Advances in Experimental Medicine and Biology 1402

Brianne K. Connizzo
Lin Han
Robert L. Sah *Editors*

Electromechanobiology of Cartilage and Osteoarthritis

A Tribute to Alan Grodzinsky on his 75th Birthday

OPEN ACCESS

Springer

Advances in Experimental Medicine and Biology

Volume 1402

Advances in Experimental Medicine and Biology provides a platform for scientific contributions in the main disciplines of the biomedicine and the life sciences. This series publishes thematic volumes on contemporary research in the areas of microbiology, immunology, neurosciences, biochemistry, biomedical engineering, genetics, physiology, and cancer research. Covering emerging topics and techniques in basic and clinical science, it brings together clinicians and researchers from various fields.

Advances in Experimental Medicine and Biology has been publishing exceptional works in the field for over 40 years, and is indexed in SCOPUS, Medline (PubMed), EMBASE, BIOSIS, Reaxys, EMBiology, the Chemical Abstracts Service (CAS), and Pathway Studio.

2021 Impact Factor: 3.650 (no longer indexed in SCIE as of 2022)

Brianne K. Connizzo • Lin Han
Robert L. Sah
Editors

Electromechanobiology of Cartilage and Osteoarthritis

A Tribute to Alan Grodzinsky on his 75th Birthday

Springer

Editors
Brianne K. Connizzo
Department of Biomedical Engineering
Boston University
Boston, MA, USA

Lin Han
School of Biomedical Engineering,
Science and Health Systems
Drexel University
Philadelphia, PA, USA

Robert L. Sah
Department of Bioengineering
& Institute of Engineering in Medicine
University of California, San Diego
La Jolla, CA, USA

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-031-25587-8 ISBN 978-3-031-25588-5 (eBook)
https://doi.org/10.1007/978-3-031-25588-5

This work was supported by Department of Biomedical Engineering, Boston University
Northeastern University
Universitätsklinikum Freiburg
Christian-Albrechts-Universität zu Kiel
Clemson University
Cornell University
Weill Cornell Medicine, New York
Harvard Medical School
College of Engineering, Boston University
Drexel University

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

It is a great pleasure and honor to introduce this festschrift for the celebration of the 75th birthday of our mentor, **Professor Alan J. Grodzinsky** (Fig. 1). On Saturday, November 13, 2021, colleagues, collaborators, and trainees gathered in Cambridge, Massachusetts to celebrate this joyous occasion. First, a scientific symposium was held in his honor with presentations describing the work that was influenced and shaped by Al's mentorship and collaboration. With the simmering COVID-19 pandemic, the presentations were not only projected in person at the Massachusetts Institute of Technology but also streamed around the world. After the symposium, a celebratory dinner and reception were held at a local restaurant and included reflections. This book is representative of that celebration, with the first half containing ten chapters from Al's students, former trainees, and international collaborators that range from basic science and engineering, to animal models, and clinical applications. The second half of this book contains such reflections with personal tributes, notes of gratitude, and stories that represent Al's character and the impact he has had on all that surround him.

In 1971, Al received his bachelor's and master's degrees from MIT in Electrical Engineering. Just three years later, he received his doctoral degree, a Sc.D. also from MIT, focusing his dissertation on the electromechanics of deformable polyelectrolyte membranes with his thesis advisor, James Melcher, and co-supervisor Ioannis Yannas. Immediately after finishing his doctorate, Al started his academic career as Assistant Professor in Electrical Engineering and Computer Science (EECS), but it was a sabbatical year in 1976–1977 under the mentorship of Melvin Glimcher, Chief of Orthopaedic Surgery at Boston Children's Hospital, that initiated Al's scientific obsession with cartilage biology. Thereafter, he quickly rose to the forefront of research on cartilage electromechanobiology and osteoarthritis.

Al is one of the world's most prominent biomedical scientists. His research has been continuously funded by the National Institute of Health since 1980, including the highly prestigious NIH MERIT award from 1995 to 2005. In total, his work has resulted in over 350 publications with more than 40,000 citations, giving Al an h-index of 105. Over his five-decade career in musculoskeletal research, Al has transformed the field in both basic science and translational topics.

Al introduced the theory of electromechanics to biomedical engineering, establishing electromechanics as a cornerstone of the modern biomechanics framework. Al was also a pioneer in multiscale biomechanics and mechano-

Fig. 1 Alan J. Grodzinsky poses with his viola in the summer of 2021. (Photo by Webb Chappell)

biology. He was the first to develop theoretical models that connect tissue nanoscale integrity with macroscopic functions. He also established oscillatory atomic force microscopy at high frequencies as the state-of-the-art paradigm to probe the multiscale mechanics of biomolecules, cells, and tissues. His discovery that dynamic compression can stimulate chondrocyte anabolism is now used as a standard practice in cartilage tissue engineering.

Al also established novel cartilage explant models to simulate the inflammation and joint injury responses *in vivo*, models which have become standard tools in cartilage research. Al developed several novel biomaterials to improve the repair of articular cartilage, including innovative nanocarriers to promote drug delivery to local cartilage defects. These advances helped to overcome obstacles in cartilage therapeutics arising from its avascular and dense nature. Al's most recent project is sending human cartilage explants to the outer space. We are waiting for Al to tell us what space and its microgravity will do to our knees.

The scientific contributions from the Grodzinsky Lab have been recognized worldwide by the American Society of Mechanical Engineering (ASME), Society for Physical Regulation in Biology and Medicine, the American Society for Biomechanics (ASB), the American Academy of Orthopaedic Surgeons (AAOS), the International Cartilage Regeneration and Joint Preservation Society (ICRS), the Osteoarthritis Research Society International (OARSI), the Orthopaedic Research Society (ORS), and many more. Most recently, Al was honored with the 2019 ORS/OREF Distinguished Investigator Award and the 2021 OARSI Lifetime Achievement Award. These are the highest-level awards possible at their respective societies, demonstrating just how impactful Al's research on cartilage has been.

While making forefront advances in research, Al has mentored many trainees to success. Over his career, he has mentored 27 post-doctoral researchers, 53 doctoral theses, 53 master's theses, and 64 bachelor's theses. Al has also served on over 130 doctoral committees, including numerous ones from institutions abroad, and hosted over 100 visiting scientists in his laboratory. But these are just the formal mentees; everyone who knows Al knows that he is extremely generous with his time and his mentorship. His door is always open no matter how junior or senior the person seeking advice is. Despite his success, he always exudes humility and gratitude. Al was recently awarded the 2018 ORS Outstanding Achievement in Mentoring Award, a well-deserved recognition of his impact.

Al is a dedicated, enthusiastic, and passionate educator. Starting in the EECS department, he initially taught a wide array of required EE courses to undergraduates for over 20 years. He then taught biomechanics in the Biological Engineering Department. But perhaps his most seminal contribution is the development of his "Fields, Forces, and Flows" graduate class which has been offered at MIT in some form since its inception in 1975 (known locally as "FFF"). This course has been taught to Mechanical, Biological, Chemical, Electrical, and Materials Science and Engineering students. He redesigned the FFF course significantly to focus on biological systems in the late 1990s and wrote a textbook to accompany the course; the textbook is used in many courses at other universities, including some taught by his former trainees. Al has received local and national accolades for his teaching efforts. Tributes from former students and personal anecdotes from his classes abound in the second half of this book and clearly demonstrate the impact he has made on student learning. The reach of Al's teaching is far beyond the walls of MIT, a true testament to his legacy as a professor.

With all of these accomplishments and recognition with prestigious awards, it would surprise anyone to know just how humble and down to earth that Al truly is. While many know him as a giant in the field and the "father of modern cartilage mechanics," to us he is a mentor, a colleague, and a friend. His commitment to his students, his endless excitement in the lab, his patience, and his unwavering positive outlook on life—those are just a few of the reasons we are inspired by Al. We hope that reading the tributes from his

staff, students, colleagues, and collaborators in the second half of this book will elaborate on our commentary. It has truly been an honor to work with Al, and we wish him the absolute best for the future.

Happy Birthday, Al!

Boston, MA, USA — Brianne K. Connizzo

Philadelphia, PA, USA — Lin Han

La Jolla, CA, USA — Robert L. Sah

Contents

Editors and Contributors

About the Editors

Brianne K. Connizzo, Ph.D., is an Assistant Professor at Boston University in the Department of Biomedical Engineering. She obtained her Ph.D. from the University of Pennsylvania in Philadelphia, where she focused on novel techniques to measure the multiscale mechanical function of tendons and ligaments under the mentorship of Dr. Louis Soslowsky. She continued and expanded upon this work during her postdoctoral training with Dr. Alan Grodzinsky at the Massachusetts Institute of Technology, using AFM-based assays to establish the poroviscoelastic properties of murine tendon. With time, her research interests evolved toward a focus on tendon and ligament cell biology. More recently, her group is making strides toward understanding the mechanobiology of aging with the ultimate goal of extending the number of healthy, active years for older adults.

Lin Han, Ph.D., is an Associate Professor in the School of Biomedical Engineering, Science and Health Systems at Drexel University. He obtained his B.E. from Tsinghua University in Beijing, P. R. China, and his Ph.D. from the Massachusetts Institute of Technology in the area of Bio- and Polymeric Materials under the mentorship of Dr. Alan Grodzinsky. After graduation, he worked as a quantitative analyst at Aristeia Capital, LLC, responsible for developing and testing statistical models. He then completed his postdoctoral training in the Department of Materials Science and Engineering and the Center for Biomedical Engineering at MIT. His research interests focus on exploring the nanoscale structure-property relationships of biomaterials, which aim to provide important insights into the application of disease diagnostics, tissue regeneration, and bio-inspired material design. Dr. Han recently received the 2021 Kappa Delta Young Investigator Award for research on the structure and function of cartilage extracellular matrix (ECM) and its impact on tissue regeneration and disease evolution in osteoarthritis (OA).

Robert L. Sah, M.D., Sc.D., is a Professor of Bioengineering at the University of California, San Diego and a Professor of the Howard Hughes Medical Institute. He received his B.S. and M.S. in Electrical Engineering and Sc.D. in Medical Engineering from the Massachusetts Institute of Technology, working with Dr. Alan Grodzinsky. He obtained his M.D. from

Harvard Medical School. He joined UCSD Bioengineering in 1992 and was promoted to Professor in 2001. Dr. Sah has received a Young Investigator Award (National Science Foundation), a Hulda Irene Duggan Investigator Award (Arthritis Foundation), three Kappa Delta Awards (American Academy of Orthopaedic Surgeons), and the Van C. Mow Medal (American Society of Mechanical Engineers), At UCSD, Dr. Sah has served as Vice Chair of Bioengineering, and he is currently Co-Director of the Center for Musculoskeletal Research of the Institute of Engineering in Medicine.

Contributors

Ambika Bajpayee Department of Bioengineering, Northeastern University, Boston, MA, USA

Rebecca M. Black Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

Lawrence J. Bonassar Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA

Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, USA

Linda D. Bragman Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

Joseph A. Buckwalter Department of Orthopedics and Rehabilitation, University of Iowa, Iowa City, IA, USA

Susan Chubinskaya Departments of Pediatrics, Orthopaedic Surgery, and Medicine, Rush University, Chicago, IL, USA

Brianne K. Connizzo Department of Biomedical Engineering, Boston University, Boston, MA, USA

Rodolfo E. De la Vega Musculoskeletal Gene Therapy Laboratory, Mayo Clinic, Rochester, MN, USA

Atte S. A. Eskelinen Department of Technical Physics, University of Eastern Finland, Kuopio, Finland

Amir Esrafilian Department of Technical Physics, University of Eastern Finland, Kuopio, Finland

Christopher H. Evans Musculoskeletal Gene Therapy Laboratory, Mayo Clinic, Rochester, MN, USA

Cristina Florea Department of Technical Physics, University of Eastern Finland, Kuopio, Finland

Bruce S. Gardiner Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, WA, Australia

Lin Han School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA

Melanie L. Hart G.E.R.N. Research Center for Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Faculty of Medicine, Medical Center—Albert-Ludwigs-University of Freiburg, Freiburg im Breisgau, Germany

Vincent C. Hascall Department of Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH, USA

Han-Hwa Hung Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

Elizabeth R. Kahle School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA

Byumsu Kim Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA

Young-Jo Kim Harvard Medical School, Boston, MA, USA

Boston Children's Hospital, Boston, MA, USA

Rami K. Korhonen Department of Technical Physics, University of Eastern Finland, Kuopio, Finland

Bodo Kurz Department of Anatomy, Christian-Albrechts-University, Kiel, Germany

Qing Li School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA

Stefan Lohmander Orthopaedics, Department of Clinical Sciences Lund, Faculty of Medicine, Lund University, Lund, Sweden

Rachel E. Miller Department of Internal Medicine, Rush University, Chicago, IL, USA

Saeed Miramini Department of Infrastructure Engineering, The University of Melbourne, Melbourne, VIC, Australia

Meghan M. Moran Department of Anatomy and Cell Biology, Rush University Medical Center, Chicago, IL, USA

Christopher V. Nagelli Musculoskeletal Gene Therapy Laboratory, Mayo Clinic, Rochester, MN, USA

Patrik Önnerfjord Rheumatology and Molecular Skeletal Biology, Department of Clinical Sciences Lund, Faculty of Medicine, Lund University, Lund, Sweden

Gustavo A. Orozco Department of Technical Physics, University of Eastern Finland, Kuopio, Finland

Department of Biomedical Engineering, Lund University, Lund, Sweden

Anna H. K. Plaas Department of Internal Medicine (Rheumatology), Rush University Medical Center, Chicago, IL, USA

Bernd Rolauffs G.E.R.N. Research Center for Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Faculty of Medicine, Medical Center—Albert-Ludwigs-University of Freiburg, Freiburg im Breisgau, Germany

Martin Rydén Orthopaedics, Department of Clinical Sciences Lund, Faculty of Medicine, Lund University, Lund, Sweden

Robert L. Sah Department of Bioengineering & Institute of Engineering in Medicine, University of California, San Diego, La Jolla, CA, USA

John D. Sandy Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL, USA

Sandra L. Shefelbine Departments of Bioengineering and Mechanical Engineering, Northeastern University, Boston, MA, USA

David W. Smith School of Physics, Mathematics and Computing, The University of Western Australia, Perth, WA, Australia

Petri Tanska Department of Technical Physics, University of Eastern Finland, Kuopio, Finland

Stephen B. Trippel Cell Biology & Physiology, Indiana University School of Medicine, Indianapolis, IN, USA
Department of Biomedical Engineering, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA

Chao Wang School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA

Lihai Zhang Department of Infrastructure Engineering, The University of Melbourne, Melbourne, VIC, Australia

Hannah M. Zlotnick Departments of Bioengineering and Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, USA

Part I

Scientific Impact

1 Aggrecan and Hyaluronan: The Infamous Cartilage Polyelectrolytes – Then and Now

Anna H. K. Plaas, Meghan M. Moran, John D. Sandy, and Vincent C. Hascall

Abstract

Cartilages are unique in the family of connective tissues in that they contain a high concentration of the glycosaminoglycans, chondroitin sulfate and keratan sulfate attached to the core protein of the proteoglycan, aggrecan. Multiple aggrecan molecules are organized in the extracellular matrix via a domain-specific molecular interaction with hyaluronan and a link protein, and these high molecular weight aggregates are immobilized within the collagen and glycoprotein network. The high negative charge density of glycosaminoglycans provides hydrophilicity, high osmotic swelling pressure and conformational flexibility, which together function to absorb fluctuations in biomechanical stresses on cartilage during movement of an articular joint. We have summarized information on the history and current knowledge obtained by biochemical and genetic approaches, on cell-mediated regulation of aggrecan metabolism and its role in skeletal development, growth as well as during the development of joint disease. In addition, we describe the pathways for hyaluronan metabolism, with particular focus on the role as a "metabolic rheostat" during chondrocyte responses in cartilage remodeling in growth and disease.

Future advances in effective therapeutic targeting of cartilage loss during osteoarthritic diseases of the joint as an organ as well as in cartilage tissue engineering would benefit from 'big data' approaches and bioinformatics, to uncover novel feed-forward and feed-back mechanisms for regulating transcription and translation of genes and their integration into cell-specific pathways.

Keywords

Cartilage · Aggrecan · Hyaluronan · Extracellular matrix

A. H. K. Plaas (✉)
Department of Internal Medicine (Rheumatology), Rush University Medical Center, Chicago, IL, USA

M. M. Moran
Department of Anatomy and Cell Biology, Rush University Medical Center, Chicago, IL, USA

J. D. Sandy
Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL, USA

V. C. Hascall
Department of Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH, USA

1.1 Introduction

Cartilages are unique in the family of connective tissues in that they contain a high concentration of the glycosaminoglycans (GAG),

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_1

chondroitin sulfate (CS) and keratan sulfate (KS) that are attached to the core protein of the proteoglycan, aggrecan. Aggrecan is organized in the extracellular matrix via a domain-specific molecular interaction with hyaluronan (HA) and with a link protein, and it is present throughout the collagen and glycoprotein network.

The high concentration of these organized GAGs have a well-documented essential role for articular cartilages to absorb alterations in biomechanical stresses during movement of an articular joint. At the structural level this is due to their biophysical characteristics at physiological pH, which include hydrophilicity and high osmotic swelling pressure due to the negative charges on their carbohydrate subunits (carboxyl and sulfate groups) and on their conformational flexibility and efficiency at filling space due to their sizes.

In this chapter we review the history and current knowledge of the cell-mediated regulation of aggrecan metabolism (Fig. 1.1) including: (a) the posttranslational modification of the core protein with CS and KS and its extracellular organization into 'aggregates' with HA and link proteins; (b) the proteolytic processing of the core protein by a specific set of extracellular proteases (ADAMTSs and MMPs); and (c) the function of hyaluronan (HA) metabolism in the context of serving as a "metabolic rheostat" during chondrocyte responses in cartilage remodeling during growth and disease.

Throughout the Chapter, components of the metabolic pathways that have been shown to be affected by biomechanical perturbation of tissues will be highlighted. In this research area, the Grodzinsky lab, together with an extensive network of collaborators, spearheaded in vitro bioreactor experiments using cartilage explants or chondrocytic cell constructs, to delineate the effects of static and dynamic compression, and of sheer stress, on the illustrated pathways in aggrecan post-translational processing. This set in motion a research approach used by multiple laboratories to extend our understanding of mechanotransduction pathways in chondrocytes and progenitor cells for cartilage engineering purposes ([77, 106, 144, 228, 239, 279] and references therein). In addition, throughout the comprehensive list of key references in the covered research areas the publications from the Grodzinsky lab and its past members are annotated in the Bibliography.

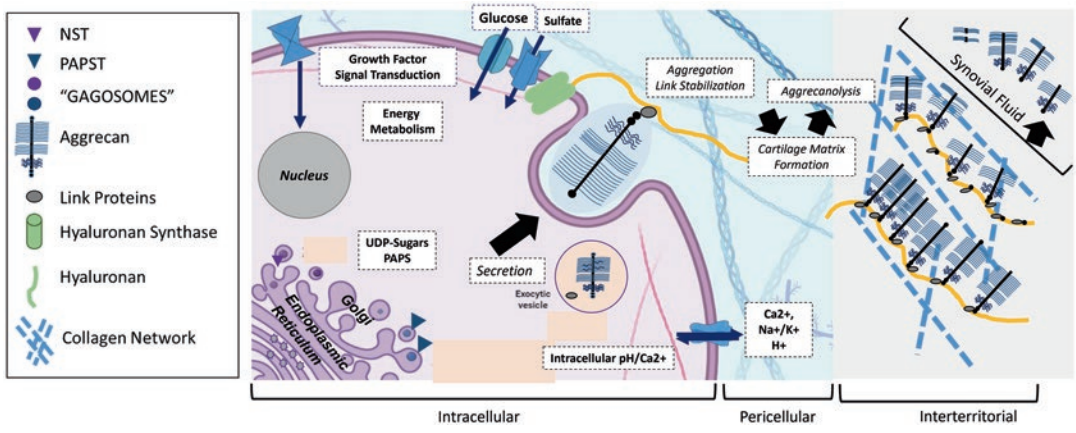


Fig. 1.1 Schematic of topographical organization of components involved in intracellular aggrecan synthesis and extracellular matrix organization

1.2 Chondroitin Sulfate and Keratan Sulfate Fine Structure on Aggrecan

Core protein linkage regions for synthesis and polymerization of CS and KS on the aggrecan core protein domains are illustrated in Fig. 1.2. CS is O-glycosidically linked to the serine residues along the CS rich regions 1 and 2 of the core protein via a linkage region oligosaccharide (-Xyl-Gal-Gal-GlcA) followed by unbranched chains consisting of disaccharides, (→4)β-GlcA (1→3)βGalNAc(1→), in which the amino sugar can be substituted on the C4 and/or C6 by a sulfate ester.

KS on aggrecan, also known as 'skeletal' KS, [180, 214] is O-linked to a serine or threonine in the KS domain, via a mucin core-2 linkage structure, (-GalNAc β(1–6)GlcNAc(1→). The GAG polymer is based on a polylactosamine backbone, with repeated disaccharides of (→4) βGal β (1–3) GlcNAc (1→). Both sugars in the disaccharide repeat can be sulfated on their C6 carbon, and an additional fucose can be substituted on the GlcNAc-6S. Many of these chains also capped with a sialic acid at the non-reducing terminal.

1.2.1 Aggrecan CS Chain Length and Sulfation Are Different in Skeletal Growth and Mature Cartilages

It is well established that chain length of CS and the type of sulfation on the C-4 or C-6 position of GalNAc residues in CS can vary with cartilage source depending on species and anatomical location. Detailed analyses of aggrecan CS fine structures in cartilage growth and maturation have provided more insights into conserved adaptations of CS biosynthesis to altered biophysical and biomechanical demands of a particular cartilage type.

Thus, examination of the GAG fine structure on growth and mature cartilage aggrecan core protein GAG domains using HPLC [163, 199]

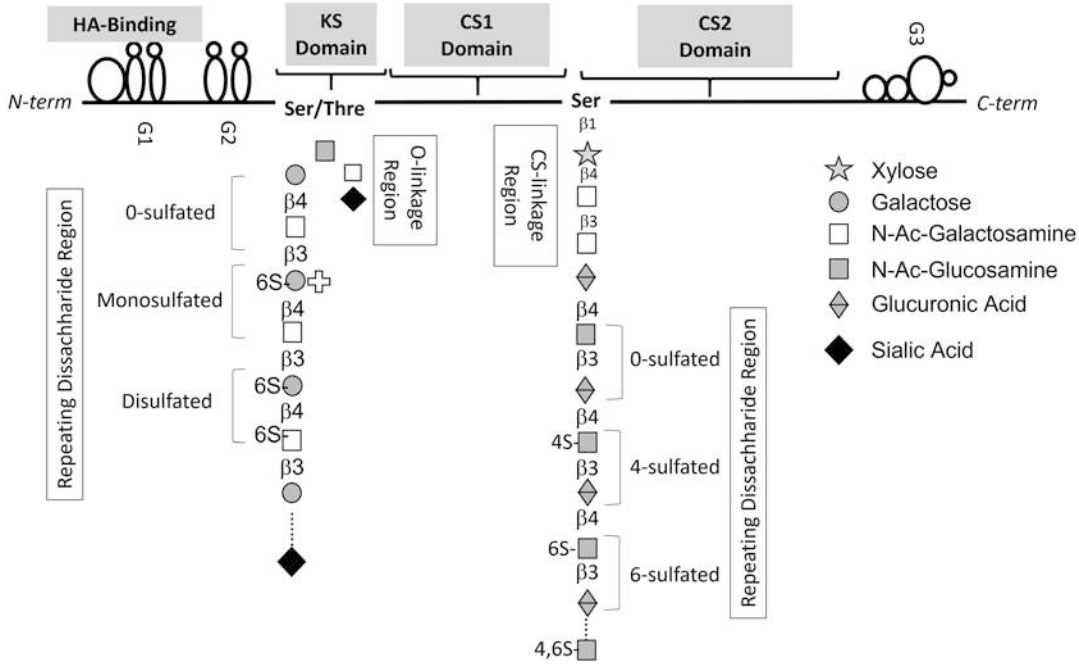


Fig. 1.2 Schematic of Aggrecan Core Protein Domains: G1, HA binding; KS, KS or O-linked oligosaccharide substituted domain, CS1/CS2, CS attachment domains

and FACE analyses [33, 201] established both location and age-related changes. For example, CS fine structure analyses of fetal growth plate cartilage aggrecan revealed a gradient in CS composition from the reserve zone to the hypertrophic zone, characterized by a marked increase in chain length accompanied by increased 6-sulfation and a concomitant decrease in 4-sulfation [55]. Furthermore, major changes in both CS chain length and sulfation pattern during postnatal maturation of human knee cartilage from the epiphyseal growth to a mature articular phenotype [200, 214, 285] were also detected. Upon skeletal maturation, chain length decreased by as much as 50%, and transitioned from an equal abundance of 4- and 6-sulfated GalNAc residues in growth cartilage to a predominance of 6-sulfated GalNAc residues. In addition CS chains in the CS2 region were shorter than those in the CS1 domain and carried a non-reducing terminal 4, 6-disulfated GalNAc residue instead of a 4S-GalNAc residue. A similar pattern in decreased chain length and increased 6-sulfation of both internal and terminal GalNAc residues was also observed by analyses of equine carpal articular cartilage CS [27].

1.2.2 GAG Biosynthesis Is a Multienzyme Process That Takes Place During Core Protein Trafficking Through the ER and Golgi

Studies to-date have shown that the conserved heterogeneity in GAG fine structures, unlike protein synthesis, do not follow a template, and it is regulated by individual cell phenotypes as well as by the structure of the proteoglycan core proteins that provide the acceptors. It is now recognized that conserved GAG structures are generated by transcriptional [124, 164, 288] and topographical [127, 238, 248, 249] control of the numerous enzymes responsible for linkage region synthesis and by GAG polymerization and sulfation (Table 1.1).

1.2.3 Skeletal Disorders Caused by Defective Genes Encoding Biosynthetic Enzymes for Sulfated Glycosaminoglycans

The generation of knock out mouse strains deficient in these enzymes revealed that many had an embryonic lethal phenotype due to defective cell proliferation and organ development, or altered neuronal function. However, they did not reveal a specific function for their role in cartilage growth and maturation (Table 1.2). On the other hand, human genetic studies revealed that defects in GAG-biosynthetic glycosyltransferases, epimerases or sulfotransferases cause distinct phenotypes of congenital disorders in cartilage growth, such as skeletal dysplasia, chondrodysplasia, multiple exostoses, and Ehlers-Danlos syndrome. This has furthered our understanding of the functional importance in the CS substitution on the aggrecan core protein (Table 1.3). In addition to the studies listed, individuals with either Kashin–Beck disease (KBD) [84], who show a dysfunction of CS sulfation enzymes, or a rare polymorphism in the aggrecan core protein [61, 122] are pre-disposed to the development of multi-joint or hand osteoarthritis, respectively.

1.2.4 Intracellular Localization and Topographical Organization of Enzymes for Aggrecan GAG Synthesis

The initiation of the linkage region by xylosyltransferases (I or II) [80, 203, 247] using UDP-xylose for addition of xylose to CS-region serine residues on aggrecan has been shown to occur in a pre-Golgi compartment, either at endoplasmic reticulum (ER) exit sites or in the ER-Golgi intermediate compartment [115, 174, 267]. However, the locations of these enzymes are also proteoglycan core protein and/or cell type specific since xylosyltransferases (I and II) were identified in the cis-Golgi region in rat liver cells and

Table 1.1 Chondroitin sulfate synthetic enzymes

Enzyme	Human gene name https://www.ncbi.nlm.nih.gov/gene	Human mRNA accession #	Gene records for other species
Linkage region			
Xylosyltransferase 1 (XylT-1)	*XYLT1*	NM_022167	Mouse, Rat, Dog, Pig
Xylosyltransferase 2 (XylT-2)	*XYLT2*	NM_007255	Mouse, Rat, Dog, Bovine
Beta-1,4-Galactosyltransferase 1 (GalT-I)	*B4GALT7*	NM_080605	Mouse, Rat
Beta-1,4-Galactosyltransferase 2 (GalT-II)	*B3GALT6*	NM_012200	Mouse, Rat, Pig
Beta-1,3-Glucuronyltransferase 1 (GlcAT-I)	*B3GAT3*	NM_014864	Mouse, Rat, Pig
Repeating disaccharide region			
Beta-1,4-Glucuronyltransferase 1 (GlcAT-II) Beta-1,3 NAcetyl Galactosaminyl transferase II (GalNAcTII)	*CHSY1* *CHSY2 (CSS3)* *CHSY3 (CHPF2)*	NM_014918 NM_175856 NM_019015	Mouse, Rat, Bovine — Mouse, Rat, Bovine
Chondroitin Polymerizing Factor (GalNAcT-II, CS-GlcAT-II)	*CHPF (CSS2)*	NM_024536	Mouse, Rat
Chondroitin N-GalNAc transferase (GalNAcT-I; GalNAcT-II)	*CSGALNACT1* *CSGALNACT2*	NM_018371 NM_018590	Mouse, Rat, Bovine, Pig, Horse Mouse, Rat, Bovine, Pig, Horse
Chondroitin 4-O-Sulfotransferase	*CHST11 (C4ST-1)* *CHST12 (C4ST-2)* *CHST13 (C4ST-3)*	NM_018413 NM_018641 NM_152889	Mouse, Rat Mouse, Rat, Bovine, Pig, Horse Mouse, Rat, Bovine, Pig
Chondroitin 6-O-Sulfotransferase	*CHST3 (C6ST-1)*	NM_004273	Mouse, Rat, Bovine, Pig, Horse
N-Acetylgalactosamine 4-Sulfate 6-O-Sulfotransferase	*CHST15*	NM_015892	Mouse, Rat, Bovine, Pig

chondrosarcoma cells [149, 181]. Glycosyl- and sulfotransferases for extension and sulfation of the CS chains in the C4 or C6 positon of the GalNAc residues takes place in the Golgi stacks and extends into the trans-Golgi network (TGN) [249, 264].

Much less is known about the topographical location of the O-linked KS synthesis enzymes, largely impeded by the fact that their activity rapidly declines when tissues or cells are maintained ex vivo [75, 179]. For example, it has not been determined whether CS and KS synthesis occur simultaneously or whether GAG-specific enzymes are segregated in Golgi subcompartments, or whether there is a regulated temporal recruitment as the core protein is trafficked through the secretory pathway enzymes in the same compartment.

Table 1.2 Genetic deletion of CS-synthesis enzymes in mice and associated phenotypes

Enzyme	Knock-out Mouse strains	Major phenotype
Linkage region		
Xylosyltransferase 2	Ferencz et al. [69]	Increased weight differences of lung, heart, and spleen.
Beta-1,4-Galactosyltransferase 1	Kido et al. [117] and Nakamura et al. [178]	Altered brain development
Beta-1,4-Galactosyltransferase 2	Asano et al. [7]	Defective proliferation and differentiation of epithelial cells; growth retardation Embryonic lethality, growth retardation
Beta-1,3-Glucuronyltransferase 1	Izumikawa et al. [108], Yada et al. [290], and Gotoh et al. [79]	Embryonic lethality due to failed cytokinesis
Repeating disaccharide region		
Chondroitin N-GalNAc transferase	Inada et al. [104], Watanabe et al. [278], Sato et al. [226], Shimbo et al. [234], and Adhikara et al. [1]	Defective neuronal plasticity and axon regeneration Defective cartilage growth and collagen organization; defective enchondral ossification; chondrodysplasia; impaired macrophage action
Chondroitin 6-O-Sulfotransferase	Ito et al. [107]	Enhanced motor function recovery after spinal cord injury
Chondroitin 4-O-Sulfotransferase	**Not available**	Abnormal CS elongation shown in sog9 murine L cell mutant
GalNAc4-Sulfate 6-O-Sulfotransferase	Habuchi et al. [83], Kitazawa et al. [126] and Ohtake-Niimi et al. [186]	Enhanced liver fibrosis; abnormal perineuronal net; altered bone marrow derived mast cells; altered dermal repair

Table 1.3 Human skeletal disorders caused by genetic abnormalities in CS-synthesis

Gene/protein	MIM No	Clinical features of resulting skeletal defects
XYLT1 (Xylosyl transferase 1) Desbuquios dysplasia type II Baratola Scott syndrome	**61577** **608124** **300681**	Short stature, joint laxity, hand abnormalities Short stature, patellar dislocation, facial abnormalities
SLC26A2 (Sulfate Transporter) Achondrogenesis type IB Diastrophic Dyplasia Multiple Epiphyseal Dysplasia	**600972** **222600** **226900**	Pre- or early post-natal lethal chondrodysplasia with underdeveloped skeleton Epiphyseal Dysplasia, early onset of Osteoarthritis
PAPSS2 (PAPS Synthase-2) Spondyloepimetaphyseal dysplasia	**612847**	Short bowed lower limbs, enlarged knee joints, short trunk, scoliosis
SLC35D1 (UDP-GlcA/UDP-GalNAc transporter) Schneckbecken dysplasia	**269250**	Neonatal lethal chondrodysplasia short long bones, deformed vertebral bodies
B4GALT7 (GalT-I) EDS, progeroid form	**130070**	Short stature, cranial dysmorphism, osteopenia, aged appearance
B3GALT6 (GalT-II) Ehlers Danlos Syndrome Spondylodysplatic type 2	**615349** **615291**	Short stature, joint laxity and dislocation, spondylodysplasia
B3GAT3 (GlcAT-I) Larsen-like Syndrome	**245600**	Joint dislocations mainly at elbow, scoliosis
CHSY1 (Chondroitin Synthase 1) Temtamy preaxial brachydactyl syndrome	**605282** **608183**	Short stature, limb malformations, growth retardation
CSGALNACT1 (GalNAcT-II, Mild Skeletal Dysplasia	**616615**	Brachydoctyly, joint lacity, mild facial deformations
CHST3 (CS6 sulfotransferase) Spondyloepiphyseal dysplasia	**143095** **603799**	Short stature, dislocation of large joints, kyphoscoliosis, osteoarthritis of elbow, wrist and knee
CHT11 (CS4 sulfotransferase) Osteochondrodysplasia brachydactyly	**610128** **618167**	Brachydactyly, clinosymphalangism in hands and feet, syndactyly and hexadactyly in feet, scoliosis, dislocated patellae, and fibulae and pectus

1.2.5 ER/Golgi Topography and Organelle Microenvironment of GAG Synthesizing Enzymes

The ER/Golgi membrane localization of the GAG synthesis enzymes has been confirmed from their protein sequences, but details of their arrangement in these compartments are still debated [66]. For example, it has been proposed that the enzymes are at different membrane locations throughout the Golgi, and in that configuration, they would randomly synthesize chains depending on overall luminal availability of UDP-sugars and PAPS substrates. More recently, studies with chemically modified xylosides that serve as "substitute" acceptors for CS synthesis in the Golgi [43, 269] suggest that distinct functional macromolecular assemblies of elongation and sulfation enzymes, termed "GAGOSOMES", are present. These complexes would concurrently catalyze the UDP-sugar addition and sulfate transfer to generate diverse GAG chain structures. This type of mechanism could indeed account for the differences in CS chain structures present on the CS1 and CS2 domains of aggrecan. The need for a specialized configuration of the Golgi compartment to achieve coordinated glycosylation reactions has also been suggested from genetic mutations in proteins such as COG4, CORAB and GOG8 associated with Golgi subdomains. These proteins have been shown to cause congenital disorders of glycosylation, including GAG biosynthesis, due to mis-localization of the transferase enzymes [2, 99, 167]. Topographical organization of the GAG biosynthetic enzymes is also a necessary prerequisite for targeted transport of nucleotide sugar precursors [242] for glycosylation and PAPS for sulfation [18, 57] from their production sites in the cytosol into the ER/Golgi lumen. In this regard, genetic deletion of the nucleotide sugar transporter Slc35d1 caused a skeletal defect in the knockout mice, and this was due to a sparse substitution of significantly shortened CS chains on aggrecan [98]. Other factors that could influence a functional Golgi membrane structure and luminal environment, and thereby regulate core protein glycosylation, include pH [213], ionic strength, [137] and cellular stress responses [225].

1.2.6 Alterations in CS Fine Structure by Biomechanical Stimuli – What Parts of the Post-translational Pathway Are They Targeting?

While there have been studies on the effects of growth factors (e.g. TGFβ1, IGF1) and cytokines on cartilage GAG synthesis, [161] and on CS synthesis [22, 171, 188], there have been relatively few studies to determine the effects of biomechanical stimuli on modulation of CS and KS synthesis enzymes. Cyclic compression of bovine cartilage explants in vitro resulted in the synthesis of CS chains with increased GalNAc6-sulfation and a concomitant decrease in GalNAc4-sulfation, and with fewer chains terminating with disulfated GalNAc4,6S [28, 227]. In vivo treadmill exercise in horses [28] increased CS chain size, which was accompanied by a greater proportion of un-sulfated regions in the chains, suggesting a differential effect on the supply of UDP-precursors and PAPS to the CS-synthesizing enzymes, or a selective decrease in activity of the sulfotransferases.

However, a considerable number of studies have reported structural changes in the cytoskeleton and intracellular organelles, such as mitochondria, ER/Golgi [145] and the nucleus, and in structures in response to biomechanical stimuli, including compression, hydrostatic and osmotic pressure [29, 32, 53, 56, 64, 82, 95, 123, 125, 128, 137–139, 145, 168, 169, 253]. Likewise such mechanical perturbations of the tissues or the cells is expected to modify ion channel activity, Ca2+ signaling [53, 101, 196, 299, 300] and glucose transport and utilization [138, 160, 241, 276, 286] that can affect steps in glycosylation pathways.

In summary, mechano-signal transduction, [77] which targets the aggrecan GAG substitution pathways, is likely to induce changes in the GAG precursor synthesis and/or topographical organization of the GAG synthesis enzymes,

rather than in transcriptional regulation of the GAG biosynthetic enzymes (Fig. 1.1).

1.3 Aggrecan Metabolic Turnover in the ECM of Healthy and Osteoarthritic Cartilages

The cartilage ECM composition changes in order to adapt to various postnatal stages of growth and maturation, and is also affected by arthritic diseases. The mechanisms that such metabolic turnover events have on aggrecan have been well studied. For example, Maroudas and coworkers [156, 268] measured the D/LAsp ratio and the advanced glycation end product, pentosidine, in aggrecan purified from adult human cartilages and reported a half-life of ~3 years in vivo. A different approach [91] utilized an in vitro cartilage explant culture method with medium supplemented with 35S radiolabel to tag the CS-bearing region of newly synthesized aggrecan. By quantitating both the matrix retention and release into the culture medium of newly synthesized and resident CS-core protein fragments, turnover constants and half-lives for both pools of aggrecan in vitro were determined to be between 6-20 days. This method was subsequently used by others [35] to show that the half-life of aggrecan in the ECM can be prolonged by the inclusion of serum or anabolic growth factors [35, 172] or was shortened by proinflammatory stimulators [88] in the culture medium. It is also influenced by the type of cartilage [197] or the disease state [37, 219], and can be modulated by biomechanical perturbations [58, 133, 191, 205–207, 217].

1.3.1 Enzymatic Mechanism of Aggrecanolysis

Explant culture experiments demonstrated that a cell-dependent process generates aggrecan species that can no longer bind to HA and therefore diffuse from the tissue. This in turn motivated a research area to determine the molecular mechanism for the “aggrecanolysis”.

Our understanding of “aggrecanolysis” in the human joint was clarified by detailed analysis of aggrecan intermediates in chondrocyte and cartilage culture medium [103, 222], and this was shown to occur naturally in human cartilage and synovial fluids [220] (Fig. 1.3). The most studied aspect has been the proteolysis of the interglobular domain (IGD) of aggrecan with the release of the glycosaminoglycan (GAG)-attachment regions which is destructive to the tissue biomechanical function [20, 21] as it causes loss of the CS from the cartilage ECM.

Although there had been much debate around data suggesting a role for MMP3 (Stromelysin) in aggrecanolysis, a team of scientists at the pharmaceutical company DuPont [258] purified the aggrecan degrading proteolytic enzymes from the medium of catabolically stimulated bovine cartilage explant cultures. They belonged to the “A Disintegrin and Metalloproteinase with the ThromboSpondin motifs” (ADAMTS) family of metalloproteinases. They were termed aggrecanase-1 (ADAMTS-4) and aggrecanase-2 (ADAMTS-5).

1.3.2 Targeted Inhibition of Aggrecanolysis – A Potential Treatment for Human Osteoarthritis?

Given that aggrecan depletion of the articular cartilage is a hallmark of chronic OA and that ADAMTS5 has been proposed as the primary aggrecanase responsible for the destructive cleavages [73, 78, 246], it appeared likely that inhibitors of this enzyme would have therapeutic value as a Disease Modifying OA Drug (DMOAD).

A number of preclinical studies with in vitro explant cultures and/or animal models of OA using small molecular weight inhibitors of ADAMTS5 [25, 41, 45, 46] and catalytic-site directed neutralizing antibodies [192] showed promising results, and several of these potential therapeutics were tested in clinical trials (Table 1.4). However to-date, although showing promising DMOAD activity in pre-clinical models of OA [40, 134, 166], none were effec-

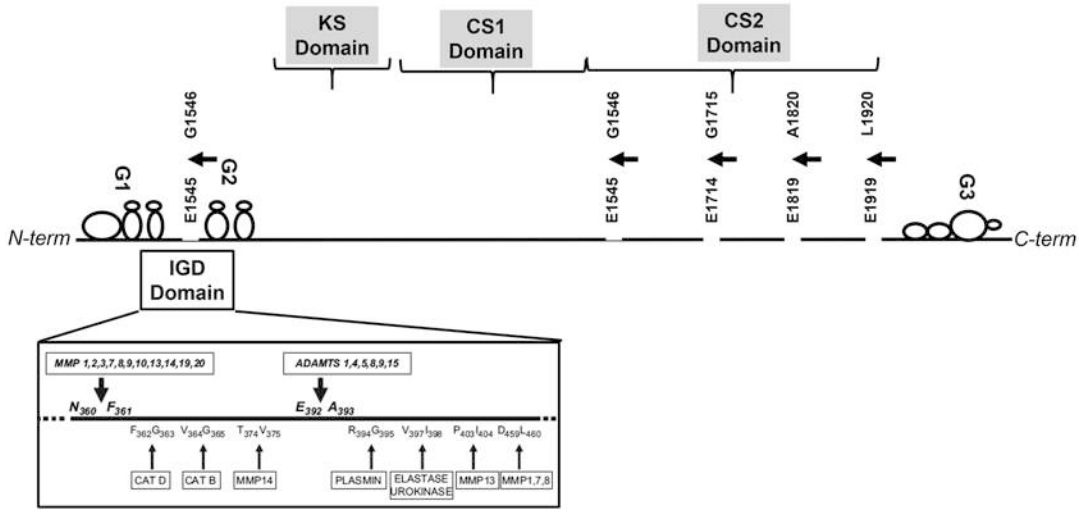


Fig. 1.3 Proteolysis sensitive sites in the human aggrecan core protein: The amino acid sequences in the sciscle bonds were either identified by protein sequencing of fragments isolated from human cartilages or synovial fluids (for MMPs, N-F and for ADAMTS, E-A, E-G and E-L) [220] or predicted from the published aggrecan core protein sequences [60]

Table 1.4 ADAMTS-5 inhibitors advancing into human clinical trials

Drug	Clinical trial ID and duration	Outcome Measures and Study Subjects	Published Data
Small molecule inhibitors			
AGG-523 Wyeth	NCT00454298 Phase I (2007–2009)	Evidence for aggrecan catabolism in urine, blood, or the knee joint Pharmacokinetics and safety profile after taking the drug either once a day or twice a day for 4 weeks. Healthy and OA patients	NO Data available
AGG-523 Wyeth	NCT00427687; Phase I (Feb 2007–June 2007)	The effect of AGG-523 on biomarkers related to osteoarthritis	NO Data available
GLPG1972 Galapagos	NCT02612246; Phase I (April 2016–July 2016)	Toxicity, pharmacokinetics, pharmacodynamics Healthy and OA patients	[25]
GLPG1972 Galapagos	NCT03595618 Phase II (August 2018–July 2020)	Reduction in cartilage loss was assessed by cartilage thickness as measured in the medial cMTFC of the target knee using qMRI. OA Patients	www.fiercebiotech.com/biotech/galapagos Shows no DMOAS activity
Antibodies			
M6495 Ablynx	NCT03583346 Phase I (August 2018–July 2019)	In participants with symptomatic knee OA to explore the safety, tolerability, immunogenicity, pharmacokinetics (PK), and pharmacodynamics (PD)	NO Data available
M6495 Ablynx	NCT03224702 Phase I	Healthy Male Subjects	NO Data available

tive in the human disease, or showed detrimental side effects and thus not approved for clinical use. For example, in human OA explants, a humanized ADAMTS-5-selective monoclonal antibody (GSK2394002) was able to decrease the levels of aggrecan fragments released. However, toxicity studies of this antibody in a primate model of OA showed impairment of cardiovascular function as a side effect, and clinical trial studies were not developed. A novel type of therapeutic anti-ADAMTS-5 antibody, the Nano-body (M6495, Ablynx) blocked OA progression in mice following destabilization of the medial meniscus (DMM) surgery and reduced circulating levels of aggrecanase-generated aggrecan fragments when administered in a primate model [26]. A different set of antibodies that inhibited either the ability of ADAMTS5 for auto-activation or its interaction with an activating factor, such as LRP1, have also been shown to protect against aggrecanolysis in vitro [223, 224]. However, no information is available if they were investigated for their clinical therapeutic usefulness.

In summary, future plans for the generation of aggrecanase inhibitors as clinically sound therapeutics for targeted mitigation of aggrecan depletion from the cartilage ECM during OA pathogenesis may remain impeded by the findings that these enzymes have multi-tissue and organ distributions and functions. For example ADAMTS5 is essential for dermal wound healing [266], maintenance of tendon fibrillar structure/function [275], regulation of metabolic health by adipose tissue [17], and cardiovascular homeostasis [16]. An alternative future approach to restoring the aggrecan-dependent physiochemical and biomechanical properties of the cartilage matrix may require the cartilage-targeted delivery of engineered cleavage-resistant aggrecan-or GAG-mimetics, singly or in molecular complexes with other components. Such an approach could develop from technological advances made to-date in chemo-enzymatic synthesis of functional GAG structures and domains [175, 240].

1.4 Hyaluronan Metabolism and Its Relevance to Cartilage Structure and Function

Hyaluronan is a high-molecular weight polysaccharide composed of repeating disaccharide units, (→4) β-GlcA (1→3) βGlcNAc (1→) with a wide range of structural and metabolic functions in all tissues and body fluids [89]. These functions include lubrication, water homeostasis, macromolecular filtering, interactions with "hyaladherins" in matrix organization [49, 158, 274, 303] and regulation of cellular activities during development and in a range of pathologies [76, 92, 130, 194, 257]. This section provides a brief summary of the extensive research into the role of HA in cartilage structure/function and follow with highlights of recent advances in HA metabolism that could be incorporated into studying the cell biological responses of tissues under mechanical perturbations.

1.4.1 Hyaluronan in Cartilage Matrix Structure and Articular Joint Mechanics

The role of HA in cartilage has largely been considered in the light of its physical properties, namely for organizing aggrecan throughout the extracellular cartilage matrix A first report of a specific interaction of aggrecan with HA was reported by Hardingham and Muir [86, 87, 260], followed by more detailed analyses of the role of HA chemistry [90] and the role of the link glycoproteins in stabilization of the protein carbohydrate interactions [23, 67, 182, 252]. The biochemical analyses was later confirmed by electron microscopic methodology to visualize the structural arrangement of aggrecan monomers [96, 215] and link proteins [30, 31, 173] along the extended HA polymer backbone. In vitro cell biological studies with rat chondrosarcoma cells, and with pig and rabbit articular chondrocytes, confirmed that the ternary com-

plex between aggrecan, link protein and hyaluronan was formed extracellularly, soon after secretion of the glycosylated proteins from the cell [120, 121, 198, 209, 216].

A different protein-HA modification, first discovered in the cumulus oophorus extracellular matrix [74] has also been identified in the extracellular matrix of OA cartilage [296]. These macromolecular HA complexes are formed in the extracellular matrix by covalent transfer of heavy chains (HCs) from inter-alpha-inhibitor (ITI) to HA. ITI is a modified CS proteoglycan with a core protein, bikunin that has 1, 2 or 3 HCs attached by an ester linkage between an aspartate in the HC and the 6-OH of a GalNAc in the CS chain [150]. The HC is transferred to the 6-OH on GlcNAc in HA [301] by tumor necrosis factor-induced protein-6 (TSG-6) [48, 176]. Subsequent investigations have identified the formation of such HC-HA matrices as part of a cellular response in tissue inflammations in a wide range of chronic diseases [136, 274], including asthma [250] Crohn's disease [195], diabetic nephropathy [141], and degenerative suspensory ligament desmitis [202]. In both, OA and RA, HA-HC complexes are abundantly present in synovial fluid aspirates from patients [116, 229, 293, 296] and in animal models [68, 135] likely having been shed into the fluid after formation in inflamed synovium and/or degenerated cartilage.

In addition to the role of HA in organization of tissue and cell-specific extracellular matrices, it generates the viscoelastic properties of synovial fluid [185, 251], and in cooperation with the mucin-like molecule, PRG4 (aka Superficial Zone Protein or Lubricin), it provides boundary lubrication of the articular cartilage surfaces in diarthrodial joints [230]. Notably, in both OA and RA, decreased size and increased polydispersity of molecular the weight distribution of HA polymers in synovial fluid have been reported [12, 13] in keeping with the proposed impaired cartilage boundary lubrication in degenerative joint diseases [24]. Such observations led to the wide clinical use of intra-articular injections of high molecular weight HA as potential therapeutic 'viscosupplementation' for arthritic joints [4, 10, 11, 211].

1.4.2 Engagement of Hyaluronan Receptors Modulates Cell Responses

The studies of HA receptors, CD44, RHAMM, LYVE, Layilin and Stabilin2 and their downstream effects on cellular functions have been extensively investigated, particularly in the areas of development, cancer and respiratory diseases, as well as neuro- and vascular pathologies. A number of comprehensive recent reviews on this topic are available [76, 111, 131, 146, 162, 193, 263, 281]. Several of these receptors, in particular CD44, have also been shown to be active in cartilage matrix development and inflammatory pathologies, and those reports are summarized in Table 1.5. In the context of biomechanical effects

Table 1.5 Reported in vivo and in vitro functions of HA receptors in mechanosentive joint tissues

Receptor	Cartilage/Synovium	Bone
CD44	Immobilization of pericellular HA [129]; Cell adhesion [132, 147]; Endocytosis of HA [3]; Modulation of BMP7 signaling [151]	Unloading and inflammation induced bone loss [94, 143] Osteoclast multinucleation [51]
RHAMM	Localized in epiphyseal cartilage, articular fibrocartilage [62]; Modulation of expression of transcription factor Nrf2 in chondrocytes [189] Decreased IL6 and IL8 production, decreased migration of synoviocytes [287]	Differentiation of osteoblasts [93]
Layilin	Modulation of cytokine expression [8] Inhibition of IL-1β-induced MMP-1 and MMP-13 production in synoviocytes [177]	**No reports**
LYVE	Synovial biomarker for joint inflammation [102]; Lymphatic and blood **ve**ssel ingrowth in endplate cartilage [218] Increased lymphatics in OA and RA synovium [289]	Deficient lymphatics in peri-implant membrane [65]

on receptor HA interactions it is notable that ligand responses to tensile or flow stresses have been reported [14, 15, 184, 208], which would imply that application of physiological forces, such as tensile stress, sheer stress and fluid flow can affect receptor-HA interactions. This would provide an important function of these cell/matrix interactions as force sensing mechanisms [71, 148].

1.5 HA Metabolism Pathways Support Cell Survival

The biophysical, structural and cell biological roles of HA polymers reviewed above should be viewed in relation to their biosynthesis and degradation pathways. Over the past 5 decades many laboratories contributed research data that have built a comprehensive picture of these pathways (see Fig. 1.4).

1.5.1 Enzymatic Pathways in HA Synthesis and Catabolism

The first insights into the mechanism of HA synthesis were reported in 1959, using Streptococcus membranes [154] that contained an enzyme activity (HA synthase (HAS)), which uses GlcA-UDP and GlcNAc-UDP as substrates to polymerize HA chains, and its gene was cloned in 1993 [52]. This was followed by identification of mammalian HAS genes (HAS1, HAS2 and HAS3) from a number of laboratories (reviewed in [282, 284]). They are transmembrane proteins, and have similar domain organizations that allows the direct translocation of the HA polymer into the extracellular space during HAS-catalyzed synthesis [153, 280]. Rates of polymer synthesis and size of the extruded HA chain are dependent on expression, translation and plasma membrane targeting of the enzyme proteins [255] as well as

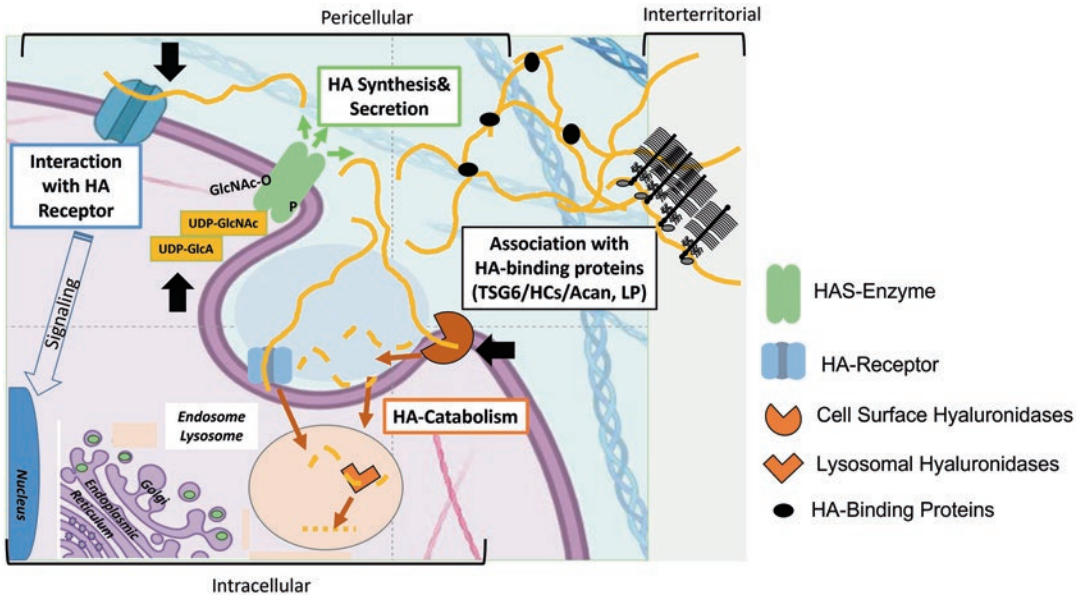


Fig. 1.4 Schematic illustration of coordination of HA synthesis, catabolism and HA-protein interactions: HA-Synthesis steps include HAS1, 2 or 3 protein transcription, modification and translocation to the plasma membrane, polymerization of HA chains using cytosolic UDP-GlcA and UDP-GlcNAc precursors and extrusion into the extracellular space. Cell signaling can be induced by HA/cell surface receptor interactions (CD44, RHAMM, Layilin). Interaction of HA with binding proteins (Acan, LP, TSG6, HCs) in the pericellular and interterritorial matrix generate specialized macromolecular complexes. HA-catabolism is mediated either by receptor mediated internalization (via LYVE-1 or Stabilin-2) of high molecular weight polymer or of low molecular weight fragments generated by cell surface hyaluronidases (TMEM2 or CEMIP) and completed in the lysosomal compartment by resident hyaluronidases (HYAL1, HYAL2 or HYAL3)

on the supply of the UDP-sugar precursors from the cytoplasm [92, 112]. A detailed study of HAS2 has revealed additional levels of post-translational control, including phosphorylation, [270], O-GlcNAcylation, [112], ubiquitination and dimerization [114]. Furthermore, the establishment of HAS knock out mouse strains provided important insights into the distinct roles of the three HAS proteins in development, growth and pathologies (summarized in Table 1.6).

In addition to the biosynthetic pathways, the degradative mechanisms for HA in tissues is also becoming more clearly defined. The existence of lysosomal hyaluronidases has long been established [110, 259], and their involvement following receptor mediated endocytosis via CD44 [47, 85], LYVE-1 [204] and HARE (Stabilin 1) [283]. However, extracellular hyaluronidase activities remained elusive until the identification of two extracellular hyaluronidase activities: (1) TMEM2, a type II transmembrane protein with hyaluronidase activity at neutral pH, [105, 256, 291] is expressed widely in adult mouse tissues, including vascular and lymphatic endothelial cells and liver, the major sites of HA clearance; and (2) KIAA1199 (CEMIP) [294, 295]. CEMIP was initially described as having a pivotal role in cancer cells, aiding their migration during tissue invasion and metasis [72, 262]. However, a number of recent reports have demonstrated its involvement in both cartilage pathologies [54, 59, 235, 299, 300] and osteoblast differentiation [39] making this an interesting candidate gene and protein to examine in relation to biomechanical stimulants imposed on cartilage and bone tissues (see Fig. 1.4).

Table 1.6 Genetic deletion of HA synthases and hyaluronidases in mice

Gene/protein	Phenotype knock-out mouse strains
Has1 (hyaluronan synthase 1)	Defective formation of retrocanal Bursa [237] Increased Synovial Fibrosis, Osteopenia [38]
Has2 (hyaluronan synthase 2)[a]	Impaired skeletal development [159, 170] Increased airway hypersensity in asthma [233]
Has3 (hyaluronan synthase 3)	Altered neuronal activity [6] Decreased neointimal hyperplasia [118, 231] Increased tumor cell invasion in human mammary parenchymal tissues [140]
Hyal1 (hyaluronidase 1)[b]	Accelerated thinning of knee joint cartilage in aging Prolonged fertility [157]
Hyal2 (hyaluronidase 2)	Severe cardiopulmonary dysfunction, Anemia, Mild craniofacial abnormalities [42]
Hyal3 (hyaluronidase 2)	No detectable phenotype [9]
Tmem2[c] (Transmembrane protein 2; aka CEMIP2)	Increased levels of circulating HA, active on the surface of endothelial cells in the lymph nodes and liver [256]
Cemip (aka KIAA1199)	Impaired learning and memory ability due to decreased dendritic spine density in dentate gyrus granule cell [297]

[a]Conditional and Heterozygous Knockout Strains only; complete Knockout is embryonically lethal due to failure of heart development [34]
[b]Human Mucopolysaccharidosis Type IX is due to a mutation in the HYAL1 gene
[c]Conditional Knockout Strains

1.5.2 Synergy Between Glucose Metabolism and HA Synthesis Adjusts the Cellular Energy Status

More recent studies on HA metabolism in cancer biology and diabetes have clearly demonstrated that biosynthesis of the HA is closely linked to intracellular glucose metabolism. This is through both aerobic and anaerobic glycolysis for energy production [265], and by the generation of the two sugar nucleotides, UDP-GlcNAc and UDP-GlcA. Together these sugar nucleotides regulate HA production by modification of both the biosynthetic activity [272, 304] and the half-lives of the membrane-associated HAS enzymes [271].

