A MODEL TO STUDY ARTICULAR CARTILAGE MECHANICAL AND BIOLOGICAL RESPONSES TO SLIDING AND ROLLING LOADS

A thesis submitted to attain the degree of

DOCTOR OF SCIENCES of ETH ZURICH

(Dr. sc. ETH Zurich)

presented by

OLIVER ROBERT SCHÄTTI

M.Sc. Human Movement Sciences, ETH Zurich

born on 26.09.1984

citizen of Lachen, SZ, Switzerland

accepted on the recommendation of

Prof. Dr. Jess G. Snedeker

Prof. Dr. Luigi M. Gallo

Dr. Peter A. Torzilli

2016
Acknowledgments

I would like to express my special thanks to all the people that directly or indirectly contributed to this work. This entire research project would not have been possible without their support.

First of all I, would like to sincerely thank Prof. Luigi Gallo for giving me the possibility to work on such an interesting project. With his invaluable support and assistance he allowed me to scientifically and personally grow. I also want to express my deep gratitude to Dr. Peter Torzilli. His knowledge and passion were a great source of motivation and inspiration. He is not only an excellent teacher and mentor but also a great human being. Thank you for letting me work in your lab and for all your invaluable guidance and support. Many thanks to Prof. Jess Snedeker for giving me the opportunity to do my dissertation at ETH Zurich and supervising my work. I am very thankful to all my colleagues at Hospital for Special Surgery for their help and support. In particular I would like to thank Dr. Patrick Donnelly, Dr. Tony Chen, Dr. Miguel Otero, Dr. Supansa Yodmuang and Camila Carballo for answering all my questions, helping me to establish research protocols and for being friends. Moreover, many thanks to Lilly Ying who would always help me with histological analyses. Likewise, I want to thank the members of the KFS team for their support and help in particular Dr. Vera Colombo, Michala Markova, Stefan Erni, Eveline Studer, Dominique Waldvogel and Pia Hawkins for being such great people to work with. In addition, many thanks to Prof. Franz Weber, Dr. Chafik Ghayor and all the members of the Oral Biotechnology Lab for their great support.

Finally, I am deeply indebted to my parents for their unconditional support, encouragement and trust over all these years. Equally, I owe my deepest gratitude to my wife Nadja and her tireless support, understanding and unconditional love. Thank you for being the best partner one can imagine.
Abstract

Articular cartilage is a tissue with a highly specialized extracellular matrix (ECM) that covers the end of long bones. The interplay of collagen fibers, proteoglycan macromolecules and interstitial fluid within the ECM allow for redistribution of complex mechanical loads and wear resistant and low-frictional motion. Under normal circumstances, the tissue withstands millions of cycles of loading during one’s lifetime. However, overuse or traumatic incidents are often associated with progressive ECM breakdown and loss of function eventually resulting in joint diseases such as osteoarthritis (OA). As OA is accompanied with a high social and economic burden, research has been conducted in order to understand the pathological processes from a mechanical and biological point of view.

Various in vitro and in vivo models have been developed aiming to simulate the mechanical processes leading to cartilage ECM degradation under mechanical load. Mostly uni-axial compression or shear forces have been applied to small cartilage explant before investigating the cartilage’s biological response. While these studies undoubtedly revealed many basic mechanism of joint degeneration, their uni-axial modeling of joint mechanics is a limitation. As has become increasingly clear, articular joints are subjected to a complex combination of compressive and tribological stimuli.

Therefore, the goal of the present thesis was to apply multi-axial mechanical stimuli to the surface of articular cartilage explants. Focus was put on a thorough analysis of the strains and stresses imposed on the cartilage ECM which was subsequently correlated with the studied biological changes. The multi-axial load consisted of the application of simultaneous compression and transversal motion of an indenter over the cartilage surface in order to mimic the complex mechanical environment of an articular joint. Focus was put on the investigation of different axial forces, sliding speeds and indenter curvatures and their effect on the mechanobiological response of the tissue. After the application of the load, the biological response of the tissue was quantified by analyzing matrix integrity, gene expression changes and the loss of matrix components. Attempts were made to correlate the mechanical impact on the ECM with the observed biological changes in order to determine loading schemes that have a detrimental effect on the cartilage tissue. This thesis encloses three studies; one was conducted on bovine nasal septum cartilage and the other two with articular cartilage obtained from bovine femoral
condyles. The latter two are considered an improvement over the first study as the account for the curvature of articular cartilage.

We found that an increase in axial load resulted in increasing ECM strains, contact stresses and effective moduli. In addition, according to the poro-elastic nature of the cartilage, increasing the sliding speed of the indenter from 1 mm/s to 20 mm/s resulted in decreasing deformation and increasing contact stresses and effective moduli. However, no changes in these parameters were found once sliding speeds where further increased. Furthermore, reducing the curvature of the indenter resulted in similar results. From a biological perspective, sliding resulted in alterations of gene expression patterns with the regulation of catabolic markers and the loss of proteoglycan molecules. This response was particularly observed at high deformations in combination with high contact stresses around 10 MPa.

In conclusion, this thesis presents a new model to investigate mechanically-driven degenerative changes in articular cartilage. The application of physiologically more relevant cues is a clear advantage over previous studies. The results obtained will provide further knowledge to understand articular cartilage’s reaction to mechanical loads. A profound understanding of the tribological characteristics of articular motion is essential to understand degenerative joint diseases, and to develop therapies for their treatment.
Zusammenfassung

Der Gelenkknorpel ist ein Gewebe mit einer hochspezialisierten extrazellulären Matrix (EZM) und ein essentieller Bestandteil jedes Gelenkes. Das Zusammenspiel von Kollagenfasern, Proteoglykannen und Interstitialflüssigkeit ermöglicht die Verteilung von komplexen mechanischen Kräften sowie eine widerstandsfähige und reibungslose Artikulation.

Unter normalen Umständen hält das Gewebe Millionen von Belastungszyklen stand. Traumatische Vorfälle und Überbelastung sind jedoch häufige Faktoren, die zu einem progressiven Abbau der EZM und dadurch zum Verlust ihrer Funktionen führt, was in schwerwiegenden Fällen in Osteoarthrose (OA) resultieren kann. OA kann zu einer grossen Einschränkung der Lebensqualität und hohen finanziellen Kosten führen. Aus diesem Grund wurde in den letzten Jahren viel Forschung betrieben um die Entstehungsmechanismen der Knorpeldegeneration aus einer mechanischen und biologischen Perspektive besser zu verstehen.

Mit dem Ziel die Vorgänge zu simulieren, die unter mechanischer Last zum Knorpelabbau führen, wurden verschiedenartige in vitro und in vivo Modelle erarbeitet. Das Prinzip der Modelle besteht darin, uniaxiale Kompression und Scherkräfte auf Knorpelexplantate zu übertragen um die darauffolgend biologischen Veränderungen im Gewebe zu analysieren. Während diese Studien viel dazu beitrugen grundlegende Mechanismen der Knorpeldegeneration zu verstehen, haben sie den Nachteil, dass sie die multi-axiale Kräfte welche auf den Knorpel wirken nur eingeschränkt repräsentieren.


Die multi-axiale Gelenksbewegung wurde durch einen Indenter erzeugt, welcher auf eine Knorpeleoberfläche gepresst und gleichzeitig horizontal verschoben wird. Bei unseren Versuchen beschränkten wir uns auf den Einfluss von axialer Kraft, Gleitgeschwindigkeit und Indentergeometrie auf die biomechanische Reaktion des Knorpels. Gleich nach dem mechanischen Test wurde die biologische Antwort des Gewebes mithilfe von gängigen Techniken wie histologischen Färbungen, Genexpressionsanaly-


Contents

1 Introduction ............................................. 1
  1.1 Articular Cartilage Composition and Structure ......................... 1
    1.1.1 Articular Cartilage Composition .................................. 1
      1.1.1.1 Chondrocytes: The Local Cell Type .......................... 2
      1.1.1.2 Collagen .................................................. 2
      1.1.1.3 Proteoglycans .......................................... 3
      1.1.1.4 Water .................................................... 3
      1.1.1.5 Non-collagenous Matrix Proteins ............................ 4
    1.1.2 Articular Cartilage Structure .................................... 5
      1.1.2.1 Superficial Zone ......................................... 5
      1.1.2.2 Middle Zone ............................................ 6
      1.1.2.3 Deep Zone ............................................. 6
      1.1.2.4 Calcified Zone ......................................... 7
      1.1.2.5 Subchondral Bone ...................................... 7
  1.2 Articular Cartilage Biomechanical Properties and Function .............. 7
    1.2.1 Biomechanical Properties ...................................... 7
    1.2.2 Lubrication Mechanisms ....................................... 9
      1.2.2.1 Fluid Film or Hydrodynamic Lubrication ..................... 9
      1.2.2.2 Boundary Lubrication ................................ 10
      1.2.2.3 Articular Cartilage-Specific Lubrication Modes .......... 11
      1.2.2.4 Summary of Cartilage Lubrication .......................... 12
  1.3 Articular Cartilage Degeneration and Osteoarthritis ....................... 13
    1.3.1 Osteoarthritis ............................................... 13
    1.3.2 Post-Traumatic Osteoarthritis .................................. 14
    1.3.3 Pathomechanics Due to Joint Injury ................................ 14
1.3.4 Osteoarthritic Changes in Articular Cartilage ........................................... 15
1.4 Experimental Models for Mechanically-Induced Osteoarthritis .................. 18
  1.4.1 Mechanobiological Models and Loading Devices ................................. 18
    1.4.1.1 Uni-Axial Loading Systems ..................................................... 18
    1.4.1.2 Multi-Axial Loading Systems .................................................. 19
  1.4.2 Biomechanical Degeneration ............................................................... 20
  1.4.3 Biochemical Degeneration ................................................................. 21
1.5 Motivation and Aim of Thesis ................................................................. 21

2 Mechanical Loading of Cartilage Explants with Compression and Sliding Motion Modulates Gene Expression of Lubricin and Catabolic Enzymes ........ 25
  2.1 Abstract ........................................................................................................ 25
  2.2 Introduction .................................................................................................... 26
  2.3 Methods ......................................................................................................... 27
    2.3.1 Cartilage Explants .................................................................................. 27
    2.3.2 Mechanical Loading ............................................................................... 28
    2.3.3 Calculations ............................................................................................ 28
    2.3.4 Gene Expression Analysis ...................................................................... 30
    2.3.5 Statistics ................................................................................................. 31
  2.4 Results ........................................................................................................... 32
    2.4.1 Initial Loading Phase ............................................................................. 32
    2.4.2 Mechanical Parameters During Sliding Phase ....................................... 33
    2.4.3 Correlative Analysis ............................................................................. 33
  2.5 Discussion ....................................................................................................... 34

3 A Model to Study Articular Cartilage Mechanical and Biological Responses To Sliding Loads ................................................................................. 41
  3.1 Abstract ........................................................................................................... 41
  3.2 Introduction ..................................................................................................... 42
  3.3 Materials and Methods ................................................................................... 44
    3.3.1 Device and Components ...................................................................... 44
    3.3.2 DACTS Validation ............................................................................... 44
    3.3.3 Stiffness/Compliance of the System .................................................... 45
    3.3.4 Corrections for the Curvature of the Condyle ....................................... 45
5 General Discussion

5.1 The Influence of Axial Load, Speed and Curvature on Cartilage Mechanics ............. 76
  5.1.1 Axial Load and Mechanical Parameters ............................................. 76
  5.1.2 Sliding Speed and Mechanical Parameters ......................................... 77
  5.1.3 Indenter Curvature and Mechanical Parameters .................................... 80

5.2 The Influence of Load, Speed and Curvature on Cartilage Biology ................. 80
  5.2.1 The Influence of Axial Load and Translation Speed on Cartilage Biology .. 81
  5.2.2 The Influence of Indenter Curvature on Cartilage Biology .................... 83

5.3 Limitations ............................................................................................... 84

5.4 Summary and Conclusions ......................................................................... 85

5.5 Perspectives ............................................................................................... 86
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Structural components of an aggrecan molecule</td>
<td>4</td>
</tr>
<tr>
<td>1.2</td>
<td>Collagen-aggrecan network</td>
<td>5</td>
</tr>
<tr>
<td>1.3</td>
<td>Depth-dependent cartilage morphology</td>
<td>7</td>
</tr>
<tr>
<td>1.4</td>
<td>Schematic drawing of different lubrication regimes</td>
<td>12</td>
</tr>
<tr>
<td>1.5</td>
<td>Healthy vs. osteoarthritic cartilage</td>
<td>17</td>
</tr>
<tr>
<td>2.1</td>
<td>Loading setup of the rolling plowing explant test system (RPETS)</td>
<td>29</td>
</tr>
<tr>
<td>2.2</td>
<td>Biological sampling area</td>
<td>30</td>
</tr>
<tr>
<td>2.3</td>
<td>Cell viability of explanted bovine nasal cartilage</td>
<td>32</td>
</tr>
<tr>
<td>2.4</td>
<td>Typical stress-strain diagrams of the initial loading/compression-phase</td>
<td>33</td>
</tr>
<tr>
<td>3.1</td>
<td>Dynamic articular cartilage test system (DACTS)</td>
<td>45</td>
</tr>
<tr>
<td>3.2</td>
<td>Drawing to illustrate the correction for the condylar curvature</td>
<td>46</td>
</tr>
<tr>
<td>3.3</td>
<td>Explanted bovine femoral condyle with sampling locations</td>
<td>49</td>
</tr>
<tr>
<td>3.4</td>
<td>Variations in strain due to changes in axial load and sliding speed</td>
<td>51</td>
</tr>
<tr>
<td>3.5</td>
<td>Variations in contact stress due to changes in axial load and sliding speed</td>
<td>51</td>
</tr>
<tr>
<td>3.6</td>
<td>Variations in dynamic effective modulus due to changes in axial load and sliding speed</td>
<td>52</td>
</tr>
<tr>
<td>3.7</td>
<td>Scatterplots showing gene expression vs. individual mechanical parameters</td>
<td>53</td>
</tr>
<tr>
<td>3.8</td>
<td>Strain, contact stress and dynamic effective modulus during dynamic sliding</td>
<td>57</td>
</tr>
<tr>
<td>4.1</td>
<td>Setup of test system using two different-sized indenters and sampling locations on condyle</td>
<td>63</td>
</tr>
<tr>
<td>4.2</td>
<td>Calculated mechanical parameters</td>
<td>67</td>
</tr>
<tr>
<td>4.3</td>
<td>Relative gene expression levels of the genes investigated</td>
<td>70</td>
</tr>
<tr>
<td>4.4</td>
<td>Proteoglycan loss for individual condyles</td>
<td>71</td>
</tr>
</tbody>
</table>
# List of Tables

2.1 Forward and reverse primers (study 1) ........................................... 31
2.2 Duration of individual phases of the loading/unloading cycle ............ 32
2.3 Calculated mechanical parameters ............................................... 34
2.4 Multiple linear regression: coefficients ....................................... 35
2.5 Multiple linear regression: significances ..................................... 36
3.1 Forward and reverse primers (Study 2) ........................................ 49
3.2 DACTS: Load accuracy during dynamic loading ............................ 50
4.1 Forward and reverse primers (Study 3) ........................................ 66
4.2 Calculated mechanical parameters for individual condyles (gene analysis) ............................................. 68
4.3 Calculated mechanical parameters for individual condyles (PG loss analysis) ............................................ 69
4.4 Univariate regression analysis ................................................... 73
Chapter 1

Introduction

1.1 Articular Cartilage Composition and Structure

1.1.1 Articular Cartilage Composition

Diarthrodial joints such as hip, knee and jaw are the basis of our ability to move and provide the agility for many tasks of daily living. Based on their location in an enclosed joint capsule which is filled with synovial fluid they are also termed synovial joints. As natural bearings, articular joints provide almost frictionless motion attributed to the functional properties of the cartilage extracellular matrix (ECM) and its interaction with the other synovial components. Articular cartilage covers the end of the long bones that form the joints. It is a compliant tissue that enables an efficient distribution of the complex mechanical loads acting across the joint during movement. Studies on articular cartilage date back to 1742 [Hunter, 1742]. The adult tissue is only barely populated by cells and lacks the presence of blood vessels and neural innervation, and thus articular cartilage was erroneously regarded as a simple tissue with low metabolic activity. However, after research intensified and new techniques became available, a complex heterogeneous and anisotropic ECM structure was found that is tightly regulated by the local cells. Different zones in the ECM depth have been determined and led to the conclusion that the articular cartilage is in fact a mechanically, biologically and rheologically complex structure [Benninghoff, 1925, McCutchen, 1962, Muir et al., 1970, Stockwell, 1971]. The unique mechanobiological properties of articular cartilage are related to the hydrogel-like structure composed of collagen fibers and proteoglycan macromolecules that forms a specialized ECM. Together with the synovial fluid that surrounds the matrix, the tissue attains the functional properties which makes it perfectly adapted to withstand the harsh physical environment present in articular joints. The following sections will briefly introduce the main components of the ECM, how they integrate into the overall structure and their role for articular cartilage function.
1.1.1.1 Chondrocytes: The Local Cell Type

Despite recent evidence for stem/progenitor cells in articular cartilage [Jiang and Tuan, 2015], the chondrocyte is the predominant cell type found in articular cartilage. During chondrogenesis in embryonic tissue, chondrocytes form from mesenchymal progenitor cells and synthesize the cartilaginous matrix that serves as a template for skeletal growth [Goldring et al., 2006]. Chondrocytes are normally roundly shaped but depending on their location within the ECM, their shape, number and size can vary [Buckwalter and Mankin, 1997a]. Cells at the surface of the tissue are more numerous and flatter than their counterparts in deeper layers of the tissue. In adult tissue, the main function of the chondrocytes is to maintain and repair the ECM [Archer and Francis-West, 2003]. Chondrocytes are present in very low numbers in adult tissue with an average 14,000 cells/mm$^3$ [Stockwell, 1971]. Hence, each chondrocyte is responsible for maintaining a relatively large matrix area. Due to their spacing, chondrocytes synthesize individual micro-environments, what is referred to as cytoplasmic isolation [Archer and Francis-West, 2003]. An integral part of this micro-environment is the pericellular matrix (PCM). This region around the chondrocyte is characterized by high concentration of collagen type VI and proteoglycans and is thought to have an influence on the mechanical sensation of the cell [Alexopoulos et al., 2005]. Furthermore, since articular cartilage is not innervated and avascular, the chondrocytes depend on diffusion through the articular surface for nutrition and waste transport and operates at very low oxygen tensions (10 % at cartilage surface and 1 % in deep layers) [Silver, 1975]. As metabolically active cells, chondrocytes are known to react to a variety of different stimuli including growth factors and electrical, mechanical and pressure gradients from shear and compressive forces [Grodzinsky et al., 2000, Mow et al., 1999]. Depending on the stimuli the chondrocyte senses, it modifies the matrix by synthesizing proteins to maintain and remodel the ECM in its vicinity and to provide it with the competence to withstand mechanical forces.

1.1.1.2 Collagen

Collagen is the major structural component of the cartilage ECM, accounting for 60 - 70 % of the dry weight of the tissue. By far the most abundant collagen is Type II, representing 90 - 95 % of the total collagen in articular cartilage [Mayne, 1989]. The orientation of collagen fibers varies with tissue depth. Collagen fibers are oriented parallel to the surface in the superficial zone, randomly oriented in the transitional zone and perpendicular to the surface in the deep zone [Benninghoff, 1925]. Type II collagen has a very long turnover time, of up to several hundred years, leading to low capacity for remodeling after initial deposition [Eyre et al., 2006]. Together with collagen Type IX and XI it forms the fibrillar structure made visible by electron microscope [Eyre, 2002]. Collagen Type X expression is restricted
to the hypertrophic cells located in the calcified zone of articular cartilage. It plays an important role to anchor the articular cartilage to the underlying subchondral bone [Kuettner, 1992]. Collagen fibers are responsible for the tensile resistance of the tissue such as during tangential joint sliding [Mow and Mansour, 1977]. However a recent study has reported that the collagen fiber orientation has less influence on articular cartilage shear properties than its density where a twofold change in the collagen volume fraction resulted in a 100-fold change in shear modulus [Silverberg et al., 2014].

1.1.3 Proteoglycans

Proteoglycans are the second largest group of macromolecules found in articular cartilage and account for 5 - 15 % of the dry weight. They consist of a protein core to which several highly negatively charged and linear glycosaminoglycans (GAG) chains are covalently attached [Roughley, 2006]. One end of the protein core forms a globular structure and is linked to hyaluronic acid via link proteins [Heinegård and Hascall, 1974]. The largest and most abundant proteoglycan in articular cartilage is aggrecan. The aggrecan aggregate is composed of a core filament of HA where up to 100 aggrecan molecules are attached. The principal GAGs in aggrecan are keratan sulfate and chondroitin sulfate (Figure 1.1). Due to this large size and the entrapment within the collagen fibers the aggrecan aggregates get retained in the ECM [Roughley, 2006]. The proteoglycans contribute to the swelling pressure of the tissue from a combined effect of the fixed charge density (FCD) and the Donnan osmotic pressure [Mow et al., 1992]. The FCD comes from the repulsive forces of the closely packed GAG molecules which is correlated with their location in the tissue [Maroudas et al., 1969]. In consequence, these negative charges then regulate tissue hydration by attracting water via the Donnan effect (see Section 1.1.4) and therefore cause the tissue to swell. The resulting interstitial swelling pressure is balanced against the fibrillar network of collagen fibers. The swelling pressure significantly contributes to the compressive stiffness of the articular cartilage ECM [Maroudas, 1979, Mow et al., 1980], mainly due to the GAG chains on the proteoglycan molecules. In addition, the GAG chains produce a high drag force on the interstitial fluid [Comper et al., 1990] which controls the hydraulic permeability [Muir et al., 1970]. The content of proteoglycans is low in the superficial zone and increases towards deeper zones.

1.1.4 Water

Water contributes 75-80% of the total wet weight of the tissue and plays an essential part in its deformational response to mechanical loads [Mow et al., 1984, 1980]. It contains ions and metabolites, of which a small amount is bound to the collagen molecules while most is freely movable [Linn and Sokoloff, 1965]. Cationic ions present in the water are attracted by the negatively charged proteoglycans and
Figure 1.1: Structural components of an aggrecan molecule. Keratan sulfate and chondroitin sulfate are the principal glycosaminoglycans of aggrecan and bind to a core protein. The core protein binds to hyaluronic acid via a link protein and together with other aggrecan molecules results in large aggrecan aggregates containing up to 100 single molecules.

lead to a net increase of inorganic ions. This results in an osmotic pressure gradient that draws in water and causes an osmotic swelling pressure known as the Donnan osmotic pressure [Mow et al., 1990]. The Donnan osmotic pressure is in the range of 0.1 to 0.2 MPa and may contribute up to 50 % of the cartilage’s compressive stiffness [Mow et al., 1990].

1.1.1.5 Non-collagenous Matrix Proteins

Other extracellular proteins that are neither collagens or proteoglycans are present in articular cartilage. One is fibronectin, a 490 kDa glycoprotein with known roles in tissue repair and mechano-transduction. Fibronectin is known to be accumulated in osteoarthritic tissue [Burton-Wurster and Lust, 1985] and its fragments have been reported to cause an inflammatory response [Barilla and Carson, 2000]. Another non-collagenous matrix protein found in articular cartilage is cartilage oligomeric matrix protein (COMP). Its function is not entirely clear but recent evidence suggest that COMP is involved in catalyzing collagen fibrillogenesis [Halasz et al., 2007]. COMP is a mechano-sensitive protein and therefore regulated by mechanical load [Giannoni et al., 2003]. Endurance-trained runners who expose their joints to repetitive cyclic loading over extensive time periods have shown increased serum COMP levels compared to non-running healthy subjects [Neidhart et al., 2000]. As elevated COMP levels have also been reported in patients with knee injury and osteoarthritis (OA)[Lohmander et al., 1994], the regulation of this protein is of clinical interest.
1.1.2 Articular Cartilage Structure

Based on the nature of ECM components, articular cartilage can be divided into two main phases: a fluid phase and a solid phase. The fluid phase contains the interstitial water and dissolved ions. The solid phase is mostly a composite network of the collagen fibers and proteoglycan macromolecules [Buckwalter and Mankin, 1997a, Kuettner, 1992] that creates a densely packed network with low permeability and a porosity in the order of 2-6 nm [Maroudas, 1979, Mow et al., 1984] (Figure 1.2). The mechanical behavior of cartilage also depends on 2 phases and is often modeled as a biphasic [Mow and Mansour, 1977] or even triphasic material [Mow et al., 1990], where the charged ions are included as a third phase. Articular cartilage is an anisotropic tissue. With respect to chondrocyte morphology and macromolecular distribution and orientation, five distinct zones can be recognized throughout the thickness of the tissue from the cartilage surface to the underlying bone: (1) superficial tangential zone, (2) middle zone, (3) deep zone, (4) calcified zone and (5) subchondral bone (Figure 1.3). Collagen content and orientation, proteoglycan content and water content vary with depth of the tissue and anatomical site [Kuettner, 1992]. This depth- and location-dependent distribution of components leads to heterogeneous mechanical properties throughout the ECM, which becomes particularly obvious when loaded in compression and tension [Jurvelin et al., 2003, Buckley et al., 2008].

