior of these tissues.

Therefore, this thesis aims to comprehensively characterize and compare the mechanical material properties of knee ligaments and patellar tendon, determine and compare the compositional characteristics, bring novel information on structure–function relationships and develop a new material model that would account for the complex viscoelastic behavior. The results of this thesis may be utilized for improved computational modeling of the knee joint, when estimating the effect of compositional changes on ligament and tendon function and when developing clinical imaging methods for tissue evaluation. Moreover, the results may be useful in tissue engineering, artificial ligament and tendon design, knee joint replacement design, and computational modeling of fiber-reinforced materials. The new information obtained in this thesis, together with other efforts, may eventually lead to better joint disease prevention, diagnostics and treatment, thus improving the quality of life of people with knee problems.
2 KNEE LIGAMENTS AND PATELLAR TENDON

2.1 ANATOMY AND FUNCTIONAL ROLE

The main structure of the knee joint consists of bones and soft connective tissues (Figure 2.1). The movements of femur, tibia, and patella are stringently mediated by the ligaments and tendons, together with medial and lateral menisci and femoral, tibial and patellar articular cartilages. All of these tissues act together during normal locomotion. Articular cartilages enable nearly frictionless translations and rotation of bones relative to each other, while the menisci help to distribute loads.

The knee joint has four primary ligaments, anterior cruciate ligament (ACL), lateral collateral ligament (LCL), medial collateral ligament (MCL), and posterior cruciate ligament (PCL). ACL and PCL are located at the center of the knee inside the synovial pouch and are thus intracapsular ligaments. The MCL and LCL are located on the medial and lateral sides of the knee, outside the synovial pouch, and are therefore extracapsular ligaments. Ligaments are collagen-rich soft connective tissues, connecting bone to bone, and these knee ligaments connect the femur to the tibia. The patellar tendon (PT) connects patella to the tibia, and its main function is to transmit forces from muscle to bone.

![Figure 2.1: Antero-medial view of the knee joint.](image)

The ligaments act primarily in tension, transmitting forces, stabilizing the joint, restricting and guiding joint movement, and serving as mechanical dampers. The primary and secondary functions of knee ligaments and PT are summarized in
Figure 2.2. The primary function of the ACL is to restrict anterior tibial translation [5,28] while secondarily stabilize internal tibial rotation [28]. The main function of the LCL is to restrict varus angulation [29,30] and the secondary functions are to provide restraint to anterior and posterior tibial translations [31] and internal and external tibial rotations [29]. The MCL restricts valgus angulation and external tibial rotation [29,32], while also restraining anterior tibial translation [5]. The PCL primarily limits posterior tibial translation [5,31], and secondarily external tibial rotation [31]. The PT transmits forces from quadriceps muscle to tibia [33], but also partly limits posterior tibial translation [34]. Though these tissues act mainly in tension, they may experience compressive, transverse and shear loads locally [6,35].
<table>
<thead>
<tr>
<th>Ligament</th>
<th>Primary function(s)</th>
<th>Secondary function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Cruciate Ligament</td>
<td>Anterior tibial translation</td>
<td>Internal tibial rotation</td>
</tr>
<tr>
<td>ACL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral Collateral Ligament</td>
<td>Varus angulation</td>
<td>Tibial translations Tibial rotations</td>
</tr>
<tr>
<td>LCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial Collateral Ligament</td>
<td>Valgus angulation External tibial rotation</td>
<td>Anterior tibial translation</td>
</tr>
<tr>
<td>MCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior Cruciate Ligament</td>
<td>Posterior tibial translation</td>
<td>External tibial rotation</td>
</tr>
<tr>
<td>PCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patellar tendon</td>
<td>Transmit forces from quadriceps muscle</td>
<td>Posterior tibial translation</td>
</tr>
<tr>
<td>PT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.2:** Functions of the primary knee joint ligaments and patellar tendon, shown for the right knee.
2.2 BIOCHEMICAL COMPOSITION

Ligaments and tendons are composed of fibroblasts or tenocytes, water, collagen, elastin, proteoglycans, and a small proportion of other proteins, such as fibrillin I and II (Figure 2.3) [3,36,37]. Cells account for a small percentage of the total volume [38] and they synthesize and secrete extracellular matrix components. Water accounts for 50-72 % of the ligament or tendon wet weight [3,12,39–41], and is commonly calculated from the difference between wet and dry weights, following freeze-drying [8,12,39–41]. Collagen, primarily type I, constitutes 56-87 % of dry weight [3,11–13] and is the main load-bearing constituent in ligaments and tendons. Collagen molecules are triple-helical polypeptide chains, consisting mainly of sequences of amino acids glycine, proline and hydroxyproline [42]. The collagen content is usually determined by quantifying the amount of hydroxyproline [8,11–13,15,43–45], and assuming it constitutes a certain percentage of collagen (for example 14 % in tendons [39]). Elastic fiber elastin, present at 2-11 % of dry weight [13,16], has been suggested to restore tissue to its original shape after deformation [37], affects the ligament stress-strain behavior in the toe region [41], mediates transverse tensile and shear responses [46,47], and may influence the Young’s modulus of the ligaments and tendons [48,49]. Elastin is a fiber-like structure consisting of crosslinked tropoelastin molecules, embedded in fibrillin scaffolds [50]. Fibrillin I and II have been suggested to act together with elastin [37,51]. Elastin content may be determined directly without surrogate contents. Proteoglycans (PGs) constitute up to 2.5 % of the dry weight [52]. Proteoglycans attract water [53–55], separate, lubricate and facilitate sliding of collagen fibrils [56,57] and may minorly affect shear properties [58]. Proteoglycans are molecules consisting of a protein core (such as decorin, biglycan, fibromodulin, lumican, aggrecan and versican), to which glycosaminoglycan side chains (such as chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, hyaluronan and heparin) are attached [55]. Uronic acid is present in all glycosaminoglycans except keratan sulfate [55]. The amount of proteoglycans is often evaluated by determining the content of glycosaminoglycans [11–13,16,56] or uronic acid [55,57,59,60].
Figure 2.3: Biochemical composition of ligaments and tendons as a percentage of total weight (wet weight).
2.3 STRUCTURAL ORGANIZATION

Ligaments and tendons are organized in a directional and hierarchical manner (Figure 2.4) [61,62]. Starting from the highest level of the hierarchy, ligaments and tendons divide into distinct fascicles, separated by interfascicular matrix. Depending on their anatomical location, ligaments or tendons may also have distinct bundles or subtendons, which then consist of fascicles [62]. Fascicles are made up of fibers, which in turn are formed from collagen fibrils. Fibrils are formed from microfibrils, consisting of tropocollagen molecules, which are triple-helical polypeptide chains.

The hierarchical structure affects the mechanics of the tissue. The fascicles may slide with respect to one another and divide or merge together [63]. Fibers may slide relative to each other, and this mechanism is suggested to be the primary mechanism of relaxation within tendons [64–66]. Within fibers, fibrils may also slide relative to each other, with this sliding being facilitated by proteoglycans [56]. It is however not fully understood how the load is transferred between the different hierarchical levels [67,68].

Figure 2.4: Hierarchical structural organization of ligaments and tendons.
3 BIOMECHANICS AND MODELING

Knee joint ligaments and patellar tendon primarily function in tension in the direction of the collagen fibers, and thus in this thesis, only tensile properties in that direction are considered. However, also localized compressive, transverse, and shear loads may be present [6,35]. Experimentally tensile properties are commonly obtained via tensile testing, in which either force or displacement is controlled and the other one measured.

3.1 QUASI-STATIC PROPERTIES

Quasi-static tensile material properties may be obtained via tensile testing at velocities so slow that viscous effects can be considered negligible. From the resulting force-displacement relationship, the stress-strain curve of the material may be obtained by calculating the stress and strain by some definitions, such as engineering stress and strain, or true stress and logarithmic strain. The stress-strain curve describes the fundamental mechanical behavior of the material. The stress-strain curve of ligaments and tendons is characterized by a nonlinear toe region, followed by a linear region, ending up in a yield and failure region (Figure 3.1a) [9,10,76,77,31,69–75]. The toe region is thought to originate from a gradual straightening of the collagen crimp pattern, resulting in the recruitment of collagen fibers with increasing strain. Fiber realignment may also influence the toe region [78,79]. In the linear region, collagen fibers are essentially straight, whereas at yield, microstructural damage starts to occur, followed by a catastrophic failure. After failure, the main load-bearing components are ruptured, but often some interfascicular matrix is left to bind the remnants together but not bearing major loads.

Various parameters may be extracted from the stress-strain curve, such as toe region end point ($\varepsilon_{toe}$, $\sigma_{toe}$), Young’s modulus $E$, yield point ($\epsilon_{yield}$, $\sigma_{yield}$), ultimate strength ($\epsilon_{ult}$, $\sigma_{ult}$) and toughness (energy density) at yield or failure (Figure 3.1a).

3.2 VISCOELASTIC PROPERTIES

Viscoelastic, or time-dependent, properties of ligaments and tendons may be demonstrated in various ways: strain rate stiffening, in which a higher strain rate leads to a higher modulus [73,80,81], stress-relaxation in which stress decreases with time at constant strain [26,49,86–91,64–66,77,82–85], creep in which strain increases with time under a constant stress [82,90,92,93], and hysteresis in which energy is dissipated during loading-unloading or during sinusoidal loading [26,41,82,87,92]. In this thesis, the viscoelasticity is examined in terms of sinusoidal loading (Figure
Sinusoidal loading is characterized by phase difference and dynamic modulus (Figure 3.1b). Typical incremental stress-relaxation of ligaments and tendons show fast and slow relaxation, i.e., two-relaxation-time behavior [64,66,85,86,91], and an increase in the relaxation magnitude with increasing strains [64,66,84–86,91] (Figure 3.1c). The author hypothesizes that fast initial relaxation originates principally from between-fiber sliding [64,66], and slow relaxation mainly from the fibril level [64,83], possibly from fluid and molecular interactions inside the fibrils [94].

**Figure 3.1:** Schematic figures of results of experimental testing by (a) ultimate test until tissue failure, (b) sinusoidal testing and (c) stress-relaxation testing. Certain important parameters and phenomena are highlighted. $E$=Young’s modulus, $\sigma$=stress, $\varepsilon$=strain, $E_{dy}n$=dynamic modulus, $A_{\sigma}$=stress amplitude, $A_{\varepsilon}$=strain amplitude, $\Delta t$=time difference, $T$=period, $\gamma$=phase difference.
3.3 FIBRIL-REINFORCED POROVISCOELASTIC MODELING

Various types of tissue-level continuum material models of ligaments and tendons have been developed and used. For example, ligaments and tendons have been previously modelled as transversely isotropic poroelastic [95], fiber-reinforced hyperelastic [96–99], fiber-reinforced hyperelastic with dispersed fibers (Gasser-Ogden-Holzapfel model [100]) [101,102] and fibril-reinforced poroelastic or poroviscoelastic [102–105]. The poroelastic and fibril-reinforced poroelastic and poroviscoelastic material models are presented in the next sections in detail.

3.3.1 Poroelastic theory

A biphasic poroelastic material model comprises of solid and fluid phases (Figure 3.2). The pores of the solid phase are fully saturated with fluid, and the total stress in the material is given by

\[ \sigma_{tot} = \sigma_s + \sigma_{fl}, \]

(3.1)

where \( \sigma_s \) is the solid and \( \sigma_{fl} \) is the fluid stress tensor. They are defined as follows:

\[ \sigma_s = -n_s p I + \sigma_{eff}, \]

(3.2)

\[ \sigma_{fl} = -n_f p I, \]

(3.3)

where \( n_s \) and \( n_f \) are the solid and fluid volume fractions, \( p \) is the pressure of the fluid, \( I \) is the unit tensor and \( \sigma_{eff} \) is the effective solid stress. Combining the above equations, the total stress becomes

\[ \sigma_{tot} = \sigma_{eff} - p I. \]

(3.4)

Figure 3.2: Schematic figure of a poroelastic material filled with fluid, highlighting the possibility of fluid flow within and through the material.
3.3.2 Neo-Hookean poroelastic material model

The effective solid stress was calculated using a neo-Hookean material model, suitable for modeling large deformations. The effective solid stress is thus given by

\[ \sigma_{\text{eff}} = \sigma_{\text{neo-Hooke}} = K \frac{\ln J}{J} I + \frac{G}{J} (F F^T - J^{2/3} I) , \]  

(3.5)

where \( F \) is the deformation gradient tensor, \( J \) is the determinant of the deformation gradient tensor and bulk modulus \( K \) and shear modulus \( G \) are calculated as

\[ K = \frac{E}{3(1-2v)} , \]  

(3.6)

\[ G = \frac{E}{2(1+v)} , \]  

(3.7)

where \( E \) and \( v \) are the Young’s modulus and Poisson’s ratio. When considering fluid flow within a porous material (Figure 3.2), a pressure gradient \( \nabla p \) causes fluid to flow, which can be modeled using Darcy’s law [106]

\[ q = -k \nabla p , \]  

(3.8)

where \( q \) is the fluid flow rate and \( k \) is the hydraulic permeability. The deformation changes the permeability of the material. The permeability was assumed to be void ratio dependent according to [107]

\[ k = k_0 \left( \frac{1+e}{1+e_0} \right)^M , \]  

(3.9)

where \( k_0 \) is the initial permeability, \( e_0 \) is the initial void ratio, \( e \) is the current void ratio and \( M \) is a coefficient describing permeability deformation dependency.