Biosynthesis of the two nucleotide precursors takes place in the cytoplasm (Fig. 1.5) and is driven by the availability of intracellular glucose taken up by the cell from the interstitial fluid by glucose transporters and its subsequent conversion to Glc6P [36]. UDP-GlcNAc is then synthesized

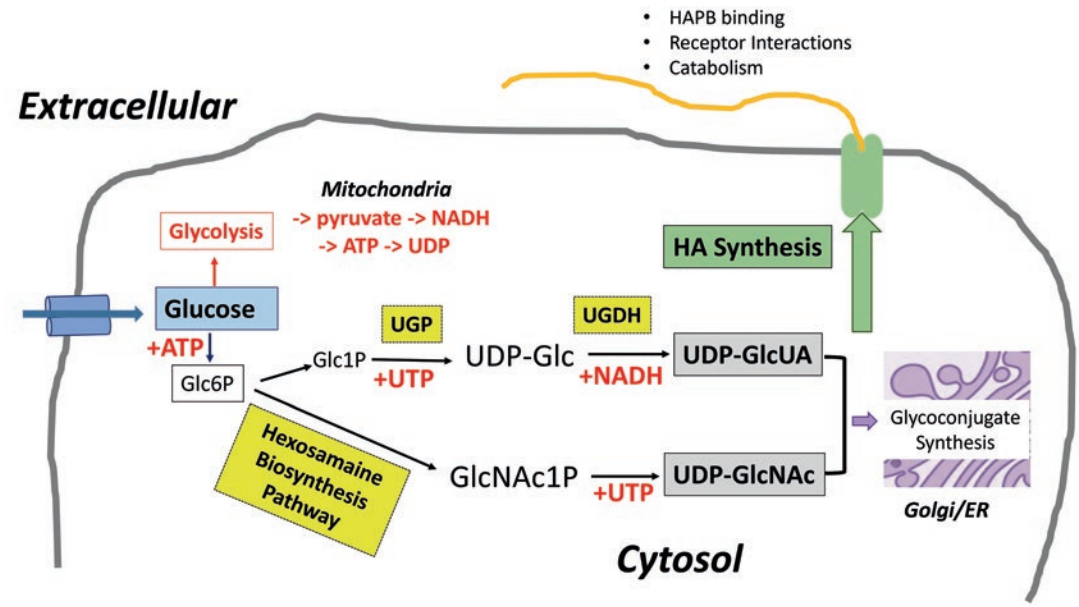


Fig. 1.5 Schematic Illustration of Integration of Glucose Metabolism for Cytosolic Production of HA Biosynthesis Precursors UDP-GlcNAc and UDP-GlcUA: Extracellular glucose is transported into the cytoplasm by specific glucose transporters, where it is shunted for energy production via glycolysis and for production of the HA synthesis precursors UDP-GlcNAc and UDP-GlcUA via the hexosamine biosynthetic pathway or by UDP-glucose pyrophosphorylase/UDP-glucose dehydrogenase, respectively. Potential regulatory sites for mechanical stimuli of cells/tissues are indicated by bold black arrows. It should be noted that HA synthases have 'direct' access to cytosolic UDP-precursors, whereas UDP precursors for the chondroitin and keratan polymerases, or for other enzymes of glycol-conjugate synthesis, require an additional translocation/transport step into the ER/Golgi compartments

via the hexosamine biosynthetic pathway [187], that also engages products from amino acid metabolism (glutamine) and lipid metabolism (Acetyl-CoA). UDP-GlcA biosynthesis on the other hand, depends on the activity of two enzymes, UDP-Glucose pyrophosphorylase (UPP), which uses glucose-1-phosphate (Glc1P) and UTP to generate UDP-Glc for conversion to UDP-GlcUA by UDP-Glucose dehydrogenase (UGDH) [244, 304]. Both enzymes show a wide tissue distribution, including cartilages [44, 152].

To date, the mechanistic linkage of glucose metabolism and HA synthesis has not been studied in detail in the context of cartilage during growth, maturation and pathologies, with only one recent review pointing to its importance in the developmental biology of the tissue [100]. An interest in the importance of the HBP in OA pathology was initiated by the observations that high concentrations of extracellular glucosamine or mannosamine could inhibit in vitro cytokine-induced aggrecan degradation by ADAMTS proteinases [190, 221] and inhibit disease progression in animal models of OA [183, 273]. Clinical use of oral dosages of glucosamine as a potential DMOAD [19, 70, 109, 165, 212] is still debated.

1.5.3 Are Biophysical Stressors Important in Regulation of HA Metabolism by Chondrocytes?

The subject of biomechanical effects on HA metabolism has been most broadly studied in endothelial cells and their response to sheer stresses generated by blood flow [81, 155, 277], as well as in epithelial cells in the alveolar lining [97]. Other mechanical perturbances, such as cyclic mechanical stretch or strain, shear stress, surface motion or mechanical injury [63, 119, 138, 142, 210, 254, 298] imposed on connective tissue cells, including fibrochondrocytes and articular

chondrocytes, have also been shown to modulate HA production. The later studies have not provided any information on potential transduction pathways for stimulated HA production, but likely mechanisms could come from the newly emerging databases on cartilage "metabolomics' [5, 50, 232, 236, 243]. Key regulatory points would include glucose transport [168, 169, 241], subsequent Glc6P shunting to aerobic [113] or anaerobic glycolysis [292, 302] for energy production, and/or synthesis of UDP-GlcNAc and UDP-GlcUA to regulate HAS activities. Given the critical structural and cell regulatory roles of HA reviewed above, a more detailed understanding of HA metabolism and its response under biomechanical perturbation of tissues and cells would provide novel opportunities to uncover treatment of cartilage pathologies [261], as well as optimization of procedures for the production of tissue engineered cartilages [160, 245].

1.6 Conclusion

Despite the extensive knowledge base in cartilage extracellular matrix structure and metabolism in health and diseases, there remain multiple opportunities to apply 'big data' generation and bioinformatics mining approaches to gain further insights to the feed-forward and feed-back mechanisms between genes, their products and cellular pathways. These goals could be achieved by applying such approaches to examine engineered tissues, animal models and clinical biorepositories.

References

1. Adhikara IM, Yagi K, Mayasari DS, Suzuki Y, Ikeda K, Ryanto GRT, Sasaki N, Rikitake Y, Nadanaka S, Kitagawa H, Miyata O, Igarashi M, Hirata KI, Emoto N (2021) Chondroitin Sulfate N-acetylgalactosaminyltransferase-2 impacts foam cell formation and atherosclerosis by altering macrophage glycosaminoglycan chain. Arterioscler Thromb Vasc Biol 41(3):1076–1091
2. Adusumalli R, Åsheim HC, Lupashin V, Blackburn JB, Prydz K (2021) Proteoglycan synthesis in conserved oligomeric Golgi subunit deficient HEK293T cells is affected differently, depending on the lacking subunit. Traffic 22(7):230–239
3. Aguiar DJ, Knudson W, Knudson CB (1999) Internalization of the hyaluronan receptor CD44 by chondrocytes. Exp Cell Res 252(2):292–302
4. Altman R, Hackel J, Niazi F, Shaw P, Nicholls M (2018) Efficacy and safety of repeated courses of hyaluronic acid injections for knee osteoarthritis: a systematic review. Semin Arthritis Rheum 48(2):168–175
5. Anderson JR, Phelan MM, Foddy L, Clegg PD, Peffers MJ (2020) Ex vivo equine cartilage explant osteoarthritis model: a metabolomics and proteomics study. J Proteome Res 19(9):3652–3667
6. Arranz AM, Perkins KL, Irie F, Lewis DP, Hrabe J, Xiao F, Itano N, Kimata K, Hrabetova S, Yamaguchi Y (2014) Hyaluronan deficiency due to Has3 knockout causes altered neuronal activity and seizures via reduction in brain extracellular space. J Neurosci 34(18):6164–6176
7. Asano M, Furukawa K, Kido M, Matsumoto S, Umesaki Y, Kochibe N, Iwakura Y (1997) Growth retardation and early death of beta-1,4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. EMBO J 16(8):1850–1857
8. Asano K, Arito M, Kurokawa MS, Omoteyama K, Okamoto K, Suematsu N, Yudoh K, Nakamura H, Beppu M, Kato T (2014) Secretion of inflammatory factors from chondrocytes by layilin signaling. Biochem Biophys Res Commun 452(1):85–90
9. Atmuri V, Martin DC, Hemming R, Gutsol A, Byers S, Sahebjam S, Thliveris JA, Mort JS, Carmona E, Anderson JE, Dakshinamurti S, Triggs-Raine B (2008) Hyaluronidase 3 (HYAL3) knockout mice do not display evidence of hyaluronan accumulation. Matrix Biol 27:653–660
10. Balazs EA (2004) Viscosupplementation for treatment of osteoarthritis: from initial discovery to current status and results. Surg Technol Int 12:278–289
11. Balazs EA, Denlinger JL (1993) Viscosupplementation: a new concept in the treatment of osteoarthritis. J Rheumatol Suppl 39:3–9
12. Balazs EA, Watson D, Duff IF, Roseman S (1967) Hyaluronic acid in synovial fluid. I. Molecular parameters of hyaluronic acid in normal and arthritis human fluids. Arthritis Rheum 10(4):357–376
13. Band PA, Heeter J, Wisniewski HG, Liublinska V, Pattanayak CW, Karia RJ, Stabler T, Balazs EA, Kraus VB (2015) Hyaluronan molecular weight distribution is associated with the risk of knee osteoarthritis progression. Osteoarthr Cartil 23(1):70–76
14. Bano F, Banerji S, Howarth M, Jackson DG, Richter RP (2016) A single molecule assay to probe monovalent and multivalent bonds between hyaluronan and its key leukocyte receptor CD44 under force. Sci Rep 6:34176
15. Bano F, Tammi MI, Kang DW, Harris EN, Richter RP (2018) Single-molecule unbinding forces between the polysaccharide hyaluronan and its binding proteins. Biophys J 114(12):2910–2922

16. Barallobre-Barreiro J, Radovits T, Fava M, Mayr U, Lin WY, Ermolaeva E, Martínez-López D, Lindberg EL, Duregotti E, Daróczi L, Hasman M, Schmidt LE, Singh B, Lu R, Baig F, Siedlar AM, Cuello F, Catibog N, Theofilatos K, Shah AM, Crespo-Leiro MG, Doménech N, Hübner N, Merkely B, Mayr M (2021) Extracellular matrix in heart failure: role of ADAMTS5 in proteoglycan remodeling. Circulation 144(25):2021–2034
17. Bauters D, Bedossa P, Lijnen HR, Hemmeryckx B (2018) Functional role of ADAMTS5 in adiposity and metabolic health. PLoS One 13(1):e0190595
18. Bexiga MG, Simpson JC (2013) Human diseases associated with form and function of the Golgi complex. Int J Mol Sci 14:18670–18681
19. Block JA, Oegema TR, Sandy JD, Plaas A (2010) The effects of oral glucosamine on joint health: is a change in research approach needed? Osteoarthr Cartil 18(1):5–11
20. Bonassar LJ, Jeffries KA, Paguio CG, Grodzinsky AJ (1995a) Cartilage degradation and associated changes in biochemical and electromechanical properties. Acta Orthop Scand Suppl 266:38–44
21. Bonassar LJ, Frank EH, Murray JC, Paguio CG, Moore VL, Lark MW, Sandy JD, Wu JJ, Eyre DR, Grodzinsky AJ (1995b) Changes in cartilage composition and physical properties due to stromelysin degradation. Arthritis Rheum 38(2):173–183
22. Bonassar LJ, Grodzinsky AJ, Srinivasan A, Davila SG, Trippel SB (2000) Mechanical and physicochemical regulation of the action of insulin-like growth factor-I on articular cartilage. Arch Biochem Biophys 379(1):57–63
23. Bonnet F, Dunham DG, Hardingham TE (1985) Structure and interactions of cartilage proteoglycan binding region and link protein. Biochem J 228(1):77–85
24. Bonnevie ED, Bonassar LJ (2020) A century of cartilage tribology research is informing lubrication therapies. J Biomech Eng 142(3):031004
25. Brebion F, Gosmini R, Deprez P, Varin M, Peixoto C, Alvey L, Jary H, Bienvenu N, Triballeau N, Blanque R, Cottereaux C, Christophe T, Vandervoort N, Mollat P, Touitou R, Leonard P, De Ceuninck F, Botez I, Monjardet A, van der Aar E, Amantini D (2021) Discovery of GLPG1972/S201086, a potent, selective, and orally bioavailable ADAMTS-5 inhibitor for the treatment of osteoarthritis. J Med Chem 64(6):2937–2952
26. Brenneis C, Serruys B, Van Belle T et al (2018) Structural and symptomatic benefit of a half-live extended, systemically applied anti-ADAMTS-5 inhibitor (M6495). Osteoarthr Cartil 26:S299–S300
27. Brown MP, West LA, Merritt KA, Plaas AH (1998) Changes in sulfation patterns of chondroitin sulfate in equine articular cartilage and synovial fluid in response to aging and osteoarthritis. Am J Vet Res 59(6):786–791
28. Brown MP, Trumble TN, Plaas AH, Sandy JD, Romano M, Hernandez J, Merritt KA (2007) Exercise and injury increase chondroitin sulfate chain length and decrease hyaluronan chain length in synovial fluid. Osteoarthr Cartil 15(11):1318–1325
29. Browning JA, Saunders K, Urban JP, Wilkins RJ (2004) The influence and interactions of hydrostatic and osmotic pressures on the intracellular milieu of chondrocytes. Biorheology 41(3–4):299–308
30. Buckwalter JA, Rosenberg LC (1988) Electron microscopic studies of cartilage proteoglycans. Electron Microsc Rev 1(1):87–112
31. Buckwalter JA, Rosenberg LC, Tang LH (1984) The effect of link protein on proteoglycan aggregate structure. An electron microscopic study of the molecular architecture and dimensions of proteoglycan aggregates reassembled from the proteoglycan monomers and link proteins of bovine fetal epiphyseal cartilage. J Biol Chem 259(9):5361–5363
32. Buschmann MD, Hunziker EB, Kim YJ, Grodzinsky AJ (1996) Altered aggrecan synthesis correlates with cell and nucleus structure in statically compressed cartilage. J Cell Sci 109(Pt 2):499–508
33. Calabro A, Midura R, Wang A, West L, Plaas A, Hascall VC (2001) Fluorophore-assisted carbohydrate electrophoresis (FACE) of glycosaminoglycans. Osteoarthr Cartil 9(Suppl A):S16–S22
34. Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro A Jr, Kubalak S, Klewer SE, McDonald JA (2000) Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. J Clin Invest 106(3):349–360
35. Campbell MA, Handley CJ, Hascall VC, Campbell RA, Lowther DA (1984) Turnover of proteoglycans in cultures of bovine articular cartilage. Arch Biochem Biophys 234(1):275–289
36. Caon I, Parnigoni A, Viola M, Karousou E, Passi A, Vigetti D (2021) Cell energy metabolism and hyaluronan synthesis. J Histochem Cytochem 69(1):35–47
37. Carney SL, Billingham ME, Muir H, Sandy JD (1984) Demonstration of increased proteoglycan turnover in cartilage explants from dogs with experimental osteoarthritis. J Orthop Res 2(3):201–206
38. Chan DD, Li J, Luo W, Predescu DN, Cole BJ, Plaas A (2018) Pirfenidone reduces subchondral bone loss and fibrosis after murine knee cartilage injury. J Orthop Res 36(1):365–376
39. Chen L, Shi K, Andersen TL, Qiu W, Kassem M (2019) KIAA1199 is a secreted molecule that enhances osteoblastic stem cell migration and recruitment. Cell Death Dis 10(2):126
40. Chiusaroli R, Visentini M, Galimberti C, Casseler C, Mennuni L, Covaceuszach S, Lanza M, Ugolini G, Caselli G, Rovati LC, Visintin M (2013) Targeting of ADAMTS5′s ancillary domain with the recombinant mAb CRB0017 ameliorates disease progression in a spontaneous murine model of osteoarthritis. Osteoarthr Cartil 21(11):1807–1810
41. Chockalingam PS, Sun W, Rivera-Bermudez MA, Zeng W, Dufield DR, Larsson S, Lohmander LS,

Flannery CR, Glasson SS, Georgiadis KE, Morris EA (2011) Elevated aggrecanase activity in a rat model of joint injury is attenuated by an aggrecanase specific inhibitor. Osteoarthr Cartil 19(3):315–323
42. Chowdhury B, Hemming R, Hombach-Klonisch S, Flamion B, Triggs-Raine B (2013) Murine hyaluronidase 2 deficiency results in extracellular hyaluronan accumulation and severe cardiopulmonary dysfunction. J Biol Chem 288:520–528
43. Chua JS, Kuberan B (2017) Synthetic Xylosides: probing the glycosaminoglycan biosynthetic machinery for biomedical applications. Acc Chem Res 50(11):2693–2705
44. Clarkin CE, Allen S, Kuiper NJ, Wheeler BT, Wheeler-Jones CP, Pitsillides AA (2011) Regulation of UDP-glucose dehydrogenase is sufficient to modulate hyaluronan production and release, control sulfated GAG synthesis, and promote chondrogenesis. J Cell Physiol 226(3):749–761
45. Clement-Lacroix P, Little CB, Smith MM, Cottereaux C, Merciris D, Meurisse S, Mollat P, Touitou R, Brebion F, Gosmini R, De Ceuninck F, Botez I, Lepescheux L, van der Aar E, Christophe T, Vandervoort N, Blanqué R, Comas D, Deprez P, Amantini D (2021) Pharmacological characterization of GLPG1972/S201086, a potent and selective small-molecule inhibitor of ADAMTS5. Osteoarthr Cartil:S1063–S4584
46. Clement-Lacroix P, Little CB, Smith MM, Cottereaux C, Merciris D, Meurisse S, Mollat P, Touitou R, Brebion F, Gosmini R, De Ceuninck F, Botez I, Lepescheux L, van der Aar E, Christophe T, Vandervoort N, Blanqué R, Comas D, Deprez P, Amantini D (2022) Pharmacological characterization of GLPG1972/S201086, a potent and selective small-molecule inhibitor of ADAMTS5. Osteoarthr Cartil 30(2):291–301
47. Culty M, Nguyen HA, Underhill CB (1992) The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. J Cell Biol 116(4):1055–1062
48. Day AJ, Milner CM (2019) TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biol 78-79:60–83
49. Day AJ, Sheehan JK (2001) Hyaluronan: polysaccharide chaos to protein organisation. Curr Opin Struct Biol 11(5):617–622
50. De Sousa EB, Dos Santos GC Jr, Duarte MEL, Neto MV, Aguiar DP (2017) Metabolomics as a promising tool for early osteoarthritis diagnosis. Braz J Med Biol Res 50(11):e6485
51. de Vries TJ, Schoenmaker T, Beertsen W, van der Neut R, Everts V (2005) Effect of CD44 deficiency on in vitro and in vivo osteoclast formation. J Cell Biochem 94(5):954–966
52. DeAngelis PL, Papaconstantinou J, Weigel PH (1993) Molecular cloning, identification, and sequence of the hyaluronan synthase gene from group A Streptococcus pyogenes. J Biol Chem 268(26):19181–19184
53. Degala S, Williams R, Zipfel W, Bonassar LJ (2012) Calcium signaling in response to fluid flow by chondrocytes in 3D alginate culture. J Orthop Res 30(5):793–799
54. Deroyer C, Charlier E, Neuville S, Malaise O, Gillet P, Kurth W, Chariot A, Malaise M, de Seny D (2019) CEMIP (KIAA1199) induces a fibrosis-like process in osteoarthritic chondrocytes. Cell Death Dis 10(2):103
55. Deutsch AJ, Midura RJ, Plaas AH (1995) Structure of chondroitin sulfate on aggrecan isolated from bovine tibial and costochondral growth plates. J Orthop Res 13(2):230–239
56. Di Federico E, Bader DL, Shelton JC (2020) 3D models of chondrocytes within biomimetic scaffolds: Effects of cell deformation from loading regimens. Clin Biomech (Bristol, Avon) 79:104972
57. Dick G, Grøndahl F, Prydz K (2008) Overexpression of the 3′-phosphoadenosine 5′-phosphosulfate (PAPS) transporter 1 increases sulfation of chondroitin sulfate in the apical pathway of MDCK II cells. Glycobiology 18(1):53–65
58. DiMicco MA, Patwari P, Siparsky PN, Kumar S, Pratta MA, Lark MW, Kim YJ, Grodzinsky AJ (2004) Mechanisms and kinetics of glycosaminoglycan release following in vitro cartilage injury. Arthritis Rheum 50(3):840–848
59. Ding QH, Qi YY, Li XM, Chen WP, Wang XH, Ji XW (2019) Knockdown of KIAA1199 suppresses IL-1β-induced cartilage degradation and inflammatory responses in human chondrocytes through the Wnt/β-catenin signaling pathway. Int Immunopharmacol 73:203–211
60. Doege KJ, Sasaki M, Kimura T, Yamada Y (1991) Complete coding sequence and deduced primary structure of the human cartilage large aggregating proteoglycan, aggrecan. Human-specific repeats, and additional alternatively spliced forms. J Biol Chem 266(2):894–902
61. Doege KJ, Coulter SN, Meek LM, Maslen K, Wood JG (1997) A human-specific polymorphism in the coding region of the aggrecan gene. Variable number of tandem repeats produce a range of core protein sizes in the general population. J Biol Chem 272(21):13974–13979
62. Dowthwaite GP, Edwards JC, Pitsillides AA (1998) An essential role for the interaction between hyaluronan and hyaluronan binding proteins during joint development. J Histochem Cytochem 46(5):641–651
63. Dowthwaite GP, Ward AC, Flannely J, Suswillo RF, Flannery CR, Archer CW, Pitsillides AA (1999) The effect of mechanical strain on hyaluronan metabolism in embryonic fibrocartilage cells. Matrix Biol 18(6):523–532
64. Early JO, Fagan LE, Curtis AM, Kennedy OD (2021) Mitochondria in injury, inflammation and disease of articular skeletal joints. Front Immunol 12:695257
65. Edwards J, Schulze E, Sabokbar A, Gordon-Andrews H, Jackson D, Athanasou NA (2008) Absence of lymphatics at the bone-implant interface: impli-

cations for periprosthetic osteolysis. Acta Orthop 79(2):289–294
66. Esko JD, Selleck SB (2002) Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu Rev Biochem 71:435–471
67. Faltz LL, Caputo CB, Kimura JH, Schrode J, Hascall VC (1979) Structure of the complex between hyaluronic acid, the hyaluronic acid-binding region, and the link protein of proteoglycan aggregates from the swarm rat chondrosarcoma. J Biol Chem 254(4):1381–1387
68. Fasanello DC, Su J, Deng S, Yin R, Colville MJ, Berenson JM, Kelly CM, Freer H, Rollins A, Wagner B, Rivas F, Hall AR, Rahbar E, DeAngelis PL, Paszek MJ, Reesink HL (2021) Hyaluronic acid synthesis, degradation, and crosslinking in equine osteoarthritis: TNF-α-TSG-6-mediated HC-HA formation. Arthritis Res Ther 23(1):218
69. Ferencz B, Condac E, Poudel N, Munteanu MC, Sivasami P, Choudhury B, Naidu NN, Zhang F, Breshears M, Linhardt RJ, Hinsdale ME (2020) Xylosyltransferase 2 deficiency and organ homeostasis. Glycoconj J 37(6):755–765
70. Fernández-Martín S, González-Cantalapiedra A, Muñoz F, García-González M, Permuy M, López-Peña M (2021) Glucosamine and chondroitin sulfate: is there any scientific evidence for their effectiveness as disease-modifying drugs in knee osteoarthritis preclinical studies?-A systematic review from 2000 to 2021. Animals (Basel) 11(6):1608
71. Ferrari LF, Araldi D, Bogen O, Levine JD (2016) Extracellular matrix hyaluronan signals via its CD44 receptor in the increased responsiveness to mechanical stimulation. Neuroscience 324:390–398
72. Fink SP, Myeroff LL, Kariv R, Platzer P, Xin B, Mikkola D, Lawrence E, Morris N, Nosrati A, Willson JK, Willis J, Veigl M, Barnholtz-Sloan JS, Wang Z, Markowitz SD (2015) Induction of KIAA1199/CEMIP is associated with colon cancer phenotype and poor patient survival. Oncotarget 6(31):30500–30515
73. Fosang AJ, Rogerson FM (2010) Identifying the human aggrecanase. Osteoarthr Cartil 18(9):1109–1116
74. Fülöp C, Szántó S, Mukhopadhyay D, Bárdos T, Kamath RV, Rugg MS, Day AJ, Salustri A, Hascall VC, Glant TT, Mikecz K (2003) Impaired cumulus mucification and female sterility in tumor necrosis factor-induced protein-6 deficient mice. Development 130(10):2253–2261
75. Funderburgh JL (2000) Keratan sulfate: structure, biosynthesis, and function. Glycobiology 10(10):951–958
76. Garantziotis S, Savani RC (2019) Hyaluronan biology: a complex balancing act of structure, function, location and context. Matrix Biol 78-79:1–10
77. Gilbert SJ, Bonnet CS, Blain EJ (2021) Mechanical cues: bidirectional reciprocity in the extracellular matrix drives mechano-signalling in articular cartilage. Int J Mol Sci 22:13595
78. Glasson SS, Askew R, Sheppard B et al (2005) Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 434:644–648
79. Gotoh M, Yada T, Sato T, Akashima T, Iwasaki H, Mochizuki H, Inaba N, Togayachi A, Kudo T, Watanabe H, Kimata K, Narimatsu H (2002) Molecular cloning and characterization of a novel chondroitin sulfate glucuronyltransferase that transfers glucuronic acid to N-acetylgalactosamine. J Biol Chem 277:38179–38188
80. Götting C, Kuhn J, Zahn R, Brinkmann T, Kleesiek K (2000) Molecular cloning and expression of human UDP-d-Xylose:proteoglycan core protein beta-d-xylosyltransferase and its first isoform XT-II. J Mol Biol 304(4):517–528
81. Gouverneur M, Spaan JA, Pannekoek H, Fontijn RD, Vink H (2006) Fluid shear stress stimulates incorporation of hyaluronan into endothelial cell glycocalyx. Am J Physiol Heart Circ Physiol 290(1):H458–H452
82. Guilak F (1995) Compression-induced changes in the shape and volume of the chondrocyte nucleus. J Biomech 28(12):1529–1541
83. Habuchi H, Ushida T, Habuchi O (2016) Mice deficient in N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase exhibit enhanced liver fibrosis and delayed recovery from fibrosis in carbon tetrachloridetreated mice. Heliyon 2(8):e00138
84. Han J, Li D, Qu C, Wang D, Wang L, Guo X, Lammi MJ (2017) Altered expression of chondroitin sulfate structure modifying sulfotransferases in thearticular cartilage from adult osteoarthritis and Kashin- Beck disease. Osteoarthr Cartil 25(8):1372–1375
85. Harada H, Takahashi M (2007) CD44-dependent intracellular and extracellular catabolism of hyaluronic acid by hyaluronidase-1 and -2. J Biol Chem 282:5597–5607
86. Hardingham TE, Muir H (1972) The specific interaction of hyaluronic acid with cartilage proteoglycans. Biochim Biophys Acta 279(2):401–405
87. Hardingham TE, Muir H (1974) Hyaluronic acid in cartilage and proteoglycan aggregation. Biochem J 139:565–581
88. Hardingham TE, Bayliss MT, Rayan V, Noble DP (1992) Effects of growth factors and cytokines on proteoglycan turnover in articular cartilage. Br J Rheumatol 31(Suppl 1):1–6
89. Hascall VC (2019) The journey of hyaluronan research in the Journal of Biological Chemistry. J Biol Chem 294(5):1690–1696
90. Hascall VC, Heinegard D (1974) Hyaluronic acid in cartilage and proteoglycan aggregation. J Biol Chem 249(13):I4232–I4241
91. Hascall VC, Handley CJ, McQuillan DJ, Hascall GK, Robinson HC, Lowther DA (1983) The effect of serum on biosynthesis of proteoglycans by bovine

articular cartilage in culture. Arch Biochem Biophys 224(1):206–223
92. Hascall VC, Wang A, Tammi M, Oikari S, Tammi R, Passi A, Vigetti D, Hanson RW, Hart GW (2014) The dynamic metabolism of hyaluronan regulates the cytosolic concentration of UDP-GlcNAc. Matrix Biol 35:14–17
93. Hatano H, Shigeishi H, Kudo Y, Higashikawa K, Tobiume K, Takata T, Kamata N (2012) Overexpression of receptor for hyaluronan-mediated motility (RHAMM) in MC3T3-E1 cells induces proliferation and differentiation through phosphorylation of ERK1/2. J Bone Miner Metab 30(3):293–303
94. Hayer S, Steiner G, Görtz B, Reiter E, Tohidast-Akrad M, Amling M, Hoffmann O, Redlich K, Zwerina J, Skriner K, Hilberg F, Wagner EF, Smolen JS, Schett G (2005) CD44 is a determinant of inflammatory bone loss. J Exp Med 201(6):903–914
95. He Y, Makarczyk MJ, Lin H (2020) Role of mitochondria in mediating chondrocyte response to mechanical stimuli. Life Sci 15:263
96. Heinegård D, Lohmander S, Thyberg J (1978) Cartilage proteoglycan aggregates. Electron-microscopic studies of native and fragmented molecules. Biochem J 175(3):913–919
97. Heise RL, Stober V, Cheluvaraju C, Hollingsworth JW, Garantziotis S (2011) Mechanical stretch induces epithelial-mesenchymal transition in alveolar epithelia via hyaluronan activation of innate immunity. J Biol Chem 286(20):17435–17444
98. Hiraoka S, Furuichi T, Nishimura G, Shibata S, Yanagishita M, Rimoin DL, Superti-Furga A, Nikkels PG, Ogawa M, Katsuyama K, Toyoda H, Kinoshita-Toyoda A, Ishida N, Isono K, Sanai Y, Cohn DH, Koseki H, Ikegawa S (2007) Nucleotide-sugar transporter SLC35D1 is critical to chondroitin sulfate synthesis in cartilage and skeletal development in mouse and human. Nat Med 13(11):1363–1367
99. Hirschberg CB, Robbins PW, Abeijon C (1998) Transporters of nucleotide sugars, ATP, and nucleotide sulfate in the endoplasmic reticulum and Golgi apparatus. Annu Rev Biochem 67:49–69
100. Hollander JM, Zeng L (2019) The emerging role of glucose metabolism in cartilage development. Curr Osteoporos Rep 17(2):59–69
101. Huang W, Nagasaka M, Furukawa KS, Ushida T (2020) Local strain distribution and increased intracellular Ca2+ signaling in bovine articular cartilage exposed to compressive strain. J Biomech Eng 142(6):061008
102. Hulme CH, Peffers MJ, Harrington GMB, Wilson E, Perry J, Roberts S, Gallacher P, Jermin P, Wright KT (2021) Identification of candidate synovial fluid biomarkers for the prediction of patient outcome after microfracture or osteotomy. Am J Sports Med 49(6):1512–1523
103. Ilic MZ, Handley CJ, Robinson HC, Mok MT (1992) Mechanism of catabolism of aggrecan by articular cartilage. Arch Biochem Biophys 294(1):115–122
104. Inada R, Miyamoto K, Tanaka N, Moriguchi K, Kadomatsu K, Takeuchi K, Igarashi M, Kusunoki S (2021) Chondroitin sulfate N-acetylgalactosyltransferase-1 knockout shows milder phenotype in experimental autoimmune encephalomyelitis than in wild type. Glycobiology 31(3):260–265
105. Irie F, Tobisawa Y, Murao A, Yamamoto H, Ohyama C, Yamaguchi Y (2021) The cell surface hyaluronidase TMEM2 regulates cell adhesion and migration via degradation of hyaluronan at focal adhesion sites. J Biol Chem 296:100481
106. Iseki T, Rothrauff BB, Kihara S, Sasaki H, Yoshiya S, Fu FH, Tuan RS, Gottardi R (2019) Dynamic compressive loading improves cartilage repair in an in vitro model of microfracture: comparison of 2 mechanical loading regimens on simulated microfracture based on fibrin gel scaffolds encapsulating connective tissue progenitor cells. Am J Sports Med 47(9):2188–2199
107. Ito Z, Sakamoto K, Imagama S, Matsuyama Y, Zhang H, Hirano K, Ando K, Yamashita T, Ishiguro N, Kadomatsu K (2010) N-acetylglucosamine 6-O-sulfotransferase-1-deficient mice show better functional recovery after spinal cord injury. J Neurosci 30(17):5937–5947
108. Izumikawa T, Kitagawa H (2010) Mice deficient in glucuronyltransferase-I. Prog Mol Biol Transl Sci 93:19–34
109. Jackson CG, Plaas AH, Sandy JD, Hua C, Kim-Rolands S, Barnhill JG, Harris CL, Clegg DO (2010) The human pharmacokinetics of oral ingestion of glucosamine and chondroitin sulfate taken separately or in combination. Osteoarthr Cartil 18(3):297–302
110. Jadin L, Wu X, Ding H, Frost GI, Onclinx C, Triggs-Raine B, Flamion B (2008) Skeletal and hematological anomalies in HYAL2-deficient mice: a second type of mucopolysaccharidosis IX? FASEB J 22(12):4316–4312
111. Johnson LA, Jackson DG (2021) Hyaluronan and its receptors: key mediators of immune cell entry and trafficking in the lymphatic system. Cell 10(8):2061
112. Jokela TA, Makkonen KM, Oikari S, Kärnä R, Koli E, Hart GW, Tammi RH, Carlberg C, Tammi MI (2011) Cellular content of UDP-N-acetylhexosamines controls hyaluronan synthase 2 expression and correlates with O-linked N-acetylglucosamine modification of transcription factors YY1 and SP1. J Biol Chem 286(38):33632–33640
113. Kan S, Duan M, Liu Y, Wang C, Xie J (2021) Role of Mitochondria in physiology of chondrocytes and diseases of osteoarthritis and rheumatoid arthritis. Cartilage 13(2_suppl):1102S–1121S
114. Karousou E, Kamiryo M, Skandalis SS, Ruusala A, Asteriou T, Passi A, Yamashita H, Hellman U, Heldin CH, Heldin P (2010) The activity of hyaluronan synthase 2 is regulated by dimerization and ubiquitination. J Biol Chem 285(31):23647–23654

115. Kearns AE, Vertel BM, Schwartz NB (1993) Topography of glycosylation and UDP-xylose production. J Biol Chem 268:11097–11104
116. Kida D, Yoneda M, Miyaura S, Ishimaru T, Yoshida Y, Ito T, Ishiguro N, Iwata H, Kimata K (1999) The SHAP-HA complex in sera from patients with rheumatoid arthritis and osteoarthritis. J Rheumatol 26(6):1230–1238
117. Kido M, Asano M, Iwakura Y, Ichinose M, Miki K, Furukawa K (1999) Normal levels of serum glycoproteins maintained in beta-1, 4-galactosyltransferase I-knockout mice. FEBS Lett 464(1–2):75–79
118. Kiene LS, Homann S, Suvorava T, Rabausch B, Müller J, Kojda G, Kretschmer I, Twarock S, Dai G, Deenen R, Hartwig S, Lehr S, Köhrer K, Savani RC, Grandoch M, Fischer JW (2016) Deletion of Hyaluronan synthase 3 inhibits Neointimal Hyperplasia in Mice. Arterioscler Thromb Vasc Biol 36(2):e9–e16
119. Kim YJ, Grodzinsky AJ, Plaas AH (1996) Compression of cartilage results in differential effects on biosynthetic pathways for aggrecan, link protein, and hyaluronan. Arch Biochem Biophys 328(2):331–340
120. Kimura JH, Hardingham TE, Hascall VC, Solursh M (1979) Biosynthesis of proteoglycans and their assembly into aggregates in cultures of chondrocytes from the Swarm rat chondrosarcoma. J Biol Chem 254(8):2600–2609
121. Kimura JH, Hardingham TE, Hascall VC (1980) Assembly of newly synthesized proteoglycan and link protein into aggregates in cultures of chondrosarcoma chondrocytes. J Biol Chem 255(15):7134–7143
122. Kirk KM, Doege KJ, Hecht J, Bellamy N, Martin NG (2003) Osteoarthritis of the hands, hips and knees in an Australian twin sample–evidence of association with the aggrecan VNTR polymorphism. Twin Res 6(1):62–66
123. Kisiday JD, Jin M, DiMicco MA, Kurz B, Grodzinsky AJ (2004) Effects of dynamic compressive loading on chondrocyte biosynthesis in self-assembling peptide scaffolds. J Biomech 37(5):595–604
124. Kitagawa H, Uyama T, Sugahara K (2001) Molecular cloning and expression of a human chondroitin synthase. J Biol Chem 276:38721–38726
125. Kitagawa H, Izumikawa T, Uyama T, Sugahara K (2003) Molecular cloning of a chondroitin polymerizing factor that cooperates with chondroitin synthase for chondroitin polymerization. J Biol Chem 278:23666–23671
126. Kitazawa K, Nadanaka S, Kadomatsu K, Kitagawa H (2021) Chondroitin 6-sulfate represses keratinocyte proliferation in mouse skin, which is associated with psoriasis. Commun Biol 4(1):114
127. Kjellén L, Lindahl U (2018) Specificity of glycosaminoglycan-protein interactions. Curr Opin Struct Biol 50:101–108
128. Knight MM, Bomzon Z, Kimmel E, Sharma AM, Lee DA, Bader DL (2006) Chondrocyte deformation induces mitochondrial distortion and heterogeneous intracellular strain fields. Biomech Model Mechanobiol 5(2-3):180–191
129. Knudson W, Aguiar DJ, Hua Q, Knudson CB (1996) CD44-anchored hyaluronan-rich pericellular matrices: an ultrastructural and biochemical analysis. Exp Cell Res 228(2):216–228
130. Kobayashi T, Chanmee T, Itano N (2020) Hyaluronan: metabolism and function. Biomolecules 10(11):1525
131. Krolikoski M, Monslow J, Puré E (2019) The CD44-HA axis and inflammation in atherosclerosis: a temporal perspective. Matrix Biol 78-79:201–218
132. Kurtis MS, Tu BP, Gaya OA, Mollenhauer J, Knudson W, Loeser RF, Knudson CB, Sah RL (2001) Mechanisms of chondrocyte adhesion to cartilage: role of beta1-integrins, CD44, and annexin V. J Orthop Res 19(6):1122–1130
133. Kurz B, Jin M, Patwari P, Cheng DM, Lark MW, Grodzinsky AJ (2001) Biosynthetic response and mechanical properties of articular cartilage after injurious compression. J Orthop Res 19(6):1140–1146
134. Larkin J, Lohr TA, Elefante L, Shearin J, Matico R, Su JL, Xue Y, Liu F, Genell C, Miller RE, Tran PB, Malfait AM, Maier CC, Matheny CJ (2015) Translational development of an ADAMTS-5 antibody for osteoarthritis disease modification. Osteoarthr Cartil 8:1254–1266
135. Lauer ME, Glant TT, Mikecz K, DeAngelis PL, Haller FM, Husni ME, Hascall VC, Calabro A (2013) Irreversible heavy chain transfer to hyaluronan oligosaccharides by tumor necrosis factor-stimulated gene-6. J Biol Chem 288(1):205–214
136. Lauer ME, Loftis J, de la Motte C, Hascall VC (2015) Analysis of the heavy-chain modification and TSG-6 activity in pathological hyaluronan matrices. Methods Mol Biol 1229:543–548
137. Lee TH, Linstedt AD (1999) Osmotically induced cell volume changes alter anterograde and retrograde transport, Golgi structure, and COPI dissociation. Mol Biol Cell 10(5):1445–1462
138. Lee RB, Wilkins RJ, Razaq S, Urban JP (2002) The effect of mechanical stress on cartilage energy metabolism. Biorheology 39(1–2):133–143
139. Lee JH, Fitzgerald JB, Dimicco MA, Grodzinsky AJ (2005) Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression. Arthritis Rheum 52(8):2386–2395
140. Lee WJ, Tu SH, Cheng TC, Lin JH, Sheu MT, Kuo CC, Changou CA, Wu CH, Chang HW, Chang HL, Chen LC, Ho YS (2021) Type-3 Hyaluronan synthase attenuates tumor cells invasion in human mammary parenchymal tissues. Molecules 26(21):6548
141. Lewis A, Steadman R, Manley P, Craig K, de la Motte C, Hascall V, Phillips AO (2008) Diabetic nephropathy, inflammation, hyaluronan and interstitial fibrosis. Histol Histopathol 23(6):731–739
142. Li Z, Yao S, Alini M, Grad S (2007) Different response of articular chondrocyte subpopulations to surface motion. Osteoarthr Cartil 15(9):1034–1041

143. Li Y, Zhong G, Sun W, Zhao C, Zhang P, Song J, Zhao D, Jin X, Li Q, Ling S, Li Y (2015) CD44 deficiency inhibits unloading-induced cortical bone loss through downregulation of osteoclast activity. Sci Rep 5:16124
144. Li K, Zhang C, Qiu L, Gao L, Zhang X (2017) Advances in application of mechanical stimuli in bioreactors for cartilage tissue engineering. Tissue Eng Part B Rev 23(4):399–411
145. Li J, Ahata E, Wang Y (2019) Golgi structure and function in health, stress, and diseases. Results Probl Cell Differ 67:441–485
146. Liang J, Jiang D, Jung Y, Xie T, Ingram J, Church T, Degan S, Leonard M, Kraft M, Noble PW (2011) Role of hyaluronan and hyaluronan-binding proteins in human asthma. J Allergy Clin Immunol 128(2):403–411
147. Lin H, Zhou J, Shen L, Ruan Y, Dong J, Guo C, Chen Z (2014) Biotin-conjugated anti-CD44 antibody-avidin binding system for the improvement of chondrocyte adhesion to scaffolds. J Biomed Mater Res A 102(4):1140–1148
148. Lintuluoto M, Horioka Y, Hongo S, Lintuluoto JM, Fukunishi Y (2021) Molecular dynamics simulation study on allosteric regulation of CD44-Hyaluronan binding as a force sensing mechanism. ACS Omega 6(12):8045–8055
149. Lohmander LS, Shinomura T, Hascall VC, Kimura JH (1989) Xylosyl transfer to the core protein precursor of the rat chondrosarcoma proteoglycan. J Biol Chem 264:18775–18780
150. Lord MS, Melrose J, Day AJ et al (2020) The Inter-α-Trypsin inhibitor family: versatile molecules in biology and pathology. J Histochem Cytochem 68:907–927
151. Luo N, Knudson W, Askew EB, Veluci R, Knudson CB (2014) CD44 and hyaluronan promote the bone morphogenetic protein 7 signaling response in murine chondrocytes. Arthritis Rheum 66(6):1547–1558
152. Magee C, Nurminskaya M, Linsenmayer TF (2001) UDP-glucose pyrophosphorylase: up-regulation in hypertrophic cartilage and role in hyaluronan synthesis. Biochem J 360(Pt 3):667–674
153. Maloney FP, Kuklewicz J, Corey RA, Bi Y, Ho R, Mateusiak L, Pardon E, Steyaert J, Stansfeld PJ, Zimmer J (2022) Structure, substrate recognition and initiation of hyaluronan synthase. Nature; Mar 30. Epub ahead of print
154. Markovitz A, Cifonelli JA, Dorfman A (1959) The biosynthesis of hyaluronic acid by group A Streptococcus. VI. Biosynthesis from uridine nucleotides in cell-free extracts. J Biol Chem 234:2343–2350
155. Maroski J, Vorderwülbecke BJ, Fiedorowicz K, Da Silva-Azevedo L, Siegel G, Marki A, Pries AR, Zakrzewicz A (2011) Shear stress increases endothelial hyaluronan synthase 2 and hyaluronan synthesis especially in regard to an atheroprotective flow profile. Exp Physiol 96(9):977–986
156. Maroudas A, Bayliss MT, Uchitel-Kaushansky N, Schneiderman R, Gilav E (1998) Aggrecan turnover in human articular cartilage: use of aspartic acid racemization as a marker of molecular age. Arch Biochem Biophys 350(1):61–71
157. Martin DC, Atmuri V, Hemming RJ, Farley J, Mort JS, Byers S, Hombach-Klonisch S, Csoka AB, Stern R, Triggs-Raine BL (2008) A mouse model of human mucopolysaccharidosis IX exhibits osteoarthritis. Hum Mol Genet 17(13):1904–1915
158. Mason RM, Crossman MV, Sweeney C (1989) Hyaluronan and hyaluronan-binding proteins in cartilaginous tissues. CIBA Found Symp 143:107–116
159. Matsumoto K, Li Y, Jakuba C, Sugiyama Y, Sayo T, Okuno M, Dealy CN, Toole BP, Takeda J, Yamaguchi Y, Kosher RA (2009) Conditional inactivation of Has2 reveals a crucial role for hyaluronan in skeletal growth, patterning, chondrocyte maturation and joint formation in the developing limb. Development 136(16):2825–2835
160. McCorry MC, Kim J, Springer NL, Sandy J, Plaas A, Bonassar LJ (2019) Regulation of proteoglycan production by varying glucose concentrations controls fiber formation in tissue engineered menisci. Acta Biomater 100:173-183.
161. Melching LI, Roughley PJ (1999) Modulation of keratan sulfate synthesis on lumican by the action of cytokines on human articular chondrocytes. Matrix Biol 18(4):381–390
162. Messam BJ, Tolg C, McCarthy JB, Nelson AC, Turley EA (2021) RHAMM is a multifunctional protein that regulates cancer progression. Int J Mol Sci 22(19):10313
163. Midura RJ, Calabro A, Yanagishita M, Hascall VC (1995) Nonreducing end structures of chondroitin sulfate chains on aggrecan isolated from Swarm rat chondrosarcoma cultures. J Biol Chem 270(14):8009–8015
164. Mikami T, Kitagawa H (2013) Biosynthesis and function of chondroitin sulfate. Biochim Biophys Acta 1830(10):4719–4733
165. Miller KL, Clegg DO (2011) Glucosamine and chondroitin sulfate. Rheum Dis Clin N Am 37(1):103–118
166. Miller RE, Tran PB, Ishihara S, Larkin J, Malfait AM (2016) Therapeutic effects of an anti-ADAMTS-5 antibody on joint damage and mechanical allodynia in a murine model of osteoarthritis. Osteoarthr Cartil 24(2):299–306
167. Mizumoto S, Yamada S (2021) Congenital disorders of deficiency in Glycosaminoglycan biosynthesis. Front Genet 12:717535
168. Mobasheri A, Carter SD, Martín-Vasallo P, Shakibaei M (2002a) Integrins and stretch activated ion channels; putative components of functional cell surface mechanoreceptors in articular chondrocytes. Cell Biol Int 26(1):1–18
169. Mobasheri A, Neama G, Bell S, Richardson S, Carter SD (2002b) Human articular chondrocytes express three facilitative glucose transporter iso-

forms: GLUT1, GLUT3 and GLUT9. Cell Biol Int 26(3):297–300
170. Moffatt P, Lee ER, St-Jacques B, Matsumoto K, Yamaguchi Y, Roughley PJ (2011) Hyaluronan production by means of Has2 gene expression in chondrocytes is essential for long bone development. Dev Dyn 240(2):404–412
171. Morales TI (1994) Transforming growth factor-beta and insulin-like growth factor-1 restore proteoglycan metabolism of bovine articular cartilage after depletion by retinoic acid. Arch Biochem Biophys 315(1):190–198
172. Morales TI, Hascall VC (1989) Factors involved in the regulation of proteoglycan metabolism in articular cartilage. Arthritis Rheum 32(10):1197–1201
173. Mörgelin M, Paulsson M, Heinegård D, Aebi U, Engel J (1995) Evidence of a defined spatial arrangement of hyaluronate in the central filament of cartilage proteoglycan aggregates. Biochem J 307(Pt 2):595–601
174. Moriarity JL, Hurt KJ, Resnick AC, Storm PB, Laroy W, Schnaar RL, Snyder SH (2002) UDP-glucuronate decarboxylase, a key enzyme in proteoglycan synthesis: cloning, characterization, and localization. J Biol Chem 277(19):16968–16975
175. Morla S (2019) Glycosaminoglycans and Glycosaminoglycan mimetics in cancer and inflammation. Int J Mol Sci 20(8):1963
176. Mukhopadhyay D, Asari A, Rugg MS, Day AJ, Fülöp C (2004) Specificity of the tumor necrosis factor-induced protein 6-mediated heavy chain transfer from inter-alpha-trypsin inhibitor to hyaluronan: implications for the assembly of the cumulus extracellular matrix. J Biol Chem 279(12):11119–11128
177. Murata M, Yudoh K, Shimizu H, Beppu M, Nakamura H, Kato T, Masuko K (2013) Layilin, a Talin-binding hyaluronan receptor, is expressed in human articular chondrocytes and synoviocytes and is down-regulated by interleukin-1β. Mod Rheumatol 23(3):478–488
178. Nakamura N, Yamakawa N, Sato T, Tojo H, Tachi C, Furukawa K (2001) Differential gene expression of beta-1,4-galactosyltransferases I, II and V during mouse brain development. J Neurochem 76(1):29–38
179. Nakazawa K, Takahashi I, Yamamoto Y (1998) Glycosyltransferase and sulfotransferase activities in chick corneal stromal cells before and after in vitro culture. Arch Biochem Biophys 359(2):269–282
180. Nieduszynski IA, Huckerby TN, Dickenson JM, Brown GM, Tai GH, Bayliss MT (1990) Structural aspects of skeletal keratan sulphates. Biochem Soc Trans 18(5):792–793
181. Nuwayhid N, Glaser JH, Johnson JC, Conrad HE, Hauser SC, Hirschberg CB (1986) Xylosylation and glucuronosylation reactions in rat liver Golgi apparatus and endoplasmic reticulum. J Biol Chem 261:12936–12941
182. Oegema TR Jr, Hascall VC, Dziewiatkowski DD (1975) Isolation and characterization of proteoglycans from the swarm rat chondrosarcoma. J Biol Chem 250(15):6151–6159
183. Oegema TR Jr, Deloria LB, Sandy JD, Hart DA (2002) Effect of oral glucosamine on cartilage and meniscus in normal and chymopapain-injected knees of young rabbits. Arthritis Rheum 46(9):2495–2503
184. Ogino S, Nishida N, Umemoto R, Suzuki M, Takeda M, Terasawa H, Kitayama J, Matsumoto M, Hayasaka H, Miyasaka M, Shimada I (2010) Two-state conformations in the hyaluronan-binding domain regulate CD44 adhesiveness under flow condition. Structure 18(5):649–656
185. Ogston AG, Stanier JE (1950) On the state of hyaluronic acid in synovial fluid. Biochem J 46(3):364–376
186. Ohtake-Niimi S, Kondo S, Ito T, Kakehi S, Ohta T, Habuchi H, Kimata K, Habuchi O (2010) Mice deficient in N-acetylgalactosamine 4-sulfate 6-o-sulfotransferase are unable to synthesize chondroitin/dermatan sulfate containing N-acetylgalactosamine 4,6-bissulfate residues and exhibit decreased protease activity in bone marrow-derived mast cells. J Biol Chem 285(27):20793–20805
187. Oikari S, Makkonen K, Deen AJ, Tyni I, Kärnä R, Tammi RH, Tammi MI (2016) Hexosamine biosynthesis in keratinocytes: roles of GFAT and GNPDA enzymes in the maintenance of UDP-GlcNAc content and hyaluronan synthesis. Glycobiology 26(7):710–722
188. Olander B, Eklund E, Todorova L, Bengtsson M, Maccarana M, Westergren-Thorsson G, Malmström A (2005) Regulation of the chondroitin/dermatan fine structure by transforming growth factor-beta1 through effects on polymer-modifying enzymes. Glycobiology 12:1277–1285
189. Onodera Y, Teramura T, Takehara T, Fukuda K (2015) Hyaluronic acid regulates a key redox control factor Nrf2 via phosphorylation of Akt in bovine articular chondrocytes. FEBS Open Bio 5:476–484
190. Patwari P, Kurz B, Sandy JD, Grodzinsky AJ (2000) Mannosamine inhibits aggrecanase-mediated changes in the physical properties and biochemical composition of articular cartilage. Arch Biochem Biophys 374(1):79–85
191. Patwari P, Cook MN, DiMicco MA, Blake SM, James IE, Kumar S, Cole AA, Lark MW, Grodzinsky AJ (2003) Proteoglycan degradation after injurious compression of bovine and human articular cartilage in vitro: interaction with exogenous cytokines. Arthritis Rheum 48(5):1292–1301
192. Pereira J, Ottevaere I, Serruys B et al (2018) Pharmacokinetic and pharmacodynamic modelling of the novel anti-ADAMTS-5 nanobody M6495 using the neoepitope ARGS as a biomarker. Osteoarthr Cartil 26:S176
193. Peters A, Sherman LS (2020) Diverse roles for Hyaluronan and Hyaluronan receptors in the developing and adult nervous system. Int J Mol Sci 21(17):5988

194. Petrey AC, de la Motte CA (2014) Hyaluronan, a crucial regulator of inflammation. Front Immunol 5:101
195. Petrey AC, de la Motte CA (2019) Hyaluronan in inflammatory bowel disease: cross-linking inflammation and coagulation. Matrix Biol 78–79:314–323
196. Pingguan-Murphy B, El-Azzeh M, Bader DL, Knight MM (2006) Cyclic compression of chondrocytes modulates a purinergic calcium signalling pathway in a strain rate- and frequency-dependent manner. J Cell Physiol 209(2):389–397
197. Plaas AH, Sandy JD (1993) A cartilage explant system for studies on aggrecan structure, biosynthesis and catabolism in discrete zones of the mammalian growth plate. Matrix 13(2):135–147
198. Plaas AH, Sandy JD, Kimura JH (1988) Biosynthesis of cartilage proteoglycan and link protein by articular chondrocytes from immature and mature rabbits. J Biol Chem 263(16):7560–7566
199. Plaas AH, Hascall VC, Midura RJ (1996) Ion exchange HPLC microanalysis of chondroitin sulfate: quantitative derivatization of chondroitin lyase digestion products with 2-aminopyridine. Glycobiology 6(8):823–829
200. Plaas AH, Wong-Palms S, Roughley PJ, Midura RJ, Hascall VC (1997) Chemical and immunological assay of the nonreducing terminal residues of chondroitin sulfate from human aggrecan. J Biol Chem 272(33):20603–20610
201. Plaas AH, West LA, Midura RJ (2001) Keratan sulfate disaccharide composition determined by FACE analysis of keratanase II and endo-beta-galactosidase digestion products. Glycobiology 11(10):779–790
202. Plaas A, Sandy JD, Liu H, Diaz MA, Schenkman D, Magnus RP, Bolam-Bretl C, Kopesky PW, Wang VM, Galante JO (2011) Biochemical identification and immunolocalization of aggrecan, ADAMTS5 and inter–trypsin-inhibitor in equine degenerative suspensory ligament desmitis. J Orthop Res 29:900–906
203. Pönighaus C, Ambrosius M, Casanova JC, Prante C, Kuhn J, Esko JD, Kleesiek K, Götting C (2007) Human xylosyltransferase II is involved in the biosynthesis of the uniform tetrasaccharide linkage region in chondroitin sulfate and heparan sulfate proteoglycans. J Biol Chem 282(8):5201–5206
204. Prevo R, Banerji S, Ferguson DJ, Clasper S, Jackson DG (2001) Mouse LYVE-1 is an endocytic receptor for hyaluronan in lymphatic endothelium. J Biol Chem 276(22):19420–19430
205. Quinn TM, Grodzinsky AJ, Buschmann MD, Kim YJ, Hunziker EB (1998a) Mechanical compression alters proteoglycan deposition and matrix deformation around individual cells in cartilage explants. J Cell Sci 111(Pt 5):573–583
206. Quinn TM, Grodzinsky AJ, Hunziker EB, Sandy JD (1998b) Effects of injurious compression on matrix turnover around individual cells in calf articular cartilage explants. J Orthop Res 16(4):490–499
207. Ragan PM, Chin VI, Hung HH, Masuda K, Thonar EJ, Arner EC, Grodzinsky AJ, Sandy JD (2000) Chondrocyte extracellular matrix synthesis and turnover are influenced by static compression in a new alginate disk culture system. Arch Biochem Biophys 15; 383(2):256–264
208. Raman PS, Alves CS, Wirtz D, Konstantopoulos K (2011) Single-molecule binding of CD44 to fibrin versus P-selectin predicts their distinct shear-dependent interactions in cancer. J Cell Sci 124(Pt 11):1903–1910
209. Ratcliffe A, Hughes C, Fryer PR, Saed-Nejad F, Hardingham T (1987) Immunochemical studies on the synthesis and secretion of link protein and aggregating proteoglycan by chondrocytes. Coll Relat Res 7(6):409–421
210. Reiprich S, Akova E, Aszódi A, Schönitzer V (2021) Hyaluronan synthases' expression and activity are induced by fluid shear stress in bone marrow-derived mesenchymal stem cells. Int J Mol Sci 22(6):3123
211. Richardson C, Plaas A, Block JA (2019) Intra-articular hyaluronan therapy for symptomatic knee osteoarthritis. Rheum Dis Clin N Am 45(3):439–451
212. Riegger J, Baumert J, Zaucke F, Brenner RE (2021) The Hexosamine biosynthetic pathway as a therapeutic target after cartilage Trauma: modification of chondrocyte survival and metabolism by glucosamine derivatives and PUGNAc in an ex vivo model. Int J Mol Sci 22(14):7247
213. Rivinoja A, Pujol FM, Hassinen A, Kellokumpu S (2012) Golgi pH, its regulation and roles in human disease. Ann Med 44(6):542–554
214. Rodriguez E, Roland SK, Plaas A, Roughley PJ (2006) The glycosaminoglycan attachment regions of human aggrecan. J Biol Chem 281(27):18444–18450
215. Rosenberg L, Hellmann W, Kleinschmidt AK (1975) Electron microscopic studies of proteoglycan aggregates from bovine articular cartilage. J Biol Chem 250(5):1877–1883
216. Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD (1990) Effects of tissue compression on the hyaluronate-binding properties of newly synthesized proteoglycans in cartilage explants. Biochem J 267(3):803–808
217. Sah RL, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD (1991) Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants. Arch Biochem Biophys 286(1):20–29
218. Salo J, Kaigle Holm A, Indahl A, Mackiewicz Z, Sukura A, Holm S, Jämsen E, Konttinen YT (2008) Expression of vascular endothelial growth factor receptors coincide with blood vessel in-growth and reactive bone remodelling in experimental intervertebral disc degeneration. Clin Exp Rheumatol 26(6):1018–1026
219. Sandy JD, Plaas AH (1986) Age-related changes in the kinetics of release of proteoglycans from normal rabbit cartilage explants. J Orthop Res 4(3):263–272