1.1.2.1 Superficial Zone

The superficial zone (SZ) is the tissue part that is in direct contact with the opposing articulating surface. It plays a crucial role for load transmission and for the biomechanical (deformational) behavior of the deeper zones. The chondrocytes have a flattened shape and are generally smaller than in deeper zones. They also show a different metabolic response to loading [Vanderploeg et al., 2008] with the expression
of elastin [Mansfield et al., 2009] and superficial zone protein [Schumacher et al., 1994]. This region is richer in collagens than the deeper zones but has a very low proteoglycan content. In this zone, the collagen fibers are densely packed and oriented parallel to the surface [Benninghoff, 1925]. There are two main structural and functional modalities that give the SZ its special character. First, it has been shown that the compressive modulus of the SZ is roughly 20 times lower than the deep zone [Schinagl et al., 1997, Laasanen et al., 2003]. Second, due to the large amount and dense packing, the collagen fibers in the SZ form a layer with low permeability. Consequently, this zone has an important influence on the fluid redistribution and deformational parameters of the tissue under compressive loads. It has been demonstrated that the removal of the SZ leads to increased tissue permeability and therefore faster equilibration time (seconds compared to hours) and greater peak compressive strains immediately after compression [Torzilli, 1984, Setton et al., 1993]. Computational models highlighted these findings and have shown that under 0.2 MPa compression, the rapid collapse of the surface due to its low compressive modulus decreased the permeability by 88% and effectively trapped the interstitial fluid in the middle and deep zone what resulted in pressurization of the interstitial fluid [Guo et al., 2015]. Similar to the compressive properties, shear properties of the ECM vary throughout the tissue depth. The shear modulus has been found to exhibit a minimum value on the deep end of the superficial zone (in 50 - 250 μm depth from surface) [Buckley et al., 2008]. The authors proposed that axial compression possibly leads to buckling of the collagen fibers, which under tension must first have to align before they can oppose the shear strains. This is thought to act as a mechanism that makes them less vulnerable to shear-induced damage by minimizing the strain on the fibers.

1.1.2.2 Middle Zone

Chondrocytes in the middle zone (MZ) of articular cartilage are rounded chondrocytes with more synthetic organelles than the cells in the SZ; they synthesize collagen fibers with fibrils that are thicker in diameter [Benninghoff, 1925, Muir et al., 1970]. The change in orientation of these fibers results in more random orientation in this zone. In addition, the proteoglycan content is higher and results in a slightly increased swelling ratio [Lipshitz et al., 1976] and increased compressive stiffness [Kempson and Muir, 1970]. This zone is also characterized by the lowest volume density of the chondrocytes, roughly 1800 cells/mm³[Poole et al., 1991].

1.1.2.3 Deep Zone

In the deep zone (DZ) of articular cartilage, the rounded chondrocytes have a columnar alignment perpendicular to cartilage surface. The collagen fibrils have the largest diameter of all zones, are per-
pendicular to the cartilage and bone surfaces and then the cartilage to the underlying subchondral bone through a layer of calcified tissue [Clarke, 1971]. Proteoglycan content remains high and in combination with the fluid provides effective support for compressive loads in order to minimize ECM strains [Soltz and Ateshian, 2000].

1.1.2.4 Calcified Zone

At the deep end of the DZ, the boundary between the uncalcified cartilage and the the calcified cartilage is termed the tidemark. Specific for the calcified zone are small chondrocytes that synthesize collagen X. Some of the chondrocytes can be seen as completely surrounded by calcified matrix, mainly with crystals of calcium apatite. This zone anchors the cartilage to the underlying subchondral bone and was reported to be important for nutrient transport in immature cartilage [Arkill and Winlove, 2008].

1.1.2.5 Subchondral Bone

The subchondral bone provides the mechanical support for the relatively compliant articular cartilage that attaches to it. Its stiffness is an important factor for cartilage health [Radin and Rose, 1986] with the main function of acting as a shock absorber and therefore to attenuate the load acting on the overlying cartilage [Pugh et al., 1974, Radin and Paul, 1970].

![Figure 1.3: Depth-dependent chondrocyte morphology (a) and collagen orientation (b) of mature articular cartilage. Adapted from Buckwalter et al. [1994].](image)

1.2 Articular Cartilage Biomechanical Properties and Function

1.2.1 Biomechanical Properties

Articular cartilage is a heavily loaded tissue, has to withstand several thousand cycles of loading a day and is subjected to a combination of compressive, tensile and shear forces. Both the structural macromolecules of the solid and fluid phases, and the intrinsic material properties of each phase define
the biomechanical response of the tissue to mechanical forces [Mow et al., 1980]. During mechanical compression of the cartilage matrix, interstitial fluid is squeezed out from the loaded areas into the joint capsule or unloaded adjacent tissue areas. However, due to the low porosity of the solid matrix and the frictional drag of the interstitial fluid this is not an instant process but rather happens over time, thus creating a high internal fluid pressure [Setton et al., 1993]. As a consequence, the resulting fluid pressure resists a significant amount of the applied load and reduces the stresses on the solid phase of the ECM, that is transmitted by collagens and proteoglycans. The fluid phase therefore shields the cells, prevents solid-solid contact of matrix components and provides efficient lubrication (for lubrication mechanisms see 2.3). Upon removal of the load rehydration of the loaded area occurs due to the existing swelling pressure. This mechanism contributes to the longevity of articular cartilage and has been described in several studies, both theoretical and experimental [Mow and Mansour, 1977, Soltz and Ateshian, 1998, Krishnan et al., 2004, Park et al., 2003, Eckstein et al., 1999]. If the compressive load is applied over an extended period of time, the fluid is continuously re-distributed until it is in an equilibrium with the surrounding solid tissue areas. During this time, the fluid pressurization slowly ceases and transfers the load from the fluid component to the solid component of the matrix [Mow and Mansour, 1977, Park et al., 2003]. This results in more stress on the solid matrix, decreasing hydrodynamic lubrication and increasing the coefficient of friction (COF) [Nickel et al., 2004, 2006]. A loss of fluid pressurization is prevented when the loaded area is temporarily unloaded and rehydrated. In vitro studies have shown that an unloading phase as short as 1 s results in a short-term reduction in COF [McCutchen, 1962, ? Forster and Fisher, 1999]. Other studies provided evidence that the COF could be kept sustainably low by cyclically translating the contact zone over the tissue surface [Caligaris and Ateshian, 2008] and that under such circumstances loads were mainly born by the fluid component (greater than 70%) [Park et al., 2003, Bonnevie et al., 2012, Caligaris and Ateshian, 2008].

Joint motion is characterized by the relative motion of the two joint surfaces against each other where the load oscillates axially and tangentially by sliding and rolling over the tissue surface. During such moving contact motion, mechanical parameters such as strain, stress, modulus and contact radius become functions of axial load, loading time, surface geometries and moving speed (translation speed). In recent studies it was found that increasing sliding speed (0.05–5 mm/s) results in decreasing tissue deformations and friction coefficients while increasing the effective stiffness of the tissue [Accardi et al., 2011, Bonnevie et al., 2011]. Research from in vivo studies might provide insight into physiological loading dimensions and the mechanical behavior of the tissue. By using fluoroscopy, the absolute amount of contact zone translation has been investigated in vivo in the knee. It was determined that under weight-bearing conditions in normal (asymptomatic) knees, the medial part of the
femoral condyle moves more than 21 mm in anterior-posterior direction [Dennis et al., 2005]. In other joints such as the temporomandibular joint (TMJ), the contact area moves ± 6 mm medial-lateral during mouth opening/closing with translation speeds up to 70 mm/s [Gallo et al., 2000]. Since the contact zone is moving along the articular surface, loading (deformation) and unloading (recovery) alternately occur. In the knee it was found that total cartilage deformation was between 2.4% and 8% after 50 knee bends and needed more than 90 minutes of unloading to recover [Eckstein et al., 1999]. In fact, in in situ measurement of human femoral-patellar joints loaded with 1.5 times body weight, strains of up to 57% (44% median) have been observed [Herberhold et al., 1999]. The fact that tissue deformation response is different from its recovery response implies that, depending on the moving speed, the tissue might not have enough time to recover between loading cycles. Thus, the overall deformation of cartilage depends not only on the amount of applied load but also on the loading frequency of an area and the time it has to recover. Moreover, when sliding and rolling motions are applied to poro-elastic surfaces, deformation of the leading edge is different from recovery of the trailing edge [Margetson, 1972]. All these factors result in a consistently changing physical environment for the cells throughout the loading period depending on their location within the matrix.

1.2.2 Lubrication Mechanisms

In particular, the joints of the lower body sustain loads several times body weight during daily activities such as walking, stair climbing or while rising from a chair. Joints of the upper body and fingers also sustain very high loads. During prolonged standing, loads are static or applied at very low speeds. In healthy joints, the COF of articular joints is extremely low and wear of the cartilage surfaces are negligible when they slide and roll against each other. Typically, the COF is in the range of 0.005 [Charnley, 1960, Linn, 1968] and several times lower than the best man-made bearing materials. To guarantee such low coefficients for several decades, efficient lubrication modes need to be in effect. In order to describe the phenomena from a tribological standpoint, various types of lubrication modes were described for articular cartilage. They can be classified in two main categories, fluid film lubrication and boundary lubrication [Dowson, 1967].

1.2.2.1 Fluid Film or Hydrodynamic Lubrication

Fluid film lubrication can be divided into three different modes [Dowson, 1967]. (1) Hydrodynamic lubrication describes a mechanism where two rigid moving surfaces are completely separated by a fluid film at all times. As soon as the two surfaces start moving relative to each other, the fluid that is present will be drawn into the gap between the surfaces due to viscous forces. In consequence, the
resulting fluid pressure bears the applied load. (2) Elasto-hydrodynamic lubrication employs the same principles as hydrodynamic lubrication with the difference that the moving surfaces are (or become) deformable. When the surfaces deform, the geometry changes and increases the congruency and area of contact. This results in a greater volume of fluid drawn into the loading zone (gap) which increases fluid pressurization and load bearing capacity. Mini-elasto-hydrodynamic (mini-EHL) is an extension of this theory to the molecular level where locally generated pressures have the potential to flatten out and smooth the initially rough cartilage surface [Dowson and Jin, 1986].(3) The third mechanism is called squeeze-film lubrication. Contrary to the other two mechanisms, there is no tangential motion but only normal approach. The compressive forces squeeze the fluid between the surfaces but this process is counteracted by viscous forces and the resulting pressurization of the fluid serves as bearing fluid. The fluid film is usually less than 15 μm thick, and is created with the relative motion of one surface against the other by drawing the viscous fluid into the contact zone.

1.2.2.2 Boundary Lubrication

The second main category is called boundary lubrication and is generally considered to happen when requirements for hydrodynamic lubrication are not met, such as low translation speeds, low viscosity of the fluid and, very high loads. The fluid film between the surfaces reaches a thickness that is comparable to the size of the fluid molecules and surface asperities where both surfaces make contact. In consequence, the frictional characteristics rely on molecular and biochemical interactions rather than physical properties of the lubricant. In this case molecules attached to the surface may play a crucial role when it comes to chondro-protection and prevention of wear. Several molecules have been considered as boundary lubricants and three of the most common ones are discussed in the next paragraph.

Hyaluronic acid (HA) is a polymer composed of repeating disaccharide units of glucuronic acid and acetylglucosamine [Fraser et al., 1997]. In healthy synovial fluid it is present at a concentration of 0.1 - 5 mg/mL and relatively heavy with an average molecular weight of $7 \times 10^6$ Da [Swann et al., 1974, Fraser et al., 1997]. Due to its large size and relatively high concentration, HA is responsible for the viscosity of the synovial fluid. Therefore, it has been proposed to be one of the molecules responsible for the low friction in a boundary lubrication regime [Mabuchi et al., 1994, 1999]. However, even by degrading HA and reducing its viscosity, no loss of the boundary lubrication ability was found. This suggest that HA most likely acts synergistically with other synovial fluid components rather than serving as a boundary lubricant on its own. However, its exact function is still unclear. Due to its high negative charges (repulsive forces), HA does not bind to cartilage surfaces (also negatively charged), a requirement for boundary lubrication [Chang et al., 2008]. Some studies have suggested that HA gets
physically trapped in the loading zone where it then serves as a lubrication agent [Yu et al., 2012].

Another molecule associated with boundary lubrication is a 227 kDa proteoglycan called lubricin. Its concentration in synovial fluid is in the order of 200 μg/mL [Elsaid et al., 2005]. Lubricin is one of several proteins that are encoded by the prg4 gene, others being superficial zone protein (SZP), proteoglycan-4 (PRG4) and megakaryocyte stimulating factor (MSF). All these proteins share a common primary structure with differences in glycosylation that is post-translationally modified [Flannery et al., 1999]. Unfortunately, in the literature, the names of these proteins are sometimes (confusingly) used interchangeably with the only difference reported in the molecular weight due to the glycosylation. In joint tissue, the protein is synthesized by synovial lining cells and by chondrocytes from the superficial zone but not from chondrocytes of middle and deep zones [Schumacher et al., 1994]. Different biological functions of lubricin were reported such as: cyto-protection, lubricating, self-aggregating and matrix-binding. It has been shown that inflammatory cytokines such as IL-1 beta inhibit lubricin biosynthesis [Flannery et al., 1999]. The synthesis of lubricin in degenerated cartilage is unclear as both up- and down-regulation of lubricin has been associated with the pathogenesis of OA [Neu et al., 2010, Elsaid et al., 2008]. As mentioned above, boundary lubricants must be capable of binding to the surface while also generating repulsion. Unlike HA, lubricin is composed of several domains. The N- and C-terminus of the molecule is thought the bind to the surface whereas the central mucine like domain contributes to the lubricating action by repulsion forces. It has been shown that upon removal of the mucin like domain, the COF increased [Jay, 2004]. It has also been shown that surface motion (shear/sliding) up-regulates SZP and that the superficial zone chondrocytes are mainly responsible for the synthesis [Grad et al., 2005, Schätti et al., 2015b].

Surface active phospholipids (SAPLs) is another category of molecules associated with boundary lubrication. SAPLs are constituents of synovial fluid and are found to have the ability to reduce the surface tension of water, and bind to hydrophilic surfaces, making them hydrophobic [Hills and Butler, 1984]. However, evidence of their boundary lubricating ability is sparse and controversial.

1.2.2.3 Articular Cartilage-Specific Lubrication Modes

Weeping and boosting lubrication are the two main lubrication mechanisms proposed for the characteristics of articular cartilage. The weeping lubrication mechanism [McCutchten, 1959, Lewis and McCutchten, 1959] predicts that under mechanical load fluid is pressed out of the tissue and flows into the inter-articular space. There it creates a layer between the contacting bodies, thus serving as a lubricant to partially bear the load. In contrast, in the boosted lubrication theory, the hydrostatic pressure built up during compression forces the interstitial water into the articular cartilage, the superficial zone
serving as a membrane only permeable to water and small ions. The water present in synovial fluid gets filtered into the tissue whereas large molecules such as HA cannot go through and get trapped. The synovial fluid components left on the articular surface form a gel-like and viscous layer that provides the low-friction lubrication [Walker et al., 1968].

1.2.2.4 Summary of Cartilage Lubrication

To summarize, a synovial joint has to withstand many complex motion patterns that include static and dynamic compressive and shear forces, and translational rolling and sliding. Translational sliding speeds can range from very high (e.g. during sprinting) to very low (during standing), and compressive forces alternating between minimal load during sleep up to several times bodyweight from muscle action. In order to explain sustainably low friction and excellent bearing capacity of articular cartilage under these various loading cases, multiple lubrication modes are probably simultaneously in effect [Schmidt et al., 2007] (Figure 1.4). The prevalence of any of these modes depends on many different factors including the kinematics and contact parameters, as well as tissue composition, structure and geometry. It is predicted that even during a single walking cycle a mixed lubrication regime has to be in effect and that the load support is shared by a fluid film and contacting asperities is proposed [Dowson, 1967, Schmidt et al., 2007, Neu et al., 2008].

![Figure 1.4: Schematic drawing of different lubrication regimes. Weeping and boosted lubrication are cartilage specific lubrication regimes. Since the physiologically relevant mechanisms are still not well understood, it is likely that a combination of these regimes play a role. The presence of each of these mechanisms depends on factors such as articular cartilage composition and structure, compressive forces and translation speed. Adapted from Neu et al. [2008].](image-url)
1.3 Articular Cartilage Degeneration and Osteoarthritis

1.3.1 Osteoarthritis

Osteoarthritis (OA) is regarded as a whole-joint disease. It not only affects the articular cartilage but also other synovial joint structures including the subchondral bone, synovial tissue and joint capsule [Poole, 2012]. OA is the most severe form of a degenerated joint, known to affect joints such as the knee, the hip and TMJ. OA can be classified into two major subgroups. Primary OA (idiopathic OA) refers to OA that develops over an extensive period of time (i.e. normal aging) and without a known cause, while secondary OA has a known cause that induced cartilage degeneration, such as a traumatic incidence. Primary OA is generally observed in the elderly population where age, sex, genetic and environmental factors are considered to influence its initiation and progression but the exact triggers still remain widely unknown. Since articular cartilage is a mechanically loaded tissue, altered and abnormal mechanical forces, which affect ECM morphology and biomechanical properties, are generally thought to be key factors associated with the initiation and propagation of tissue breakdown. Joint morphology is a genetic factor that in combination with mechanical loads influences the stress distribution in the cartilage ECM. Unfavorable curvatures and geometries have been reported to increase peak stresses on the tissue and potentially induce OA [Bullough, 1980]. On the other hand, epidemiological studies found that OA also occurs frequently in patients with a history of joint injury or trauma [Anderson et al., 2011], hence the name post-traumatic OA (PTOA). In both cases, the disease rarely becomes symptomatic until it reaches its end stage. Even if detected at an early stage, it has not been possible to attenuate or stop its progression. It has been proposed that OA should be considered less a disease process and more as an “organ failure” caused by altered mechanical joint loading and propagated by biological factors [Radin et al., 1990, 1991]. OA ultimately results in the loss of articular cartilage, sclerosis of the underlying bone and inflammation of the synovium (see section 3.4). The consequence is pain, disability and a reduction in the quality of life. It is estimated that currently 10-12% of the adult population have symptomatic OA and that the number of people affected will increase by 50% over the next 20 years [Hunter et al., 2014]. This will have a significant socio-economic impact with direct and indirect costs being in the range of billions [Chen et al., 2012]. In conclusion, OA has a substantial impact on life quality, productivity and imposes tremendous costs on the health care systems world-wide [Hunter et al., 2014].
1.3.2 Post-Traumatic Osteoarthritis

PTOA is characterized by joint degeneration and pain that develops after traumatic incidents that affect any of the joint structures. Unlike the idiopathic form of OA that usually develops in elderly people, PTOA can affect persons of all age groups. Whereas elderly patients can be effectively treated with state of the art medical techniques such as joint replacement and joint fusion, these methods do not provide acceptable solutions for younger and active patients and therefore impose even bigger social, economic and clinical challenges. Several joint-related injuries have been shown to be related with cartilage injuries and PTOA. Many of them are related to the lower extremity and include but are not limited to meniscal tears, anterior cruciate ligament (ACL) tears [Lohmander et al., 2007, Englund et al., 2003], joint dislocations [de Bont and Stegenga, 1993] and intra-articular fractures [Laird, 2005, Weigel and Marsh, 2002, Anderson et al., 2011]. Brown et al., [2006] reported that from a patient group with symptomatic hip, knee and ankle OA, 1.6% (hip), 9.8% (knee) and 79.5% (ankle) showed a history of traumatic incidents. In addition, to the joints of the lower body, other joint structures are also susceptible to injury and the degeneration of its cartilaginous structures can have severe influence on the patients quality of life. Such a joint is the TMJ where the bony components as well as the fibrocartilaginous surfaces can be impacted and severely compromised by incidents such as fractures [Giannakopoulos et al., 2009].

1.3.3 Pathomechanics Due to Joint Injury

As mentioned in section 3.2, traumatic incidents can affect the passive stabilizing structures of joints. In healthy joints, muscles, ligaments, menisci and tendons control the kinematic patterns of the joint [Torzilli et al., 1994]. However, once these stabilizing structures are compromised, kinematics change affecting the mechanical and consequently the biological environment. Anterior cruciate ligament (ACL) tears can lead to alterations in relative femoral-tibial translation [Dennis et al., 2005, Torzilli et al., 1994, Beynnon et al., 2002, Andriacchi and Dyrby, 2005]. The majority of these studies report an increase in anterior-posterior (AP) translation of the tibia relative to the femur in ACL deficient knees. The absolute translation range depends on knee angle, measurement techniques and ACL condition but generally ranges from 3 mm [Beynnon et al., 2002] up to 21 mm [Dennis et al., 2005]. An increase in loading path length due to ligamentous transections was also observed in a sheep model [Beveridge et al., 2013]. Sheep that underwent transections not only showed significantly increased path lengths but also a 10-fold increase in tibio-femoral translation velocity. In general, a change in loading path length is accompanied by a change in contact locations. Since it is known that spatial variations in cartilage mechanical properties are due to different functional requirements [Wong and Carter, 2003,
Moore and Burris, 2015], a shift in the contact areas could possibly lead to mechanical loading of cartilage areas unsuitable for load bearing [Andriacchi et al., 2009]. A change in translation speed influences the deformational response of the tissue due to its poro-elasticity. As cartilage exhibits a non-linear behavior as a function of loading frequency [Lee et al., 1981], an increase in translation speed results in increased tissue stiffness, decreased deformation and concomitant higher contact stresses [Mow and Lai, 1979, Bonnevie et al., 2011]. Other joints also experience changes in loading kinematics and patterns in diseased states. In the TMJ, it has been shown that during mouth opening/closing the medio-lateral component of the stress-field trajectory is significantly larger in diseased joints than in controls [Gössi et al., 2004]. Furthermore, when the TMJ disc is displaced the energy density in the tissue is significantly increased, which could correlate with its fatigue [Gallo et al., 2015]. Mechanically induced degenerative changes occur in a variety of different joints. They can negatively impact the cartilaginous structures directly or induce kinematic changes, which in turn leads to altered and potentially detrimental loading patterns.

1.3.4 Osteoarthritic Changes in Articular Cartilage

Osteoarthritis is regarded as a whole-joint disease and affects all synovial joint structures including articular cartilage, subchondral bone, synovial tissue and the joint capsule [Poole, 2012]. However, major osteoarthritic changes include the articular cartilage and subchondral bone [Radin and Rose, 1986, Buckwalter and Mankin, 1997b] and involve alterations of the biochemical composition and structural changes that consequently influence the biomechanical properties of the tissue. According to Buckwalter et al. [1997b], the process of articular cartilage degeneration can be divided into three overlapping stages: (1) disruption and fragmentation of the ECM, (2) changes in the biosynthetic response of the chondrocytes due to the ECM damage, and (3) progressive loss of the tissue.