3.3.3 Fibril-reinforced poroviscoelastic material model

Inspired by the composition of ligaments and tendons, the solid part of the material is divided into a non-fibrillar matrix and a network formed by the collagen fibrils (Figure 3.3). The effective solid stress may then be calculated as

\[ \sigma_{\text{eff}} = \sigma_{nf} + \sigma_f , \]  

(3.10)

where \( \sigma_{nf} \) and \( \sigma_f \) are the nonfibrillar matrix and fibrillar network stress tensors. The total stress then becomes

\[ \sigma_{\text{tot}} = \sigma_{nf} + \sigma_f - p I . \]  

(3.11)

In this model, the nonfibrillar matrix was modeled as a neo-Hookean material and the stress tensor \( \sigma_{nf} \) was calculated using equation (3.5). The fibrillar network was modeled using a rheological element oriented to the longitudinal direction, acting
only in tension, \( i.e. \sigma_f = 0 \) when fibril strain is negative \( (\varepsilon_f < 0) \). The different rheological elements used in this study, along with their mathematical formulations are presented in detail in the following section.

**Figure 3.3:** Schematic figure of a fibril-reinforced poroviscoelastic material.

### 3.3.4 Fibrillar network formulations

The simplest fibrillar network formulation is linear elastic (Table 3.1, linear elastic), which has been previously applied for ligaments [22], tendon [105] and cartilage [108–111]. However, a nonlinear elastic formulation (Table 3.1, nonlinear elastic) may be better able to capture the gradual recruitment of collagen with the increasing strain (straightening of collagen crimp). Nearly similar representation has been used previously for ligaments [112], meniscus [113], and cartilage [109,114–116]. These linear or nonlinear elastic fibrillar network formulations do not take into account the inherent viscoelasticity of the collagen, observed experimentally [64,83,117,118].

The viscoelasticity of the collagen fibrils may be incorporated by including a Maxwell element in the fibrillar network representation, in parallel with the linear elastic spring (Table 3.1, linear viscoelastic). The spring in the Maxwell element is nonlinear. This formulation was earlier applied for cartilage [119–122]. To better capture collagen fibril recruitment with strain, the linear elastic spring was changed to a nonlinear elastic spring (Table 3.1, nonlinear viscoelastic), which results in a new fibrillar network formulation, presented for the first time in this study. As an alternative to a quadratic formulation of the springs, a traditional exponential formulation may be used (Table 3.2, exponential viscoelastic). This type of fibrillar network has been used for the Achilles tendon [102–104] and cartilage [123,124].

In order to capture the two-relaxation-time behavior of fibrillar network observed experimentally [64,66,83], two Maxwell elements were introduced, in parallel with a nonlinear spring (Table 3.2, nonlinear two-relaxation-time viscoelastic). Finally, in order to incorporate increased relaxation with increasing strain observed in experimental studies [64,66,84–86], the spring moduli of the Maxwell element springs were assumed to depend linearly on fibril strain (Table 3.2, nonlinear two-relaxation-time strain-recruited viscoelastic). This formulation recruits the
viscoelasticity with increasing strain. These last two fibrillar network formulations, with two-relaxation-time viscoelastic formulation, are new fibrillar networks, which are presented for the first time in this study.
**Table 3.1:** Fibrillar network formulations and equations of fibril stress for elastic and viscoelastic fibrils. See Appendix A for the derivation of equations. $\sigma_f =$ fibril stress, $\varepsilon_f =$ fibril strain, $E_0, E_1, E_2 =$ fibrillar network moduli, $\eta_0 =$ damping coefficient of the fibrillar network.

<table>
<thead>
<tr>
<th>Fibrillar network formulation</th>
<th>Rheological model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linear elastic</strong></td>
<td>![Linear elastic diagram]</td>
<td>$\sigma_f = E_0 \varepsilon_f$</td>
</tr>
<tr>
<td><strong>Nonlinear elastic</strong></td>
<td>![Nonlinear elastic diagram]</td>
<td>$\sigma_f = E_1 \varepsilon_f^2$</td>
</tr>
<tr>
<td><strong>Linear viscoelastic</strong></td>
<td>![Linear viscoelastic diagram]</td>
<td>$\begin{align*} \sigma_f &amp;= E_0 \varepsilon_f - \frac{\eta_0}{2 \sqrt{(\sigma_f - E_0 \varepsilon_f)E_2}} \dot{\varepsilon}_f \ &amp;+ \left( \frac{\eta_0}{2 \sqrt{(\sigma_f - E_0 \varepsilon_f)E_2}} \right) \dot{\varepsilon}_f \end{align*}$</td>
</tr>
<tr>
<td><strong>Nonlinear viscoelastic</strong></td>
<td>![Nonlinear viscoelastic diagram]</td>
<td>$\begin{align*} \sigma_f &amp;= E_1 \varepsilon_f^2 + \eta_0 \left( \dot{\varepsilon}_f - \frac{\dot{\varepsilon}_f - 2E_1 \varepsilon_f \dot{\varepsilon}_f}{2 \sqrt{(\sigma_f - E_1 \varepsilon_f^2)E_2}} \right) \end{align*}$</td>
</tr>
</tbody>
</table>
Table 3.2: Fibrillar network formulations and equations of fibril stress for exponential viscoelastic and two-relaxation-time viscoelastic fibrils, in the view of numerical implementation. See Appendix A for the derivation of equations. $\sigma_f =$ fibril stress, $A_1, B_1, A_2, B_2 =$ constants of the exponential springs $E_1, E_3, E_4, E_5, E_6 =$ fibrillar network moduli, $\eta_0, \eta_1, \eta_2 =$ damping coefficients of the fibrillar network, $\sigma_{1,old}, \sigma_{2,old} =$ stress of the Maxwell element from the previous time increment, $\Delta t =$ time increment.

<table>
<thead>
<tr>
<th>Fibrillar network formulation</th>
<th>Rheological model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exponential viscoelastic</strong></td>
<td>$\sigma_f = A_1(\text{e}^{B_1\varepsilon_f} - 1)$</td>
<td>$\sigma_f = A_1(\text{e}^{\eta_0 \dot{\varepsilon}_f} - 1) + \frac{\dot{\varepsilon}_f \eta_0}{2B_1\Delta t} - \frac{\eta_0}{2B_1} A_2$</td>
</tr>
<tr>
<td>$\varepsilon_e$</td>
<td>$\varepsilon_v$</td>
<td>$\frac{1}{2} \left( -\dot{\varepsilon}_f \eta_0 + \frac{\eta_0}{B_1\Delta t} + A_2 \right)^2 + 4\dot{\varepsilon}<em>f \eta_0 A_2 + 4 \frac{\eta_0 \sigma</em>{1,old}}{B_1\Delta t}$</td>
</tr>
<tr>
<td><strong>Nonlinear two-relaxation-time viscoelastic</strong></td>
<td>$\sigma_f = E_1 \varepsilon_f^2 + E_3 \eta_1 \Delta t + \eta_1 \dot{\varepsilon}_f$</td>
<td>$\sigma_f = E_1 \varepsilon_f^2 + \frac{E_3 \eta_1 \Delta t}{E_3 \Delta t + \eta_1} \dot{\varepsilon}_f$</td>
</tr>
<tr>
<td>$\varepsilon_e$</td>
<td>$\varepsilon_v$</td>
<td>$+ \frac{\eta_1 \sigma_{1,old}}{E_3 \Delta t + \eta_1} + \frac{E_4 \eta_2 \Delta t}{E_4 \Delta t + \eta_2} \dot{\varepsilon}_f$</td>
</tr>
<tr>
<td>$\sigma = E_3 \varepsilon_{\sigma 1}$</td>
<td>$\sigma = E_4 \varepsilon_{\sigma 2}$</td>
<td>$+ \frac{\eta_2 \sigma_{2,old}}{E_4 \Delta t + \eta_2}$</td>
</tr>
<tr>
<td><strong>Nonlinear two-relaxation-time strain-recruited viscoelastic</strong></td>
<td>$\sigma_f = E_1 \varepsilon_f^2 + \frac{E_5 \varepsilon_f \eta_1 \Delta t}{E_5 \varepsilon_f \Delta t + \eta_1} \dot{\varepsilon}_f$</td>
<td>$\sigma_f = E_1 \varepsilon_f^2 + \frac{E_5 \varepsilon_f \eta_1 \Delta t}{E_5 \varepsilon_f \Delta t + \eta_1} \dot{\varepsilon}_f$</td>
</tr>
<tr>
<td>$\varepsilon_e$</td>
<td>$\varepsilon_v$</td>
<td>$+ \frac{\eta_1 \sigma_{1,old}}{E_5 \varepsilon_f \Delta t + \eta_1} + \frac{E_6 \varepsilon_f \eta_2 \Delta t}{E_6 \varepsilon_f \Delta t + \eta_2} \dot{\varepsilon}_f$</td>
</tr>
<tr>
<td>$\sigma = E_5 \varepsilon_{\sigma 1}$</td>
<td>$\sigma = E_6 \varepsilon_{\sigma 2}$</td>
<td>$+ \frac{\eta_2 \sigma_{2,old}}{E_6 \varepsilon_f \Delta t + \eta_2}$</td>
</tr>
</tbody>
</table>
4 CLINICAL RELEVANCE

The knee joint ligaments and PT suffer from injuries and diseases, which cause pain and impairment in joint function. Ligaments may completely rupture or partially tear as a result of high loads occurring for example in sports or accidents. A complete rupture or partial tear of the ACL is a common injury, which leads to 32-38 repair surgeries per 100 000 people annually in Sweden, Norway and Denmark (71-91 among young people aged between 15-39) [125–127]. For PCL injuries the corresponding numbers were between 3.6-10.6 per 100 000 annually [128]. A study on MCL injuries in young athletic population showed an incidence of 727 injuries per 100 000 people [129], and in general population MCL was the second most commonly injured ligament after ACL [130]. LCL injuries are more rare, but showed similar occurrences to PCL [130]. PT may suffer from gradually developing overuse injury, patellar tendinopathy, often referred to as “jumper’s knee”. Repetitive overloading causes microtrauma, leading to pain and altered tendon composition [131]. Complete rupture of the patellar tendon is rather rare, with an incidence of 0.68 annually per 100 000 people in UK [132].

As a result of ligament or PT injury, or patellar tendinopathy, the normal mechanical function of the knee is disrupted. This abnormal condition may result in excessive loads in the other knee joint tissues, such as articular cartilage, and possibly lead to post-traumatic osteoarthritis. Ligaments may also start to adapt to the abnormal loading [133]. All these conditions and diseases may be understood and treated better when the basic mechanical function, biochemical composition, and their relationships are better understood and modeled.

As an example, ACL rupture is commonly treated by replacing the ruptured ACL with a PT autograft. Correct stiffness of the graft is crucial for the outcome [7], and thus when planning the replacement, the knowledge of the mechanical material properties and how they compare between tissues is merited. As another example, different imaging methods are important tools in diagnostics, but they mainly reflect the composition and structure of the tissue. Linking the composition to the mechanical properties is thus necessary in evaluating the function and health of the tissue. This way, for example, healing of an MCL rupture could be quantitatively monitored. Furthermore, at the level of the whole knee, computational tools enable evaluation of the joint function and, in the future, could be used to predict disease progression and effect of different treatments in the knee [134–141]. However, accurate material modeling and material properties are needed, especially in ligaments [21,22,27], to provide reliable outcomes.
5 AIMS

Although the mechanical material properties of individual ligaments have been previously characterized, it is poorly understood how the quasi-static and viscoelastic properties compare between knee ligaments and patellar tendon from the same set of skeletally mature knees. Moreover, the literature is conflicting and limited on how the biochemical composition compares between these tissues and how it relates to mechanical properties. Furthermore, the current tissue level material models of ligaments that distinguish fibers, matrix and fluid, are inherently limited in their viscoelastic representation and are unable to accurately capture the complex stress-relaxation behavior. To investigate these matters, bovine knee joint ligaments and patellar tendons were subjected to tensile testing to determine the mechanical material properties, their biochemical composition was measured, and a material model was developed.