220. Sandy JD, Flannery CR, Neame PJ, Lohmander LS (1992) The structure of aggrecan fragments in human synovial fluid. Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond of the interglobular domain. J Clin Invest 89(5):1512–1516
221. Sandy JD, Gamett D, Thompson V, Verscharen C (1998) Chondrocyte-mediated catabolism of aggrecan: aggrecanase-dependent cleavage induced by interleukin-1 or retinoic acid can be inhibited by glucosamine. Biochem J 335(Pt 1):59–66
222. Sandy JD, Thompson V, Doege K, Verscharen C (2000) The intermediates of aggrecanase-dependent cleavage of aggrecan in rat chondrosarcoma cells treated with interleukin-1. Biochem J 351(Pt 1):161–166
223. Santamaria S, Yamamoto K, Botkjaer K, Tape C, Dyson MR, McCafferty J, Murphy G, Nagase H (2015) Antibody-based exosite inhibitors of ADAMTS-5 (aggrecanase-2). Biochem J 471(3):391–401
224. Santamaria S, Fedorov O, McCafferty J, Murphy G, Dudhia J, Nagase H, Yamamoto K (2017) Development of a monoclonal anti-ADAMTS-5 antibody that specifically blocks the interaction with LRP1. MAbs 9(4):595–602
225. Sasaki K, Yoshida H (2019) Golgi stress response and organelle zones. FEBS Lett 593(17):2330–2340
226. Sato T, Gotoh M, Kiyohara K, Akashima T, Iwasaki H, Kameyama A, Mochizuki H, Yada T, Inaba N, Togayachi A, Kudo T, Asada M, Watanabe H, Imamura T, Kimata K, Narimatsu H (2003) Differential roles of two N-acetylgalactosaminyltransferases, CSGalNAcT-1 and a novel enzyme CSGalNAcT-2 Initiation and elongation in synthesis of chondroitin sulfate. J Biol Chem 278:3063–3071
227. Sauerland K, Plaas AH, Raiss RX, Steinmeyer J (2003) The sulfation pattern of chondroitin sulfate from articular cartilage explants in respo2 to mechanical loading. Biochim Biophys Acta 1638(3):241–248
228. Sauerland K, Wolf A, Schudok M, Steinmeyer J (2021) A novel model of a biomechanically induced osteoarthritis-like cartilage for pharmacological in vitro studies. J Cell Mol Med 25(24):11221–11231
229. Scavenius C, Poulsen EC, Thøgersen IB, Roebuck M, Frostick S, Bou-Gharios G, Yamamoto K, Deleuran B, Enghild JJ (2019) Matrix-degrading protease ADAMTS-5 cleaves inter-α-inhibitor and releases active heavy chain 2 in synovial fluids from arthritic patients. J Biol Chem 294(42):15495–15504
230. Schmidt TA, Gastelum NS, Nguyen QT, Schumacher BL, Sah RL (2007) Boundary lubrication of articular cartilage: role of synovial fluid constituents. Arthritis Rheum 56(3):882–891
231. Schneckmann R, Suvorava T, Hundhausen C, Schuler D, Lorenz C, Freudenberger T, Kelm M, Fischer JW, Flögel U, Grandoch M (2021) Endothelial Hyaluronan synthase 3 augments postischemic arteriogenesis through CD44/eNOS signaling. Arterioscler Thromb Vasc Biol 41(10):2551–2562
232. Senol O, Gundogdu G, Gundogdu K, Miloglu FD (2019) Investigation of the relationships between knee osteoarthritis and obesity via untargeted metabolomics analysis. Clin Rheumatol 38(5):1351–1360
233. Sherpa MT, Kiwamoto T, Matsuyama M, Tsunoda Y, Yazaki K, Yoshida K, Nakajima M, Matsuno Y, Morishima Y, Ishii Y, Hizawa N (2022) Has2 regulates the development of Ovalbumin-induced airway remodeling and steroid insensitivity in mice. Front Immunol 12:770305
234. Shimbo M, Suzuki R, Fuseya S, Sato T, Kiyohara K, Hagiwara K, Okada R, Wakui H, Tsunakawa Y, Watanabe H, Kimata K, Narimatsu H, Kudo T, Takahashi S (2017) Postnatal lethality and chondrodysplasia in mice lacking both chondroitin sulfate N-acetylgalactosaminyltransferase-1 and -2. PLoS One 12(12):e0190333
235. Shimizu H, Shimoda M, Mochizuki S, Miyamae Y, Abe H, Chijiiwa M, Yoshida H, Shiozawa J, Ishijima M, Kaneko K, Kanaji A, Nakamura M, Toyama Y, Okada Y (2018) Hyaluronan-binding protein involved in Hyaluronan depolymerization is up-regulated and involved in Hyaluronan degradation in human osteoarthritic cartilage. Am J Pathol 188(9):2109–2119
236. Showiheen SAA, Sun AR, Wu X, Crawford R, Xiao Y, Wellard RM, Prasadam I (2019) Application of metabolomics to osteoarthritis: from basic science to the clinical approach. Curr Rheumatol Rep 21(6):26
237. Sikes KJ, Renner K, Li J, Grande-Allen KJ, Connell JP, Cali V, Midura RJ, Sandy JD, Plaas A, Wang VM (2018) Knockout of hyaluronan synthase 1, but not 3, impairs formation of the retrocalcaneal bursa. J Orthop Res 36(10):2622–2632
238. Silbert JE, DeLuca S (1968) Formation of chondroitin sulfate by a microsomal preparation from chick embryo epiphyseal cartilage. Biochem Biophys Res Commun 31(6):990–995
239. Smith RL, Carter DR, Schurman DJ (2004) Pressure and shear differentially alter human articular chondrocyte metabolism: a review. Clin Orthop Relat Res (427 Suppl):S89–S95
240. Sodhi H, Panitch A (2020) Glycosaminoglycans in tissue engineering: a review. Biomolecules 29; 11(1):29
241. Somemura S, Kumai T, Yatabe K, Sasaki C, Fujiya H, Niki H, Yudoh K (2021) Physiologic mechanical stress directly induces bone formation by Activating Glucose Transporter 1 (Glut 1) in Osteoblasts, inducing signaling via NAD+-Dependent Deacetylase (Sirtuin 1) and Runt-Related Transcription Factor 2 (Runx2). Int J Mol Sci 22(16):9070
242. Song Z (2013) Roles of the nucleotide sugar transporters (SLC35 family) in health and disease. Mol Asp Med 34(2-3):590–600

243. Southan J, McHugh E, Walker H, Ismail HM (2020) Metabolic signature of articular cartilage following mechanical injury: an integrated transcriptomics and metabolomics analysis. Front Mol Biosci 7:592905
244. Spicer AP, Kaback LA, Smith TJ, Seldin MF (1998) Molecular cloning and characterization of the human and mouse UDP-glucose dehydrogenase genes. J Biol Chem 273(39):25117–25124
245. Stampoultzis T, Karami P, Pioletti DP (2021) Thoughts on cartilage tissue engineering: a 21st century perspective. Curr Res Transl Med 69(3):103299
246. Stanton H, Rogerson FM, East CJ et al (2005) ADAMTS-5 is the major aggrecanase in mouse cartilage in vivo and in vitro. Nature 434:648–652
247. Stoolmiller AC, Horwitz AL, Dorfman A (1972) Biosynthesis of the chondroitin sulfate proteoglycan. Purification and properties of xylosyltransferase. J Biol Chem 10; 247(11):3525–3532
248. Sugumaran G, Coburn JN, Silbert JE (1986) Simultaneous sulfation of endogenous chondroitin sulfate and chondroitin-derived oligosaccharides. Studies with separate 4-sulfating and 6-sulfating microsomal systems. J Biol Chem 261(27):12659–12664. 45–48
249. Sugumaran G, Katsman M, Silbert JE (1992) Effects of brefeldin A on the localization of chondroitin sulfate-synthesizing enzymes. Activities in subfractions of the Golgi from chick embryo epiphyseal cartilage. J Biol Chem 267:8802–8806
250. Swaidani S, Cheng G, Lauer ME, Sharma M, Mikecz K, Hascall VC, Aronica MA (2013) TSG-6 protein is crucial for the development of pulmonary hyaluronan deposition, eosinophilia, and airway hyperresponsiveness in a murine model of asthma. J Biol Chem 288(1):412–422
251. Swann DA, Radin EL, Nazimiec M, Weisser PA, Curran N, Lewinnek G (1974) Role of hyaluronic acid in joint lubrication. Ann Rheum Dis 33(4):318–326
252. Swann DA, Powell S, Broadhurst J, Sordillo E, Sotman S (1976) The formation of a stable complex between dissociated proteoglycan and hyaluronic acid in the absence of a link protein. Biochem J A157(2):503–506
253. Szafranski JD, Grodzinsky AJ, Burger E, Gaschen V, Hung HH, Hunziker EB (2004) Chondrocyte mechanotransduction: effects of compression on deformation of intracellular organelles and relevance to cellular biosynthesis. Osteoarthr Cartil 12(12):937–946
254. Takemura M, Itoh H, Sagawa N, Yura S, Korita D, Kakui K, Kawamura M, Hirota N, Maeda H, Fujii S (2005) Cyclic mechanical stretch augments hyaluronan production in cultured human uterine cervical fibroblast cells. Mol Hum Reprod 11(9):659–665
255. Tammi RH, Passi AG, Rilla K, Karousou E, Vigetti D, Makkonen K, Tammi MI (2011) Transcriptional and post-translational regulation of hyaluronan synthesis. FEBS J 278(9):1419–1428
256. Tobisawa Y, Fujita N, Yamamoto H, Ohyama C, Irie F, Yamaguchi Y (2021) The cell surface hyaluronidase TMEM2 is essential for systemic hyaluronan catabolism and turnover. J Biol Chem 297(5):101281
257. Toole BP (2000) Hyaluronan is not just a goo! J Clin Invest 106(3):335–336
258. Tortorella MD, Burn TC, Pratta MA et al (1999) Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. Science 284:1664–1666
259. Triggs-Raine B, Natowicz MR (2015) Biology of hyaluronan: insights from genetic disorders of hyaluronan metabolism. World J Biol Chem 6(3):110–120
260. Tsiganos CP, Hardingham TE, Muir H (1972) Aggregation of cartilage proteoglycans. Biochem J 128(4):121P
261. Tsuchiya S, Ohashi Y, Ishizuka S, Ishiguro N, O'Rourke DP, Knudson CB, Knudson W (2020) Suppression of murine osteoarthritis by 4-methylumbelliferone. J Orthop Res 38(5):1122–1131
262. Tsuji S, Nakamura S, Yamada T, de Vega S, Okada Y, Inoue S, Shimazawa M, Hara H (2021) HYBID derived from tumor cells and tumor-associated macrophages contribute to the glioblastoma growth. Brain Res 1764:147490
263. Turley EA, Noble PW, Bourguignon LY (2002) Signaling properties of hyaluronan receptors. J Biol Chem 277(7):4589–4592
264. Uhlin-Hansen L, Yanagishita M (1993) Defferential effect of brefeldin A on the biosynthesis of heparan sulfate and chondroitin/dermatan sulfate proteoglycans in rat ovarian granulosa cells in culture. J Biol Chem 268:17370–17376
265. Vaupel P, Multhoff G (2021) Revisiting the Warburg effect: historical dogma versus current understanding. J Physiol 599(6):1745–1757
266. Velasco J, Li J, DiPietro L, Stepp MA, Sandy JD, Plaas A (2011) Adamts5 deletion blocks murine dermal repair through CD44-mediated aggrecan accumulation and modulation of transforming growth factor β1 (TGFβ1) signaling. J Biol Chem 286(29):26016–26027
267. Vertel BM, Walters LM, Flay N, Kearns AE, Schwartz NB (1993) Xylosylation is an endoplasmic reticulum to Golgi event. J Biol Chem 268:11105–11112
268. Verzijl N, DeGroot J, Bank RA, Bayliss MT, Bijlsma JW, Lafeber FP, Maroudas A, TeKoppele JM (2001) Age-related accumulation of the advanced glycation endproduct pentosidine in human articular cartilage aggrecan: the use of pentosidine levels as a quantitative measure of protein turnover. Matrix Biol 20(7):409–417
269. Victor XV, Nguyen TK, Ethirajan M, Tran VM, Nguyen KV, Kuberan B (2009) Investigating the elusive mechanism of glycosaminoglycan biosynthesis. J Biol Chem 284(38):25842–25853

270. Vigetti D, Clerici M, Deleonibus S, Karousou E, Viola M, Moretto P, Heldin P, Hascall VC, De Luca G, Passi A (2011) Hyaluronan synthesis is inhibited by adenosine monophosphate-activated protein kinase through the regulation of HAS2 activity in human aortic smooth muscle cells. J Biol Chem 286(10):7917–7924
271. Vigetti D, Deleonibus S, Moretto P, Karousou E, Viola M, Bartolini B, Hascall VC, Tammi M, De Luca G, Passi A (2012) Role of UDP-N-acetylglucosamine (GlcNAc) and O-GlcNAcylation of hyaluronan synthase 2 in the control of chondroitin sulfate and hyaluronan synthesis. J Biol Chem 287(42):35544–35555
272. Vigetti D, Karousou E, Viola M, Deleonibus S, De Luca G, Passi A (2014) Hyaluronan: biosynthesis and signaling. Biochim Biophys Acta 1840(8):2452–2459
273. Wang SX, Laverty S, Dumitriu M, Plaas A, Grynpas MD (2007) The effects of glucosamine hydrochloride on subchondral bone changes in an animal model of osteoarthritis. Arthritis Rheum 56(5):1537–1548
274. Wang A, de la Motte C, Lauer M, Hascall V (2011) Hyaluronan matrices in pathobiological processes. FEBS J 278(9):1412–1418
275. Wang VM, Bell RM, Thakore R, Eyre DR, Galante JO, Li J, Sandy JD, Plaas A (2012) Murine tendon function is adversely affected by aggrecan accumulation due to the knockout of ADAMTS5. J Orthop Res 30(4):620–626
276. Wang Y, Li Q, Liu F, Jin S, Zhang Y, Zhang T, Zhu Y, Zhou Y (2018) Transcriptional activation of glucose transporter 1 in orthodontic tooth movement-associated mechanical response. Int J Oral Sci 15; 10(3):27
277. Wang G, Kostidis S, Tiemeier GL, Sol WMPJ, de Vries MR, Giera M, Carmeliet P, van den Berg BM, Rabelink TJ (2020) Shear stress regulation of endothelial glycocalyx structure is determined by glucobiosynthesis. Arterioscler Thromb Vasc Biol 40(2):350–364
278. Watanabe Y, Takeuchi K, Higa Onaga S, Sato M, Tsujita M, Abe M, Natsume R, Li M, Furuichi T, Saeki M, Izumikawa T, Hasegawa A, Yokoyama M, Ikegawa S, Sakimura K, Amizuka N, Kitagawa H, Igarashi M (2010) Chondroitin sulfate N-acetylgalactosaminyltransferase-1 is required for normal cartilage development. Biochem J 432(1):47–55
279. Weber JF, Perez R, Waldman SD (2015) Mechanobioreactors for cartilage tissue engineering. Methods Mol Biol 1340:203–219
280. Weigel PH (2015) Hyaluronan synthase: the mechanism of initiation at the reducing end and a Pendulum model for Polysaccharide Translocation to the cell exterior. Int J Cell Biol 2015:367579
281. Weigel PH (2019) Discovery of the liver Hyaluronan Receptor for endocytosis (HARE) and its progressive emergence as the multi-ligand scavenger receptor Stabilin-2. Biomolecules. 9(9):454
282. Weigel PH, DeAngelis PL (2007) Hyaluronan synthases: a decade-plus of novel glycosyltransferases. J Biol Chem 282(51):36777–36781
283. Weigel JA, Weigel PH (2003) Characterization of the recombinant rat 175-kDa hyaluronan receptor for endocytosis (HARE). J Biol Chem 278(44):42802–42811
284. Weigel PH, Hascall VC, Tammi M (1997) Hyaluronan synthases. J Biol Chem 272(22):13997–13400
285. West LA, Roughley P, Nelson FR, Plaas AH (1999) Sulphation heterogeneity in the trisaccharide (GalNAcSbeta1, 4GlcAbeta1, 3GalNAcS) isolated from the non-reducing terminal of human aggrecan chondroitin sulphate. Biochem J 342:223–229
286. Windhaber RA, Wilkins RJ, Meredith D (2003) Functional characterization of glucose transport in bovine articular chondrocytes. Pflugers Arch 446(5):572–577
287. Wu J, Qu Y, Zhang YP, Deng JX, Yu QH (2018) RHAMM induces progression of rheumatoid arthritis by enhancing the functions of fibroblast-like synoviocytes. BMC Musculoskelet Disord D19(1):455
288. Wu ZY, He YQ, Wang TM, Yang DW, Li DH, Deng CM, Cao LJ, Zhang JB, Xue WQ, Jia WH (2021) Glycogenes in Oncofetal Chondroitin Sulfate biosynthesis are differently expressed and correlated with immune response in placenta and colorectal cancer. Front Cell Dev Biol 9:763875
289. Xu H, Edwards J, Banerji S, Prevo R, Jackson DG, Athanasou NA (2003) Distribution of lymphatic vessels in normal and arthritic human synovial tissues. Ann Rheum Dis 62(12):1227–1229
290. Yada T, Gotoh M, Sato T, Shionyu M, Go M, Kaseyama H, Iwasaki H, Kikuchi N, Kwon YD, Togayachi A, Kudo T, Watanabe H, Narimatsu H, Kimata K (2003) Chondroitin sulfate synthase-2. Molecular cloning and characterization of a novel human glycosyltransferase homologous to chondroitin sulfate glucuronyltransferase which has dual enzymatic activities. J Biol Chem 278:30235–30247
291. Yamamoto H, Tobisawa Y, Inubushi T, Irie F, Ohyama C, Yamaguchi Y (2017) A mammalian homolog of the zebrafish transmembrane protein 2 (TMEM2) is the long-sought-after cell-surface hyaluronidase. J Biol Chem 292(18):7304–7313
292. Yao Q, Parvez-Khan M, Schipani E (2020) In vivo survival strategies for cellular adaptation to hypoxia: HIF1α-dependent suppression of mitochondrial oxygen consumption and decrease of intracellular hypoxia are critical for survival of hypoxic chondrocytes. Bone 140:115572
293. Yingsung W, Zhuo L, Morgelin M, Yoneda M, Kida D, Watanabe H, Ishiguro N, Iwata H, Kimata K (2003) Molecular heterogeneity of the SHAP-hyaluronan complex. Isolation and characterization of the complex in synovial fluid from patients with rheumatoid arthritis. J Biol Chem 278(35):32710–32718
294. Yoshida H, Nagaoka A, Kusaka-Kikushima A, Tobiishi M, Kawabata K, Sayo T, Sakai S,

Sugiyama Y, Enomoto H, Okada Y, Inoue S (2013a) KIAA1199, a deafness gene of unknown function, is a new hyaluronan binding protein involved in hyaluronan depolymerization. Proc Natl Acad Sci U S A 110(14):5612–5617

295. Yoshida H, Nagaoka A, Nakamura S, Sugiyama Y, Okada Y, Inoue S (2013b) Murine homologue of the human KIAA1199 is implicated in hyaluronan binding and depolymerization. FEBS Open Bio 3:352–356
296. Yoshihara Y, Plaas A, Osborn B, Margulis A, Nelson F, Stewart M, Rugg MS, Milner CM, Day AJ, Nemoto K, Sandy JD (2008) Superficial zone chondrocytes in normal and osteoarthritic human articular cartilages synthesize novel truncated forms of inter-alpha-trypsin inhibitor heavy chains which are attached to a chondroitin sulfate proteoglycan other than bikunin. Osteoarthr Cartil 16(11):1343–1355
297. Yoshino Y, Shimazawa M, Nakamura S, Inoue S, Yoshida H, Shimoda M, Okada Y, Hara H (2018) Targeted deletion of HYBID (hyaluronan binding protein involved in hyaluronan depolymerization/KIAA1199/CEMIP) decreases dendritic spine density in the dentate gyrus through hyaluronan accumulation. Biochem Biophys Res Commun 503(3):1934–1940
298. Yung S, Thomas GJ, Davies M (2000) Induction of hyaluronan metabolism after mechanical injury of human peritoneal mesothelial cells in vitro. Kidney Int 58(5):1953–1962
299. Zhang K, Wang L, Liu Z, Geng B, Teng Y, Liu X, Yi Q, Yu D, Chen X, Zhao D, Xia Y (2021a) Mechanosensory and mechanotransductive processes mediated by ion channels in articular chondrocytes: Potential therapeutic targets for osteoarthritis. Channels (Austin) 15(1):339–359
300. Zhang W, Yin G, Zhao H, Ling H, Xie Z, Xiao C, Chen Y, Lin Y, Jiang T, Jin S, Wang J, Yang X (2021b) Secreted KIAA1199 promotes the progression of rheumatoid arthritis by mediating hyaluronic acid degradation in an ANXA1-dependent manner. Cell Death Dis 12(1):102
301. Zhao M, Yoneda M, Ohashi Y, Kurono S, Iwata H, Ohnuki Y, Kimata K (1995) Evidence for the covalent binding of SHAP, heavy chains of inter-alpha-trypsin inhibitor, to hyaluronan. J Biol Chem 270(44):26657–26663
302. Zheng L, Zhang Z, Sheng P, Mobasheri A (2021) The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis. Ageing Res Rev 66:101249
303. Zhuo L, Hascall VC, Kimata K (2004) Inter-alpha-trypsin inhibitor, a covalent protein-glycosaminoglycan-protein complex. J Biol Chem 279(37):38079–38082
304. Zimmer BM, Barycki JJ, Simpson MA (2021) Integration of sugar metabolism and proteoglycan synthesis by UDP-glucose dehydrogenase. J Histochem Cytochem 69(1):13–23

2 Understanding the Influence of Local Physical Stimuli on Chondrocyte Behavior

Byumsu Kim and Lawrence J. Bonassar

Abstract

Investigating the mechanobiology of chondrocytes is challenging due to the complex micromechanical environment of cartilage tissue. The innate zonal differences and poroelastic properties of the tissue combined with its heterogeneous composition create spatial- and temporal-dependent cell behavior, which further complicates the investigation. Despite the numerous challenges, understanding the mechanobiology of chondrocytes is crucial for developing strategies for treating cartilage related diseases as chondrocytes are the only cell type within the tissue. The effort to understand chondrocyte behavior under various mechanical stimuli has been ongoing over the last 50 years. Early studies examined global biosynthetic behavior under unidirectional mechanical stimulus. With the technological development in high-speed confocal imaging techniques, recent studies have focused on investigating real-time individual and collective cell responses to multiple / combined modes of mechanical stimuli. Such efforts have led to tremendous advances in understanding the influence of local physical stimuli on chondrocyte behavior. In addition, we highlight the wide variety of experimental techniques, spanning from static to impact loading, and analysis techniques, from biochemical assays to machine learning, that have been utilized to study chondrocyte behavior. Finally, we review the progression of hypotheses about chondrocyte mechanobiology and provide a perspective on the future outlook of chondrocyte mechanobiology.

Keywords

Chondrocyte · Mechanobiology · Cartilage

2.1 Introduction

The central physiological role of cartilage is purely mechanical. Cartilage cushions mechanical joint loading to facilitate smooth movement. The composition and structure of cartilage tissue have evolved to accommodate the complex *in vivo* multiaxial loading that the tissue experiences. Such composition and structure generate a

B. Kim
Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA

L. J. Bonassar (✉)
Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA

Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, USA
e-mail: lb244@cornell.edu

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_2

unique micromechanical environment for chondrocytes during mechanical loading. Over the last 50 years, researchers have been investigating the relationship between the unique structure and the mechanical response of cartilage at multiple scales. Understanding how chondrocytes respond to such complex micromechanical environment under load is crucial for describing the bulk mechanical behavior of the tissue. As such, the field of chondrocyte mechanobiology, which seeks to understand how mechanically driven physical stimuli influence cell behavior, emerged as an important area of biomedical engineering about 30 years ago.

Mechanobiology is particularly important for chondrocytes and cartilage because chondrocytes are the only cell type to generate cartilage without blood vessels or nerves. In addition, the sole purpose of the tissue is to bear *in vivo* mechanical loads. Therefore, mechanical damage to cartilage is detrimental as the tissue continuously degenerates, ultimately leading to debilitating joint movements. Thus, investigating the influence of local physical stimuli on chondrocytes is crucial for understanding cartilage-related diseases, such as osteoarthritis, and for developing potential treatments to prevent or stop cartilage degeneration.

Studying cartilage mechanobiology is exceptionally challenging due to the three-dimensional chondrocyte-matrix interaction and the innate electrochemical-mechanical properties of the tissue. Even a simple compressive boundary condition can generate interstitial fluid flow, change in fixed charge density, and heterogenous matrix deformation, which are all coupled. Such coupled local physical stimuli are sensed by chondrocytes and influence the cells' behavior.

This chapter aims to review the history of mechanobiology studies in chondrocytes and describe experimental techniques that have been utilized. In addition, we describe the progress of hypotheses and important local physical factors that can influence chondrocyte behavior and provide an outlook for the future of chondrocyte mechanobiology.

2.2 Static Stimulus

Initial studies on the effects of mechanical forces on the chondrocyte mechanism were performed under static loading conditions. Because cartilage is poroelastic, static loading must be applied by imposing weight or displacement on the sample and waiting for hydrostatic pressure and fluid flow to be dissipated [1–6]. Under constant load conditions, cartilage experiences poroelastic creep or stress relaxation [7, 8]. Such poroelastic behavior is caused by interstitial fluid flow. In addition, confined and unconfined boundary conditions can be imposed on the samples [9]. A confined boundary condition is accomplished by placing a cartilage sample in an impermeable and enclosed chamber with permeable porous platen compressing the tissue, while an unconfined boundary condition is achieved using an open chamber with an impermeable platen. These boundary conditions affect the direction of interstitial fluid flow caused by the imposed static compression. Under confined compression, fluid escapes against the loading direction through the permeable porous platen. Meanwhile, unconfined compression (Fig. 2.1a, d) forces the fluid to escape radially.

The effects of these static compressive loading methods and variable boundary conditions on chondrocyte biosynthetic activities have been thoroughly studied for more than 30 years [1–6, 10]. At the tissue level, cartilage biosynthetic activity, defined by proline and sulfate tissue intake, is suppressed monotonically with increasing stress and strain [1] (Fig. 2.1 and Table 2.1). Notably, this is the first study to observe biosynthetic change in cartilage under load. Given the complexity of the mechanical response of cartilage, this topic has remained an important area of investigation for the past three decades. The first mechanism of compression-induced biosynthetic change explored was changes in pH that occur under static compression. Compressing cartilage concentrates negative charges within the matrix, which requires increase in interstitial counterions, including H^+ and K^+. An increase in H^+ concentration reduces intersti-

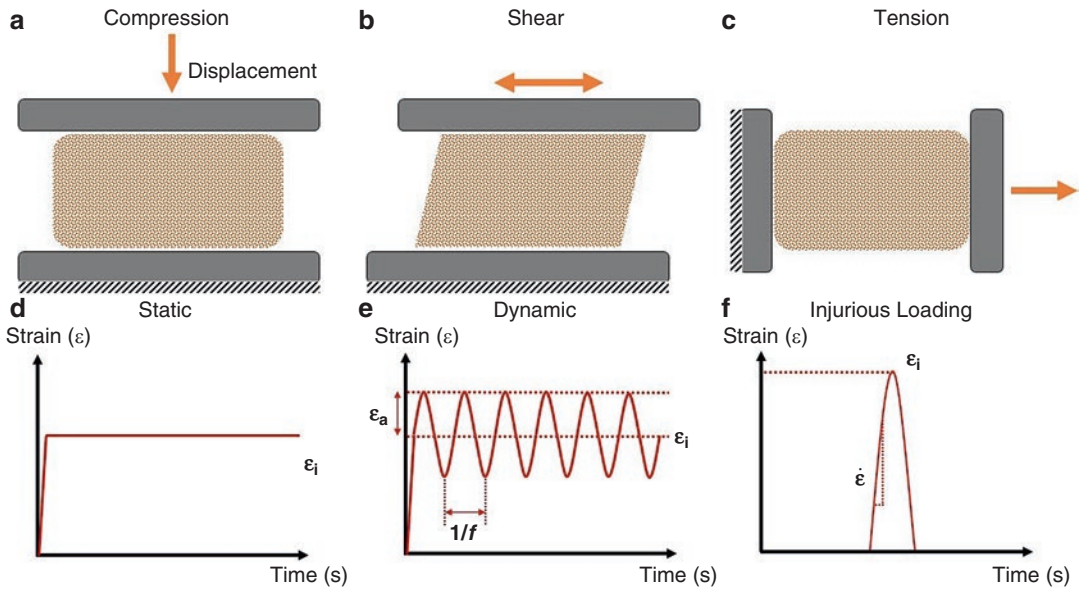


Fig. 2.1 Modes of mechanical stimulation to study cartilage mechanobiology. (**a**) Compression. (**b**) Shear. (**c**) Tension. Regimes of loading rates. (**d**) Static, ε_i represents imposed strain. (**e**) Dynamic, ε_i represents imposed strain, ε_a represents strain amplitude, and *f* represents frequency. (f) Injurious loading. ε_i represents imposed strain, and $\dot{\varepsilon}$ represents strain rate

Table 2.1 Ranges of imposed strain, strain amplitude, frequency, and strain rate of static, dynamic and injurious loading regimes used in mechanical stimulation studies

	Imposed strain, $\varepsilon_i \pm$ strain amplitude ε_a	Frequency *f* (Hz)	Strain rate $\dot{\varepsilon}$ (s^{-1})
Static [1–6, 10–12, 14, 15, 22, 65, 66]	0–0.67	0	0
Dynamic [6, 10, 12, 13, 22–27, 36, 66–73]	0–0.5 ± 0.005–0.17	0.0001–2.6	2×10^{-6} – 1.77
Injurious loading [43–48, 52, 54–59, 74, 75]	0.5–0.8	$3.5*10^{-5}$ – 3.2	7×10^{-5} – 4

tial pH, and the reduction of interstitial pH reduces the biosynthesis level. Remarkably, lowering the media's pH produces the comparable interstitial pH of compressed tissue and reduces biosynthetic levels. Overall, this highlights the important role of counterions in chondrocyte biosynthesis.

The second mechanism of compression-induced regulation of chondrocyte biosynthesis is alteration of molecular transport. Compression with an impermeable boundary condition causes solute transport to occur radially and leads to a decrease in the pore size of the matrix. Further research [2, 10] has shown that biosynthetic activity under static compression is location-dependent. In general, increased static compression decreases location-specific biosynthetic activities uniformly across the construct. Tissue at the radial edge consistently expresses a higher synthetic level, but static compression decreases the overall biosynthesis. The biosynthetic activity forms a radial gradient as the level decreases gradually towards the center. More interestingly, the synthesis level at the radial edge of the free swelling sample is slightly higher than in the rest of the sample. These phenomena were thought to be caused by the limitation of molecular transport due to the compression-induced decrease in tissue diffusivity. The compaction of the matrix around chondrocytes reduces the characteristic pore size, hindering the delivery of macromolecules to the cells [11]. Therefore, at the center of

the sample, nutrients are not as readily available as at the radial edge, leading to a radial gradient of the biosynthetic level.

However, chondrocytes embedded in agarose respond differently to those present in native tissue [12]. Agarose-chondrocyte constructs held at 5% static strain do not display a statistically significant difference in biosynthetic levels compared to those of free-swelling constructs. This indicates that other factors might play a more important role than molecular transport in depression of biosynthesis. Notably, agarose gel is significantly more diffusive than the native cartilage matrix. Therefore, 5% static strain may not hinder the diffusivity of agarose-chondrocyte constructs as much as that of native cartilage matrix. Collectively, these data highlight the unique nature of cartilage with respect to the consequences of static compression. In the absence of transport restriction and mechanochemical effects, the primary effect of static compression on chondrocytes is due to deformation. Interestingly, biosynthetic activities seem to depend on the deformation of cells in agarose systems.

At the length scale of a single cell, chondrocyte biosynthesis is primarily concentrated in the pericellular matrix [13]. Biosynthetic levels are approximately uniformly distributed around the cell in the absence of any physical stimuli [6]. Chondrocytes undergo morphological changes under static compression [3]. Cell volume and surface area decrease as higher levels of compression are imposed. The cell radius decreases in the direction of compression, while the radius in the direction perpendicular to compression remains unchanged. This deformation creates directional strain within the chondrocyte, resulting in the highest levels of biosynthesis in directions perpendicular to that of the applied compression. The directional dependence of biosynthesis becomes even more pronounced in the radial edge of the tissue compared to the center. On the other hand, the deformation pattern and magnitude of each cell remain relatively uniform across the construct.

Studies performed at the tissue and cell level suggest that alterations to chemical composition, diffusivity, and cell volume due to applied physical stimuli are the leading factors that influence chondrocyte behavior. Under static compression, cartilage tissue volume decreases due to compaction of collagen matrix. Such decrease in volume forces co-ions such as sulfate and proline to escape, increasing the concentration of counterions such as K^+ and H^+. Changes in electrochemical composition cause a decrease in interstitial pH, leading to a reduction in chondrocyte biosynthesis. In addition, molecular transport into the tissue is slowed due to a compression-induced decrease in pore size [14, 15]. As pore size decreases, transport of nutrients needed for biosynthesis becomes limited at the center of the tissue. Such limitation generates a spatially dependent biosynthetic pattern in which the radial edge displays a consistently higher biosynthetic level than the center. Furthermore, changes in cell volume in response to applied stimuli create directionally dependent cell biosynthetic activity. These phenomena explain observed changes in biosynthesis levels in cartilage tissue under static compression.

2.2.1 Mechanical Anchoring and Substrate Stiffness

Even in the absence of external stimuli, chondrocytes are sensitive to the mechanics of the surrounding extracellular matrix (ECM). Chondrocyte adhesion indicated by phenotype increases dramatically over substrate stiffness ranging from 25 kPa to 150 kPa [16]. These effects are dependent on interactions with integrins, suggesting that the cells are actively probing the matrix mechanics. Active mechanical sensing is further reiterated by studies in which cell behavior is altered by mechanical anchoring of the substrate. Static compression studies indicate that alterations to molecular transport, chemical composition, and cell volume are the major factors that influence chondrogenic biosynthesis. However, a recent study [17] has shown that mechanical properties of ECM, such as stress relaxation time and stiffness, have a significant impact on chondrocyte behavior. Chondrocytes embedded in hydrogel with faster

stress relaxation can produce up to 3 times more interconnected cartilage matrix volume and proliferate up to 6 times more than those in hydrogel with slower stress relaxation time. The effects of ECM stiffness on chondrocyte biosynthesis are not yet clear due to conflicting results [17, 18]. In addition, tissue-engineered menisci constructs that were mechanically anchored during the culture are 3 times stiffer, and collagen fibers were 50% more aligned than those that were not anchored [19].

Passive physical stimuli, such as substrate stiffness, do not alter the physicochemical properties of the tissue or chondrocyte, yet they still influence the behavior of chondrocytes. These results indicate that the chondrocyte-matrix interaction is another significant factor that impacts chondrocyte behavior, complementing static compression studies that demonstrate the importance of molecular transport, interstitial pH level, and cell deformation.

2.3 Dynamic Stimuli

2.3.1 Dynamic Compression

Studies of static stimuli on cartilage provide insights into chondrocyte behavior, but dynamic stimulus is a more physiologically realistic representation of *in vivo* loading. Superimposing cyclic loading on top of static load introduces different factors such as fluid flow, hydrostatic pressure, and streaming potential. Previous *in vivo* joint loading studies have suggested that dynamic loading may play a critical role in proteoglycan synthesis and content [20, 21]. Dynamic loading experiments (Fig. 2.1a, e) can mimic the *in vivo* loading environment of the articular cartilage and better simulate chondrocyte behavior *in vitro*. These loading conditions inherently impose both static and cyclic components where the tissue would experience the magnitude of strain and frequency. Utilizing the base knowledge and hypotheses formed from static compression studies, the influence of frequency on chondrocyte biosynthesis can be differentiated from the static component of the dynamic physical stimuli.

During a single compression-release cycle, the interstitial fluid escapes during the compression and enters the tissue during the release [22]. On this short time scale, consistent with the static physical stimulus, the proline and sulfate content in chondrocytes decreases down to 50% during the compression. However, during the release, the uptake increases up to 100%, indicating that the biosynthesis rate exceeds the pre-compression level following applied stimulus. These phenomena led to an interest in studying the effect of prolonged cyclic compression on cartilage metabolism.

Consistent and prolonged dynamic loading has different effects than single or couple compression release cycles. Sub-physiologic (0.0001 Hz) to physiologic (1 Hz) frequencies are often used for prolonged experiments (Table 2.1). Cyclic compression studies suggest that stimulus-induced amplification of biosynthesis displays a strain and frequency threshold. Frequencies of 0.01–1 Hz combined with strain amplitude of 1–5% stimulated biosynthesis levels up to 40%. Furthermore, a spatially dependent biosynthesis level is also present in dynamically stimulated tissues. At a lower frequency of 0.01 Hz with 4–7% strain, the biosynthesis rate is uniformly distributed across the tissue [23]. However, at a higher frequency of 0.1 Hz, the cartilage tissue at the radial edge has a 50% higher biosynthetic level than at the center, consistent with the observation of statically compressed samples [10]. At frequencies lower than 0.1 Hz, the interstitial pressure is uniformly distributed across the construct, creating a uniform fluid flow from the center to the outer ring of the explant. As the frequency increases, the interstitial fluid does not have adequate time to escape, and the center of the tissue becomes incompressible, causing the fluid flow to concentrate in the outer ring. Spatially dependent fluid flow creates a spatially dependent biosynthesis level. Concentration of fluid flow is further confirmed by an increase in the streaming potential in response to an increase in frequency [24]. Dynamic compression induces counterion sep-

aration, and co-ions from the separation are transported out of the tissue leading to increase in streaming potential. Collectively, these results indicate that biosynthesis stimulation is highly correlated with local interstitial fluid flow.

At the cell level, the biosynthesis level increases with dynamic loading compared to cells under free swelling condition. Frequency and spatially dependent chondrocyte biosynthesis levels are consistent with the tissue level data [6]. Chondrocytes under 0.01 Hz compression display a relatively uniform increase in biosynthesis level across constructs [10]. On the other hand, chondrocytes under 0.1 Hz display a 50% increase in biosynthesis at the radial edge, while no change is observed at the center. This trend in the biosynthetic level matches the theoretical interstitial fluid velocity and is consistent with the findings from static compression cell-level data.

Dynamic compression data collected at both the tissue and cell level indicate that interstitial fluid flow might be the most important factor in stimulating the biosynthesis of cartilage tissue. In general, dynamic compression induces interstitial fluid flow, resulting in increased streaming potential and ultimately accelerates the chondrocyte biosynthesis. There is evidence that dynamic compression helps molecular incorporation into constructs [25, 26]. In addition, biosynthesis stimulation through dynamic compression is temporally dependent [27]. Tissues under alternate day loading display up to a 30% increase in proteoglycan synthesis and a suppression of proline synthesis down to 40% compared to a continuous loading regime. This indicates that proteoglycan and proline synthesis are differently stimulated under dynamic compression. Such finding is extremely valuable as proteoglycan provides compressive mechanical strength to cartilage while collagen provides shear strength. Furthermore, biosynthesis levels vary significantly depending on the type of matrix in which chondrocytes are embedded, pointing toward the importance of chondrocyte-matrix interaction. Collectively, dynamic compression studies reveal that fluid flow is an important stimulus of chondrocyte biosynthesis.

2.3.2 Oscillatory Shear and Tension

Static and dynamic compression studies suggest that interstitial fluid flow and matrix deformation are the prominent factors that influence chondrocyte biosynthesis. These two factors are coupled under dynamic compression, as the volume change that occurs under compression generates interstitial fluid flow. In contrast, dynamic shear generates high matrix deformation with minimal interstitial fluid flow [28]. As such, imposing dynamic simple shear (Fig. 2.1b, e) can be used to differentiate the effects of matrix deformation and interstitial fluid flow on the biosynthetic activity of chondrocytes. Indeed, dynamic shear influences the biosynthetic activity of chondrocytes differently than compression. Notably, dynamic shear strain stimulates collagen synthesis two-fold more than proteoglycan synthesis. Further, tissue biosynthetic activity does not show spatial dependence [28–30], unlike the static and dynamic compressive stimuli. Importantly, dynamic shear does not promote molecular transport within the tissue, as observed under dynamic compression [30]. Collectively, these findings suggest that (1) the shear-induced ECM deformation stimulates collagen synthesis and (2) fluid flow induced by compression stimulates proteoglycan synthesis and enhances molecular transport.

Studies of the effects of dynamic tension on chondrocyte behavior have utilized a hydrogel culture system, partly due to the challenges in imposing tension on intact cartilage. Based on findings from applications of compression and shear stimuli on intact tissue, chondrocytes exposed to oscillatory tension (Fig. 2.1c, e) are expected to express an increase in collagen and proteoglycan synthesis, as this type of loading generates both matrix deformation and interstitial fluid flow [31]. Surprisingly, chondrocytes embedded in fibrin hydrogels experience a stimulation in proteoglycan synthesis with no change in collagen synthesis under dynamic tension [32]. In addition, chondrocytes harvested from different regions (superficial, middle, and deep) display different levels of biosynthetic activity in response to the same physical stimulus.

Furthermore, recent studies have suggested that the mechanical properties of cartilage zones are depth-dependent [26–29]. The zone-specific mechanical properties generate a unique micromechanical environment for chondrocytes in each zone. In fact, these differences in mechanical properties lead to differences in local strain [33], which are directly related to chondrocyte behavior [34, 35]. Differences in chondrocyte behavior, both in various cartilage zones and in native tissue versus fibrin hydrogels, confirm that chondrocytes sense matrix density and mechanics. Collectively, these findings underscore the importance of the micromechanical environment on the response of chondrocytes to external mechanical stimuli.

Dynamic stimulation studies highlight the complexity of the micromechanical environment and chondrocyte biological responses to external stimuli. In general, chondrocyte biosynthesis depends heavily on the local physical environment (Fig. 2.2a). Tissue regions that experience high levels of compression and associated interstitial fluid flow tend to show stimulated proteoglycan synthesis, while regions with high matrix deformation tend to display stimulated collagen synthesis. In fact, chondrocytes within the same construct have shown differential matrix synthesis depending on the local physical stimulus (Fig. 2.2b) [36]. In these studies, chondrocytes under local tensile strain synthesize more collagen with organized fibers. Those under the local compressive strain synthesize more proteoglycans than collagen, and the formed collagen does not contain organized fibers. Overall, oscillatory tension and shear data further confirm the critical influence of local physical environment on chondrocyte behavior.

2.3.3 Impact/Injurious Loading

It is well known that the avascular nature of cartilage hinders the tissue's natural repair capabilities. Such innate limitation in natural repair results in continuous cartilage degeneration following injuries, ultimately leading to osteoarthritis [37, 38]. Previous studies of dynamic compression reveal that compressive strain rate increases both hydrostatic pressure and matrix synthesis [10, 23, 24, 39]. In addition, chondrocytes contained within different zones react differently to the same physical stimuli [32, 40]. These findings, coupled with the ability of chondrocytes to probe the micromechanical environment, suggest that impact loading (Fig. 2.1a, f) can offer a unique perspective on the role of chondrocytes in cartilage degeneration following injurious loading.

At the tissue level, chondrocyte survivability depends heavily on the strain rate. Physical stimuli resulting in a strain rate higher than the matrix diffusion rate causes chondrocytes death at the superficial zone [41, 42], while at a strain rate lower than the matrix diffusion rate, cell death is distributed throughout the tissue [43]. Interestingly, a higher relative strain rate decreases tissue biosynthesis by 33% compared to that of a lower strain rate [44]. In addition, a higher strain rate causes surface fissures and disrupts the collagen network. Such damage results in GAG loss in tissue within 24 h following the impact [45]. During the impact, the superficial zone acts as a protective layer, where tissue without superficial zone loses three times more GAG than that with superficial zone [46]. The relationship between peak stress and total GAG loss is still unclear [45, 47]. Despite GAG loss within the tissue, impact does not affect proteoglycan synthesis. However, collagen synthesis is most likely stimulated by collagen network disruption [44]. Other factors such as insulin-like growth factor and synoviocyte co-culture can reduce GAG loss and collagen network disruption (Fig. 2.2c) [48, 49], while cytokines can accentuate tissue damage [50]. Overall, the results suggest that the injurious impact disrupts and damages the collagen network, resulting in GAG loss (Fig. 2.2d).

At the length scale of a single cell, strain imposed by impact loading is highly correlated with cell death [42]. In addition, chondrocyte death develops within 2 h after the impact and is concentrated at the superficial zone of the tissue. When the surface region is removed, chondrocyte death is distributed towards the deeper zone. This

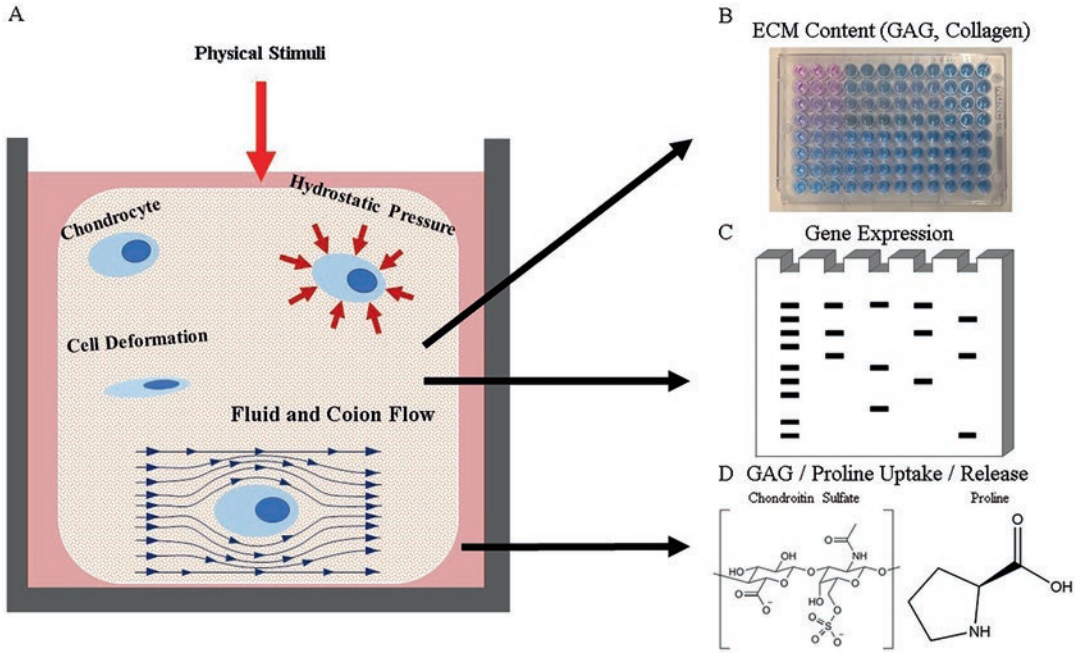


Fig. 2.2 Bulk mechanical behavior and biosynthetic analyses for cartilage. (**a**) Physical stimuli are applied to whole cartilage samples, which induce many changes including: cell deformation; increase in hydrostatic pressure; and interstitial fluid/coion flow. Changes in ECM content are determined through bulk biochemical assays and radio labeling (**b**), and changes in chondrocyte gene expression can be analyzed via Western Blot or *in situ* hybridization (**c**). Media contents of ECM components can be quantified by using biochemical assays or radio labeling (**d**)

data is consistent with tissue level data [43] and further confirms the protective role of the superficial zone [46]. Numerous studies demonstrate that such high-speed impact induces cell death over time, mostly through apoptosis [44, 51–55]. At 1 s^{-1} strain rate, 5–20% of the total cells undergo apoptosis depending on the age of the subject [56], and up to 97% of the dead cells undergo apoptosis [57], demonstrating that preventing apoptosis can potentially stop the development of post-traumatic osteoarthritis. Various factors can influence the apoptotic process. Following injury, the immune system produces pro-inflammatory cytokines, like tumor necrosis factor alpha (TNF-α), and such cytokines can induce further GAG loss [55]. On the contrary, the response to impact injury can also induce expression of several factors such as vascular endothelial growth factor, hypoxia-inducible factor, and matrix metalloproteinase [38, 52, 54, 58]. Additionally, anti-inflammatory cytokines like interleukin-10 can reduce GAG loss and apoptosis [59]. Furthermore, estrogen and antioxidants significantly reduce impact-induced cell death [46, 56], suggesting potential effects from gender and age. Despite numerous injury studies [38, 58], the mechanisms by which impact induces apoptosis are not clear.

Recent technological developments in high-speed confocal microscopy and soft tissue impact testing devices (Fig. 2.3a) enable further investigation of phenomena upstream of apoptosis. Additionally, these techniques facilitate the assessment of spatially dependent behavior of single cells on physiologic time scales (Fig. 2.3b, c). With such advances, a recent study [60] demonstrates that impact-induced chondrocyte apoptosis is caused by mitochondrial dysfunction, and mitoprotective therapy can prevent chondrocytes from undergoing apoptosis [61]. Further

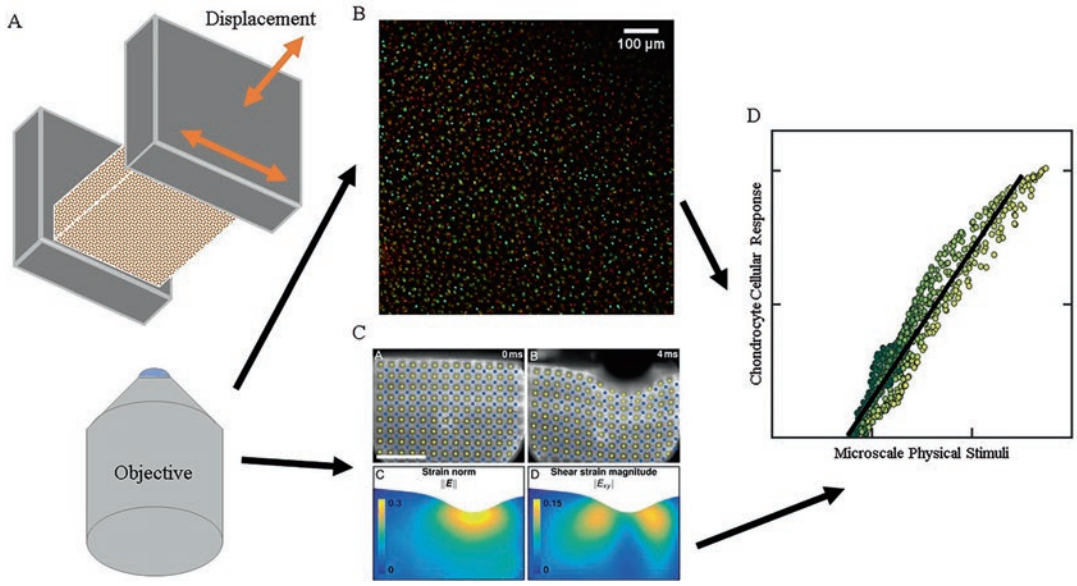


Fig. 2.3 Microscale behavior and cellular responses for cartilage. Physical stimuli are imposed (**a**) and cellular responses (**b**) and local micromechanical environment (**c**) can be measured in real-time via microscopy. Comparing physical stimuli and cellular response (**d**) enables high throughput assessment of chondrocyte mechanobiology (adapted from [42])

investigation reveals that calcium signaling, inter- and intra-cellular communication that activates mitochondrial dysfunction in response to physical stimuli, occurs within milliseconds after the impact [62]. The impact-induced chondrocyte death mechanism remains under active investigation and developing a greater understanding of this phenomenon could inform therapeutic options to prevent post-traumatic osteoarthritis.

2.4 Future Direction

2.4.1 Combined Loading

Cartilage experiences a complicated *in vivo* mechanical environment wherein mixed modes of loading are applied to the tissue. Unidirectional mechanical testing, such as compression, tension, and shear, grants only a limited understanding of the influence of local physical stimuli on chondrocyte behavior. In addition, cartilage tissue has shown that the consequence of a mode of loading can affect the tissue behavior under another mode of loading. For example, impact loading increases the surface roughness of cartilage tissue two-fold, causing the friction coefficient to increase [63], and dynamic shear can increase the secretion of lubricating molecules [35]. Understanding chondrocyte behavior under combined loading is particularly important to halt the development and progression of osteoarthritis.

Several studies have investigated the effect of combined loading on the tissue level [41, 44, 50, 64]. In general, dynamic compression followed by an injurious impact slightly promotes biosynthesis [44, 50], but only up to a threshold amplitude of 20%. In addition, injured cartilage displays elevated shear strain [64], and dynamic shear after an injury exacerbates the apoptotic behavior [41]. Chondrocyte behavior under combined loading is most likely spatially and temporarily dependent, as indicated by previous studies [3, 23, 62]. Understanding the temporally and spatially dependent chondrocyte response to combined loading could identify the mechanism of osteoarthritis progression and enable development of therapeutic options to stop the progression of osteoarthritis.

2.4.2 Big Data/Machine Learning

Recent technological development in high-speed confocal imaging techniques has enabled the capture of individual and collective cell responses to multiple modes of physical stimuli at a higher frame rate. This development has led to an explosion in the number of collectable data sets. In the late 1980s, a single cartilage explant could provide only two data points, sulfate and proline uptake [1, 22, 24]. With the advent of high-speed confocal imaging, a single sample can provide more than 2000 individual cell data points [42]. This exponential increase in collectable data sets makes individual data analysis inefficient. Utilizing machine learning would enable efficient data analysis and the categorization of cellular behavior under various types of loading. In fact, a recent study has shown the efficacy of machine learning in analyzing cell signaling and mitochondrial depolarization [62]. The combination of machine learning algorithms and mechanobiology is an uncharted territory. The innate complexity of chondrocyte behavior makes machine learning an attractive candidate for data analysis.

2.5 Conclusion

Five decades of research have led to a much greater understanding of the influence of local physical stimuli on chondrocyte behavior. The innate zonal differences and poroelastic properties of cartilage tissue create spatial- and temporal-dependent cell behavior under various types of loads. This chapter covered the progression of hypotheses for chondrocyte behavior under load and the development of associated experimental techniques. Early studies investigated cartilage biosynthesis at the tissue level under static stimulus. Long-term biosynthesis was suppressed the most at the center and the least at the edge of the tissue, revealing a spatially dependent response. Dynamic stimulus tends to increase the biosynthetic level. The spatially dependent response still exists, but only when the stimulus is at a high frequency (>0.1 Hz). Findings from studies with static and dynamic stimuli generally indicate that the micromechanical environment plays a critical role in chondrocyte behavior. Along with the development of imaging and mechanical loading techniques for soft tissues, further understanding of chondrocyte behavior has been achieved. Impact loading, combined with advanced confocal imaging techniques, indicates that chondrocyte behavior is not only spatially dependent, but also possesses temporal characteristics. Under impact loading, most of the chondrocyte death is concentrated at the superficial zone, and the apoptosis process starts within 2 h after the applied stimulus. Further research in combined loading accompanied by machine learning is required to understand chondrocyte behavior during the onset and progression of osteoarthritis. Such understanding will give insight into prevention and treatment possibilities for post-injury cartilage degeneration.

Acknowledgments We thank Serafina Lopez for providing biochemical assay image for Fig. 2.2b, Jingyang Zheng for providing chondrocyte behavior image for Fig. 2.3c, and Cara Robertus for helpful feedback on the manuscript.