The earliest degenerative changes that are observed in osteoarthritic cartilage appear at the articular surface and are related to a change in collagen orientation and arrangement and proteoglycan content. This is not surprising as this is the location with direct tribological and mechanical impact. The development of OA is characterized by a gradual loss of proteoglycans from the ECM [Mankin et al., 1971] with the process starting in the superficial zone [Saarakkala et al., 2010]. Whereas the total collagen content does not change until advanced stages of OA, the collagen network has been shown to be compromised [Wut et al., 1991]. In particular, the fibers of the superficial zone of early-stage OA cartilage were more randomly oriented (less parallel to the surface) than in healthy tissue [Saarakkala et al., 2010, Bi et al., 2005]. As a consequence, the macromolecular network is disrupted and damage of the superficial zone occurs. In fact, fibrillation of the articular surface is one of the first signs of
OA [Buckwalter and Mankin, 1997b]. With the progression of OA, the fibrillation can extend into the deep zone of the tissue reaching to the subchondral bone. This whole process of macromolecular unraveling leads to roughening of the articular surface and fragmentation of ECM components, leading to their release into the joint cavity. At the same time, the combination of changes affecting the proteoglycan and collagen framework results in an increased water content and tissue swelling [Mankin and Thrasher, 1975]. Furthermore, decreased proteoglycan aggregation and increased water content increase the hydraulic permeability of the ECM [Grenier et al., 2014]. These events significantly influence the tissue’s ability for fluid pressurization, load bearing and lubrication thus increasing its vulnerability to mechanical damage [Guo et al., 2015, Saarakkala et al., 2010, Thambyah and Broom, 2007] (Figure 3).

A change in the biochemical composition and structure of the ECM macromolecules influences the load bearing capacity of articular cartilage. As a result, alterations in strains, stresses and osmolarity impose a change in the local mechanical environment of the chondrocytes and they react with the synthesis of anabolic and catabolic mediators. Interleukin-1 has been found to be one of the major inflammatory cytokines in OA [Towle et al., 1997] and has been shown to stimulate the synthesis of matrix metalloproteinases (MMPs-1, -3 and -13) and aggrecanases (ADAMTS-4 and -5, a disintegrin and metalloproteinase with thrombospondin-like motifs) [Lin et al., 2004, Chevalier, 1997, Tung et al., 2002]. These enzymes degrade collagens and proteoglycans and result in further degradation of the ECM. ADAMTS were found to be the first active enzymes followed by MMPs [Van Meurs et al., 1999b]. In addition, OA and trauma are now classified as inflammatory arthritis, as a result of inflamed synovial tissues, which contribute to the degeneration of the articular cartilage. Increased numbers of immune cells in the synovium lead to the induction of interleukin-1 (IL-1) which in turn stimulate the production of MMPs and ADAMTS [Tung et al., 2002]. An up-regulation of those catabolic enzymes results in more ECM breakdown products, which potentially trigger further immune reactions [Goldring and Otero, 2011]. Furthermore, not only has the up-regulation of fibronectin synthesis been associated with degenerative changes [Chevalier, 1993] but also the presence of its fragments that in turn stimulate IL-1 synthesis and increase proteoglycan depletion [Homandberg et al., 1997, Homandbergs et al., 1992, Homandberg et al., 1993]. During this phase of the process, the chondrocytes try to compensate for the degradation and loss of ECM components with the synthesis of new macromolecules such as collagens and proteoglycans [Cs-Szabó et al., 1995, Mankin, 1974, Mankin et al., 1981, Poole et al., 1992] and the synthesis of protease inhibitors such as tissue inhibitor metalloproteinases (TIMPs) [Lohmander et al., 1993]. The chondrocytes are also reported to undergo proliferation at this stage, probably in an attempt to further increase matrix production Goldring and Marcu [2009]. In addition, cartilage oligomeric matrix protein (COMP) and SZP are synthesized in order to catalyze collagen fibrillogenesis.
and promote surface lubrication, respectively [Neu et al., 2010, Halasz et al., 2007]. In general, the second phase of the disease process aims at reversing the effects of the mechanical and enzymatic degradation as the cells try to repair the damaged ECM.

Failure to repair the damaged matrix and restore its biomechanical properties ultimately lead to the third phase, the progressive loss of articular cartilage. It is thought that chondrocytes undergo apoptosis and necrosis due to the lack of protection from the disintegrating ECM. In consequence, the synthesis of matrix macromolecules is further decreased and eventually, articular cartilage is completely lost. This results in OA that is accompanied with loss of articular function and pain.

As the articular cartilage acts as a cushion the attenuate and distribute mechanical forces, the subchondral bone is also affected by the loss of the cartilage ECM and vice versa. Radin and Rose [Radin and Rose, 1986] suggest that the most likely cause of subchondral stiffening is due to compromised cartilage not able to properly distribute compressive loads what results in high peak stresses on the bone. This in turn leads to adaptation and stiffening of the bone that again adds to the large stiffness gradient. Also, deformation of the underlying bone causes deformation of the cartilage layer what could lead to incongruent joint surfaces and therefore high maximum peak stresses in certain areas. They also proposed that the health of articular cartilage depends on the mechanical properties of the subchondral bone and in particular a large stiffness gradient between cartilage and bone could result in the initiation of cartilage degeneration.

**Figure 1.5:** Depth-dependent structural differences of healthy cartilage (left) and osteoarthritic cartilage (right). Adapted from Goldring and Marcu [2012].
1.4 Experimental Models for Mechanically-Induced Osteoarthritis

1.4.1 Mechanobiological Models and Loading Devices

Excessive mechanical forces are known to be a major contributor for OA [Radin et al., 1990, 1991]. Injury can happen through repetitive loading over an extensive time period, e.g. due to joint instabilities which result in non-physiological loading patterns, or through a high-energy single impact as in a car accident. To study the mechanical and biological factors during such traumatic incidents, researchers have tried to recreate these events with cartilage explant models \textit{in vitro}. Since joint kinematics and the factors leading to cartilage injury are complex, these models can only provide limited insight into the events occurring during injury. Nevertheless, important knowledge on the mechanical competence and response of articular cartilage under various loading patterns could be gained. Likewise, by using live cartilage explants, cellular and tissue specific degradation processes were uncovered and linked with the mechanical input.

Joint kinematics are a complex combination of compressive, shear, sliding and rolling forces resulting in different strains, stresses and impact energies depending on the loading rate and the anatomical location they are applied to. The most common factors that were investigated in combination with articular cartilage defects were contact stress and stress rate, strain and strain rate, and impact energy [Ewers et al., 2001, Milentijevic and Torzilli, 2005, Jeffrey et al., 1995, Quinn et al., 2001, Chen et al., 1999, Ding et al., 2010]. Whereas it is most likely that a combination of all these factors have an influence on the kind and severity of an injury, it is impossible to take all these variables into account with one loading system. The model becomes increasingly complex when more variables are included, and accurate predictions on the outcome and the causality between mechanical input and biomechanical response become difficult. Therefore, most loading systems are uni-axial where impact variables can be varied on a single axis. More complex multi-axial devices exist where an axial load (perpendicular to the cartilage surface) is simultaneously applied with a sliding/rolling motion that is tangential to the surface. In consequence, the ideal design of the loading device strongly depends on the hypothesis of the study and due to differences in the loading setup, outcomes can sometimes be difficult to compare.

1.4.1.1 Uni-Axial Loading Systems

The most common type of loading system used in biomechanical studies is designed to apply uni-axial compression to the surface of articular cartilage explants. The compression can be applied as a single impact [Verteramo and Seedhom, 2007] or over a longer time period in a static [Sah et al., 1990, Fitzgerald et al., 2004] or dynamic manner [Thibault et al., 2002, Lin et al., 2004]. Energy-, displacement- and
Load-controlled systems can be distinguished. The first, and simplest, systems used to investigate cartilage injury due to mechanical loads are called drop-tower devices [Repo and Finlay, 1977, Verteramo and Seedhom, 2007, Jeffrey et al., 1995]. Here, a known weight is dropped from a defined height to impact the surface with a controlled amount of energy. By dropping a weight onto the cartilage surface, the load is applied in a very short amount of time and with extremely high stress and strain rates similar to the ones occurring during road accidents [Repo and Finlay, 1977, Jeffrey et al., 1995].

In displacement-controlled devices, the amount of deformation is controlled in order to achieve a desired deformation (strain) of the articular surface [Kurz et al., 2001, Quinn et al., 1998]. Similarly, in load-controlled systems, a defined load is applied onto the cartilage in order to reach a wanted maximum load or maximum contact stress [Torzilli et al., 1999, Milentijevic et al., 2003]. Depending on the total loading time, the number of applied cycles and the loading frequency, different loading scenarios can be generated in both the displacement- and load controlled systems, and mechanical [Korhonen et al., 2002a, Mow et al., 1980] and biological [Wong et al., 1999, Buschmann et al., 1995] aspects investigated. Load-controlled experiments are generally considered more physiological due to the fact that during joint motion a resulting load acts onto the cartilage surface which, in turn deforms according to its mechanical properties.

1.4.1.2 Multi-Axial Loading Systems

Multi-axial loading systems are designed to account for the fact that in joints a variety of forces simultaneously act in different directions. As already mentioned in section 2.1, joint motion is a complex combination of compression, shear, sliding and rolling loads. In order to more realistically mimic joint kinematics, the device needs to have the capability to control loads and forces in different axis. Shear loading devices apply shear forces (tangential to the surface) superimposed on an offset strain (perpendicular to the surface). The offset compression can be static [Jin et al., 2001] or dynamic [Fitzgerald et al., 2006]. By applying shear load, the biosynthetic response of the cartilage [Jin et al., 2001, Fitzgerald et al., 2006] as well as shear properties [Buckley et al., 2008, 2010] have been investigated. These studies do have in common that the area of cartilage-indenter contact remains constant and shear is induced by oscillating (rotating) the indenter in place. Newer models have been developed the study the biomechanical response of cartilage to compression and shear induced by sliding (plowing) and rolling over the articular surface [Colombo et al., 2011, Schätti et al., 2015a]. With these loading systems, the contact point between indenter and cartilage tangentially translated over the tissue surface, which has been shown to be an important feature for sustainably joint function [Caligaris and Ateshian, 2008, Soltz and Ateshian, 1998].
To summarize, uni-axial as well as multi-axial loading systems have their advantages and disadvantages and depending on the hypothesis asked one might be chosen over another. In general, uni-axial systems allow for greater control of the test environment. By using only small tissue explants (biopsy punches) with known or easily testable properties, strains and stresses can simply be controlled. On the other hand, applying uni-axial loading does only very limitedly represent the complex kinematic environment present in joints. Multi-axial systems do reproduce this complex environment more realistically. In particular in devices with a transulatory movement (sliding/rolling) dimensions of contacts are in the physiological range and applied over an extended area on the cartilage surface, similar as it happens in vivo. This in turn requires the use of bigger cartilage explants such as nasal septum cartilage or femoral condyles. The advantage of such large specimens is that upon explantation, most of the ECM stays intact. The downside of using large motion paths over intact cartilage specimens is that the material properties can vary over the loading area. This results in the fact that the biomechanical environment becomes more uncontrollable and confounding factors can be introduced.

1.4.2 Biomechanical Degeneration

Traumatic incidents such as sport injuries or car accidents cause high peak stresses and stress rates on articular cartilage. The impact can lead to direct cartilage breakdown through ECM failure and chondrocyte death. From impact loading studies, it is known that high levels of mechanical loading of articular cartilage influence chondrocyte viability, ECM water content and ECM protein composition. Chen et al. [2003] showed that cell death occurred in the superficial zone (SZ) of articular cartilage after as little as 1 min of cyclic loading at 5 MPa and that the same stress levels resulted in broken collagen fibers in the SZ after 1 hour of loading. In another study, cartilage explants were impact loaded at different stress levels [Torzilli et al., 1999]. No matrix damage was found with impact loads up to 15 MPa, surface fibrillation occurred at 25 MPa, and 45 MPa resulted in complete matrix disruption. The authors concluded that the morphological changes in the tissue matrix are associated to collagen rupture. Even though 15 - 20 MPa is needed to see matrix damage [Chen et al., 1999, Torzilli et al., 1999], 5 MPa seemed sufficient to detect mechanically damaged collagen fibers [Chen et al., 1999]. Furthermore, a peak contact stress of 14 MPa applied at strain rates of 0.5 and 0.7 s\(^{-1}\) was found to significantly increase proteoglycan release into the medium [Quinn et al., 2001]. In general, it is known that the effect of mechanical loads depends on their magnitude and rate [Ewers et al., 2001, Jeffrey et al., 1995, Quinn et al., 2001, Milentijevic et al., 2005]. High peak stresses and extended loading durations result in changes of water content and increased depth of cell death from the articular surface [Milentijevic and Torzilli, 2005, Chen et al., 1999].
1.4.3 Biochemical Degeneration

As described above, mechanical overload can lead to tissue wear and cell death. However, acting forces can be below-threshold for direct cell and tissue damage. Nevertheless, such loads can still disturb articular cartilage homeostasis and induce detrimental changes in chondrocyte metabolism. In healthy cartilage, tissue anabolism and catabolism are tightly controlled in order to maintain a functional ECM [Aurich et al., 2002]. From in vitro studies, it is known that mechanical loading of cartilage explants results in an altered biosynthetic response of chondrocytes [Grodzinsky et al., 2000]. Static loads were found to result in a significant decrease in metabolic rate [Guilak et al., 1994, Buschmann et al., 1995, Burton-Wurster et al., 1993]. Dynamic loading, on the other hand, increases metabolic activity and the biological response of the cells has been found to depend on peak-stress, stress-rate and loading duration [Chen et al., 1999]. Subject to the stimulus, the response can be anabolic or catabolic and is most commonly assessed by investigating changes in gene expression [Lee et al., 2005, Valhmu et al., 1998, Torzilli et al., 2011, Wong et al., 1999, Fitzgerald et al., 2006] and protein synthesis [Burton-Wurster et al., 1993, Buschmann et al., 1999, Jin et al., 2001, Parkkinen et al., 1993, Sah et al., 1989]. Physiological stress levels result in balanced homeostasis of the main matrix components, such as collagen type II and proteoglycan [Buschmann et al., 1999, Jin et al., 2001, Parkkinen et al., 1992]. Non physiological stresses, such as over- but also under-loading, result in the production of catabolic enzymes such as matrix metalloproteinases (MMPs) and aggrecanases also called a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). MMPs-1, -2, -3, -9 and -13 and ADAMTS-4 and -5 (aggrecanas 1 and 2) are the most common types associated with cartilage metabolism [Lee et al., 2005, Murphy et al., 2002, Porter et al., 2005, Schätti et al., 2015b, Correro-Shahgaldian et al., 2011, 2015]. Mechanical loading of articular cartilage has also shown to increase fibronectin synthesis [Farquhar et al., 1996, Burton-Wurster et al., 1993, Burton-Wurster and Lust, 1985] that in turn is thought to be cleaved by MMPs [Ding et al., 2014]. The resulting fragments stimulate IL-1 production and further MMP and ADAMTS synthesis [Homandbergs et al., 1992, Homandberg et al., 1993, 1997, Chevalier, 1997]. Furthermore, there is novel evidence that aggrecan fragments can directly excite nociceptive neurons [Miller et al., 2015].

1.5 Motivation and Aim of Thesis

The understanding of articular cartilage mechanobiology in health and disease is still limited. A lot of research has been conducted to unravel the basic mechanisms underlying articular cartilage’s functional properties, wear resistance and response to mechanical load. Nevertheless, knowledge about exact
processes leading to the initiation and progression of OA are still very limited. In consequence, there is a lack of treatments and therapies to attenuate or reverse progressive cartilage degeneration that occurs in diseases such as OA. In order to establish effective therapies or being able to fully restore functional and resilient cartilage tissue after it has degenerated, a profound knowledge on the reason for its degeneration is inevitable. Significant progress has been made in the past three decades due to new analytical techniques and experimental models. By using different loading systems and computational models, important knowledge about the mechanical and rheological properties of the tissue under load and their influence on the initiation of OA could be revealed. Similarly, the use of mechanical loading systems in combination with live tissue explants resulted in new knowledge about the biosynthetic response of the living tissue to a variety of different loads. However, due to the complexity of articular cartilage it is exceedingly difficult the link changes of kinematics with biological consequences.

The main motivation of this thesis is to establish a new test model to further improve our understanding of articular cartilage degeneration in response to mechanical load. The use of a new multi-axial loading system allows us to apply mechanical loads that more accurately mimic the complex in vivo situation, both in health and disease. This is a significant improvement over the vast majority of studies previously performed where relatively simple loading regimens of compression and shear applied onto stationary contact points. Most cartilage explants used for testing were biopsy punches and rarely exceeded a few millimeters in diameter. While this allowed for a large amount of samples to be tested and relatively easy control of mechanical parameters, it might compromise the validity of the results in a physiological context. In particular, as it became obvious that articular cartilage gains its outstanding mechanical properties to a large part due to its macro-structural organization and its ability to transfer and attenuate loads over large tissue areas. Two important mechanisms that are clearly impaired when using isolated tissue plugs. Furthermore, as mentioned in section 2.1, under physiological conditions articular joints engage the cartilaginous structures in relative sliding and rolling against each other which results in a moving point of contact. This translation results in previously loaded areas being able to recover (imbibe fluid). As a consequence, the recovered tissue is hydrated for the next loading cycle and consequently is capable to provide efficient lubrication and load bearing for an extended time period. Furthermore, the resulting fluid flow plays an important role for chondrocyte metabolism. Since cartilage is avascular, it receives its nutrients either through diffusion [Maroudas et al., 1968] or mechanical pumping [Salter and Field, 1960]. The moving loading patterns constantly redistribute the interstitial fluid and force it through the tissue, determining the pathways by which the chondrocytes receive their nutrients. According to this, joint loading influences the chondrocytes directly by generating a pressure differential between intra- and extracellular compartments, in the order of tens of kPa.
Therefore, the aim of this thesis is to describe and test a model to investigate mechanical and biological changes emerging from moving contact due to sliding and rolling. The use of new loading devices (RPETS and DACTS) with the ability to mimic translating motion patterns, gives us the opportunity to analyze articular cartilage mechanobiology from a more physiological perspective. Not only do such sliding and rolling loads more accurately mimic complex in vivo kinematics but, due to the translative motion, also take into account the articular cartilage’s heterogeneity in mechanical properties [Moore and Burris, 2015].

Specific Aims:

1. Investigate the influence of axial load and sliding speed on the mechanobiological response of flat cartilage explants.
2. Establish a model to investigate the mechanobiological response of curved articular cartilage explants to a combination of translation and axial compression.
3. Investigate the mechanobiological response of articular cartilage explants to different indenter curvatures under simultaneous compression and translation of the indenters.

A correlative analysis will serve to directly correlate the mechanics with the biology. In consequence, this allows us to make more accurate predictions about the influence of certain mechanical stimuli on the biological response depending on the material properties of the cartilage. In our studies, we aim to apply physiological loading parameters in terms of compressive force, relative translation speed and loading distance. As physiological loading distances cover different surface areas (up to 20 mm), the response of the tissue at several location can be investigated in respect to the local material properties, curvature and external variables such as compressive force, sliding speed and resulting mechanical parameters (deformation, strain, contact stress etc.).

**Significance** Synovial joint deficiencies (such as anterior cruciate ligament tears / disc displacement in temporomandibular disorders) change kinematic patterns and accelerate initiation and progression of joint degenerative processes. Findings of this study will help to identify mechanical and biological cues (biomarkers) detrimental for cartilage health and provide strategies for prevention and treatment of mechanically induced OA. Furthermore, the knowledge gained from these experiments will also help to understand what mechanisms and competences are important for tissue engineered constructs. In the future, our loading devices can be used to mechanically condition such constructs in order to keep the cells differentiated (chondrocytes) or induce their differentiation (mesenchymal stem cells) to engineer mechanically and biologically functional cartilage tissue.
Chapter 2

Mechanical Loading of Cartilage Explants with Compression and Sliding Motion Modulates Gene Expression of Lubricin and Catabolic Enzymes

Oliver R. Schätti\textsuperscript{1,2,3}, Michala Marková\textsuperscript{1,4}, Peter A. Torzilli\textsuperscript{3}, and Luigi M. Gallo\textsuperscript{1}

\textsuperscript{1}Laboratory of Physiology and Biomechanics of the Masticatory System, Center for Oral Medicine, Dental and Maxillo-Facial Surgery, University of Zurich, Plattenstrasse, Zurich, Switzerland
\textsuperscript{2}Institute for Biomechanics, Swiss Federal Institute of Technology, ETH Zentrum, Zurich, Switzerland
\textsuperscript{3}Laboratory for Soft Tissue Research, Hospital for Special Surgery, New York, NY, USA
\textsuperscript{4}Laboratory of Biomechanics, Department of Mechanics, Biomechanics and Mechatronics, Faculty of Mechanical Engineering, Czech Technical University in Prague, Czech Republic

Keywords: Mechanobiology, Chondrocytes, Cells, Sliding contact, Lubricin, Degradative enzymes

This manuscript has been archived after Cartilage 6:185–193, Apr 2015. The final publication is available at Sage via http://dx.doi.org/10.1177/1947603515581680.

2.1 Abstract

Objective. Translation of the contact zone in articulating joints is an important component of joint kinematics, yet rarely investigated in a biological context. This study was designed to investigate how sliding contact areas affect cartilage mechanobiology. We hypothesized that higher sliding speeds would
lead to increased extracellular matrix mechanical stress and the expression of catabolic genes. **Design.** A cylindrical Teflon indenter was used to apply 50 or 100 N normal forces at 10, 40, or 70 mm/s sliding speed. Mechanical parameters were correlated with gene expressions using a multiple linear regression model. **Results.** In both loading groups there was no significant effect of sliding speed on any of the mechanical parameters (strain, stress, modulus, tangential force). However, an increase in vertical force (from 50 to 100 N) led to a significant increase in extracellular matrix strain and stress. For 100 N, significant correlations between gene expression and mechanical parameters were found for TIMP-3 \(r^2 = 0.89\), ADAMTS-5 \(r^2 = 0.73\), and lubricin \(r^2 = 0.73\). **Conclusions.** The sliding speeds applied do not have an effect on the mechanical response of the cartilage, this could be explained by a partial attainment of the “elastic limit” at and above a sliding speed of 10 mm/s. Nevertheless, we still found a relationship between sliding speed and gene expression when the tissue was loaded with 100 N normal force. Thus despite the absence of speed-dependent mechanical changes (strain, stress, modulus, tangential force), the sliding speed had an influence on gene expression.

### 2.2 Introduction

Articular cartilage is responsible for the low friction, reduced wear, and redistribution of complex mechanical loads for millions of cycles during one’s lifetime. These outstanding functional properties are based on a highly specialized extracellular matrix (ECM), which can be divided into a solid phase and a fluid phase [Mow et al., 1980]. The solid phase is primarily composed of collagen fibers, proteoglycans, and cells. The fluid phase contains interstitial water and mobile ions. During mechanical compression, the porous solid matrix hinders interstitial fluid escape, creating an internal pressure to resist the applied load and shield the cells within the solid matrix. The importance of this mechanism during physiological joint function has been previously investigated, both theoretically and experimentally [Soltz and Ateshian, 1998, Krishnan et al., 2004, Park et al., 2003, Eckstein et al., 1999].

When a static load is applied, fluid exudes from the loaded area to adjacent areas, resulting in a decrease in interstitial fluid pressure, a transition of the load from the fluid component to the solid matrix, and an elevated coefficient of friction. During physiological joint motion, loads are applied intermittently and result in multidirectional sliding of the joint surfaces and varied contact areas between cartilage surfaces [Kizuki et al., 1995, Gallo et al., 2006]. This results in fluid flow between high- and low-pressure regions of the ECM and a continuous rehydration of the previously loaded areas. As a consequence, high fluid pressurization (and therefore high fluid load support) and a low coefficient of friction can be sustained over hours of loading under continuously changing contact locations [Caligaris and Ateshian, 2008]. The influence of different loading parameters on the mechanical re-
The biological response of cartilage has already been extensively explored [Bonnevie et al., 2011, 2012, Accardi et al., 2011, Forster and Fisher, 1999]. However, little is known about the effect of migrating contacts on the biological response of cartilage. It is known that the biological response of chondrocytes to mechanical forces depends on the magnitude of the force, where high forces cause an up-regulation of catabolic genes, such as collagen- or proteoglycan-degrading enzymes [Fitzgerald et al., 2004, 2006, Lee et al., 2005]. However, these studies applied relatively simple loading regimens, typically uniaxial compressive and/or shear forces alone or in combination. A previous study from our laboratory found that increasing tractional forces, induced by sliding and plowing, induced catabolic changes within the cartilage [Correro-Shahgaldian et al., 2011].

This study was designed to explore the influence of sliding on the biological response of cartilage. The mechanical response of the cartilage was measured during the application of 2 physiological sliding loads of 50 and 100 N for 2 hours. Mechanical parameters were then correlated with the cellular expression of catabolic genes. We hypothesized that increased sliding speeds would result in elevated stresses and regulate the expression of catabolic marker genes.

2.3 Methods

Unless otherwise indicated, all chemicals were purchased from Life Technologies, CH-6300 Zug, Switzerland.