The specific aims of this thesis can be summarized as follows:

- To comprehensively characterize and compare the mechanical material properties of the knee joint ligaments and the patellar tendon in tension at the mesoscopic level.
- To determine and compare the water, hydroxyproline, uronic acid, and elastin contents of the knee joint ligaments and the patellar tendon and to correlate them with the mechanical material properties.
- To investigate the performance of different tissue-level continuum material models in capturing anterior cruciate ligament stress-relaxation behavior in tension, and further to develop a fibril-reinforced poroviscoelastic model to accurately represent this behavior.
6 MATERIALS AND METHODS

6.1 SAMPLE PREPARATION

The four primary knee ligaments, cranial cruciate ligament (CrCL), caudal cruciate ligament (CaCL), medial collateral ligament (MCL), lateral collateral ligament (LCL), and the patellar tendon (PT) were carefully dissected from 10 bovine stifle (knee) joints (Figure 6.1a). The CrCL and CaCL in the bovine stifle joint correspond to the anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) in the human knee joint, and the human terms are used throughout this thesis. The joints were obtained from an abattoir (Atria Oyj, Seinäjoki, Finland) as a by-product of meat production; the animals were skeletally mature, aged 14 to 22 months. Immediately after dissection, the ligaments were immersed in phosphate-buffered saline (PBS) solution and stored in a freezer (-20 °C) in plastic containers. Before the measurements, the samples were allowed to thaw at room temperature, and dumbbell-shaped tensile test pieces were cut from the mid-substance of the tissues, with collagen fibers running along the longitudinal axis (Figure 6.1b). First, a slice was cut using two parallel razor blades, separated by approximately 1.8 mm, to obtain a sheet of desired thickness. Then, a custom punch tool was used to cut the sheet to the desired dumbbell shape, with approximately 2 mm in width and 10 mm in measurement length. Samples of similar size and shape have been used earlier for ligaments and tendons [25,41,76,87,142,143]. Sample thickness and width were measured with a microscope (4.6x magnification), and the cross-sectional area was calculated assuming an elliptical shape [144–146]. This shape assumption was verified as being accurate, with an error of 0.15 % in the area compared to the direct microscopy measurement of a cut cross-section. Custom double-sided sandpapers (Mirox P80, Mirka Oy, Uusikaarlepyy, Finland) were attached to the sample ends using glue (Loctite Precision, Henkel AG, Düsseldorf, Germany), and the sample was placed between custom jaw-type tensile testing clamps (Figure 6.1c). Clamp screws were tightened to a moment of 4 Nm, in order to avoid slipping and to ensure constant clamping for all the samples. The samples were immersed in PBS at room temperature during the mechanical measurement.
Figure 6.1: Workflow of studies I-III. Dumbbell-shaped tensile test specimens were cut from the mid-substance of bovine knee ligaments (a, b and c). Stress-relaxation, sinusoidal and ultimate testing was conducted (d, e and f). The sample was divided into two parts (g) and biochemical composition was determined (h). A finite element model of the stress-relaxation experiment was created (i).
6.2 TENSILE TESTING

Tensile testing was carried out using a uniaxial material testing system, which consists of a 25 lb load cell (Model 31/AL311BL, Honeywell, Columbus, OH, USA), a linear actuator (Newport, Irvine, CA, USA; resolution 0.1 µm) and a controller (Newport, Irvine, CA, USA), which are controlled by a custom LabView software (version 10.0, National Instruments Corporation, Austin, TX, USA). The zero-load length was established using a tensile stress of 0.05 MPa [41]. Preconditioning was performed with 10 cycles of loading-unloading to 2 % strain with 0.05 mm/s velocity. The sample was allowed to recover for 2 minutes after which the zero-load length was re-established. This protocol was repeated five times to stabilize the zero-load length and mechanical behavior [147]. After preconditioning, a 10-minute recovery period was held, after which the zero-load length was re-verified, and the experiment was started.

A four-step stress-relaxation test in tension was conducted using steps of 2 % strain (i.e. steps at 2, 4, 6, and 8 % strain), ramp speed of 0.1 mm/s (1 %/s), and 30 minutes of relaxation at each step (Figure 6.1d). After this, at 8 % strain, a sinusoidal loading test was conducted with 0.1, 0.5 and 1 Hz frequencies using 0.5 % strain amplitude [148], with 20 cycles at each frequency (Figure 6.1e). The sample was returned to zero-load length (0 % strain) and allowed to recover for 1 hour, after which an ultimate tensile test until tissue failure was conducted with 0.005 mm/s (0.05 %/s) velocity (Figure 6.1f).

6.3 BIOCHEMICAL ANALYSES

After the tensile testing, the sample piece (n=50) was divided into two parts of 7 – 38 mg in wet weight and placed in a freezer (-20 °C) (Figure 6.1g). Water, hydroxyproline and uronic acid contents were determined from one part, and the elastin content from the other (Figure 6.1h). The samples were thawed at room temperature and were in a fully rehydrated state before the biochemical analyses.

The water content was calculated from the difference in wet and dry weights, each measured three times and averaged. The dry weight was obtained after freeze-drying in a lyophilizer for 24 hours. Then, the hydroxyproline content, indicative of the amount of collagen, and the uronic acid content, reflecting the amount of proteoglycans, were determined. We assumed the level of proline hydroxylation to be similar among ligaments and PT [1], i.e. hydroxyproline constitutes the same constant proportion of collagen in these tissues, and therefore the hydroxyproline was used to indicate and compare the collagen content. We used uronic acid content as a surrogate for proteoglycan content [55,57,59,60], as it presumably reflects the proteoglycan content similarly in these tissues, enabling comparisons between them. First, in order to digest the proteoglycans, the samples were incubated at a pH of 5.8 and 60 °C for 16 h in a 1 mg/ml concentration of papain in 150 mM sodium acetate,
which included 5 mM L-cysteine and 15 mM EDTA [60]. Next, to deactivate the enzyme, the samples were boiled for 10 minutes. The hydroxyproline content was quantified from the papain digested samples after hydrolysis with a spectrophotometric assay [149]. The uronic acid content was measured from the ethanol-precipitated samples [150]. The determination of the hydroxyproline and uronic acid contents was performed three times and averaged, and the contents were normalized by dry and wet weights.

The elastin content was determined using Fastin Elastin Assay (Biocolor Ltd., Carrickfergus, County Antrim, United Kingdom) according to the instructions from the assay manufacturer. Briefly, the samples were boiled for one hour in 0.25 M oxalic acid to extract elastin. This was repeated three times to ensure that all elastin had been recovered (verified in our pilot measurements). A precipitation reagent was added to two aliquots of pooled extracts, and the precipitated elastin was dyed. After 90 minutes of reaction with the dye, the red-brown elastin-dye complex was dissociated, and the solution placed in a microwell plate. Absorbance was read at 513 nm and compared against a standard curve to determine the elastin content. Finally, the content was normalized by dry and wet weights.

**6.4 DATA ANALYSIS**

Sinusoidal and ultimate tensile test data were analyzed in terms of true stress \((\sigma = \frac{F}{A_0} \frac{L}{L_0})\), where \(F\) is the measured force, \(A_0\) the initial cross-sectional area, \(L\) the current length and \(L_0\) the initial length) and logarithmic strain \((\varepsilon = \ln \frac{L}{L_0})\). The true stress equation assumes an incompressible material (constant volume). Logarithmic strain, i.e., true strain, was used to quantify the strain state as strains were relatively large. The dynamic modulus and phase angle were obtained by fitting a sinusoidal function

\[
\begin{align*}
  z &= A \sin(2\pi ft + \varphi) + z_0, \\
  \end{align*}
\]

(6.1)

to stress-time and strain-time signals. In the above equation, \(z\) is the value of the stress or strain, \(A\) is the oscillation amplitude, \(f\) is the oscillation frequency, \(t\) is the time, \(\varphi\) is the phase angle and \(z_0\) is the constant term. The dynamic modulus is then calculated as

\[
E_{dy} = \frac{A_\sigma}{A_\varepsilon},
\]

(6.2)

and phase difference as

\[
\gamma = \varphi_\sigma - \varphi_\varepsilon.
\]

(6.3)

The ultimate tensile test data were analyzed from 0.2 MPa stress onwards, with the purpose of excluding the portion in which the tissue was slack from the analysis.
The Young’s modulus was determined by making multiple linear fits to the stress-strain curve, and picking the maximum tangent modulus [151] from these fits. At each data point in the curve, a linear fit was made on the subsequent data points covering an 8 % strain interval. The linear fit with the maximum modulus was then used to determine also the toe region end and yield points. They (\( \varepsilon_{\text{toe}}, \sigma_{\text{toe}} \) and \( \varepsilon_{\text{yield}}, \sigma_{\text{yield}} \)) were determined by the points where the stress-strain curve deviated from the linear fit by 0.6 % strain [152]. Ultimate strength (\( \varepsilon_{\text{ult}}, \sigma_{\text{ult}} \)) was defined as the point where the maximum stress of the experiment was reached. The length of the linear region was then calculated as \( \varepsilon_{\text{linear}} = \varepsilon_{\text{yield}} - \varepsilon_{\text{toe}} \). Energy density, i.e., toughness, was defined as the integral of the stress-strain curve up to ultimate strain. Additionally, we calculated energy density up to the yield point:

\[
K_{\text{yield/ult}} = \int_{0}^{\varepsilon_{\text{yield/ult}}} \sigma \, d\varepsilon .
\] (6.4)

The nonlinear toe region, \( 0 < \varepsilon < \varepsilon_{\text{toe}} \), can be characterized using a second-order formula [152]

\[
\sigma = A\varepsilon^2 + B\varepsilon + C ,
\] (6.5)

where \( A, B \) and \( C \) are constants, or an exponential formula [153]

\[
\sigma = D(e^{F\varepsilon} - 1) ,
\] (6.6)

where \( D \) and \( F \) are constants. In the above equations, \( A \) and \( F \) represent the strain dependence of the modulus, while \( B \) describes the initial modulus.

### 6.5 FIBRIL-REINFORCED POROVISCOELASTIC MODELING

To investigate different material models, and to further develop a fibril-reinforced poroviscoelastic model, they were implemented in a finite element (FE) model of the stress-relaxation experiment (Figure 6.1i). One representative model geometry was created from the average dimensions of 10 ACL samples, and assuming an elliptical cross-sectional shape. ACL was chosen as a representative ligament because of its clinical importance. Due to the symmetrical geometry in the experiment, only a quarter ligament was implemented in the FE model. It was created with Abaqus (v. 2018, Dassault Systèmes, Johnston, RI, USA) using 2592 C3D8P elements. The mesh size was chosen based on mesh convergence analysis. Boundary conditions were applied according to the stress-relaxation experiment described in section 6.2. At the bottom nodes, all displacements were restricted, while at the top nodes, lateral displacements were constrained. Vertical displacement was assigned to the top nodes to replicate the stress-relaxation experiment. In the symmetry planes, displacements perpendicular to the plane were restricted. Zero pore pressure was assigned to the outer surface to enable a free flow of fluid. The average water content
of these 10 ACL samples was 77.6 % (see section 7.2) and thus the initial void ratio $e_0$ was fixed to 3.46.

For each material model outlined in Table 6.1, the force-time output of the model was fitted to the experimentally measured force-time curve by optimizing the material parameters. One experimental force-time curve was used, created by averaging the force-time output of 10 ACL samples. The three first steps of the relaxation test were used in the optimization, in order to keep the response in the toe-region. The optimized parameters of each model are shown in Table 6.1, and fixed parameters in Table 6.2. In the material parameter optimization, the function $f = \sqrt{1 - R^2}$ was minimized to attain the coefficient of determination as close to 1 as possible. A built-in algorithm, fminsearch, was used in MATLAB R2017b (The MathWorks, Inc., Natick, MA, USA) for optimization. Material models were evaluated based on their $R^2$-value.

In order to investigate the effect of initial permeability $k_0$, permeability deformation dependency coefficient $M$, water content and Poisson’s ratio of the non-fibrillar matrix, a parametric analysis was conducted using two models. This revealed the effect of $k_0$ and $M$ to be negligible on the reaction force-time curve in tensile stress-relaxation, when the Poisson’s ratio was 0.48, and thus they were fixed to $k_0 = 2.942 \times 10^{-15} \text{m}^4\text{N}^{-1}\text{s}^{-1}$ and $M = 7.988$ [154] for models 4-8. Even with larger volume changes (Poisson’s ratio 0.9), their effect was shown to be small. The effect of water content was negligible on the overall response. The Poisson’s ratio of the non-fibrillar matrix had an effect only with large negative values. It was fixed to $\nu_{nf} = 0.48$ since a bi-directional video showed approximately constant volume for a subset of these samples, which is in line with the results of Vergari et al. [155]. The results of the parametric analysis are shown in Appendix B.
Table 6.1: Optimized material parameters of different material models. See Section 3.3.4 for fibrillar network formulations.