References

1. Gray ML, Pizzanelli AM, Grodzinsky AJ, Lee RC (1988) Mechanical and physicochemical determinants of the chondrocyte biosynthetic response. J Orthop Res 6:777–792. https://doi.org/10.1002/jor.1100060602
2. Buschmann MD, Hunziker EB, Kim YJ, Grodzinsky AJ (1996) Altered aggrecan synthesis correlates with cell and nucleus structure in statically compressed cartilage. J Cell Sci 109:499–508. https://doi.org/10.1242/jcs.109.2.499
3. Wong M, Wuethrich P, Buschmann MD, Eggli P, Hunziker E (1997) Chondrocyte biosynthesis correlates with local tissue strain in statically compressed adult articular cartilage. J Orthop Res 15:189–196. https://doi.org/10.1002/jor.1100150206
4. Chen AC, Sah RL (1998) Effect of static compression on proteoglycan biosynthesis by chondrocytes transplanted to articular cartilage in vitro. J Orthop Res 16:542–550. https://doi.org/10.1002/jor.1100160504
5. Ragan PM, Chin VI, Hung H-HK, Masuda K, Thonar EJ-MA, Arner EC, Grodzinsky AJ, Sandy JD (2000) Chondrocyte extracellular matrix syn-

thesis and turnover are influenced by static compression in a new alginate disk culture system. Arch Biochem Biophys 383:256–264. https://doi.org/10.1006/abbi.2000.2060
6. Quinn TM, Grodzinsky AJ, Buschmann MD, Kim YJ, Hunziker EB (1998) Mechanical compression alters proteoglycan deposition and matrix deformation around individual cells in cartilage explants. J Cell Sci 111:573–583. https://doi.org/10.1242/jcs.111.5.573
7. Nia HT, Han L, Li Y, Ortiz C, Grodzinsky A (2011) Poroelasticity of cartilage at the nanoscale. Biophys J 101:2304–2313. https://doi.org/10.1016/j.bpj.2011.09.011
8. Han L, Frank EH, Greene JJ, Lee H-Y, Hung H-HK, Grodzinsky AJ, Ortiz C (2011) Time-dependent nanomechanics of cartilage. Biophys J 100:1846–1854. https://doi.org/10.1016/j.bpj.2011.02.031
9. Eisenberg SR, Grodzinsky AJ (1985) Swelling of articular cartilage and other connective tissues: electromechanochemical forces. J Orthop Res 3:148–159. https://doi.org/10.1002/jor.1100030204
10. Kim YJ, Sah RLY, Grodzinsky AJ, Plaas AHK, Sandy JD (1994) Mechanical regulation of cartilage biosynthetic behavior: physical stimuli. Arch Biochem Biophys 311:1–12. https://doi.org/10.1006/abbi.1994.1201
11. Bonassar LJ, Grodzinsky AJ, Srinivasan A, Davila SG, Trippel SB (2000) Mechanical and physicochemical regulation of the action of insulin-like growth factor-I on articular cartilage. Arch Biochem Biophys 379:57–63. https://doi.org/10.1006/abbi.2000.1820
12. Buschmann MD, Gluzband YA, Grodzinsky AJ, Hunziker EB (1995) Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. J Cell Sci 108:1497–1508. https://doi.org/10.1242/jcs.108.4.1497
13. Quinn TM, Maung AA, Grodzinsky AJ, Hunziker EB, Sandy JD (1999) Physical and biological regulation of proteoglycan turnover around chondrocytes in cartilage explants: implications for tissue degradation and repair. Ann N Y Acad Sci 878:420–441. https://doi.org/10.1111/j.1749-6632.1999.tb07700.x
14. Quinn TM, Morel V, Meister JJ (2001) Static compression of articular cartilage can reduce solute diffusivity and partitioning: implications for the chondrocyte biological response. J Biomech 34:1463–1469. https://doi.org/10.1016/S0021-9290(01)00112-9
15. Quinn TM, Kocian P, Meister J-J (2000) Static compression is associated with decreased diffusivity of dextrans in cartilage explants. Arch Biochem Biophys 384:327–334. https://doi.org/10.1006/abbi.2000.2077
16. Genes NG, Rowley JA, Mooney DJ, Bonassar LJ (2004) Effect of substrate mechanics on chondrocyte adhesion to modified alginate surfaces. Arch Biochem Biophys 422:161–167. https://doi.org/10.1016/j.abb.2003.11.023
17. Lee H, Gu L, Mooney DJ, Levenston ME, Chaudhuri O (2017) Mechanical confinement regulates cartilage matrix formation by chondrocytes. Nat Mater 16:1243–1251. https://doi.org/10.1038/nmat4993
18. Lee CR, Grodzinsky AJ, Spector M (2001) The effects of cross-linking of collagen-glycosaminoglycan scaffolds on compressive stiffness, chondrocyte-mediated contraction, proliferation and biosynthesis. Biomaterials 22:3145–3154. https://doi.org/10.1016/S0142-9612(01)00067-9
19. Puetzer JL, Koo E, Bonassar LJ (2015) Induction of fiber alignment and mechanical anisotropy in tissue engineered menisci with mechanical anchoring. J Biomech 48:1436–1443. https://doi.org/10.1016/j.jbiomech.2015.02.033
20. Bricca A, Juhl CB, Grodzinsky AJ, Roos EM (2017) Impact of a daily exercise dose on knee joint cartilage – a systematic review and meta-analysis of randomized controlled trials in healthy animals. Osteoarthr Cartil 25:1223–1237. https://doi.org/10.1016/j.joca.2017.03.009
21. Kiviranta I, Tammi M, Jurvelin J, Säämänen A-M, Helminen HJ (1988) Moderate running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee joint of young beagle dogs. J Orthop Res 6:188–195. https://doi.org/10.1002/jor.1100060205
22. Sah RL-Y, Kim Y-J, Doong J-YH, Grodzinsky AJ, Plass AHK, Sandy JD (1989) Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 7:619–636. https://doi.org/10.1002/jor.1100070502
23. Buschmann MD, Kim Y-J, Wong M, Frank E, Hunziker EB, Grodzinsky AJ (1999) Stimulation of Aggrecan synthesis in cartilage explants by cyclic loading is localized to regions of high interstitial fluid Flow1. Arch Biochem Biophys 366:1–7. https://doi.org/10.1006/abbi.1999.1197
24. Kim Y-J, Bonassar LJ, Grodzinsky AJ (1995) The role of cartilage streaming potential, fluid flow and pressure in the stimulation of chondrocyte biosynthesis during dynamic compression. J Biomech 28:1055–1066. https://doi.org/10.1016/0021-9290(94)00159-2
25. Bonassar LJ, Grodzinsky AJ, Frank EH, Davila SG, Bhaktav NR, Trippel SB (2001) The effect of dynamic compression on the response of articular cartilage to insulin-like growth factor-I. J Orthop Res 19:11–17. https://doi.org/10.1016/S0736-0266(00)00004-8
26. DiDomenico CD, Xiang Wang Z, Bonassar LJ (2017) Cyclic mechanical loading enhances transport of antibodies into articular cartilage. J Biomech Eng 139:011012. https://doi.org/10.1115/1.4035265
27. Kisiday JD, Jin M, DiMicco MA, Kurz B, Grodzinsky AJ (2004) Effects of dynamic compressive loading on chondrocyte biosynthesis in self-assembling peptide scaffolds. J Biomech 37:595–604. https://doi.org/10.1016/j.jbiomech.2003.10.005
28. Jin M, Frank EH, Quinn TM, Hunziker EB, Grodzinsky AJ (2001) Tissue shear deformation stimulates proteoglycan and protein biosynthesis in bovine cartilage explants. Arch Biochem Biophys 395:41–48. https://doi.org/10.1006/abbi.2001.2543
29. Frank EH, Jin M, Loening AM, Levenston ME, Grodzinsky AJ (2000) A versatile shear and compres-

sion apparatus for mechanical stimulation of tissue culture explants. J Biomech 33:1523–1527. https://doi.org/10.1016/S0021-9290(00)00100-7
30. Jin M, Emkey GR, Siparsky P, Trippel SB, Grodzinsky AJ (2003) Combined effects of dynamic tissue shear deformation and insulin-like growth factor I on chondrocyte biosynthesis in cartilage explants. Arch Biochem Biophys 414:223–231. https://doi.org/10.1016/S0003-9861(03)00195-4
31. Grodzinsky AJ, Roth V, Myers E, Grossman WD, Mow VC (1981) The significance of electromechanical and osmotic forces in the nonequilibrium swelling behavior of articular cartilage in tension. J Biomech Eng 103:221–231. https://doi.org/10.1115/1.3138284
32. Vanderploeg EJ, Wilson CG, Levenston ME (2008) Articular chondrocytes derived from distinct tissue zones differentially respond to in vitro oscillatory tensile loading. Osteoarthr Cartil 16:1228–1236. https://doi.org/10.1016/j.joca.2008.02.016
33. Wong BL, Bae WC, Chun J, Gratz KR, Lotz M, Sah RL (2008) Biomechanics of cartilage articulation: effects of lubrication and degeneration on shear deformation. Arthritis Rheum 58:2065–2074. https://doi.org/10.1002/art.23548
34. Bonnevie ED, Delco ML, Bartell LR, Jasty N, Cohen I, Fortier LA, Bonassar LJ (2018) Microscale frictional strains determine chondrocyte fate in loaded cartilage. J Biomech 74:72–78. https://doi.org/10.1016/j.jbiomech.2018.04.020
35. Nugent GE, Aneloski NM, Schmidt TA, Schumacher BL, Voegtline MS, Sah RL (2006) Dynamic shear stimulation of bovine cartilage biosynthesis of proteoglycan 4. Arthritis Rheum 54:1888–1896. https://doi.org/10.1002/art.21831
36. Puetzer JL, Bonassar LJ (2016) Physiologically distributed loading patterns drive the formation of zonally organized collagen structures in tissue-engineered meniscus. Tissue Eng A 22:907–916. https://doi.org/10.1089/ten.tea.2015.0519
37. Varady NH, Grodzinsky AJ (2016) Osteoarthritis year in review 2015: mechanics. Osteoarthr Cartil 24:27–35. https://doi.org/10.1016/j.joca.2015.08.018
38. Kurz B, Lemke AK, Fay J, Pufe T, Grodzinsky AJ, Schünke M (2005) Pathomechanisms of cartilage destruction by mechanical injury. Ann Anat 187:473–485. https://doi.org/10.1016/j.aanat.2005.07.003
39. Orozco GA, Tanska P, Florea C, Grodzinsky AJ, Korhonen RK (2018) A novel mechanobiological model can predict how physiologically relevant dynamic loading causes proteoglycan loss in mechanically injured articular cartilage. Sci Rep 8:1–16. https://doi.org/10.1038/s41598-018-33759-3
40. Jones ARC, Chen S, Chai DH, Stevens AL, Gleghorn JP, Bonassar LJ, Grodzinsky AJ, Flannery CR (2009) Modulation of lubricin biosynthesis and tissue surface properties following cartilage mechanical injury. Arthritis Rheum 60:133–142. https://doi.org/10.1002/art.24143
41. Ayala S, Delco ML, Fortier LA, Cohen I, Bonassar LJ (2021) Cartilage articulation exacerbates chondrocyte damage and death after impact injury. J Orthop Res 39:2130–2140. https://doi.org/10.1002/jor.24936
42. Bartell LR, Fortier LA, Bonassar LJ, Cohen I (2015) Measuring microscale strain fields in articular cartilage during rapid impact reveals thresholds for chondrocyte death and a protective role for the superficial layer. J Biomech 48:3440–3446. https://doi.org/10.1016/j.jbiomech.2015.05.035
43. Morel V, Quinn TM (2004) Cartilage injury by ramp compression near the gel diffusion rate. J Orthop Res 22:145–151. https://doi.org/10.1016/S0736-0266(03)00164-5
44. Kurz B, Jin M, Patwari P, Cheng DM, Lark MW, Grodzinsky AJ (2001) Biosynthetic response and mechanical properties of articular cartilage after injurious compression. J Orthop Res 19:1140–1146. https://doi.org/10.1016/S0736-0266(01)00033-X
45. DiMicco MA, Patwari P, Siparsky PN, Kumar S, Pratta MA, Lark MW, Kim Y-J, Grodzinsky AJ (2004) Mechanisms and kinetics of glycosaminoglycan release following in vitro cartilage injury. Arthritis Rheum 50:840–848. https://doi.org/10.1002/art.20101
46. Imgenberg J, Rolauffs B, Grodzinsky AJ, Schünke M, Kurz B (2013) Estrogen reduces mechanical injury-related cell death and proteoglycan degradation in mature articular cartilage independent of the presence of the superficial zone tissue. Osteoarthr Cartil 21:1738–1745. https://doi.org/10.1016/j.joca.2013.07.007
47. Patwari P, Cheng DM, Cole AA, Kuettner KE, Grodzinsky AJ (2007) Analysis of the relationship between peak stress and proteoglycan loss following injurious compression of human post-mortem knee and ankle cartilage. Biomech Model Mechanobiol 6:83–89. https://doi.org/10.1007/s10237-006-0037-y
48. Lee CM, Kisiday JD, McIlwraith CW, Grodzinsky AJ, Frisbie DD (2013) Synoviocytes protect cartilage from the effects of injury in vitro. BMC Musculoskelet Disord 14:54. https://doi.org/10.1186/1471-2474-14-54
49. Li Y, Wang Y, Chubinskaya S, Schoeberl B, Florine E, Kopesky P, Grodzinsky AJ (2015) Effects of insulin-like growth factor-1 and dexamethasone on cytokine-challenged cartilage: relevance to post-traumatic osteoarthritis. Osteoarthr Cartil 23:266–274. https://doi.org/10.1016/j.joca.2014.11.006
50. Li Y, Frank EH, Wang Y, Chubinskaya S, Huang H-H, Grodzinsky AJ (2013) Moderate dynamic compression inhibits pro-catabolic response of cartilage to mechanical injury, tumor necrosis factor-α and interleukin-6, but accentuates degradation above a strain threshold. Osteoarthr Cartil 21:1933–1941. https://doi.org/10.1016/j.joca.2013.08.021
51. Loening AM, James IE, Levenston ME, Badger AM, Frank EH, Kurz B, Nuttall ME, Hung H-H, Blake SM, Grodzinsky AJ, Lark MW (2000) Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Arch Biochem Biophys 381:205–212. https://doi.org/10.1006/abbi.2000.1988

52. Lee JH, Fitzgerald JB, DiMicco MA, Grodzinsky AJ (2005) Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression. Arthritis Rheum 52:2386–2395. https://doi.org/10.1002/art.21215
53. Kisiday JD, Vanderploeg EJ, McIlwraith CW, Grodzinsky AJ, Frisbie DD (2010) Mechanical injury of explants from the articulating surface of the inner meniscus. Arch Biochem Biophys 494:138–144. https://doi.org/10.1016/j.abb.2009.11.022
54. Patwari P, Cook MN, DiMicco MA, Blake SM, James IE, Kumar S, Cole AA, Lark MW, Grodzinsky AJ (2003) Proteoglycan degradation after injurious compression of bovine and human articular cartilage in vitro: interaction with exogenous cytokines. Arthritis Rheum 48:1292–1301. https://doi.org/10.1002/art.10892
55. Sui Y, Lee JH, DiMicco MA, Vanderploeg EJ, Blake SM, Hung H-H, Plaas AHK, James IE, Song X-Y, Lark MW, Grodzinsky AJ (2009) Mechanical injury potentiates proteoglycan catabolism induced by interleukin-6 with soluble interleukin-6 receptor and tumor necrosis factor α in immature bovine and adult human articular cartilage. Arthritis Rheum 60:2985–2996. https://doi.org/10.1002/art.24857
56. Kurz B, Lemke A, Kehn M, Domm C, Patwari P, Frank EH, Grodzinsky AJ, Schünke M (2004) Influence of tissue maturation and antioxidants on the apoptotic response of articular cartilage after injurious compression. Arthritis Rheum 50:123–130. https://doi.org/10.1002/art.11438
57. Patwari P, Gaschen V, James IE, Berger E, Blake SM, Lark MW, Grodzinsky AJ, Hunziker EB (2004) Ultrastructural quantification of cell death after injurious compression of bovine calf articular cartilage. Osteoarthr Cartil 12:245–252. https://doi.org/10.1016/j.joca.2003.11.004
58. Pufe T, Lemke A, Kurz B, Petersen W, Tillmann B, Grodzinsky AJ, Mentlein R (2004) Mechanical overload induces VEGF in cartilage discs via hypoxia-inducible factor. Am J Pathol 164:185–192. https://doi.org/10.1016/S0002-9440(10)63109-4
59. Behrendt P, Preusse-Prange A, Klüter T, Haake M, Rolauffs B, Grodzinsky AJ, Lippross S, Kurz B (2016) IL-10 reduces apoptosis and extracellular matrix degradation after injurious compression of mature articular cartilage. Osteoarthr Cartil 24:1981–1988. https://doi.org/10.1016/j.joca.2016.06.016
60. Delco ML, Bonnevie ED, Bonassar LJ, Fortier LA (2018) Mitochondrial dysfunction is an acute response of articular chondrocytes to mechanical injury. J Orthop Res 36:739–750. https://doi.org/10.1002/jor.23651
61. Delco ML, Bonnevie ED, Szeto HS, Bonassar LJ, Fortier LA (2018) Mitoprotective therapy preserves chondrocyte viability and prevents cartilage degeneration in an ex vivo model of posttraumatic osteoarthritis. J Orthop Res 36:2147–2156. https://doi.org/10.1002/jor.23882
62. Zheng J, Jackson T, Fortier LA, Bonassar LJ, Delco ML, Cohen I (2020) Establishing the peracute relationship between calcium signaling and mitochondrial depolarization after impact injury to articular cartilage. ORS 2020 Annual Meeting No 0191
63. Bonnevie ED, Delco ML, Galesso D, Secchieri C, Fortier LA, Bonassar LJ (2017) Sub-critical impact inhibits the lubricating mechanisms of articular cartilage. J Biomech 53:64–70. https://doi.org/10.1016/j.jbiomech.2016.12.034
64. Wong BL, Kim SHC, Antonacci JM, McIlwraith CW, Sah RL (2010) Cartilage shear dynamics during tibio-femoral articulation: effect of acute joint injury and tribosupplementation on synovial fluid lubrication. Osteoarthr Cartil 18:464–471. https://doi.org/10.1016/j.joca.2009.11.008
65. Gray ML, Pizzanelli AM, Lee RC, Grodzinsky AJ, Swann DA (1989) Kinetics of the chondrocyte biosynthetic response to compressive load and release. Biochim Biophys Acta Gen Subj 991:415–425. https://doi.org/10.1016/0304-4165(89)90067-6
66. Davisson T, Kunig S, Chen A, Sah R, Ratcliffe A (2002) Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. J Orthop Res 20:842–848. https://doi.org/10.1016/S0736-0266(01)00160-7
67. Grodzinsky AJ, Levenston ME, Jin M, Frank EH (2000) Cartilage tissue remodeling in response to mechanical forces. Annu Rev Biomed Eng 2:691–713. https://doi.org/10.1146/annurev.bioeng.2.1.691
68. Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH, Hunziker EB (1992) Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. J Orthop Res 10:745–758. https://doi.org/10.1002/jor.1100100602
69. Thibault M, Robin Poole A, Buschmann MD (2002) Cyclic compression of cartilage/bone explants in vitro leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments. J Orthop Res 20:1265–1273. https://doi.org/10.1016/S0736-0266(02)00070-0
70. Hunter CJ, Mouw JK, Levenston ME (2004) Dynamic compression of chondrocyte-seeded fibrin gels: effects on matrix accumulation and mechanical stiffness. Osteoarthr Cartil 12:117–130. https://doi.org/10.1016/j.joca.2003.08.009
71. Mouw JK, Connelly JT, Wilson CG, Michael KE, Levenston ME (2007) Dynamic compression regulates the expression and synthesis of chondrocyte-specific matrix molecules in bone marrow stromal cells. Stem Cells 25:655–663. https://doi.org/10.1634/stemcells.2006-0435
72. Lee B, Han L, Frank EH, Chubinskaya S, Ortiz C, Grodzinsky AJ (2010) Dynamic mechanical properties of the tissue-engineered matrix associated with individual chondrocytes. J Biomech 43:469–476. https://doi.org/10.1016/j.jbiomech.2009.09.053
73. Zhang L, Miramini S, Smith DW, Gardiner BS, Grodzinsky AJ (2015) Time evolution of deformation in a human cartilage under cyclic loading. Ann Biomed Eng 43:1166–1177. https://doi.org/10.1007/s10439-014-1164-8

74. Lee CM, Kisiday JD, McIlwraith CW, Grodzinsky AJ, Frisbie DD (2013) Development of an in vitro model of injury-induced osteoarthritis in cartilage explants from adult horses through application of single-impact compressive overload. Am J Vet Res 74:40–47. https://doi.org/10.2460/ajvr.74.1.40

75. Rolauffs B, Muehleman C, Li J, Kurz B, Kuettner KE, Frank E, Grodzinsky AJ (2010) Vulnerability of the superficial zone of immature articular cartilage to compressive injury. Arthritis Rheum 62:3016–3027. https://doi.org/10.1002/art.27610

Multiscale *In Silico* Modeling of Cartilage Injuries

3

Rami K. Korhonen, Atte S. A. Eskelinen, Gustavo A. Orozco, Amir Esrafilian, Cristina Florea, and Petri Tanska

Abstract

Injurious loading of the joint can be accompanied by articular cartilage damage and trigger inflammation. However, it is not well-known which mechanism controls further cartilage degradation, ultimately leading to post-traumatic osteoarthritis. For personalized prognostics, there should also be a method that can predict tissue alterations following joint and cartilage injury. This chapter gives an overview of experimental and computational methods to characterize and predict cartilage degradation following joint injury. Two mechanisms for cartilage degradation are proposed. In (1) biomechanically driven cartilage degradation, it is assumed that excessive levels of strain or stress of the fibrillar or non-fibrillar matrix lead to proteoglycan loss or collagen damage and degradation. In (2) biochemically driven cartilage degradation, it is assumed that diffusion of inflammatory cytokines leads to degradation of the extracellular matrix. When implementing these two mechanisms in a computational *in silico* modeling workflow, supplemented by *in vitro* and *in vivo* experiments, it is shown that biomechanically driven cartilage degradation is concentrated on the damage environment, while inflammation via synovial fluid affects all free cartilage surfaces. It is also proposed how the presented *in silico* modeling methodology may be used in the future for personalized prognostics and treatment planning of patients with a joint injury.

Keywords

Cartilage · Injury · Modeling · Loading · Degradation

3.1 Introduction

Abnormal loading of the joint is one of the most common risk factors of osteoarthritis (OA) (Fig. 3.1). Injurious loading of the joint may cause damage to articular cartilage or other joint tissues, possibly resulting in excessive forces or deformations in specific regions of the joint surfaces. Subsequently, these processes may lead to articular cartilage degeneration and post-traumatic OA [2, 3]. Joint injury can also trigger inflammation and increase expression of aggrecanases (such as a dis-

R. K. Korhonen (✉) · A. S. A. Eskelinen
A. Esrafilian · C. Florea · P. Tanska
Department of Technical Physics, University of Eastern Finland, Kuopio, Finland
e-mail: rami.korhonen@uef.fi

G. A. Orozco
Department of Technical Physics, University of Eastern Finland, Kuopio, Finland

Department of Biomedical Engineering, Lund University, Lund, Sweden

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_3

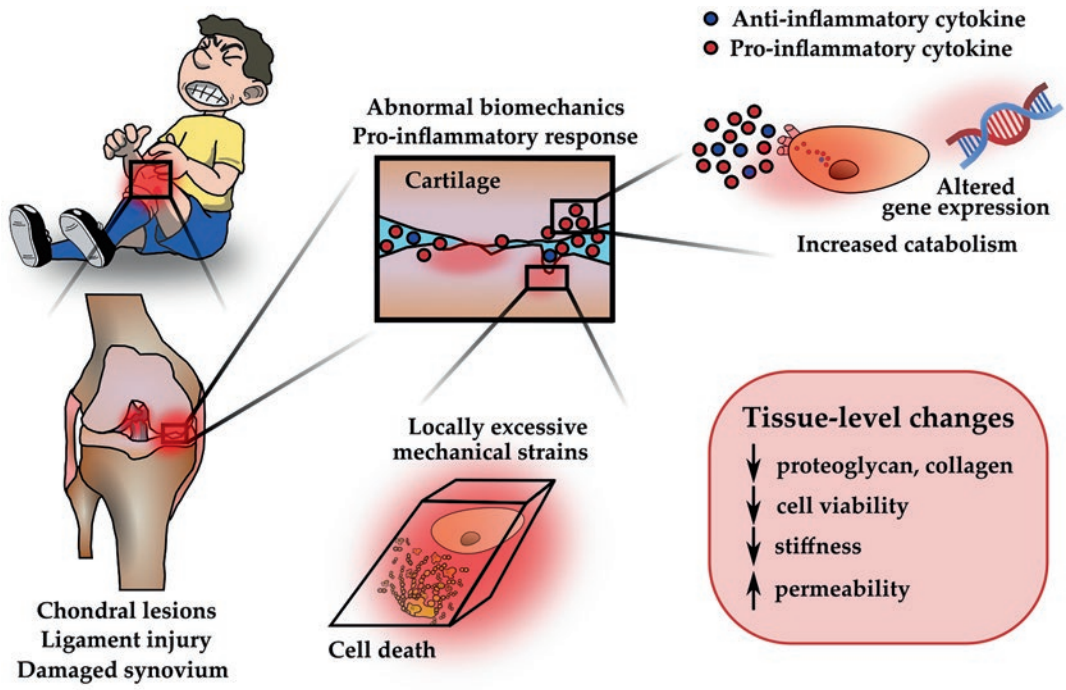


Fig. 3.1 Overview of cartilage degradation mechanisms triggered by a joint injury. An injury may result in lesions on articular cartilage surfaces, ligament tearing, and synovium damage. Together, these damages promote a catabolic joint environment encompassing abnormal biomechanical loading patterns and pro-inflammatory cytokines diffusing into cartilage. The former could lead to locally elevated mechanical strains or stresses, suggested to lead to cell death, collagen network damage and PG loss. It can also lead to release of reactive oxygen species, and cell death due to necrosis (acute) and apoptosis (persisting abnormal loading). The latter mechanism upregulates catabolic and suppresses anabolic gene expression in chondrocytes. Ultimately, injured cartilage exhibits loss of PG and collagen contents, lower cell viability, smaller stiffness, and higher permeability compared to healthy cartilage [15, 26, 46]

integrin and metalloproteinase with thrombospondin motifs, ADAMTS-4,5) [35] and collagenases (such as matrix metalloproteinase, MMP-1,13) [58], degrading the extracellular matrix of cartilage, particularly collagen and proteoglycans (PGs). However, the relationship between biomechanically and biochemically driven deterioration of injured cartilage and progression of post-traumatic OA is not well known. Moreover, prevention and personalized treatment of OA is possible only if the disease progression can be predicted. In this chapter, we provide evidence for both degeneration mechanisms through multiscale *in vitro* and *in vivo* experiments and *in silico* finite element (FE) modeling. We also showcase *in silico* modeling approaches for personalized prediction of OA progression. Generally, for more detailed understanding, we refer to specific publications in each sub-chapter.

3.2 Experiments to Study Tissue Alterations Following Cartilage Injury

3.2.1 General

In order to understand biomechanically and biochemically driven mechanisms leading to cartilage degradation in detail, *in vitro* experiments have often been conducted [8, 23]. In contrast to *in vivo* animal model experiments or clinical studies, in *in vitro* measurement setups one can fully control both biomechanical and biochemical environments of the samples.

3.2.2 Setup

A typical *in vitro* measurement setup to study tissue alterations following cartilage injury has been described in Fig. 3.2. Here, articular cartilage plugs were subjected to injurious loading under unconfined compression (50–65% strain amplitude, 100–400%/s strain rate), often producing small cracks on the cartilage surface [9, 11, 21, 38, 40, 53]. This was followed by cyclic (dynamic) loading (10–30% strain amplitude, 0.5–1 Hz loading frequency, haversine waveform) and interleukin (IL)-1-challenge (1 ng/ml) for up to 24 days, both separately and combined. For the cyclic loading, 1 h loading periods with 3–10 h resting periods were applied [9, 23, 38].

3.2.3 Analysis of Structure and Composition

There are several methods to analyze alterations in cartilage structure and composition following injury. Biochemical methods have often been used to analyze glycosaminoglycan and collagen contents of the samples (dimethylmethylene blue and hydroxyproline assays, respectively [24]). Polarized light microscopy has been used to determine changes in the collagen fibril network, namely collagen fibril orientation. Fourier transform infrared imaging has been performed to quantify the spatial collagen content in cartilage, while digital densitometry analysis of Safranin-O-stained sections is suitable for evaluation of

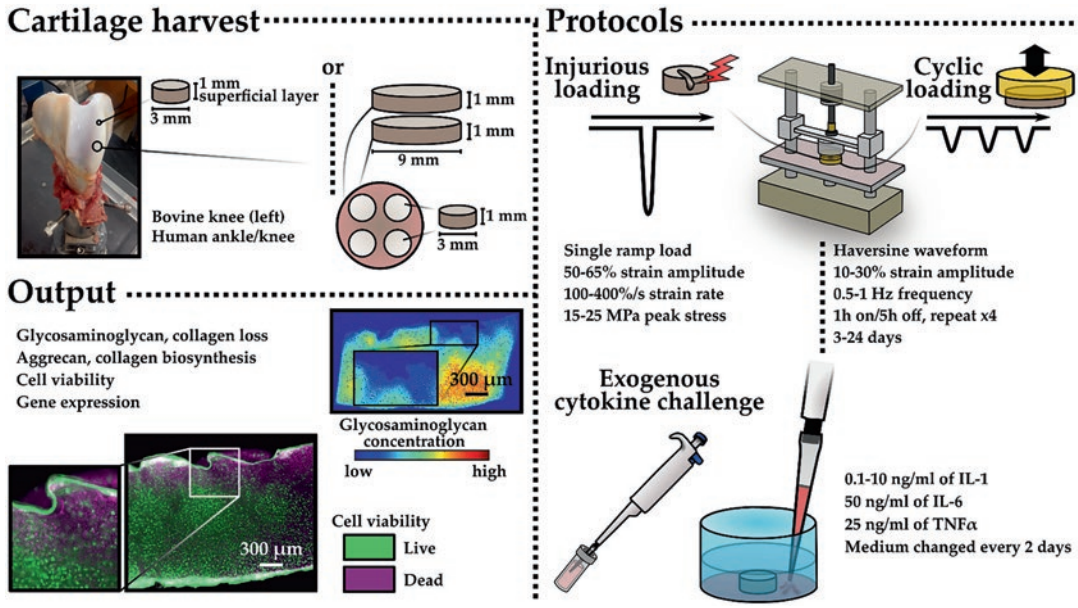


Fig. 3.2 Experimental tissue explant models of post-traumatic osteoarthritis. Cylindrical articular cartilage plugs (thickness 1 mm, diameter 3 mm) have typically been harvested from knee and ankle joints of calves and humans *post mortem*. Two controlled biomechanical loading protocols have widely been used in the *in vitro* models. The first is single injurious compressive loading in unconfined compression, leading into formation of cartilage cracks in the superficial zone. The second is cyclic (dynamic) loading mimicking daily walking, exhibiting physiological strain amplitudes and loading frequencies. To induce biochemical degradation and inflammation, exogenous administration of interleukin (IL)-1, IL-6, and/or tumor necrosis factor α (TNFα) has been used. After subjecting cartilage plugs to biomechanical loading, their PG and collagen contents and depth-wise distributions, collagen network architecture, aggrecan and collagen biosynthesis rates, cell viability, and gene expression, focusing on genes such as aggrecan and IL-1, can be analyzed [8, 9, 23, 25, 38]

the spatial PG content of the tissue. For more details, see for instance [27, 36].

3.2.4 Biological Analysis

Cell viability assays (fluorescent staining) have been used to analyze the percentage of dead cells. Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) is a technique for investigation of gene expression in cartilage, targeting factors such as aggrecan and IL-1 [23]. On the other hand, aggrecan and collagen biosynthesis rates can be analyzed by ^{35}S-sulfate and ^{3}H-proline incorporation [45].

3.3 *In Silico* Models for Understanding Mechanisms Leading to Cartilage Degeneration

3.3.1 General

There are several constitutive material models in the literature that can characterize cartilage mechanics in different loading scenarios. Briefly, traditional poroelastic and biphasic models can distinguish between solid and fluid phases [32, 48]. When combined with anisotropic properties of the solid matrix, these models can also characterize tension–compression nonlinearity and high fluid pressurization under rapid loading conditions. Later developed fibril-reinforced poroelastic and poroviscoelastic models are able to separate the fibrillar network from the non-fibrillar matrix, and can even consider swelling of cartilage due to fixed charge density (FCD) of PGs [20, 60]. In the latter model, the total stress is given by

$$\begin{aligned}\boldsymbol{\sigma}_{\mathrm{tot}} &= \boldsymbol{\sigma}_{\mathrm{f}} + \boldsymbol{\sigma}_{nf} - p\mathbf{I} - T_{\mathrm{c}}\mathbf{I} \\ &= \boldsymbol{\sigma}_{\mathrm{f}} + \boldsymbol{\sigma}_{nf} - \Delta\pi\mathbf{I} - \mu^{\mathrm{f}}\mathbf{I} - T_{\mathrm{c}}\mathbf{I},\end{aligned} \tag{3.1}$$

where $\boldsymbol{\sigma}_{\mathrm{tot}}$ is the total stress tensor, $\boldsymbol{\sigma}_{\mathrm{f}}$ and $\boldsymbol{\sigma}_{\mathrm{nf}}$ are the stress tensors of the fibrillar and non-fibrillar matrices, respectively, p and $\Delta\pi$ are the hydrostatic and swelling pressures, respectively, $\mathbf{I}$ is the unit tensor, μ^{f} is the chemical potential of water, and T_{c} is the chemical expansion stress. In this equation, $\boldsymbol{\sigma}_{\mathrm{f}}$ is directly affected by the collagen volume fraction.

These highly nonlinear material models have been implemented using finite element (FE) analysis and recently applied to generate adaptive algorithms for prediction of tissue alterations due to abnormal biomechanical or biochemical environment of knee joint, cartilage, and chondrocytes [11, 17, 31, 55]. In these models, it is first assumed that the amount of a certain constituent of the tissue (particularly collagen and PGs, or FCD of PGs, or their biomechanical properties) can change over time depending on the local mechanical (stress or strain) or biochemical (amount of inflammatory cytokines) environment. A brief overview of biomechanically and biochemically driven cartilage degradation mechanisms is given in the following.

3.3.2 Theory

Part I — Biomechanically driven degradation: Biomechanically driven degradation models of cartilage first assume that overloading (stress or strain) can lead to cell death, altered tissue properties and OA [31, 47, 49]. In this approach, excessive shear or deviatoric strains of over 30% have been suggested to lead to cell death and FCD loss or non-fibrillar matrix softening, while excessive collagen fibril strains (>8%) or maximum principal stresses (>7 MPa) have been suggested to lead to collagen fibril damage and softening. The former affects directly $\Delta\pi$ and T_{c} in Eq. (3.1) and reduces swelling pressure in the tissue or softens the tissue by reducing $\boldsymbol{\sigma}_{\mathrm{nf}}$. The latter mechanism reduces $\boldsymbol{\sigma}_{\mathrm{f}}$ in the same equation. See more detailed mechanisms and implementation from [16, 31, 38].

In the degradation and damage algorithms, collagen fibrils can also adapt to the changing mechanical environment and bend toward maximum principal strain directions [55], simulating collagen fibril reorientation in OA. In addition,

PGs can be released directly through the tissue surface through fluid expulsion, particularly through a lesion surface where the collagen network is damaged [38, 57].

Part II — Diffusion-based biochemical degradation: In this model, the inflammatory cytokines are assumed to regulate the behavior of chondrocytes and subsequently the cartilage constituent biosynthesis and degradation [17]. The cytokines bind to corresponding receptors on the cell surface. This triggers signaling cascades within the cell which results in increased expression of aggrecanases (such as ADAMTS-4,5) and collagenases (such as MMP-1,13) which can then act in the pericellular and extracellular matrices [28, 35, 58]. Furthermore, there are tissue inhibitors of metalloproteinases (TIMPs), which inhibit the activity of ADAMTS and MMPs [35]. However, the activity of TIMPs either remains unchanged or is down-regulated by the cytokines [54]. Ultimately, when the degrading factors outweigh the matrix biosynthesis and repair, this biochemical process leads to accelerated loss of aggrecan and/or collagen.

These biochemical processes have been implemented in mechanobiological models by using reaction–diffusion partial differential equations [11, 17], which can be written as:

$$\frac{\partial C_i}{\partial t} = D_i \nabla^2 C_i \pm R_i, \tag{3.2}$$

where C_i is concentration of the constituent i (*e.g.,* chondrocyte, aggrecan, collagen, cytokine), D_i is the effective diffusivity of chemical species i, and R_i is the corresponding source–sink term, which describes the rate of generation/repair or degradation/apoptosis/consumption of individual species. Aggrecan and collagen concentration can then be linked with FCD and collagen volume fraction in Eq. (3.1), affecting directly $\Delta\pi$ and T_c or $\boldsymbol{\sigma}_f$, respectively.

In Fig. 3.3, see an example of implementation of these two degradation mechanisms in a mechanobiological model and how the model has shown to produce results comparable to experimental findings.

3.4 From *In Vitro* to *In Vivo*

3.4.1 General

In silico modeling of cartilage lesions *in vivo* includes several multiscale steps. First, clinical imaging is needed to generate the model geometry. For loading input, motion capture is needed and supplemented by musculoskeletal (MS) modeling. *In vitro* data and validated soft tissue models can then be implemented to capture biomechanically and biochemically driven degradation mechanisms of cartilage. Finally, the FE model is generated and simulated based on the input information, and the predictions are compared with literature or personalized imaging data. To get a better idea of the workflow, an example is given below (see also Fig. 3.4).

3.4.2 *In Vivo* Experiments

In a study by [37], magnetic resonance imaging (MRI) and motion analysis were conducted for subjects with anterior cruciate ligament (ACL) injury and reconstruction. Changes in $T_{1\rho}$ and T_2 relaxation times and kinematics of the subjects' knees were followed for 3 years post-surgery. $T_{1\rho}$ is generally assumed to relate with PG content, while T_2 has often been associated with collagen orientation of cartilage [41, 52]. Cone-beam computed tomography (CBCT) has also been used to image cartilage injuries [18, 43]. It can provide better resolution than MRI but has not shown capabilities for specific evaluation of cartilage structure and composition.

3.4.3 *In Vivo* FE Analysis

MRI and motion capture data at the 1-year follow-up time point were used to generate computational MS-FE models of knees [37]. Cartilage was modeled similarly as in the *in vitro* model, including biomechanically (excessive shear strains) and biochemically (diffusion of IL-1) driven degradation mechanisms. Simulation results of FCD loss

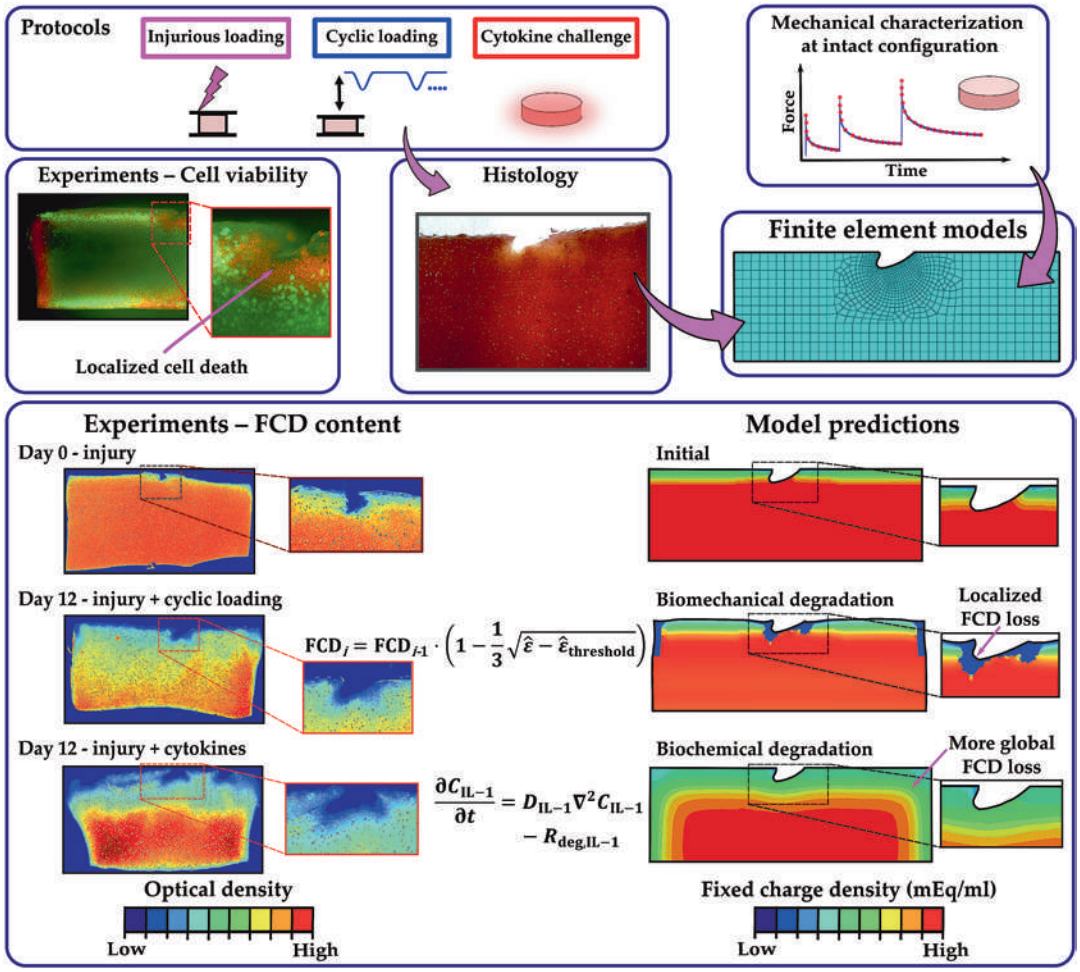


Fig. 3.3 Tissue-level *in vitro* modeling of cartilage injuries. In these examples, injurious loading experiments were simulated by an adaptive fibril-reinforced poroelastic finite element model [11, 38, 60]. Two cartilage degradation mechanisms were implemented. Biomechanically driven degradation assumed that shear strains over a threshold of 32–50% induce apoptosis and fixed charge density (FCD) loss. Biochemically driven degradation simulated diffusion of pro-inflammatory cytokine interleukin (IL)-1 (1 ng/ml) into cartilage and subsequent FCD loss. Simulated and experimental FCD losses were compared [11, 38]. (Material from: Orozco et al. [38])

were compared with changes in $T_{1\rho}$ and T_2 times during the follow-up. Similarly, *in vivo* CBCT imaging has been used to generate FE models of knees for evaluation of altered biomechanics related to cartilage injuries [34].

3.4.4 Summary from *In Vitro* and *In Vivo* Studies

Based on these selected experimental and computational studies, *in vitro* and *in vivo* results showed local FCD loss around cartilage lesions when the biomechanically driven cartilage degradation was applied. On the other hand, IL-1 diffusion via synovial fluid and subsequent FCD loss were more global and observed on the free cartilage surfaces [10, 11, 37, 38]. Therefore, it was suggested that biomechanically and biochemically driven cartilage degradation mechanisms occur simultaneously in post-traumatic OA, but they affect cartilage structure and composition differently in a location-specific manner. These two mechanisms may also have a different

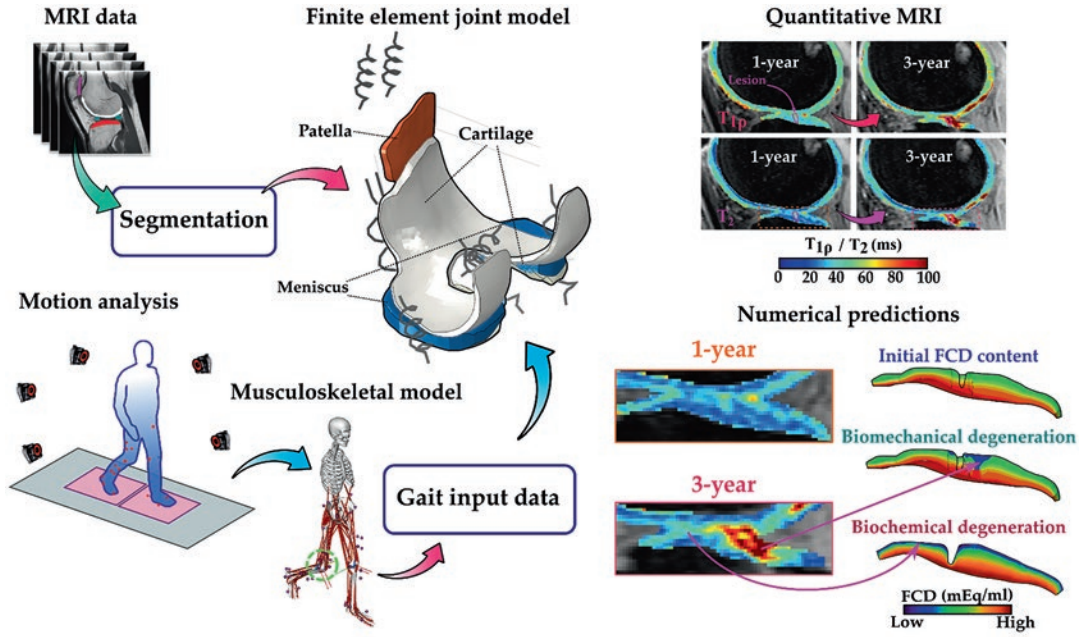


Fig. 3.4 Multiscale *in vivo* modeling of cartilage injuries. Based on *in vitro* data, validated soft tissue models and degradation mechanisms, loading scenarios, and clinical imaging, an MS-FE model was developed [37]. As can be seen on the bottom-right, biomechanically and biochemically driven degradation mechanisms predicted different locations for fixed charge density (FCD) loss (very localized *vs.* more global, respectively). These results suggest that altered biomechanics regulates tissue composition around the cartilage injury while pro-inflammatory cytokines affect all surfaces in contact with synovial fluid. (Material from: Orozco et al. [37, 39])

time-dependent response since the concentrations of cytokines vary greatly between the early acute phase after injury compared to possible later chronic phase. The introduced model could be used to estimate the effect of biomechanical and biochemical interventions on the subsequent cartilage degradation.

3.5 Toward a Clinical Assessment Tool to Aid Decision Making

Modeling workflows presented in this chapter do not yet provide any aid for clinicians to support their decision making. For this reason, all the steps in model generation and simulation should become fast and reliable. For this task, all modeling steps, including generation of the model geometry and mesh, implementation of loading and material properties, and simulation, should be automatic or at the very least semi-automatic.

Incorporating the aforementioned and complex material models requires a well-structured and precise FE mesh to be able to correctly implement different tissue constituents (*e.g.*, collagen fibril orientation and density, and fluid fraction), and also to successfully converge the FE analysis. In addition, the numerical convergence of an FE model that includes several contact-pairs, complex geometries and loading conditions, and especially large deformations of highly nonlinear materials, depends heavily on the mesh quality. Therefore, there have been attempts to develop rapid state-of-the-art MS-FE modeling and simulation pipelines, potentially feasible for clinical applications to investigate joint- and tissue-level knee mechanics in different functional activities. One of those approaches is an atlas-based FE modeling toolbox [30] along with an electromyography (EMG)-assisted, muscle force-driven MS-FE analysis workflow [12]. In this approach, based on certain anatomical

dimensions of the joint, the existing template model is scaled to match the corresponding dimensions of an individual patient. This process provides a personalized model geometry and mesh and takes only a few minutes, underlining the potential clinical applicability. The generated model is then supplemented by muscle forces, joint contact forces, and moments, as well as automatic implementation of the material properties of the soft tissues. To showcase the usability of the pipeline to estimate joint cartilage stresses and strains, indicative of tissue health and degradation, examples of simulation results of daily activities and rehabilitation exercises are given in Fig. 3.5. For more details, see Refs. [12, 13].

When supplementing this pipeline with adaptive modeling of cartilage health and degradation, as shown in previous sections, one can design personalized daily activity or rehabilitation protocols to avoid further cartilage degradation and progression of osteoarthritis.

3.6 Future Plans

In addition to the aforementioned mechanisms of cartilage degradation, high shear strains near chondral lesions may also lead to necrosis [51] and apoptosis via abrupt and excessive deformation of cell membrane and increased levels of reactive oxygen species (ROS) [5, 29]. Evidence suggests that these cell death mechanisms also result ultimately in PG loss via release of damage-associated molecular patterns and aggrecanases, ROS-amplified oxidative stress, and inflammatory response [1, 22, 29]. In the light of the cell-level experimental findings, it is now widely accepted that elevated pro-inflammatory factors and subsequent catabolic cell responses play a key role in the pathogenesis of post-traumatic OA [61]. There is also evidence that the pericellular matrix acts as a transducer of biochemical and biomechanical signals for chondrocytes, regulating their metabolic activity in response to environmental signals [6, 7, 14]. Alterations in the pericellular matrix properties and cell–matrix interactions may also contribute to OA initiation and progression. Currently, next-generation *in silico* models are under development considering both cell death and ROS-activity, as well as other introduced mechanisms in this chapter, and these models could help better understand post-traumatic OA progression and possible recovery of the PG content in temporally changing mechanobiological environments [19, 33].

No consensus exists whether there is an association between symptomatic and radiographic OA [50, 59]. Since cartilage does not have nerves, pain is often not associated with the structural progression of OA until at later disease stages, but is rather related to other tissues, such as bone and ligaments, or to inflammation. However, mechanisms of pain are still an unexplored topic in the field of computational modeling, and they should be known before implementing them in any *in silico* modeling framework.

While the development and validation of high-fidelity and highly detailed predictive models is essential to improve the understanding of mechanisms leading to OA, the development of artificial intelligence (AI)-based models is needed for fast prediction. There are sophisticated AI-based methods for diagnosis of OA [4, 44, 56] and real-time simulation of joint contact forces [42]. Fed by personalized information, such methods could be applied for fast and even real-time prediction of OA progression and simulation of the effects of interventions, pushing towards a more low-fidelity and simpler, but as accurate as the high-fidelity, tool for clinical use. When supplemented with rapid X-ray imaging, wearables, and 2D video imaging rather than MRI and extensive 3D motion capture, the future *in silico* models could provide a means for an out-of-lab setting where clinical environment would not be needed to obtain prognosis and enable monitoring. This could best enable informed patient participation in self-management of lifestyle and physical activity interventions, which is a crucial factor in prevention or delay of the progression of OA and even more importantly in improving the patients' quality of life.

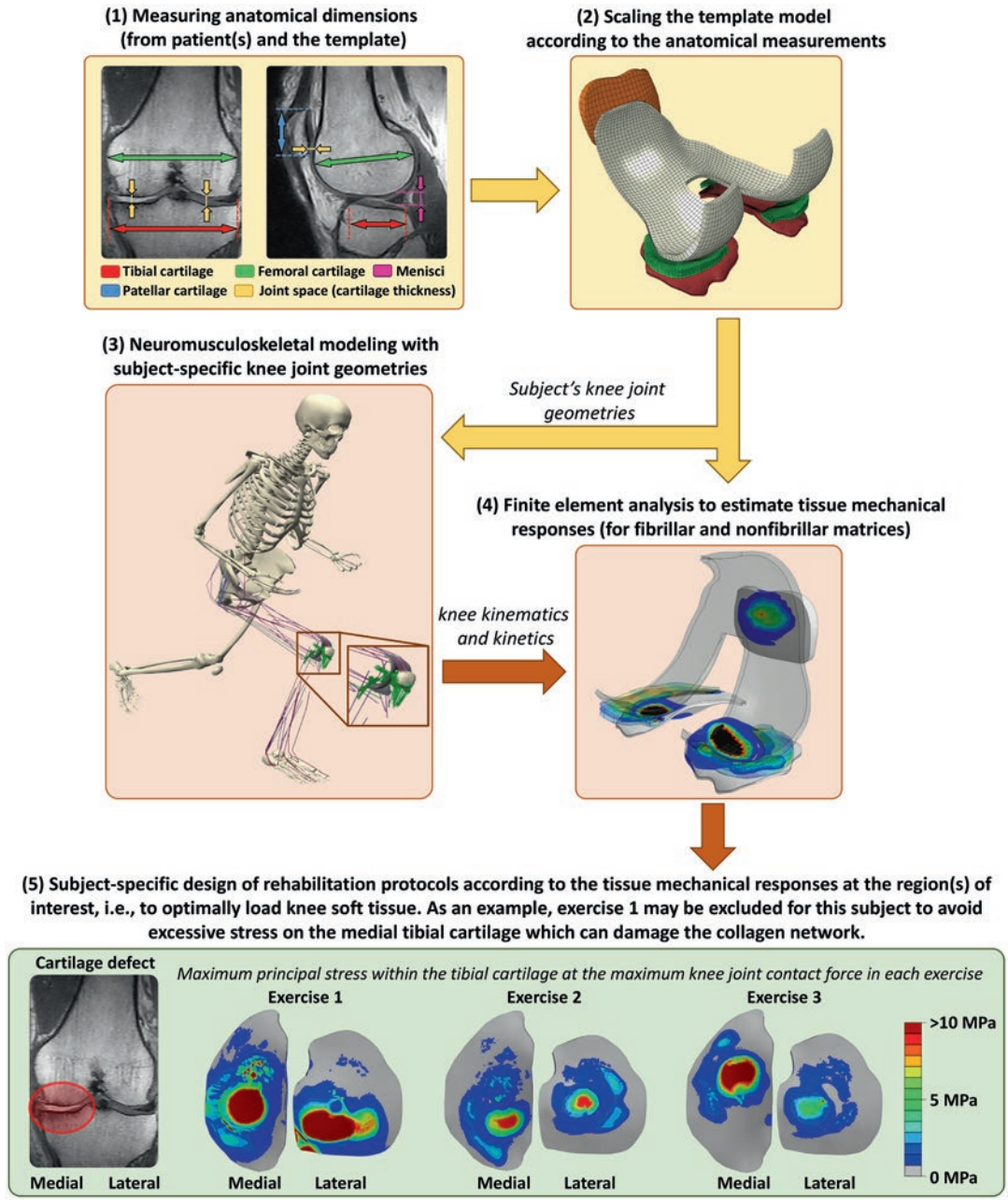


Fig. 3.5 Atlas-based rapid MS-FE modeling, toward a clinical assessment tool to aid decision making. (1): Anatomical dimensions are measured from subject's and the template's medical images, such as MRI. (2): The template FE model (*i.e.*, meshed geometries) are anisotropically scaled according to the anatomical dimensions. Note that the template FE model contains the fibril-reinforced poroviscoelastic material model, contact pairs, *etc.*, enabling rapid generation of the subject's FE model. (3): Neuromusculoskeletal modeling is used to estimate subject's kinematics, muscle forces, and joint contact forces to provide the FE model with subject-specific inputs. The MS model can incorporate subject's muscle activation patterns (*i.e.*, measured by electromyography) and subject's knee joint geometries (obtained from the scaled FE model) within the analysis. (4): Using joint kinematics and kinetics from neuromusculoskeletal modeling, FE analysis is used to estimate tissue-level joint mechanics for fibrillar (collagen network) and non-fibrillar (PGs) matrices. (5): The estimated tissue mechanics in different rehabilitation exercises can be used to assist clinicians with decision making, *i.e.*, designing subject-specific rehabilitation protocols to avoid excessive loading and accelerated degradation of the joint cartilage regions with defects. (For more details, see Refs. [12, 13])

References

1. Ansari MY, Ahmad N, Haqqi TM (2020) Oxidative stress and inflammation in osteoarthritis pathogenesis: role of polyphenols. Biomed Pharmacother 129:110452. https://doi.org/10.1016/j.biopha.2020.110452
2. Arokoski JPA, Jurvelin JS, Väätäinen U, Helminen HJ (2000) Normal and pathological adaptations of articular cartilage to joint loading. Scand J Med Sci Sports 10:186–198. https://doi.org/10.1034/j.1600-0838.2000.010004186.x
3. Bader DL, Salter DM, Chowdhury TT (2011) Biomechanical influence of cartilage homeostasis in health and disease. Arthritis 979032:1–16. https://doi.org/10.1155/2011/979032
4. Brahim A, Jennane R, Riad R et al (2019) A decision support tool for early detection of knee OsteoArthritis using X-ray imaging and machine learning: data from the OsteoArthritis initiative. Comput Med Imaging Graph 73:11–18. https://doi.org/10.1016/j.compmedimag.2019.01.007
5. Brouillette MJ, Ramakrishnan PS, Wagner VM et al (2014) Strain-dependent oxidant release in articular cartilage originates from mitochondria. Biomech Model Mechanobiol 13:565–572. https://doi.org/10.1007/s10237-013-0518-8
6. Chery DR, Han B, Li Q et al (2020) Early changes in cartilage pericellular matrix micromechanobiology portend the onset of post-traumatic osteoarthritis. Acta Biomater 111:267–278. https://doi.org/10.1016/j.actbio.2020.05.005
7. Chery DR, Han B, Zhou Y et al (2021) Decorin regulates cartilage pericellular matrix micromechanobiology. Matrix Biol 96:1–17. https://doi.org/10.1016/j.matbio.2020.11.002
8. DiMicco M, Patwari P, Siparsky P et al (2004) Mechanisms and kinetics of glycosaminoglycan release following in vitro cartilage injury. Arthritis Rheum 50:840–848. https://doi.org/10.1002/art.20101
9. Eskelinen A, Florea C, Tanska P et al (2022) Cyclic loading regime considered beneficial does not protect injured and interleukin-1-inflamed cartilage from post-traumatic osteoarthritis. J Biomech 141:111181. https://doi.org/10.1016/j.jbiomech.2022.111181
10. Eskelinen ASA, Mononen ME, Venäläinen MS et al (2019) Maximum shear strain-based algorithm can predict proteoglycan loss in damaged articular cartilage. Biomech Model Mechanobiol 18:753–778. https://doi.org/10.1007/s10237-018-01113-1
11. Eskelinen ASA, Tanska P, Florea C et al (2020) Mechanobiological model for simulation of injured cartilage degradation via proinflammatory cytokines and mechanical stimulus. PLoS Comput Biol 16:1–25. https://doi.org/10.1371/journal.pcbi.1007998
12. Esrafilian A, Stenroth L, Mononen ME et al (2020a) 12 degrees of freedom muscle force driven fibril-reinforced poroviscoelastic finite element model of the knee joint. IEEE Trans Neural Syst Rehabil Eng 29:123–133. https://doi.org/10.1109/tnsre.2020.3037411; Esrafilian A, Stenroth L, Mononen ME, Vartiainen P, Tanska P, Karjalainen PA, Suomalainen JS, Arokoski J, Saxby DJ, Lloyd DG, Korhonen RK (2022) An EMG-assisted muscle-force driven finite element analysis pipeline to investigate joint- and tissue-level mechanical responses in functional activities: towards a rapid assessment toolbox. IEEE Trans Biomed Eng 69(9):2860–2871. https://doi.org/10.1109/TBME.2022.3156018. Epub 2022 Aug 19.PMID: 35239473
13. Esrafilian A, Stenroth L, Mononen ME et al (2020b) EMG-assisted muscle force driven finite element model of the knee joint with fibril-reinforced poroelastic cartilages and menisci. Sci Rep 10:3026. https://doi.org/10.1038/s41598-020-59602-2; Esrafilian A, Stenroth L, Mononen ME, Vartiainen P, Tanska P, Karjalainen PA, Suomalainen JS, Arokoski JPA, Saxby DJ, Lloyd DG, Korhonen RK (2022) Toward tailored rehabilitation by implementation of a novel musculoskeletal finite element analysis pipeline. IEEE Trans Neural Syst Rehabil Eng 30:789–802. https://doi.org/10.1109/TNSRE.2022.3159685. Epub 2022 Mar 31.PMID: 35286263
14. Guilak F, Nims R, Dicks A et al (2018) Osteoarthritis as a disease of the cartilage pericellular matrix. Matrix Biol 71–72:40–50. https://doi.org/10.1016/j.matbio.2018.05.008
15. Henao-Murillo L, Pastrama MI, Ito K, van Donkelaar CC (2019) The relationship between proteoglycan loss, overloading-induced collagen damage, and cyclic loading in articular cartilage. Cartilage 1–12:1501S–1512S. https://doi.org/10.1177/1947603519885005
16. Hosseini S, Wilson W, Ito K, van Donkelaar C (2014) A numerical model to study mechanically induced initiation and progression of damage in articular cartilage. Osteoarthr Cartil 22:95–103. https://doi.org/10.1016/j.joca.2013.10.010
17. Kar S, Smith DW, Gardiner BS et al (2016) Modeling IL-1 induced degradation of articular cartilage. Arch Biochem Biophys 594:37–53. https://doi.org/10.1016/j.abb.2016.02.008
18. Kokkonen HT, Suomalainen JS, Joukainen A et al (2014) In vivo diagnostics of human knee cartilage lesions using delayed CBCT arthrography. J Orthop Res 32:403–412. https://doi.org/10.1002/jor.22521
19. Koli J, Multanen J, Kujala UM et al (2015) Effects of exercise on patellar cartilage in women with mild knee osteoarthritis. Med Sci Sports Exerc 47:1767–1774. https://doi.org/10.1249/MSS.0000000000000629
20. Korhonen RK, Julkunen P, Wilson W, Herzog W (2008) Importance of collagen orientation and depth-dependent fixed charge densities of cartilage on mechanical behavior of chondrocytes. J Biomech Eng 130:021003. https://doi.org/10.1115/1.2898725
21. Kurz B, Jin M, Patwari P et al (2001) Biosynthetic response and mechanical properties of articular cartilage after injurious compression. J Orthop

Res 19:1140–1146. https://doi.org/10.1016/S0736-0266(01)00033-X
22. Li S, Cao J, Caterson B, Hughes CE (2012) Proteoglycan metabolism, cell death and Kashin-Beck disease. Glycoconj J 29:241–248. https://doi.org/10.1007/s10719-012-9421-2
23. Li Y, Frank EH, Wang Y et al (2013) Moderate dynamic compression inhibits pro-catabolic response of cartilage to mechanical injury, tumor necrosis factor-α and interleukin-6, but accentuates degradation above a strain threshold. Osteoarthr Cartil 21:1933–1941. https://doi.org/10.1016/j.joca.2013.08.021
24. Li Y, Wang Y, Chubinskaya S et al (2015) Effects of insulin-like growth factor-1 and dexamethasone on cytokine-challenged cartilage: relevance to post-traumatic osteoarthritis. Osteoarthr Cartil 23:266–274. https://doi.org/10.1016/j.joca.2014.11.006
25. Lu YC, Evans CH, Grodzinsky AJ (2011) Effects of short-term glucocorticoid treatment on changes in cartilage matrix degradation and chondrocyte gene expression induced by mechanical injury and inflammatory cytokines. Arthritis Res Ther 13:R142. https://doi.org/10.1186/ar3456
26. Mäkelä J, Han S, Herzog W, Korhonen R (2015) Very early osteoarthritis changes sensitively fluid flow properties of articular cartilage. J Biomech 48:3369–3376. https://doi.org/10.1016/j.jbiomech.2015.06.010
27. Mäkelä JTA, Rezaeian ZS, Mikkonen S et al (2014) Site-dependent changes in structure and function of lapine articular cartilage 4 weeks after anterior cruciate ligament transection. Osteoarthr Cartil 22:869–878. https://doi.org/10.1016/j.joca.2014.04.010
28. Martel-Pelletier J, McCollum R, DiBattista J et al (1992) The interleukin-1 receptor in normal and osteoarthritic human articular chondrocytes. Identification as the type I receptor and analysis of binding kinetics and biologic function. Arthritis Rheum 35:530–540. https://doi.org/10.1002/art.1780350507
29. Martin JA, McCabe D, Walter M et al (2009) N-acetylcysteine inhibits post-impact chondrocyte death in osteochondral explants. J Bone Jt Surg Am 91:1890–1897. https://doi.org/10.2106/JBJS.H.00545
30. Mononen M, Liukkonen M, Korhonen R (2019) Utilizing atlas-based modeling to predict knee joint cartilage degeneration: data from the osteoarthritis initiative. Ann Biomed Eng 47:813–825. https://doi.org/10.1007/s10439-018-02184-y
31. Mononen M, Tanska P, Isaksson H, Korhonen R (2016) A novel method to simulate the progression of collagen degeneration of cartilage in the knee: data from the osteoarthritis initiative. Sci Rep 6:21415. https://doi.org/10.1038/srep21415
32. Mow VC, Kuei SC, Lai WM, Armstrong CG (1980) Biphasic creep and stress relaxation of articular cartilage in compression: theory and experiments. J Biomech Eng 102:73–84. https://doi.org/10.1115/1.3138202
33. Munukka M, Waller B, Rantalainen T et al (2016) Efficacy of progressive aquatic resistance training for tibiofemoral cartilage in postmenopausal women with mild knee osteoarthritis: a randomised controlled trial. Osteoarthr Cartil 24:1708–1717. https://doi.org/10.1016/j.joca.2016.05.007
34. Myller KAH, Korhonen RK, Töyräs J et al (2019) Computational evaluation of altered biomechanics related to articular cartilage lesions observed in vivo. J Orthop Res 37:1042–1051. https://doi.org/10.1002/jor.24273
35. Nagase H, Kashiwagi M (2003) Aggrecanases and cartilage matrix degradation. Arthritis Res Ther 5:94–103. https://doi.org/10.1186/ar630
36. Ojanen SP, Finnilä MAJ, Mäkelä JTA et al (2020) Anterior cruciate ligament transection of rabbits alters composition, structure and biomechanics of articular cartilage and chondrocyte deformation 2 weeks post-surgery in a site-specific manner. J Biomech 98:109450. https://doi.org/10.1016/j.jbiomech.2019.109450
37. Orozco GA, Eskelinen ASA, Kosonen JP et al (2022) Shear strain and inflammation-induced fixed charge density loss in the knee joint cartilage following ACL injury and reconstruction: a computational study. J Orthop Res 40:1505–1522. https://doi.org/10.1002/jor.25177
38. Orozco GA, Tanska P, Florea C et al (2018) A novel mechanobiological model can predict how physiologically relevant dynamic loading causes proteoglycan loss in mechanically injured articular cartilage. Sci Rep 8:1–16. https://doi.org/10.1038/s41598-018-33759-3
39. Orozco GA et al (2020) Prediction of local fixed charge density loss in cartilage following ACL injury and reconstruction: A computational proof-of-concept study with MRI follow-up. J Orthop Res 39(5):1064–1081. [John Wiley and Sons]
40. Patwari P, Gaschen V, James I et al (2004) Ultrastructural quantification of cell death after injurious compression of bovine calf articular cartilage. Osteoarthr Cartil 12:245–252. https://doi.org/10.1016/j.joca.2003.11.004
41. Pedoia V, Su F, Amano K et al (2017) Analysis of the articular cartilage T1ρ and T2 relaxation times changes after ACL reconstruction in injured and contralateral knees and relationships with bone shape. J Orthop Res 35:707–717. https://doi.org/10.1002/jor.23398
42. Pizzolato C, Reggiani M, Saxby DJ et al (2017) Biofeedback for gait retraining based on real-time estimation of tibiofemoral joint contact forces. IEEE Trans Neural Syst Rehabil Eng 25:1612–1621. https://doi.org/10.1109/TNSRE.2017.2683488
43. Ramdhian-Wihlm R, Le Minor JM, Schmittbuhl M et al (2012) Cone-beam computed tomography arthrography: an innovative modality for the evaluation of wrist ligament and cartilage injuries. Skelet Radiol 41:963–969. https://doi.org/10.1007/s00256-011-1305-1
44. Riad R, Jennane R, Brahim A et al (2018) Texture analysis using complex wavelet decomposition for knee osteoarthritis detection: data from the

osteoarthritis initiative. Comput Electr Eng 68:181–191. https://doi.org/10.1016/j.compeleceng.2018.04.004
45. Sah R, Kim Y, Doong J et al (1989) Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 7:619–636. https://doi.org/10.1002/jor.1100070502
46. Sauerland K, Raiss R, Steinmeyer J (2003) Proteoglycan metabolism and viability of articular cartilage explants as modulated by the frequency of intermittent loading. Osteoarthr Cartil 11:343–350. https://doi.org/10.1016/S1063-4584(03)00007-4
47. Seedhom BB (2006) Conditioning of cartilage during normal activities is an important factor in the development of osteoarthritis. Rheumatology 45:146–149. https://doi.org/10.1093/rheumatology/kei197
48. Simon BR (1992) Multiphase poroelastic finite element models for soft tissue structures. ASME Appl Mech Rev 45:191–218. https://doi.org/10.1115/1.3121397
49. Smith RL, Carter DR, Schurman DJ (2004) Pressure and shear differentially alter human articular chondrocyte metabolism: a review. Clin Orthop Relat Res 427:S89–S95
50. Son KM, Hong JI, Kim D et al (2020) Absence of pain in subjects with advanced radiographic knee osteoarthritis. BMC Musculoskelet Disord 21:640
51. Stolberg-Stolberg JA, Furman BD, William Garrigues N et al (2013) Effects of cartilage impact with and without fracture on chondrocyte viability and the release of inflammatory markers. J Orthop Res 31:1283–1292. https://doi.org/10.1002/jor.22348
52. Su F, Hilton JF, Nardo L et al (2013) Cartilage morphology and T1ρ and T2 quantification in ACL-reconstructed knees: a 2-year follow-up. Osteoarthr Cartil 21:1058–1067. https://doi.org/10.1016/j.joca.2013.05.010
53. Sui Y, Lee JH, DiMicco MA et al (2009) Mechanical injury potentiates proteoglycan catabolism induced by interleukin-6 with soluble interleukin-6 receptor and tumor necrosis factor α in immature bovine and adult human articular cartilage. Arthritis Rheum 60:2985–2996. https://doi.org/10.1002/art.24857
54. Takahashi K, Goomer RS, Harwood F et al (1999) The effects of hyaluronan on matrix metalloproteinase-3 (MMP-3), interleukin-1β (IL-1β), and tissue inhibitor of metalloproteinase-1 (TIMP- 1) gene expression during the development of osteoarthritis. Osteoarthr Cartil 7:182–190. https://doi.org/10.1053/joca.1998.0207
55. Tanska P, Julkunen P, Korhonen R (2018) A computational algorithm to simulate disorganization of collagen network in injured articular cartilage. Biomech Model Mechanobiol 17:689–699. https://doi.org/10.1007/s10237-017-0986-3
56. Tiulpin A, Klein S, Bierma-Zeinstra SMA et al (2019) Multimodal machine learning-based knee osteoarthritis progression prediction from plain radiographs and clinical data. Sci Rep 9:20038. https://doi.org/10.1038/s41598-019-56527-3
57. Venäläinen M, Mononen M, Salo J et al (2016) Quantitative evaluation of the mechanical risks caused by focal cartilage defects in the knee. Sci Rep 6:37538. https://doi.org/10.1038/srep37538
58. Vincenti M, Brinckerhoff C (2002) Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. Arthritis Res 4:157–164. https://doi.org/10.1186/ar401
59. Wang K, Kim HA, Felson DT et al (2018) Radiographic knee osteoarthritis and knee pain: cross-sectional study from five different racial/ethnic populations. Sci Rep 8:1–8. https://doi.org/10.1038/s41598-018-19470-3
60. Wilson W, Van Donkelaar CC, Van Rietbergen B, Huiskes R (2005) A fibril-reinforced poroviscoelastic swelling model for articular cartilage. J Biomech 38:1195–1204. https://doi.org/10.1016/j.jbiomech.2004.07.003
61. Zhao R, Dong Z, Wei X et al (2021) Inflammatory factors are crucial for the pathogenesis of post-traumatic osteoarthritis confirmed by a novel porcine model: "idealized" anterior cruciate ligament reconstruction and gait analysis. Int Immunopharmacol 99:107905. https://doi.org/10.1016/j.intimp.2021.107905

4 *In Vitro* Models and Proteomics in Osteoarthritis Research

Martin Rydén and Patrik Önnerfjord

Abstract

This review summarizes and exemplifies the current understanding of osteoarthritis *in vitro* models and describes their relevance for new insights in the future of osteoarthritis research. Our friend and highly appreciated colleague, Prof. Alan Grodzinsky has contributed greatly to the understanding of joint tissue biology and cartilage biomechanics. He frequently utilizes *in vitro* models and cartilage explant cultures, and recent work also includes proteomics studies. This review is dedicated to honor his 75-year birthday and will focus on recent proteomic *in vitro* studies related to osteoarthritis, and within this topic highlight some of his contributions to the field.