2.3.1 Cartilage Explants

Cartilage was obtained from bovine nasal septum (BNS) of 20 young calves (12-18 months) provided by a local abattoir within 4 hours of slaughter. BNS is a homogenous and isotropic hyaline cartilage containing chondrocytes in their natural environment, which was easily cut into a rectangular shape for mounting onto our test apparatus.

Cartilage was explanted under sterile conditions by first removing the perichondrium and then cutting into experimental (70 × 17 × 2 mm³) and control (70 × 12 × 2 mm³) (L × W × H) sizes from the same nasal septum using a custom designed cutter. The specimens were washed in phosphate buffered saline (PBS), transferred to culture medium (Dulbecco’s modified eagle’s medium [DMEM] supplemented with 10% fetal bovine serum, 4 mM L-glutamine, 10 mM Hepes buffer, 100 units/mL penicillin, 100 µg/mL streptomycin, 1 mM sodium pyruvate, and 0.25 µg/mL amphotericin B) and equilibrated for 72 hours before sliding tests were performed.

Cell viability of explanted constructs was assessed immediately before loading using an inclusion/exclusion double-label assay kit containing calcein-acetoxyethyl ester (CAM) and propidium
iodide (PI) (Fluka, Buchs, Switzerland). CAM passes through membranes of viable cells and emits a strong green fluorescence once it enters the cell nucleus. PI can only pass through damaged cell membranes (i.e., dead cells) and in combination with the DNA double helix emits a red fluorescence. Cartilage sections (~1 mm thick) were manually cut with a microtome blade, washed in PBS (2 × 5 minutes), and incubated in both stains for 15 minutes at 37 °C. After incubation, the cartilage sections were washed in PBS for another 3 minutes before imaging using a fluorescent microscope.

2.3.2 Mechanical Loading

Ten millimeters of the cartilage specimen were attached to a Plexiglas plate (130 × 34 × 1.5 mm³) with cyanoacrylate glue (Renfert, Hilzingen, Germany) as shown in Figure 2.1. The rest of the cartilage was free to move. The plate and cartilage were placed into a tank filled with culture medium at 37 °C. Control specimens were free and allowed to swell in culture medium at 37 °C.

A cylindrical, nonrotating Teflon indenter (25 mm diameter by 50 mm length) was used to apply a normal force of 50 or 100 N to the cartilage. On reaching the target force, the force was held constant while the indenter slid over the cartilage for 50 mm at a constant speed of 10, 40, or 70 mm/s. After 50 mm translation, the indenter lifted off and returned to the start position to begin another load-sliding cycle (Fig. 2.1). The load was cycled for 120 minutes and the positions and forces of the indenter in the horizontal (x-) and vertical (z-) directions recorded at 100 Hz (minutes 0-5 and minutes 115-120) or 20 Hz (minutes 5-115) using linear variable differential transducers (LVDTs) and load sensors, respectively. The combination of 2 normal forces and 3 sliding speeds resulted in a total of 6 different experimental groups. For each group, at least 3 specimens were tested and for each loading condition a separate cartilage strip (and corresponding control) was used. For detailed information on the loading apparatus see previous work Colombo et al. [2011].

2.3.3 Calculations

The strain, stress, and elastic modulus of the cartilage were continually calculated along the sliding path. Strain (ε) was calculated as a change in thickness (Δl), as measured by the LVDTs, divided by the initial thickness (l₀) of the cartilage specimen

$$\varepsilon = \frac{\Delta l}{l_0}$$

(2.1)

The contact area and stress were calculated using the Hertzian theory of elastic deformation. The semicontact width a of the contact rectangle was calculated using
Figure 2.1: Illustration of the loading setup. The experimental cartilage strip is glued to the Plexiglas plate that is fixed to a Teflon tank filled with culture medium. The entire setup is placed in a flow hood to guarantee sterile conditions. Arrows show the unidirectional motion of the indenter in z- and x-direction. The first 10 mm of the cartilage strip are attached to the Plexiglas by medical glue. The biological sampling area is between \( x = 30 \) mm and \( x = 45 \) mm.

\[
a = \sqrt{r^2 - (r - \delta)^2}
\]  

(2.2)

where \( r \) is the radius of the indenter (12.5 mm) and \( \delta \) the depth of indentation.

The modulus of the cartilage (\( E_{\text{cartilage}} \)) was calculated from

\[
E_{\text{cartilage}} = \frac{1}{E'} \left( 1 - \frac{\nu_{\text{cartilage}}}{E_{\text{Teflon}}} \right)
\]  

(2.3)

where the reduced modulus (\( E' \)) is

\[
E' = \frac{4 \times F \times r}{a^2 \times \pi \times L}
\]  

(2.4)

where \( F \) is the normal force and \( L \) the width of the cartilage strip (17 mm). The Poisson’s ratio for BNS cartilage (\( \nu_{\text{cartilage}} \)) was 0.24 [Colombo et al., 2013] and for the Teflon indenter (\( \nu_{\text{Teflon}} \)) was 0.45. The elastic modulus for Teflon (\( E_{\text{Teflon}} \)) is 500 MPa. The cartilage modulus during sliding will subsequently be referred to as an “apparent modulus” since it may not represent the true compressive elastic modulus of cartilage. The maximum stress under the indenter was then calculated from

\[
\sigma_{\text{max}} = \sqrt{\frac{F \times E_{\text{cartilage}}}{L \times \pi \times R}}
\]  

(2.5)
2.3.4 Gene Expression Analysis

After loading for 2 hours, a 15 mm wide region (30-45 mm from the starting point of the indenter) was removed from the loaded and control specimens for analyses (Figs. 2.1 and 2.2). Three specimens were taken from this area. One specimen was immediately analyzed for gene expression after loading and the other 2 specimens after 4 and 8 hours in culture media at 37 °C. The samples were snap frozen in liquid nitrogen and stored at ~80 °C until RNA extraction. Frozen specimens were pulverized using a biopulverizer (Biospec, Bartlesville, OK, USA), transferred into 1 mL Trizol reagent, centrifuged at 12,000 × g for 10 minutes at 4 °C, supernatant collected, 200 µL chloroform added, vigorously shaken and centrifuged at 12,000 × g for 15 minutes at 4 °C. The aqueous layers were then extracted with 200 µL chloroform (as described above), 500 µL isopropanol added and further centrifugation at 12,000 × g for 10 minutes at 4 °C formed RNA pellets. The pellets were washed with 75% ethanol and resuspended in RNase-free water. RNA was purified using RNeasy mini kit (Qiagen GmbH, CH-8634 Hombrechtikon, Switzerland) and reverse transcribed with QuantiTect Reverse Transcription Kit (Qiagen GmbH, Hombrechtikon, Switzerland) according to the manufacturer’s instructions. Real-time quantitative polymerase chain reaction (RT-PCR) was performed on an iCycler detection system (iQ5, BioRad Laboratories, Inc, Hercules, CA, USA) on 96-well plates with a QuantiFast SYBR Green RT-PCR Kit (Qiagen GmbH, Hombrechtikon, Switzerland).

Figure 2.2: The area between x-position 30 mm to 45 mm was used for harvesting cartilage to analyze gene expression by means of PCR analysis. The sampled strips are shown in dark grey. The indenter speed in the chosen area is constant.

Genes, commonly associated with degenerative states of cartilage, were analyzed and divided in 3 groups: genes degrading collagens and proteoglycans (MMP-3, MMP-13, ADAMTS-4, ADAMTS-5), genes inhibiting the degrading enzymes (TIMP-1, TIMP-3), and the gene for Lubricin, a surface protein, known to be up-regulated with sliding motion and in degenerated states of cartilage. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene (previously verified using a
Table 2.1: Forward and reverse primer sequences (bovine) used for gene expression analysis with real-time PCR (annealing temperature ~60 °C).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-3</td>
<td>CACTCAACCGAACGTGAAGCT</td>
<td>CGTACAGGAACTGAATGCGGT</td>
</tr>
<tr>
<td>MMP-13</td>
<td>TCCAGGAGATGAAGACCCCT</td>
<td>CAGCCGCCAGAAGAATCTGT</td>
</tr>
<tr>
<td>ADAMTS-4</td>
<td>CAT CCTACGCCGGAGAGTC</td>
<td>CATGGAATGCCGCCATCTTG</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>CCTGCCCAGCTAACGGTAAAAA</td>
<td>GGGCAGGACACCAGCATATT</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>TCCCTGGAACAGCATGAGTTC</td>
<td>TGTGCGCTCTGCAGTTTGCA</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>ACTTTGGAGACTCGAGCAGC</td>
<td>CTTGGCTCGGATCAGCATGT</td>
</tr>
<tr>
<td>Lubricin</td>
<td>GAGCAGACCTGAATCCGTGTATT</td>
<td>GGTGGGTTCCTGTTGTAAGTGTA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>ATCAAGAAGGGTGGAAGCAGG</td>
<td>TGAGTGTCGCTGTTGAGTCG</td>
</tr>
</tbody>
</table>

published algorithm [Andersen et al., 2004]. Forward and reverse primers for each gene can be found in Table 2.1. Delta-delta Ct (ΔΔCt) values were calculated for each gene followed by the fold change in expression with the formula \(2^{-\Delta\Delta Ct}\). Primers for MMP-3, TIMP-1, GAPDH, and Lubricin were taken from the literature [Fitzgerald et al., 2006, Grad et al., 2005]. Primer sequences for MMP-13, ADAMTS-4/-5, and TIMP-3 were designed using GeneBank database.

2.3.5 Statistics

A multiple linear regression model was used to express the mRNA regulation as a function of 8 experimental parameters. The linear regression equation for each gene was modeled by

\[
gene\ expression = \sum_{i=1}^{9} b_i - 1 \times x_i - 1
\]

and the following experimental parameters: sliding speed (\(b_1\)), sampling time point (\(b_2\)), cycle number (\(b_3\)), strain (\(b_4\)), x-force (\(b_5\)), z-force (\(b_6\)), modulus (\(b_7\)), and maximum stress (\(b_8\)). The values for each time step were averaged for the biological sampling area for the 2-hour loading period. The experimental parameters were treated as independent variables and plotted against the gene expression. The influence of each factor was determined from the \(r^2\) and P values with a significance level of \(\alpha = 0.05\). A Kolmogorov-Smirnov test was used to determine if the residuals were equally distributed and the Durbin-Watson test to check for autocorrelations. An analysis of variance (ANOVA) was also performed to assess the gene expression changes with regard to the main controlled input variables: z-force, sliding speed, and sampling time. Differences between individual groups were determined with the Fisher’s least significant difference (LSD) test at \(\alpha\)-level = 0.05. A total of 20 BNS were tested with
N ≥ 3 for each experimental group.

2.4 Results

Viability assays showed that cell viability is generally very high and dead cells are only sparsely located throughout the tissue section. A roughly 75 μm thick, acellular outer layer can be found (Fig. 2.3).

Each cartilage specimen was subjected to a sliding load of 50 or 100 N at a constant speed of 10, 40, or 70 mm/s for 2 hours. Table 2.2 illustrates how differences in sliding speed resulted in different cycle times and number of cycles (or impacts) over the 2-hour loading period. For a speed of 10 mm/s, there were 425 cycles; 40 mm/s had a total of 539 cycles or 26.8% more impacts. At 70 mm/s sliding speed, there were 585 cycles and an increase of 37.6% and 8.5% impacts compared with 10 and 40 mm/s, respectively.

Table 2.2: Duration of individual phases of the loading/unloading cycle. Cycle No. refers to the number of cycles during the 2 hour loading time.

<table>
<thead>
<tr>
<th>Sliding Speed (mm/s)</th>
<th>Total Cycle (sec)</th>
<th>Sliding Phase (sec)</th>
<th>Sampling Region (sec)</th>
<th>Unloading (sec)</th>
<th>Total Cycle No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>16.71 ± 0.84</td>
<td>5.06 ± 0.05</td>
<td>1.530</td>
<td>11.60 ± 0.02</td>
<td>425</td>
</tr>
<tr>
<td>40</td>
<td>13.16 ± 0.72</td>
<td>1.33 ± 0.02</td>
<td>0.399</td>
<td>11.82 ± 0.05</td>
<td>539</td>
</tr>
<tr>
<td>70</td>
<td>12.13 ± 1.17</td>
<td>0.76 ± 0.04</td>
<td>0.228</td>
<td>11.29 ± 0.09</td>
<td>585</td>
</tr>
</tbody>
</table>

2.4.1 Initial Loading Phase

Before every sliding cycle, the target load was applied to the edge of the glued cartilage region (x-position = 0 mm, Fig. 2.1) and a stress–strain curve obtained. This provided an estimate of the initial

![Figure 2.3](image-url) CAM-PI staining of a representative cartilage section before the load is applied. The image shows the left border (A), middle section (B) and right border of the cartilage strip (C). The nuclei of live and dead cells are stained green and red, respectively. Scale bar = 250 μm.
mechanical properties of the cartilage and any changes during the 2-hour loading time. Figure 2.4 shows typical stress-strain curves (cycles 100, 200 and 400) for the 50 N and 100 N loads at 10 mm/s. Notice that on unloading, the cartilage regained its initial height after each loading cycle while the stress-strain curve continuously shifted to the right, the latter an indication of ECM softening.

![Figure 2.4](image)

**Figure 2.4:** Typical stress-strain diagrams of the initial loading/compression-phase. Graphs are displayed for sliding speed = 10 mm/s. Curves for ongoing cycle numbers are shown and reveal a shift to the right and flattening of the curve.

### 2.4.2 Mechanical Parameters During Sliding Phase

All mechanical parameters investigated are summarized in Table 2.3. The numbers represent averaged values for the biological sampling area (x-position: 30-45 mm) for the total number of cycles over the entire 2-hour loading period. Significantly higher \((P \leq 0.05)\) strains and maximum stresses were found for samples loaded with 100 N compared with 50 N. No significant differences were observed for apparent moduli and tangential (shear) forces between 50 and 100 N. No significant differences in any parameters were found between different sliding speeds at the same normal force.

### 2.4.3 Correlative Analysis

Analysis of variance between all controlled input parameters (z-force, sliding speed, and sampling time) and gene expression showed no significant differences. However, when the 50 N load data was removed and the ANOVA performed for cartilage loaded with 100 N, ANOVA revealed significant differences between 10 and 70 mm/s sliding speed for Lubricin \((P = 0.012)\) and ADAMTS-5 \((P = 0.049)\). No significant differences were found for different time points with all genes.

Linear correlations with 50 N revealed low \(r^2\) values for all gene expressions and mechanical parameters, and thus only the results for the 100 N normal force are given in Tables 2.4 and 2.5. Significant
Table 2.3: Mean values ± SD for the calculated mechanical parameters for the biological sampling area averaged over the 2 hour loading period. * Indicates P < 0.05 as compared to the same sliding speed loaded with 50 N.

<table>
<thead>
<tr>
<th>Mechanical Parameter</th>
<th>50 N</th>
<th>100 N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mm/s</td>
<td>40 mm/s</td>
</tr>
<tr>
<td>z-force (N)</td>
<td>55.03 ± 2.9</td>
<td>63.66 ± 7.8</td>
</tr>
<tr>
<td>x-force (N)</td>
<td>1.36 ± 0.8</td>
<td>2.12 ± 0.9</td>
</tr>
<tr>
<td>Strain (%)</td>
<td>28.11 ± 3.1</td>
<td>28.10 ± 5.2</td>
</tr>
<tr>
<td>Stress (MPa)</td>
<td>0.51 ± 0.04</td>
<td>0.61 ± 0.14</td>
</tr>
<tr>
<td>Modulus (MPa)</td>
<td>3.16 ± 0.4</td>
<td>4.16 ± 1.7</td>
</tr>
</tbody>
</table>

correlations between mechanical parameters and gene expression were found for TIMP-3 ($r^2 = 0.89$), ADAMTS-5 ($r^2 = 0.73$), and Lubricin ($r^2 = 0.73$). Positive and negative correlations of experimental parameters varied with different genes. All residuals for each gene were normally distributed and the Durbin-Watson test did not reveal any autocorrelation between the mechanical parameters.

2.5 Discussion

Cartilaginous surfaces of human joints, such as the knee or temporomandibular joint (TMJ), spend most of their time in relative motion with a continuous translation of the contact zone [Gallo et al., 2006, Katta et al., 2008]. Several research groups have investigated joint tribology from a purely mechanical point of view [Caligaris and Ateshian, 2008, Bonnevie et al., 2011, 2012, Accardi et al., 2011, Forster and Fisher, 1999]. Mechanobiological studies do not incorporate such migrating contacts into their loading regimens, however they do approximate the in vivo joint with compressive and/or shear forces. Dependent variables usually include strain rate and stress [Fitzgerald et al., 2004, Quinn et al., 2001] as well as temporal patterns [Lee et al., 2005]. By allowing the contact area to migrate over the cartilage surface, new variables such as sliding speed, cycle number, and tangential force are introduced. Thus, strain and stress become dynamic parameters depending on the speed applied. Our mechanobiological study was designed to address the complexity and multiaxial nature of joint motion with the goal to investigate the biological response in terms of catabolic gene expression. Lubricin was included into the study since it is considered a joint lubricant and its gene expression has been shown to increase with surface motion Grad et al. [2005], Nugent et al. [2006] as well as being increased in patients with degenerated joint diseases Neu et al. [2010].

We used a mechanical test system capable of simultaneously applying a normal force and tangen-
Table 2.4: Gene expression = $b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + b_7x_7 + b_8x_8$. Table presents values representing the correlation coefficients for each experimental parameter. Cycle No. refers to the number of cycles during the 2-hour loading time.

<table>
<thead>
<tr>
<th>Gene</th>
<th>b0</th>
<th>b1: Sliding Speed (mm/s)</th>
<th>b2: Time Point (hr)</th>
<th>b3: Cycle No.</th>
<th>b4: Strain (%)</th>
<th>b5: x-force (N)</th>
<th>b6: z-force (N)</th>
<th>b7: Modulus (MPa)</th>
<th>b8: Max. Stress (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubricin</td>
<td>-10.14</td>
<td>0.47</td>
<td>0.25</td>
<td>0.26</td>
<td>18.10</td>
<td>4.11</td>
<td>0.38</td>
<td>31.81</td>
<td>339.88</td>
</tr>
<tr>
<td>MMP-3</td>
<td>316.29</td>
<td>-2.06</td>
<td>-3.76</td>
<td>1.20</td>
<td>-597.98</td>
<td>-61.16</td>
<td>2.41</td>
<td>82.18</td>
<td>-1205.99</td>
</tr>
<tr>
<td>MMP-13</td>
<td>20.12</td>
<td>-0.05</td>
<td>-0.33</td>
<td>0.04</td>
<td>-70.53</td>
<td>-5.57</td>
<td>0.55</td>
<td>3.46</td>
<td>-74.81</td>
</tr>
<tr>
<td>ADAMTS-4</td>
<td>15.48</td>
<td>0.04</td>
<td>-0.11</td>
<td>-0.01</td>
<td>-64.12</td>
<td>-1.93</td>
<td>0.88</td>
<td>9.58</td>
<td>-135.14</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>12.56</td>
<td>-0.03</td>
<td>0.16</td>
<td>0.03</td>
<td>-38.06</td>
<td>-3.19</td>
<td>0.07</td>
<td>-2.52</td>
<td>3.09</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>9.24</td>
<td>0.04</td>
<td>-0.04</td>
<td>-0.02</td>
<td>-20.31</td>
<td>-0.92</td>
<td>-0.03</td>
<td>-5.49</td>
<td>44.33</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>3.82</td>
<td>0.15</td>
<td>0.05</td>
<td>-0.07</td>
<td>-19.58</td>
<td>-1.37</td>
<td>-0.45</td>
<td>-21.55</td>
<td>210.40</td>
</tr>
</tbody>
</table>
Table 2.5: R²-values indicate the fit of the regression equation to explain the change in gene expression. The numbers below the mechanical parameters represent p-values for each regression coefficient. The Gene column refers to the number of cycles during the 2 hour loading time.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sliding Speed (mm/s)</th>
<th>Time Point (hrs)</th>
<th>Cycle No.</th>
<th>Strain (%)</th>
<th>x-force (N)</th>
<th>z-force (N)</th>
<th>Modulus (MPa)</th>
<th>Max. Stress (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubricin</td>
<td>0.002</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.473</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>MMP-1</td>
<td>0.876</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>MMP-2</td>
<td>0.616</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>MMP-13</td>
<td>0.132</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>0.032</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>0.73</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>MMP-4</td>
<td>0.73</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>MMP-1</td>
<td>0.616</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
<tr>
<td>MMP-13</td>
<td>0.132</td>
<td>0.012</td>
<td>0.060</td>
<td>0.050</td>
<td>0.014</td>
<td>0.033</td>
<td>0.89</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Gene R² Sliding Speed (mm/s) Time Point (hrs) Cycle No. Strain (%) x-force (N) z-force (N) Modulus (MPa) Max. Stress (MPa)
tial sliding velocity, as previously described [Colombo et al., 2011]. Physiological loading conditions were established by combining 50 and 100 N normal forces with previously reported functional sliding speeds of 10, 40, and 70 mm/s [Gallo et al., 2006, Gilbert et al., 2014]. Since most biomaterials, such as hydrogels, are mechanically weak and unable to resist physiological sliding/shear loads, a cell-scaffold system could not be used. In addition, the isolation of chondrocytes prior to adding them to a scaffold makes them prone to dedifferentiation, which can change the biological response [Müller et al., 1977]. Because of its size and availability, BNS was found to be a suitable hyaline cartilage model to investigate the response of chondrocytes to such mechanical forces. Most important, BNS incorporates the chondrocytes within their natural ECM environment. BNS can be shaped into sufficiently large and flat specimens allowing the application of translations over large sliding paths. It is abundantly available and shown to have viscoelastic behavior, with similar mechanical properties to a variety of other cartilaginous tissues [Colombo et al., 2013]. In addition, the alignment of collagen fibers provides homogeneity in the plane used for the experiments [Xia et al., 2012].

For this study, contact stress and apparent modulus were calculated from the Hertzian theory of elastic deformation. Since the indenter is moving along the horizontal plane, the point of contact is dynamic and changes with time. To calculate contact stress and modulus, values for each time step were calculated and averaged over the biological sampling area. While these values do not necessarily represent the true elastic properties of the material, they are representative of the mechanical response and present a valid tool to compare relative changes with other sliding speeds and normal forces. Moore and Burris [2014] recently developed an analytical model to determine functional parameters, such as contact area and stress under migrating contact conditions. However, their approach is limited to small contacts of a sphere on a plane surface and is therefore not directly applicable to our study.

After each loading cycle, the initially loaded cartilage region (at x = 0) recovered to its initial thickness whereas the deformation in the sampled sliding region (x = 30-45 mm) continually increased. With the assumption of a recovery to its initial thickness after each loading cycle in the sliding region, the increasing deformation indicates a softening of the cartilage with increasing sliding load duration. However, the fact that the tissue is not attached and gets temporarily stretched during loading could lead to a different response. Because of the averaging of the mechanical values (for the 2-hour load) used in the regression model, this information was not taken into account when analyzing the gene response.

As expected, there was increased strain, contact stress, apparent modulus, and tangential force with 100 N compared with 50 N. However, sliding speed did not significantly affect the response of these mechanical parameters, which could be explained by a partial attainment of the “elastic limit” at and
above 10 mm/s sliding speed. The finding of a slight increase in some of the experimental parameters between 10 and 40 mm/s, but not between 40 and 70 mm/s, further supports this and indicates that the cartilage is in transition from a viscoelastic to an elastic regime. This is in agreement with Bonnevie et al., [2011] who found that sliding speeds greater than 1 mm/s did not cause changes in cartilage deformation.

In the present study, sliding contact stresses were in the order of 0.6 MPa (50 N) and 0.9 MPa (100 N) as calculated from Equation 2.5. Compared with physiological stresses in cartilage, these levels are on the lower limit. The lack of significant changes in gene expression for the 50 N specimens could be regarded as a low-stress response similar to no loading, and less as a metabolic response due to the applied loading regimen. However, by analyzing only the 100 N loaded cartilage, we found several significant correlations for Lubricin, ADAMTS-5, and TIMP-3. Lubricin gene expression was significantly influenced by sliding speed, time point, cycle number, horizontal (shear) force, modulus, and maximum stress, though all these parameters are not completely independent (Table 2.5). The absence of gene expression changes with strain may be due to the small number and range of sliding speeds and normal loads used. ANOVA with the 100 N samples did show significantly higher Lubricin and ADAMTS-5 gene expression between the 10 and 70 mm/s groups, which further emphasizes the results from the regression analysis and highlights the importance of sliding speed. Also, finite element analysis has shown that different sliding speeds result in dissimilar patterns of fluid flow [Guo et al., 2012]. This is likely to affect the micro- and nano-environment of the chondrocytes. Ateshian et al. [2007] calculated that fluid shear stresses acting on the chondrocyte surface differ by several orders of magnitude from the shear stresses in the solid matrix. Thus, differences in interstitial hydrostatic pressures and osmotic changes might account for an alteration in gene expression, as already proposed in other studies [Mow et al., 1999, Parkkinen et al., 1993, Lippiello et al., 1985].