<table>
<thead>
<tr>
<th>Material model</th>
<th>Optimized parameters</th>
<th>Non-fibrillar matrix</th>
<th>Fluid flow</th>
<th>Fibrillar network</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Poroelastic (PE)</td>
<td></td>
<td>$E_{nf}$</td>
<td>$k_0$</td>
<td>$M$</td>
</tr>
<tr>
<td>2. PE + linear elastic fibrillar network (FN)</td>
<td></td>
<td>$E_{nf}$</td>
<td>$k_0$</td>
<td>$M$ $E_0$</td>
</tr>
<tr>
<td>3. PE + nonlinear elastic FN</td>
<td></td>
<td>$E_{nf}$</td>
<td>$k_0$</td>
<td>$M$ $E_1$</td>
</tr>
<tr>
<td>4. PE + linear viscoelastic FN</td>
<td></td>
<td>$E_{nf}$</td>
<td></td>
<td>$E_0$ $E_2$ $\eta_0$</td>
</tr>
<tr>
<td>5. PE + nonlinear viscoelastic FN</td>
<td></td>
<td>$E_{nf}$</td>
<td></td>
<td>$E_1$ $E_2$ $\eta_0$</td>
</tr>
<tr>
<td>6. PE + exponential viscoelastic FN</td>
<td></td>
<td>$E_{nf}$</td>
<td>$A_1$ $B_1$ $A_2$ $B_2$ $\eta_0$</td>
<td></td>
</tr>
<tr>
<td>7. PE + two-relaxation-time viscoelastic FN</td>
<td></td>
<td>$E_{nf}$</td>
<td>$E_1$ $E_3$ $E_4$ $\eta_1$ $\eta_2$</td>
<td></td>
</tr>
<tr>
<td>8. PE + two-relaxation-time strain-recruited viscoelastic FN</td>
<td></td>
<td>$E_{nf}$</td>
<td>$E_1$ $E_5$ $E_6$ $\eta_1$ $\eta_2$</td>
<td></td>
</tr>
</tbody>
</table>

PE=poroelastic, FN=fibrillar network, $E_{nf}$=Young’s modulus of the non-fibrillar matrix, $k_0$=initial permeability, $M$=permeability strain-dependency coefficient, $E_0$, $E_1$, $E_2$, $E_3$, $E_4$, $E_5$, $E_6$=fibrillar network moduli, $A_1$, $B_1$, $A_2$, $B_2$=constants of the exponential springs, $\eta_0$, $\eta_1$, $\eta_2$=damping coefficients of the fibrillar network.

Table 6.2: Fixed material parameters of different material models.

<table>
<thead>
<tr>
<th>Models</th>
<th>Fixed parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>$\nu_{nf} = 0.48$</td>
</tr>
<tr>
<td>4-8</td>
<td>$\nu_{nf} = 0.48$</td>
</tr>
<tr>
<td></td>
<td>$k_0 = 2.942 \times 10^{-15}$ m$^4$N$^{-1}$s$^{-1}$</td>
</tr>
</tbody>
</table>

$\nu_{nf}$=Poisson’s ratio of the non-fibrillar matrix, $e_0$=initial void ratio, $k_0$=initial permeability, $M$=permeability deformation dependency coefficient.
6.6 STATISTICAL METHODS

The data are shown as mean ± standard deviation (SD) in the figures and tables. Statistical comparison between ligament types and PT in mechanical and biochemical parameters were conducted using a linear mixed model [156] in IBM SPSS Statistics 25.0.0.1 (SPSS Inc., IBM Company, Armonk, NY, USA). The model takes into account the fact that the samples originate from the same collection of knees. In this statistical comparison, performed for each mechanical and biochemical parameter, the tissue type (ACL, LCL, MCL, PCL, and PT) was set as a fixed variable and the knee as a random variable. Statistical significance was set to 0.05.

Structure–function relationships, i.e. relationships between biochemical contents and mechanical properties, were examined using 1) the significant differences between tissues and 2) multiple linear regression. Significant differences between tissues in the mechanical properties were mirrored against significant differences in biochemical contents, and similarities in the trends were investigated. In multiple linear regression analyses, conducted in R [157], for each mechanical property a regression model was formulated. The hydroxyproline, uronic acid and elastin contents normalized by dry weight, as well as tissue type were used to predict the mechanical property. Water content correlated strongly with uronic acid (dry weight) and was omitted as a predictor. Backward elimination was performed according to the Akaike information criterion to remove uninformative predictors. The same analysis was performed also with hydroxyproline, uronic acid and elastin contents normalized by wet weight and tissue type as predictors.
7 RESULTS

7.1 MECHANICAL PROPERTIES

Dynamic modulus was highest in MCL at all tested frequencies, with significant differences compared with ACL, LCL ($p<0.05$), and PT ($p<0.01$) (Table 7.1). At 0.1 Hz loading, PCL had a higher dynamic modulus than PT ($p<0.05$). At 0.1 Hz loading, LCL had a higher phase difference than ACL, PCL and PT ($p<0.01$), and MCL had a higher phase difference than PCL ($p<0.05$) (Figure 7.1). At 0.5 Hz loading, LCL had a higher phase difference than all other ligaments and PT ($p<0.05$ compared with ACL and MCL, $p<0.01$ as compared with PCL and PT). At 1 Hz loading, the ligaments and PT did not display any significant differences in phase difference.

**Table 7.1:** Dynamic modulus $E_{dyn}$ (MPa) for ACL, LCL, MCL, PCL, and PT at different sinusoidal loading frequencies shown as mean ± SD.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>ACL</th>
<th>LCL</th>
<th>MCL</th>
<th>PCL</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 Hz</td>
<td>163.94 ± 73.98 *</td>
<td>181.66 ± 135.26 *</td>
<td>288.51 ± 102.56</td>
<td>214.26 ± 126.21 °</td>
<td>115.77 ± 93.03 **</td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>168.73 ± 76.84 *</td>
<td>187.80 ± 140.20 *</td>
<td>296.60 ± 105.48</td>
<td>220.15 ± 130.55</td>
<td>119.38 ± 97.18 **</td>
</tr>
<tr>
<td>1 Hz</td>
<td>171.14 ± 78.43 *</td>
<td>190.08 ± 142.14 *</td>
<td>298.56 ± 105.96</td>
<td>222.37 ± 130.26</td>
<td>120.45 ± 99.14 **</td>
</tr>
</tbody>
</table>

Significant differences with respect to MCL are indicated with * ($p<0.05$) and ** ($p<0.01$). Additionally, PCL and PT were significantly different, indicated with ° ($p<0.05$).

**Figure 7.1:** Phase difference at different frequencies for knee ligaments and PT.
Parameter $A$, describing strain-dependent modulus at the toe region, was higher in MCL compared with ACL, LCL ($p<0.05$) and PT ($p<0.01$), and was higher in PCL compared with PT ($p<0.05$) (Table 7.2). Parameter $F$, also describing toe region nonlinearity, was significantly higher in MCL compared with LCL and PT ($p<0.05$).

**Table 7.2**: Characterization of the toe region with $\sigma = A\epsilon^2 + B\epsilon + C$ and $D\epsilon^2 + E\epsilon + F$ (Equations 6.5 and 6.6). Significant differences with respect to MCL are indicated with * ($p<0.05$) and ** ($p<0.01$). Additionally, PCL and PT were significantly different indicated with ° ($p<0.05$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ACL</th>
<th>MCL</th>
<th>LCL</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width (mm)</td>
<td>2.32 ± 0.32</td>
<td>2.17 ± 0.36</td>
<td>2.32 ± 0.32</td>
<td>2.09 ± 0.31</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>1.99 ± 0.32</td>
<td>1.96 ± 0.29</td>
<td>1.99 ± 0.29</td>
<td>1.94 ± 0.29</td>
</tr>
<tr>
<td>Zero-load length (mm)</td>
<td>10 16 ± 0.35</td>
<td>10 02 ± 0.35</td>
<td>9.88 ± 0.38</td>
<td>9.94 ± 0.41</td>
</tr>
<tr>
<td>$F$</td>
<td>24.73 ± 6.33</td>
<td>19.91 ± 10.90</td>
<td>30.27 ± 8.23</td>
<td>24.73 ± 6.33</td>
</tr>
</tbody>
</table>

Significant differences with respect to MCL are indicated with * ($p<0.05$) and ** ($p<0.01$). Additionally, PCL and PT were significantly different indicated with ° ($p<0.05$).
Other toe region fit parameters or linear region length showed no significant differences. The zero-load length, thickness and width were included for reference, and they showed no significant differences (Table 7.2).

The toe region end point showed no significant differences in stress ($\sigma_{\text{toe}}$), but strain ($\varepsilon_{\text{toe}}$) was lower in PCL as compared with either LCL ($p<0.05$) or PT ($p<0.01$), and lower in MCL as compared with PT ($p<0.05$) (Figure 7.2). Yield stress ($\sigma_{\text{yield}}$) was higher in MCL in comparison with ACL and PT ($p<0.05$) (Figure 7.2). MCL had a higher ultimate strength ($\sigma_{\text{ult}}$) than ACL ($p<0.05$) and PT ($p<0.01$) (Figure 7.2). Strain at ultimate strength ($\varepsilon_{\text{ult}}$) was lower in ACL compared with LCL and PT ($p<0.05$).

MCL had a higher Young’s modulus than ACL and PT ($p<0.05$) (Figure 7.3). Energy density at yield ($K_{\text{yield}}$) was higher in MCL as compared with PT ($p<0.05$). Energy density at failure ($K_{\text{ult}}$) was higher in MCL than in ACL and PT ($p<0.05$). In ultimate testing, 24 failures occurred at the mid-substance, 14 near the clamps and for 12 samples the rupture location was indistinguishable (Figures 7.4 and 7.5).

![Figure 7.2: Toe region end point, yield point and ultimate strength for knee ligaments and PT. Data are shown as mean ± SD and significant differences are indicated with * (p<0.05) and ** (p<0.01).](image)
Figure 7.3: Young’s modulus (left scale) and toughnesses (right scale) for knee ligaments and PT. Data are shown as mean ± SD and significant differences are indicated with * (p<0.05) and ** (p<0.01).

Figure 7.4: Rupture locations of tensile test samples for knee ligaments and PT.
Figure 7.5: Tensile test of an MCL sample highlighting the difficulty in determining the rupture location. Figure shows the sample at the initial state (left), immediately after rupture (middle), and far beyond rupture (right). Rupture can be clearly seen from the biomechanical data.

7.2 BIOCHEMICAL ANALYSES

The water content was higher in ACL as compared with MCL and LCL \((p<0.01)\) (Figure 7.6). The water content was higher in PT than in MCL \((p<0.05)\). PT had a lower hydroxyproline content per dry weight when compared with all ligaments \((p<0.01)\) (Figure 7.6). ACL and PT had a lower hydroxyproline content per wet weight than the other ligaments \((p<0.05)\). Cruciate ligaments had a significantly higher uronic acid content per dry weight as compared with collateral ligaments \((p<0.05)\). PCL had a higher uronic acid content per wet weight than LCL and MCL \((p<0.05)\). PCL had a higher elastin content per dry weight in comparison with ACL and LCL \((p<0.05)\). ACL had lower elastin content per wet weight than MCL, PCL and PT \((p<0.05)\). The uronic acid content per dry weight correlated positively with the water content \((r=0.62, p<0.01)\). The hydroxyproline content per wet weight correlated negatively with the water content \((r=-0.91, p<0.01)\).
Figure 7.6: Biochemical contents of knee ligaments and patellar tendon. The ligaments and patellar tendon are highlighted in (a). The water (b), hydroxyproline (c), uronic acid (d) and elastin contents (e), per dry and wet weights are shown as mean ± SD. Significant differences are indicated with * ($p<0.05$) and ** ($p<0.01$).

7.3 STRUCTURE–FUNCTION RELATIONSHIPS

The significant differences between ligaments in Young’s modulus, yield stress, toughness at yield, ultimate strength, and toughness at failure were similarly reflected in the water and hydroxyproline (wet weight) contents (Figures 7.2, 7.3 and 7.6, Table 7.3). Toughness at yield showed a difference similar to hydroxyproline per dry weight (Figures 7.3 and 7.6, Table 7.3).

With contents normalized by dry weight, uronic acid predicted the dynamic moduli, strain dependency coefficient $A$, Young’s modulus, yield stress, and toughness at yield (all $p<0.05$), with a higher uronic acid content predicting lower values (Table 7.4). When analysed according to dry weight, the ligament type was a predictor of phase differences at 0.1 and 0.5 Hz, dynamic moduli and strain dependency coefficients $A$ and $F$ (all $p<0.05$).
When the contents were normalized by wet weight, the hydroxyproline content predicted the phase difference at 0.1 Hz, toe region strain, and coefficient $D$ (all $p<0.05$) (Table 7.5). The elastin content was found to predict dynamic moduli ($p<0.01$), strain dependency coefficients $A$ ($p<0.01$) and $F$ ($p<0.05$), and Young's modulus ($p<0.01$). The ligament type predicted phase difference at 0.1 and 0.5 Hz loading frequencies (both $p<0.05$).

**Table 7.3**: Similar trends between mechanical properties and biochemical contents. Arrows indicate that significant differences between ligaments and PT in a mechanical property were observed similarly in the biochemical content. The direction of the arrow indicates the trend: □ higher biochemical content associated with a lower value in the biomechanical property, ▲ higher biochemical content associated with a higher value in the biomechanical property.