Keywords

Proteomics · Osteoarthritis · Cartilage

M. Rydén
Orthopaedics, Department of Clinical Sciences Lund, Faculty of Medicine, Lund University, Lund, Sweden

P. Önnerfjord (✉)
Rheumatology and Molecular Skeletal Biology, Department of Clinical Sciences Lund, Faculty of Medicine, Lund University, Lund, Sweden
e-mail: patrik.onnerfjord@med.lu.se

4.1 Introduction

Osteoarthritis (OA), the most common degenerative joint disease, is a major source of pain, disability, and socioeconomic cost worldwide [17]. OA is a complex disease affecting the whole joint and multiple molecular and clinical phenotypes of OA seem to exist [38]. The pathologic changes seen in OA joints include degradation of the articular cartilage, thickening of the subchondral bone, osteophyte formation, variable degrees of synovial inflammation, degeneration of ligaments and, in the knee, the menisci, and hypertrophy of the joint capsule [30]. The degradation of articular cartilage is caused by an increased proteolytic activity of matrix degrading enzymes such as matrix metalloproteinases (MMPs) and aggrecanases. However, as the detailed molecular mechanisms involved in OA initiation and progression remain poorly understood, no effective Disease-Modifying OA Drugs (DMOADs) are currently available.

Human tissue samples for the study of osteoarthritis are often collected at the time of joint replacement when disease progression is in late stage, and limit researchers' ability to study the early development factors that contribute to the disease. The inherent variability of OA disease progression and onset of symptoms between individuals also presents challenges for studying OA pathophysiology. To overcome these limitations, *in vitro* models have been extensively used

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_4

to address queries related to pathological changes, drug-target interactions, molecular pathways and to investigate the roles of pro-inflammatory cytokines in certain conditions.

Proteomics is the analysis of the entire protein complement of a cell, tissue, or organism under a specific, defined set of conditions. Proteomics applications in the field of OA has become rather common [19] as the technology can analyze complex samples in a more discovery-based approach than traditional specific methods such as enzyme-linked immunosorbent assays (ELISA). In this review, we have limited the content and searched the literature for proteomics applications (by mass spectrometry) within OA using in vitro experiments that are further discussed below.

4.2 *In Vitro* Models in Osteoarthritis

One of the main challenges in osteoarthritis research is to find a model that accurately represents the mechanisms of the disease. Different models have been developed, each with its own advantages and disadvantages [12, 22, 49, 50], but there is currently no consensus or gold standard approach. The two most common *in vitro* models are cell cultures and explant cultures.

Cell culture models typically target the chondrocyte as the cell type of interest for OA research but also synoviocytes and other joint cells have been targeted. Monolayer cell culture models are inexpensive, easy to use and allow for many replicates to be made from a single source of tissue. The layout of cells as they are put on a flat surface in a culture flask exposes them to an equal amount of growth factors in the surrounding media. Typical applications of monolayer cultures are the effect of cytokine stimulation, osmotic pressure, or the role of synovium in OA [12]. Like monolayer cell culture models, co-culture models are also used to study the effect of cytokines and osmotic pressure but have the added benefit of allowing cell-cell interactions and crosstalk between cell types to be investigated. However, co-culture experiments can be costly, and are limited because different cell types can require different conditions and cells may de-differentiate depending on the co-culture system. A limitation of cell cultures is the potential loss of chondrogenic phenotype as the chondrocyte is isolated from the extracellular matrix, due to their sensitivity to their molecular environment [11, 22].

Explant models are derived directly from *in vivo* tissue and for the study of OA pathophysiology, they have the unmistakable advantage of being a better representation of the *in vivo* tissue compared to cell cultures, as the overall characteristics of the tissue are maintained. An advantage of explant models over *in vivo* models is that they, to a greater extent, allow standardization and controlled variables [56]. Explant models are relatively inexpensive and easy to set up and can be used to study both inflammatory processes and biomechanical loading of the tissue. Despite the benefits over cell culture models, the use of tissue explants also has disadvantages such as chondrocyte death at the edge of the explant and limited number of cells. Explant models are also limited in that few replicates are available from the tissue source [50, 56]. Due to the limitations in sample availability, much of the published work is performed using animals, which is a limitation as not all findings can be directly translated to humans [12].

4.3 Inflammatory Models

The development of inflammatory processes in OA involves the increased expression of catabolic proteins in chondrocytes following cytokine exposure [22]. Cytokines are signaling proteins involved in inflammation response and are produced by nearly all cell types [39]. The balance and interplay of cytokines is increasingly being recognized to have a central role in OA disease progression [60]. To study these effects, models of pro- and anti-inflammatory cytokines are implemented in cell culture or explant models. Some of the most important pro-inflammatory cytokines used for induction of OA-like biological changes in these models are IL-1α, IL-1β, TNF-α, IL-6, leukemia inhibitory factor (LIF), oncostatin-M (OSM), IL-15, IL-17, and IL-18 [18, 40, 60]. In contrast, the major

anti-inflammatory cytokines, which are involved in OA pathogenesis by inhibiting actions of catabolic cytokines, are IL-4, IL-10, IL-11 and IL-13, IL-1 receptor antagonist (IL-1Ra), and interferon (IFN)- γ [18, 60].

Investigations to compare different pro-inflammatory cytokines were made in a cell culture model using RNA-seq [42]. In this study, the authors studied the effect of IFN-γ, IL-1β, IL-4 and IL-17 on gene expression in OA chondrocytes and found that 2800 genes were altered in chondrocytes treated with IL-1β. The mechanisms of IL-1β related to inflammation in OA were also studied in an *in vitro* cell culture model where human articular chondrocytes were cultured with or without recombinant IL-1β [24]. Known proteome changes following IL-1β stimulation, such as activation of the NFKB pathway, and subsequent synthesis of IL-1β, IL-6, IL-8, MMP-13, and ADAMTS-5, were validated by mass spectrometry on articular cartilage from three donors. After the cells had been stimulated with recombinant IL-1β for 20 h, IL-1β, IL-6, MMP-13 and IL-8 were upregulated. The authors also demonstrated that microRNA-140 inhibits the activation of the NFKB pathway, meaning a possible increase in cartilage repair and decrease of cartilage breakdown.

4.4 Mechanical Loading

In addition to biochemical factors, the development of osteoarthritis is affected by the biomechanical homeostasis of the joint. While mechanical loading is essential for the function and maintenance of healthy joints, mechanical *overload* induces molecular events similar to those stimulated by pro-inflammatory cytokines [13, 20]. It is becoming increasingly clear that impact-induced injuries not only cause cell damage, but also initiate progressive tissue damage and are recognized as a risk factor for OA. With this in mind, load-based models using tissue explants are effective systems for simulating the development of post-traumatic OA.

A study of post-traumatic OA has demonstrated a relationship between injury-induced oxidative damage and progressive matrix degradation [34]. Further, *in vitro* models have shown that while moderate intermittent compression have anti-catabolic effects on cartilage homeostasis, the cellular response to high compression involves degradation and decreased biosynthesis of ECM, and upregulation of pro-inflammatory enzymes [27, 29].

Due to variations in experimental protocols defined as injurious compression, direct comparisons of outcomes from models can be difficult. Indeed, mechanical injury can be induced in a multitude of ways; short repetitive loading, cyclic loading over a longer period of time, cartilage can be injured from a weight which drops down from a defined height, and explants can be compressed under confined conditions such that bulging of the explants do not occur [25].

4.5 Proteomics

The advances in mass spectrometry (MS) over the last decade has enabled this powerful technology to be frequently used in proteomics applications [1, 33]. The most common application "bottom-up proteomics" includes an enzymatic digestion of proteins e.g. extracted from cells, tissue or released into cell/tissue culture media. Proteins are digested with a sequence-specific enzyme like trypsin to generate a complex mixture of peptides. Peptides are usually separated by liquid chromatography, after which they enter a mass spectrometer where peptide ions are measured, to enable their identification and quantification. The data acquisition methods can be either discovery based or targeted against a predetermined list of peptides. Both approaches have been utilized for *in vitro* studies in osteoarthritis research, see Table 4.1. The discovery approach has the advantage of not being biased against any particular proteins, although in practice there is some bias towards medium and high abundant proteins as the precursor selection for MSMS experiments are based on top N most intense peptides i.e. data-dependent acquisition (DDA), and thereby low abundant proteins can be missed out. There is another discovery approach using data-independent acquisition (DIA) where all precursors within a certain m/z

range, sequential isolation windows of typical 20–25 Da, are selected for fragmentation to cover a typical mass range for proteolytic digests. This results in highly complex and data information-rich datasets that typically are matched against spectral libraries using a targeted analysis [61]. This latter approach combines an untargeted data collection with a targeted data analysis, reducing missing values and in addition enables data to be re-analyzed against more comprehensive libraries.

The traditional targeted approach, multiple reaction monitoring (MRM) is usually performed on triple quadrupole instruments using preset precursor mass filter (Q1), collision cell for fragmentation (Q2) and precursor fragment mass filter (Q3) with combined settings called peptide transitions which can be identified and optimized for highest sensitivity in pilot experiment [16]. The targeted approach has the advantage of high sensitivity, better reproducibility and fewer missing values and it is suitable for absolute quantification using heavy-isotope labeled standards while having limitations in a number of precursors as well as being limited to the pre-selection of targets missing out on novel findings (non-discovery). The development of parallel reaction monitoring (PRM) does increase the number of targets available and also results in full MSMS scans as the third quadrupole is replaced with a high-resolution mass analyzer (orbitrap) [43].

4.6 Proteomics Applications Using *In Vitro* Models

Mass spectrometry-based proteomics is a powerful technique that has increasingly enabled insights generated from OA *in vitro* experiments and has been used for characterizing biological elements relevant to OA pathophysiology, such as extracellular matrix components and structure. Cartilage is the most used tissue in OA *in vitro* studies and in proteomics application special considerations must be taken into account due to its physical characteristics. Articular cartilage is an avascular tissue with few cells and an extensive extracellular matrix (ECM) with major components being collagen (mainly type II) and proteoglycans (mainly aggrecan). The crossed-linked collagen network results in poor extractability with only a minor part (soluble fraction) being measured [41]. The high level of aggrecan can cause problems as the highly negatively charged glycosaminoglycan chains can interfere in the sample preparation steps ultimately affecting the chromatography performance [21]. However, despite these difficulties, more than 1000 additional proteins can be identified using current proteomics technologies [8].

Earlier in this review, we described a typical bottom-up proteomics workflow and here, we will demonstrate some of its applications using *in vitro* experiments related to OA. An experimental workflow for an in vitro model using proteomics is shown in Fig. 4.1.

In early proteomics studies, a commonly applied technique was two-dimensional gel electrophoresis (2D SDS-PAGE), which separated complex mixtures of a sample by isoelectric point (pI) and molecular weight. Following this, spots of interest and subsequent differentially expressed proteins could be identified by MS while quantification was usually performed using image analysis and intensities of matching protein spots [23]. Some limitations of this technique include low sensitivity, labor intensive work and poor ability for automation [48]. A simpler alternative was the more straightforward "salami" approach where sample lanes from 1D gels were cut into multiple bands followed by LC-MS analysis. However, due to these limitations and overall technological developments in the field, separation by liquid chromatography coupled with MS (gel-free proteomics) has been the preferred method in recent studies.

4.6.1 Targeted Proteomics Applications

4.6.1.1 Explant Cultures

A cartilage explant study by Melin-Fürst et al. [14] used MRM, a targeted approach, to

Table 4.1 In vitro studies in osteoarthritis using proteomics applications

Culture model type	OA model type	Treatments	Species	Analytical methods	MS method	Reference
Cartilage explant	Inflammation	± (IL-1β/TNF-α)	Equine	SDS-PAGE, LC-MS, NMR metabolomics	Discovery (DDA)	[2]
Cartilage explant	Inflammation	± (OSM/TNF-α, IL-17A)	Bovine	ELISA, LC-MS	Discovery (DDA)	[51]
Cell culture	Inflammation	± IL-1β	Human	SILAC, SDS-PAGE, LC-MS (MALDI)	MALDI MSMS	[10]
Cell culture	Inflammation	± IL-1β, ± nicotine	Human	SILAC, 2D LC-MS (MALDI)	MALDI MSMS	[31]
Cell culture, cartilage explant	OA secretome	Macrosopically normal	Human	SILAC, SDS-PAGE, LC-MS	Discovery (DDA)	[44]
Cartilage-synovium coculture	Tissue crosstalk, inflammation	± IL-1α, ± IL-1Ra	Bovine	LC-MS	Discovery (DDA)	[37]
Cell culture	OA secretome	Chondrogenesis BMSCs	Human	SILAC, SDS-PAGE, LC-MS	Discovery (DDA)	[46]
Cartilage, meniscus explant	Inflammation	± IL-1α, ± IL-1β	Porcine	MMP activity, NO, sGAG release, aggregate modulus, permeability	No MS	[36]
Cartilage explant	Inflammation	± IL-1β, ± carprofen	Canine	SDS PAGE, LC-MS, machine learning	Discovery (DDA)	[54]
Cartilage explant	PTOA, inflammation	± IL-1α	Bovine	MMP activity assay, SDS PAGE, LC-MS	Discovery (DDA)	[45]
Cartilage explant	Inflammation	± IL-1α, wt, Adamts5Δcat	Mouse	SDS-PAGE, LC-MS, microarray	Discovery (DDA)	[59]
Cell culture	Inflammation	± IL-1β	Human	2D SDS-PAGE, MALDI	MALDI MSMS	[47]
Cell culture	Inflammation	± IL-1β, ± miRNA-140	Human	RT-qPCR, LC-MS, Western blot	Discovery (DDA)	[24]
Osteochondral explants	Inflammation	±LPS, ± TGF-β RI inhibitor	Human	ELISA	No MS	[15]
Cartilage explant	Inflammation	± (IL-1β/TNF-α)	Equine	sGAG, RT-qPCR, Western blot	No MS	[35]
Cell culture	Inflammation, drug response	IL-1β + chondroitin sulfate	Human	LC, MALDI	MALDI MSMS	[9]
Cell culture	OA secretome	Different zones (N, UOA, WOA)	Human	2D SDS-PAGE, LC-MS	Discovery (DDA)	[26]
Cell culture	OA proteome	N vs OA chondrocytes	Human	SDS-PAGE, LC-MS	Discovery (DDA)	[57]
Cartilage explant	Dynamic loading	Loading ± IGF-1 or ± TGF-β	Bovine	ELISA	No MS	[13]
Cartilage explant	OA secretome	Different zones (N, UOA, WOA)	Human	iTRAQ, 2D LC-MS	MALDI MSMS	[32]
Cartilage explant	PTOA, inflammation	± (injury, TNF-α, IL-1α)	Bovine	SDS PAGE, LC-MS	Discovery (DDA)	[52]
Cartilage explant	PTOA, inflammation	± injury, ± (IL-6/TNF/sIL6R), ± Dex	Bovine	LC-MS	Discovery (DDA)	[7]
Cell culture	Inflammation	± IL-1β (N vs OA synoviocytes)	Human	IHC, LC-MS	Discovery (DIA)	[55]
Cartilage explant	PTOA, inflammation	± (injury, TNF-α, IL-1α)	Bovine	2D LC-MS	ITRAQ	[53]
Cartilage explant	PTOA, inflammation	± injury, ± (IL-6/TNF/sIL6R)	Human	LC-MS	Targeted (MRM)	[58]
Cartilage explant	PTOA, inflammation	± injury, ± (IL-6/TNF/sIL6R), ± Dex	Bovine, Human	LC-MS	Discovery (DDA)	[6]
Cartilage explant	Inflammation	± IL-1α	Bovine	ELISA, LC-MS	Targeted (MRM)	[14]

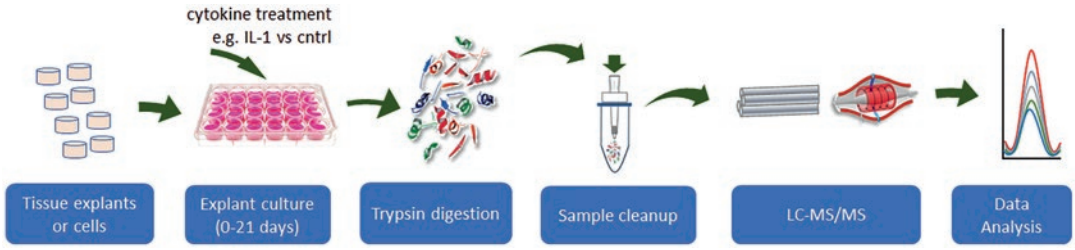


Fig. 4.1 Schematic workflow of an *in vitro* model using proteomics. Tissue explants or cells are kept in culture and exposed to different treatment conditions e.g. cytokines vs control. Cell culture media is replaced every 2–3 days and various time points are collected for further MS sample preparation (reduction, alkylation, ethanol precipitation, trypsin digestion, excess GAG removal, desalting) before peptides are separated using reversed phase liquid chromatography (nano-LC) and finally analyzed by mass spectrometry. The data generated is processed using specific software and searched against a protein sequence database or spectral library for identification and quantification

characterize the inflammatory processes involved cartilage degradation, induced by IL-1α in bovine cartilage explants, and monitor interactions with the complement system. Following cytokine stimulation, the authors found a decrease in proteoglycan and collagen content in the cartilage, and activation of the complement.

MRM was also used in a study to characterize cartilage response to mechanical injury and cytokine treatment [58]. The targeted approach allowed the authors to monitor a predefined set of potential molecular biomarkers including cleavage neoepitopes, see also Fig. 4.2. In this work, both the explant culture media (individual time points) and the final explant, representing the sum of events during the entire culture period, were measured. The neo-epitope measurements give extra biological value as it represents an active proteolytic event being measured in addition to the overall protein release.

4.6.2 Discovery Proteomics Applications

4.6.2.1 Cell Cultures

Several comparative proteomic analyses that aim to describe the proteome of OA have been conducted as cell culture models analyzed using discovery-based acquisition methods. One such study used 2D SDS-PAGE and subsequent discovery MS to analyze the proteome of human articular chondrocytes and described the phosphorylation status of differentially expressed proteins in OA progression [26]. Another study reported on the role of hypertrophy-like alterations in chondrocytes in OA using high resolution MS [57].

Secretome analysis can provide information on the mechanisms behind remodeling of ECM in response to drug treatment or mechanical load and thereby provide insights into the pathogenesis of OA. The effect of chondroitin sulfate in the presence of IL-1β on proteins in chondrocyte secretome was examined in a study from 2012 by 2D SDS-PAGE and SILAC in a discovery workflow [9]. The study found 75 proteins in the secretome, 18 of which were modulated by chondroitin sulfate, and provided evidence of its anti-angiogenic, anti-inflammatory, and anti-catabolic properties.

Using bovine cartilage explant monoculture and cartilage-synovium co-culture, a recent study investigated the role of cartilage-synovium crosstalk in a discovery-based mass spectrometry experiment [37]. Sustained doses of IL-1Ra were shown to suppress cytokine-induced catabolism in cartilage more effectively in the presence of synovium, which was associated with endogenous production of anti-catabolic factors.

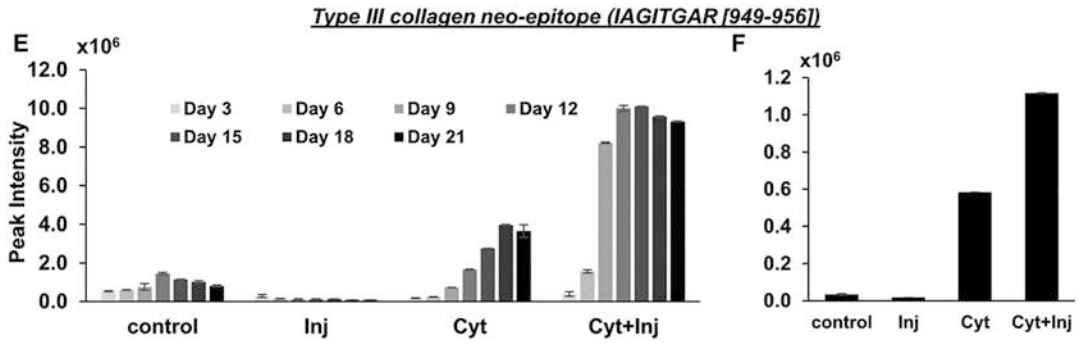


Fig. 4.2 Collagen type III cleavage neo-epitope release into explant culture media over 21 days (E) and the remaining peptide left in explant after the complete culture period (F). Values are average peak intensities of two technical replicates using targeted proteomics (MRM). Modified figure reprinted with permission from [58]

Though most of the discovery workflows relied on data dependent acquisition (DDA), one study, focusing on the difference in the phosphoproteome of OA and acute joint fracture in synovial tissue, used data independent acquisition (DIA) in an IL-1β–treated human synoviocyte (HS) *in vitro* model to verify their results [55]. The study found that IL-1β could induce HS to secrete proteins associated with the endosomal/vacuolar pathway, endoplasmic reticulum/Golgi secretion, complement activation, and collagen degradation.

4.6.2.2 Explant Cultures

Cartilage degradation is a well-established process in OA pathogenesis, and as such, a commonly studied model is the degradation of cartilage upon cytokine stimulation in explant culture. Several proteomic studies have employed this model to measure the effects of pro-inflammatory cytokines on articular cartilage. One study from 2016 investigated the chondrocyte response to IL-1α within native cartilage tissue and its secretome using discovery LC-MS and whole-genome expression profiling using microarray [59]. The study was the first to report on the effects of IL-1α in native cartilage and cartilage lacking the catalytic domain of ADAMTS5 (aggrecanase) and identified more than 150 proteins modulated by IL-1α. Additionally, the combined LC-MS and microarray analysis permitted the authors to differentiate between proteins modulated by IL-1α on gene expression level and those which were a product of ECM degradation.

Discovery LC-MS using DDA was used in a metabolomics and proteomics study published in 2020, where Anderson et al. studied OA pathogenesis using an equine cartilage explant model [2]. In the study, nine potential novel OA neoepitope peptides were discovered.

Unbiased labeled approaches such as Isobaric Tag for Relative and Absolute Quantitation (ITRAQ) were used in two studies. Stevens et al. used traumatic injury, IL-1β or TNF-α compared to control showing increased levels of MMPs and proteins of the innate immunity while the mechanical injury mainly led to an increased release of intracellular proteins [53]. Lourido et al. conducted a comparative profiling of proteins in early- versus late-stage OA, and subsequent clustering analysis and found upregulation of periostin and downregulation of osteoprotegerin in OA [32]. In a recent study a cartilage injury model was described using articular cartilage explants treated with cytokines and mechanical injury [7]. The addition of dexamethasone was shown to rescue the catabolic response but not the anabolic dysregulation. The treatment effects in this model are illustrated in a heatmap (Fig. 4.3).

4.7 Concluding Remarks and Future Perspectives

In this review, we have shown an increased use of proteomics applications within *in vitro* models during the last decade and this trend will

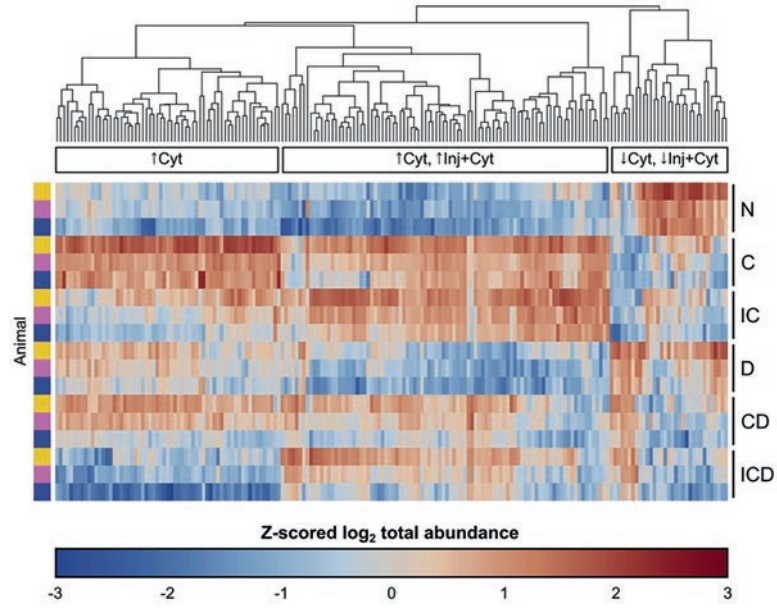


Fig. 4.3 Heatmap of proteins significantly affected by disease treatment. Treatment effects were evaluated by pairwise comparisons of MS abundance data of different disease treatments within each animal replicate. Proteins were selected that had a differential effect of C or IC treatments and that were present in at least three time points across at least one consistent treatment condition between all three biological replicates, resulting in 188 selected proteins. The raw abundance value for each filtered protein was summed over all time points and $\log_2$-transformed. For visualization, the $\log_2$-transformed values were normalized via z-scoring across all treatment conditions, excluding injury alone and injury with Dex: control (N), cytokine (C), injury + cytokines (IC), Dex (D), cytokines + Dex (CD), and injury + cytokines + Dex (ICD). Proteins are plotted on the horizontal axis and ordered based on their hierarchical clustering (Euclidian distance) across all six selected treatment conditions. Each individual replicate is plotted on the vertical axis, ordered by treatment condition and then by animal. The clustering reveals three major patterns of protein release: increased release by cytokines alone (↑Cyt), an increase by both cytokines and injury + cytokines (↑Cyt, ↑Inj + Cyt), and decreased release by cytokines and injury + cytokines (↓Cyt, ↓Inj + Cyt). (Figure reproduced from [7] under a Creative Common license)

most likely continue. The high sensitivity and selectivity offered by current and future MS instruments allow identification and quantification of a large number of proteins in highly complex samples. These information-rich data sets further allow much more detailed results to be obtained including individual peptide levels (relative abundances), post-translational modifications (e.g. measuring catabolic proteolytic events, phosphorylation states and protein synthesis), hence making this a very attractive technology. Novel developments in MS instrumentation, data acquisition methods as well as improved tools for data processing and evaluation tools will enable an increased depth of the proteome improving the bioinformatic evaluation of the data to identify proteins involved in specific functional pathways and interpretation of protein-protein association networks. Clustering analysis can also identify proteins following similar patterns e.g. grouping proteins according to their kinetic release profile [6].

In comparison to cell culture models, explant models better represent the extracellular matrix environment *in vivo* and also possess the ability to perform mechanical loading experiments. The kinetics of specific proteins release can easily be addressed as the culture medium needs to be replaced every 2–3 days creating a longitudinal experiment that can reflect both early-, mid- and late stages in the model. This is a great potential

when healthy donors are available as early changes can be investigated. *In vitro* models are also well suited for molecular therapeutic interventions e.g. applying potential drug candidates for treatment purposes. One example is the glucorticoid dexamethasone that has been extensively studied with somewhat conflicting results in the past [5] but in explant culture, the combination of dexamethasone with insulin-like growth factor-1 inhibited both the loss of soluble proteoglycan (sGAG) and collagen, rescued the suppression of matrix biosynthesis and inhibited loss of chondrocyte viability induced by IL-1α treatment [28]. Drug delivery is an important aspect to this as without a carrier, intra-articular injections of the drug is rapidly cleared from the joint cavity. The Grodzinsky group has circumvented this drawback by linking the drug to a basic carrier enabling rapid uptake and sustained delivery both in vitro [3] and in vivo [4]. The Dex treatment effect was further investigated using proteomics in a post-traumatic *in vitro* model where it suppressed most of the proteins affected by cytokine+injury treatment versus control [6, 7].

The ground-breaking work of Prof. Grodzinsky and his contributions to the research community has been widely recognized by numerous awards. He is considered as a world leader in his field and in 2021, he received the most prestigious Lifetime Achievement Award by the Osteoarthritis Research Society International.

References

1. Aebersold R, Mann M (2016) Mass-spectrometric exploration of proteome structure and function. Nature 537:347–355. https://doi.org/10.1038/nature19949
2. Anderson JR, Phelan MM, Foddy L, Clegg PD, Peffers MJ (2020) Ex vivo equine cartilage explant osteoarthritis model: a metabolomics and proteomics study. J Proteome Res 19:3652–3667. https://doi.org/10.1021/acs.jproteome.0c00143
3. Bajpayee AG, Quadir MA, Hammond PT, Grodzinsky AJ (2016) Charge based intra-cartilage delivery of single dose dexamethasone using Avidin nano-carriers suppresses cytokine-induced catabolism long term. Osteoarthr Cartil 24:71–81. https://doi.org/10.1016/j.joca.2015.07.010
4. Bajpayee AG, Scheu M, Grodzinsky AJ, Porter RM (2014) Electrostatic interactions enable rapid penetration, enhanced uptake and retention of intra-articular injected avidin in rat knee joints. J Orthop Res 32:1044–1051. https://doi.org/10.1002/jor.22630
5. Black R, Grodzinsky A (2019) Dexamethasone: chondroprotective corticosteroid or catabolic killer? Eur Cell Mater 38:246–263. https://doi.org/10.22203/ecm.v038a17
6. Black RM, Wang Y, Struglics A, Lorenzo P, Chubinskaya S, Grodzinsky AJ, Önnerfjord P (2021) Proteomic clustering reveals the kinetics of disease biomarkers in bovine and human models of post-traumatic osteoarthritis. Osteoarthr Cartil Open 3:100191. https://doi.org/10.1016/j.ocarto.2021.100191
7. Black RM, Wang Y, Struglics A, Lorenzo P, Tillgren V, Rydén M, Grodzinsky AJ, Önnerfjord P (2020) Proteomic analysis reveals dexamethasone rescues matrix breakdown but not anabolic dysregulation in a cartilage injury model. Osteoarthr Cartil Open 2:100099. https://doi.org/10.1016/j.ocarto.2020.100099
8. Bundgaard L, Åhrman E, Malmström J, dem Keller UA, Walters M, Jacobsen S (2021) Effective protein extraction combined with data independent acquisition analysis reveals a comprehensive and quantifiable insight into the proteomes of articular cartilage and subchondral bone. Osteoarthr Cartil 30:137–146. https://doi.org/10.1016/j.joca.2021.09.006
9. Calamia V, Lourido L, Fernández-Puente P, Mateos J, Rocha B, Montell E, Vergés J, Ruiz-Romero C, Blanco FJ (2012) Secretome analysis of chondroitin sulfate-treated chondrocytes reveals anti-angiogenic, anti-inflammatory and anti-catabolic properties. Arthritis Res Ther 14:R202–R202. https://doi.org/10.1186/ar4040
10. Calamia V, Rocha B, Mateos J, Fernández-Puente P, Ruiz-Romero C, Blanco FJ (2011) Metabolic labeling of chondrocytes for the quantitative analysis of the Interleukin-1-beta-mediated modulation of their intracellular and extracellular proteomes. J Proteome Res 10:3701–3711. https://doi.org/10.1021/pr200331k
11. Caron MMJ, Emans PJ, Coolsen MME, Voss L, Surtel DAM, Cremers A, van Rhijn LW, Welting TJM (2012) Redifferentiation of dedifferentiated human articular chondrocytes: comparison of 2D and 3D cultures. Osteoarthr Cartil 20:1170–1178. https://doi.org/10.1016/j.joca.2012.06.016
12. Cope PJ, Ourradi K, Li Y, Sharif M (2019) Models of osteoarthritis: the good, the bad and the promising. Osteoarthr Cartil 27:230–239. https://doi.org/10.1016/j.joca.2018.09.016
13. Engstrøm A, Gillesberg FS, Groen SS, Frederiksen P, Bay-Jensen A-C, Karsdal MA, Thudium CS (2021) Intermittent dynamic compression confers anabolic effects in articular cartilage. Appl Sci 11:7469. https://doi.org/10.3390/app11167469

14. Fürst CM, Åhrman E, Bratteby K, Waldemarson S, Malmström J, Blom AM (2016) Quantitative mass spectrometry to study inflammatory cartilage degradation and resulting interactions with the complement system. J Immunol 197:3415–3424. https://doi.org/10.4049/jimmunol.1601006
15. Geurts J, Jurić D, Müller M, Schären S, Netzer C (2018) Novel ex vivo human osteochondral explant model of knee and spine osteoarthritis enables assessment of inflammatory and drug treatment responses. Int J Mol Sci 19:1314. https://doi.org/10.3390/ijms19051314
16. Gillet LC, Navarro P, Tate S, Röst H, Selevsek N, Reiter L, Bonner R, Aebersold R (2012) Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol Cell Proteomics 11(6). https://doi.org/10.1074/mcp.o111.016717
17. Gillette MA, Carr SA (2013) Quantitative analysis of peptides and proteins in biomedicine by targeted mass spectrometry. Nat Methods 10:28–34. https://doi.org/10.1038/nmeth.2309
18. Glyn-Jones S, Palmer AJR, Agricola R, Price AJ, Vincent TL, Weinans H, Carr AJ (2015) Osteoarthritis. Lancet 386:376–387. https://doi.org/10.1016/s0140-6736(14)60802-3
19. Goldring MB (2000) Osteoarthritis and cartilage: the role of cytokines. Curr Rheumatol Rep 2:459–465. https://doi.org/10.1007/s11926-000-0021-y
20. Haartmans MJJ, Emanuel KS, Tuijthof GJM, Heeren RMA, Emans PJ, Cillero-Pastor B (2021) Mass spectrometry-based biomarkers for knee osteoarthritis: a systematic review. Expert Rev Proteomics 18:693–706. https://doi.org/10.1080/14789450.2021.1952868
21. Henrotin Y, Pesesse L, Sanchez C (2011) Mechanical stretch and cytokines. Springer, pp 223–233. https://doi.org/10.1007/978-94-007-2004-6_10
22. Hsueh M-F, Khabut A, Kjellström S, Önnerfjord P, Kraus VB (2016) Elucidating the molecular composition of cartilage by proteomics. J Proteome Res 15:374–388. https://doi.org/10.1021/acs.jproteome.5b00946
23. Johnson CI, Argyle DJ, Clements DN (2016) In vitro models for the study of osteoarthritis. Vet J 209:40–49. https://doi.org/10.1016/j.tvjl.2015.07.011
24. Joshi K, Patil D (2017) Innovative approaches in drug discovery. Elsevier, pp 273–294. https://doi.org/10.1016/b978-0-12-801814-9.00009-x
25. Karlsen TA, de Souza GA, Ødegaard B, Engebretsen L, Brinchmann JE (2016) microRNA-140 inhibits inflammation and stimulates chondrogenesis in a model of interleukin 1β-induced osteoarthritis. Mol Ther Nucleic Acids 5:e373. https://doi.org/10.1038/mtna.2016.64
26. Kurz B, Lemke AK, Fay J, Pufe T, Grodzinsky AJ, Schünke M (2005) Pathomechanisms of cartilage destruction by mechanical injury. Ann Anat 187:473–485. https://doi.org/10.1016/j.aanat.2005.07.003
27. Lambrecht S, Verbruggen G, Verdonk PCM, Elewaut D, Deforce D (2008) Differential proteome analysis of normal and osteoarthritic chondrocytes reveals distortion of vimentin network in osteoarthritis. Osteoarthr Cartil 16:163–173. https://doi.org/10.1016/j.joca.2007.06.005
28. Li Y, Frank EH, Wang Y, Chubinskaya S, Huang H-H, Grodzinsky AJ (2013) Moderate dynamic compression inhibits pro-catabolic response of cartilage to mechanical injury, tumor necrosis factor-α and interleukin-6, but accentuates degradation above a strain threshold. Osteoarthr Cartil 21:1933–1941. https://doi.org/10.1016/j.joca.2013.08.021
29. Li Y, Wang Y, Chubinskaya S, Schoeberl B, Florine E, Kopesky P, Grodzinsky AJ (2015) Effects of insulin-like growth factor-1 and dexamethasone on cytokine-challenged cartilage: relevance to post-traumatic osteoarthritis. Osteoarthr Cartil 23:266–274. https://doi.org/10.1016/j.joca.2014.11.006
30. Lin W, Klein J (2021) Recent Progress in cartilage lubrication. Adv Mater 33:2005513. https://doi.org/10.1002/adma.202005513
31. Loeser RF, Goldring SR, Scanzello CR, Goldring MB (2012) Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 64:1697–1707. https://doi.org/10.1002/art.34453
32. Lourido L, Calamia V, Fernández-Puente P, Mateos J, Oreiro N, Blanco FJ, Ruiz-Romero C (2016) Secretome analysis of human articular chondrocytes unravels catabolic effects of nicotine on the joint. Proteomics Clin Appl 10:671–680. https://doi.org/10.1002/prca.201400186
33. Lourido L, Calamia V, Mateos J, Fernández-Puente P, Fernández-Tajes J, Blanco FJ, Ruiz-Romero C (2014) Quantitative proteomic profiling of human articular cartilage degradation in osteoarthritis. J Proteome Res 13:6096–6106. https://doi.org/10.1021/pr501024p
34. Ludwig C, Gillet L, Rosenberger G, Amon S, Collins BC, Aebersold R (2018) Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial. Mol Syst Biol 14:e8126. https://doi.org/10.15252/msb.20178126
35. Martin JA, McCabe D, Walter M, Buckwalter JA, McKinley TO (2009) N-acetylcysteine inhibits post-impact chondrocyte death in osteochondral explants. J Bone Joint Surg Am 91:1890–1897. https://doi.org/10.2106/jbjs.h.00545
36. Matta C, Fellows CR, Quasnichka H, Williams A, Jeremiasse B, Allaway D, Mobasheri A (2021) Clusterin secretion is attenuated by the proinflammatory cytokines interleukin-1β and tumor necrosis factor-α in models of cartilage degradation. J Orthop Res 39:1017–1029. https://doi.org/10.1002/jor.24814
37. McNulty AL, Rothfusz NE, Leddy HA, Guilak F (2013) Synovial fluid concentrations and relative potency of interleukin-1 alpha and beta in cartilage and meniscus degradation. J Orthop Res 31:1039–1045. https://doi.org/10.1002/jor.22334

38. Mehta S, Akhtar S, Porter RM, Önnerfjord P, Bajpayee AG (2019) Interleukin-1 receptor antagonist (IL-1Ra) is more effective in suppressing cytokine-induced catabolism in cartilage-synovium co-culture than in cartilage monoculture. Arthritis Res Ther 21:238. https://doi.org/10.1186/s13075-019-2003-y
39. Mobasheri A, Saarakkala S, Finnilä M, Karsdal MA, Bay-Jensen A-C, van Spil WE (2019) Recent advances in understanding the phenotypes of osteoarthritis. F1000Res 8:F1000 Faculty Rev-2091. https://doi.org/10.12688/f1000research.20575.1
40. Noakes PS, Michaelis LJ (2013) Diet, immunity and inflammation, Part I: Immunity and inflammation: an introduction. Elsevier, pp 3–33. https://doi.org/10.1533/9780857095749.1.3
41. Olson SA, Horne P, Furman B, Huebner J, AlRashid M, Kraus VB, Guilak F (2014) The role of cytokines in posttraumatic arthritis. J Am Acad Orthop Surg 22:29–37. https://doi.org/10.5435/jaaos-22-01-29
42. Önnerfjord P, Khabut A, Reinholt FP, Svensson O, Heinegård D (2012) Quantitative proteomic analysis of eight cartilaginous tissues reveals characteristic differences as well as similarities between subgroups*. J Biol Chem 287:18913–18924. https://doi.org/10.1074/jbc.m111.298968
43. Pemmari A, Leppänen T, Hämäläinen M, Moilanen T, Moilanen E (2021) Chondrocytes from osteoarthritis patients adopt distinct phenotypes in response to central TH1/TH2/TH17 cytokines. Int J Mol Sci 22:9463. https://doi.org/10.3390/ijms22179463
44. Peterson AC, Russell JD, Bailey DJ, Westphall MS, Coon JJ (2012) Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics*. Mol Cell Proteomics 11:1475–1488. https://doi.org/10.1074/mcp.o112.020131
45. Polacek M, Bruun J-A, Johansen O, Martinez I (2010) Differences in the secretome of cartilage explants and cultured chondrocytes unveiled by SILAC technology: chondrocytes secretome by SILAC. J Orthop Res 28:1040–1049. https://doi.org/10.1002/jor.21067
46. Ravindra KC, Ahrens CC, Wang Y, Ramseier JY, Wishnok JS, Griffith LG, Grodzinsky AJ, Tannenbaum SR (2018) Chemoproteomics of matrix metalloproteases in a model of cartilage degeneration suggests functional biomarkers associated with posttraumatic osteoarthritis. J Biol Chem 293:11459–11469. https://doi.org/10.1074/jbc.m117.818542
47. Rocha B, Calamia V, Casas V, Carrascal M, Blanco FJ, Ruiz-Romero C (2014) Secretome analysis of human mesenchymal stem cells undergoing Chondrogenic differentiation. J Proteome Res 13:1045–1054. https://doi.org/10.1021/pr401030n
48. Ruiz-Romero C, Carreira V, Rego I, Remeseiro S, López-Armada MJ, Blanco FJ (2008) Proteomic analysis of human osteoarthritic chondrocytes reveals protein changes in stress and glycolysis. Proteomics 8:495–507. https://doi.org/10.1002/pmic.200700249
49. Ruiz-Romero C, Fernández-Puente P, Calamia V, Blanco FJ (2015) Lessons from the proteomic study of osteoarthritis. Expert Rev Proteomics 12:433–443. https://doi.org/10.1586/14789450.2015.1065182
50. Salgado C, Jordan O, Allémann E (2021) Osteoarthritis in vitro models: applications and implications in development of intra-articular drug delivery systems. Pharmaceutics 13:60. https://doi.org/10.3390/pharmaceutics13010060
51. Samvelyan HJ, Hughes D, Stevens C, Staines KA (2021) Models of osteoarthritis: relevance and new insights. Calcif Tissue Int 109:243–256. https://doi.org/10.1007/s00223-020-00670-x
52. Sinkeviciute D, Aspberg A, He Y, Bay-Jensen A-C, Önnerfjord P (2020) Characterization of the interleukin-17 effect on articular cartilage in a translational model: an explorative study. BMC Rheumatol 4:30. https://doi.org/10.1186/s41927-020-00122-x
53. Stevens AL, Wishnok JS, Chai DH, Grodzinsky AJ, Tannenbaum SR (2008) A sodium dodecyl sulfate–polyacrylamide gel electrophoresis–liquid chromatography tandem mass spectrometry analysis of bovine cartilage tissue response to mechanical compression injury and the inflammatory cytokines tumor necrosis factor α and interleukin-1β. Arthritis Rheum 58:489–500. https://doi.org/10.1002/art.23120
54. Stevens AL, Wishnok JS, White FM, Grodzinsky AJ, Tannenbaum SR (2009) Mechanical injury and cytokines cause loss of cartilage integrity and upregulate proteins associated with catabolism, immunity, inflammation, and repair*. Mol Cell Proteomics 8:1475–1489. https://doi.org/10.1074/mcp.m800181-mcp200
55. Swan AL, Hillier KL, Smith JR, Allaway D, Liddell S, Bacardit J, Mobasheri A (2013) Analysis of mass spectrometry data from the secretome of an explant model of articular cartilage exposed to pro-inflammatory and anti-inflammatory stimuli using machine learning. BMC Musculoskelet Disord 14:349. https://doi.org/10.1186/1471-2474-14-349
56. Tang S, Deng S, Guo J, Chen X, Zhang W, Cui Y, Luo Y, Yan Z, He Q-Y, Shen S, Wang T (2018) Deep coverage tissue and cellular proteomics revealed IL-1β can independently induce the secretion of TNF-associated proteins from human Synoviocytes. J Immunol 200:821–833. https://doi.org/10.4049/jimmunol.1700480
57. Tarafder S, Park G, Lee CH (2019) Explant models for meniscus metabolism, injury, repair, and healing. Connect Tissue Res 61:1–12. https://doi.org/10.1080/03008207.2019.1702031
58. Tsolis KC, Bei ES, Papathanasiou I, Kostopoulou F, Gkretsi V, Kalantzaki K, Malizos K, Zervakis M, Tsezou A, Economou A (2015) Comparative proteomic analysis of hypertrophic chondrocytes in osteoarthritis. Clin Proteomics 12:12. https://doi.org/10.1186/s12014-015-9085-6
59. Wang Y, Li Y, Khabut A, Chubinskaya S, Grodzinsky AJ, Önnerfjord P (2017) Quantitative proteomics

analysis of cartilage response to mechanical injury and cytokine treatment. Matrix Biol 63:11–22. https://doi.org/10.1016/j.matbio.2016.12.004
60. Wilson R, Golub SB, Rowley L, Angelucci C, Karpievitch YV, Bateman JF, Fosang AJ (2016) Novel elements of the chondrocyte stress response identified using an in vitro model of mouse cartilage degradation. J Proteome Res 15:1033–1050. https://doi.org/10.1021/acs.jproteome.5b01115
61. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D (2014) The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediat Inflamm 2014:561459. https://doi.org/10.1155/2014/561459

5 Nanomechanics of Aggrecan: A New Perspective on Cartilage Biomechanics, Disease and Regeneration

Chao Wang, Elizabeth R. Kahle, Qing Li, and Lin Han

Abstract

Articular cartilage is a hydrated macromolecular composite mainly composed of type II collagen fibrils and the large proteoglycan, aggrecan. Aggrecan is a key determinant of the load bearing and energy dissipation functions of cartilage. Previously, studies of cartilage biomechanics have been primarily focusing on the macroscopic, tissue-level properties, which failed to elucidate the molecular-level activities that govern cartilage development, function, and disease. This chapter provides a brief summary of Dr. Alan J. Grodzinsky's seminal contribution to the understanding of aggrecan molecular mechanics at the nanoscopic level. By developing and applying a series of atomic force microscopy (AFM)-based nanomechanical tools, Grodzinsky and colleagues revealed the unique structural and mechanical characteristics of aggrecan at unprecedented resolutions. In this body of work, the "bottle-brush"-like ultrastructure of aggrecan was directly visualized for the first time. Meanwhile, molecular mechanics of aggrecan was studied using a physiological-like 2D biomimetic assembly of aggrecan on multiple fronts, including compression, dynamic loading, shear, and adhesion. These studies not only generated new insights into the development, aging, and disease of cartilage, but established a foundation for designing and evaluating novel cartilage regeneration strategies. For example, building on the scientific foundation and methodology infrastructure established by Dr. Grodzinsky, recent studies have elucidated the roles of other proteoglycans in mediating cartilage integrity, such as decorin and perlecan, and evaluated the therapeutic potential of biomimetic proteoglycans in improving cartilage regeneration.

Keywords

Proteoglycan · Nanomechanics · AFM · Aggrecan · Decorin

5.1 Introduction

Articular cartilage is the soft tissue at the end of bones that enables joint locomotion, energy dissipation, and lubrication [40]. These functions are endowed by the specialized extracellular matrix (ECM) of cartilage, a hydrated composite of col-

C. Wang · E. R. Kahle · Q. Li · L. Han (✉)
School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA
e-mail: lh535@drexel.edu

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_5

lagens and proteoglycans that are synthesized by residing chondrocytes [26] (Fig. 5.1a). Osteoarthritis (OA), the most prevalent musculoskeletal disease, is characterized by the irreversible breakdown of cartilage ECM, resulting in severe joint pain and limited motion [45]. Due to its avascular and dense nature, cartilage has very limited self-repair capabilities, and regenerative therapies often fail to restore the structure and function of healthy tissue [29]. This renders it crucial to understand the establishment, homeostasis, and disease-induced degeneration of cartilage ECM. The ECM mainly consists of ~65–70% w/w water, ~20–30% collagens, ~5–10% proteoglycans [40] as well as DNAs and other minor proteins/proteoglycans (Fig. 5.1a–c) [28]. *In vivo*, the collagen network is primarily responsible for cartilage tensile stiffness, while aggrecan and its negative fixed charges are the key determinants of cartilage compressive resistance and fluid flow-associated energy dissipative properties [40].

In the past few decades, there have been many attempts in understanding cartilage biomechanics in health and disease, in the hope to gain new insights into disease progression and functional regeneration. In the 1980's, Mow and colleagues applied the biphasic poroelasticity theory [2] to interpret the time-dependent, energy dissipative properties of cartilage. This theory addressed the key role of molecular friction arising from water-solid matrix interactions during fluid flow in contributing to energy dissipation [42]. Grodzinsky and colleagues further integrated the concept of electrical streaming potential with cartilage mechanical deformation and underscored the crucial role of fixed charges in overt tissue biomechanics [16, 17]. These seminal studies established the foundation of modern cartilage biomechanics theory. At the same time, numerous experimental tools have been implemented to delineate the contributions of each matrix constituent to cartilage mechanical properties, including confined and unconfined compression, indentation, dynamic oscillatory loading, and shear [39]. For instance, investigating the biomechanics of normal and glycosaminoglycan (GAG)-depleted cartilage have shown that aggrecan and its fixed charges directly contribute to ~50% compressive modulus of cartilage [60].

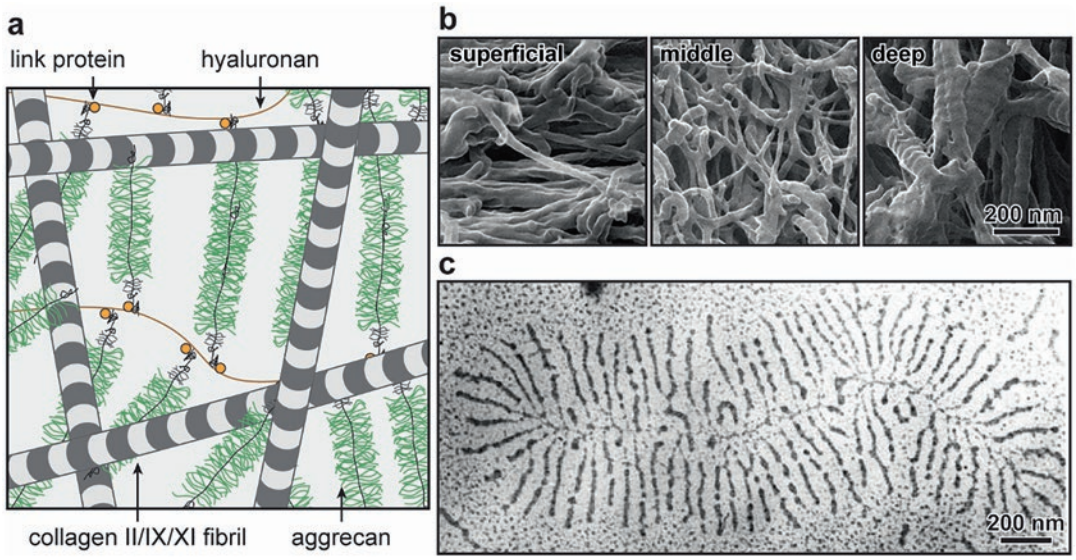


Fig. 5.1 An overview of articular cartilage extracellular matrix (ECM). (**a**) Schematic illustration of the major constituents in cartilage ECM: type II/IX/XI collagen fibril network and aggrecan-hyaluronan (HA) aggregates. (**b**) Depth-dependent nanostructure of collagen fibril network visualized by helium ion microscopy on proteoglycan-removed rabbit cartilage. Adapted with permission from Ref. [56]. (**c**) Nanostructure of aggrecan-HA aggregates imaged by transmission electron microscopy. (Adapted with permission from Ref. [3])

Despite these advances in tissue-level studies, disease intervention and regeneration remain elusive. This is at least partly because the tissue-level investigatory approaches are unable to account for the salient heterogeneity and a high level of complexity of cartilage structural hierarchy from nm-to-mm scales. For example, the collagen network is dominated by types II/IX/XI collagen heterotypic fibrils [15], with a minor amount of type III collagen co-assembling on the surface of collagen II fibrils [58]. The collagen fibrils (diameter ~ 30–80 nm) vary in orientation and diameter with depth in the tissue [9], from being transverse in the superficial layer, to random in the middle layer, then predominantly perpendicular in the deep layer, accompanied by an increasing gradient of fibril diameter and proteoglycan concentration (Fig. 5.1b) [56]. Aggrecan, the major proteoglycan, has a "bottle-brush" structure, and is decorated with highly negatively charged chondroitin sulfate (CS) and keratan sulfate (KS) GAGs along its core protein [26]. *In vivo*, aggrecan is end-attached to the linear hyaluronan (HA) molecules via its G1 domain at the N-terminal [27], and this interaction is further stabilized by link protein [4]. In the ECM, these supramolecular aggregates are entrapped within ~100 nm nanopores between collagen fibrils at ~50% molecular compressive strain [59], thereby adopting a highly compacted configuration that endows the tissue with its high fixed charge density and osmotic swelling pressure. The electron microscopy study by Buckwalter and Rosenberg highlighted the complexity in the assembly and retention of aggrecan *in vivo*, and provided the first direct visual evidence of the aggrecan-HA aggregation (Fig. 5.1c) [3]. Given these complexities, understanding the ECM from the molecular level is necessary for developing effective disease intervention and tissue regeneration.

Dr. Grodzinsky is the pioneer in studying the molecular mechanics of cartilage ECM constituents. Through collaboration with Dr. Christine Ortiz, a world-renowned scientist in polymer nanomechanics and atomic force microscopy, this team has made numerous transformative discoveries on the nanostructure and nanomechanics of cartilage, with a focus on the major proteoglycan, aggrecan. This chapter provides a brief summary of Grodzinsky's contributions to the understanding of aggrecan within the context of cartilage function, disease, and regeneration. This chapter begins with the summary of the ultrastructural and nanomechanical studies of native aggrecan (Sect. 5.2), followed by the overview of applying the knowledge of aggrecan to understanding cartilage aging, disease and tissue engineering (Sect. 5.3), and then, the discussion of more recent studies on other native and biomimetic proteoglycans that were directly inspired by the Grodzinsky's work (Sect. 5.4), and finally, concludes with a summary and future outlook (Sect. 5.5).