Finally, different sliding speeds obviously changed the number of cycles and therefore, these 2 factors seem to be inherently correlated with each other. However, for TIMP-3 gene expression, we found a positive correlation with sliding speed but a negative correlation with cycle number. It is also important to keep in mind that an up-/down-regulation of genes does not necessarily imply an up./down-regulation of proteins. The fact that BNS is not a loaded tissue and does not have the zonal arrangement present in articular cartilage could be regarded as a potential limitation of the study. Therefore it is reasonable to assume that articular cartilage would react slightly different under the same loads.

In conclusion, this study demonstrates the importance of applying migrating contact loads to cartilage explants for studying the biological responses of the tissue. Even though we found no obvious
macroscopic changes in the mechanical variables by comparing different sliding speeds, changes in the mRNA expression pattern still occur, possibly evoked by the migrating contact points. Therefore, applying such multiaxial forces is important when simulating joint kinematics in mechanobiological studies. In a first step a profound mechanical analysis of the behavior of the tissue under the applied mechanical stimuli is vital. Second, the mechanical parameters need to be directly linked to the biological response. This will improve our understanding of how chondrocytes react to such dynamic and multi axial forces during articulation.

Author Contributions

This is to declare that all authors contributed to the conception and design of this study and approved the submission of this manuscript. OS: data acquisition, data analysis, data interpretation, writing of the manuscript. PT, LG, MM: data interpretation, critical review. LG: obtaining funding.

Acknowledgments

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was supported by the Swiss National Science Foundation; Grant No. 325230-130715. Swiss National Science Foundation promotes scientific research in Switzerland.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
Chapter 3

A Model to Study Articular Cartilage Mechanical and Biological Responses To Sliding Loads

Oliver R. Schätti¹,²,³, Luigi M. Gallo², Peter A. Torzilli¹

¹Laboratory for Soft Tissue Research, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021, USA
²Laboratory of Physiology and Biomechanics of the Masticatory System, Center for Oral Medicine, Dental and Maxillo-Facial Surgery, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland;
³Institute for Biomechanics, Swiss Federal Institute of Technology, ETH Zentrum, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

Keywords: Mechanobiology, Cartilage mechanics, Dynamic loading, Biotribology, Gene expression

This manuscript has been archived after Annals of Biomedical Engineering 1-12, Dec 2015. The final publication is available at Springer via http://dx.doi.org/10.1007/s10439-015-1543-9.

3.1 Abstract

In physiological conditions, joint function involves continuously moving contact areas over the tissue surface. Such moving contacts play an important role for the durability of the tissue. It is known that in pathological joints these motion paths and contact mechanics change. Nevertheless, limited information exists on the impact of such physiological and pathophysiological dynamic loads on cartilage mechanics and its subsequent biological response. We designed and validated a mechanical device
capable of applying simultaneous compression and sliding forces onto cartilage explants to simulate moving joint contact. Tests with varying axial loads (1 - 4 kg) and sliding speeds (1 - 20 mm/s) were performed on mature viable bovine femoral condyles to investigate cartilage mechanobiological responses. High loads and slow sliding speeds resulted in highest cartilage deformations. Contact stress and effective cartilage moduli increased with increasing load and increasing speed. In a pilot study, changes in gene expression of extracellular matrix proteins were correlated with strain, contact stress and dynamic effective modulus. This study describes a mechanical test system to study the cartilage response to reciprocating sliding motion and will be helpful in identifying mechanical and biological mechanisms leading to the initiation and development of cartilage degeneration.

3.2 Introduction

The integrity of the extracellular matrix (ECM) of articular cartilage is controlled by a balance of anabolism and catabolism. Normal physiological forces lead to adaptation and physiological remodeling of the matrix so that it can distribute compressive loads and enable a low-friction motion. On the contrary, abnormal or non-physiological forces can result in over-expression of matrix enzymes such as matrix metalloproteinases (MMPs) and disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS') that lead to tissue destruction as observed in joint diseases and osteoarthritis (OA) [Heinegård and Saxne, 2011]. In OA, the equilibrium between anabolism and catabolism is shifted towards catabolic processes which impairs ECM integrity [Grodzinsky et al., 2000]. A persistent predominance of catabolic stimuli results in tissue deterioration, compromises its ability to cope with mechanical stresses and ultimately results in inferior mechanical properties what expedites further degeneration and tissue breakdown [Andriacchi et al., 2004, Carter et al., 2004]. The balance between mechanics and biology led researchers to investigate the gene expression response of ECM genes and degradative enzymes to mechanical forces. Applied loads in the physiological range generally stimulated chondrocytes to up-regulate genes for matrix proteins such as collagen type II and aggrecan [Valhmu et al., 1998, Wong et al., 2003]. On the other hand, supra-physiological loads resulted in over-expression of catabolic enzymes and inflammatory cytokines [Lee et al., 2005]. While these studies offer important insight into mechanobiological processes, the mechanical loading was applied to isolated explants over a stationary contact area. It is known however, that joint surfaces move relative to each other and the area of contact stress is in continuous motion [Gallo et al., 2000, Kizuki et al., 1995]. From a tribological standpoint, these moving contacts/stress-fields are an important component of joint kinematics to produce high fluid pressurization and a sustainably low coefficient of friction [Ateshian, 2009, McCutchen, 1962, Soltz and Ateshian, 1998].
Pathological changes in joints can alter these stress-field kinematics and result in changes in loading paths and patterns [Gallo et al., 2006, Kizuki et al., 1995]. Such altered kinematics can have negative consequences for articular cartilage biomechanics. They potentially expose previously unloaded or less conditioned cartilage areas to mechanical forces [Andriacchi et al., 2004]. In the knee joint, anteroposterior (AP) translation can be as high as 6 mm in healthy joints but almost double that after anterior cruciate ligament (ACL) injury [Kizuki et al., 1995]. In addition, the relative sliding velocity between the femur and tibia has been shown to significantly increase after ACL section in a dog model [Anderst and Tashman, 2009]. This in turn changes loading frequencies and evokes an altered deformational response due to the tissue’s poroelasticity. Some of these changes have already been proposed as possible inducers for OA [Anderst and Tashman, 2009, Beveridge et al., 2013]. For this reason, parameters such as sliding speed, loading time, path length and contact area have already been investigated from a mechanical perspective [Beveridge et al., 2013, Bonnevie et al., 2011, Caligaris and Ateshian, 2008]. However, the use of this fundamental knowledge of joint kinematics in a mechanobiological model is missing. Questions still remain on how variations in sliding speed, loading paths and contact geometries influence cartilage mechanics and biology. This paper describes a test system for examination of such moving stress-fields on cartilage. Our goal was to investigate articular cartilage mechanical and biological responses to contact forces applied over a relatively large area, such as a bovine knee condyle. For that reason, a new mechanical test system was developed and preliminary mechanical and biological results on cartilage explants are presented.

We hypothesized that (1) high axial forces in combination with low speeds would result in increased cartilage strain, contact stress and dynamic effective modulus, and (2) that these increases would be positively correlated with the gene expression of catabolic enzymes.

To test hypothesis (1) we applied 1, 2, 3 and 4 kg axial loads in combination with 1, 2, 5, 10 and 20 mm/s sliding speeds and measured the resulting cartilage strains, contact stresses and dynamic effective moduli during dynamic loading. For hypothesis (2), we applied the different axial loads to bovine femoral condyles at a sliding speed of 10 mm/s and measured the gene expression of anabolic (collagen type II, aggrecan, sox 9, fibronectin) and catabolic (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5) proteins. We found that high loads and slow sliding speeds resulted in the highest cartilage deformations, while the contact stress and effective cartilage moduli increased with increasing load and speed. In addition, changes in gene expression of extracellular matrix proteins correlated with increases in strain, contact stress and dynamic effective moduli. However, the gene expression for degradative enzymes was not correlated with the changes in dynamic loading.
3.3 Materials and Methods

3.3.1 Device and Components

A mechanical test apparatus for mechanobiological studies of articular cartilage was designed and constructed. The device, called “Dynamic Articular Cartilage Test System” (DACTS) was designed to apply dynamic sliding or rolling and axial force onto the articular surface of a cartilage explants. The DACTS mimicked the migrating contact area during articular joint motion where two opposing cartilage surfaces slide or roll against each other under the compressive force of muscles, ligaments and other soft tissues, and body weight. The goal of using this type of test apparatus was to investigate the mechanical and biological response of articular cartilage to such sliding and rolling forces. Even though the DACTS was designed to apply sliding and rolling, this study only focused on the application of sliding loads onto articular cartilage explants.

To load the cartilage, we used a spherical Delrin ball (diameter = 2.54 cm) to apply a constant normal (perpendicular) force onto the surface of the articular cartilage layer of a femoral condyle. In brief, the DACTS is composed of a dead-weight load-frame attached by movable linear bearings to two parallel, vertically aligned rods. To control the applied dead–weight force, the frame is suspended by a 111.2 N load cell (MLP-25, sensitivity 2 mV/V, Transducer Techniques, Temecula, CA) and a frictionless air cylinder (E16DU, Airpot, Norwalk, CT) (Fig. 3.1). The Delrin sphere is attached to a horizontal rod, which was fixed to prevent it from rolling in order to have pure sliding contact with the cartilage. The test specimen (condyle) is held using a vise clamp mounted in a tank attached to a linear stage (Design Components Inc., Palatine, IL) driven by a stepper motor (LE57-51, Parker Compumotor, Rohnert Park, CA). The stepper motor displacement (X-direction, 1 μm resolution), velocity and acceleration are controlled via a computer-interface (Laboratory Technologies Corporation, Wilmington, MA). The X-displacement was monitored using a ± 50.8 mm linear variable differential transformer (LVDT) (Sensotec, Columbus, OH; model VL7A, 10 μm/mV resolution) and the vertical Y-displacement using two ± 5.08 mm LVDTs (model DS400A, 1 μm/mV resolution).

3.3.2 DACTS Validation

To validate the system’s performance (accuracy and precision), tests were run with different loads (1, 2, 3 and 4 kg) and speeds (1, 10 and 40 mm/s). The different test cases were applied to a metal femoral condyle, with the x-displacement = ± 12 mm. Twenty cycles were run for each load/speed-combination to determine accuracy and precision of the system. The applied velocity profile was trapezoidal with equal times for acceleration and deceleration. Acceleration/deceleration for all test cases was chosen...
3.3.3 Stiffness/Compliance of the System

In order to correct for the system’s stiffness, the compliance was measured and subtracted from the raw data before analysis. The indenter sphere was lowered onto a rigid metal plate with increasing load and the load and Y-displacement continuously recorded. The Y-displacement was plotted against the load and a polynomial compliance curve fitted to the resulting load-displacement response.

3.3.4 Corrections for the Curvature of the Condyle

To obtain the curvature of the specimen, the indenter was repeatedly lowered onto the surface, yielding force-deformation responses, which were used to determine the surface geometry (at contact) and initial effective modulus ($E_0$), calculated from Hertzian theory (see Equation 3.4). First, the compliance of the system was subtracted from the raw data and the values from both Y-LVDTs averaged. The data obtained from the LVDTs represent the location of the center of the indenter as it moves along the surface. All coordinates of the ball center at surface contact were then fitted using a 3rd order polynomial. Displacements and contact forces were then corrected according to the slope of the condyle (Fig. 3.2).
The slope of the curvature at each point along the X-axis was derived from the polynomial and the corrected X- and Y-displacement – the real contact point of indenter and cartilage - calculated via the sine of the angle at any given location. A second polynomial was then fitted to the corrected X- and Y-coordinates representing the true cartilage surface. After completion of the loading experiments, the cartilage was removed from the bone with a surgical blade and the bone surface was identically mapped in order to obtain the spatial variation of the cartilage thickness.

To calculate contact stresses and elastic moduli, Hertzian theory of elastic deformation was used to calculate a single reduced radius (R’) for the curved surfaces of the indenter and the condyle. The reduced radius was calculated with \( 1/R' = 1/R_a + 1/R_b \) where \( a \) (body \( a \)) represents the Delrin indenter and \( b \) (body \( b \)) the condyle. Since the Delrin indenter is a sphere, \( R_a = 1.27 \) cm. For the condyle, the \( R_b \) was calculated from the polynomial fit to the cartilage surface given by

\[
R_b = \left[ 1 + \left( \frac{dy}{dx} \right)^2 \right]^{\frac{3}{2}}. \tag{3.1}
\]

To obtain the initial cartilage thickness, the shortest distance between the cartilage surface and bone was calculated with a Matlab (MathWorks, Natick, MA) routine.

For each cycle during the loading process, a 3rd order polynomial was obtained according to the procedure described above. These polynomials represented the cartilage contact surface at any given time under the applied loading conditions. With this information, cartilage deformation (\( \delta \)), total strain (\( \varepsilon \)), maximum dynamic contact stress (\( \sigma_{\text{max}} \)) and dynamic effective modulus (\( E^* \)) could be calculated at each location along the condyle for each individual cycle using Hertzian theory. The bone was considered incompressible.
### 3.3.5 Calculations of Mechanical Parameters

Total strain ($\varepsilon$) was calculated by a change in thickness (deformation, $\delta$) as measured by the LVDTs, divided by the initial thickness ($l_0$) of the cartilage specimen

$$\left( \varepsilon = \frac{\delta}{l_0} \right).$$

By using Hertzian theory of elastic deformation [Johnson, 1987] the contact radius ($a$) between the indenter and the cartilage was calculated with

$$a = \sqrt{\delta \cdot R'}$$

and the effective modulus ($E^*$) by using

$$E^* = \frac{3 \cdot F \cdot R'^{-0.5} \cdot \delta^{-1.5}}{4}.$$ (3.4)

The axial force ($F$) was measured by the load cell and $R'$ and $\delta$ calculated as described in paragraph 3.3.4. Note that $E^*$ is not a true material property. Amongst others, it depends on material properties (elastic modulus, permeability) of the cartilage explant and Delrin sphere, the sliding speed (deformation rate), and the reduced radius. However it does provide a valid tool to measure relative changes between the different loading conditions used in this study.

We modeled the cartilage layer as a thin elastic compressible layer bonded to a rigid substrate (bone). Using the formulas from Jaffar [2006], the maximum contact stress ($\sigma_{\text{max}}$) was calculated with

$$p_{\text{max}} = \frac{2 \cdot F}{\pi \cdot a}.$$ (3.5)

### 3.3.6 Loading of Cartilage Explants

Unless otherwise indicated, all chemicals were purchased from Life Technologies, Grand Island, NY.

Mature bovine knees were obtained from a local abattoir within 24 hours of death. Viable femoral condyles were removed, rinsed with sterile PBS to remove blood and bone marrow, and either incubated overnight in culture medium (Dulbecco’s modified eagle’s medium (DMEM) supplemented with 1% antibiotic-antimycotic and 10 mM Hepes buffer) for live studies or wrapped in PBS (phosphate buffered saline)-soaked gaze and frozen at -80° C for mechanical tests. All tests were performed at room temperature.
3.3.6.1 Mechanical Response to Sliding Loads

Frozen bovine condyles were thawed overnight at 4°C and mounted into the DACTS, the cartilage kept wet by a continuous flow of PBS for the duration of the experiment. The indenter was fixed to prevent it from rotating, therefore only allowing a sliding motion. The cartilage surface was mapped as described above using 12 N load, yielding force-deformation curves which were converted to stress-strain using Hertzian theory. Following a 20-minute recovery period, the sphere was lowered onto the apex of the condyle and cyclically slid over the cartilage (x = ± 15 mm amplitude in X-direction), at speeds of 1, 2, 5, 10 or 20 mm/s with applied loads of 1, 2, 3 or 4 kg for 25 cycles. After each test the cartilage surface was checked for visual cartilage damage with India ink (Speedball Art, Statesville, NC), rinsed and left unloaded to recover for 20 minutes before the next test was started. Prior the start of each test the indenter was lowered to touch the cartilage surface (x=0) in order to guarantee full recovery of the tissue to its initial height. After the cartilage was loaded with all speed and load combinations, the cartilage layer was removed and the bone surface mapped.

3.3.6.2 Mechanobiological Response to Sliding Loads

For the mechanobiological study, explants were removed from the culture medium immediately before the mechanical loading was applied and kept wet by a continuous flow of culture medium. The indenter was prevented from rotating and the cartilage surface was mapped as previously described. Following the 20-minute recovery period, the indenter was cyclically slid on the cartilage (x = ± 18 mm) at 10 mm/s and 1.2, 2.4, 3.6 or 4.8 kg for 400 cycles (48 minutes loading time) at room temperature. After each test, 6 mm diameter full-depth cartilage samples (N_{total} = 15) were harvested along the loading path (± 18 mm), incubated for 72 minutes in culture medium at 37°C (loading + incubation = 2 hours) and processed for mRNA analysis. The entire cartilage layer was then removed to map the bone surface, as described above. At each biological sampling location (Fig. 3.3), the following mechanical parameters were calculated: initial cartilage thickness, initial effective cartilage modulus, applied normal force, deformation, strain, stress and dynamic effective modulus. For each mRNA sample, the gene response was analyzed by real-time quantitative polymerase chain reaction (RT-qPCR). A linear regression model was used to correlate the mechanical parameters with mRNA. Adjacent unloaded cartilage specimens were used as controls. Input values for the mechanical parameters were the final values after 400 cycles.

3.3.7 Gene Expression

Genes, commonly associated with degenerative states of cartilage, were analyzed and divided in two groups: anabolic genes for the ECM constituents (collagen type II, aggrecan, fibronectin, sox 9) and
catabolic genes for degrading ECM constituents (MMP-3, MMP-13, ADAMTS-4, ADAMTS-5). Glyceraldehyde-3-phosphatde dehydrogenase (GAPDH) and ribosomal protein L13a (RPL13a) were used as housekeeping genes. Forward and reverse primers for each gene can be found in Table 3.1. Delta-delta Ct ($\Delta \Delta Ct$) values were calculated for each gene followed by the fold change in expression with the formula $2^{-\Delta \Delta Ct}$.

**Table 3.1:** Forward and reverse primer sequences (bovine) used for gene expression analysis with real-time PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-3</td>
<td>GCAAGCCCATTAAGACCACATCA</td>
<td>TTCTAGATATTGCTGAACAAAGCTCC</td>
</tr>
<tr>
<td>MMP-13</td>
<td>TCCAGTTTGCAGAGAGCTACC</td>
<td>CTGCCAGTCACCTCTAAGCC</td>
</tr>
<tr>
<td>ADAMTS-4</td>
<td>CATCCTACGCGGAAGAGATC</td>
<td>CATGGAATGCCGCCATCTTG</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>TGGAAAGGGACGATTCGCTG</td>
<td>AGAGGTCAAGAGACTGCCAGC</td>
</tr>
<tr>
<td>Collagen Type II</td>
<td>GCTTCCACTTCACTATGGA</td>
<td>CAGGGAACTTCTCCACGA</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>GGGGAGGAGACGACTGCAATC</td>
<td>CCCATTCCGTCTTGTCTTCT</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>CTACCCTCACTGTGGTGGGAC</td>
<td>TTCCAGGAACACTGGGAACCTGT</td>
</tr>
<tr>
<td>Sox 9</td>
<td>ACGCCGAGCTCAGCAAGA</td>
<td>CACGAACGGGCTTCTT</td>
</tr>
<tr>
<td>RPL13a</td>
<td>GCCGTACTGGAAGCTTGGCT</td>
<td>GCCGTACCTGCTGGACCTTT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GGTGTCATCATTCTCTGACACCT</td>
<td>GGTCATAAGTCCACCAAG</td>
</tr>
</tbody>
</table>

### 3.3.8 Statistics

The relationship between the mechanical parameters and the biological response was assessed with univariate and multiple linear regressions using the r-squared ($r^2$) value to determine the best fit model. For each regression coefficient an $\alpha$-level = 0.05 was considered statistically significant.
3.4 Results

3.4.1 System Validation

System compliance was determined by fitting a 2nd order polynomial to the load vs. Y-deformation using the full weight of the load frame (4828 ± 43.2 g). The X-displacement vs. time profile used was a triangle waveform with constant acceleration of 400 mm/s², the speeds of 1, 10 and 40 mm/s reached their maximum value in 0.1 sec., 0.025 sec., and 0.0025 sec, respectively. Calculations of the actual speed from 20 cycles at 1, 10 and 40 mm/s input (at full load, 4.8 kg normal load) was 1.012 mm/s ± 0.004 mm/s, 10.08 mm/s ± 0.09 mm/s and 39.45 mm/s ± 1.25 mm/s, respectively. The air cylinder was able to control the dead-weight loads for all speeds with an accuracy >93% and the coefficient of variation < 7% (Table 3.2).

Table 3.2: Load accuracy during dynamic loading; Mean ± SD

<table>
<thead>
<tr>
<th>Load (g)</th>
<th>1 mm/s</th>
<th>10 mm/s</th>
<th>40 mm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>929.92 ± 21.37</td>
<td>1031.59 ± 71.03</td>
<td>973.37 ± 37.4</td>
</tr>
<tr>
<td>2000</td>
<td>1950.04 ± 10.75</td>
<td>1914.87 ± 94.58</td>
<td>2039.31 ± 31.99</td>
</tr>
<tr>
<td>3000</td>
<td>2991.52 ± 14.96</td>
<td>3016.35 ± 66.79</td>
<td>2994.33 ± 38.02</td>
</tr>
<tr>
<td>4000</td>
<td>3989.47 ± 11.00</td>
<td>4026.40 ± 36.03</td>
<td>3992.54 ± 16.44</td>
</tr>
</tbody>
</table>

3.4.2 Mechanical Response to Sliding Loads

One condyle was used to measure cartilage deformation for each load and speed combination. Results are presented only for the X-position = 0 (X=0). However the same responses were found for any given location along the condylar surface. All condyles underwent creep deformation (Fig. 3.4) that was more evident at higher loads. The application of different axial forces led to a variety of strains, contact stresses and dynamic effective moduli (Figs. 3.5 and 3.6, respectively). The initial cartilage thickness at x=0 was 1.48 mm while the mean thickness at the start of all tests was 1.43 mm ± 0.0856 mm. No visual cracks were observed with Indian ink after the tests were performed. An increase in axial load led to increased deformation of the tissue and higher strains (Fig. 3.4). Total strain increased from the first loading cycle, reaching a plateau after 5-10 cycles, independent of the load and sliding speed applied. An increase in sliding speed resulted in decreased deformational response of the tissue and lower strains. Additionally, an increase in axial load as well as sliding speed resulted in higher contact stresses and an increased dynamic effective modulus (Figs. 3.5 and 3.6).
Figure 3.4: Strain at X-location = 0 mm during 25 cycles of loading for axial loads of 1 kg (A), 2 kg (B), 3 kg (C) and 4 kg (D) at 1 mm/s, 2 mm/s, 5 mm/s, 10 mm/s and 20 mm/s sliding speed.

Figure 3.5: Calculated contact stress at X-location = 0 mm during 25 cycles of loading for axial loads of 1 kg (A), 2 kg (B), 3 kg (C) and 4 kg (D) at 1 mm/s, 2 mm/s, 5 mm/s, 10 mm/s and 20 mm/s sliding speed.
3.4.3 Mechanobiological Response to Sliding Loads

Regression analysis between mechanical parameters and gene expression (N = 15) found several significant correlations. Contact stress, strain and dynamic effective modulus had the largest effect on gene expression of ECM molecules. Results are presented for univariate linear regression in Figure 3.7. The multiple regression analysis showed that only fibronectin gene expression was significantly influenced by more than one mechanical parameter. Sixty-six percent of the increase in fibronectin gene expression was due to the increase in strain (e) and initial effective modulus (E₀) where fibronectin gene expression = 1.303 – 0.0569 e [%] + 0.1120 E₀ [MPa].