<table>
<thead>
<tr>
<th>Mechanical property</th>
<th>Symbol</th>
<th>Unit</th>
<th>Water content</th>
<th>Hydroxyproline (dry weight)</th>
<th>Hydroxyproline (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young's modulus</td>
<td>$E$</td>
<td>MPa</td>
<td>↘</td>
<td>↗</td>
<td>↗</td>
</tr>
<tr>
<td>Yield stress</td>
<td>$\sigma_{yield}$</td>
<td>MPa</td>
<td>↘</td>
<td>↗</td>
<td>↗</td>
</tr>
<tr>
<td>Toughness at yield</td>
<td>$K_{yield}$</td>
<td>mJ/mm$^3$</td>
<td>↘</td>
<td>↗</td>
<td>↗</td>
</tr>
<tr>
<td>Ultimate strength</td>
<td>$\sigma_{ult}$</td>
<td>MPa</td>
<td>↘</td>
<td>↗</td>
<td>↗</td>
</tr>
<tr>
<td>Toughness at failure</td>
<td>$K_{ult}$</td>
<td>mJ/mm$^3$</td>
<td>↘</td>
<td>↗</td>
<td>↗</td>
</tr>
</tbody>
</table>
Table 7.4: Results of the multiple linear regression analysis with biochemical contents normalized by dry weight. A number is shown if the biochemical content is an informative predictor of the biomechanical property according to the Akaike Information Criterion. The number shows the change in the mechanical property if the biochemical content increases by one standard deviation. If ligament type is an informative predictor, it is indicated with “Yes”. For each regression model, the adjusted $R^2$ value is shown. Statistically significant predictors are indicated with a dark grey background ($p<0.05$).

<table>
<thead>
<tr>
<th>Mechanical property</th>
<th>Symbol</th>
<th>Unit</th>
<th>Hydroxyproline (dry weight)</th>
<th>Uronic acid (dry weight)</th>
<th>Elastin (dry weight)</th>
<th>Type</th>
<th>$R^2_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase difference (0.1 Hz)</td>
<td>$\gamma_{0.1Hz}$</td>
<td>deg</td>
<td>0.2 ± 0.2</td>
<td>-0.2 ± 0.1</td>
<td>-0.2 ± 0.1</td>
<td>Yes ($p&lt;0.05$)</td>
<td>0.25</td>
</tr>
<tr>
<td>Phase difference (0.5 Hz)</td>
<td>$\gamma_{0.5Hz}$</td>
<td>deg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>Phase difference (1 Hz)</td>
<td>$\gamma_{1Hz}$</td>
<td>deg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Dynamic modulus (0.1 Hz)</td>
<td>$E_{\text{dyn,0.1Hz}}$</td>
<td>MPa</td>
<td>-35.2 ± 18.1</td>
<td>-36.2 ± 16.1</td>
<td>24.3 ± 16.0</td>
<td>Yes ($p&lt;0.05$)</td>
<td>0.27</td>
</tr>
<tr>
<td>Dynamic modulus (0.5 Hz)</td>
<td>$E_{\text{dyn,0.5Hz}}$</td>
<td>MPa</td>
<td>-36.2 ± 18.7</td>
<td>-37.3 ± 16.7</td>
<td>25.1 ± 16.6</td>
<td>Yes ($p&lt;0.05$)</td>
<td>0.27</td>
</tr>
<tr>
<td>Dynamic modulus (1 Hz)</td>
<td>$E_{\text{dyn,1Hz}}$</td>
<td>MPa</td>
<td>-36.5 ± 18.9</td>
<td>-37.8 ± 16.8</td>
<td>24.9 ± 16.8</td>
<td>Yes ($p&lt;0.05$)</td>
<td>0.26</td>
</tr>
<tr>
<td>Toe region strain</td>
<td>$\varepsilon_{\text{toe}}$</td>
<td>%</td>
<td>-0.9 ± 0.5</td>
<td></td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Toe region stress</td>
<td>$\sigma_{\text{toe}}$</td>
<td>MPa</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Strain dependency coefficient, $\lambda$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain dependent modulus</td>
<td>$A$</td>
<td>MPa</td>
<td>-189.2 ± 117.4</td>
<td>-212.8 ± 98.9</td>
<td>195.7 ± 98.4</td>
<td>Yes ($p&lt;0.05$)</td>
<td>0.27</td>
</tr>
<tr>
<td>Initial modulus</td>
<td>$B$</td>
<td>MPa</td>
<td></td>
<td>-3.4 ± 2.0</td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td>$C$</td>
<td>MPa</td>
<td></td>
<td>-0.03 ± 0.02</td>
<td></td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td>$D$</td>
<td>MPa</td>
<td>0.7 ± 0.4</td>
<td></td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Strain dependency coefficient</td>
<td>$F$</td>
<td></td>
<td>-2.7 ± 1.6</td>
<td>2.5 ± 1.3</td>
<td></td>
<td>Yes ($p&lt;0.05$)</td>
<td>0.17</td>
</tr>
<tr>
<td>Young’s modulus</td>
<td>$E$</td>
<td>MPa</td>
<td>-33.1 ± 18.3</td>
<td>-35.3 ± 18.4</td>
<td>24.0 ± 15.3</td>
<td>Yes</td>
<td>0.19</td>
</tr>
<tr>
<td>Yield strain</td>
<td>$\varepsilon_{\text{yield}}$</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Yield stress</td>
<td>$\sigma_{\text{yield}}$</td>
<td>MPa</td>
<td></td>
<td>5.1 ± 2.2</td>
<td></td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Toughness at yield</td>
<td>$K_{\text{yield}}$</td>
<td>mJ/mm$^3$</td>
<td>0.4 ± 0.2</td>
<td></td>
<td></td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Ultimate strain</td>
<td>$\varepsilon_{\text{ult}}$</td>
<td>%</td>
<td>-1.2 ± 0.7</td>
<td></td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Ultimate strength</td>
<td>$\sigma_{\text{ult}}$</td>
<td>MPa</td>
<td></td>
<td>-5.0 ± 2.7</td>
<td></td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Toughness at failure</td>
<td>$K_{\text{ult}}$</td>
<td>mJ/mm$^3$</td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>
Table 7.5: Results of the multiple linear regression analysis with biochemical contents normalized by wet weight. A number is shown if the biochemical content is an informative predictor of the biomechanical property according to the Akaike Information Criterion. The number shows the change in the mechanical property if the biochemical content increases by one standard deviation. If ligament type is an informative predictor, it is indicated with “Yes”. For each regression model, the adjusted $R^2$ value is shown. Statistically significant predictors are indicated with a dark grey background ($p<0.05$).

<table>
<thead>
<tr>
<th>Mechanical property</th>
<th>Symbol</th>
<th>Unit</th>
<th>Hydroxyproline (wet weight)</th>
<th>Uronic acid (wet weight)</th>
<th>Elastin (wet weight)</th>
<th>Type</th>
<th>$R^2_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase difference (0.1 Hz)</td>
<td>$\gamma_{0.1Hz}$</td>
<td>deg</td>
<td>$0.3 \pm 0.2$ (p&lt;0.05)</td>
<td>$-0.2 \pm 0.1$</td>
<td>$-0.2 \pm 0.1$</td>
<td>Yes</td>
<td>0.26</td>
</tr>
<tr>
<td>Phase difference (0.5 Hz)</td>
<td>$\gamma_{0.5Hz}$</td>
<td>deg</td>
<td>$0.2 \pm 0.2$</td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Phase difference (1 Hz)</td>
<td>$\gamma_{1Hz}$</td>
<td>deg</td>
<td>$-0.3 \pm 0.2$</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Dynamic modulus (0.1 Hz)</td>
<td>$E_{dyn 0.1Hz}$</td>
<td>MPa</td>
<td>$45.4 \pm 15.3$ (p&lt;0.01)</td>
<td></td>
<td></td>
<td>Yes</td>
<td>0.29</td>
</tr>
<tr>
<td>Dynamic modulus (0.5 Hz)</td>
<td>$E_{dyn 0.5Hz}$</td>
<td>MPa</td>
<td>$47.1 \pm 15.8$ (p&lt;0.01)</td>
<td></td>
<td></td>
<td>Yes</td>
<td>0.29</td>
</tr>
<tr>
<td>Dynamic modulus (1 Hz)</td>
<td>$E_{dyn 1Hz}$</td>
<td>MPa</td>
<td>$47.2 \pm 16.0$ (p&lt;0.01)</td>
<td></td>
<td></td>
<td>Yes</td>
<td>0.29</td>
</tr>
<tr>
<td>Toe region strain</td>
<td>$\varepsilon_{toe}$</td>
<td>%</td>
<td>$-1.0 \pm 0.5$ (p&lt;0.05)</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Toe region stress</td>
<td>$\sigma_{toe}$</td>
<td>MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Strain dependency coefficient, strain dependent modulus</td>
<td>$A$</td>
<td>MPa</td>
<td>$299.0 \pm 92.1$ (p&lt;0.01)</td>
<td></td>
<td></td>
<td>Yes</td>
<td>0.32</td>
</tr>
<tr>
<td>Initial modulus</td>
<td>$B$</td>
<td>MPa</td>
<td>$-3.1 \pm 2.0$</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Coefficient</td>
<td>$C$</td>
<td>MPa</td>
<td>$-0.03 \pm 0.02$</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Coefficient</td>
<td>$D$</td>
<td>MPa</td>
<td>$1.3 \pm 0.4$ (p&lt;0.01)</td>
<td></td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>Strain dependency coefficient</td>
<td>$F$</td>
<td>-</td>
<td>$2.9 \pm 1.3$ (p&lt;0.05)</td>
<td>Yes</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young’s modulus</td>
<td>$E$</td>
<td>MPa</td>
<td>$40.5 \pm 14.8$ (p&lt;0.01)</td>
<td></td>
<td></td>
<td>Yes</td>
<td>0.20</td>
</tr>
<tr>
<td>Yield strain</td>
<td>$\varepsilon_{y}^\text{yield}$</td>
<td>%</td>
<td>$-1.0 \pm 0.6$</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Yield stress</td>
<td>$\sigma_{y}^\text{yield}$</td>
<td>MPa</td>
<td>$4.6 \pm 2.4$</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Toughness at yield</td>
<td>$K_{y}^\text{yield}$</td>
<td>mJ/m$^3$</td>
<td>$0.3 \pm 0.2$</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Ultimate strain</td>
<td>$\varepsilon_{u}^\text{ult}$</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Ultimate strength</td>
<td>$\sigma_{u}^\text{ult}$</td>
<td>MPa</td>
<td>$5.2 \pm 2.9$</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Toughness at failure</td>
<td>$K_{u}^\text{ult}$</td>
<td>mJ/m$^3$</td>
<td>$0.7 \pm 0.3$</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
</tr>
</tbody>
</table>
7.4 POROELASTIC AND FIBRIL-REINFORCED POROVISCOELASTIC MATERIAL MODELS

The isotropic poroelastic model poorly represented the complex relaxation behavior of the ACL ($R^2=0.806$, Figure 7.7, Table 7.6). Adding linear elastic fibrillar network to the poroelastic ground matrix did not improve the fitting ($R^2=0.803$), whereas a nonlinear elastic fibrillar network captured better the nonlinear elastic behavior ($R^2=0.938$). These models consider fluid flow as the only mechanism of relaxation and showed a small amount of relaxation, approximately 0.002 N, while experiments showed the relaxation to be roughly 100 times higher. The initial permeability $k_0$ and the permeability deformation dependency coefficient $M$, which control the fluid flow related relaxation, optimized to dissimilar values with these models and had little effect on the overall response.

The poroelastic model with a viscoelastic fibrillar network (model 4) captured the relaxation behavior better ($R^2=0.815$), but not the nonlinear elastic response. Replacing the linear elastic spring with a nonlinear one in model 5 represented better the experimental behavior ($R^2=0.978$). Defining the fibrillar network springs with an exponential function (model 6) performed almost as well as model 5 ($R^2=0.976$). Adding two-relaxation-time behavior to the fibrillar network representation (model 7) was able to capture the rapid and long-term relaxations ($R^2=0.978$). Model 8 with two-relaxation-time strain-recruited viscoelastic fibrillar network was able to reproduce the rapid and long-term relaxations, as well as the strain dependency of the peak forces and relaxation ($R^2=0.997$).
Figure 7.7: The eight material models (solid lines) fitted to experimental data (blue circles). Relaxation of the first three models is magnified. The eighth model captured best the experimental relaxation behavior ($R^2=0.997$). FN=fibrillar network.
Table 7.6: Material parameter values of different models obtained by optimization. Refer to section 3.3.4 for fibrillar network representations. $\beta$ = coefficient of determination, $\beta$ = coefficient of the exponential springs (MPa), $\beta$ = constant of the exponential springs, $\beta$ = Young's modulus of the non-fibrillar matrix (MPa), $\beta$ = initial permeability (10^-15 m^4 Pa^-1 s^-1), $\beta$ = permeability deformation dependency coefficient, $\beta$ = Young's modulus of the fibrillar network module (MPa), $\beta$ = fibrillar network module moduli (MPa), $\beta$ = constants of the exponential springs, $\beta$ = damping coefficients.