5.2 Ultrastructure and Nanomechanics of Aggrecan

One seminal contribution by Grodzinsky and colleagues is the direct visualization of the ultrastructure of aggrecan and its GAG side chains, which was the first of its kind [43]. In this study, Ng et al. deposited aggrecan molecules extracted from fetal epiphyseal and mature nasal bovine cartilage samples onto 3-aminopropyltriethoxysilane (APTES)-treated, positively charged, atomisticly flat mica surfaces. The nanostructure of aggrecan was then revealed via tapping mode AFM-imaging at a spatial resolution of ~2 nm. Imaging aggrecan at such unprecedented resolution enabled not only visualization of its "bottle-brush"-like molecular architecture, but also direct quantification of its structural parameters (Fig. 5.2a). This includes the core protein contour length, L_c, end-to-end distance, R_{ee}, degree of extension, R_{ee}/L_c, and the packing density and length of GAG bristles. In turn, the persistence length, L_p, as calculated from the worm-like chain model (~110 nm for fetal epiphyseal aggrecan), illustrated the highly extended conformation of aggrecan in its equilibrium state. Conversely, this study also showed that *in vivo*, aggrecan adapted a highly compacted conformation, for that the *in vivo* concentration is at least 40× higher than the densely packed form imaged on mica (Fig. 5.2b). To this end, the contrast

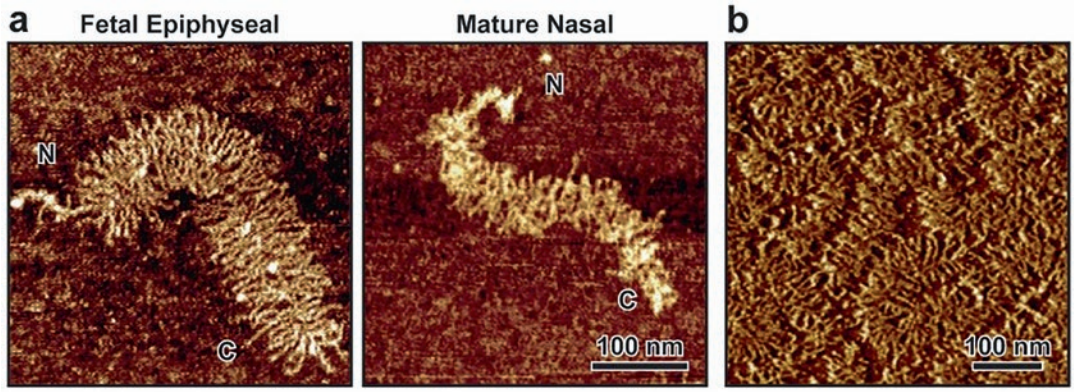


Fig. 5.2 Ultrastructure of aggrecan via tapping mode atomic force microscopy (AFM) imaging. (**a**) Nanostructure of individual fetal bovine epiphyseal and mature bovine nasal aggrecan deposited on atomicly flat mica surface. (**b**) Nanostructure of densely packed fetal bovine epiphyseal aggrecan monomers, illustrating the highly compressed conformation of aggrecan *in vivo* (~ 40× higher than the aggrecan packing imaged here). (Panels (**a**) and (**b**) are adapted with permission from Ref. [43])

between aggrecan from fetal epiphyseal versus mature nasal cartilage highlighted the large variation of its ultrastructure with tissue source and age (Fig. 5.2a). Furthermore, these high resolution images clearly illustrated the dominating role of longer CS-GAGs in contributing to the molecular conformation of aggrecan. Indeed, a follow-up study by Lee et al. compared the structure and conformation of aggrecan from a 29-year-old human donor subjected to chondroitinase ABC and keratanase II treatment, and confirmed that the extension and conformation of aggrecan is predominantly governed by the longer CS-GAGs, rather than the shorter KS-GAG chains [36].

In addition to nanostructure, Grodzinsky and colleagues also, for the first time, assessed the nanomechanics of aggrecan under multiple deformation modes, including compression, energy dissipation, shear, and adhesion. Building on earlier work of CS-GAG nanomechanics [51–53], Dean et al. chemically functionalized aggrecan with thiol-groups at its N-terminal, and end-attached thiol-functionalized aggrecan onto gold-coated planar silicon substrates and microspherical AFM colloidal tips ($R \approx 2.5$ μm). This set-up established a 2D biomimicry assembly of aggrecan at ~50 mg/mL, near its physiological packing density (~20–80 mg/mL), thus enabling the studies of aggrecan interactions under *in vivo*-like conditions. With this biomimetic system, the team performed an in-depth analysis of key molecular mechanical behaviors of aggrecan. First, compressive nanomechanics was quantified using force spectroscopy and contact mode AFM imaging in aqueous solutions with varied ionic strength (IS) conditions [10, 11]. As expected, the long-range repulsion force between two opposing aggrecan layers extended to >1 μm in IS = 0.001 M solution, while the distance and magnitude of compression resistance decreased drastically with increasing IS from 0.001 to 1.0 M (Fig. 5.3a). Applying Poisson-Boltzmann-based models, this study confirmed that the electrical double layer (EDL) repulsion interactions arising from CS-GAGs play a dominating role in the compressive nanomechanics of aggrecan, in comparison to other non-electrostatic factors such as steric hindrance and conformational entropy. Importantly, under *in vivo* conditions (IS = 0.15 M), given that the Debye length, $\kappa^{-1} \approx 0.8$ nm, is at the same order of GAG-GAG packing distance (~ 2–3 nm), the salient heterogeneity in electrical potential was a key factor in determining the magnitude of repulsion. While the continuum Donnan model substantially overestimated the repulsion force, both the unit cell model [5] and charged rod model [12], which accounted for the nanoscale spatial heterogeneity of electrical potential, accurately predicted the aggrecan-aggrecan repulsion [10].

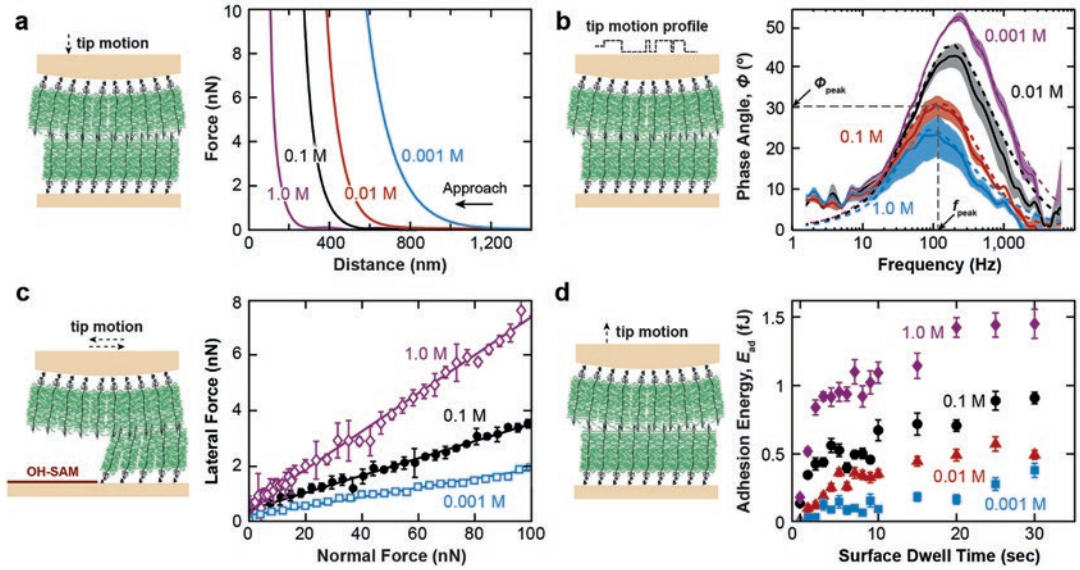


Fig. 5.3 Nanomechanics of aggrecan measured between two opposing layers of aggrecan via atomic force microscopy (AFM)-based nanomechanical modalities. Experiments were performed applying aggrecan functionalized colloidal tips ($R \approx 2.5$ μm, except that $R \approx 22.5$ μm for panel (**b**) to aggrecan-functionalized planar substrates in NaCl aqueous solutions at different ionic strengths (IS = 0.001–1.0 M). Left panels: Schematics of experimental set-ups. For panel (**c**), the substrate was prepared via micro-contact printing to form micropatterned surface of hydroxyl-terminated self-assembled monolayer (OH-SAM, $HS(CH_2)_{11}OH$) and aggrecan monolayer. Right panels: (**a**) Compressive force-versus distance curves as a function of bath IS via colloidal molecular force spectroscopy. Adapted with permission from Ref. [10]. (**b**) The magnitude of phase angle, ϕ, of newborn human aggrecan as a function of dynamic frequency and bath IS via AFM-based nanorheometric test (mean ± 95% CI, $n = 6$). Adapted with permission from Ref. [44]. (**c**) Lateral versus applied normal force curves as a function of bath IS via lateral force microscopy (mean ± SD, $n = 8$). Adapted with permission from Ref. [24]. (**d**) The total aggrecan-aggrecan adhesion energy as a function of surface dwell time and bath IS via molecular force spectroscopy (mean ± SEM, $n \geq 30$). (Adapted with permission from Ref. [23])

Following the studies of elasticity, Nia et al. probed the energy dissipative, poroelastic nanomechanics of aggrecan using the custom-built AFM-nanorheometer [44]. Similar to the case of elastic modulus, the energy dissipation of aggrecan layer is also largely governed by the EDL repulsion, as illustrated by the salient dependence of phase angle on bath IS (Fig. 5.3b). Meanwhile, the dynamic oscillatory loading responses of three specimens were compared: normal cartilage, GAG-depleted cartilage, and the end-attached aggrecan monolayers. In the low frequency elastic domain, the modulus of GAG-depleted cartilage, E_L, was ~1.5 × lower than that of the normal cartilage, while the modulus of aggrecan layer is ~7 × lower. Despite this much lower modulus, the aggrecan monolayer had comparable hydraulic permeability, k, to the native cartilage, while that of the GAG-depleted cartilage was ~24 × higher. Such contrast underscored the direct contribution of aggrecan and its sGAGs to cartilage fluid flow and pressurization capabilities. This is because the closely spaced GAG chains of aggrecan, with an effective pore size ~2–4 nm, provide the main resistance to intra-tissue fluid flow in cartilage, as manifested in the GAG-GAG nanomolecular model of hydraulic permeability [14], while the collagen network has a much larger pore size (~100 nm), resulting in elevated hydraulic permeability. Similar to the case of elasticity, a more complex structural model was needed to capture the magnitude of aggrecan-endowed energy dissipation. The fiber-reinforced model [55] or transversely isotropic model [54], which accounted for cartilage tension-compression asymmetry, were able

to quantitatively capture the degree of energy dissipation, while an isotropic poroelastic model would markedly underestimate these values.

Applying lateral force microscopy (LFM), Han et al. elucidated the shear nanomechanics of both single and two opposing aggrecan layers [24, 25]. The shear resistance of aggrecan was quantified as a function of aggrecan layer height and applied normal force under varied IS. The lateral linearity ratio, μ (= $dF_{lateral}/dF_{normal}$), was found to vary significantly with both IS (Fig. 5.3c) and lateral displacement rate, suggesting that the shear resistance was also largely governed by both EDL repulsion and fluid flow. At lower IS, given the dominance of EDL repulsion, aggrecan exhibited a more extended conformation. Therefore, a lower lateral proportional coefficient could be attributed to the minimal interdigitation between opposing aggrecan and strong water hydration effects surrounding negative charges, similar to the highly lubricative case of negatively charged synthetic polyelectrolytes. To this end, the shear of two opposing aggrecan layers also yielded a lower lateral coefficient, due to stronger EDL repulsion than just one single layer. In addition, divalent Ca^{2+} ions (~2–4 mM in cartilage [40]) were also found to mediate the shear behavior through extra screening of EDL repulsion and potentially the ion bridging effect. Collectively, EDL repulsion dominates not only the compressive, but also shear nanomechanics of aggrecan. It is also worth noting that the low lateral coefficient, μ, observed here does not imply a role of aggrecan in cartilage lubrication, as the concentration of aggrecan on cartilage surface is very low, and the synovial fluid is dominated by fragmented aggrecan, which lacks the G1-domain that enables its binding to HA [50].

Interestingly, despite the dominance of strong EDL repulsion, aggrecan also exhibited marked adhesive interactions with adjacent aggrecan molecules [23], and with collagen II fibrils [49]. When compressed at physiological-like molecular strain (~50% [59]) for 0–30 seconds, aggrecan was found to undergo pronounced adhesion, with a magnitude at ~1 pN between per pair of aggrecan-aggrecan, and ~ 0.3 pN per aggrecan molecule versus collagen II fibrils in physiological-like solution. Such adhesion was attributed to non-specific interactions, such as hydrogen bonding, hydrophobicity, ionic interactions as well as physical entanglement. Increasing EDL repulsion by lowering IS effectively limited the intermolecular contact and reduced the adhesion between the layers (Fig. 5.3d). On the other hand, Ca^{2+}-mediated ion bridging further enhanced the adhesion by providing additional ionic linkage. Given that the highly compressed state of aggrecan mimics the physiological molecular strain in unloaded cartilage, these non-specific interactions were hypothesized to be an important biophysical factor that helps stabilizing the retention of fragmented aggrecan in healthy cartilage and contributes to the integrity of cartilage ECM.

5.3 Implications for Aging, Disease and Regeneration

Following these fundamental studies of aggrecan, Grodzinsky and colleagues further applied the experimental paradigm to gain new molecular insights into cartilage disease pathogenesis and regenerative medicine. One important application was to assess age-associated changes of aggrecan polymorphism using human cartilage samples. Applying tapping mode AFM imaging and force spectroscopy, Lee et al. compared aggrecan molecules from newborn and 38-year-old adult donors [36]. Aggrecan from newborn cartilage exhibited superior nanostructure and compressive nanomechanics relative to that from adult cartilage. First, the adult aggrecan population consisted of substantially more fragmented monomers that did not have the G1 or G3 globular domain (Fig. 5.4a, left panel). Such observation illustrated that aggrecan fragmentation could be a normal homeostasis process during growth and aging, and is a prevalent feature even in healthy adult cartilage. It also indicated that the retention of aggrecan *in vivo* may require additional mechanisms beyond the aggrecan-HA association, such as the aggrecan-aggrecan and aggrecan-collagen II adhesion [23, 49]. And, even for the sub-population of full length aggre-

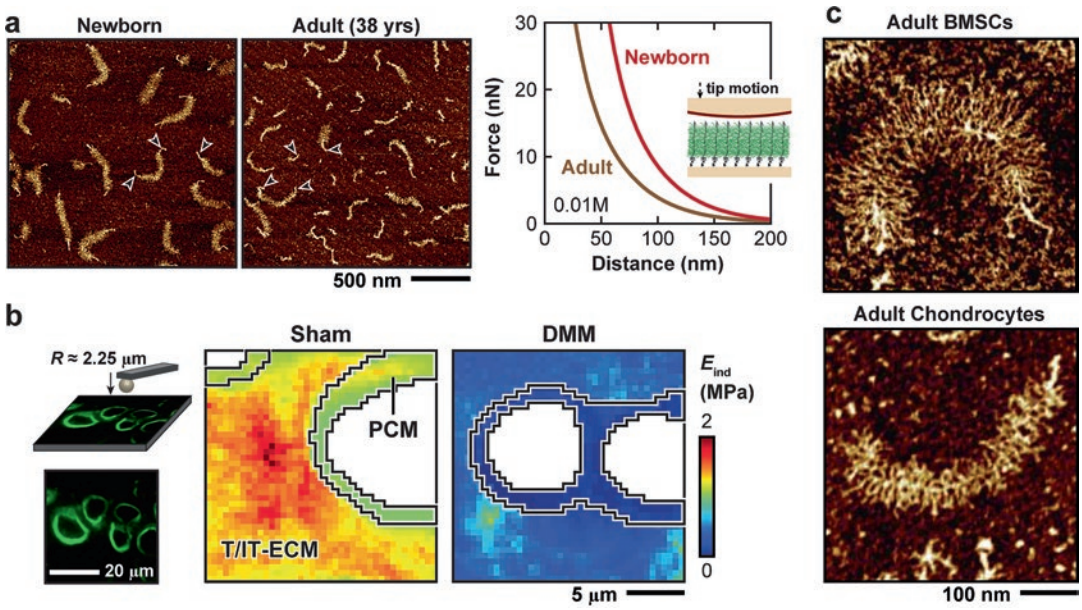


Fig. 5.4 Applications of AFM in the studies of aggrecan in cartilage aging, disease initiation and tissue engineering. (**a**) Left panel: Tapping mode AFM height images of newborn and adult (38-year-old) human aggrecan monomers. Arrow heads: globular domains. Right Panel: Compression resistance curves of end-attached newborn and adult human aggrecan monolayer measured at 0.01 M ionic strength via colloidal force spectroscopy. (Adapted with permission from Ref. [36]). (**b**) Left panel: Schematic illustration of immunofluorescence (IF)-guided AFM nanomechanical mapping on mature murine cartilage cryosection using a microspherical colloidal tip; the pericellular matrix (PCM) is immunolabeled with collagen VI. Right panel: Representative indentation modulus maps show the early reduction of PCM and territorial/inter-territorial extracellular matrix (T/IT-ECM) modulus at 1 week after applying the destabilization of the medial meniscus (DMM) surgery to 3-month-old male wild-type mice, relative to the Sham control. (Adapted with permission from Ref. [6]). (**c**) Tapping mode AFM height images of aggrecan ultrastructure synthesized by adult equine bone marrow stromal cells (BMSCs) and chondrocytes. (Adapted with permission from Ref. [33])

can, the newborn aggrecan exhibited longer core protein length, longer CS-GAG length, and denser packing of CS-GAGs, contributing to much stronger compression resistance (Fig. 5.4a, right panel). Taken together, these results provide direct molecular-level evidence about the effects of age on cartilage matrix changes, which may assist the intervention of age-associated cartilage degeneration and OA initiation.

Despite being part of the natural homeostatic process, aggrecan fragmentation is more aggravated during the initiation of OA. Inspired by the molecular-level studies by Grodzinsky, Chery et al. investigated how aggrecan degeneration alters the micromechanics of pericellular matrix (PCM), the immediate microenvironment of chondrocytes, and in turn, the mechanotransduction of chondrocytes in post-traumatic OA. In the destabilization of the medial meniscus (DMM) murine model [18], the micromodulus of PCM was measured by immunofluorescence (IF)-guided AFM nanomechanical mapping (Fig. 5.4b), and was found to show significant reduction as early as 3 days post-surgery relative to the Sham control. This reduction preceded both changes of overt tissue-level mechanical properties measured by classical AFM-nanoindentation (1 week after) [13], and appearance of histological cartilage damage (4–8 weeks after) [18]. This weakening of the PCM can be attributed to accelerated aggrecan degradation in OA, as the aggrecan neo-epitopes (e.g., VDIPEN) were found to be mainly localized in the pericellular domain at this early stage. In alignment with the PCM degeneration, at 3 days after DMM, chondrocytes also exhibited

demoted intracellular calcium signaling, $[Ca^{2+}]_i$, activities, one of the earliest, fundamental cell responses to mechanical stimuli [8]. This effect was most pronounced under hypo-osmotic stimuli, which simulate the amplified GAG-GAG EDL repulsion and increased cell strain during compressive joint loading. Conversely, when the aggravated catabolism was attenuated by small molecule inhibitor, GM6001, the reduction of PCM modulus and disruption of $[Ca^{2+}]_i$ activities could be effectively rescued. Thus, aggravated aggrecan degradation represents a key molecular event in the initiation of OA, which not only impacts the tissue-level mechanical properties, but disrupts chondrocyte mechanotransduction by impairing the PCM.

Understanding the molecular aspects of aggrecan in normal aging and disease initiation also shed light on the development of novel tissue engineering and regeneration strategies. In tissue engineering, the use of primary chondrocytes is challenged by the limited amount of cells and donor site morbidity. Bone marrow stromal cells (BMSCs) are often used as the alternative cell source [41]. When undergoing chondrogenesis, BMSCs were found to synthesize full length aggrecan within 1–2 weeks of chondrogenic culture [33, 37]. Adult equine BMSCs undergoing chondrogenesis within hydrogel cultures could synthesize aggrecan molecules having CS-GAG chains that were almost 2× longer than the CS-GAGs synthesized by primary chondrocytes harvested from those same horses (Fig. 5.4c). Importantly, it was also discovered via fluorophore-assisted carbohydrate electrophoresis (FACE) analysis that the aggrecan made by these adult BMSCs demonstrated CS-GAG sulfation patterns typical of those observed in newborn growth cartilage, even though these cells were originated from adult animals. The BMSC-derived aggrecan also showed higher compressive stiffness, close to that of newborn human aggrecan as seen in Fig. 5.4a. On the other hand, in comparison to primary chondrocytes, BMSCs had a lower synthesis rate of collagen and proteoglycans, as well as a lower retention rate of newly synthesized aggrecan in its neo-matrix. This resulted in a lesser assembled matrix with lower sGAG content in the BMSC neo-matrix [1]. When assessed via AFM-nanorheometer, BMSC neo-matrix showed a higher degree of energy dissipation and similar elastic modulus at lower frequencies, but lower modulus at high frequency relative to that of chondrocyte neo-matrix [34, 35]. Therefore, despite its capability of synthesizing more superior aggrecan, BMSCs may also have inferior capabilities in biosynthesis and neo-matrix assembly. These factors need to be considered and modulated at both molecular and cellular levels to enhance the quality of regenerative tissues.

5.4 Other Native and Biomimetic Proteoglycans

In addition to aggrecan, cartilage matrix also consists of many other proteoglycans and glycoproteins, including small leucine rich proteoglycans (SLRPs), perlecan, lubricin, matrilins and cartilage oligomeric matrix protein (COMP) [28]. These molecules are present at minor quantities, and thus, do not directly contribute to tissue biomechanics. However, they could have important roles in regulating matrix assembly or cell-matrix interactions through specific interactions with other matrix molecules, cell surface receptors, and/or cytokines [32]. Regulatory roles of individual proteoglycans have been studied by assessing the phenotype of various genetic knockout murine models [28]. Previously, analysis of murine cartilage phenotype has been mainly limited to gross-level assays, such as bulk chemistry, histology, immunohistochemistry, and micro-computed tomography (μCT). Assessment of the functional relevance of these molecules was challenged by the small volume and irregular shape of murine cartilage, which renders conventional biomechanical tools not applicable.

The nanomechanical paradigm established by Grodzinsky and colleagues enabled direct quantification of murine cartilage biomechanical properties, providing a new path for pinpointing the activities of individual proteoglycans [22]. For example, a recent study by Han et al. investigated the role of decorin in cartilage biomechanical function and OA progression [21]. Decorin is a class I SLRP containing ~40 kDa leucine rich

core protein with one CS- or dermatan sulfate (DS)-GAG chain attached near its N-terminal. In human cartilage, the concentration of decorin is ≈ 15 nmol/ml, comparable to that of aggrecan (≈ 20 nmol/ml) [47], which implies its potential importance to cartilage integrity. In both decorin-null (*Dcn*$^{-/-}$) and inducible decorin knockout mice (*Dcn*$^{f/f}$/*Rosa26Cre*^{ER}, or *Dcn*^{iKO}), loss of decorin resulted in reduced aggrecan and sGAG content in the ECM (Fig. 5.5a). When tested under the AFM-nanorheometer, decorin-deficient cartilage demonstrated compromised biomechanical properties, including lower modulus, higher hydraulic permeability and reduced fluid pressurization (Fig. 5.5b, c). These observations highlighted a crucial role of decorin in regulating the integrity of aggrecan in cartilage ECM. This hypothesis was supported by molecular-level

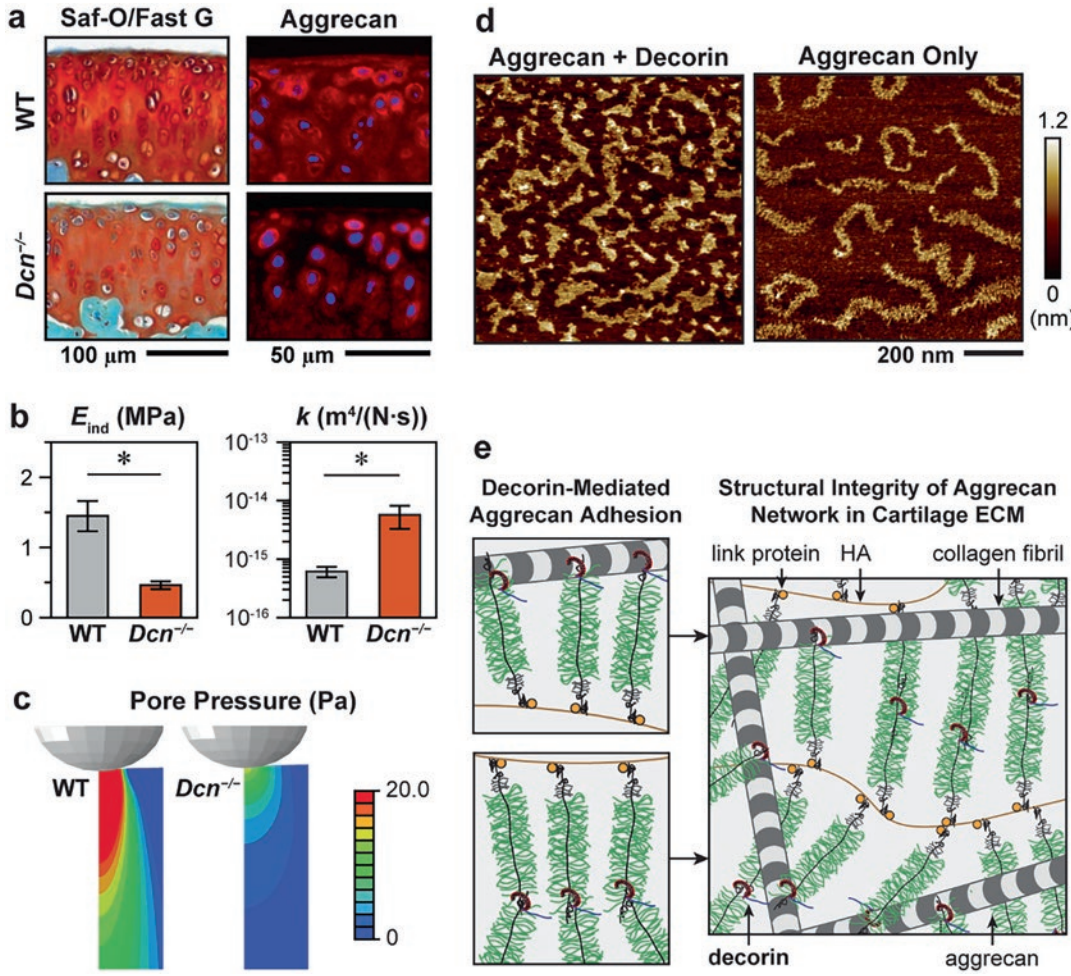


Fig. 5.5 Decorin regulates the integrity of aggrecan and biomechanics of cartilage ECM. a-c) Structural and biomechanical phenotype of *Dcn*$^{-/-}$ murine cartilage relative to the wild-type (WT) control at 3 months of age. (**a**) Safranin-O/Fast Green histology and IF images show the reduction of sulfated glycoaminoglycans (sGAGs) and aggrecan in *Dcn*$^{-/-}$ cartilage. (**b**) AFM-based nanoindentation and nanorheometric tests show the reduction of indentation modulus, E_{ind}, and the increase of hydraulic permeability, k, of cartilage. (**c**) Maximum pore pressure calculated from the fibril-reinforced poroelastic finite element model at the peak frequency (~10 Hz) corresponding to maximum phase angle. (**d**) Tapping mode AFM height imaging shows the formation of interconnected supramolecular network when aggrecan is reconstituted with free decorin protein, and individual aggrecan monomers when reconstituted without. (**e**) Schematic illustration of the structural role of decorin in regulating the molecular adhesion of aggrecan-aggrecan and aggrecan-collagen fibrils, and thus, the integrity of cartilage ECM. (Panels (**a**)–(**e**) are adapted with permission from Ref. [21])

nanomechanical experiments. First, when free decorin protein was added to the solution, molecular adhesions between two opposing aggrecan layers, and between aggrecan and collagen II fibrils, were both significantly increased. Second, when decorin protein and aggrecan monomers were reconstituted on a mica surface, they formed interconnected supramolecular networks, despite the presence of strong EDL repulsion (Fig. 5.5d). These results corroborate the observation that *Dcn*$^{-/-}$ chondrocytes synthesized a similar amount of sGAGs, but a lesser portion was retained in the neo-matrix. Therefore, in cartilage, decorin could serve as a "physical linker", which in turn, strengthens the aggrecan-aggrecan and aggrecan-collagen II molecular adhesion, enhancing the integration of aggrecan network in cartilage (Fig. 5.5e) [21].

The impact of decorin on aggrecan integrity also regulates chondrocyte mechanotransduction. Applying immunofluorescence (IF)-guided AFM, Chery et al. showed that the PCM of *Dcn*$^{-/-}$ cartilage was impaired during post-natal growth, leading to demoted chondrocyte intracellular calcium signaling, $[Ca^{2+}]_i$, activities in situ [7]. This study further confirmed that such impairment can be attributed to the reduction of aggrecan and sGAG content in the PCM, supporting the role of decorin in mediating chondrocyte mechanobiology through regulating the integrity of aggrecan in the PCM. In the DMM model, both *Dcn*$^{-/-}$ and *Dcn*^{iKO} mice exhibited accelerated loss of sGAGs and fibrillation of cartilage surface, contributing to more severe OA relative to the control [38]. The mediation of aggrecan assembly was also found to be specific to decorin. Biglycan is another class I SLRP, whose core protein has ~57% structural homology to that of decorin, but harbors two, rather than one, CS/DS-GAG side chains near its N-terminal [30]. In contrast, such aggravated OA was not detected in biglycan inducible knockout mice (*Bgn*^{fff}*/Rosa26Cre*^{ER}) subjected to DMM surgery [20]. Therefore, building on the foundation established by Grodzinsky, these recent studies highlighted an indispensable role of decorin in regulating the integrity of aggrecan network in cartilage matrix, and thus, the ECM biomechanics and chondrocyte mechanotransduction. Meanwhile, decorin also contributes to the slowdown of OA progression by attenuating the loss of fragmented aggrecan and inhibiting cartilage fibrillation.

Besides decorin, the impact of perlecan on cartilage development and homeostasis has also been studied from the nanomechanics perspective. Perlecan is a basement membrane-specific heparan sulfate proteoglycan (HSPG, M_w ~ 470 kDa), and contains three heparan sulfate (HS)-GAG or CS-GAG chains near its N-terminal. In cartilage, perlecan is localized in the PCM, and is suggested to interact with collagens VI and XI to stabilize the matrix compartment [62]. It also directly regulates cell surface mechanosensing [19] and activation of fibroblast growth factor-2 (FGF-2) [57]. Applying IF-guided AFM, Wilusz et al. demonstrated direct contribution of perlecan and its HS-GAGs to PCM integrity. Heparinase III digestion was shown to increase the micromodulus of porcine cartilage PCM, but not that of the bulk ECM [61]. It was hypothesized that the HS-GAG chain of perlecan could contribute to the local fixed charges and osmotic swelling pressure, while its enzymatic removal may reduce the swelling of PCM and in turn, increase the apparent local modulus. Furthermore, in newborn perlecan knockdown mice (*Hspg2*$^{+/-}$), Xu et al. observed reduced cartilage matrix stiffness as well as defective PCM formation. Production of an abundance of matrix proteins was elevated, including atypical sGAGs, which was hypothesized to compensate for the loss of perlecan, illustrating an important role of perlecan in mediating initial matrix assembly [63].

Marcolongo and colleagues synthesized a family of biomimetic proteoglycans (BPGs) to recapitulate the biophysical characteristics of native aggrecan at the molecular level [48]. Specifically, BPG10 is a synthetic polymer consisting of a ~ 10 kDa synthetic poly(acrylic acid) (PAA) core, decorated with ~5–7 CS-GAG bristles (Fig. 5.6a). Similar to that of aggrecan, BPG10 exhibits the "bottle-brush" architecture, with CS-GAGs packed at 3–4 nm spacing along the PAA core [48], comparable to the 2–3 nm spacing of CS-GAGs along aggrecan core protein [43]. When infiltrated into bovine cartilage

explants, these BPGs were able to localize in the PCM and territorial domain [46] (Fig. 5.6b). Kahle et al. applied IF-guided AFM to BPG10-augmented bovine cartilage explant, and showed that its localization increased the micromodulus of PCM without altering properties of the matrix bulk (Fig. 5.6c) [31]. Such effect was attributed to the increased fixed charge density within the

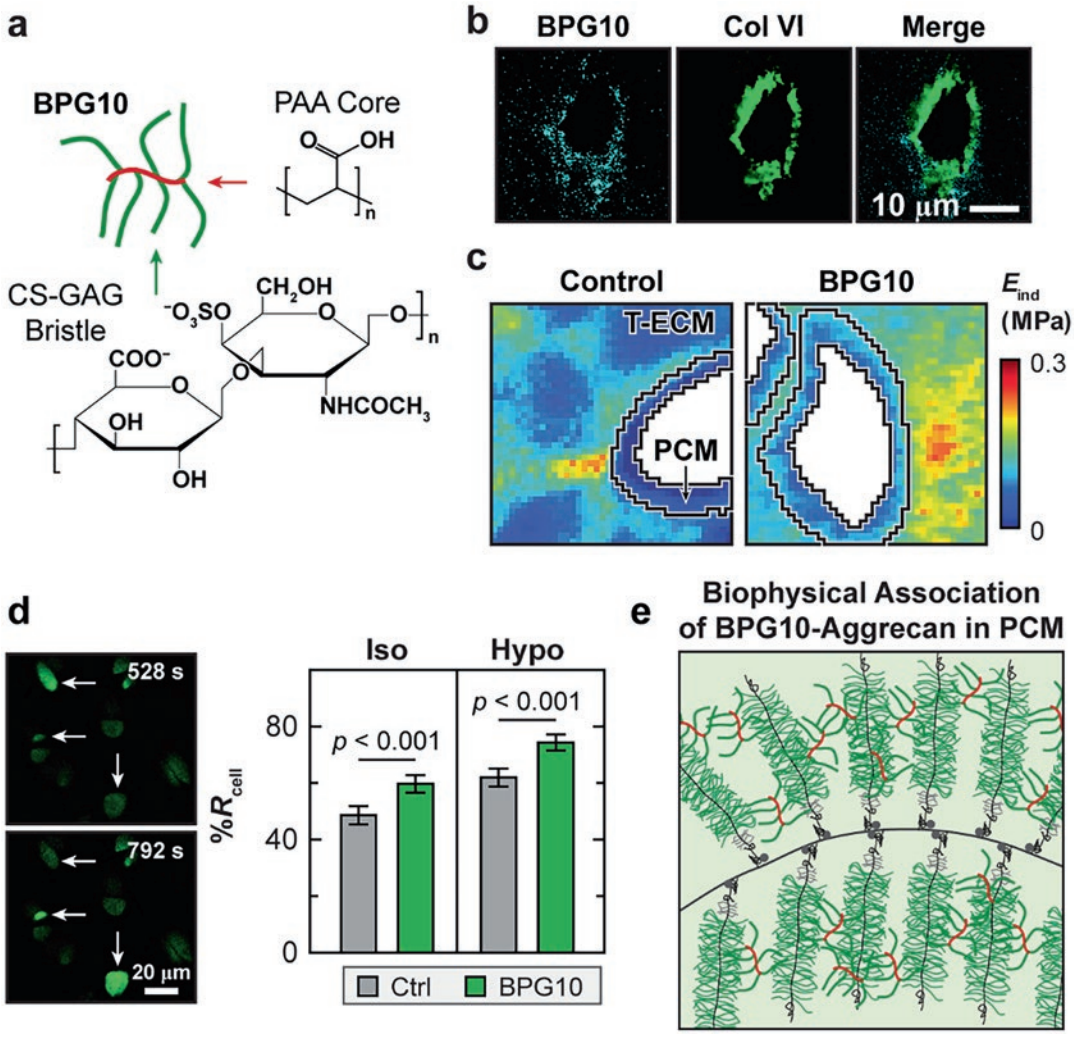


Fig. 5.6 Biomimetic proteoglycan, BPG10, strengthens cartilage pericellular matrix (PCM) and modulates chondrocyte mechanotransduction through integrating with the aggrecan network in the PCM. (**a**) BPG10 is synthesized by grafting natural chondroitin sulfate glycosaminoglycan (CS-GAG) bristles to an enzymatically resistant, synthetic poly (acrylic acid) (PAA) core. (**b**) IF images of adult bovine cartilage explants infiltrated with fluorescently-labeled BPG10 and co-stained with collagen VI demonstrate the preferred distribution of BPG10 within the PCM and nearby territorial domain. (**c**) Representative indentation modulus, E_{ind}, maps of control and BPG10-treated cartilage in 20 × 20 μm^2 regions of interest (ROIs) containing well-defined PCM rings (40 × 40 indents) via IF-guided AFM nanomechanical mapping illustrate the increase of PCM micromodulus by the infiltration of BPG10. (**d**) Left panel: Representative IF images of intracellular calcium signaling, $[Ca^{2+}]_i$, of adult bovine chondrocytes in situ. BPG10 enhances mechanosensing of chondrocytes in both isotonic and hypotonic (osmotically-simulated compression) conditions, as illustrated by an increase in the percentage of responding cells, $\%R_{cell}$ (mean ± 95% CI, ≥ 445 cells from n = 3 animals). (**e**) Schematic illustration of biophysical adhesion interactions between BPG10 and aggrecan, which enables the integration of BPG10 with the aggrecan-enriched cartilage PCM, and thus, the preferred localization of BPG10 in the PCM. (Panels (**a**)–(**e**) are adapted with permission from Ref. [31])

PCM due to the localization of BPG10. In turn, residing chondrocytes in BPG10-augmented PCM exhibited enhanced *in situ* $[Ca^{2+}]_i$ activities (Fig. 5.6d). When tested by molecular force spectroscopy, these BPG10 molecules demonstrated the capability of undergoing molecular adhesion with other BPG10 molecules and with native aggrecan, at a similar adhesion magnitude as aggrecan-aggrecan self-adhesion. Thus, it was hypothesized that by mimicking the "brush-like" ultrastructure and polyanionic nature of aggrecan, BPG10 can integrate with aggrecan in native cartilage through biophysical adhesions (Fig. 5.6e), and thus, has the potential to be used for harnessing cell mechanoresponses and modifying disease progression [31].

5.5 Summary and Outlook

This chapter summarized the transformative impact of Dr. Grodzinsky's contributions to the understanding of aggrecan molecular mechanics at the nanoscale. By developing and applying an array of AFM-based nanomechanical modalities to cartilage molecules, cells, and tissues, this body of work established a new front in understanding the origins of cartilage ECM functions, cell-ECM interactions, and disease initiation events. In addition, as discussed in this chapter, this nanotechnology paradigm established by Dr. Grodzinsky opened the door for further in-depth studies on the roles of other minor proteoglycans and proteins in cartilage biomechanics and mechanobiology, as well as the evaluation of novel molecular therapeutic strategies for OA treatment. It is expected that many future studies will benefit immensely from this molecular foundation established by Dr. Grodzinsky, which is one of the many fronts that he has contributed in musculoskeletal research.

Acknowledgements The authors would like to thank Alan J. Grodzinsky for his mentorship, inspiration and invaluable support over the past decades. We would also like to acknowledge the support from National Science Foundation Grant CMMI-1751898 and National Institutes of Health Grant AR074490.

References

1. Babalola OM, Bonassar LJ (2010) Effects of seeding density on proteoglycan assembly of passaged mesenchymal stem cells. Cell Mol Bioeng 3:197–206
2. Biot MA (1956) Theory of propagation of elastic waves in a fluid-saturated porous solid. I Low-frequency range. J Acoust Soc Am 28:168–178
3. Buckwalter JA, Rosenberg L (1983) Structural changes during development in bovine fetal epiphyseal cartilage. Coll Relat Res 3:489–504
4. Buckwalter JA, Rosenberg LC, Tang L-H (1984) The effect of link protein on proteoglycan aggregate structure – an electron microscopic study of the molecular architecture and dimensions of proteoglycan aggregates reassembled from the proteoglycan monomers and link proteins of bovine fetal epiphyseal cartilage. J Biol Chem 259:5361–5363
5. Buschmann MD, Grodzinsky AJ (1995) A molecular model of proteoglycan-associated electrostatic forces in cartilage mechanics. J Biomech Eng 117:179–192
6. Chery DR, Han B, Li Q, Zhou Y, Heo SJ, Kwok B, Chandrasekaran P, Wang C, Qin L, Lu XL, Kong D, Enomoto-Iwamoto M, Mauck RL, Han L (2020) Early changes in cartilage pericellular matrix micromechanobiology portend the onset of post-traumatic osteoarthritis. Acta Biomater 111:267–278
7. Chery DR, Han B, Zhou Y, Wang C, Adams SM, Chandrasekaran P, Kwok B, Heo SJ, Enomoto-Iwamoto M, Lu XL, Kong D, Iozzo RV, Birk DE, Mauck RL, Han L (2021) Decorin regulates cartilage pericellular matrix micromechanobiology. Matrix Biol 96:1–17
8. Clapham DE (2007) Calcium signaling. Cell 131:1047–1058
9. Clark IC (1971) Articular cartilage: a review and scanning electron microscopy study. I The interterritorial fibrillar architecture. J Bone Joint Surg Br 53B:732–750
10. Dean D, Han L, Grodzinsky AJ, Ortiz C (2006) Compressive nanomechanics of opposing aggrecan macromolecules. J Biomech 39:2555–2565
11. Dean D, Han L, Ortiz C, Grodzinsky AJ (2005) Nanoscale conformation and compressibility of cartilage aggrecan using microcontact printing and atomic force microscopy. Macromolecules 38:4047–4049
12. Dean D, Seog J, Ortiz C, Grodzinsky AJ (2003) Molecular-level theoretical model for electrostatic interactions within polyelectrolyte brushes: applications to charged glycosaminoglycans. Langmuir 19:5526–5539
13. Doyran B, Tong W, Li Q, Jia H, Zhang X, Chen C, Enomoto-Iwamoto M, Lu XL, Qin L, Han L (2017) Nanoindentation modulus of murine cartilage: a sensitive indicator of the initiation and progression of post-traumatic osteoarthritis. Osteoarthr Cartil 25:108–117

14. Eisenberg SR, Grodzinsky AJ (1988) Electrokinetic micromodel of extracellular matrix and other polyelectrolyte networks. Physicochem Hydrodyn 10:517–539
15. Eyre DR, Weis MA, Wu J-J (2006) Articular cartilage collagen: an irreplaceable framework? Eur Cell Mater 12:57–63
16. Frank EH, Grodzinsky AJ (1987a) Cartilage electromechanics – I. Electrokinetic transduction and the effects of electrolyte pH and ionic strength. J Biomech 20:615–627
17. Frank EH, Grodzinsky AJ (1987b) Cartilage electromechanics – II. A continuum model of cartilage electrokinetics and correlation with experiments. J Biomech 20:629–639
18. Glasson SS, Blanchet TJ, Morris EA (2007) The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. Osteoarthr Cartil 15:1061–1069
19. Gubbiotti MA, Neill T, Iozzo RV (2017) A current view of perlecan in physiology and pathology: a mosaic of functions. Matrix Biol 57–58:285–298
20. Han B, Li Q, Wang C, Chandrasekaran P, Zhou Y, Qin L, Liu XS, Enomoto-Iwamoto M, Kong D, Iozzo RV, Birk DE, Han L (2021) Differentiated activities of decorin and biglycan in the progression of post-traumatic osteoarthritis. Osteoarthr Cartil 29:1181–1192
21. Han B, Li Q, Wang C, Patel P, Adams SM, Doyran B, Nia HT, Oftadeh R, Zhou S, Li CY, Liu XS, Lu XL, Enomoto-Iwamoto M, Qin L, Mauck RL, Iozzo RV, Birk DE, Han L (2019) Decorin regulates the aggrecan network integrity and biomechanical functions of cartilage extracellular matrix. ACS Nano 13:11320–11333
22. Han B, Nia HT, Wang C, Chandrasekaran P, Li Q, Chery DR, Li H, Grodzinsky AJ, Han L (2017) AFM-nanomechanical test: an interdisciplinary tool that links the understanding of cartilage and meniscus biomechanics, osteoarthritis degeneration and tissue engineering. ACS Biomater Sci Eng 3:2033–2049
23. Han L, Dean D, Daher LA, Grodzinsky AJ, Ortiz C (2008) Cartilage aggrecan can undergo self-adhesion. Biophys J 95:4862–4870
24. Han L, Dean D, Mao P, Ortiz C, Grodzinsky AJ (2007a) Nanoscale shear deformation mechanisms of opposing cartilage aggrecan macromolecules. Biophys J 93:L23–L25
25. Han L, Dean D, Ortiz C, Grodzinsky AJ (2007b) Lateral nanomechanics of cartilage aggrecan macromolecules. Biophys J 92:1384–1398
26. Han L, Grodzinsky AJ, Ortiz C (2011) Nanomechanics of the cartilage extracellular matrix. Annu Rev Mater Res 41:133–168
27. Hardingham TE, Muir H (1972) The specific interaction of hyaluronic acid with cartilage proteoglycans. Biochim Biophys Acta 279:401–405
28. Heinegård D (2009) Proteoglycans and more – from molecules to biology. Int J Exp Pathol 90:575–586
29. Huey DJ, Athanasiou KA (2011) Maturational growth of self-assembled, functional menisci as a result of TGF-beta 1 and enzymatic chondroitinase-ABC stimulation. Biomaterials 32:2052–2058
30. Iozzo RV (1998) Matrix proteoglycans: from molecular design to cellular function. Annu Rev Biochem 67:609–652
31. Kahle ER, Han B, Chandrasekaran P, Phillips ER, Mulcahey MK, Lu XL, Marcolongo MS, Han L (2022) Molecular engineering of pericellular microniche via biomimetic proteoglycans modulates cell mechanobiology. ACS Nano 16:1220–1230
32. Karamanos NK, Piperigkou Z, Theocharis AD, Watanabe H, Franchi M, Baud S, Brézillon S, Götte M, Passi A, Vigetti D, Ricard-Blum S, Sanderson RD, Neill T, Iozzo RV (2018) Proteoglycan chemical diversity drives multifunctional cell regulation and therapeutics. Chem Rev 118:9152–9232
33. Kopesky PW, Lee H-Y, Vanderploeg EJ, Kisiday JD, Frisbie DD, Plaas AHK, Ortiz C, Grodzinsky AJ (2010) Adult equine bone marrow stromal cells produce a cartilage-like ECM mechanically superior to animal-matched adult chondrocytes. Matrix Biol 29:427–438
34. Lee B, Han L, Frank EH, Chubinskaya S, Ortiz C, Grodzinsky AJ (2010a) Dynamic mechanical properties of the tissue-engineered matrix associated with individual chondrocytes. J Biomech 43:469–476
35. Lee B, Han L, Frank EH, Grodzinsky AJ, Ortiz C (2015) Dynamic nanomechanics of individual bone marrow stromal cells and cell-matrix composites during chondrogenic differentiation. J Biomech 48:171–175
36. Lee H-Y, Han L, Roughley PJ, Grodzinsky AJ, Ortiz C (2013) Age-related nanostructural and nanomechanical changes of individual human cartilage aggrecan monomers and their glycosaminoglycan side chains. J Struct Biol 181:264–273
37. Lee H-Y, Kopesky PW, Plaas AHK, Sandy JD, Kisiday J, Frisbie D, Grodzinsky AJ, Ortiz C (2010b) Adult bone marrow stromal cell-based tissue-engineered aggrecan exhibits ultrastructure and nanomechanical properties superior to native cartilage. Osteoarthr Cartil 18:1477–1486
38. Li Q, Han B, Wang C, Tong W, Wei Y, Tseng WJ, Han LH, Liu XS, Enomoto-Iwamoto M, Mauck RL, Qin L, Iozzo RV, Birk DE, Han L (2020) Mediation of cartilage matrix degeneration and fibrillation by decorin in post-traumatic osteoarthritis. Arthritis Rheumatol 72:1266–1277
39. Lu XL, Mow VC (2008) Biomechanics of articular cartilage and determination of material properties. Med Sci Sports Exerc 40:193–199
40. Maroudas A (1979) Physicochemical properties of articular cartilage. In: Freeman MAR (ed) Adult Articular Cartilage. Pitman, Tunbridge Wells, pp 215–290
41. Mauck RL, Yuan X, Tuan RS (2006) Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. Osteoarthr Cartil 14:179–189

42. Mow VC, Kuei SC, Lai WM, Armstrong CG (1980) Biphasic creep and stress relaxation of articular cartilage in compression: theory and experiments. J Biomech Eng 102:73–84
43. Ng L, Grodzinsky AJ, Patwari P, Sandy J, Plaas A, Ortiz C (2003) Individual cartilage aggrecan macromolecules and their constituent glycosaminoglycans visualized via atomic force microscopy. J Struct Biol 143:242–257
44. Nia HT, Han L, Bozchalooi IS, Roughley P, Youcef-Toumi K, Grodzinsky AJ, Ortiz C (2015) Aggrecan nanoscale solid-fluid interactions are a primary determinant of cartilage dynamic mechanical properties. ACS Nano 9:2614–2625
45. Pearle AD, Warren RF, Rodeo SA (2005) Basic science of articular cartilage and osteoarthritis. Clin Sports Med 24:1–12
46. Phillips ER, Haislup BD, Bertha N, Lefchak M, Sincavage J, Prudnikova K, Shallop B, Mulcahey MK, Marcolongo MS (2019) Biomimetic proteoglycans diffuse throughout articular cartilage and localize within the pericellular matrix. J Biomed Mater Res A 107:1977–1987
47. Poole AR, Rosenberg LC, Reiner A, Ionescu M, Bogoch E, Roughley PJ (1996) Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. J Orthop Res 14:681–689
48. Prudnikova K, Yucha RW, Patel P, Kriete AS, Han L, Penn LS, Marcolongo MS (2017) Biomimetic proteoglycans mimic macromolecular architecture and water uptake of natural proteoglycans. Biomacromolecules 18:1713–1723
49. Rojas FP, Batista MA, Lindburg CA, Dean D, Grodzinsky AJ, Ortiz C, Han L (2014) Molecular adhesion between cartilage extracellular matrix macromolecules. Biomacromolecules 15:772–780
50. Sandy JD, Flannery CR, Neame PJ, Lohmander LS (1992) The structure of aggrecan fragments in human synovial fluid. Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond of the interglobular domain. J Clin Invest 89:1512–1516
51. Seog J, Dean D, Plaas AHK, Wong-Palms S, Grodzinsky AJ, Ortiz C (2002) Direct measurement of glycosaminoglycan intermolecular interactions via high-resolution force spectroscopy. Macromolecules 35:5601–5615
52. Seog J, Dean D, Rolauffs B, Wu T, Genzer J, Plaas AHK, Grodzinsky AJ, Ortiz C (2005) Nanomechanics of opposing glycosaminoglycan macromolecules. J Biomech 38:1789–1797
53. Seog J, Dean DM, Frank EH, Ortiz C, Grodzinsky AJ (2004) Preparation of end-grafted polyelectrolytes on nanoscale probe tips using an electric field. Macromolecules 37:1156–1158
54. Soltz MA, Ateshian GA (2000) A conewise linear elasticity mixture model for the analysis of tension-compression nonlinearity in articular cartilage. J Biomech Eng 122:576–586
55. Soulhat J, Buschmann MD, Shirazi-Adl A (1999) A fibril-network-reinforced biphasic model of cartilage in unconfined compression. J Biomech Eng 121:340–347
56. Vanden Berg-Foels WS, Scipioni L, Huynh C, Wen X (2012) Helium ion microscopy for high-resolution visualization of the articular cartilage collagen network. J Microsc 246:168–176
57. Vincent TL, McLean CJ, Full LE, Peston D, Saklatvala J (2007) FGF-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer. Osteoarthr Cartil 15:752–763
58. Wang C, Brisson BK, Terajima M, Li Q, Hoxha K, Han B, Goldberg AM, Sherry Liu X, Marcolongo MS, Enomoto-Iwamoto M, Yamauchi M, Volk SW, Han L (2020) Type III collagen is a key regulator of the collagen fibrillar structure and biomechanics of articular cartilage and meniscus. Matrix Biol 85–86:47–67
59. Wight TN, Heinegård DK, Hascall VC (1991) Cell biology of extracellular matrix. Plenum press, New York
60. Williamson AK, Chen AC, Sah RL (2001) Compressive properties and function-composition relationships of developing bovine articular cartilage. J Orthop Res 19:1113–1121
61. Wilusz RE, Defrate LE, Guilak F (2012) A biomechanical role for perlecan in the pericellular matrix of articular cartilage. Matrix Biol 31:320–327
62. Wilusz RE, Sanchez-Adams J, Guilak F (2014) The structure and function of the pericellular matrix of articular cartilage. Matrix Biol 39:25–32
63. Xu X, Li Z, Leng Y, Neu CP, Calve S (2016) Knockdown of the pericellular matrix molecule perlecan lowers in situ cell and matrix stiffness in developing cartilage. Dev Biol 418:242–247

6 Computational Modelling for Managing Pathways to Cartilage Failure

Saeed Miramini, David W. Smith, Bruce S. Gardiner, and Lihai Zhang

Abstract

Over several decades the perception and therefore description of articular cartilage changed substantially. It has transitioned from being described as a relatively inert tissue with limited repair capacity, to a tissue undergoing continuous maintenance and even adaption, through a range of complex regulatory processes. Even from the narrower lens of biomechanics, the engagement with articular cartilage has changed from it being an interesting, slippery material found in the hostile mechanical environment between opposing long bones, to an intriguing example of mechanobiology in action. The progress revealing this complexity, where physics, chemistry, material science and biology are merging, has been described with increasingly sophisticated computational models. Here we describe how these computational models of cartilage as an integrated system can be combined with the approach of structural reliability analysis. That is, causal, deterministic models placed in the framework of the probabilistic approach of structural reliability analysis could be used to understand, predict, and mitigate the risk of cartilage failure or pathology. At the heart of this approach is seeing cartilage overuse and disease processes as a 'material failure', resulting in failure to perform its function, which is largely mechanical. One can then describe pathways to failure, for example, how homeostatic repair processes can be overwhelmed leading to a compromised tissue. To illustrate this 'pathways to failure' approach, we use the interplay between cartilage consolidation and lubrication to analyse the increase in expected wear rates associated with cartilage defects or meniscectomy.

Keywords

Computational modelling · Biomechanics · Cartilage · Injury

S. Miramini · L. Zhang
Department of Infrastructure Engineering, The University of Melbourne, Melbourne, VIC, Australia
e-mail: s.miramini@unimelb.edu.au; lihzhang@unimelb.edu.au

D. W. Smith
School of Physics, Mathematics and Computing, The University of Western Australia, Perth, WA, Australia
e-mail: david.smith@uwa.edu.au

B. S. Gardiner (✉)
Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, WA, Australia
e-mail: b.gardiner@murdoch.edu.au

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_6

6.1 Introduction

Over the past ~40 years, incredible advances have been made in cartilage biology. We are thinking about the finding that transient compressive stiffness arises from low hydraulic conductivity of cartilage and collagen network stiffness, how the equilibrium cartilage compressive stiffness arises primarily from repulsion between negatively charged aggrecan, the interplay between the aggrecan compression and the collagen tension to maintain normal cartilage stiffness, and how turnover of these ECM components is regulated by chondrocytes via chemical, electromechanical and mechanical signals [1–8]. These insights, often gained by patient work on individual processes or isolated molecules, have been integrated to present a compelling story of how this tissue functions in both the short term (to an individual loading) and the long-term (to repeated cyclic loadings), and potential pathways to pathology [8]. With the insight made possible by understanding this integrated system, we are only just beginning to develop rational strategies to intervene at the tissue scale to maintain or reverse cartilage damage.

Meanwhile great advances have also occurred in imaging and computational biomechanics, genetics and genetic manipulations that may soon enable patient (or cohort) specific data to be incorporated into cartilage treatment strategies [9, 10]. How best to do this is still in its infancy, and so largely an open question, but it holds such promise. Our belief is that this way forward will no doubt be computational (*i.e.* based on deterministic quantitative mechanistic models of cartilage tissue turnover in response to its mechanical and chemical environment), but will also necessarily include statistical aspects (*e.g.* variable loadings and model tissue parameters over time and/or population). More to our point, we believe the concepts of risk analysis borrowed from reliability engineering, provides a promising framework to shepherd our hard-won understanding of cartilage biology into the clinic [11, 12]. Here we will expand on these ideas. In doing so we will necessarily review some cartilage biology. However, the focus will be on identifying strategies cartilage uses to maintain and repair itself, and the pathways to cartilage 'failure', however failure may be defined. This approach promises to enable the risk associated with various 'disease pathways' in an individual or patient cohort to be rationally quantified, and then managed.

6.2 Articular Cartilage, an Extraordinary Tissue

Articular cartilage faces extraordinary mechanical challenges during daily physical activities. For example, knee cartilages in adults experience contact forces up to 5 times the body weight during stair climbing [13], leading to contact stresses up to 18 MPa. To get this loading in perspective, we note that a large stiletto heel exerts about 10 MPa pressure on the ground, and this contact stress is well-known for damaging some wooden floors—while cartilage tissue repeatedly experiences stresses that are almost twice as great. Probably due to this harsh mechanical environment, cartilage is an avascular tissue with a sparse chondrocyte to extracellular matrix (ECM) distribution, which limits its repair capacity. All tissues that normally repair quickly have abundant blood supplies, while in contrast, cartilage relies solely on diffusion/advection of nutrients and oxygen from synovial fluid that bathes its contact surface, with the subchondral bone- cartilage interface generally considered impermeable in healthy joints. Consequently, articular cartilage function and homeostasis largely rely on complex interactions between its main extracellular components: interstitial fluid, aggrecan and a Type II collagen network [5, 8]. For example, the rate of tissue strain under sustained load largely depends on the interstitial fluid movement through the cartilage tissue and across the cartilage surfaces, influencing the mechanical and chemical microenvironments continually being sensed by chondrocytes, while the fluid contributes to synovial joint lubrication [5, 14]. When cartilage is subjected to compressive loading, it consolidates. The load is initially carried by the fluid phase, which is slowly squeezed out of the

extracellular matrix, helping to sustain very low frictional force between two opposing cartilage surfaces. As cartilage interstitial fluid exudes through the tissue surface, load is gradually transferred to the solid matrix, resulting in a gradual increase in friction at contacting surfaces.

6.3 Cartilage Damage Mechanics

The ability of cartilage to maintain its physiological function in this hostile mechanical environment depends on the tissue's ability to continually synthesize extracellular matrix components, while avoiding excessive strain, an attribute normally conferred by its composite structure. Aggrecans are negatively charged molecules that have counter ions in a diffuse double-layer to maintain overall electroneutrality. Overlapping double-layers repel and so normally expand and imbibe water. However, this expansion is resisted by the collagen network [15]. The aggrecan molecules within the collagen network normally provide the equilibrium compressive stiffness for cartilage tissue [16], and ensure a very small hydraulic permeability which delays consolidation of the tissue to its equilibrium state. Consequently, when loaded, a long consolidation time follows (e.g. up to three or more hours).

As shown schematically in Fig. 6.1, a damaged collagen network is not able to effectively retain a high enough concentration of aggrecan molecules within the collagen network, which leads to cartilage softening [17]. Reduced aggrecan content also leads to larger hydraulic conductivity, more rapid consolidation and larger strains. This means the aggregate, collagen tissue network and chondrocytes are more likely to be damaged by excessive strain (following even normal load), leading to further loss of aggrecan [18]. This positive feedback cycle is just one important pathway that can lead to disease such as osteoarthritis. One can imagine many more and so osteoarthritis is not just one disease [8, 19]. Its management is likely to also differ depending on the etiology.