3.5 Discussion

In this paper we present the design and validation of a new cartilage testing system (DACTS) capable of applying a constant sliding load to the surface of articular cartilage of osteochondral explants of different geometric shape. This system is an approach to more realistically mimic the in vivo joint kinematics where relative sliding and rolling between articulating surfaces induces moving contact points. The capability of loading curved surfaces [Caligaris and Ateshian, 2008] is an advantage over other devices which load flat tissue sections [Colombo et al., 2011, Schätti et al., 2015b, Whitney et al., 2015]. Velocity profiles had good accuracy between input and output speeds with the largest difference.
Figure 3.7: Scatterplots showing gene expression vs. individual mechanical parameters. The gene expression was normalized (fold change) to the unloaded control (dashed lines). Significant correlations ($p < 0.05$) are shown for fibronectin vs. strain (A) and initial effective modulus (B), collagen type II vs. contact stress (C), and initial effective modulus (D) and aggrecan vs. dynamic effective modulus (E). The fit of the linear model is denoted $R^2$ and the slope of the line is equal to $a$. The units of the slope are fold expression (FE) per unit of the mechanical parameter.
at 40 mm/s speed with 3.2%. The use of an air cylinder allowed consistent application of constant loads with variations of less than 7%.

The mechanical analysis of bovine condyles subjected to different sliding loads has found a load- and speed-dependent deformational response of the articular cartilage (Fig. 3.4). Beside an expected increase in deformation (strain) with increasing axial load, we also found decreased deformation (strain) with increasing speed. A four-fold increase in load (from 1 kg to 4 kg) had a larger effect on deformation than a twenty-fold increase in speed (1 mm/s to 20 mm/s).

The speed-dependency of the deformation can be explained by the poroelastic nature of articular cartilage. According to the biphasic theory, the compressive behavior of articular cartilage depends on the resistance or permeability of fluid flow through the solid matrix [Mow et al., 1980]. Increased cyclic contact speeds result in higher loading frequencies and loading rates where the fluid component has less time to escape the solid matrix. This results in higher tissue stiffness (dynamic effective modulus) (Fig. 3.6) and consequently less strain. This nonlinear behavior has already been described in compression experiments [Lee et al., 1981] and microtribology studies [Bonnevie et al., 2011] over a wide range of loading frequencies and speeds. Since contact speed has a direct influence on loading rate and instantaneous compressive modulus, it will also affect other mechanical parameters such as contact area and contact stress. Contact stress depends on the contact area, therefore it is imperative to understand the deformational properties of the tissue under the indenter in order to make accurate assumptions on the area in contact. According to Ateshian and Wang [1995] the symmetry of the contact depends on the Peclet number (Pe) and is defined as Pe = (V.A) / (Ha.x) where V is the sliding velocity, A the cartilage thickness, Ha the aggregate modulus and x the permeability of the cartilage. The contact becomes perfectly symmetrical at Pe > 100. In our case, Pe increased from ~1,500 (1 mm/s) up to ~30,000 (20 mm/s) based on an aggregate modulus of 1 MPa and a permeability of 0.001 mm4/Ns [Mow et al., 1980], leading to symmetric contact areas. Since higher speeds produced less deformation, the contact stress increases due to a smaller contact area. In fact, by increasing the sliding speed we found decreased contact radii (data not shown) and increasing contact stresses for all loads applied (Fig. 3.5).

In order to investigate the mechanobiological response of the tissue, we quantified and correlated the mechanical response with the biological response. As can be seen in Figure 3.8, a short-term application of cyclic compressive sliding applied to the articular surface resulted in substantial location- and time-dependent changes in strain, contact stress and dynamic effective modulus. Amongst other factors, both location- and time dependence is due to varying intrinsic, geometric material and mechanical properties of the cartilage ECM along the load path (surface), which also caused different magnitudes.
of the applied force due to changing sphere-surface contact angles. The fact that different locations within a joint can have different reactions to mechanical loads has already been demonstrated by other researchers [Moore and Burris, 2015]. Another factor that needs to be taken into consideration is that due to the cyclic, reciprocating motion, different locations will have different loading/unloading patterns. Whereas the apex of the condyle (x = 0) has a symmetric loading/unloading pattern, locations further away from the apex, towards the end of the loading path (x = -18/+18 in our case), generally have shorter times between two loadings or “hits” in the same cycle and longer recovery times in between (minimum and maximum at the ends, respectively). These are factors, which influence the mechanical response over time and might ultimately determine the biological outcome. Therefore, it is safe to assume that chondrocytes in the ECM were subjected to different strains and stresses depending on their location and the morphological, material and mechanical properties of their surrounding ECM.

Mechanical parameters such as strain and contact stress are known to influence chondrocyte gene expression [Wong et al., 2003, Lee et al., 2005]. We performed a multiple regression analysis in order to determine what mechanical parameters influenced the gene expression. As found in previous studies, we found an increase in collagen type II and aggrecan expression with dynamic loading [Correro-Shahgaldian et al., 2011, Valhmu et al., 1998], suggesting an attempt by the chondrocytes to adapt the ECM in response to the dynamic loading conditions. Fibronectin was the only gene that showed a significant correlation with multiple parameters; 66% of the fibronectin regulation in our model was correlated with strain (extrinsic) and the initial effective modulus (intrinsic). A higher initial effective modulus (stiffer material) resulted in lower fibronectin gene expression, whereas an increase is generally considered an indicator for cartilage degeneration [Burton-Wurster and Lust, 1985]. No genes for matrix degradation had a significant correlation with the dynamic mechanical loading in this study. This is consistent with a study reporting no change in MMP gene expression with compressive dynamic loading up to 12 MPa [Fehrenbacher et al., 2003]. It may be that the contact stresses and strains were not high enough to reach a threshold to influence the gene expression. Even the highest measured contact stress in this study (~ 8 MPa) is still considered physiological, and might not induce catabolism. It may also be that the genes studied are not influenced by the mechanical parameters we investigated. In this study, only one speed was used. Different speeds would provide us with different stress rates and loading frequencies, two factors known to be major regulators for the biological response of cartilage [Chen et al., 1999].

In our study we loaded the articular surface of bovine articular cartilage using a Delrin sphere. While this does not represent in vivo human joint conditions, it is an easy-to-quantify mechanical environment. One major limitation of the study was that the cartilage loading was performed at room
temperature. It has been shown that temperature can influence gene expression in isolated chondrocytes [Ito et al., 2015]. Thus it is possible that at physiological temperature the gene expression response to the mechanical loading will be different than found here, both in absolute magnitude and relative expression of the ECM proteins studied. For future studies it will be important to address this limitation by controlling the temperature, such as placing the entire test system in an incubator or using a small enclosure around the specimen.

Another limitation of our test system was the inability to measure the cartilage rebound or recovery after each loading cycle. The cartilage deformations (strains) reported are relative to the initial (unloaded) cartilage surface (thickness) and not the actual real-time cyclic strains per cycle (to the previous loading cycle). As such, the stress and dynamic effective modulus, calculated via deformation (strain), do not necessarily represent the true stresses and moduli. In addition, the mechanobiological study used one sliding speed (10 mm/s) to eliminate confounding variables. Future studies will need to address such issues, and experiments with different speeds must be performed. Also, the difference between sliding (no rotation) and rolling (free rotation) is of interest and will be studied in future experiments.

To summarize, modeling dynamic contact parameters to mimic gait is complex, and DACTS is a simplified model to investigate cyclic sliding and rolling contact mechanics applied to articular cartilage. DACTS is a versatile loading apparatus, which in addition to mechanobiological studies, could also be used to test implants and their behavior in the host tissue. By introducing migrating contacts into mechanical and biological studies, the analysis becomes more physiological but also more complex. Not only does the application of moving contacts in a mechanobiological study need a relatively large tissue surface (availability of tissue for sampling) but also these larger tissue areas are likely to have dissimilar tissue properties at different spatial locations along the path of motion. Thus, a profound analysis of spatial and temporal changes in articular cartilage mechanics and biology are imperative to fully characterize and understand the mechanobiology of chondrocytes during physiological joint motion. This study describes a new approach to investigate the effect of sliding joint contact on cartilage mechanics and biology. It provides valuable insight into how cartilage responds to reciprocating motion, as occurs during gait. This information will be helpful in identifying mechanical and biological mechanisms that lead to the initiation and development of cartilage degeneration and ultimately OA, as after traumatic events such as ACL tears.
Figure 3.8: Typical strain, contact stress and dynamic effective modulus during 400 cycles of dynamic sliding with a 3.6 kg axial load at 10 mm/s sliding speed. X-position represents the location along the femoral condyle with respect to the apex (X-position = 0).

Supplement

Gene expression was analyzed according to the following protocol: after harvesting, cartilage samples were put into RNAlater and stored at -80 °C until RNA extraction. Specimens were transferred into 1 mL Trizol reagent and homogenized using a TissueRuptor (Qiagen, Valencia, CA). The cartilage is then centrifuged at 9500 × g for 10 min at 4 °C, supernatant collected, 200 μL chloroform added, vigorously shaken and centrifuged at 12,000 × g for 15 min at 4 °C. The aqueous layer was then collected, 500 μL iso-propanol added and incubated for 2 h at -80 °C for RNA precipitation. After centrifugation at 12,000 × g for 10 min at 4 °C RNA pellets were washed once with 7% ethanol and resuspended in RNase-free water. RNA was purified using RNeasy mini Elute CleanUp Kit (Qiagen Valencia, CA) and reverse transcribed with High Capacity cDNA Reverse Transcription Kit according to the manufacturer’s instructions. RT-PCR was performed on an Opticon 2 detection system (BioRad Laboratories, Inc., Hercules CA) on 96-well plates with Maxima SYBR Green/ROX qOCR Master Mix (Thermo Scientific, Waltham, MA).

Author Contributions

This is to declare that all authors contributed to the conception and design of this study and approved the submission of this manuscript. OS: data acquisition, data analysis, data interpretation, writing of the manuscript. PT: data interpretation. PT and LG critical review, obtaining funding.
Acknowledgments

The study was supported by the Swiss National Science Foundation Grant 325230-130715 (LMG) and National Institutes of Health Grants (NIAMS) R21- AR059203 (PAT), AR 066635 (PAT) and (NCRR) C06- RR12538-01.
Chapter 4

Articular Cartilage Response to a
Sliding Load Using Two Different-Sized
Spherical Indenters

Oliver R. Schätti\textsuperscript{1,2,3}, Vera Colombo\textsuperscript{2}, Peter A. Torzilli\textsuperscript{1}, Luigi M. Gallo\textsuperscript{2}

\textsuperscript{1}Laboratory for Soft Tissue Research, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021, USA
\textsuperscript{2}Laboratory of Physiology and Biomechanics of the Masticatory System, Center for Oral Medicine, Dental and Maxillo-Facial Surgery, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland;
\textsuperscript{3}Institute for Biomechanics, Swiss Federal Institute of Technology, ETH Zentrum, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

Keywords: Cartilage, Contact mechanics, Indenter Size, Mechanobiology, Gene regulation

This manuscript is currently under revision (November 19, 2016)

4.1 Abstract

Objective: To correlate the mechanical and cellular response of articular cartilage to dynamic reciprocating sliding motion when loaded with two different-sized spherical indenters. Methods: Articular cartilage explants were subjected to a reciprocating sliding load using a 17.6 mm or 30.2 mm spherical ball for 2000 cycles at 10 mm/s and 4 kg axial load. Deformation of the cartilage was recorded and contact mechanics parameters were calculated according to Hertzian theory. After mechanical loading cartilage samples were collected and analyzed for extracellular matrix (ECM) collagen damage and
proteoglycan (PG) loss, and gene regulation of common matrix proteins and enzymes. The biological response was correlated with the mechanical parameters. **Results:** Significantly higher ECM deformation, strain and contact stress and lower dynamic effective modulus were found for explants loaded with the smaller diameter indenter whereas contact radius and stress remained unaffected. Mechanical loading with the 17.6 mm indenter induced no visible ECM damage but did result in increased PG loss and induce significant up-regulation of aggrecan, fibronectin, cartilage oligomeric matrix protein (COMP) collagen type II, MMP-3 and ADAMTS-5 genes as compared to the 30.2 mm indenter. **Conclusion:** Sliding loads that increase ECM load/stress and deformation/strain were found to induce enzyme-mediated catabolic processes in articular cartilage explants. These observations provide further understanding of how changes in cartilage contact mechanics under dynamic conditions can affect the cellular response of chondrocytes. This knowledge has potential value for the development of and improvement of tissue-engineered constructs for cartilage repair.

### 4.2 Introduction

From decades of research on the mechanical and biological responses of cartilage to various stimuli, it is clear that mechanical factors have considerable influence on cartilage health and disease [Grodzinsky et al., 2000, Radin et al., 1990, Carter et al., 2004]. Strains and stresses have long been identified as parameters influencing chondrocyte metabolism and extracellular matrix (ECM) integrity. Forces within a physiological range are associated with normal ECM turnover and an effort of the chondrocytes to adapt the ECM in response to the loading conditions. [Buschmann et al., 1999, Jin et al., 2001]. On the other hand, non-physiological forces have been shown to lead to ECM damage that manifests itself in proteoglycan (PG) loss and collagen fiber disruption [Saarakkala et al., 2010] and an increase in catabolic enzymes such as matrix metalloproteinases (MMPs) and disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS') [Goldring and Marcu, 2009, Lee et al., 2005, Schätti et al., 2015b, Correro-Shahgaldian et al., 2015]. Also, the presence of other proteins that are associated with degenerative changes of the ECM have been reported, such as fibronectin and cartilage oligomeric matrix protein (COMP) [Heinegård and Saxne, 2011, Farquhar et al., 1996, Lohmander et al., 1994]. How the chondrocytes within the cartilage ECM respond to mechanical forces, that is anabolic or catabolic biosynthesis, will depend on the magnitude of the strains and stresses within the ECM. From biomechanical studies it is known that these parameters are functions of loading rate and duration and also the location-dependent mechanical properties of the ECM [Moore and Burris, 2015, Lee et al., 1981, Schätti et al., 2015a, Chen et al., 1999]. A further factor influencing contact mechanics and local ECM strains and stresses is the geometry of the contacting cartilage surfaces [Bullough, 1980]. The two
cartilage surfaces engaging in relative motion are never perfectly congruent, and as a consequence morphological differences will influence the deformational behavior and stress distribution throughout the ECM, ultimately influencing the mechanobiological response of the tissue. In fact, studies have shown that the degree of congruence between the opposing cartilage surfaces can influence cartilage contact parameters in the ECM, and even small variations in morphology can lead to significant changes in contact mechanics which have been correlated with osteoarthritis (OA)-related changes [Bullough, 1980, Harris et al., 2012]. However, the vast majority of such morphological studies are patient-specific and based on imaging techniques in combination with finite element methods (FEM).

While these data provide valuable epidemiological information, general information on how the mechanobiological reaction of articular cartilage to changing contact geometries during joint motion is difficult to obtain. A limited number of in vitro studies have analyzed the mechanical response of articular cartilage to different indenter curvatures under dynamic sliding loads. These found that by using spherical indenters, the contact radius was a function of indenter curvature and sliding speed [Bonnevie et al., 2012, Margetson, 1972, Goryacheva and Sadeghi, 1995]. Hence, indenter shape and size are crucial factors governing the contact mechanics, and the change of either can lead to alterations in ECM strains and stresses. Thus, the aim of this study was to investigate the influence of indenter curvature (spherical radius) on contact mechanics (strains and stresses) during reciprocating sliding motion and how this affected the metabolic response of the chondrocytes evoked through changes in the mechanical environment.

We hypothesized that the application of a sliding load with a small diameter spherical indenter, compared to a large diameter spherical indenter, would result in increased cartilage damage, catabolic gene up-regulation and PG loss. We postulate that the main mechanical mechanisms for this mechanobiological response are the increased ECM strains and stresses that arise due to the reduced contact areas between the smaller diameter indenter and the cartilage surface [Johnson, 1987].

In order to test our hypotheses we applied a dynamic reciprocating sliding load on the surface of articular cartilage explants using two different-sized spherical indenters. We then calculated the contact mechanical parameters of the tissue during loading and correlated these results with the biological responses. We believe that the findings from this type of dynamic in vitro mechanobiological model will be useful for studying degenerative changes occurring during the early phases of OA.

### 4.3 Methods

Unless otherwise indicated, all chemicals were purchased from Thermo Fisher Scientific, Waltham, MA.
4.3.1 Tissue Acquisition

Knees from skeletally mature bovine (>18 months) were obtained from a local abattoir within twenty-four hours of death. The intact joint capsule was opened and the femoral condyles covered with gauze soaked in phosphate buffered saline (PBS) containing 1% antibiotic/antimycotic (ab/am) to prevent drying and degradation. The condyles were then removed using an electrical saw; the condyles rinsed with PBS + 1% ab/am to remove blood and bone marrow, covered with gauze soaked in culture medium (Dulbecco’s modified eagle’s medium (DMEM) without phenol red, supplemented with 1% ab/am, 10 mM Hepes buffer, 1mM sodium pyruvate and 4mM L-glutamin), and stored in an incubator overnight at 37 °C for testing on the next day.

4.3.2 Sliding Experiments

As previously described, a condyle was mounted into a custom-designed dynamic articular cartilage test system (DACTS) [Schätti et al., 2015a] with a continuous flow of sterile culture medium applied to the cartilage surface for the duration of the experiment. To obtain the curvature of the specimen, the indenter was repeatedly lowered onto the cartilage surface yielding a force-deformation response. Force in the y-direction and displacements in the x- and y-directions were recorded using a load cell and two linear variable differential transducers (LVDTs), respectively (Fig. 4.1A). The resulting force-deformation curve at each location along the surface was used to calculate the surface location (at contact). After a 20-minute recovery period the sliding protocol was applied by lowering the indenter onto the apex (x = 0) of the condyle. A 39.2 N axial force was applied in order to achieve contact stresses on the upper limit of the physiological range. Then the indenter cyclically slid over the cartilage for 40 mm (x = ± 20 mm) in the x-direction at 10 mm/s sliding speed for 2000 cycles (Fig. 4.1). To obtain the true cartilage surface, the coordinates of the ball center at surface contact along the path of motion were fitted using a 3rd order polynomial and used correct for the curvature of the condyle, as previously described [2015a]. Two different-sized indenters, 17.6 mm and 30.2 mm diameters, were used to load the cartilage (Fig. 4.1B and C). Indenter diameters were approximated from curvatures of human mandibular condylar heads (5th and 95th percentile). Immediately after 2000 cycles, full-depth cartilage specimens were removed for analyses (Fig. 4.1D). Thereafter, the entire cartilage layer was removed and the bone surface mapped as described above.

4.3.3 Mechanical Analysis

For each loading cycle a 3rd order polynomial was fit to the deformed articular surface and corrected for curvature as described above. The initial thickness and deformation of the cartilage during the cyclic
loading was calculated using a Matlab (MathWorks, Natick, MA) routine to determine the minimum
distance between the cartilage surface and underlying subchondral bone. Total strain ($\varepsilon$) was calculated
from the deformation ($\delta$) divided by the initial thickness ($l_0$)

$$
\varepsilon = \frac{\delta}{l_0}
$$

(4.1)

Contact radius, maximum stress and dynamic effective modulus were calculated using Hertzian theory
of elastic deformation [Johnson, 1987]. The contact radius ($a$) between the indenter and the cartilage
was calculated using

$$
a = \sqrt{\delta \cdot R'}
$$

(4.2)

where the reduced radius ($R'$) for the indenter (radius $R_a$) and condyle (radius $R_b$) is given by $1/R' =
1/R_a + 1/R_b$. The condyle radius was calculated from the polynomial fit to the cartilage surface given
by

$$
R_b = \left[1 + \left(\frac{dy}{dx}\right)^2\right]^{\frac{1}{2}}
$$

(4.3)
The maximum contact stress ($\sigma_{\text{max}}$) and dynamic effective modulus ($E^*$) were then calculated at each location along the sliding path on the condyle for each individual cycle using Hertzian theory. We modeled the cartilage layer as a thin elastic compressible layer bonded to a rigid substrate (bone), which was considered incompressible. The effective modulus ($E^*$) was calculated using

$$E^* = \frac{3 \cdot F \cdot R_0}{4} \cdot \delta^{-1.5}$$

(4.4)

and the maximum contact stress ($\sigma_{\text{max}}$) calculated from

$$\sigma_{\text{max}} = \frac{2 \cdot F}{\pi \cdot a}$$

(4.5)

both formulas for spherical indentation of a thin layer on a rigid substrate from Jaffar [2006]. While the effective modulus $E^*$ is not a true material property for cartilage (it depends on several factors, such as the material properties of the cartilage and Delrin sphere, the sliding speed, deformation rate, and the reduced radius), it does provide a measure for relative changes between the different indenters in this study.

4.3.4 Biological Analysis

Eight knees (16 condyles) were used in this study whereof four were used for PG loss analysis (knee 1-4) and four for gene expression analysis (knee 5-8). For each indenter, a medial or lateral condyle was loaded, with the contralateral unloaded condyle serving as a control. To investigate the biological response of the cartilage to the two different-sized indenters, a 5 mm cartilage specimen for histological staining was removed from the apex of the condyle immediately after loading and put in 10% buffered formalin (Pharmco-Aaper, Brookfield, CT) + 1% cetylpyridinium chloride (Sigma-Aldrich, St. Louis, MO). In order to correlate the mechanical parameters to chondrocyte gene expression and PG loss along the loading path, ten 3 mm diameter cartilage specimens were removed from the loading path and incubated in culture medium at 37 °C as described below (Fig. 4.1). The specimens were collected at known locations.

4.3.4.1 Histology

Immediately after collection cartilage specimens were put into formalin for 3 hours, washed 3 times with tap water and stored in 70% ethanol at 4 °C for further processing. The samples were dehydrated using a gradient of ethanol from 70% to 100%, 2 steps of xylene and 3 steps of hot molten paraffin. The tissue was embedded in paraffin, cut at 5 μm thickness and mounted on a coverslip. In order to enhance
attachment of the section to the glass slide, they were heated to 56 °C overnight. Before staining, paraffin sections are rehydrated with 2 washes of xylene and a gradient of ethanol from 100% to 70%. Following staining, sections were rehydrated in a gradient of ethanol up to 100%, washed in xylene, air-dried, and cover-slipped using mounting medium. Finally, the sections were viewed via light microscopy with a polarizing filter added to view the collagen structure in the picrosirius red stained sections [Junqueira et al., 1979].

**Safranin-O**  Sections were stained in Mayer’s Hematoxylin for 7 minutes and washed in running tap water. Next, sections were counterstained in Fast Green (Sigma-Aldrich, St. Louis, MO) solution for 15 minutes, and quickly rinsed with 1% acetic acid solution for 15 seconds before staining in 0.1% safranin-O (Sigma-Aldrich) solution for 20 minutes.

**Picrosirius red**  Sections were stained in Fast Green for 10 minutes, transferred to 1% acetic acid for 2 minutes and quickly rinsed in tap water. Next, sections were stained in picrosirius red (Sigma-Aldrich) for 30 minutes before being rinsed in tap water.

### 4.3.4.2 Proteoglycan Loss

To investigate proteoglycan loss, the 3 mm cartilage specimens were incubated in 700 ul culture medium at 37 °C for 48 hours. After 24 hours, medium was collected, frozen at -20 °C for further analysis and replaced with new culture medium. After 48 hours, culture medium was again collected and the cartilage specimen digested in 1 mg/mL proteinase K (MP Biomedical, Santa Ana, CA) for 16 hours at 56 °C. Digests and conditioned media samples were used to determine the proteoglycan content using the dimethylmethylene blue (DMMB) assay [Farndale et al., 1986]. The initial proteoglycan concentration of each sample was calculated by adding the loss after 24 and 48 hours to the proteoglycan measured in the tissue.

### 4.3.4.3 Gene Expression Analysis

Cartilage specimens for gene expression analysis were incubated in culture medium at 37 °C for 4 hours after loading was applied and subsequently frozen in liquid nitrogen for further analysis. Total RNA was extracted using the phenol-chloroform extraction method [Chomczynski and Sacchi, 1987]. Genes for ECM proteins (aggrecan, type II collagen, fibronectin, cartilage oligomeric matrix protein (COMP)) and catabolic enzymes (MMP-3/-13 and ADAMTS-4/-5) were analyzed. Glyceraldehyde-3-phosphoate dehydrogenase (GAPDH) and ribosomal protein L13a (RPL13a) were used as calibrator genes.
and delta–delta Ct (ΔΔCt) values were calculated for each gene. Forward and reverse primers for all
genes used in this study can be found in Table 4.1.