<table>
<thead>
<tr>
<th>Model</th>
<th>Non-fibrillar matrix</th>
<th>Fibrillar network</th>
<th>Fluid flow</th>
<th>Lea matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Poisson's</td>
<td>0.806</td>
<td>495.6</td>
<td>19.06</td>
<td>9.53</td>
</tr>
<tr>
<td>2. Linear elastic FN</td>
<td>0.803</td>
<td>493.33</td>
<td>22.83</td>
<td>393.33</td>
</tr>
<tr>
<td>3. Nonlinear elastic FN</td>
<td>0.938</td>
<td>498.15</td>
<td>513.21</td>
<td>561.46</td>
</tr>
<tr>
<td>4. Linear viscoelastic FN</td>
<td>0.815</td>
<td>42.19</td>
<td>1186.27</td>
<td>13.00</td>
</tr>
<tr>
<td>5. Nonlinear viscoelastic FN</td>
<td>0.978</td>
<td>0.15</td>
<td>42.19</td>
<td>8.62</td>
</tr>
<tr>
<td>6. Exponential viscoelastic FN</td>
<td>0.976</td>
<td>0.89</td>
<td>0.15</td>
<td>42.19</td>
</tr>
<tr>
<td>7. Two-relaxation-time viscoelastic FN</td>
<td>0.96</td>
<td>0.08</td>
<td>0.89</td>
<td>12261.28</td>
</tr>
<tr>
<td>8. Two-relaxation-time viscoelastic FN</td>
<td>0.96</td>
<td>0.51</td>
<td>0.08</td>
<td>12261.28</td>
</tr>
</tbody>
</table>

FN = fibrillar network, $\beta$ = Young's modulus of the non-fibrillar matrix (MPa), $\beta$ = initial permeability (10^-15 m^4 Pa^-1 s^-1), $\beta$ = permeability deformation dependency coefficient, $\beta$ = Young's modulus of the fibrillar network module (MPa), $\beta$ = fibrillar network module moduli (MPa), $\beta$ = constants of the exponential springs, $\beta$ = damping coefficients.
8 DISCUSSION

8.1 MECHANICAL PROPERTIES

The phase difference results showed that LCL was more viscous than the other ligaments at low-frequency loads. If one assumes that mechanical material properties are adapted to the loading encountered by the tissue, this indicates that at low strain rates, or close to static loads, the viscous behavior of LCL might be beneficial, providing a damping effect for the joint, or stabilization [1,2]. This could be an adaptation of the LCL to frontal plane sway, as its frequency is comparable to that used in this study [158], and this type of loading may occur in large quantities as cattle are standing for a considerable proportion of their time [159]. The observed behavior could be typical for bovine knees, and it should be clarified whether this also occurs in human knees. To the author’s knowledge, comparisons of sinusoidal test results have not been previously performed for the knee ligaments and PT. The values measured in this study were comparable to the phase difference observed for human MCL [26,87]. Higher phase difference at 0.1 Hz loading was related to the hydroxyproline (wet weight) content, and collagen viscoelasticity may be the underlying cause for this relationship. In cartilage, collagen viscoelasticity strongly influenced the relaxation behavior in tension [160]. The effect of water is possibly more important in compression [92,160] and was not observed to correlate with the phase difference. Structurally, collateral ligaments contain more small-diameter fibrils than the cruciate ligaments or the PT [12]. This may lead to a larger surface interaction area between the fibrils and the other constituents, which could influence the viscoelastic behavior.

Dynamic moduli were slightly smaller than Young’s modulus of the linear region from the quasi-static test until failure. This was unexpected, as the values should be higher due to stiffening as a function of the strain rate, as is the situation in viscoelastic tissues and seen also in the values between frequencies. The possible reason is that some samples have been in the toe region of the stress-strain curve during the sinusoidal test, whereas the Young’s modulus was always calculated from the linear portion. This could explain why the PCL had significantly higher dynamic modulus at 0.1 Hz compared with the PT, as the PCL had also a shorter toe region, placing the PCL sinusoidal test more often into the linear region. Young’s moduli of the ultimate test were not significantly different between PCL and PT, supporting that the sinusoidal test might have been more often conducted in the toe region in PT. Nevertheless, an 8 % strain where the test was performed is well within the physiological operating range.

MCL was the most non-linear at the toe region (strain dependent modulus, coefficient $F$), had the highest dynamic moduli, Young’s modulus, yield stress, ultimate strength, energy density at yield, and energy density at failure. MCL was
thus the stiffest, strongest and absorbed the most energy until yield and failure. MCL had a higher Young’s modulus than ACL, which is consistent with earlier studies [76,80,161–164]. On the other hand, MCL had higher Young’s modulus than PT, contrary to earlier studies [8,9,165]. The results suggest that MCL is well adapted to its loading environment and geometrical constraints. Its size and shape must allow for proper flexion and extension of the knee while maintaining the capability for force transmission and adequate stiffness to regulate valgus rotation.

The patellar tendon had the highest toe region strain and the smallest strain dependent modulus, which could imply that there are larger crimp angles compared with the cruciate and collateral ligaments. Bovine PT might require a long toe region for normal operation, as a large strain range could be needed at rest (flexed joint in bovines), standing and walking. Based on earlier studies [8,9,70–72,165–169], patellar tendon could be expected to have a larger Young’s modulus compared with other ligaments. Our results contradict this, but the values are similar to those of Rupp et al. [170] and Wilson et al. [171]. The difference in values and comparison with respect to Eleswarapu et al. [8] may be attributed to the age of the specimens, as we had skeletally mature knees, whereas they used one-week-old calves. The difference in comparison with studies using human PT [9,70–72,165–169] could originate from differences in anatomy. Lateral and medial patellotibial ligaments may have a more pronounced role in transmission of force from quadriceps muscle to tibia in bovine. Additionally, typical ranges of motion are different [172]. Nevertheless, animal models, notably bovine, remain typical in musculoskeletal biomechanics studies [173], due to easy availability of tissues and when considering the large animal models, the bovine knee ligaments display a good resemblance to the anatomy of the human knee ligaments [172].

The toe region strains measured in this study were higher than values observed for human [9,70,72,73,76,87] or rabbit [174] ligaments or PT but were similar to those obtained for porcine MCL [41]. These differences likely originate from compositional or structural characteristics, such as a combination of a higher elastin content and crimp angles, which may be typical for quadruped animals.

This study was the first in which coefficient $A$ (Eq. 6.5) describing the strain dependent modulus was determined for ligaments. As one might expect, the values for ligaments and PT (mean values 754–1765 MPa) were considerably higher than for bovine meniscus ($224.78 \pm 136.08$ MPa) or cartilage ($2.29 \pm 1.30$ MPa) [152]. Similarly to $A$, coefficient $F$ (Eq. 6.6) describes the nonlinearity at the toe region and both coefficients exhibited the highest value in MCL. The value of $F$ was comparable to goat MCL ($48.4 \pm 23.1$ [88]) and bovine meniscus ($31.79 \pm 9.64$ [152]), but higher as compared to bovine cartilage ($5.11 \pm 1.82$ [152]). Coefficient $D$ (Eq. 6.6), present in the exponential formula describing the toe region, exhibited similar magnitudes with goat MCL ($4.0 \pm 4.2$ MPa [88]). However, it was higher than in bovine meniscus ($0.34 \pm 0.06$ MPa [152]) or cartilage ($0.17 \pm 0.04$ MPa [152]).
The lengths of the linear region were similar among ligaments and PT, suggesting that straight collagen fibers withstand similar straining before yielding. LCL had the highest ultimate strain, though it was not as high and not as clearly different from the other ligaments as described by Smeets et al. [10], where the ultimate strain was close to 40 % in LCL but around 23 % in MCL. The energy densities measured in this study were consistent with earlier reports [9,162,165,175], but variation was high. The difference between MCL and LCL reported by Smeets et al. [10] was not observed here, possibly due to the fact that they evaluated old human samples (74 ± 7 years) whereas bovine tissues were utilized in this study.

8.2 BIOCHEMICAL CONTENTS

The water content in PT was similar to that in ACL and PCL, but higher than in MCL or LCL. This contradicts an earlier study conducted with ovine knees [12], where the water content in PT was significantly lower than in other ligaments. In addition, the water contents reported in their study (mean values ranging from 53.1% in PT to 67.7% in PCL) were lower than our values (mean values 72.0–77.6 %). Different species may contribute to these differences, as well as the small sample size in the ovine study or PBS preservation of our samples prior to the wet weight measurement. Tendons have been reported to swell in PBS [176,177], which could have elevated the water content in the present study. In addition, we used ruptured samples for the water content measurement, and consequently the tissue may have imbibed additional water. Nevertheless, these effects may be considered to affect similarly all of the samples, allowing comparisons within the present study.

Relative to dry weight, PT had significantly lower hydroxyproline content when compared with other ligaments. This finding suggests that PT has more of some other constituent, not quantified here, since the uronic acid or elastin contents (dry weight) were not higher in PT than in the other ligaments. Cruciate and collateral ligaments exhibited similar hydroxyproline (dry weight) contents, consistent with the findings of Kharaz et al. [13] where no differences were found between MCL and ACL in canine ligaments. However, Rumian et al. [12] detected a higher collagen content in collateral ligaments and PT as compared with the cruciate ligaments, but those differences were not statistically significant. The collagen content (dry weight) has been observed to be higher in PT when compared with ACL in humans [14], and when compared with MCL and the cruciate ligaments in rabbits [11]. However, Suzuki et al. [15] found no differences between PT and ACL in humans, and PT was not different from other ligaments in immature bovine specimens [8]. The comparison of the collagen content (dry weight) between knee ligaments and PT thus seems to depend on species and maturation.

If one considers the physiological function in the knee, the ligaments and PT are in a hydrated state. Therefore, the contents normalized by wet weight are of relevance. Hydroxyproline contents normalized by wet weight suggest that collateral
ligaments demand a more densely arranged collagen for normal operation. The collateral ligaments are limited in size and shape in order to enable efficient function of the knee, while transmitting forces and controlling for the varus and valgus angulations. Interestingly, PCL had a significantly higher hydroxyproline (wet weight) content than ACL, which could originate from different anatomical restrictions or different physiological loadings. Our results are similar to those reported by Mommersteeg et al. [45] in humans, where the LCL, MCL and PCL had higher hydroxyproline densities than the ACL. In bovine knees, the ACL and PCL had a lower collagen content than the LCL, MCL and PT [8], but these tissues were taken from immature animals.

The uronic acid content, reflecting the proteoglycan (PG) content, was higher in the cruciate ligaments compared with collateral ligaments, with normalization to both dry and wet weights. This most clearly highlights the differences between cruciate and collateral ligaments. PG content was concluded to facilitate fibril sliding [56], which may be required more in ACL and PCL because of the diverse loading environment. We observed uronic acid (dry weight) content to correlate positively with the water content, which implies the capability to attract water, as has also been suggested for other tissues [53–55]. Our results are in agreement with those of Kharaz et al. [13] where MCL had a lower sulphated glycosaminoglycan (sGAG) content as compared with ACL in canine knees, as well as with the results of Amiel et al. [11] where MCL and PT had lower GAG contents than cruciate ligaments in rabbits. In contrast to our values, Smith et al. [16] detected a higher sGAG content in ACL as compared with PCL in canine tissues. These discrepancies may be attributed to different species. One may argue that the discrepancy between studies may originate from different methods of estimating the PG content. However, the PG composition (PG types) in the studied tissues is likely highly similar, as they primarily act in tension [55], and therefore uronic acid and GAGs should reflect the amount of PGs in a similar manner.

The elastin content in ACL was, surprisingly, among the lowest per dry weight and the lowest per wet weight. ACL could experience more shear loading than collateral ligaments, which could then be reflected as a higher elastin content, as elastin has been postulated to govern the response to shear loading [46,47], but the opposite was observed. The elastin content was highest in PCL, which implies that PCL may encounter more transverse and shear loads, especially in comparison to the ACL. PCL and the PT had comparable elastin contents, which may indicate that in bovine PT, it is necessary to have a high restorative ability as a result of a large operating strain range. Earlier studies contradict our results, as the elastin content in canine knees was not different between the ACL and PCL [16], the MCL had a lower elastin content than the ACL [13], and the elastin content in human was higher in the ACL as compared with the PT [14].
8.3 STRUCTURE–FUNCTION RELATIONSHIPS

The significant differences observed between ligaments in Young’s modulus, yield stress, toughness at yield, ultimate strength, and toughness at failure were similarly observed in the water and hydroxyproline (wet weight contents). The MCL stands out as the stiffest ligament, having the highest collagen and lowest water content. In the phase difference results, the LCL exhibited the most viscosity at low-frequency loads, but this does not seem to be attributable to its water content. The phase difference displays a rather similar trend with collagen (wet weight), and the behavior may originate from the viscoelasticity of the collagen fibrils, supported by the positive correlation observed between phase difference and hydroxyproline (wet weight) content at 0.1 Hz loading. Viscoelasticity of collagen fibrils is a well-known property [64,83,117,118,160,178], which possibly originates from molecular interactions and fluid inside the fibrils [94]. Collagen molecules may unwind and slide relative to each other, and water molecules may flow out or rearrange inside the fibrils [83]. These findings suggest that the hydroxyproline content of wet weight, in preference to dry weight, is an important factor determining the mechanical function of the ligaments and the PT. Our results concur with the study by Mommersteeg et al. [45] where the hydroxyproline density variation was coherent with the variation in Young’s modulus observed in other studies in the ACL, PCL and MCL. In our study, however, all the significant differences between ligaments and PT in the biochemical constituents were not mirrored as significant differences in mechanical properties. This suggests that other properties, such as collagen structural organization, may affect the mechanical properties.