6.4 Role of Computational Modelling to Capture Complex Interactions

A key attraction of computational modeling is that it can be employed to reveal the spatial and temporal distribution of tissue microenvironments experienced by chondrocytes embedded within articular cartilage. This involves interpolating sparse experimental data sets, often measured at tissue boundaries, to define local conditions experienced by chondrocytes throughout the cartilage tissue. This immediately opens up the possibility of beginning to define previously inaccessible variables that are likely to be driving local ECM damage and chondrocyte repair processes within articular cartilage. This new capability, together with experimentally calibrated computational damage and repair functions, then naturally leads to predictions about the integrity of cartilage under various short and long-term scenarios.

Through this process, computational modeling can provide a pathway from laboratory data to quantitative predictions about future tissue states under various scenarios. This should be of great interest to clinicians, as they are in the business of advising patients as to the optimal path to follow in response to their problem. The process of building and calibrating computational models of cartilage tissue also offers up the possibility of a new pathway to more effective use of the new data being generated by 'precision medicine' for individual patients.

There are many chemical molecules and mechanical cues that regulate articular cartilage homeostasis. Therefore, to realistically define cartilage microenvironments requires consideration of interactions between many specific and rather detailed computational modules (involving multi-physics and multiphasic modelling). The theory of porous media has been widely employed to simulate the mechanical behaviour of biological soft tissues, such as articular cartilage [20–25] and fracture callus [26–28]. Here the extracellular matrix and interstitial fluid interaction contributes to the time-dependent observed tissue stiffness and deformation behaviour. By

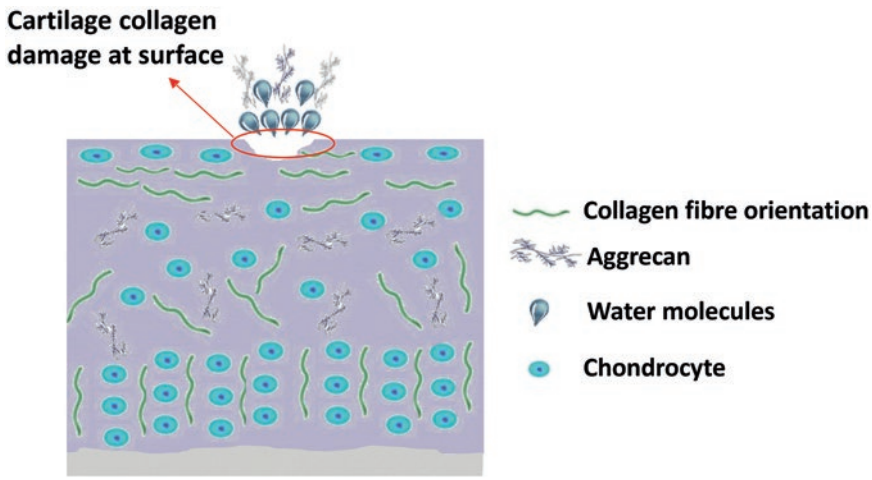


Fig. 6.1 Schematic shows damage to superficial collagen network results in escape of aggrecans and water molecules from the cartilage tissue, which may significantly increase strain locally. This can lead to local damage of the extracellular matrix and chondrocytes

combining the porous media theory with transport models and chemical reactions, we can also simulate three-dimensional diffusion and advection of different molecules in cartilage, and use this information to predict different cellular activities [14, 29–36]. For example, insulin-like growth factors mediate cartilage cellular activities such as cell proliferation, differentiation, apoptosis and synthesis of extracellular matrix [32, 37, 38]. We have built a spatial model for IGF in cartilage tissue that includes many simultaneous chemical reactions, as well as transport parameters [36]. Each of these parameters depends on the actual chemical structure of IGF and its binding proteins, as these determine the chemical rate constants that define their interactions. These rate constants depend on the amino acid sequence in each molecule, which in turn depends on the genetic code in that individual. If the genetic code is known, as revealed by precision medicine, it should be possible to predict the effect of that person's genetic code on the chemical rate constants, enabling the creation of customized computational models for each individual's IGF system for their cartilage. By this means, we can bridge the information gap between genetic data and what this data actually means in the context of a tissue.

This same principle can be applied to all the other chemical molecules found in cartilage tissue. For example, the inflammatory cytokine IL-1a is known to modulate biochemical degradation of cartilage tissue following a traumatic joint injury, so we built a detailed model of IL-1beta in cartilage, and calibrated the model using detailed experimental data generated exclusively in the Grodzinsky lab [37]. The model simulated the experimental observation of biochemical degradation of bovine articular cartilage explants. The developed model can help improve our understanding of in vivo events after a joint injury and potentially be employed for assessing the influence of different therapeutic molecules on osteoarthritis management [39, 40].

Since the biochemical signaling pathways are influenced by the mechanical microenvironment of cartilage, we built a mechanical model of cartilage. We have developed and published a state-of-the-art biphasic model of cartilage mechanics that is validated against ex-vivo mechanical experiments on human osteochondral plugs subject to cyclic loading [22]. The model takes into account tensile loading being carried by the collagen network, and compressive load carried by water and aggrecan. The model has a non-linear compressive stiffness and non-linear hydraulic

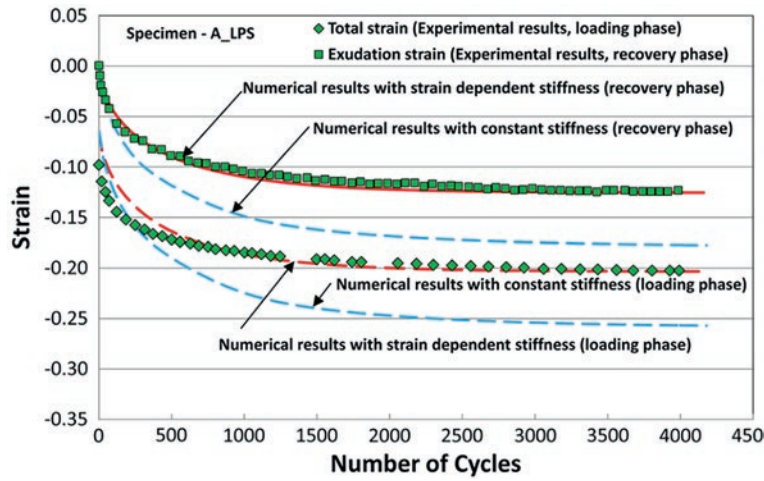


Fig. 6.2 Our cartilage mechanics model can accurately reproduce experimental data of time-course of deformation in both the loading and recovery phase of the experiment. (Figure adapted from Zhang et al. [22] with permission)

permeability based on the aggrecan concentration, which changes as the cartilage tissue deforms. As such this model represents a new constitutive model of articular cartilage, which helps provide a sound foundation for new models describing cartilage damage and repair modelling (Fig. 6.2).

6.5 Using Models to Investigate Pathways to Cartilage Failure

Synovial joint lubrication is one of the key roles of articular cartilage. Synovial joints can experience very small frictional force, with initial friction coefficient ranging from 0.005 to 0.02, while bearing extremely large mechanical stress [41, 42]. However, experimental studies have shown that the cartilage friction coefficient can rise with time (~minutes) under loading [43, 44]. Our computational modelling in conjunction with experimental observations have shown that there is a strong correlation between cartilage friction coefficient and the degree of cartilage consolidation. This suggests that after prolonged period of loading, in particular stationary activities (e.g. standing), consolidation has occurred and friction coefficient rises (Fig. 6.3). The cartilage surface can experience relatively large friction coefficient (*e.g.* 0.2–0.3, with subsequent additional cartilage damage likely as motion recommences).

It is known that large frictional force at the joints can result in elevated cartilage surface wear and damage and cartilage delamination. A well calibrated and patient-specific computational modelling can help us simulate the likely impact of physical activities on synovial joint health and thereby design patient-specific physical therapy activities for management of osteoarthritis We have incorporated different joint states such as meniscectomy and cartilage surface defects, and simulated cartilage time-dependent lubrication. As shown in Fig. 6.4, the response of damaged cartilage, to the same loading conditions, was a faster rate of consolidation and quicker increase in surface friction coefficient. The expectation then is these compromised joints will experience a higher average friction coefficient, than a healthy cartilage, and so a higher surface wear rate. Knowing this, from medical history (*e.g.* observation of defects in MRI) it is possible to devise activities that minimize the likelihood of these adverse situations occurring, thereby increasing the likelihood cartilage will maintain its functional integrity.

Although we have shown above that defects and meniscectomies can increase consolidation and extend the time a cartilage surface experiences high friction, we still do not know how critical this is in a particular individual. The load the joint experiences depends on factors such as body weight, joint size and shape, limb geometry and lifestyle. Some of these biomechanical

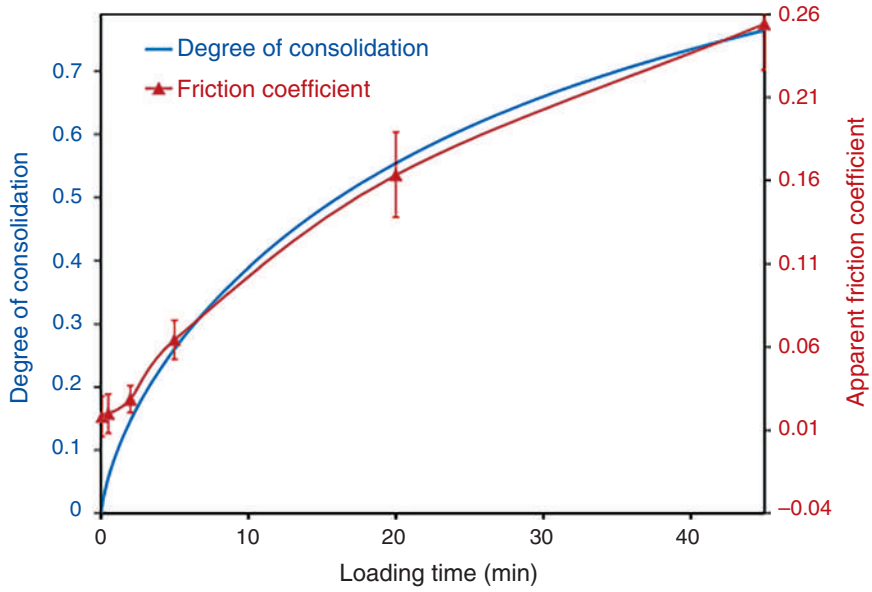


Fig. 6.3 There is a strong correlation between degree of consolidation of articular cartilage and friction coefficient in synovial joint. (This figure has been reproduced from Miramini et al. [45] with permission)

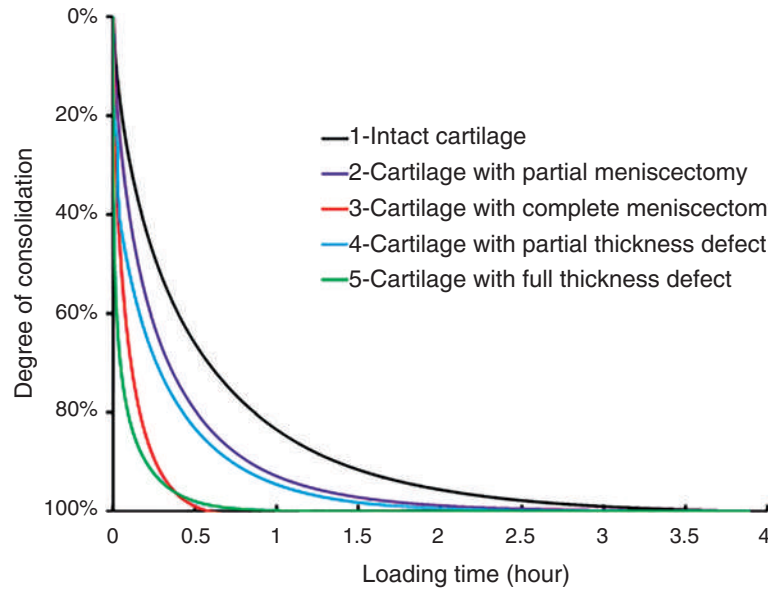


Fig. 6.4 A faster consolidation is predicted for cartilage with meniscectomy and the one with a full thickness defect. (This figure has been adapted from Miramini et al. [45] with permission)

aspects can be incorporated for an individual through a combination of imaging and gait analysis to predict joint loads. In addition, the functional mechanical properties of the cartilage tissue are also expected to vary in a population due to genetic and environmental histories. These factors can be also incorporated in patient -specific simulation.

Computational modelling can also assist clinicians in assessment of cartilage health. For example, we can assess cartilage tissue functional properties by combined fluoroscopic and MRI imaging of the knee in a standing still posture and measuring the degree of joint closure over time [46]. The calculated degree of consolidation of knee joint together with computational modelling

enable evaluation of knee joint ability to sustain interstitial fluid pressure and so experience a normal low surface friction coefficient [45].

6.6 Probabilistic Modelling and Osteoarthritis Risk Assessment

As alluded to above, there are numerous uncertainties and variability associated with the parameters affecting cartilage behaviour. For example, cartilage loading condition depends on many factors including physical activity, body weight and joint anatomy. In addition, the physical properties of cartilage also remain uncertain and depend on factors such as age, joint health and genetic factors. Therefore, it is of critical importance to consider the uncertainty and variability of different factors when simulating cartilage behaviors. Probabilistic analysis has been traditionally developed and employed for reliability assessment of engineering structure such as bridges and nuclear power stations. Compared with a deterministic approach that adopts a discrete value for a specific model parameter, a probabilistic modelling approach takes into account the distribution of environmental factors and model parameters in the deterministic calculation and therefore generates a distribution of tissue trajectories (including pathways to disease) and therefore outcomes. In the context of engineering, we can define the 'probability of failure' as the likelihood of exceeding some pre-determined state critical to the functional performance of the engineered structure. For example, it might be the probability of a load exceeding the structure's (*e.g.* a bridge's) strength, or the probability of a load exceeding a certain level of deformation in the structure, or the probability of environmental factors causing a certain level of material damage (*e.g.* component fatigue damage, irradiation damage, or corrosion damage). Reliability can be defined as one minus the probability of failure. In the context of loading a structure, the probability of failure can be defined as the overlap between the probability density function of a 'generalized loading' applied on the structure and probability density function of the structure 'generalized resistance', as shown in Fig. 6.5.

Recently, we have used this approach to predict the likelihood of knee osteoarthritis [9]. This was done on the basis of a simple model of chondrocyte ECM synthesis in response to loading and the possibility for chondrocyte apoptosis under that load. Failure was defined by the ability to resist a test load (i.e. not exceed a maximum strain threshold). The model predicted that low activity leads to low ECM synthesis and so a gradual softening of the tissue. High activity increases ECM synthesis but also exposure to excessive loads. The probabilistic predictions had the median time to onset of failure occurred earlier in the low activity model, and with a relatively narrow uncertainty in onset time. The high activity level had a delayed median onset, but had

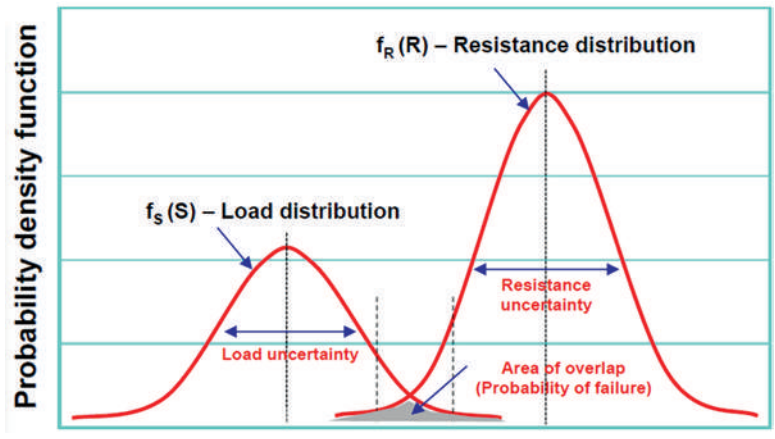


Fig. 6.5 The probability of failure of a structure can be estimated by finding the overlapping area under the probability density functions of generalized load and generalized resistance. (Figure adapted with permission from Miramini and Yang [47])

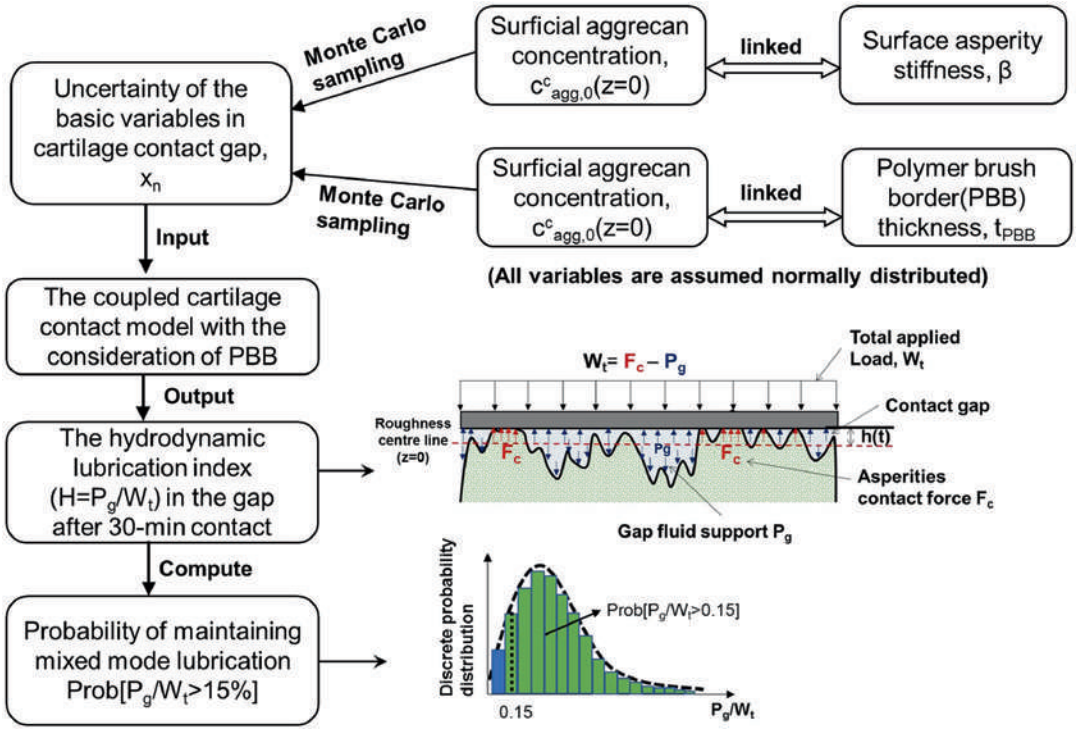


Fig. 6.6 Probabilistic computational model of cartilage lubrication. (Figure adapted with permission from Liao et al. [48])

much wider distribution of failure onset, relative to the low activity predictions [9].

In addition, we have recently developed a multi-scale probabilistic computational model (Fig. 6.6) to simulate the cartilage lubrication behaviour by incorporating the uncertainties associated with the key variables governing cartilage contact gap mechanics [48–50]. The model takes into account the internal relation between different variables and their correlated influence on cartilage lubrication. The simulation results show that an increase of polymer brush border thickness at the cartilage surface improves the hydrodynamic lubrication of cartilage, while the increasing surficial GAG content of the cartilage and increasing asperity stiffness could negatively affect hydrodynamic lubrication. Finally, we note that this probabilistic approach has also been adopted to estimate the probability of delayed bone fracture healing [47, 51].

6.7 Conclusion

To conclude, we are reaching a stage where it is now possible to connect all the pieces together into a whole picture of articular cartilage homeostasis and to identify pathways to disease. Computational modeling seems to be the natural platform upon which to integrate, into their proper context, the many interacting processes involved. Here we presented various mechanistic sub-models describing aspects of articular cartilage health. However we have also advocated for the merging of these mechanistic sub-models

with the statistical-based models or approaches from (structural) reliability engineering. This then can provide a 'bridge' between the molecular and cell biology, biomechanics and epidemiology of osteoarthritis to give a rational basis for patient specific treatments. Although all the computational approaches are present to make this approach possible, the barriers to its adoption depends, not the least, on the adoption by clinicians.

Acknowledgements The authors would like to thank Alan J. Grodzinsky for his enthusiasm, openness and endless support and encouragement to us over many, many years. We would also like to acknowledge the support of Australian Research Council (ARC DP180100915).

References

1. Kisiday J et al (2002) Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. Proc Natl Acad Sci 99(15):9996–10001
2. Buschmann MD, Gluzband YA, Grodzinsky AJ, Hunziker EB (1995) Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. J Cell Sci 108(4):1497–1508
3. Vunjak-Novakovic G et al (1999) Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. J Orthop Res 17(1):130–138
4. Buckwalter JA, Mankin HJ, Grodzinsky AJ (2005) Articular cartilage and osteoarthritis, vol 54. Instructional Course Lectures-American Academy of Orthopaedic Surgeons, p 465
5. Grodzinsky AJ, Levenston ME, Jin M, Frank EH (2000) Cartilage tissue remodeling in response to mechanical forces. Annu Rev Biomed Eng 2(1):691–713
6. Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH, Hunziker EB (1992) Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. J Orthop Res 10(6):745–758
7. Gray ML, Pizzanelli AM, Grodzinsky AJ, Lee RC (1988) Mechanical and physicochemical determinants of the chondrocyte biosynthetic response. J Orthop Res 6(6):777–792
8. Smith DW, Gardiner BS, Zhang L, Grodzinsky AJ (2019) Articular cartilage dynamics. Springer, Singapore
9. Gardiner BS et al (2016) Predicting knee osteoarthritis. Ann Biomed Eng 44(1):222–233
10. Fregly BJ et al (2012) Grand challenge competition to predict in vivo knee loads. J Orthop Res 30(4):503
11. Melchers RE (1999) Structural reliability analysis and prediction, 2nd edn. Wiley, England
12. Tobias P, Trindade D (2012) Applied reliability, 3rd edn. Taylor & Francis, Boca Raton
13. Taylor WR, Heller MO, Bergmann G, Duda GN (2004) Tibio-femoral loading during human gait and stair climbing. J Orthop Res 22(3):625–632
14. Zhang L, Gardiner BS, Smith DW, Pivonka P, Grodzinsky A (2008) A fully coupled poroelastic reactive-transport model of cartilage. Mol Cell Biomech 5(2):133
15. Smith DW, Gardiner BS, Davidson J, Grodzinsky AJ (2016) Computational model for the analysis of cartilage and cartilage tissue constructs. J Tissue Eng Regen Med 19(10):1160–1170
16. Basser PJ, Schneiderman R, R. A. Bank, Wachtel E, Maroudas A (1998) Mechanical properties of the collagen network in human articular cartilage as measured by osmotic stress technique. Arch Biochem Biophys 351(2):207–219
17. Poole A et al (1993) Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and reorganization. Agents Actions Suppl 39:3–13
18. Loening AM et al (2000) Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Arch Biochem Biophys 381(2):205–212
19. Dell'Isola A, Steultjens M (2018) Classification of patients with knee osteoarthritis in clinical phenotypes: Data from the osteoarthritis initiative. PloS One 13(1):e0191045
20. Liao J, Smith DW, Miramini S, Gardiner BS, Zhang L (2020) A coupled contact model of cartilage lubrication in the mixed-mode regime under static compression. Tribol Int 145:106185
21. Liao J, Smith DW, Miramini S, Thibbotuwawa N, Gardiner BS, Zhang L (2019) The investigation of fluid flow in cartilage contact gap. J Mech Behav Biomed Mater 95:153–164
22. Zhang L, Miramini S, Gardiner BS, Smith DW, Grodzinsky AJ (2015) Time evolution of deformation in a human cartilage under cyclic loading. Ann Biomed Eng 43(5):1166–1177
23. Ateshian G, Lai W, Zhu W, Mow V (1994) An asymptotic solution for the contact of two biphasic cartilage layers. J Biomech 27(11):1347–1360
24. Ateshian GA (2009) The role of interstitial fluid pressurization in articular cartilage lubrication. J Biomech 42(9):1163–1176
25. Soltz MA, Ateshian GA (2000) A conewise linear elasticity mixture model for the analysis of tension-

compression nonlinearity in articular cartilage. J Biomech Eng 122(6):576–586
26. Ganadhiepan G et al (2019) The Effects of Dynamic Loading on Bone Fracture Healing Under Ilizarov Circular Fixators. J Biomech Eng 141(5):051005
27. Ghimire S, Miramini S, Richardson M, Mendis P, Zhang L (2018) Role of dynamic loading on early stage of bone fracture healing. Ann Biomed Eng 46(11):1768–1784
28. Ganadhiepan G, Miramini S, Patel M, Mendis P, Zhang L (2021) Optimal time-dependent levels of weight-bearing for bone fracture healing under Ilizarov circular fixators. J Mech Behav Biomed Mater 121:104611
29. Zhang L (2011) Solute transport in cyclic deformed heterogeneous articular cartilage. Int J Appl Mech 3(03):507–524
30. Zhang L, Gardiner BS, Smith DW, Pivonka P, Grodzinsky A (2007) The effect of cyclic deformation and solute binding on solute transport in cartilage. Arch Biochem Biophys 457(1):47–56
31. Zhang L, Gardiner BS, Smith DW, Pivonka P, Grodzinsky AJ (2008) IGF uptake with competitive binding in articular cartilage. J Biol Syst 16(02):175–195
32. Zhang L, Gardiner BS, Smith DW, Pivonka P, Grodzinsky AJ (2009) Integrated model of IGF-I mediated biosynthesis in a deformed articular cartilage. J Eng Mech 135(5):439–449
33. Zhang L, Gardiner BS, Smith DW, Pivonka P, Grodzinsky AJ (2010) On the role of diffusible binding partners in modulating the transport and concentration of proteins in tissues. J Theor Biol 263(1):20–29
34. Zhang L, Gardiner BS, Smith DW, Pivonka P, Grodzinsky AJ (2010) The transport of insulin-like growth factor through cartilage. In: Vafai K (ed) Porous media: applications in biological systems and biotechnology. Taylor & Francis Group, pp 399–453
35. Zhang L, Gardiner BS, Smith DW, Pivonka P, Grodzinsky AJ (2009) Integrated model of IGF-I mediated biosynthesis in deforming articular cartilage. J Eng Mech 135(3):439–449
36. Zhang L, Smith DW, Gardiner BS, Grodzinsky AJ (2013) Modeling the insulin-like growth factor system in articular cartilage. PLoS One 8(6):e66870
37. Li Y et al (2015) Effects of insulin-like growth factor-1 and dexamethasone on cytokine-challenged cartilage: relevance to post-traumatic osteoarthritis. Osteoarthr Cartil 23(2):266–274
38. Bonassar LJ, Grodzinsky AJ, Srinivasan A, Davila SG, Trippel SB (2000) Mechanical and physicochemical regulation of the action of insulin-like growth factor-I on articular cartilage. Arch Biochem Biophys 379(1):57–63
39. Kar S, Smith DW, Gardiner BS, Li Y, Wang Y, Grodzinsky AJ (2016) Modeling IL-1 induced degradation of articular cartilage. Arch Biochem Biophys 594:37–53
40. Kar S, Smith DW, Gardiner BS, Grodzinsky AJ (2016) Systems based study of the therapeutic potential of small charged molecules for the inhibition of IL-1 mediated cartilage degradation. PLoS One 11(12):e0168047
41. Merkher Y, Sivan S, Etsion I, Maroudas A, Halperin G, Yosef A (2006) A rational human joint friction test using a human cartilage-on-cartilage arrangement. Tribol Lett 22(1):29–36. [Online]
42. Longmore RB, Gardner DL (1975) Development with age of human articular cartilage surface structure. A survey by interference microscopy of the lateral femoral condyle. Ann Rheum Dis 34(1):26–2. [Online]
43. Forster H, Fisher J (1999) The influence of continuous sliding and subsequent surface wear on the friction of articular cartilage. Proc Inst Mech Eng H J Eng Med 213(4):329–345
44. Forster H, Fisher J (1996) The influence of loading time and lubricant on the friction of articular cartilage. Proc Inst Mech Eng H 210(2):109–119. [Online]
45. Miramini S, Smith DW, Zhang L, Gardiner BS (2017) The spatio-temporal mechanical environment of healthy and injured human cartilage during sustained activity and its role in cartilage damage. J Mech Behav Biomed Mater 74:1–10
46. Hosseini A et al (2010) In-vivo time-dependent articular cartilage contact behavior of the tibiofemoral joint. Osteoarthr Cartil 18(7):909–916
47. Miramini S, Yang Y (2019) A probabilistic-based approach for computational simulation of bone fracture healing. Comput Methods Programs Biomed 180:105011
48. Liao J, Smith DW, Miramini S, Gardiner BS, Zhang L (2021) A probabilistic failure risk approach to the problem of articular cartilage lubrication. Comput Methods Prog Biomed 203:106053
49. Liao J, Miramini S, Liu X, Zhang L (2020) Computational study on synovial fluid flow behaviour in cartilage contact gap under osteoarthritic condition. Comput Biol Med 123:103915
50. Liao J, Smith DW, Miramini S, Gardiner BS, Zhang L (2021) Investigation of role of cartilage surface polymer brush border in lubrication of biological joints. Friction 10:1–18
51. Ganadhiepan G, Miramini S, Mendis P, Patel M, Zhang L (2021) A probabilistic approach for modelling bone fracture healing under Ilizarov circular fixator. Int J Numer Method Biomed Eng 37:e3466

Gene Delivery to Chondrocytes

7

Christopher V. Nagelli, Christopher H. Evans, and Rodolfo E. De la Vega

Abstract

Delivering genes to chondrocytes offers new possibilities both clinically, for treating conditions that affect cartilage, and in the laboratory, for studying the biology of chondrocytes. Advances in gene therapy have created a number of different viral and non-viral vectors for this purpose. These vectors may be deployed in an ex vivo fashion, where chondrocytes are genetically modified outside the body, or by in vivo delivery where the vector is introduced directly into the body; in the case of articular and meniscal cartilage in vivo delivery is typically by intra-articular injection. Ex vivo delivery is favored in strategies for enhancing cartilage repair as these can be piggy-backed on existing cell-based technologies, such as autologous chondrocyte implantation, or used in conjunction with marrow-stimulating techniques such as microfracture. In vivo delivery to articular chondrocytes has proved more difficult, because the dense, anionic, extra-cellular matrix of cartilage limits access to the chondrocytes embedded within it. As Grodzinsky and colleagues have shown, the matrix imposes strict limits on the size and charge of particles able to diffuse through the entire depth of articular cartilage. Empirical observations suggest that the larger viral vectors, such as adenovirus (~100 nm), are unable to transduce chondrocytes in situ following intra-articular injection. However, adeno-associated virus (AAV; ~25 nm) is able to do so in horse joints. AAV is presently in clinical trials for arthritis gene therapy, and it will be interesting to see whether human chondrocytes are also transduced throughout the depth of cartilage by AAV following a single intra-articular injection. Viral vectors have been used to deliver genes to the intervertebral disk but there has been little research on gene transfer to chondrocytes in other cartilaginous tissues such as nasal, auricular or tracheal cartilage.

Keywords

Chondrocyte · Gene therapy · Cartilage · Osteoarthritis

7.1 Introduction: Why Transfer Genes to Chondrocytes?

Gene transfer has emerged as a valuable technology serving both as a therapeutic modality and as a research tool. In the context of diseases that

C. V. Nagelli · C. H. Evans (✉) · R. E. De la Vega
Musculoskeletal Gene Therapy Laboratory, Mayo Clinic, Rochester, MN, USA
e-mail: Evans.christopher@mayo.edu

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_7

affect cartilage, genetic modification of chondrocytes promises to improve the treatment of osteoarthritis (OA) and other arthritides, as well as to promote the regeneration of damaged cartilage. As a research tool, gene transfer enables the biology of chondrocytes to be interrogated in new and unique ways. To exploit this potential, it is necessary to develop technologies allowing the efficient transfer of genes to chondrocytes and the expression of those transgenes in controlled, predictable ways. This chapter summarizes progress made in these endeavors.

7.2 A Gene Transfer Primer

7.2.1 Viral Vectors

Genes do not spontaneously enter cells in a fashion that allows their meaningful expression. Instead genes or, more usually, their complementary (c)DNA equivalents, are purposefully transferred to cells by vectors that cross the cell membrane and deliver their genetic payloads to the nucleus of the cell where the transcriptional machinery resides. The most powerful vectors for gene transfer take advantage of the natural ability of viruses to enter cells and deliver their own genomes in a manner where the virally encoded genes are expressed efficiently. Gene transfer using viruses is known as transduction.

Vectors for gene delivery have been extensively reviewed in a number of recent publications [1–3]. Although several different viruses are in pre-clinical development as a basis for gene therapy vectors [4], the main viruses that have been successfully modified for gene therapy in human clinical trials are retrovirus, adenovirus and adeno-associated virus (AAV). Two different types of retrovirus have been employed in this fashion, γ-retrovirus and lentivirus. The main relevant properties of the major viral vector groups are summarized in Table 7.1.

Retroviruses were the first viruses to be developed usefully for human gene therapy. On entering cells, their RNA genomes are reverse transcribed into DNA (hence the word retrovirus) which then integrates into genomic DNA within the host nucleus where the transferred coding sequences (transgenes) are expressed. Because integration occurs at unpredictable sites there is a finite possibility of insertional mutagenesis leading to malignant transformation. Although the likelihood of this is low, it has been observed in clinical trials [5]. Of practical concern, γ-retroviruses require host cell division for transduction to occur whereas lentiviruses transduce both dividing and non-dividing cells. Because of the safety concerns raised by insertional mutagenesis, retroviruses are unlikely to be used clinically to treat diseases affecting cartilage but they remain powerful research tools.

Adenoviruses are non-integrating DNA viruses that are relatively straightforward to construct and propagate. They transduce a wide range of dividing and non-dividing cells. Depending on the promoter used in the vector, transgene expression can be very high. Because the viral DNA remains episomal it is rapidly lost from dividing cells and adenoviral vectors tend to provide high levels of transgene expression for a limited period time. The ability of adenovirus to activate both the innate and adaptive immune systems is a disadvantage for *in vivo* applications. The innate immune system is triggered because infection of cells with adenovirus stimulates mitogen-activated protein (MAP) kinases, leading to the activation of nuclear factor kappa-B (NF-κB), a pro-inflammatory transcription factor. Adaptive immunity occurs in response to highly antigenic adenoviral capsid proteins. Cells infected with early generation adenovirus vectors express low levels of these proteins and are killed by the resulting CD8+ T-cell response. Later generation vectors have addressed this issue by removing additional viral DNA leading to the construction of high-capacity vectors (also known variously as "gutted", "gutless" or "helper-dependent" adenovirus) that lack all adenovirus coding sequences. These vectors can accommodate a DNA cargo as large as 36 kb but are difficult to manufacture.

AAV is a small parvovirus with a single-stranded DNA genome. It is attractive for human gene therapy because the wild-type virus is endemic in human populations yet causes no known disease. However, the single-stranded genome presents a limitation for gene therapy

Table 7.1 Salient properties of commonly used viral vectors

Viral vector	Advantages	Disadvantages	Other properties
Adenovirus	Easy to produce in high titers Transduces both dividing and non-dividing cells Relatively good freeze-thaw stability Easy to procure and produce (first and second generation vectors)	Immunogenic Difficult to procure and produce (third generation) Does not transduce chondrocytes in situ	~1 in every 50–100 viral particles is infectious Non-integrating Carrying capacity 8–30 kb Transient transduction of dividing cells
Adeno-associated virus (AAV)	Transduces both dividing and non-dividing cells Relatively good freeze-thaw and thermal stability Capable of transducing chondrocytes in vivo No human disease associated with AAV Multiple serotypes allow for directed tropism	Difficult to procure and produce Gene carrying capacity is small Large number of the human population have pre-existing neutralizing antibodies to certain serotypes Expensive	Depending on serotype ~1 in 50 particles is infectious Transducing capacity varies widely between serotypes, cells, species and different preparations Non-integrating
Retrovirus (Moloney murine leukemia virus derived)	Easy to produce Selection of transduced cells straightforward	Modest titers Does not transduce non-dividing cells Risk of insertional mutagenesis Does not transduce chondrocytes in vivo	~1 in every 100–1000 viral particles is infectious ~8 kb of packaging capacity Integrating
Lentivirus	Transduces both dividing and non-dividing cells Selection of transduced cells straightforward	Risk of insertional mutagenesis Does not transduce chondrocytes in vivo	~1 in every 100–1000 viral particles is infectious ~8 kb of packaging capacity Integrating

because second-strand synthesis is required within the nucleus of the host cell before gene expression can occur. In certain types of cells and in certain species second strand synthesis is very inefficient. The development of self-complementing AAV genomes comprising double-stranded DNA has overcome this problem at the expense of reducing the packaging capacity of AAV from an already modest 5 kb to 2.5 kb DNA. However, this capacity is ample for the small cytokine molecules and growth factors relevant to many aspects of chondrocyte biology. The genomes of recombinant AAV vectors are non-integrating but exist as stable, concatemeric episomes which provide the basis for long-term expression in non-dividing cells. Multiple years of transgene expression in liver and eye have been noted in human clinical trials [6]. AAV has a number of distinct serotypes, both natural and synthetic, which display different tropisms.

Practical aspects of chondrocyte transduction have been described recently by Nagelli et al. [3].

7.2.2 Non-viral Vectors

Although viral vectors are very powerful and dominate clinical application, there is also interest in non-viral vectors. Non-viral vectors promise to be simpler, less expensive and possibly safer than viral vectors; they are also less likely to have packaging constraints. Gene transfer with non-viral vectors is known as transfection.

Plasmids are the simplest of non-viral vectors. Although they do not provoke adaptive immunity in the same way as viral vectors, unmethylated cytosine-phosphate-guanine (CpG) dinucleotide motifs in DNA activate innate immunity by interacting with toll-like receptors. Moreover, transfection efficiency is inversely proportional to the

size of the construct; plasmid uptake and expression is very low with constructs >3 kb in length; it is most efficient with mini-circles of 650 bp or less [7, 8]. Transfection is very inefficient in non-dividing cells.

The negative charge of DNA impedes cell uptake because the surfaces of cells also have a net negative charge. Various cationic agents may be added to mask the repulsive electrostatic charges and may additionally facilitate uptake by condensing the DNA. Physical methods to improve uptake include electroporation, hydrodynamic injection, ultrasound, and "magnetofection". In general, transfection provides low and transient transgene expression, especially in primary cells. Non-viral gene delivery is reviewed in references [9, 10].

There is much recent interest in the use of RNA as a therapy and a research tool [11]. Delivery of mRNA serves to enhance expression of the encoded protein, albeit transiently, while RNA inhibition suppresses expression of specific transcripts. Transfection with chemically modified mRNA encoding bone morphogenetic protein-2 (BMP-2) has recently been shown to promote the formation of cartilage within an osseous defect in the rat femur [12].

7.2.3 Gene Activated Matrices

Gene activated matrices (GAMs), combining vectors with scaffolds, are of interest in the context of tissue regeneration. For most envisaged applications the GAM is implanted into a defect where host cells infiltrate the matrix during which process they become genetically modified by the associated vectors. Genes encoding regenerative products are thus expressed locally by host cells within the defect where they stimulate a reparative response. First introduced in for bone healing [13], GAMs have also been explored in the context of cartilage repair and regeneration [14]. The original formulations combined plasmid DNA with a collagen sponge, but later iterations include viral vectors, RNA and more elaborate scaffolds [15].

7.3 Gene Delivery to Chondrocytes

For *in vitro* genetic modification, chondrocytes in monolayer culture need only be incubated with the vectors of choice using techniques of the type described by Nagelli et al. [3]. A sizeable literature dating back 25 years confirms that cultures of chondrocytes can be transduced efficiently with viral vectors [16–18].

For *in vivo* genetic modification, in which genes are transferred to the articular cartilage within a joint, there is the choice of *ex vivo* or *in vivo* delivery (Fig. 7.1). For *ex vivo* delivery the cells are transduced *in vitro* and then implanted into the cartilage. For *in vivo* delivery, the vector

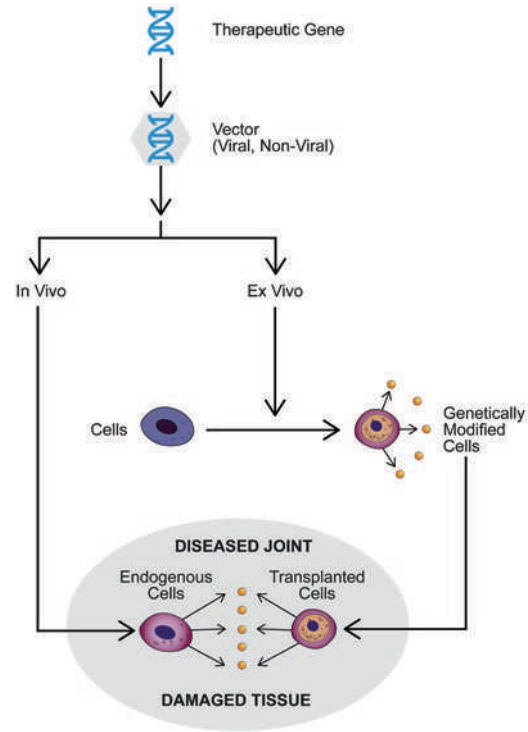


Fig. 7.1 Principles of local gene therapy to chondrocytes and cartilage. The therapeutic gene, usually in its cDNA form, is incorporated into a viral or non-viral vector and delivered to the site of cartilage disease or damage in an in vivo or ex vivo fashion. For in vivo delivery, the vector is administered directly to the relevant site. For ex vivo delivery, the vector transfers genes to cells outside the body, and the genetically modified cells are then administered to the relevant site. (Reproduced from [1])

is introduced directly into the body and transduction occurs *in situ*. *In vivo* gene delivery to articular chondrocytes has several barriers to overcome. Particles, such as vectors, delivered systemically barely enter joints and, in any case, articular cartilage is avascular. Direct injection of vectors into the joint by-passes the systemic circulation but there are two further barriers to the genetic modification of chondrocytes. The first is rapid efflux via lymphatic drainage which removes particles, including vectors, from joints [19]. The second is the dense, extra-cellular matrix (ECM) of cartilage that prevents the vectors from gaining access to the chondrocytes embedded within it (Fig. 7.2) [20].

Much of what we know about diffusion through the ECM of cartilage comes from the work of Grodzinsky and colleagues at MIT who have studied the ability of molecules to diffuse into articular cartilage from both empirical and theoretical perspectives [21–23]. The dominating parameters are the size and charge of the diffusate; shape may also be a factor. The high, fixed, negative charge of glycosaminoglycans (GAGs) within the cartilaginous ECM excludes anionic materials electrostatically, while the dense packing of proteoglycans sterically excludes particles with a Stoke's radius larger than about 15 nm. Although a positive surface charge neutralizes the electrostatic exclusion of a particle from cartilage, an excessive positive charge is counterproductive because the affinity of the particles for cartilage GAGs will be too high, in which case particles will accumulate at the surface and fail to diffuse through the full thickness of cartilage. To enter cartilage in a useful way, it is thus necessary for a vector to have a net positive charge, but one that is not too high, and an affinity for GAGs whose off-rate permits progress through the matrix. In this context, Bajpayee *et al* studied the

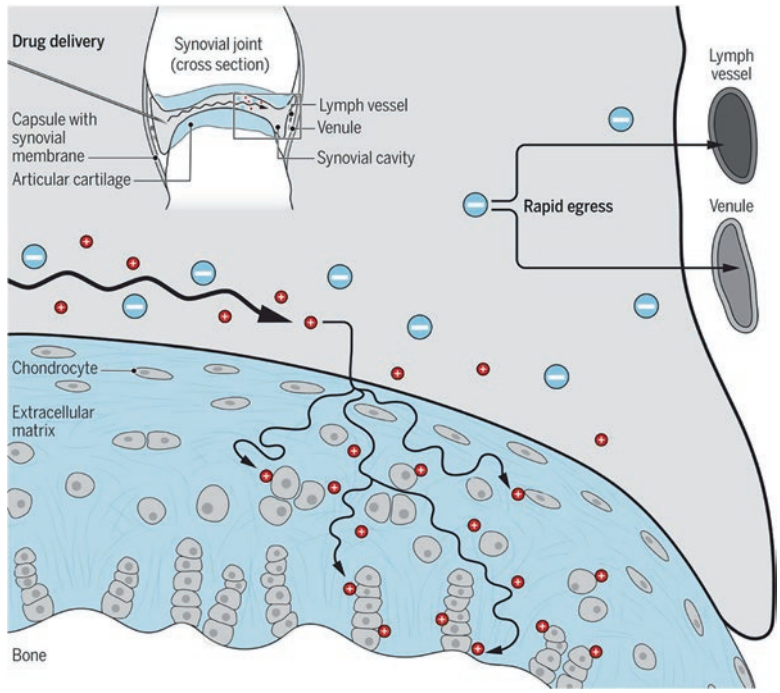


Fig. 7.2 Delivering drugs to chondrocytes in situ by intra-articular injection. Although drugs can be easily injected into joints, most materials within the joint space are rapidly removed by lymphatic drainage or by diffusion into the subsynovial capillaries. Penetration of the articular cartilage, where the chondrocytes reside, is restrained sterically and electrostatically by the high concentration of anionic (−) glycosaminoglycans (GAGs). Particles with an appropriate positive (+) surface charge bind reversibly to the anionic GAG chains enabling transport through the cartilage to the chondrocytes, where vectors can deliver their genetic payload. (Reproduced from [20])

diffusion of the cationic protein avidin through the ECM of bovine articular cartilage, noting that its weak and reversible binding to cartilage GAGs (K_D ~ 150 μM) allowed it to diffuse through the entire thickness of the matrix as it underwent sequential binding and release (Fig. 7.2) [22]. Under these circumstances, the higher concentration of GAGs in the deeper zones of the cartilage may have helped diffusion through the entire depth of the tissue. Thus a vector must satisfy strict biophysical requirements to be able to transduce chondrocytes *in situ* throughout the full thickness of cartilage.

Because *in vivo* delivery of genes to chondrocytes seemed extremely difficult, *ex vivo* gene delivery to cartilage was the early strategy of choice [16, 24]. There was initial optimism that genetically-modified chondrocytes would adhere to the surface of cartilage following intra-articular injection, especially to sites of damage, thus providing a new strategy for cartilage repair. However, subsequent research has confirmed that the injected cells do not adhere to cartilage but are rapidly cleared from the joint [25–27]. A more promising approach has been to implant genetically modified chondrocytes, or chondroprogenitors, surgically. To do this effectively it is necessary to use an appropriate scaffold, discussion of which lies beyond the scope of this chapter (see Ref. [28] for a recent review).

The prospect of *in vivo* delivery of genes to chondrocytes has been recently revisited on the basis of experiments in which AAV encoding green fluorescent protein (GFP) was injected into the joints of horses [29]. An unexpectedly high proportion of articular chondrocytes throughout the full thickness of the cartilage became GFP+ (Fig. 7.3). This had not been seen in earlier experiments using rats and rabbits, suggesting that the pharmacokinetics of the larger joints differ from those of smaller animals. The greater thickness of the equine cartilage was probably an additional major factor because the kinetics of diffusion-reaction transport through cartilage depend on the square of its thickness. In agreement with this, Bajpayee *et al* showed 5–6 times longer half-lives of avidin in rabbit cartilage than in rat cartilage following intra-articular injection [30].

AAV is an icosahedron, about 20–25 nm in size. This is larger than the 15 nm cut-off determined by Grodzinsky and colleagues [21–23], but entry into the cartilage of horse joints may be facilitated by the pumping action occurring as the horse moves and the articular cartilage intermittently bears weight; the apparatus used at MIT

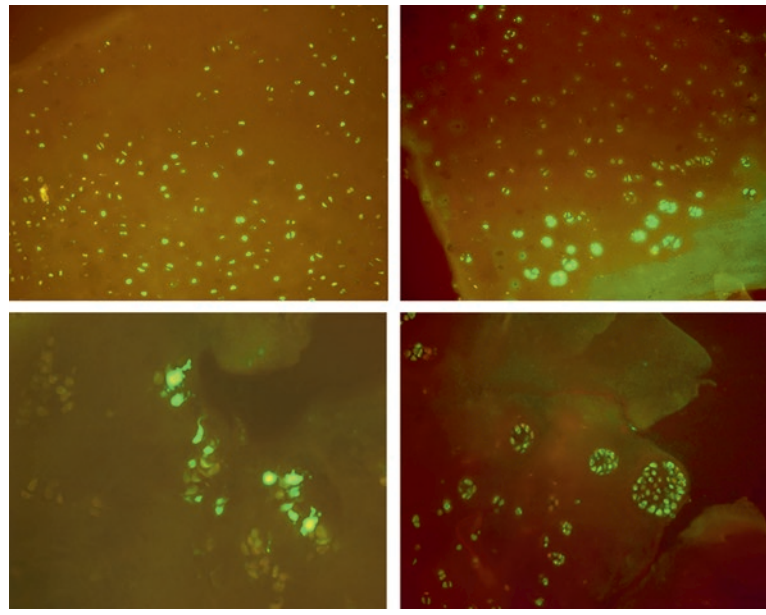


Fig. 7.3 Expression of GFP in chondrocytes of cartilage 2 weeks after the intra-articular injection of 5×10^{12} viral genomes of AAV. GFP into the intercarpal joint of the horse

provides a static system which does not subject the cartilage to loading. Age may be a second factor. The studies by Grodzinsky's group used calf cartilage in which the proteoglycan chains are very long and the fixed charge density very high. In adult horses the fixed charge density is likely to be lower and the matrix of the cartilage subjected to some degree of degradation as happens during natural aging. In a disease such as osteoarthritis (OA) the matrix is further degraded, allowing greater access to AAV and possibly other, larger vectors such as adenovirus as the disease progresses.

7.4 Progress in the Clinical Application of Gene Transfer to Chondrocytes

7.4.1 Osteoarthritis

Osteoarthritis (OA) is the one application that has advanced to clinical trials [1]. The first approach used *ex vivo* gene delivery and built on the earlier success of delivering genes in this fashion to human metacarpophalangeal joints affected by rheumatoid arthritis [31]. Almost all examples of *ex vivo* gene therapy use autologous cells as the vehicle for gene transfer. The method of Ha *et al* [32] broke new ground in using allogeneic cells, derived from the finger joints of an infant with polydactyly, to deliver a gene to human joints. Cultures of the donor chondrocytes obtained from the amputated finger were divided into two lots, one of which was retrovirally transduced to express large amounts of transforming growth factor-beta (TGF-β). Because of the potential for insertional mutagenesis, as described earlier in this paper, the transduced cells were irradiated at a dose that prevented cell division but maintained TGF-β production. Before intra-articular injection into joints of patients with knee OA, the transduced cells were mixed with untransduced chondrocytes from the same allogeneic donor finger joints.

Phase I, II and III clinical trials of this product in South Korea met their primary end points and the gene therapeutic was approved in 2017 by the Korean authorities as the drug Invossa [33]. This was the first gene therapy approved in Korea. In 2019 this approval was revoked [34]. The genetically modified cells were identified as HEK293 cells, a line of human embryonic kidney cells, not chondrocytes. The initial preclinical work had been performed with chondrocytes. HEK293 cells are used as a producer line for generating retrovirus, so it is possible that some of these cells were inadvertently introduced into the chondrocyte cultures during retroviral transduction. The high growth rate of HEK293 cells would enable them to out-compete the chondrocytes, which have a slower growth rate. At the time of writing, the fate of Invossa in Korea is uncertain. However, the FDA has allowed a Phase III trial of Invossa in knee OA (National Clinical Trial Identifier (NCT) 03291470) and a Phase I/II trial in hip OA (NCT 05276011) to proceed in the USA.

The second approach in clinical trials uses *in vivo* gene delivery by intra-articular injection into knee joints with OA. Three such trials are underway. NCT 03477487 uses a plasmid to deliver a variant of interleukin- (IL-) 10. For reasons discussed earlier in this chapter, it is unlikely to transduce chondrocytes. NCT 03477487 uses a high-capacity adenovirus to deliver the IL-1 receptor antagonist (IL-1Ra) and it is not known whether gene transfer to chondrocytes occurs. Adenoviral transduction of chondrocytes *in situ* following intra-articular injection has not be reliably observed in pre-clinical models. In particular, a detailed study by Goossens *et al* [35] using rhesus monkeys failed to observe transgene expression in cartilage following intra-articular injection of adenovirus vectors even though the adjacent synovium was transduced efficiently. Clinical trial NCT 04119687 also uses IL-1Ra as the transgene product, but with AAV2.5 as the vector. This is the same serotype vector shown to transduce chondrocytes after injection into equine joints (Fig. 7.3), so there is the expectation that human chondrocytes will be similarly transduced. This possibility is enhanced by the similar thickness of human and equine cartilage

in large joints (1.5–2 mm). Both of the trials with viral vectors are in Phase I, whereas evaluation of the plasmid vector has progressed to Phase II.

7.4.2 Cartilage Regeneration

There is considerable interest in promoting cartilage regeneration using genetically modified chondrocytes or chondroprogenitor cells [36, 37]. Data from *in vitro* experiments and preliminary studies in small animals are encouraging, but there has been limited progress towards the large animal studies that are a necessary prelude to human clinical trials.

Using equine models, Nixon and colleagues evaluated the effects of *ex vivo* gene transfer on the repair of chondral defects using a variation of the autologous chondrocyte implantation approach. Allogeneic chondrocytes were transduced *in vitro* with adenovirus vectors expressing insulin-like growth factor-1 (IGF-1) [38] or bone morphogenetic protein-7 (BMP-7) [39]. The cells were encapsulated in a fibrin gel and arthroscopically introduced into experimental chondral lesions. In both cases, early healing was greatly accelerated by gene transfer but at later time points healing by the control chondrocytes had caught up. A subsequent study in which AAV was used to deliver IGF-1 to autologous chondrocytes provided longer lasting improvement, but it is unknown whether this was due to the choice of vector or the use of autologous cells [40].

Pascher *et al* [41] developed an abbreviated *ex vivo* gene transfer method based on the technique of microfracture that is frequently used to repair damaged cartilage. Microfracture and similar marrow-activating techniques allow chondroprogenitor cells from the underlying bone marrow to enter the lesion where they produce an inferior, but often serviceable, cartilagenous repair tissue that degenerates with time. Knowing that gene transfer can enhance the chondrogenic differentiation of mesenchymal stromal cells (MSCs) derived from bone marrow [42, 43], a technique was developed whereby bone marrow is aspirated and mixed with adenovirus vectors while it clots [41]. The clotted marrow, containing transduced marrow cells as well as free virus, is then press-fit into the lesion. Sieker *et al* obtained promising results when using BMP-2 and Indian hedgehog as the transgenes [44] in an osteochondral defect model in rabbits, but similar experiments with a TGF-β transgene gave equivocal results [45]. Use of a similar TGF-β construct in a chondral defect in sheep also gave equivocal results [46].

An alternative approach to improving the microfracture technique has been pioneered by Madry and Cucchiarini [47–51]. In this method, AAV vectors are directly applied to the osteochondral lesion as the marrow enters the defect. A number of different chondrogenic genes have been applied in a rabbit model with promising results [48, 49]. Similar studies delivering fibroblast growth factor-2 (FGF-2) [51] or Sox 9 [50] in a sheep osteochondral defect, and TGF-β in a minipig have also given promising early results [47]. In a refinement of this technique, this group has developed GAMs for the delivery of these vectors to osteochondral lesions [52].

Invossa has been applied in human patients with cartilage damage (NCT 01825811). The genetically modified cells were embedded in a fibrin gel and implanted in cartilage lesions present in joints of patients with knee OA. It is not known whether the genetically modified cells were chondrocytes or HEK293 cells. The promising results from this study have been presented, but not published.

7.5 Additional Considerations

Space does not permit discussion of additional matters related to gene transfer to chondrocytes. For example, the choice of promoters that drive transgene expression is important and chondrocytes express several genes, such as COL2A1, that permit tissue specific gene expression. Sub-sets of chondrocytes may also be targeted in this way. Expression of superficial zone proteoglycan, for example, is restricted to the superficial zone chondrocytes in cartilage,

although there is also expression by synovial fibroblasts. Various constitutive and inducible promoters are also available. The repertoire of interesting gene products continues to expand and includes various types of non-coding RNA as well as the machinery of gene-editing. Payloads such as these that operate intra-cellularly will usually need to be delivered by vectors able to reach chondrocytes throughout the entire cartilage, which may be challenging.

Gene transfer to additional cartilagenous tissues such as meniscus and the intervertebral disc has also been achieved using the same sorts of approaches as discussed in this chapter. There is considerable interest in using gene transfer to treat intervertebral disc degeneration. Pre-clinical experiments have confirmed the immune privilege of the nucleus pulposus, with expression of β-galactosidase, a highly antigenic bacterial protein, for over a year in the rabbit following delivery by intra-discal injection of a first-generation adenovirus vector, itself highly antigenic [53].

7.6 Conclusions

Transfer of genes to chondrocytes promises to advance the clinical management of OA and other forms of arthritis which destroy cartilage. Several clinical trials have been initiated in the field of OA. Gene therapy also has the potential to promote cartilage regeneration; one clinical study has taken place, but the data have not been published. As a laboratory tool, the ability to manipulate the genetics of chondrocytes offer many opportunities to learn more of their biology. The tools for both *ex vivo* and, more recently, *in vivo* delivery to chondrocytes both in culture and in articular cartilage are available to further these endeavors. Other cartilaginous tissues are also amenable to these approaches.

Acknowledgements The authors' recent work in this area has been supported by the Musculoskeletal Regeneration. Partnership Fund by Mary Sue and Michael Shannon. CVN has been supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases grant T32AR56950. CHE's research is supported in part by the John and Posy Krehbiel Professorship in Orthopedics.