Table 4.1: Forward and reverse primer sequences (bovine) used for gene expression analysis with real-time PCR. Annealing temperature 57 °C or 60 °C.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>GGGTCATCATCTCTGCACCT</td>
<td>GGTCATAAGTCCCTCCACGA</td>
</tr>
<tr>
<td>RPL13a</td>
<td>GCCTACTCGAAAGTTTGCTT</td>
<td>GCCGTTACTGCCTGGTACTT</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>GGGAGGAGACGACTGCAATC</td>
<td>CCCATTCCGTCTTGTCTTCTG</td>
</tr>
<tr>
<td>Collagen Type II</td>
<td>GCTTCCACCTTCTAGCTATGG</td>
<td>CAGGTAGGCAAATGCTTGTCT</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>CTACCCTCACTGTGGTGGGAC</td>
<td>TTCCAGGAACTCGGAACCTGT</td>
</tr>
<tr>
<td>COMP</td>
<td>ACCCAGACCAGCGAAATACG</td>
<td>ATCTGAGTTGGCGACCTTGG</td>
</tr>
<tr>
<td>MMP-3</td>
<td>GCAAGCCATAAAGACCACATCA</td>
<td>TTCTAGATATTTGCTGAAACGCTCC</td>
</tr>
<tr>
<td>MMP-13</td>
<td>TCCAGTTGAGAGAGCTACC</td>
<td>CTGCCAGTCACCTCTAAGCC</td>
</tr>
<tr>
<td>ADAMTS-4</td>
<td>CATCCTAGCCGGAAGAGTC</td>
<td>CATGGAATGCGGCACCTTGT</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>TGGAAAGGGACGATTCTCGTG</td>
<td>AGAGGTCAAGAGCCTGCCAGC</td>
</tr>
</tbody>
</table>

4.3.4.4 Statistics

Two-tailed student’s t-tests were used to compare the means between loaded and control groups and
the two different indenters under the assumption that the variances of the populations are equal. The
relationship between mechanical parameters and gene expression was assessed with univariate linear
regression analysis using r-squared ($r^2$) to explain the response variability and the slope of the curve
to determine if the predictor is significant. An $\alpha$-level = 0.05 was considered significant.

4.4 Results

Each unloaded knee (medial or lateral control condyle) was analyzed for differences in PG loss and
gene expression along the loading path, and also for differences between the knees. For each knee
there were no significant differences in PG loss and gene expression found for the different sampling
locations along the loading path. However, significant differences in PG loss but not for gene expression
were found between controls of medial and lateral condyles (Fig. 4.4).

4.4.1 Mechanics

For each knee, the mechanical parameters for the loaded condyles did not differ along the sliding path,
however some differences were found between some individual condyles (see Tables 4.2 and 4.3). Figure
Figure 4.2: Calculated mechanical parameters for the small and large spherical indenters for the last cycle of the loading phase. Each bar represents the mean ± standard deviation of condyles with 11 sampling locations per condyle (N = 44).

4.2 shows the statistical analysis of mechanical parameters averaged over the sampling path (locations 1 - 10) and condyles (medial and lateral). Sliding with the smaller 17.6 mm diameter indenter resulted in larger deformations (p < 0.0001), strains (p = 0.003) and dynamic effective moduli (p = 0.0002) compared to the 30.2 mm diameter indenter, while the contact radius and stress remained unaffected by indenter size. The individual mean ± standard deviations for the mechanical parameters used for correlation with the gene expression and PG loss are given in Tables 4.2 and 4.3, respectively.

4.4.2 Biology

4.4.2.1 Histology

No differences in Safranin-O intensity (PG content) between loaded and control samples were found for either indenter. Also, sections analyzed under polarized light microscope showed no collagen disruption or change in collagen alignment and orientation between the different indenters or controls.

4.4.2.2 Gene Expression

No differences in gene regulation were found for control samples with respect to sampling location and between medial and lateral condyles. Therefore controls from different locations and corresponding condyles of each indenter group were averaged (n = 20) and the loaded samples normalized by the control mean values in order to get relative fold changes in gene expression (Figure 4.3). Gene expressions for matrix proteins were significantly increased relative to their controls when loaded with the 17.6 mm
<table>
<thead>
<tr>
<th>Knee No.</th>
<th>Condyle</th>
<th>Indenter Diameter (mm)</th>
<th>Deformation (mm)</th>
<th>Strain (%)</th>
<th>Contact Radius (mm)</th>
<th>Contact Stress (MPa)</th>
<th>Dynamic Effective Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lateral</td>
<td>76.22 ± 18.62 ± 16.44</td>
<td>5.17 ± 0.015</td>
<td>1.76 ± 0.06</td>
<td>12.65 ± 2.65</td>
<td>76.22 ± 16.44</td>
<td>1.76 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>Medial</td>
<td>66.3 ± 16.44</td>
<td>5.17 ± 0.015</td>
<td>1.76 ± 0.06</td>
<td>12.65 ± 2.65</td>
<td>76.22 ± 16.44</td>
<td>1.76 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>Lateral</td>
<td>76.22 ± 18.62 ± 16.44</td>
<td>5.17 ± 0.015</td>
<td>1.76 ± 0.06</td>
<td>12.65 ± 2.65</td>
<td>76.22 ± 16.44</td>
<td>1.76 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>Medial</td>
<td>66.3 ± 16.44</td>
<td>5.17 ± 0.015</td>
<td>1.76 ± 0.06</td>
<td>12.65 ± 2.65</td>
<td>76.22 ± 16.44</td>
<td>1.76 ± 0.06</td>
</tr>
</tbody>
</table>

**Table 4.2**: Mean ± SD of mechanical parameters from condyles loaded for gene expression analysis.
Table 4.3: Mean ± SD of mechanical parameters from condyles loaded for PG loss analysis.

<table>
<thead>
<tr>
<th>Knee No.</th>
<th>Condyle</th>
<th>Indenter Diameter (mm)</th>
<th>Deformation (mm)</th>
<th>Strain (%)</th>
<th>Contact Radius (mm)</th>
<th>Contact Stress (MPa)</th>
<th>Dynamic Effective Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lateral</td>
<td>17.6</td>
<td>0.474 ± 0.084</td>
<td>26.227 ± 4.934</td>
<td>1.736 ± 0.102</td>
<td>7.851 ± 1.002</td>
<td>35.030 ± 8.493</td>
</tr>
<tr>
<td>2</td>
<td>Medial</td>
<td>17.6</td>
<td>0.359 ± 0.060</td>
<td>27.569 ± 3.789</td>
<td>1.513 ± 0.110</td>
<td>10.557 ± 1.645</td>
<td>54.029 ± 13.001</td>
</tr>
<tr>
<td>3</td>
<td>Lateral</td>
<td>30.2</td>
<td>0.332 ± 0.130</td>
<td>30.600 ± 5.828</td>
<td>1.879 ± 0.405</td>
<td>7.950 ± 3.424</td>
<td>60.447 ± 38.796</td>
</tr>
<tr>
<td>4</td>
<td>Medial</td>
<td>30.2</td>
<td>0.230 ± 0.074</td>
<td>21.782 ± 4.924</td>
<td>1.458 ± 0.213</td>
<td>12.036 ± 4.041</td>
<td>76.839 ± 25.083</td>
</tr>
</tbody>
</table>
but not with the 30.2 mm indenter. The only enzymes that were significantly up-regulated were MMP-3 and ADAMTS-5 when loaded with the 17.6 mm indenter. No difference in the gene expressions for MMP-13 and ADAMTS-4 was observed between the loaded and control samples.

4.4.2.3 Proteoglycan Loss

Proteoglycan loss from the loaded and control cartilage samples was determined after 24 and 48 hours post-testing. After 24 hours condyles loaded with the 17.6 mm diameter indenter lost 7.7% (lateral) and 5.1% (medial) of the total proteoglycan, corresponding to a 1.6-fold and 1.4-fold PG loss compared to the unloaded control (Fig. 4.4). On the other hand, condyles loaded with the 30.2 mm indenter lost 8.1% (lateral) and 5.2% (medial) of proteoglycan to the medium after 24 hours. However, this loss was not different from the control condyles (1.1-fold for both). After 48 hours, proteoglycan loss significantly increased only for the medial condyle loaded with the smaller indenter (p = 0.04), all remaining samples were not different from the unloaded controls (data not shown).

4.5 Discussion

Under reciprocating sliding conditions, contact parameters between two opposing joint surfaces depend on intrinsic (or genetic) factors, such as material properties of the cartilage and the geometry of the joint surfaces, and also external factors such as compressive forces and relative translation speed [Schätti et al., 2015b, Correro-Shahgaldian et al., 2015, Schätti et al., 2015a, Moore and Burris, 2015, Correro-Shahgaldian et al., 2011]. The influence of compressive forces and sliding speeds has already been
investigated and deformations, contact stresses and dynamic moduli found to be functions of axial load and sliding speed [Schätli et al., 2015a]. The objective of the present study was to investigate the influence of joint geometry (curvature), modeled using two different-sized spherical indenters, on the mechanical and biological behavior of articular cartilage explants under reciprocating sliding load. The two opposing surfaces of a joint are never perfectly congruent resulting in more or less favorable geometries for optimal force distribution and attenuation [Bullough, 1980, Harris et al., 2012]. In the present study, contact parameters were calculated according to Hertz’s model of elastic deformation [Johnson, 1987] and an adjustment of the theory for elastic layers bonded to a rigid substrate (Eqns. 4.2, 4.4 and 4.5) [Jaffar, 2006]. Sliding with the smaller indenter (17.6 mm) resulted in higher ECM deformations than with the larger indenter (30.2 mm). This can be explained due to (1) the smaller indenter having a smaller contact area, larger contact stress and therefore greater ECM deformation, and (2) while we used the Hertz and Jaffar models [1987, 2006] to calculate the contact parameters, articular cartilage is not an elastic solid but rather a poroviscoelastic layer bonded to a rigid substrate (underlying bone). Therefore the subchondral bone will have a stiffening effect on the cartilage ECM that becomes more pronounced with a larger contact area, therefore restricting the total amount of ECM deformation [Hayes et al., 1972]. Thus the slightly larger contact area for the 30.2 mm diameter indenter (Figure 4.2C) might explain a ~28% higher dynamic elastic modulus (Fig 4.2E) compared to the smaller indenter (17.6 mm diameter). This trend confirms earlier results from Bonnevie et al. [2011] where they found a 30% lower modulus for their 0.8 mm indenter compared to a 3.2 mm indenter.

Due to the spherical shape of the indenter the resulting contact area between the indenter and the cartilage is a function of penetration depth and curvature of the femoral condyle (Eqn. 4.2). In general, larger deformations with the small indenter result in similar contact radii than smaller deformations with the large indenter. In a similar manner this affects the contact stresses (Eqn. 4.5) and explains the insignificant differences in the contact stresses for the different indenters. This leaves ECM deformation, strain and dynamic effective modulus as the only significantly different mechanical parameters
between the two indenters and these possibly are the parameters responsible for the biological differences observed (Figure 4.3 and 4.4).

The biological response of the articular cartilage to the sliding load applied by the two different indenter sizes was quantified by (1) direct damage to the ECM as visualized with histology staining, (2) changes in the chondrocyte’s metabolic activity by gene expression analysis, and (3) PG loss from the ECM post-sliding. Even though the sliding contact stresses were on the high end of the physiological range [Brown and Shaw, 1983, Matthews et al., 1977, Brand, 2005], no immediate damage (PG loss and collagen fiber disruption) was observed between the loaded and control groups, or between the two indenters. It is known that the deformational response highly depends on the parallel organization of the collagen fibers in the superficial zone of the cartilage [Korhonen et al., 2002b, Herzog and Federico, 2006, Guo et al., 2015]. Also, fluid redistribution may greatly differ in samples where a large portion of the ECM (relative to the loading area) is still intact (greater presence of interstitial fluid to shield the PGs and collagen fibers) [Park et al., 2003]. All of these factors potentially contribute to enhanced mechanical response of larger tissue explants.

In this study condyles loaded with the 17.6 mm indenter were found to have significant up-regulation of all genes for ECM proteins compared to controls and the 30.2 mm diameter indenter. The up-regulation of aggrecan and collagen type II suggests a possible attempt by the chondrocytes to adapt the ECM to new mechanical requirements imposed by the smaller indenter, as has been well-documented in previous studies [Buschmann et al., 1999, Smith et al., 2000]. The up-regulation of fibronectin and COMP possibly indicates a shift from an anabolic to the catabolic gene expression as these proteins are known to be present in damaged cartilage [Lohmander et al., 1994, Barilla and Carsons, 2000]. Further evidence for the initiation of catabolic processes is the enhanced presence of MMP-3 and ADAMTS-5 in the cartilage loaded with the smaller indenter. While the sole up-regulation of the genes does not automatically imply enzyme synthesis (presence or activation in the ECM), it does indicate a shift in chondrocyte phenotype [Goldring and Otero, 2011, Kurz et al., 2005]. No change in the expression of MMP-13 and ADAMTS-4 was found. As it is known that gene regulation is a time-dependent process, a post-loading incubation time of 4 hours might not be enough to show up-regulation of certain enzymes [Lee et al., 2005]. Also, these enzymes could not be affected by the magnitude of loading applied in this study. A potential indication for the presence and activation of ECM enzymes is the fact that PG loss was significantly enhanced in condyles loaded with the smaller compared to the larger indenter. Since no direct mechanical damage was histologically observed and the synthesis for new PGs takes more than [Quinn et al., 1999], it is likely that the loss of PGs detected in the culture medium was enzyme-induced.
Table 4.4: Univariate regression analysis between genes and mechanical parameters that were significantly affected by indenter size. R-squared explains the response variability while the p-value determines the significance of the predictor. Correlations with deformation and strain are positive while correlations with dynamic effective modulus are negative.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Deformation</th>
<th>Strain</th>
<th>Dynamic Effective Modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>p-value</td>
<td>r²</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>0.28</td>
<td>0.0004</td>
<td>0.07</td>
</tr>
<tr>
<td>Collagen Type 2</td>
<td>0.43</td>
<td>&lt;0.0001</td>
<td>0.71</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>0.31</td>
<td>0.0002</td>
<td>0.04</td>
</tr>
<tr>
<td>COMP</td>
<td>0.24</td>
<td>0.0014</td>
<td>0.02</td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.10</td>
<td>0.0496</td>
<td>0.10</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>0.01</td>
<td>0.6292</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Finally, even though contact stresses are known as major regulators of the biological response, we did not find any significant differences in contact stresses between the two indenter groups. When a regression analysis was performed between the mechanical parameters and the significantly up-regulated genes, several of the genes are significantly correlated with deformation (AGG, COLL2, FN, COMP, MMP-3) and dynamic effective modulus (AGG, COLL2, FN) (Table 4.4). Only collagen type II was correlated with strain.

This study has a few limitations that need to be taken into account and could have affected the study finding. First of all, the Delrin indenter is several times stiffer than the layer of cartilage that normally articulates against the femoral condyle. As a consequence, all deformations that occur in the condyle would under *in vivo* conditions be shared (what results in larger strains and the violation of hertz’ theory). In return, this setup does model cases where one part of the joint was replaced with an artificial bearing material. Also, even though the curvatures and contact dimensions are rather small for the joints of the lower body (ankle, knee hip), they represent physiological values for the temporomandibular joint (TMJ). Second, the loading tests were conducted at room temperature which has been shown to influence gene regulation [Ito et al., 2015]. But even though basal gene regulation is temperature sensitive, it is not clear to what extent temperature affects the relative expression levels between loaded and unloaded controls. Despite these limitations, this study can be regarded as a model for investigating the effect of using different indenter curvatures on contact mechanics and consequently cartilage’s metabolic response under biomimetic sliding conditions. The study clearly demonstrates that the size of the spherical indenter influences the deformational behavior of articular cartilage. Even though contact parameters were influenced to a lesser extent than hypothesized, other mechanical factors such as
deformation (strain) and dynamic elastic modulus proved to affect the biological response of cartilage under dynamic sliding conditions. The up-regulation of MMP-3 and ADAMTS-5 suggests that unfavorable contact geometries might induce enzyme-mediated catabolic processes in articular cartilage, which are common in diseases such as OA. These observations provide further understanding of cartilage contact biomechanics under dynamic conditions. These results can be particularly interesting for design of prostheses where one part needs to be replaced or to surgically alleviate stress concentrations that arose after traumatic incidents such as fractures. In addition, for tissue engineering approaches it might be useful to know what contact dimensions are important to grow functional and sustainable tissue constructs.

**Author Contributions**

We declare that all authors contributed to the conception and design of this study and approved the submission of this manuscript. OS was responsible for data acquisition and analysis with the help of PT for interpretation of the data. VC, PT and LG critically reviewed the manuscript. PT and LG obtained funding to conduct this research.

**Role of Funding Source**

The study was supported by the Swiss National Science Foundation Grant 325230-130715 (LMG) and National Institutes of Health Grants (NIAMS) R21- AR059203 (PAT), AR 066635 (PAT) and (NCRR) C06- RR12538-01. Other than financial support, the funding sources had no role in the study design, data collection and analysis and the decision to submit the manuscript for publication.

**Conflict of Interest**

The authors have no potential conflicts of interest.

**Acknowledgments**

The authors gratefully acknowledge the support from the following funding sources: Swiss National Science Foundation Grant 325230-130715 (LMG) and National Institutes of Health Grants (NIAMS) R21- AR059203 (PAT), (NIAMS) R01AR066635 (PAT) and (NCRR) C06-RR12538-01.
Chapter 5

General Discussion

Articular cartilage enables durable joint motion by providing a combination of low-friction motion and efficient stress distribution. These qualities are based on a highly specialized extracellular network of collagen fibers and proteoglycan molecules which interact with a fluid component to provide outstanding biomechanical, mechanobiological and tribological properties. Chondrocytes synthesize these and other structural proteins, together with enzymes, in order to build and remodel a mechanically competent extracellular matrix (ECM). While the metabolic activity of the chondrocytes depends on a variety of factors, mechanical forces play a crucial role in balancing anabolic and catabolic factors [Goldring and Marcu, 2009, Aurich et al., 2002].

The mechanical and biological responses of articular cartilage to compression, tensile and shear forces has been extensively studied over the past three decades [Mow et al., 1980, Fitzgerald et al., 2006, Torzilli, 1984]. From in vivo analyses of joint kinematics it is clear that joint motion engages the opposing cartilage surfaces to generate contact compressive, tensile and shear stresses, continuously during sliding and rolling along a large loading path [Dennis et al., 2005, Kizuki et al., 1995, Andriacchi and Dyrby, 2005]. Motivated by these findings, theoretical and experimental studies have shown that such moving contacts not only have extensive consequences on mechanical and tribological parameters of the ECM [Krishnan et al., 2005, Ateshian and Wang, 1995, Caligaris and Ateshian, 2008] but also are important to sustain high fluid pressurization and a low coefficient of friction [Caligaris and Ateshian, 2008, Forster and Fisher, 1999, Katta et al., 2008]. However, even though the impact of such moving contacts on cartilage mechanics has been demonstrated on the micro-scale [Bonnevie et al., 2011, Caligaris and Ateshian, 2008], their consequences on biological processes has been largely neglected.

The main motivation of this thesis was to investigate how moving contacts (such as sliding) affect the mechanical and biological response of articular cartilage. I focused on how changes in axial load, sliding speed and indenter geometry influenced cartilage mechanobiology under dynamic sliding con-
ditions. The following sections of this chapter aim to summarize the results from the three separate studies into a broader description of the mechanobiology of joint motion. Section 5.1 will discuss how axial load, sliding speed and indenter radius individually influence tissue mechanics. In Section 5.2, the influence of these mechanical factors on the biological response is discussed. Last, possible future research directions are proposed.

5.1 The Influence of Axial Load, Speed and Curvature on Cartilage Mechanics

5.1.1 Axial Load and Mechanical Parameters

Axial load itself is not a physiologically meaningful parameter when it comes to cartilage mechanobiology. Hence, its influence is usually quantified by investigating the consequences it has on the cartilage ECM e.g., deformation (strain) and contact stress. Whereas deformation (strain) can be easily obtained by a change in thickness, the calculation of stresses is more complicated and requires knowledge of contact parameters and interactions. Moreover, strain and stress not only depend on external variables such as axial load, loading rate (frequency) and indenter geometry, but also on intrinsic material properties (e.g. Young’s modulus) of the cartilage. Ultimately, a combination of external and internal factors will determine the physiological impact a specific axial load will have on the cartilage ECM. Applied to the studies in this thesis, the impact of the axial load will depend on sliding speed (loading rate) and indenter geometry (contact area).

Similar trends for the deformation (strain) response were found in study 1 and 2 (Chapter 2 and 3) when the influence of axial forces during dynamic sliding on cartilage mechanics was investigated. By increasing the axial force during the sliding motion, deformation (strain) increased at all sliding speeds tested (1 mm/s up to 70 mm/s). However, as can be seen in Figure 3.4 and Table 2.3, the deformation did not linearly increase with axial load. Whereas a 9.8 N axial load produces a strain of roughly 25 %, a 39.2 N axial load deformed the cartilage layer by only 40 to 45 %. A similar trend was found in study 1 where the axial load was increased from 50 N to 100 N but strain only increased from ~30 % to ~40 %. This is probably due to the fact that cartilage is (1) not a linearly elastic and isotropic material and (2) is supported by subchondral bone (Plexiglas plate in study 1). The influence of the underlying bone becomes more pronounced as the deformation increases and results in a stiffening of the ECM; therefore more resistance to deformation. Unfortunately, the non-linear and anisotropic nature of the cartilage and its finite thickness do not conform to the Hertzian theory of elastic deformation [Johnson, 1987]. Therefore, the calculated contact parameters might not necessarily represent true material properties.
and could be underestimated. Nevertheless, contact calculations according to Hertz’s theory present a valid tool to compare relative changes of these parameters within the same study, as is widely used.

The dynamic elastic modulus, called apparent (study 1) or effective (study 2) elastic modulus, was calculated with a quasi-static application of Hertz’s formula for the elastic modulus (Eqn. 3.4). However, instead of the equilibrium values normally used to obtain the cartilage’s elastic modulus (material property), a dynamic (instantaneous) modulus was obtained during the dynamic loading phase. Likewise, instantaneous deformations were used for calculating the deformation and contact radius. This results in the modulus being an apparent/effective modulus and therefore not a real material property of the cartilage. It rather represents the “stiffness” of the cartilage under the applied conditions and served as a tool to compare the relative changes between different loading conditions in the same study. Note that, the absolute values obtained cannot be compared between studies (in this case between study 1 and 2). It is however possible to compare trends. And as can be seen from the apparent (Chapter 3) and effective modulus (Chapter 4), both tend to increase with increasing load and deformation.

Last but not least, axial force influenced the contact stress via contact area (Eqns. 2.5 and 3.5). In order to get a more realistic estimate of the true contact stresses we accounted for the stiffening effect of the underlying bone by modeling the cartilage layer in study 3 as a thin elastic layer bonded to a rigid substrate (bone) [Jaffar, 2006]. This resulted in all calculated stresses being 33% higher than when calculated with the traditional Hertz solution. The use of rounded indenters (cylinder or sphere) resulted in the contact area being deformation-dependent. Therefore, an increase in normal force had two effects: (1) it caused increased deformation and (2) resulted in an increased area of the indenter being in contact with the cartilage. As the results in study 1 and 2 showed, I found increasing maximum contact stresses with increasing axial forces in study 1 and 2. Most likely, the stiffening effect of the bone caused the contact area to increase at a slower rate than the increasing applied force that ultimately resulted in increased contact stresses.

5.1.2 Sliding Speed and Mechanical Parameters

Unlike with increasing axial load, changes in mechanical parameters with increasing speed were different for studies 1 and 2 (Chapter 2 and 3). We found decreasing deformation (strain) and increasing contact stress and effective modulus with increasing sliding speed in study 2 (Chapter 3). The speed-dependent behavior can be explained by the poroviscoelastic nature of articular cartilage. As introduced in Chapter 1, articular cartilage is a biphasic material where the compressive (deformational) behavior depends on the resistance or permeability of fluid flow through the pores of the solid matrix [Mow et al., 1980]. The fluid flow has been demonstrated to be rate and frequency dependent. When a load
is applied at high loading rates (frequencies), the interstitial fluid within the porous network has less
time to escape from the ECM and be redistributed within the ECM, and becomes pressurized [Lee et al.,
1981]. As a consequence, the pressurized fluid resists a significant amount of the load and reduces the
depression of the solid matrix. Increasing the speed from 1 mm/s to 20 mm/s increased the rate the
cartilage deformed (loading rate). And in agreement with previously published theoretical and exper-
imental studies [Mow et al., 1980, Lee et al., 1981], I found that the deformation of articular cartilage
decreased with increasing sliding speed (Fig 3.4). Based on the same mechanism of fluid pressurization,
the observed dynamic effective modulus increased with faster sliding speeds (Figure 3.6). As briefly
discussed in the previous section, decreasing deformation is accompanied by decreased contact radius
due to the spherical shape of the indenter. Hence, since the load was kept constant, the contact stresses
increased with increasing sliding speed (Figure 3.5).