When investigated using multiple linear regression, it was found that the uronic acid content per dry weight significantly predicted the dynamic moduli, strain dependent modulus, Young’s modulus, yield stress, and toughness. A high uronic acid content was associated with lower values in the mechanical properties. A similar result was observed by Robinson et al. [179], where a high GAG content, normalized by dry weight, predicted a low Young’s modulus and strength in tail tendon fascicles of mice. Nonetheless, the GAGs were not observed to have an effect on viscoelastic or quasi-static properties in human MCL [25,26], PT [180], or rat tail tendons [148,181]. Hence, the correlation may not signify direct causality, indicating that PGs may indirectly affect these mechanical properties. PGs could regulate or be related with other properties causing the behavior, such as fibril diameters [179], fibril separation [57], fibril arrangement [182], fibril sliding or lubrication [56,57] or interfascicular matrix properties [183]. Although GAGs may facilitate fibril sliding and thus result in higher tissue-level strains, our results did not reveal any relationship between uronic acid and toe, yield, or ultimate strains. Though the collagen content (dry weight) could be expected to relate to Young’s modulus and strength [23], the hydroxyproline or elastin contents normalized by dry weight were not found to significantly predict any mechanical parameter.
With respect to wet weight, the hydroxyproline content negatively correlated with toe region strain, consistent with positive correlation with coefficient $D$ describing the nonlinearity of the toe region. This may imply that when there is more collagen, the constituent responsible for restoring the collagen crimp (potentially elastin) has probably a lower content, resulting in less crimped collagen and lower relative strains. The elastin content (wet weight) had a positive correlation with toe region nonlinearities ($A$ and $F$), in agreement with the finding of Henninger et al. [41] that elastin affects the toe region behavior. In the toe region, when the majority of the collagen fibers are crimped, elastin could be the primary load-bearing component. Although the elastin content was rather small relative to collagen, it did correlate positively with the dynamic moduli and Young’s modulus. This finding is not in agreement with Henninger et al. [41] as the Young’s modulus was not affected by digestion of elastin in porcine MCL. However, in agreement with our results, Millesi et al. [48] found that Young’s modulus was reduced by elastin digestion in human palmaris longus tendons. Our result seems to indicate that elastin is a load-bearing constituent in tension together with collagen, or alternatively that it affects the load transfer between fibrils, fibers or fascicles within knee ligaments and PT.

In the multiple linear regression analysis, the ligament type persisted as a significant predictor of many mechanical parameters. This occurred in cases where some tissue was distinguishable from the others, such as in phase difference at lower frequencies (LCL) or dynamic moduli (MCL). These findings suggest that the differences between ligaments were not completely explained by the hydroxyproline, uronic acid or elastin contents, and that other factors are involved in regulating the mechanical properties and that these are different between ligaments and PT.

### 8.4 POROELASTIC AND FIBRIL-REINFORCED POROVISCOELASTIC MATERIAL MODELS

Those models in which time-dependent behavior was modeled only by fluid flow (models 1-3, Table 6.1) were incapable of capturing the tissue relaxation. Initial permeability $k_0$ and the permeability deformation dependency coefficient $M$ had a negligible effect on the response (see Appendix B). The influence of $k_0$ and $M$ is expected to be more pronounced in compression [92], whereas in tension, as a result of the nearly unaltered tissue volume, their effect is negligible [160]. This possibly resulted in a large variation of these parameters in a tensile study conducted in rat Achilles tendons [103] that used optimization similar as utilized in this study. In effect, $k_0$ and $M$ have been quantified in compression for ligaments [154,184], articular cartilage [119,185] and meniscus [59]. In the computational models, relaxation related to fluid flow in tension could be increased with different values of Poisson’s ratios (see parametric analysis in Appendix B). The Poisson’s ratio of the non-fibrillar matrix influenced the response only with large negative values. Large
positive values would require anisotropic formulation of the non-fibrillar matrix. Reported values for ligaments and tendons show large variation, ranging from -2.12 to +4.26 [84,155,186–190]. The variation could however result from different experimental arrangements [191]. Therefore a definitive value, or even a range of values, of Poisson’s ratio is thus difficult to justify for ligaments and tendons. In this study, Poisson’s ratio was fixed to 0.48 (nearly incompressible), as for a subset of these samples, a bi-directional video showed nearly constant volume, also supported by the results of Vergari et al. [155].

Optimized values of $E_{nf}$ should be similar in the models with a fibrillar network, since the experimental data was the same for all optimizations. The discrepancy in the values results from the small effect of the non-fibrillar matrix on the mechanical response, the fibrillar network being much stiffer than the non-fibrillar matrix and inclusion of the effect of initially pre-strained fibrils in $E_{nf}$. Experimental values for compressive moduli of ligaments and tendons have been reported to range from 0.001 to 0.1 MPa [192–194], and optimized non-fibrillar matrix moduli from 0.0034 to 2.6 MPa [102–105]. Our results are thus in line with earlier fibril-reinforced poroviscoelastic optimizations.

Model 4 showed increased relaxation as it included a viscoelastic fibrillar network but did not capture the toe-region nonlinearity due to the linear elastic spring. The viscoelastic models with a nonlinear elastic spring (model 5) and exponential springs (model 6) better captured the nonlinear behavior. The relaxation results mainly from the relaxation of the fibrillar network in these models (models 4, 5 and 6), related with parameters $\eta_0$ and modulus $E_2$ or constants $A_2$ and $B_2$, and the optimized values are a compromise between rapid and slow relaxations. The two-relaxation-time behavior was captured by model 7, but it did not improve the fit ($R^2$-value) since the viscoelasticity did not increase with the increasing strain. Finally, the two-relaxation-time strain-recruited viscoelastic fibrillar network captured the complex relaxation behavior and fitted best of all models.

In the last model (model 8), the fibrillar network incorporated a nonlinear spring in parallel with two Maxwell elements, in which the spring moduli depended linearly on fibril strain. The nonlinear elastic spring models the elasticity of the collagen fibrils and straightening of the collagen crimp with increasing strain. The two Maxwell elements are however more complex to physically interpret, as the fibrillar network encompasses the fibrils and their interactions at and between all hierarchical levels, but these interactions (load transfer mechanisms) are still not completely understood in ligaments and tendons [67,68]. At the fibril level of hierarchy, it has been reported that the collagen fibrils exhibit a two-relaxation-time behavior [64,83]. At the collagen fiber level, the fibers show a small relaxation [64–66]. Between fibers, the relaxation (fiber sliding) exhibits a two-relaxation-time behavior, and it contributed most to tendon fascicle relaxation [64,66]. Additionally, the characteristic fibril relaxation times agreed with the fascicle level relaxation times [64]. The two Maxwell elements in the fibrillar network formulation, therefore,
represent the combined rapid and slow relaxation behavior at all hierarchical levels, i.e., at between-fiber and fibril levels. The damping components of the Maxwell elements affect the relaxation behavior, with higher damping coefficient values increasing the respective relaxation time. We hypothesize the rapid initial relaxation to stem mainly from between-fiber sliding [64,66], and the slow relaxation to originate principally from the fibril level [64,83], possibly from fluid and molecular interactions inside the fibrils [94].

8.5 LIMITATIONS

Samples for tensile testing were cut from the mid-substance of the tissues. The anteromedial bundle was used in ACL, but in bovine knees, the bundle division was not always clear. PCLs had no distinguishable bundles. The cutting was performed along fascicles by positioning the tool manually based on visual cues. This may lead to cutting of edge fibers and splitting of edge fascicles. Moreover, different fascicle diameters and arrangements may lead to differences in the cut-edge effects. Nonetheless, the samples included several intact fascicles, diminishing the effect of possible damaged edge fibers and fascicles. To further mitigate errors due to cutting, the same tool was used by the same individual for all the samples. Small dumbbell-shaped samples were used to obtain material properties reliably at a mesoscopic level using constant shape and size. In bone-ligament-bone complexes, cross-sectional area and length determination may be challenging, resulting in unreliable material properties.

However, the drawback of using samples without bone is that clamping in the tensile test produces a complex stress state near the clamps and the tissue may occasionally rupture outside the mid-substance. The rupture location was often difficult to determine or indistinguishable (Figure 7.5) and therefore a statistical assessment was performed for both yield and ultimate data to identify if there had been any early ruptures. These results were distributed normally, according to the Shapiro-Wilk test and according to Grubb’s test, no outliers were detected indicative of early ruptures. All samples were thus included in further analyses, but caution is needed when investigating yield and ultimate data of this study.

Bovine tissues were used in this thesis. The results may therefore not be directly valid to human knees, as the stifle joint dimensions and typical ranges of motion are different between cows and humans. Knowledge of the mechanical properties in combination with biochemical data is however very useful when investigating structure–function relationships. The biochemical composition of human and bovine knee ligaments and PT is similar. A textbook value for collagen content (dry weight) is 70-80 % [3], while immature bovine had 71-87 % [8], and in this study, we obtained 83-98 %, assuming that hydroxyproline constitutes 14 % of collagen [39]. It is worthwhile mentioning that several other studies have used similar bovine stifle joints as utilized here, when investigating cartilage and meniscus [152,160,195,196].
The bovine stifle joint was chosen for easy availability and best resemblance with the human knee joint in terms of ligament anatomy, in comparison with other common large animal models [172].

If one wishes to completely describe structure–function relationships in knee ligaments and PT, also other factors may influence the mechanical properties in addition to the water, hydroxyproline, uronic acid, and elastin contents. These factors include elastic fibers fibrillin I and II, collagen crosslinks [197,198], crimp angle, crimp length [199], collagen fibril orientation dispersion [200,201], collagen fibril diameter [12,202] and fascicle diameter. Collagen organization, and the interactions within and between different levels of hierarchy may be of high importance.

The amount of collagen was evaluated in terms of the hydroxyproline content, and it was assumed that the level of proline hydroxylation is similar among the ligaments and the PT [1]. However, the topic is not well known, and minor variations may exist. Furthermore, the storage of the samples in the freezer could arguably have changed their structural or mechanical properties. However, there were no differences in either the mid-substance PG content or mechanical properties between fresh rat Achilles tendons and specimens that had been stored frozen for 9-months [203], and therefore we consider the effect of storage to be small in our samples.

In the multiple linear regression analysis, the water content was not used as a predictor as it correlated with other contents. Thus, the results do not elucidate the importance of water in determining the tissue’s mechanical properties. For instance, when water was included in the dry weight analysis, it predicted toe region strain and coefficients describing the toe region (A and D), implying that it facilitates fibril sliding at the toe region (see supplementary material of study II).

Shear, transverse or compressive properties were not determined in this study. It may be important to consider these properties in computational modeling, and they could be more accurately implemented in the fibril-reinforced poroviscoelastic model presented here with a transversely isotropic non-fibrillar matrix [102–104], or with secondary fibrils in the fibrillar network [22,119]. The model presented in this study applies to the toe-region of the stress-strain curve, but it may be extended to the linear region by formulating the elastic part of the fibrillar network as linear after the toe region end point. In that case, the viscoelastic properties would also need to be investigated in the linear region.

The fibril-reinforced poroviscoelastic model developed in this thesis is a continuum model. It thus neglects the hierarchical structure but incorporates its effects in the fibrillar network formulation. The model is therefore unable to reveal mechanisms of force transmission or relaxation, which are likely related to different levels of hierarchy. The matrix material was modeled as a porous isotropic neo-Hookean material, though its composition may differ at different hierarchical levels [204,205] and its properties may be anisotropic or vary in the tissue [194,206]. If one wishes to perform multiscale modeling of different hierarchical levels and
interactions, then a molecular dynamics approach may be more efficient instead of continuum modeling.

### 8.6 FUTURE WORK

The finding of this study that LCL is more viscous than other ligaments at low-frequency loading should be investigated in humans. If it translates into humans, it might be important to consider for example in the modeling of the whole knee joint.

Regarding structure-function relationships, additional properties to the ones studied in this thesis should be considered to gain a more complete understanding of the origins of the mechanical material properties. These additional properties include compositional parameters, such as fibrillin I and II, and collagen crosslinks, and structural parameters, such as crimp angle and length, collagen fibril orientation dispersion, collagen fibril diameter and fascicle diameter. It is also poorly known how the load is transferred within and between different hierarchical levels.

In order to create a material model that comprehensively describes the ligament material behavior at the tissue level, also other properties than uniaxial tension should be considered. Experimental data would be needed in tension, compression and shear in all directions. Permeability and the non-fibrillar matrix are likely anisotropic [194,206], and should be modeled accordingly. As indicated earlier, these could be implemented into the current model by using, e.g., a transversely isotropic non-fibrillar matrix. Furthermore, the model should be applied to all the knee ligaments and PT to test its applicability and validity. Preliminary analyses indicate excellent suitability for all primary bovine knee ligaments and PT in uniaxial tension.
9 SUMMARY AND CONCLUSIONS

In this thesis, the mechanical and biochemical properties of bovine knee ligaments and patellar tendon were characterized and compared, and structure–function relationships were examined. A novel fibril-reinforced poroviscoelastic model was developed for the anterior cruciate ligament.

The main conclusions of this thesis may be summarized as follows:

1. Bovine LCL was the most viscous at low-frequency loads while MCL displayed the highest toe region nonlinearity, Young’s modulus, and toughness. The MCL had the highest collagen content, whereas cruciate ligaments exhibited the highest amount of proteoglycans. The findings most likely reflect adaptations to specific physiological functions, anatomical restrictions, and loading regimes.

2. The proteoglycan content, though low in magnitude, significantly predicted Young’s modulus, yield stress and toughness, while the elastin content was related to toe region nonlinearity and Young’s modulus. These findings indicate an important functional role of proteoglycans and elastin in ligaments and tendons, possibly influencing load transfer within the tissues.

3. The fibril-reinforced poroviscoelastic model with the developed nonlinear two-relaxation-time strain-recruited viscoelastic fibrillar network successfully represented the tissue level viscoelastic behavior of anterior cruciate ligament. This model may be used for ligaments in computational models of the whole knee and to investigate the effects of degradation of tissue constituents on the mechanical response.

This thesis brought novel information on the mechanical and biochemical properties, as well as on structure–function relationships of bovine knee ligaments and patellar tendon, and established a novel computational model. The results may bring improvement for computational modeling of the knee, provide more understanding of the effect of composition on ligament and tendon function and also highlight the differences among these tissues. In addition, the gained information will be useful in tissue engineering, design of artificial ligaments and tendons, design of knee joint replacements, development of clinical imaging methods and computational modeling of fiber-reinforced materials. The novel information brought by this research, together with other research efforts, will eventually lead to improved joint disease prevention, diagnostics and treatment, contributing to a better quality of life of people.
10 BIBLIOGRAPHY


G.G. Handsfield, L.C. Slane, H.R.C. Screen, Nomenclature of the tendon hierarchy: An


[106] M.H. Holmes, V.C. Mow, The nonlinear characteristics of soft gels and hydrated
connective tissues in ultrafiltration, J. Biomech. 23 (1990) 1145–1156.


APPENDICES

APPENDIX A: EQUATIONS OF FIBRIL STRESS FOR FIBRILLAR NETWORK FORMULATIONS

The derivation of the equations for fibril stress for fibrillar network formulations of section 3.3.4 is presented here for the fibrils developed in this study. While the equations for linear elastic and nonlinear elastic fibril stresses are trivial ($\sigma_f = E_0 \varepsilon_f$), for the linear viscoelastic fibril stress (model 4 in this thesis) the reader should refer to Wilson et al. [121] and for the exponential viscoelastic fibril stress (model 6) to Wilson et al. [123].

The nonlinear viscoelastic fibril (model 5) is presented in Figure A.1, with an additional linear spring in parallel, which may be required in future investigations. In this thesis, the $E_0$ was set to zero.

![Figure A.1: Nonlinear viscoelastic fibril.](image)

The fibril stress is given by

$$\sigma_f = E_1 \varepsilon_f^2 + E_0 \varepsilon_f + \eta_0 \dot{\varepsilon}_v .$$  \hspace{1cm} (A.1)

In the Maxwell element

$$\varepsilon_f = \varepsilon_e + \varepsilon_v ,$$  \hspace{1cm} (A.2)

$$\varepsilon_v = \varepsilon_f - \varepsilon_e ,$$  \hspace{1cm} (A.3)

$$\dot{\varepsilon}_v = \dot{\varepsilon}_f - \dot{\varepsilon}_e .$$  \hspace{1cm} (A.4)

To calculate $\dot{\varepsilon}_e$, in the Maxwell element the stress $\sigma_e$ is
\[
\sigma_e = E_2 \varepsilon_e^2, \quad (A.5)
\]
from which
\[
\varepsilon_e = \sqrt{\frac{\sigma_e}{E_2}}, \quad (A.6)
\]
which yields for the time derivative
\[
\dot{\varepsilon}_e = \frac{1}{2} \frac{\sigma_e^{-1/2}}{\sqrt{E_2}} \dot{\sigma}_e = \frac{\dot{\sigma}_e}{2 \sqrt{\sigma_0 E_2}}. \quad (A.7)
\]
To calculate \(\dot{\sigma}_e\), the Maxwell element stress may also be calculated as
\[
\sigma_f = E_1 \varepsilon_f^2 + E_0 \varepsilon_f + \sigma_e, \quad (A.8)
\]
\[
\sigma_e = \sigma_f - E_1 \varepsilon_f^2 - E_0 \varepsilon_f. \quad (A.9)
\]
from which
\[
\dot{\sigma}_e = \dot{\sigma}_f - 2E_1 \varepsilon_f \dot{\varepsilon}_f - E_0 \dot{\varepsilon}_f. \quad (A.10)
\]
Substituting back to equation A.1 the fibril stress becomes
\[
\sigma_f = E_1 \varepsilon_f^2 + E_0 \varepsilon_f + \eta_0 \left( \dot{\varepsilon}_f - \frac{\dot{\sigma}_f - 2E_1 \varepsilon_f \dot{\varepsilon}_f - E_0 \dot{\varepsilon}_f}{2 \sqrt{\sigma_f E_2 - E_0 \varepsilon_f}} \right). \quad (A.11)
\]
For numerical implementation it must be noted that
\[
\dot{\sigma}_f = \frac{\sigma_f - \sigma_f, \text{old}}{\Delta t}, \quad (A.12)
\]
and
\[
\dot{\varepsilon}_f = \frac{\varepsilon_f - \varepsilon_f, \text{old}}{\Delta t}, \quad (A.13)
\]
where subscript \text{old} denotes the value from the previous step and \(\Delta t\) is the time increment.

For the fibrils incorporating two-relaxation-time behavior (models 7 and 8), it is beneficial to first derive the equation for a single Maxwell element. For a Maxwell element with elastic modulus of \(E_i\) and damping coefficient \(\eta_i\), we know that
\[
\dot{\varepsilon}_f = \frac{\dot{\sigma}_f}{E_i} + \frac{\sigma_i}{\eta_i}, \quad (A.14)
\]
from which we obtain
\[
\sigma_i = \eta_i \dot{\varepsilon}_f - \frac{\eta_i \dot{\sigma}_i}{E_i}. \quad (A.15)
\]
In the view of numerical implementation, inserting \(\dot{\sigma}_i = \frac{\sigma_i - \sigma_i, \text{old}}{\Delta t}\) yields
\[
\sigma_i = \eta_i \dot{e}_f - \frac{\eta_i}{E_i \Delta t} \sigma_i + \frac{\eta_i}{E_i \Delta t} \sigma_{i,old}, \tag{A.16}
\]

which can be rearranged as
\[
\sigma_i = \frac{E_i \Delta t \eta_i \dot{e}_f + \eta_i \sigma_{i,old}}{E_i \Delta t + \eta_i}, \tag{A.17}
\]

and further as
\[
\sigma_i = \frac{E_i \Delta t \eta_i \dot{e}_f + \eta_i \sigma_{i,old}}{E_i \Delta t + \eta_i}. \tag{A.18}
\]

The nonlinear two-relaxation-time viscoelastic fibril (model 7) is presented in Figure A.2, with an additional linear spring in parallel, which may be required in future investigations. In this thesis, the \( E_0 \) was set to zero.

![Figure A.2: Nonlinear two-relaxation-time viscoelastic fibril.](image)

The fibril stress arises from the sum of the springs and Maxwell elements, and the fibril stress for model 7 is given by
\[
\sigma_f = E_0 \varepsilon_f + E_1 \varepsilon_f^2 + \frac{E_3 \eta_1 \Delta t}{E_3 \Delta t + \eta_1} \dot{e}_f + \frac{E_4 \eta_2 \Delta t}{E_4 \Delta t + \eta_2} \dot{e}_f + \frac{\eta_1 \sigma_{i,old}}{E_i \Delta t + \eta_i}. \tag{A.19}
\]

The nonlinear two-relaxation-time strain-recruited viscoelastic fibril (model 8) is presented in Figure A.3, with an additional linear spring in parallel, which may be required in future investigations. In this thesis, the \( E_0 \) was set to zero. In this strain-recruited model, the stress of a single Maxwell element is
\[
\sigma_i = \frac{E_i \varepsilon_f \eta_i \Delta t}{E_i \varepsilon_f \Delta t + \eta_i} \dot{e}_f + \frac{\eta_i \sigma_{i,old}}{E_i \varepsilon_f \Delta t + \eta_i}. \tag{A.20}
\]
The fibril stress arises from the sum of the springs and Maxwell elements, and the fibril stress for model 8 is given by

\[
\sigma_f = E_0 \varepsilon_f + E_1 \varepsilon_f^2 + \frac{E_5 \varepsilon_f \eta_1 \Delta t}{E_5 \varepsilon_f \Delta t + \eta_1} \dot{\varepsilon}_f + \frac{\eta_1 \sigma_{1,old}}{E_5 \varepsilon_f \Delta t + \eta_1} + \frac{E_6 \varepsilon_f \eta_2 \Delta t}{E_6 \varepsilon_f \Delta t + \eta_2} \dot{\varepsilon}_f + \frac{\eta_2 \sigma_{2,old}}{E_6 \varepsilon_f \Delta t + \eta_2}. \tag{A.21}
\]
APPENDIX B: EFFECT OF INITIAL PERMEABILITY, PERMEABILITY DEFORMATION DEPENDENCY COEFFICIENT, WATER CONTENT AND POISSON’S RATIO OF THE NON-FIBRILLAR MATRIX ON THE STRESS-RELAXATION RESPONSE

Figure B.1 shows a parametric study on the effect of initial permeability $k_0$ and permeability deformation dependency coefficient $M$ on the force-time output of models 4 and 8. Their effect is negligible in tensile stress relaxation. Figure B.2 shows the effect of water content and Poisson’s ratio of the non-fibrillar matrix on the force-time output of models 4 and 8. The effect of water content is negligible, whereas the Poisson’s ratio has an effect with large negative values. Figure B.3 shows a parametric study on the effect of initial permeability $k_0$ and permeability deformation dependency coefficient $M$ on the force-time output of models 4 and 8, similarly to Figure B.1. In Figure B.3, the Poisson’s ratio was set to -0.9 to investigate whether $k_0$ and $M$ have more effect when there is more volume change. The initial permeability has a pronounced effect only on very small values, which may however be unrealistic in view of the reported values of $2.942 \times 10^{-15} \text{m}^4 \text{N}^{-1} \text{s}^{-1}$ for human MCL [154] and $4.08 \times 10^{-15} \text{m}^4 \text{N}^{-1} \text{s}^{-1}$ for porcine MCL [184]. Permeability deformation dependency coefficient $M$ has a small effect on the overall response. These results further support our decision to fix these parameters for material parameter optimization of models 4-8.
Figure B.1: Parametric study on the effect of initial permeability $k_0 \left(10^{-15} \text{ m}^4 \text{N}^{-1} \text{s}^{-1}\right)$ and permeability deformation dependency coefficient $M$ on the force-time output of models 4 and 8. Small figures highlight the peak of 3rd step. The arrows show the effect of increasing the value. The effect of these parameters on the output is negligible, and thus they were fixed to $k_0 = 2.942 \times 10^{-15} \text{ m}^4 \text{N}^{-1} \text{s}^{-1}$ and $M = 7.988$ [154] for the models 4-8. FN=fibrillar network.
Figure B.2: Parametric study on the effect of water content and Poisson’s ratio of the non-fibrillar matrix on the force-time output of models 4 and 8. Small figures highlight the peak of 3rd step. The arrows show the effect of increasing the value. The effect of water content is negligible, and thus it was fixed to 0.776 based on the average water mass fraction of 10 ACL samples. Poisson’s ratio of the non-fibrillar matrix has an effect only with large negative values. It was fixed to $\nu_{nf} = 0.48$ since a bi-directional video showed approximately constant volume for a subset of samples; in line with results of Vergari et al. [155].
Figure B.3: Parametric study on the effect of initial permeability \( k_0 \) \( (10^{-15} \ m^4 N^{-1} s^{-1}) \) and permeability deformation dependency coefficient \( M \) on the force-time output of models 4 and 8, when the Poisson’s ratio is set to -0.9, inducing more volume change. The arrows show the effect of increasing the value. The initial permeability has a pronounced effect only on very small values, which may however be unrealistic in view of the reported values of \( 2.942 \times 10^{-15} \ m^4 N^{-1} s^{-1} \) for human MCL [154] and \( 4.08 \times 10^{-15} \ m^4 N^{-1} s^{-1} \) for porcine MCL [184]. Permeability deformation dependency coefficient \( M \) has a small effect on the overall response. These results further support our decision to fix these parameters for material parameter optimization of models 4-8. FN=fibrillar network.
AAPO RISTANIEMI

The structure–function relationships of knee joint ligaments and patellar tendon are incompletely understood. In this thesis, the mechanical and biochemical properties of bovine knee ligaments and patellar tendon were characterized and compared, and structure–function relationships were examined. A novel fibril-reinforced poroviscoelastic model was developed for the anterior cruciate ligament. The results may bring improvement for computational modeling of the knee, provide more understanding of the effect of composition on ligament and tendon function and also highlight the differences among these tissues.