In the first study (Chapter 2), the speed was increased up to 70 mm/s. Surprisingly, strain, contact
stress and dynamic modulus did not significantly change when the sliding speed was increased from
10 mm/s to 70 mm/s. It is possible that the 10 mm/s loading rate was high enough to induce a non-
linear elastic response. These findings seem to contradict the results from study 2, where between 10
mm/s and 20 mm/s mechanical parameters still changed. On the other hand, even though no significant
differences in mechanical parameters were found between 10, 40 and 70 mm/s, a slight change between
10 and 40 mm/s was detected. This would mean that the cartilage is in a transition form a viscoelastic to
an elastic response. Nevertheless, one needs to be careful when comparing these two studies as several
differences in the study setup could have resulted in the disagreement between the results. Namely,
(1) different type of tissue (nasal cartilage vs. femoral cartilage), (2) higher speeds were tested in the
first study therefore it is possible that the elastic limit of the cartilage was reached and no further
deformation was observed, (3) the different geometry of the indenter (cylinder vs. sphere) and (4) the
different modes of sliding (uni-directional vs. reciprocating).

The dimensionless Peclet number (Pe) is another important parameter related to fluid load support
and contact geometry under dynamic sliding conditions. The Peclet number describes the ratio between
sliding/rolling velocity and the diffusive velocity of the interstitial fluid flow. It is defined as

\[ Pe = \frac{(V \cdot A)}{(H_a \cdot k)} \]

where \( V \) is the sliding velocity, \( A \) the cartilage thickness (or another dimension of the flow
path such as the contact radius), \( H_a \) the aggregate modulus and \( k \) the permeability of the cartilage
[Ateshian and Wang, 1995]. Ateshian and Wang [1995] found that interstitial fluid load support occurs
when \( Pe > 1 \) and was predicted to be highest when \( Pe >> 1 \). Besides a measure for fluid load support the
Peclet number can also be used to obtain information about the geometry of the contact between the
indenter and cartilage surface. When \( Pe \) is very small (in the order of \( 10^{-2} \)) no fluid load support occurs.
In this case the interstitial fluid has sufficient time to displace within the matrix as the speed of the moving surface is slow enough. In addition, the part of the tissue that has been indented at the leading edge has sufficient time to recover before the trailing edge reaches the same location and therefore results in an almost perfectly symmetrical contact. Once the speed of the moving contact increases and where \( Pe = 1 \), it moves sufficiently fast to pressurize the fluid underneath but still slow enough to additionally displace some fluid. This results in incomplete recovery of the tissue between the leading and the trailing edge of the indenter and asymmetric contact [Ateshian and Wang, 1995]. On the other hand, when \( Pe \gg 1 (\gg 10^3) \) the indenter moves fast enough to efficiently pressurize the fluid underneath without allowing it to be redistributed throughout the ECM. Therefore, cartilage behaves as an almost incompressible solid with nearly instant recovery at the leading edge of the indenter resulting again in a perfectly symmetric contact. By calculating the Peclet number for our studies, \( Pe \) increased from \(-1500 (1 \text{ mm/s}) \) up to \(-140000 (70 \text{ mm/s}) \). This is at least one order of magnitude more than \( Pe = 10^2 \) and thus justifies the use of the Hertz contact model that assumes symmetric contacts and pressure distributions.

In addition, these calculated Peclet numbers suggest efficient fluid load support for all speeds applied. According to Hertz’ theory [1987], the effective modulus provides a relationship between applied load \( (F) \), reduced radius \( (R') \) and deformation \( (\delta) \) (Eqns. 5.1) and between Young’s modulus \( (E) \) and Poisson’s ratio \( (\nu) \), and is defined as follows:

\[
E^* = \frac{E}{1 - \nu} = \frac{3 \cdot F \cdot R'^{-0.5} \cdot \delta^{-1.5}}{4}.
\] (5.1)

The effective modulus \( (E^*) \) describes the modulus under the current loading conditions without the need to separately characterize the Young’s modulus and the Poisson’s ratio of the cartilage. In addition, since the effective modulus describes the instantaneous response of the cartilage it represents the load applied to the fluid and the solid components. Therefore, by transforming Equation 5.1, the fraction carried by the fluid load \( (F_{\text{fluid}}) \) can also be written by

\[
F_{\text{fluid}} = \frac{E^* - E}{E^*} = \frac{F - F_{\text{solid}}}{F}.
\] (5.2)

Therefore, the load that is carried by the fluid component \( (F_{\text{fluid}}) \) is defined as the difference between the total force \( (F) \) and the force carried by the solid matrix \( (F_{\text{solid}}) \) or as the difference between the effective modulus \( E^* \) and the equilibrium modulus \( (E) \) [Bonnevie et al., 2011]. To calculate the fluid load fraction for our experiments we calculate \( E^* \) according to Equation 5.1 and used \( E \) from literature \( (E = H_a) \) [Ateshian and Wang, 1995]. This resulted in 90 - 95 % of the load carried by the fluid component.
for all speeds used in study 2. However, only under perfectly confined loading conditions \((\nu = 0)\) could maximum fluid support reach 100 % whereas our studies were essentially unconfined. Yet, it was shown that the high tensile modulus of the collagen fibers effectively prevents expansion (perpendicular to the direction of the applied load) and therefore confines the cartilage [Cohen et al., 1998]. The maximum fluid load fraction has been calculated to be around 80–90 % [Bonnevie et al., 2012, Krishnan et al., 2004, Moore and Burris, 2015]

5.1.3 Indenter Curvature and Mechanical Parameters

In study 3, the mechanobiological response of articular cartilage explants to dynamic reciprocating sliding motion and two different-sized spherical indenters was investigated. Axial load and sliding speed were kept constant throughout the experiments and the only factor that changed was the diameter of the spherical indenters, which were 17.6 mm and 30.2 mm (71% larger). According to Hertz, the relationship between contact radius \((a)\), deformation \((\delta)\) and reduced radius \((R')\) is given by Equations 3.3 and 4.2. Under the assumption of identical condylar curvatures, contact radius would only depend on deformation and indenter radius, and would increase with deformation (constant \(R'\)) or with spherical radius (constant \(\delta\)). In addition, contact stress is a function of contact radius and according to Hertz and Jaffar [1987, 2006] is related as shown in Equation 4.5. Hence, under the assumption of a constant normal force, contact stress increases with decreasing radius \((a)\). Therefore, as long as the smaller indenter does not deform the ECM more than 71% of the deformation from the larger indenter, the contact stress is larger.

However, intrinsic properties such as thickness, elastic modulus and condylar curvature vary between condyles and even over the surface of the same condyle [Moore and Burris, 2015]. In my study, sliding with the smaller indenter resulted in larger deformations and strains compared to the larger indenter. Of interest, contact radius and consequently contact stress, remained unaffected. This could be due to several factors: (1) The deformation is larger with the small radius indenter resulting in more area of the indenter being in contact with the cartilage and (2) the curvatures of the condyles are different. An increase in one factor (deformation) could possibly compensate a decrease in the other factor (reduced radius).

5.2 The Influence of Load, Speed and Curvature on Cartilage Biology

It is well accepted that mechanical parameters such as strain and stress influence articular cartilage health and disease [Grodzinsky et al., 2000, Radin et al., 1990, Carter et al., 2004]. Mechanical loads acting on the cartilage ECM not only generates fluid flows for nutrition and waste transport but also
provide essential mechanical cues for tissue homeostasis. One of the goals of my research was to link the mechanically induced strains and stresses with subsequent biological processes such as gene regulation and ECM remodeling. Non-physiologically low or high stresses are presumed to result in mechanical ECM damage and the regulation of catabolic mediators [Saarakkala et al., 2010, Goldring and Marcu, 2009]. For decades, researchers have investigated the effect of under- and overloading cartilage and a large amount of publications exist where different model systems are presented [Torzilli and Chen, 1984].

Physiological ranges for strains and stresses are relatively wide but generally contact stresses between 0.5 and 8 MPa [Brand, 2005] and strains of up to 60% are considered normal [Herberhold et al., 1999]. Conversely, strains and stresses falling within these ranges have also been reported to cause cartilage deterioration and changes in gene and protein regulation [Chen et al., 2003, Guilak et al., 1994, Burton-Wurster et al., 1993] suggesting that the type of model system used may influence the results. Also, beside absolute strains and stresses the rate at which these are applied influences their impact, a factor that becomes increasingly important in dynamic models.

5.2.1 The Influence of Axial Load and Translation Speed on Cartilage Biology

In the first study (Chapter 2) sliding was applied with 10, 40 and 70 mm/s speed and two different axial forces of 50 and 100 N. Analyzing the changes in gene expression between different loading groups found that axial force had a strong influence on the magnitude of the expression. Cartilage loaded with 50 N did not result in any statistically significant correlations with any of the mechanical parameters investigated, both in the ANOVA and in the multiple linear regression (MLR) analyses. Since contact stresses were relatively low (0.5 - 0.6 MPa) when loaded with 50 N, the absence of biological changes could be regarded as low-stress or normal tissue response. On the other hand, an increase of the axial load to 100 N resulted in several significant changes of the gene response. First, ANOVA revealed a significant decrease of lubricin and ADAMTS-5 gene expression when loaded with 70 mm/s sliding speed as opposed to 10 mm/s. This regulation was surprising from two perspectives. First, that fact that lubricin was regulated was of interest itself since lubricin is only known to be expressed in chondrocytes of the superficial zone [Schumacher et al., 1994], whereas this zone is clearly lacking in the homogenous BNS matrix. Second, lubricin and ADAMTS-5 were correlated with speed even though no differences in strain or contact stress were found between the three speeds. Hence we assume that a change in strain- or stress-rate might have influenced the gene response since strain or stress was applied at a seven times faster rate at 70 mm/s compared to 10 mm/s sliding speed.

The MLR model was an attempt to combine all individual parameters into a single model in order to
determine their combined influence. By doing this, lubricin, ADAMTS-5 and TIMP-3 produced significant regression coefficients. These are all genes that were also previously reported to be influenced by mechanical load [Lee et al., 2005] and surface motion [Grad et al., 2005]. According to the MLR analysis, the combination of all parameters explained at least 70% of the variation in the gene regulation of these three genes. However, by interpreting the regression coefficients and the fit of the regression equation, one need to consider a few limitations of the model.

First, the fit of the regression ($R^2$) increases when more factors are added to the model. Therefore, the number of significant coefficients could potentially give a hint about how accurate the model is. The number of significant parameters found for lubricin was 6, 3 for ADAMTS-5 and 5 for TIMP-3. One could argue that due to the many significant correlation factors for lubricin (6 out of 8) and ADAMTS-5 (5 out of 8), $R^2$ is accurate and that the significant parameters account for the changes observed in gene expression.

Second, some of the parameters in the model were most likely inter-correlated. For example strain, contact stress and dynamic modulus are all calculated via the measured deformation of the cartilage under load. This is a factor known to influence the regression coefficients and could be a possible reason for the unrealistically high coefficients for strain, contact stress and dynamic modulus. In addition, such inter-correlations might influence the slope of the correlations as can be seen on the example of lubricin. For lubricin, ANOVA shows a down-regulation with increasing speed whereas the MLR model predicts a positive relation (up-regulation) with increasing speed. Lastly, it is important to note that the model assumes linear relationships between the predictor (mechanics) and the response variable (gene) yet the true relationship might be non-linear.

The cartilage samples in the study 2 (Chapter 3) were loaded with different axial loads (~9.8 - 39.2 N) at 10 mm/s sliding speed. Even though the similar mechanical parameters were recorded, a direct comparison between these is difficult due to several reasons: (1) Different types of tissues were used. The model tissue in study 1 was BNS which has a homogenous distribution of ECM components [Xia et al., 2012, Colombo et al., 2013]. For study 2, articular cartilage from bovine femoral condyles were used. This type of cartilage possesses the articular cartilage-specific depth dependent structure [Benninghoff, 1925]. (2) BNS is a normally unloaded cartilage whereas the femoral condyles exhibit daily loading. (3) The study setup is different with a longer loading time and uni-directional (as compared to reciprocating) loading in study 1. All these factors could potentially affect the mechanical impact on the cartilage and the subsequent biological response. In particular the different ECM architectures between the cartilages of the two studies might have significant influence on the stress distribution and load attenuation. Furthermore, due to the fact that BNS is normally unloaded, the cells might have a
different threshold for metabolic responses.

Only matrix specific genes and no catabolic genes were up-regulated in this study even though contact stresses of up to 9 MPa were calculated. Even though this was surprising, this is consistent with a study reporting no changes in MMP gene expression up to 12 MPa contact stress [Fehrenbacher et al., 2003]. Effective stress and strain distribution and high fluid pressurization throughout the intact femoral condyles could explain the resilient behavior in this study.

5.2.2 The Influence of Indenter Curvature on Cartilage Biology

In study 3 (Chapter 4), axial load and sliding speed were kept constant while indenter geometry was varied. The goal was to study indenter geometry influence on deformational behavior and contact parameters. While comparisons to study 1 are still difficult due to the aforementioned reasons, the results area comparable to study 2 in terms of model tissue and loading apparatus. However, changes in total loading time have to be taken into account.

Compared to the first two studies, highest contact stresses were calculated for these experiments (~12 MPa) and the cartilage was loaded for ~4.5 hours (2000 cycles). Of interest, no direct mechanical damage was observed immediately after the loading phase for both indenter sizes. A potential reason for this is the highly organized collagen network in the superficial zone of the cartilage. It has been shown that the mechanical response of the tissue highly depends on the organization of the collagen fibers in the superficial zone [Korhonen et al., 2002b, Guo et al., 2015]. It distributes the tensile stresses evoked by the sliding (plowing) over the entire tissue surface and, as mentioned under section 5.1.2, efficiently confines the cartilage ECM to allow high fluid pressurization [Cohen et al., 1998]. This means that most of the load was carried by the fluid component (interstitial fluid) and the remaining part was efficiently distributed over the large condylar surface. Even though no visible damage was detected immediately post-loading with histological stainings, the cartilage loaded with the 17.6 mm diameter indenter was found to have increased expression of collagen type II, aggrecan COMP and fibronectin compared to the 30.2 mm diameter indenter. The observed trend for collagen type II and aggrecan is well described in the literature and is commonly associated ECM remodeling in response to the mechanical load [Correro-Shahgaldian et al., 2011, Valhmu et al., 1998]. This was a similar response as for the gene expression found in study 2. On the other hand, an increase of COMP and fibronectin gene expression may indicate a catabolic shift of chondrocyte phenotype. Fibronectin is a protein known to interact with other ECM components and is responsible for proper mechano-transduction [Werb et al., 1989]. However, its synthesis in adult cartilage is generally linked to disease processes and its fragments are highly inflammatory [Burton-Wurster and Lust, 1985, Farquhar et al., 1996]. COMP is a
gene that was found to be up-regulated after intensive activities such as long-distance running and in patients with OA [Lohmander et al., 1994, Neidhart et al., 2000]. Further indication of a catabolic shift is the up-regulation of MMP-3 and ADAMTS-5 in the 17.6 mm group. MMP-3 plays a pivotal role in load-induced cartilage injury and the development of OA [Van Meurs et al., 1999a]. In addition, it is an activator for MMP-13 [Knäuper et al., 1993].

As MMPs and ADAMTS generally need several hours to be detected [Lee et al., 2005], it is possible that by the time of sampling for the PCR study (4 hours post-loading) the presence of these enzymes was not high enough to make significant differences between the study groups. However, the significant loss of PGs in the 17.6 mm group indicates that PGs were digested and lost to the culture media. This loss cannot be of direct mechanical origin since cartilage sections appeared normal immediately after the loading. Therefore it is reasonable to assume it is due to enzyme activity.

The loss of PGs is an early sign of OA-related changes. And by analyzing the culture medium after the 24- and 48-hours incubation period, significantly more PGs were found in the medium of the cartilage loaded with the 17.6 mm diameter indenter. Since no loss of PGs was observed in the histological sections, it is likely that the loss detected in the culture medium is due to translation and activation of enzymes produced during the loading.

To conclude, it is important to mention that changes in axial force, sliding speed and indenter radius can have similar effects on the tissue, e.g., increase the contact stress by decreasing contact area. However, increasing the contact stress at a certain location on the tissue with sliding might have different effects of the surrounding tissue such as a change in interstitial fluid flows, which have been shown to be crucial for cellular processes [Ateshian et al., 2007]. The difference in contact stresses between study 2 and 3 is minimal nevertheless a big difference in biological response was observed. Due to the fact that the loading duration was much longer in study 3, the total loading time could be crucial factor when it comes to an induction of catabolic response under dynamic sliding loads. Also, the gene expression response was investigated after an incubation time of 4 hours (as compared to ~1h) post-loading. This time difference could have a significant effect on the amount of RNA template present.

5.3 Limitations

The model has a few limitations worth mentioning. First of all, the use of cylindrical and spherical indenters is an idealized assumption of the contact between two joints. While it allows for relatively easy quantification of contact parameters, it is important to note the contacts in joints are never perfectly round. In addition, the plastic of the indenters is several times stiffer than the opposing cartilage. This
results in all deformations occurring in the cartilage that under \textit{in vivo} conditions would be shared between the two cartilage layers. In return, this configuration models the case where one part of the joint has been replaced by an artificial material. Also, contact areas calculated (mm$^2$) are rather small for the joints of the lower body where areas are in the range of 5-10 cm$^2$ [Brown and Shaw, 1984] though they might physiological for smaller joints such as the finger or the temporomandibular joint [Hess et al., 2013]. Last but not least, with our system, no information about cartilage rebound (recovery) between the individual loading cycles could be obtained. Therefore, only total deformation (strain) as opposed to cycle-to-cycle deformation was obtained.

5.4 Summary and Conclusions

This thesis describes a new mechanobiological approach to apply dynamic sliding onto surfaces of cartilage explants in order to model complex joint kinematics. The goal was to correlate the mechanical parameters during the sliding load with subsequently analyzed biological responses in order to determine loading patterns that are detrimental for cartilage. Two versatile testing systems (RPETS and DACTS) were used to apply dynamic sliding loads within a broad range of physiological and pathophysiological values [Herberhold et al., 1999, Brand, 2005, Brown and Shaw, 1984, Eckstein et al., 1999, 2000, Dennis et al., 2005, Kizuki et al., 1995]. A model was developed to analyze strains and stresses acting on the cartilage ECM throughout the testing. Hertz’s theory of elastic deformation was adapted to investigate the contact mechanics of dynamic mechanical loads acting on a thin layer of cartilage bonded to a rigid substrate [Johnson, 1987, Jaffar, 2006]. The model is well established and frequently used [Gerberich et al., 1998, Bonnevie et al., 2011, 2012, Moore and Burris, 2015, Moore et al., 2015] and despite being violated under certain circumstances it proved to be a reasonable tool to compare relative changes. Nevertheless, a robust theory for contact problems on anisotropic and poro-elastic surfaces needs to be developed in order to make more precise assumptions of the mechanical behavior of the cartilage.

On a daily basis, articular joints are subjected to a variety of mechanical stimuli resulting from changes in axial loads, sliding speeds and contact positions. Our test system allows for testing of such parameters under controlled conditions to investigate their individual influence on cartilage mechanobiology. To study degenerative changes of articular joints, mechanical environments were generated that accelerate metabolic and degenerative changes that normally happen over several decades.

It is important to note that changes in different parameters can have similar effects on ECM deformational properties such as increasing axial load and decreasing sliding speed, both of which result in increased deformation (strain). Nevertheless, even though the measurable impact on the ECM is
the same (increase in deformation), it does not inherently imply that a decrease in sliding speed and increase in axial load have the same effect on chondrocytes metabolism due to differently affected fluid flow patterns. It is known that such patterns determine nutrition and waste transport around chondrocytes and even small pressure gradients can have large impact on chondrocyte deformation and mechano-sensing [Ateshian et al., 2007, Kim et al., 1995]. In general, we found that the application of dynamic sliding loads resulted in alterations of ECM strains and stresses depending on the axial load, sliding speed and indenter geometry. According to their magnitude, increasing strains and stresses were associated with a metabolic reaction of the chondrocytes that manifested itself in the production of ECM components. We also found an up-regulation of catabolic enzymes for some loading scenarios, however, their absolute presence was low. Also, as histological analyses found, cartilage explants were remarkably robust to resist direct mechanical damage through the loading regimes. A potential reason for the high damage-resistance and low catabolic responses could be that most loading were supported by high fluid pressures (high Pe numbers) and therefore the solid matrix and the chondrocytes were effectively shielded from damaging stimuli. As articular cartilage is an extremely resilient material under normal circumstances, finding relatively few catabolic process confirms that the applying dynamic motion onto the surface of cartilage explants accurately mimics in vivo kinematics. Variations of loading variables will be needed in future studies to create harsher environments in order to pronounce degenerative processes. Also, analysis techniques need to be refined in order to observe spatial and temporal changes in cartilage mechanobiology under dynamic loading.

5.5 Perspectives

For future mechanobiological studies it will be important to consider external strains and stresses and also fluid flows and pressures within the ECM. Finite element models of different mechanical conditions potentially reveal differences in fluid flow patterns and internal stresses (fluid pressure, tensile stresses) that should be incorporated into correlative models. In addition, by applying long loading durations we found common patterns for the deformational response that could essentially be divided into two phases: (1) the first 20 - 50 cycles were the cartilage ECM rapidly deformed followed by (2) an equilibrium phase were the net deformation did not additionally increase. By analyzing the cellular response at the end of the loading phase, important information about the initial ~50 cycles where most of the mechanical changes happen will be lost. Therefore, a separate analysis on the mechanobiology of the initial phase could be meaningful when trying to understand degenerative processes. Furthermore, it is known that cartilage has different deformation and swelling (recovery) properties [Eckstein et al., 1999]. By finding a way to quantify cycle-to-cycle changes of deformation and recovery, one could
more accurately estimate true (real-time as opposed to total) strains and stresses at any time during the loading phase. Due to matrix compaction with loading [Grenier et al., 2014], cycle-to-cycle deformation is assumed to decrease over the course of loading that in turn impacts contact stresses. Last but not least, relative motion of the cartilage can be divided into a rolling and sliding phase. During knee motion, the knee slides from 90° to 60° of flexion and then rolls to 15°. Between 15° and 0° the lateral condyle slides on the tibia while the medial condyle rolls (if maximum extension is 0°) [Iseki and Tomatsu, 1976]. Up to now, the focus in tribological studies was mostly on sliding using simplified models [Caligaris and Ateshian, 2008, Bonnevie et al., 2011] (including the studies of this thesis). Rolling and sliding impose different mechanical impact on a cartilage surface and therefore, comparing the effects caused by rolling as opposed to sliding could be of interest from a mechanical and metabolic perspective.


95


List of Publications

Conference Abstracts

1. Schätti OR, Waldvogel DR, Gallo LM, Mechanobiological response of chondrocytes to a TMJ-specific loading pattern over time, OARSI World Congress, April 18-21, 2013

2. Schätti OR, Gallo LM, Plowing forces - as occurring in articular joints - modulate lubricin biosynthesis, ICRS 11th World Congress, September 15-18, 2013


5. Schätti OR, Marková M, Torzilli PA, Gallo LM, Mechanical loading of articular cartilage explants with joint-specific loading patterns modulates gene expression of lubricin and catabolic matrix enzymes, OARSI World Congress, April 30 - May 03, 2015

6. Schätti OR, Gallo LM, Torzilli PA, A model to study articular cartilage mechanical and biological responses to rolling and sliding loads, Summer Biomechanics, Bioengineering and Biotransport Conference, June 17-20, 2015

7. Schätti OR, Gallo LM, Torzilli PA, Mechanobiological analysis of articular cartilage responses to sliding loads, ORS Annual Meeting, March 5-8, 2016
Journal Articles