References

1. Evans CH, Ghivizzani SC, Robbins PD (2021) Orthopaedic gene therapy: twenty-five years on. JBJS Rev 9. https://doi.org/10.2106/JBJS.RVW.20.00220
2. Ghivizzani SC, Watson-Levings RS, Palmer GD, Levings PP, Dacanay AE, Evans CH (2022) Gene therapy in orthopaedics: progress and challenges in pre-clinical development and translation. Front Bioeng Biotechnol 10:901317
3. Nagelli CV, Evans CH, de la Vega, RE (2023) Viral gene delivery in chondrocytes. In: Stoddart MJ, Della Bella E, Armiento AR (eds) Cartilage tissue engineering. Methods in molecular biology, vol 2598. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-2839-3_20
4. Lundstrom K (2018) Viral vectors in gene therapy. Diseases 6:42
5. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JI, de Saint Basile G, Alexander I, Wintergerst U, Frebourg T, Aurias A, Stoppa-Lyonnet D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabesse E, Macintyre E, Sigaux F, Soulier J, Leiva LE, Wissler M, Prinz C, Rabbitts TH, Le Deist F, Fischer A, Cavazzana-Calvo M (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302:415–419
6. George LA, Monahan PE, Eyster ME, Sullivan SK, Ragni MV, Croteau SE, Rasko JEJ, Recht M, Samelson-Jones BJ, MacDougall A, Jaworski K, Noble R, Curran M, Kuranda K, Mingozzi F, Chang T, Reape KZ, Anguela XM, High KA (2021) Multiyear factor VIII expression after AAV gene transfer for Hemophilia A. N Engl J Med 385:1961–1973
7. Kreiss P, Cameron B, Rangara R, Mailhe P, Aguerre-Charriol O, Airiau M, Scherman D, Crouzet J, Pitard B (1999) Plasmid DNA size does not affect the physicochemical properties of lipoplexes but modulates gene transfer efficiency. Nucleic Acids Res 27:3792–3798
8. Yin W, Xiang P, Li Q (2005) Investigations of the effect of DNA size in transient transfection assay using dual luciferase system. Anal Biochem 346:289–294
9. Zu H, Gao D (2021) Non-viral vectors in gene therapy: recent development, challenges, and prospects. AAPS J 23:78
10. Slivac I, Guay D, Mangion M, Champeil J, Gaillet B (2017) Non-viral nucleic acid delivery methods. Expert Opin Biol Ther 17:105–118
11. Damase TR, Sukhovershin R, Boada C, Taraballi F, Pettigrew RI, Cooke JP (2021) The limitless future of RNA therapeutics. Front Bioeng Biotechnol 9:628137
12. De La Vega RE, van Griensven M, Zhang W, Coenen MJ, Nagelli CV, Panos JA, Peniche Silva CJ, Geiger J, Plank C, Evans CH, Balmayor ER (2022) Efficient healing of large osseous segmental defects using optimized chemically modified messenger RNA encoding BMP-2. Sci Adv 8:eabl6242

13. Bonadio J, Smiley E, Patil P, Goldstein S (1999) Localized, direct plasmid gene delivery in vivo: prolonged therapy results in reproducible tissue regeneration. Nat Med 5:753–759
14. Raisin S, Belamie E, Morille M (2016) Non-viral gene activated matrices for mesenchymal stem cells based tissue engineering of bone and cartilage. Biomaterials 104:223–237
15. Cam C, Segura T (2013) Matrix-based gene delivery for tissue repair. Curr Opin Biotechnol 24:855–863
16. Kang R, Marui T, Ghivizzani SC, Nita IM, Georgescu HI, Suh JK, Robbins PD, Evans CH (1997) Ex vivo gene transfer to chondrocytes in full-thickness articular cartilage defects: a feasibility study. Osteoarthr Cartil 5:139–143
17. Baragi VM, Renkiewicz RR, Jordan H, Bonadio J, Hartman JW, Roessler BJ (1995) Transplantation of transduced chondrocytes protects articular cartilage from interleukin 1-induced extracellular matrix degradation. J Clin Invest 96:2454–2460
18. Palmer GD, Gouze E, Gouze JN, Betz OB, Evans CH, Ghivizzani SC (2003) Gene transfer to articular chondrocytes with recombinant adenovirus. Methods Mol Biol 215:235–246
19. Evans CH, Kraus VB, Setton LA (2014) Progress in intra-articular therapy. Nat Rev Rheumatol 10:11–22
20. Evans CH (2018) Catering to chondrocytes. Sci Transl Med 10:eaav7043
21. Krishnan Y, Rees HA, Rossitto CP, Kim SE, Hung HK, Frank EH, Olsen BD, Liu DR, Hammond PT, Grodzinsky AJ (2018) Green fluorescent proteins engineered for cartilage-targeted drug delivery: insights for transport into highly charged avascular tissues. Biomaterials 183:218–233
22. Bajpayee AG, Wong CR, Bawendi MG, Frank EH, Grodzinsky AJ (2014) Avidin as a model for charge driven transport into cartilage and drug delivery for treating early stage post-traumatic osteoarthritis. Biomaterials 35:538–549
23. Bajpayee AG, Grodzinsky AJ (2017) Cartilage-targeting drug delivery: can electrostatic interactions help? Nat Rev Rheumatol 13:183–193
24. Baragi VM, Renkiewicz RR, Qiu L, Brammer D, Riley JM, Sigler RE, Frenkel SR, Amin A, Abramson SB, Roessler BJ (1997) Transplantation of adenovirally transduced allogeneic chondrocytes into articular cartilage defects in vivo. Osteoarthr Cartil 5:275–282
25. ter Huurne M, Schelbergen R, Blattes R, Blom A, de Munter W, Grevers LC, Jeanson J, Noel D, Casteilla L, Jorgensen C, van den Berg W, van Lent PL (2012) Antiinflammatory and chondroprotective effects of intraarticular injection of adipose-derived stem cells in experimental osteoarthritis. Arthritis Rheum 64:3604–3613
26. Horie M, Choi H, Lee RH, Reger RL, Ylostalo J, Muneta T, Sekiya I, Prockop DJ (2012) Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. Osteoarthr Cartil 20:1197–1207
27. Ozeki N, Muneta T, Koga H, Nakagawa Y, Mizuno M, Tsuji K, Mabuchi Y, Akazawa C, Kobayashi E, Matsumoto K, Futamura K, Saito T, Sekiya I (2016) Not single but periodic injections of synovial mesenchymal stem cells maintain viable cells in knees and inhibit osteoarthritis progression in rats. Osteoarthr Cartil 24:1061–1070
28. Cucchiarini M, Madry H (2019) Biomaterial-guided delivery of gene vectors for targeted articular cartilage repair. Nat Rev Rheumatol 15:18–29
29. Watson Levings RS, Broome TA, Smith AD, Rice BL, Gibbs EP, Myara DA, Hyddmark EV, Nasri E, Zarezadeh A, Levings PP, Lu Y, White ME, Dacanay EA, Foremny GB, Evans CH, Morton AJ, Winter M, Dark MJ, Nickerson DM, Colahan PT, Ghivizzani SC (2018) Gene therapy for osteoarthritis: pharmacokinetics of intra-articular self-complementary adeno-associated virus Interleukin-1 receptor antagonist delivery in an equine model, vol 29. Hum Gene Ther Clin Dev, pp 90–100
30. Bajpayee AG, Scheu M, Grodzinsky AJ, Porter RM (2015) A rabbit model demonstrates the influence of cartilage thickness on intra-articular drug delivery and retention within cartilage. J Orthop Res 33:660–667
31. Evans CH, Robbins PD, Ghivizzani SC, Wasko MC, Tomaino MM, Kang R, Muzzonigro TA, Vogt M, Elder EM, Whiteside TL, Watkins SC, Herndon JH (2005) Gene transfer to human joints: progress toward a gene therapy of arthritis. Proc Natl Acad Sci U S A 102:8698–8703
32. Ha CW, Noh MJ, Choi KB, Lee KH (2012) Initial phase I safety of retrovirally transduced human chondrocytes expressing transforming growth factor-beta-1 in degenerative arthritis patients. Cytotherapy 14:247–256
33. Evans CH, Ghivizzani SC, Robbins PD (2018) Arthritis gene therapy is becoming a reality. Nat Rev Rheumatol 14:381–382
34. Evans CH (2019) The vicissitudes of gene therapy. Bone Joint Res 8:469–471
35. Goossens PH, Huizinga TW (2002) Adenoviral-mediated gene transfer to the synovial tissue. Clin Exp Rheumatol 20:415–419
36. Graceffa V, Vinatier C, Guicheux J, Evans CH, Stoddart M, Alini M, Zeugolis DI (2018) State of art and limitations in genetic engineering to induce stable chondrogenic phenotype. Biotechnol Adv 36:1855–1869
37. Palmer G, Pascher A, Gouze E, Gouze JN, Betz O, Spector M, Robbins PD, Evans CH, Ghivizzani SC (2002) Development of gene-based therapies for cartilage repair. Crit Rev Eukaryot Gene Expr 12:259–273
38. Goodrich LR, Hidaka C, Robbins PD, Evans CH, Nixon AJ (2007) Genetic modification of chondrocytes with insulin-like growth factor-1 enhances cartilage healing in an equine model. J Bone Joint Surg Br 89:672–685
39. Hidaka C, Goodrich LR, Chen CT, Warren RF, Crystal RG, Nixon AJ (2003) Acceleration of

cartilage repair by genetically modified chondrocytes over expressing bone morphogenetic protein-7. J Orthop Res 21:573–583
40. Ortved KF, Begum L, Mohammed HO, Nixon AJ (2015) Implantation of rAAV5-IGF-I transduced autologous chondrocytes improves cartilage repair in full-thickness defects in the equine model. Mol Ther 23:363–373
41. Pascher A, Palmer GD, Steinert A, Oligino T, Gouze E, Gouze JN, Betz O, Spector M, Robbins PD, Evans CH, Ghivizzani SC (2004) Gene delivery to cartilage defects using coagulated bone marrow aspirate. Gene Ther 11:133–141
42. Palmer GD, Steinert A, Pascher A, Gouze E, Gouze JN, Betz O, Johnstone B, Evans CH, Ghivizzani SC (2005) Gene-induced chondrogenesis of primary mesenchymal stem cells in vitro. Mol Ther 12:219–228
43. Steinert AF, Palmer GD, Pilapil C, Noth U, Evans CH, Ghivizzani SC (2009) Enhanced in vitro chondrogenesis of primary mesenchymal stem cells by combined gene transfer. Tissue Eng Part A 15:1127–1139
44. Sieker JT, Kunz M, Weissenberger M, Gilbert F, Frey S, Rudert M, Steinert AF (2015) Direct bone morphogenetic protein 2 and Indian hedgehog gene transfer for articular cartilage repair using bone marrow coagulates. Osteoarthr Cartil 23:433–442
45. Nagelli CV, De La Vega RE, Coenen M, De Padilla CL, Panos JA, Tovar A, Müller SA, Evans CH (2022) Expedited gene delivery for osteochondral defect repair in a rabbit knee model: a one-year investigation. Osteoarthr Cartil Open 4:100257
46. Ivkovic A, Pascher A, Hudetz D, Maticic D, Jelic M, Dickinson S, Loparic M, Haspl M, Windhager R, Pecina M (2010) Articular cartilage repair by genetically modified bone marrow aspirate in sheep. Gene Ther 17:779–789
47. Cucchiarini M, Asen AK, Goebel L, Venkatesan JK, Schmitt G, Zurakowski D, Menger MD, Laschke MW, Madry H (2018) Effects of TGF-beta overexpression via rAAV gene transfer on the early repair processes in an osteochondral defect model in minipigs. Am J Sports Med 46:1987–1996
48. Cucchiarini M, Madry H (2014) Overexpression of human IGF-I via direct rAAV-mediated gene transfer improves the early repair of articular cartilage defects in vivo. Gene Ther 21:811–819
49. Cucchiarini M, Orth P, Madry H (2013) Direct rAAV SOX9 administration for durable articular cartilage repair with delayed terminal differentiation and hypertrophy in vivo. J Mol Med (Berl) 91:625–636
50. Lange C, Madry H, Venkatesan JK, Schmitt G, Speicher-Mentges S, Zurakowski D, Menger MD, Laschke MW, Cucchiarini M (2021) rAAV-mediated sox9 overexpression improves the repair of osteochondral defects in a clinically relevant large animal model over time in vivo and reduces perifocal osteoarthritic changes. Am J Sports Med 49:3696–3707
51. Morscheid YP, Venkatesan JK, Schmitt G, Orth P, Zurakowski D, Speicher-Mentges S, Menger MD, Laschke MW, Cucchiarini M, Madry H (2021) rAAV-mediated human FGF-2 gene therapy enhances osteochondral repair in a clinically relevant large animal model over time in vivo. Am J Sports Med 49:958–969
52. Madry H, Venkatesan JK, Carballo-Pedrares N, Rey-Rico A, Cucchiarini M (2020) Scaffold-mediated gene delivery for osteochondral repair. Pharmaceutics 12:930
53. Nishida K, Kang JD, Suh JK, Robbins PD, Evans CH, Gilbertson LG (1998) Adenovirus-mediated gene transfer to nucleus pulposus cells. Implications for the treatment of intervertebral disc degeneration. Spine (Phila Pa 1976) 23:2437–2442. discussion 2443

8 Mechanical Articular Cartilage Injury Models and Their Relevance in Advancing Therapeutic Strategies

Bodo Kurz, Melanie L. Hart, and Bernd Rolauffs

Abstract

This chapter details how Alan Grodzinsky and his team unraveled the complex electromechanobiological structure-function relationships of articular cartilage and used these insights to develop an impressively versatile shear and compression model. In this context, this chapter focuses (i) on the effects of mechanical compressive injury on multiple articular cartilage properties for (ii) better understanding the molecular concept of mechanical injury, by studying gene expression, signal transduction and the release of potential injury biomarkers. Furthermore, we detail how (iii) this was used to combine mechanical injury with cytokine exposure or co-culture systems for generating a more realistic trauma model to (iv) investigate the therapeutic modulation of the injurious response of articular cartilage. Impressively, Alan Grodzinsky's research has been and will remain to be instrumental in understanding the proinflammatory response to injury and in developing effective therapies that are based on an in-depth understanding of complex structure-function relationships that underlay articular cartilage function and degeneration.

Keywords

Cartilage · Injury · Compression · Structure-function

8.1 Introduction

As a tribute to the tremendously important work of Grodzinsky and colleagues in the context of mechanical articular cartilage injury models, the

Bodo Kurz and Melanie L. Hart contributed equally with all other contributors.

B. Kurz (✉)
Department of Anatomy, Christian-Albrechts-University, Kiel, Germany
e-mail: bkurz@anat.uni-kiel.de

M. L. Hart · B. Rolauffs (✉)
G.E.R.N. Research Center for Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Faculty of Medicine, Medical Center—Albert-Ludwigs-University of Freiburg, Freiburg im Breisgau, Germany

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_8

following text sections detail how Grodzinsky and colleagues have set out to unravel the complex and, at that time, unknown electrokinetic, biomechanical and biosynthetic characteristics of articular cartilage, implementing the cartilage injury machine as the go-to model to develop structure-function relationships. Over time, this led to model-based insights and an in-depth understanding of mechanical injury mechanisms and therapeutic strategies with fundamental clinical relevance.

8.2 From Electromechanobiological Structure-Function Relationships to Developing a Versatile Shear and Compression Model for Understanding the Injurious Response of Articular Cartilage

8.2.1 Unraveling Central Electrokinetic and Biomechanical Properties of Articular Cartilage – The Basis for Understanding Tissue Failure Under Injurious Compressive Loads

In earlier works, which began in the 1980s and preceded the arrival of the worldwide famous cartilage "injury machine", Grodzinsky and colleagues examined the compressive stiffness of articular cartilage in oscillatory (sinusoidal) confined compression over a wide frequency range including high frequencies relevant to impact loading. Interestingly, the currently well-established non-linear behavior of cartilage under load was initially found in this early study, which related this non-linear behavior of cartilage to a compression amplitude that exceeds a threshold value, which, in turn, is frequency-dependent. For linear viscoelastic behavior, stiffness defined in the usual sense was shown to depend on ionic strength and proteoglycan content, as well as the electrostatic forces between matrix charge groups over a frequency range of 0.001 to 20 Hz. Extending these findings, Grodzinsky and colleagues used the observed sinusoidal streaming potentials generated by oscillatory compression to relate the streaming potential field to the fluid velocity field [1]. These studies showed that interstitial fluid flow is significant to cartilage behavior over this entire frequency range.

Based on the knowledge that oscillatory compression of cartilage using physiological loads produces electrical potentials resulting from an electrokinetic streaming transduction mechanism, Grodzinsky and Frank reported in two parallel studies two electromechanical phenomena, namely, 'streaming current' and 'current-generated stress' [2], and subsequently formulated a continuum model for linear electrokinetic transduction in cartilage [3]. In another study, Grodzinsky and colleagues developed an electromechanical model that focused on ionic transport as the rate limiting step in chemically modulating electrical interactions between the charged macromolecules of the extracellular matrix (ECM). This aided in predicting the kinetics of changes in swelling and isometric compressive stress that occur in charged, hydrated tissues, including articular cartilage and corneal stroma, due to changes in salt concentration [4]. Not surprisingly, Grodzinsky and colleagues further advanced this topic and revealed that the modulation of ^{3}H-proline (collagen synthesis marker) incorporation by both loading and load release is faster than that of ^{35}S-sulfate (sulfated glycosaminoglycans (sGAG) synthesis marker) incorporation, and that the response to dynamic loading is not determined simply by the time average component of the dynamic load, as the response to unloading is not just the inverse of the response to loading and is characterized by an overshooting response [5]. Subsequently, this team developed an organ culture system to study the effects of static compression and physico-chemical changes [6]. Subjecting cartilage explants from the epiphyseal plate of 1 to 2-week-old calves to static compressive stresses of 0–3 MPa in unconfined compression, the Grodzinsky team demonstrated, as it is well-known today, that the ^{3}H-proline and ^{35}S-sulfate incorporation

decreases monotonically with increasing stress, which suggested in conjunction with later studies the beneficial, regenerative effects of dynamic compression over static compression. Perhaps less known today is that this study also demonstrated that ^{3}H-proline and ^{35}S-sulfate incorporation independently of mechanical compression strongly depended on pH, but was independent of $SO4^{2-}$ and K^{+} in the range studied, suggesting that compression-induced changes in local, interstitial pH may contribute to the biosynthetic response to static compression.

Using atomic force microscopy (AFM), Grodzinsky and colleagues in 2015 investigated the dynamic nanomechanical properties of murine cartilage over a wide frequency range of 1 Hz to 10 kHz [7]. Specifically, they studied the role of GAGs on the dynamic modulus and poroelastic properties of murine femoral cartilage by inducing GAG deletion. Interestingly, this study showed that poroelastic (i.e., fluid-flow-dependent) properties such as the hydraulic permeability, which is related to the resistance of the ECM matrix to fluid flow, and the high frequency modulus, which is related to fluid pressurization and the fibrillar network of the ECM, are more sensitive indicators of GAG loss induced by loss of mechanical function, compared to the equilibrium properties in which fluid flow is negligible. From this work, a fibril-reinforced finite element model was developed to estimate the poroelastic properties of mouse cartilage over a wide range of loading rates, which may be useful for understanding early cartilage aggrecan degradation relevant to mouse models of OA.

8.2.2 The Invention of a Successful In Vitro Cartilage Injury Model

In 1989, Grodzinsky, Robert Sah and colleagues designed two culture chambers for the uniaxial radially unconfined compression and mechanical testing of live cartilage explants [8]. They used one chamber inside a standard incubator and equipped the other chamber with a mechanical spectrometer to record load and displacement during compression. To the best of the knowledge of the authors, this design represents the initial prototype of Alan Grodzinsky's so-called cartilage "injury machine", which contributed to, and to no small extent, the overall understanding of tissue and cellular responses to compressive injury.

In the beginning, the focus was not injury per se. The authors used dynamic stiffness measurements of cartilage explants cut into standardized 3-mm diameter explants and identified a characteristic frequency of 0.001 Hz (cycles/s) that separated low- and high-frequency regimes [8]. At 0.0001–0.001 Hz, significant fluid was exuded from the explants, but at a frequency range of 0.01–1 Hz, the hydrostatic fluid pressure increased within explants, illustrating a frequency-dependent flow and deformation phenomena. Although the authors reported deformation of chondrocytes and matrix at all frequencies, this important early study demonstrated differential effects on dynamic compression on chondrocyte biosynthesis. Interestingly, the currently well-known effects of dynamic compression of stimulating cellular biosynthesis were shown to be present at the higher frequencies even at relatively low amplitudes of 1–5% with ^{3}H-proline and ^{35}S-sulfate incorporation increasing by ~20% and ~40%, respectively, with tissue volume remaining almost constant. In contrast, at lower frequencies of <0.001 Hz, low amplitudes of 1–5% had negligible effects and higher amplitudes were needed to induce increased biosynthesis with collagen (^{3}H-proline) exceeding sGAG (^{35}S-sulfate) incorporation. These insights are today perhaps even more relevant than they were at publication in 1989, as a rapidly growing body of literature documents the fundamental importance of biomechanical forces from the nanometer to the macroscopic scales. From today's perspective, another exciting point is that the authors noted that the reported *in vitro* findings were in general agreement with the *in vivo* studies on joint loading and motion of that time, which helped establish that *in vitro* studies on cartilage compression might aid in testing and optimizing therapeutic strategies to combat diseases of cartilage [8]. In a subsequent study, Grodzinsky and colleagues reported on the

effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants [9]. Interestingly, they demonstrated, to the best of our knowledge for the first time, that high amplitude dynamic cyclic compression (20%, 40%, and 60%) at a slow frequency (2 h of compression and 2 h of release for 24 h) induced convective fluid flow, which thereby enhanced the loss of ^{35}S- and ^{3}H-labeled macromolecules (sGAG and collagen) from the tissue into medium. In contrast, prolonged static compression induced matrix consolidation, which hindered the diffusional transport and loss of sGAG and collagen macromolecules. Thus, both early studies [8, 9] together demonstrated that the effects of dynamic compression on induced biosynthesis vs. ECM component loss from the tissue are subject to complex time-, frequency, and amplitude-dependent effects, and, importantly, that higher frequencies of 0.01–1 Hz even at low amplitudes of 1–5% induce anabolic, biosynthetic effects in articular cartilage tissue.

In the late 1990s, Grodzinsky and colleagues focused on the metabolic effects of mechanical injury, as those were and continue to be relevant to the development of strategies for cartilage repair [10, 11]. In healthy tissue, matrix deposition and turnover were spatially dependent, with the highest rates of proteoglycan deposition, turnover and the lowest rates of collagen deposition (^{3}H-proline autoradiography) occurring in the pericellular matrix. Interestingly, many of the well-known effects of injurious compression today were already reported in these studies. Hence mechanical injury of calf explants resulted in macroscopic tissue damage, led to mechanical failure, a subtotal decrease in cell viability with the emergence of an apparently inactive cell population but also containing catabolically active, abnormally large cells, and sustained, elevated rates of proteoglycan turnover in the cell-associated matrices of viable cells. The authors also formulated the idea of using the mechanical injury model as an *in vitro* model for understanding the responses of chondrocytes and the cartilage extracellular matrix to mechanical injury, which led to a range of studies using the well-known cartilage "injury machine", which was further developed and described by EH Frank et al. [12], as described below.

8.2.3 The Effects of Mechanical Compressive Injury on Articular Cartilage Biomechanics, Metabolic Behavior and Cell Viability and Their Strain-, Strain Rate- and Peak Stress-Dependency

Based on the initial studies by Sah et al. [8, 9] and Quinn et al. [10, 11], the impact of injurious compression on relevant parameters of articular cartilage integrity were studied in more detail by Grodzinsky and colleagues, using the injury machine, a specially designed computer-controlled and incubator-housed shear- and compression-device, described in [12]. In the original setup, cartilage disks of 3 mm diameter and approximately 1 mm thickness (obtained from the femoropatellar groove of 1–2 week old calves) were held between impermeable platens in an unconfined culture medium-filled chamber (Fig. 8.1). Uniaxial movement or rotation of the upper platen induced either compression or shear forces to the tissue, with displacement and load being monitored and controlled by the software.

The nature of injury-related cell death was of interest since programmed cell death might be a target for therapeutic approaches and repair mechanisms. Grodzinsky's group used an injurious compression protocol that consisted of six repetitive on/off cycles of displacement-controlled strain, ranging from 30–50%, applied at a strain rate of 1000 mm/s (=1/s). They reported that injury-induced apoptosis is maximal by 24 hours after injury and occurs at peak stresses as low as 4.5 MPa and increases dose-dependently with injurious peak stress. Moreover, a peak stress-dependent increase in tissue swelling, which was significant at 13 MPa, and GAG release, which was significant from 6 to 13 MPa peak stress, together with a decreased equilibrium and dynamic tissue stiffness, which was significant at 12 and 7 MPa peak stress, suggested

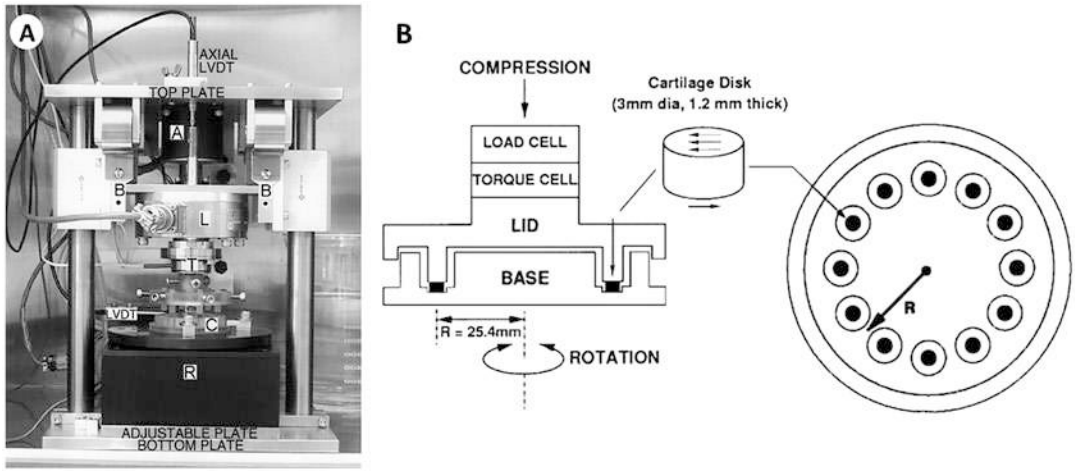


Fig. 8.1 A versatile shear and compression apparatus design by Grodzinsky and colleagues. Frank et al. described in 2000 [12], in detail, the setup of the shear/compression apparatus, which was used for the majority of the injury studies described in this chapter. Fig. **a** (left): Image of the incubator-housed loading device. An axial linear stepper motor (A) in a bearing/carriage assembly (B) applies axial compression to tissue explants located in a sample chamber (C), which is positioned on a rotary position table (R; driven by stepper motor behind the table) for application of shear forces. Load and shear stress are measured by a load (L) and torque cell (T). The adjustable plate may be moved to accommodate other fixtures. The "Linear Variable Differential Transformer" (LVDT), an electromechanical transducer that converts its displacement into a corresponding electrical signal, is placed on the left of the sample chamber (C). Fig. **b** (right): Design of the autoclavable polysulphone sample chamber with a lid and base. Cartilage explants are placed in medium-filled wells in the chamber base. The platens of the nonrotating lid compress the cartilage and rotation of the base induces shear stress to the cartilage disks/explants. The design of the sample chamber allows stimulation of up to 12 explants and single explant chambers were also designed (not shown). (Figs. **a** and **b** are reprinted from [12] with permission from Elsevier)

damage to or degradation of the collagen fibril network as well as GAG release in this range of peak stresses [13]. Thus, the peak stresses causing matrix damage and degradation were higher than those that induced apoptosis. Cell death was further investigated using a single impact of compression. While TUNEL-positive cell rates increased from 7% in unloaded controls to 33% after injury, in electron microscopy (EM) data the apoptosis rate increased from 5% in unloaded controls to 62% in injured cartilage and proved that the dead cells in injured tissue were 97% apoptotic based on cellular morphology [14].

Kurz et al. [15] investigated the effects of strain rate on cell viability, cartilage matrix biosynthesis and mechanical properties after 50% strain using a single injurious compression. A strain rate of 0.01/s resulted in no measured effect on the cells or on the ECM, although peak stresses reached levels of about 12 MPa, whereas faster strain rates of 0.1 and 1/s induced peak stresses of ~18 and ~24 MPa, increased cell death, and significantly decreased both proteoglycan and total protein biosynthesis. Comparably, increasing strain rate was associated with impaired mechanical properties and the remaining viable cells had lost their ability to have their biosynthesis stimulated by low-amplitude sinusoidal compression, suggesting an impaired reparative capability of the surviving population, in agreement with the emergence of an apparently inactive cell population in Quinn et al. [10], discussed above. This clinically relevant inability to exhibit a reparative response to dynamic compressive stimulation was most extensive after injury was applied with the highest strain rates suggesting that strain rate as well as peak stress, or strain are important parameters that define the post-injurious fate of injured cartilage.

Grodzinsky and colleagues then investigated the relationship between injurious peak stress and post-injurious proteoglycan loss in bovine

cartilage, and also in human knee and ankle cartilage. In bovine cartilage, the injury-related GAG release was highest during the first 4 h after injury, but remained higher than that in controls during the first 24 hours post-injury [16]. For experiments on human knee and ankle cartilage with no history of OA, the team applied a uniaxial unconfined injurious compression of 65% strain at 2/s, which was quicker than the bovine injurious compression model. Increased injurious peak stress (at a constant final strain and compression rate) was associated with less proteoglycan loss after injury [17], corroborating studies on bovine articular cartilage [13, 16]. When injured, fewer human ankle vs. knee cartilage explants suffered macroscopic damage and neither a post-injurious increase in proteoglycan loss from injured ankle cartilage relative to controls nor a relationship between peak stress and proteoglycan loss was observed as opposed to knee cartilage explants. Besides uncovering differences in the response of human knee and ankle cartilage to injury, this study suggested that peak stress itself did not appear to be an important cause of proteoglycan loss from human cartilage.

8.2.4 Understanding the Molecular Concept of Mechanical Injury by Studying Gene Expression, Signal Transduction and the Release of Potential Injury Biomarkers

Several studies of Grodzinsky and colleagues have used the injury model to investigate signaling pathways and gene expression patterns after mechanical overload. The first demonstrated that the angiogenesis factor VEGF (vascular endothelial growth factor) might play an important autocrine or paracrine role in the progression of post-traumatic OA (PTOA) [18]. Mechanical injury induced the expression of the transcription factor hypoxia-inducible factor-1 (HIF-1), a known promoter of VEGF expression. The subsequent expression of VEGF activated autocrine production of MMPs (MMP-1, -3 and -13) in chondrocytes, whereas tissue inhibitor of metalloproteinases-1 and -2 (TIMP-1 and -2), the inhibitors of MMPs, were reduced. Motivated by these interesting results, a more detailed study of injury-related gene expression followed [19]. mRNA levels in non-injured, free swelling bovine cartilage varied over five orders of magnitude with matrix molecules being the most highly expressed, while cytokines, MMPs (except MMP-3), aggrecanases (ADAMTS-5), and transcription factors showed lower expression levels. Specifically, the matrix molecules fibronectin and type I collagen, as well as TNF, GAPDH, and β-actin and finally IGF-1, IGF-2, and ADAMTS-4 as well as type II collagen, aggrecan, fibromodulin, link protein, and IL-1 showed little change in expression after injury vs. non-injured cartilage, whereas MMP-3 increased 250-fold, ADAMTS-5 increased 40-fold, and TIMP-1 increased 12-fold. The MMP-activating transcription factors c-fos and c-jun showed an immediate transient upregulation followed by a rapid decline within hours and a slowly increasing expression pattern was seen for most other MMPs and their inhibitors [19].

Two other studies on bovine cartilage characterized proteins lost to the medium from cartilage explant cultures after either injurious mechanical compression or treatment with IL-1β or TNFα, using mass spectrometry [20, 21]. While cytokines predominantly promoted the release of proteins that are involved in inflammation and a stress response including acute-phase and complement proteins, injury caused the release of intracellular proteins, including Grp58, Grp78, 4-actinin, pyruvate kinase, and vimentin and also caused increased release and evidence of proteolysis of type VI collagen subunits, cartilage oligomeric matrix protein, and fibronectin. These data suggested loss of cartilage integrity such as matrix damage primarily of the pericellular matrix (PCM), supporting the idea of a high turnover in the PCM or increased damage to the PCM with injury. The data also suggested cell membrane disruption, which could be responsible for reported decreases in tissue compression and shear stiffness or cell apoptosis, changes in gene expression, or for the decrease in the ability of

the remaining viable cells to up-regulate biosynthesis in response to anabolic loading as described in the above section. Although MMP-2 appeared to decrease overall in that study, mechanical injury but not cytokines increased the release of MMP-14 (MT1-MMP) and TIMP-2, which are known to interact together to activate pro-MMP-2, and many of the proteins identified as being increased in the medium in that study are in fact substrates of MMP-2 including osteopontin, galectin 1, HSP-90, and CTGF, all of which are shown to be elevated with injury or cytokine treatment. Therefore, the authors suggest a possible role for MMP-2 in overall regulation of cell surface-associated molecules in cartilage. Of the aggrecanases, only a single ADAMTS-4 peptide was identified likely because of the enzymes ADAMTS-4 and -5 being present at a very low concentration. An observed decrease in the release of C-terminal telopeptides of several collagen types following both cytokine- and injury-treatment was interpreted as decreased collagen synthesis. Another study used a targeted proteomics approach to follow the progression of matrix degradation in response to mechanical damage and cytokine treatment of human knee cartilage explants in order to study the kinetics of cartilage degradation (IL-6 and TNFα). They identified candidate proteases, including MMP-1, MMP-3, MMP-10 and MMP-13, and the absence of collagen pro-peptides and elevated levels of specific cartilage oligomeric matrix protein (COMP) and COL3A1 neo-epitopes as potential biomarkers for the earliest events in PTOA [22]. Together these studies show the differential effects of cytokines vs mechanical damage on pro-inflammatory and stress-related vs. damage-associated protein release.

8.2.5 Elucidating the Zonal, Age and Species-Dependency of Injurious Compression

Next to the impact of strain, strain rate and peak stress as described above, Grodzinsky and our two groups investigated the zonal dependence of biomechanical, biochemical, and matrix-associated changes caused by compressive injury [23]. Our teams biomechanically characterized, injured (strain: 50%, strain rate 1/s) and re-characterized cartilage explants from the superficial and deeper zones of bovine calves. Having added histology, diffraction-enhanced x-ray imaging, and texture analysis to biochemical and biomechanical methods, the study elucidated that injured superficial zone explants showed surface disruption, compaction, and importantly, immediate biomechanical impairment after injury, whereas injured deeper zone explants showed collagen crimping but remained undamaged and biomechanically intact. Moreover, superficial zone explants that appeared intact on histology exhibited textural alterations, whereas deeper zone explants showed collagen crimping but were otherwise histologically and biomechanically intact. Overall this showed that the softer superficial zone was more vulnerable to compressive injury than the deeper zones, which, in conjunction with delayed superficial proteoglycan loss, may predispose the injured articular surface to further softening and tissue damage, thus increasing the risk of development of PTOA.

In another study our groups injured bovine cartilage explants with or without the superficial zone being present. Neither the peak stresses during compression nor the rate of apoptotic cell death specifically in deeper zones were significantly different in the two groups. It was speculated that the superficial zone might be too thin and soft, and that its relative contribution to the effects measured on the total tissue in a full area-loaded and unconfined 50% compression model are negligible. However, explants with an intact superficial zone showed a different macroscopic appearance, with the lower ends showing larger swelling laterally than the upper end of the explants, probably due to the fact that superficially the fibrils are oriented parallel to the platen which may stabilize the integrity of that particular side of the explant. However, the overall release of GAG was up to five-fold lower in explants containing the superficial zone [24]. On a side note, the superficial zone harbors the majority of chondrocytes, which suggests a significant role of the superficial zone in mediating

post-injurious effects related to the tissue's cells. Another study by Grodzinsky and colleagues investigated injured superficial zone tissue alone in comparison to tissue from deeper cartilage layers and found increased lubricin biosynthesis to be an early transient response of the superficial layer of cartilage, whereas the deeper layers exhibited reduced expression after injury. Histologic and immunohistochemical analyses revealed that superficial zone explants exhibited marked cellular depletion and displayed an amorphous/swollen surface architecture with diminished GAG and collagen content after injury, whereas deeper zone explants, injured without the superficial layer, displayed some loss in GAG and collagen content, but the effect was not as prominent as for the superficial tissue alone [25]. Together these studies demonstrate a significant role of the superficial zone in mediating the effects of injury.

Together with our groups Grodzinsky also investigated age and maturation of the articular cartilage as a factor of the injurious response by using tissue from newborn calves compared to cartilage from more mature animals [26, 27]. Injurious compression induced significantly more apoptosis in newborn calves (22% of cells) than in cartilage from adult cows (2–6%), and there was less GAG loss and no significant reduction in ^{3}H-proline and ^{35}S-sulfate incorporation in cartilage from 2-year-old animals in contrast to the data from Kurz et al. [15], where a single compression induced significant GAG loss and reduction in biosynthetic activity in tissue from 2-week-old animals suggesting that immature cartilage tissue might be more vulnerable to matrix destruction after cartilage injury, which could be of clinical importance, since joint injuries in the younger, more active population are increasing. Since load stresses during compression increase with maturation of the tissue (a single axial compression of strain of 50% with a strain rate of 1 s induces mean peak stresses of 17–23 MPa in newborn tissue vs. 25 MPa in younger (6–16-month-old) tissue vs. approximately 29 MPa in 22–23-month-old tissue [26]), peak stresses do not seem to be responsible for the maturation-dependent differences in tissue response to injury, since most parameters of tissue damage increase with increasing peak stress in general.

Grodzinsky and colleagues also demonstrated a species dependency of the effects of injury by transferring the bovine *in vitro* model, whose parameters were at that time well established, to tissues of human [28] or horses [29]. The team screened specimens cultured for 28 days with subsequent histological analysis [29]. At a strain rate of 1/s the threshold strain necessary for inducing morphological and biochemical ECM changes was 60% and, thus, higher than in bovine cartilage. Patwari et al. [28] needed a uniaxial unconfined injurious compression of 65% strain at 4/s in human knee and ankle cartilage in order to induce comparable tissue damage. Both studies demonstrate that the established injury model is applicable to different species but that the strain, strain rate and peak stress leading to "injured" cartilage is species-dependent.

8.2.6 Combining Mechanical Injury with Cytokine Exposure or Co-culture Systems for Generating a More Realistic Trauma Model

A further study of Grodzinsky and colleagues investigated the effects of injury alone vs. in combination with IL-1α or TNFα on the amount of proteoglycan loss using newborn bovine as well as matched knee and ankle tissues from adult healthy human donors. The team demonstrated that in bovine cartilage MMP-3 but not MMP-13 mRNA levels increased. The proteoglycan loss, which was at that time well-known to occur after injury, was significantly increased, although its extent of only 2% of the total content and loss only over the first 3 days following injury was surely surprising. Importantly, the combination of injury with either IL-1α (1 ng/ml) or TNFα (100 ng/ml) caused, during the same time frame, substantial increases of 35% and 54% in proteoglycan loss. In human knee cartilage, comparable interactions between cytokine and injury effects were observed after injury but

with lower magnitude than in bovine cartilage. Consistent with current knowledge, there was no significant interaction between injury and IL-1α in human ankle cartilage [28]. Overall, incorporating cytokines into the *in vitro* mechanical injury model was successful and helpful for studying the interactions between mechanical forces and pro-inflammatory cytokines that may be persistently present after joint trauma, adding insight into subsequent degradative pathways of PTOA progression.

A further study demonstrated that interactions between injured cartilage and other joint tissues are important in matrix catabolism and gain more complexity into the system [30]. The authors found that mechanically injured cartilage co-cultured with the joint capsule tissue alters chondrocyte expression patterns and increases ADAMTS-5 production and subsequent GAG loss. In a related study Swärd et al. [31] found additional aggrecan fragment types released at an earlier time after injury when synovial joint tissue was present, indicative of different proteolytic pathways for aggrecan degradation under co-culture conditions, with increased aggrecanase and MMP activity toward aggrecan. On the other hand, Lee et al. [29] demonstrated that synoviocytes protect cartilage from the effects of injury *in vitro* under certain circumstances. Thus, synoviocytes extracted from normal equine synovium exerted both positive and negative effects on injured equine cartilage, but ultimately protected injured cartilage from progressing toward an OA phenotype. Co-culture of synoviocytes and injured cartilage significantly reduced the expression of ADAMTS-4 and -5, but also increased the expression of MMP-1 and reduced the expression of TIMP-1 in synoviocytes. In contrast, injured cartilage cultured with synoviocytes increased the expression of both collagen type 2 and ADAMTS-5. Moreover, an additional protective effect of synoviocytes on injured cartilage was the reduction of both focal cell loss and chondrocyte cluster formation, two major hallmarks of OA. This is supported by an early study by Kurz et al. [32] showing that articular chondrocytes are protected against the negative effects of reactive oxygen species-induced cytotoxicity and lipid peroxidation under co-culture conditions with synoviocytes indicating that more research is needed to understand the interaction between different joint cell types.

8.2.7 Predicting Articular Cartilage Properties and Injurious Damage on the Structural, Biochemical and Biomechanical Level

Throughout the years Grodzinsky and colleagues have developed several models for predicting the properties and injurious damage of articular cartilage on the structural, biochemical as well as biomechanical level**.** This began as early as 1987, as briefly discussed above, when Grodzinsky and colleagues developed an electromechanical model for predicting the kinetics of changes in swelling and isometric compressive stress that can be induced by changes in salt concentration in charged, hydrated tissues [4]. In 2015, Grodzinsky and colleagues developed a fibril-reinforced finite element model to estimate the poroelastic properties of mouse cartilage over a wide range of loading rates [7]. In 2013, Grodzinsky and our two groups demonstrated that biomechanical stress, which occurs during compressive injury, predetermines the biomechanical, biochemical, and structural consequences of articular cartilage as well as the structural and functional damage that occurs when the tissue fractures [33]. Interestingly, damage prediction in a blinded experiment using stress-vs-time grades was 100% correct and also sensitive enough to differentiate the complexity of cartilage matrix disruptions. Moreover, the injuriously dissipated energy and the maximum stress rise during injury correlated with the extent of biomechanical and biochemical damage in zonal analyses. Thus, we introduced a novel method based on the interpretation of compressive yielding for accurately predicting the extent of structural damage during injury [33].

In 2018, Orozco et al. [34] investigated the fixed charge density of proteoglycans in injured immature cartilage and subsequently dynami-

cally compressed cartilage for up to 12 days to induce biosynthesis. Based on these data they introduced a novel model that implemented deviatoric and maximum shear strain and also fluid velocity-controlled algorithms with the goal of simulating the loss of the fixed charge density of proteoglycans over time. Interestingly, the homogeneity and localization of the predicted loss of the fixed charge density depended on the degeneration algorithm being driven by fluid velocity vs. shear strain. Using a novel finite element model that incorporates (1) diffusion of the pro-inflammatory cytokine IL-1 into tissue, and (2) the effect of excessive levels of shear strain near chondral defects during physiologically relevant loading, Grodzinsky and colleagues developed this further to a computational model which simulates spatial and temporal changes of fixed charge densities in injured cartilage in order to predict the simultaneous effect of tissue inflammation and abnormal biomechanical loading on loss of cartilage proteoglycans [35]. Their data suggests that the presence of lesions plays a role in cytokine diffusion-driven degradation and also predisposes cartilage for further biomechanical degradation. These models are promising *in silico* tools for predicting disease progression, recognizing lesions at high risk, simulating treatments, and ultimately optimizing treatments to postpone the development of PTOA.

8.3 Therapeutic Modulation of the Injurious Response

Throughout the years, Grodzinsky and colleagues extended the mechanical articular cartilage injury model to test a spectrum of disease-modifying agents, which will be discussed in detail below, and have proven that an array of therapeutics can protect against injury-related responses (dexamethasone, IL-10, IGF-1, MnTMPyP antioxidant MnTMPyP, E2 estrogen, and 10–20% dynamic compressive loading) and sometimes even promote a pro-regenerative response to injury or inflammatory insult of healthy cartilage (dexamethasone, IL-10), OA-injured cartilage (IL-10) and chondrocyte-containing collagen ACI grafts (IL-10, BMP-2). Moreover, Grodzinsky and associates developed charged-nanoscale sized carrier systems to efficiently transport therapeutics (dexamethasone or IGF-1) into the cartilage, offering cartilage-targeting therapies. Some of the therapeutics advanced to clinical testing such as dexamethasone in prevention of PTOA (ClinicalTrials.gov Identifier: NCT02318433). These promising targets remain at the horizon of advancing cartilage injury-related therapeutic strategies and could pave the way forward for the development of clinical therapies that will enhance the repair of cartilage after injury (Fig. 8.2).

8.3.1 Dexamethasone and 17b-Estradiol – Steroid Hormone Treatment of Mechanically-Injured Articular Cartilage and in an In Vivo PTOA Model Lead to Clinical Assessment

A large body of work by Grodzinksy and colleagues has focused on use of dexamethasone, a corticosteroid used to treat a wide-spectrum of conditions, in preventing degenerative responses in cartilage and the onset of PTOA after injury [36–45]. In both healthy human and bovine cartilage explant mechanical injury models of injury alone or in combination with subsequent inflammatory (TNF-α alone or in combination with IL-6 and sIL-6R) insult, continuous dexamethasone treatment inhibited the production of pro-inflammatory cytokines, MMPs and nitric oxide (NO), prevented GAG loss, reduced the release of aggrecan and COMP fragments, and promoted proteoglycan synthesis [36, 38, 39, 44] demonstrating that dexamethasone protects against injury-related changes. Moreover, in non-injured IL-α stimulated bovine cartilage, dexamethasone significantly increased the mRNA expression of ACAN and COL2A1 and decreased IL-6, caspase-3, ADAMTS-4, MMP-3 and -13, and COX2 4 days after treatment [37]. These studies show that dexamethasone provides protection against not only injury-related effects but

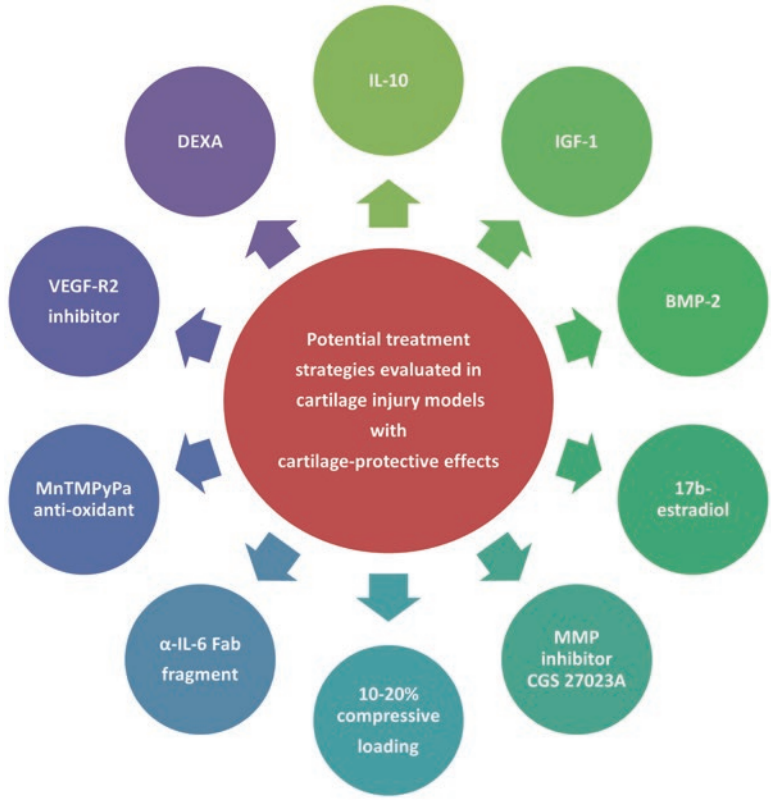


Fig. 8.2 Summary of the potential treatment strategies investigated by Grodzinsky and colleagues in cartilage injury models with cartilage-protective effects

also pro-inflammatory cytokines that may be persistently present after joint trauma.

While these studies clearly show that dexamethasone is protective against injury-related trauma and that dexamethasone could be a potential treatment to regulate many early cartilage degradative changes associated with joint injury, as reviewed by Grodzinsky and Black [46], some studies suggest that dexamethasone may have catabolic effects on the cartilage tissue by promoting apoptosis and reducing proliferation of healthy chondrocytes. However, these effects have been attributed to high doses or non-localized long-term treatment. Adverse effects have also associated with long-term systemic dexamethasone use, including stunting the growth of developing cartilage and bone and causing bone density loss thereby decreasing load potential. Therefore, Grodzinsky and other groups started to engineer biomaterial-based strategies to improve and extend the residence time of dexamethasone by preventing its joint clearance and allowing penetration of the cartilage as a means of delivering a low dose and more localized treatment strategy [43]. One such strategy developed by Grodzinsky and colleagues involved covalently linking a low dose of dexamethasone to the small, highly cationic molecule avidin. Due to avidin's net charge (+20), electrostatic interactions between the cationic avidin and anionic cartilage allow dexamethasone-nanosized carriers [45] to penetrate the full depth of the cartilage within 24 hours of application. Moreover, within thicker cartilage explants such as rabbits, as opposed to thinner rat cartilage, which better resembles the human cartilage thickness, the dexamethasone-carriers were retained within the cartilage tissue for up to 3 weeks offering a prolonged intra-articular localized treatment strategy [39–42]. Compared to a single bolus treatment, prolonged dexamethasone treatment was more effective in reducing synovial joint inflammation in rabbits by half and, whereas prolonged treatment did not prevent

MMP-3 and -13 mRNA expression and GAG loss, it was capable of significantly decreasing the mRNA expression of *IL-1β*, *MMP-1*, and *ADAMTS-5* and it restored *ACAN* to normal expression levels 3 weeks after anterior cruciate ligament transection (ACLT) injury [39, 42].

Another study investigated the effects the E2 estrogen hormone 17b-estradiol, which is the most widely clinically used estrogen in oral contraceptive pills and in hormone replacement therapy in the treatment of symptoms related to menopause, in mechanically-injured mature bovine articular cartilage. Physiological concentrations of E2 prevented mechanical injury-related cell death (nuclear blebbing and TUNEL staining; effect reversable by addition of fulvestrant, an E2 antagonist) and reduced GAG release [24] suggesting that therapeutic compounds containing the E2 estrogen may regulate and protect against joint-related trauma. Since dexamethasone and E2 both are steroid hormones, it might be speculated that higher concentrations of one or the other might trigger effects through cross-binding to different subtypes of steroid hormones.

Collectively, these studies and the work of others as summarized [46], show that dexamethasone inhibits the early processes involved in PTOA development. In view of all of this data, a pilot clinical study at the Mayo Clinic (ClinicalTrials.gov Identifier: NCT02318433) was initiated to test whether a single, intra-articular injection (4 mg) of dexamethasone given soon after intra-articular fracture of the distal radius reduces the incidence or severity of PTOA.

8.3.2 Interleukin 10 (IL-10) Treatment of Mechanically-Injured Articular Cartilage and Cell-Laden ACI Grafts

Together with our two groups, Grodzinsky investigated the therapeutic effects of the anti-inflammatory IL-10 cytokine on injured cartilage using a pre-injury [47] and post-injury [48, 49] treatment approach. In the pre-injurious treatment study, a single (10 ng/ml) dose of IL-10 significantly decreased injury-related cell death, release of GAG and NO and the mRNA expression of *NOS2*, *MMP-3* and *-13* and *ADAMT-S4* 4 days after injury of mature bovine articular cartilage [47]. In the post-injurious treatment study, continuous low doses of IL-10 were applied to mature bovine cartilage directly after injury and post-injurious effects were assessed up to 3 weeks after injury. In both non-injured and injured cartilage, IL-10 was capable of inducing the mRNA expression of *COL2A1*, *ACAN*, and *SOX9* 3 days after treatment. In injured cartilage, IL-10 treatment additionally significantly inhibited the expression of mechanical injury-induced *COL1A1* and *COL10A1*. Moreover, continuous post-injurious IL-10 treatment inhibited injury-related apoptosis, restored type 2 collagen in the ECM, and inhibited the loss of aggrecan, hyaluronic acid, and GAG 1 to 3 weeks after injury. These studies show that pre- and post-treatment of articular cartilage with low doses of IL-10 (e.g., 100 pg/ml) is highly protective against injury-related damage [48].

The effects of continuous low-dose (100 pg/ml) IL-10 treatment alone or in combination with the growth factor bone morphogenetic protein 2 (BMP-2) of post-operative material containing human chondrocytes seeded in type I/III collagen was also measured to assess the potential of IL-10 to support graft maturation in this clinically applied autologous chondrocyte implantation (ACI) transplant material (Novocart 3D®). Three weeks after injury, IL-10 significantly increased the GAG content within the grafts vs. non-treated grafts. The combination of continuous IL-10 + BMP-2 also significantly up-regulated *COL2A1*, *ACAN*, and *SOX9* and reduced injury-related *COL1A1* mRNA expression and the *COL1A1/COL2A1* ratio compared to IL-10 or BMP-2 treatment alone 3 days post-injury [48] suggesting that the combination of IL-10 and BMP-2 may enhance the repair of autologous transplanted chondrocytes after cartilage injury.

The chondro-regenerative effects of post-injurious application of IL-10 alone or in combination with lysed platelet concentrate (PC) was

additionally assessed in the treatment of mechanically-injured human OA articular cartilage and chondrocyte-containing ACI grafts from patients undergoing ACI treatment. In OA injured explants, IL-10 and PC similarly reduced apoptosis 4 days after injury. Whereas IL-10 treatment did not modulate the gene expression in OA injured cartilage explants, PC significantly increased *COL2A1* and *ACAN* expression and decreased *COL10A1* expression 3 days after injury. However, continuous IL-10 treatment had better ECM preserving effects in sGAG retention and reduction of type 1 collagen in the ECM after cartilage injury compared to PC treatment, which was less protective. Moreover, PC did not recover the loss of type 2 collagen in the superficial zone of the cartilage explants, and in fact, treatment increased type 1 collagen deposition, indicative of fibro-cartilage [49]. In the ACI samples, the combination of continuous PC and IL-10 was most effective in enhancing *COL2A1* mRNA expression but had no effect on *ACAN* expression. The combination treatment also enhanced sGAG and collagen 2 neosynthesis in the ECM. However, similar to the injured OA cartilage, PC induced *COL1A1* and *COL10*A*1* mRNA expression, which was reduced by co-treatment with IL-10 [49]. Thus, IL-10 was more potent in preserving ECM integrity and mitigating the potentially negative effects of PC suggesting that IL-10 is better in controlling injury-induced degenerative pathways.

Together these studies show that IL-10 treatment can control the post-traumatic environment when applied pre- or post-injury and that IL-10 can additionally support neo-cartilage formation, graft integration and maturation thereby enhancing cartilage repair following ACI treatment.

8.3.3 IGF-1 in Treatment of Mechanically-Injured Articular Cartilage and in an In Vivo PTOA Model

Several studies by Grodzinsky and colleagues have shown that the growth factor insulin-like growth factor 1 (IGF-1) is another potential therapeutic that protects against cartilage injury-related effects [37, 43, 50, 51]. As interleukins, such as IL-1α are typically present in the joint following joint trauma, one study investigated whether IGF-1 alone or in combination with dexamethasone could modulate moderately aggressive (high dose) cytokine IL-1α effects in young healthy bovine cartilage explants and an adult human healthy articular cartilage sample. In young bovine non-injured cartilage, continuous dexamethasone treatment more favorably reversed IL-1α-mediated effects on the mRNA level of *ACAN, COL2A1*, *IL-6*, *caspase-3*, *ADAMTS4*, *MMP-3* and *-13*, and *COX2* 4 days after treatment. However, the combination of IGF-1 and dexamethasone significantly inhibited the loss of sGAG and type II collagen, rescued the suppression of matrix (proteoglycan) biosynthesis, and inhibited the loss of chondrocyte viability caused by IL-1α treatment 1–2 weeks after continuous treatment. In adult healthy human cartilage, only IGF-1 rescued matrix biosynthesis, while dexamethasone alone inhibited sGAG loss and improved cell viability within the cartilage explants [37].

To improve the pharmacokinetics of IGF-1, nanoscale-sized cartilage-penetrating nanocarriers were developed by Grodzinsky and the Hammond group that enable the encapsulation and delivery of IGF-1 throughout the full depth of cartilage tissue [43, 50, 51]. These nanocarriers allow ionic complexation of cationic IGF-1 with anionic poly (L-glutamic acid), which has clinically been used in other FDA-approved polymer-drug conjugate systems. The surface is further modified with an excess of positive charge using cationic poly (L-arginine) that allows transport of the therapeutic growth factor across cell membranes and transport through the negatively charged cartilage ECM and full depth of cartilage [50]. Their groups further developed the nanocarriers by covalently conjugating some of the cationic side groups with polyethylene glycol (PEG) oligomers, creating a small library of nanoscale molecules with varying surface charge. With increasing surface charge and a corresponding decreasing PEGylation, increased cartilage binding was observed. Compared to free IGF-1,

which was cleared within 7 days, a single dose of the IGF-1 via the nanoscale carrier enhanced the joint residence time to 4 weeks in an in vivo rat knee PTOA model of cartilage injury (anterior cruciate ligament (ACL) transection and medial meniscus resection (ACLT1MMx)) when administered within 48 hours of injury. Moreover, a single injection of PEG-containing-IGF-1 carriers reduced synovial inflammation, the width of cartilage degeneration by 60% and volumetric osteophyte burden by 80% vs. untreated rats at 4 weeks post-surgery and was far better than free IGF-1 [51].

The results indicate the potential of a charged cartilage-targeting approach that enables delivery of IGF-1 to target cells within cartilage and over an extended period of time. Moreover, these studies show that IGF-1 is another potential early interventional therapy that could delay or prevent the onset of PTOA following joint injury.

8.3.4 Anti-IL-6 Fab-Fragment in Treatment of Mechanically-Injured Articular Cartilage

IL-6 is highly present after joint trauma, making it a relevant target for controlling injury-related responses. Since full-sized antibodies are too large to penetrate beyond the cartilage surface due to steric hindrance of the dense matrix, Grodzinsky's group investigated the transport of smaller (48 kDa) anti-IL-6 antigen Fab-fragments in healthy human and bovine cartilage [52, 53]. Uptake of the anti-IL-6 Fab significantly increased following mechanical injury, and an additional increase in uptake was observed in response to combined mechanical injury and inflammatory insult with TNFα. This may be due to a combined increase in injury-related tissue swelling which causes an increase in tissue hydration and water content and a decrease in GAG density following injury allowing the Fab-fragment to move with less hindrance within the cartilage, resulting in an increased uptake ratio [52]. While pre-treatment with the anti-IL-6 Fab-fragment had no effect on sGAG loss after injury alone or by TNFα treatment alone, the anti–IL-6 Fab-fragment partially (by approximately 20%) reduced sGAG loss due to the combination of injury plus TNFα treatment in bovine and human explants [53]. This may be attributed to the incomplete non-uniform penetration and slow diffusion of the anti–IL-6 Fab into the cartilage tissue [52]. However, this data nonetheless supports that joint trauma and the inflammatory response following joint injury play a critical role in altering the transport properties of damaged cartilage, especially if the molecules or therapeutics are smaller than 42 kDa.

8.3.5 Antioxidant Treatment of Mechanically-Injured Articular Cartilage

Inhibition of reactive oxygen species has also been explored. Apoptotic cell death due to mechanical injury was almost completely inhibited when mature bovine cartilage was either pre-treated or treated immediately after injury with a compound (manganese(III)tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride; MnTMPyP) that mimics native superoxide dismutase (SOD) and acts as a peroxynitrite and hydrogen peroxide scavenger [26]. Vitamin E (α-tocopherol) was also tested but had no effect on reducing the number of post-injury apoptotic cells. This data suggests that therapies having an antioxidant component or diets enriched in antioxidants may help decrease mechanically-induced cell death in articular cartilage.

8.3.6 MMP Inhibitors and a VEGFR-2 Kinase Inhibitor in Treatment of Mechanically-Injured Articular Cartilage

Several MMP inhibitors have also been tested. Injury-related GAG release from bovine tissue 1 to 7 days post-injury was reduced by the MMP inhibitor CGS 27023A whereas the biosynthesis inhibitor cycloheximide, MMP inhibitor GM 6001 and aggrecanase activity inhibitor SB 703704 had no effect [16]. A VEGF receptor 2

(VEGFR-2) kinase inhibitor was able to reduce the injury-dependent expression of the MMPs (MMP-1, -3, and -13), whereas TIMP-1 and -2, the inhibitors of MMPs, were reduced, which might make the HIF-1α/VEGF pathway a potential target for therapeutic approaches of PTOA [18].

8.3.7 Moderate vs. High Dynamic Compressive Loading of Mechanically-Injured Articular Cartilage

Grodzinksy's research also indirectly showed that dynamic loading of the joint following a joint trauma may be a beneficial physical therapy regime to promote healing of cartilage tissue since moderate (10% and 20% strain) but not high (30%) dynamic compression inhibited the pro-catabolic response of combined mechanical injury and subsequent persistent inflammation (TNF-α, IL-6, sIL-6R). Thus, 10% and 20% strain prevented GAG loss, diminished aggrecanase activity and decreased apoptosis in injured bovine cartilage explants. Moreover, in the presence of cytokines alone, 10% and 20% strain significantly upregulated *COL2A1* expression levels. Importantly, this study also showed that, compared to 10% and 20% strain, loading cartilage with 30% strain amplitudes significantly increased apoptosis and induced the upregulation of inflammatory (*COX-2*) and *ADAMTS-5*, the main aggrecanase involved in articular cartilage breakdown and the loss of ECM [54]. Together, this suggests that appropriate moderate loading of the joint in post-injury rehabilitation may improve cell and tissue function and generate stronger hyaline cartilage and that higher loads may be detrimental to cartilage.

8.4 Final Remarks

Alan Grodzinsky's research on the electromechanobiology of articular cartilage began more than 40 years ago with a groundbreaking publication on the compression-induced electrical potential differences between the surface and deepest regions of cartilage [55]. This important publication related the magnitude-, sign- and time-dependence of the induced electrical potentials to the, at that time, known features of cartilage mechanics and fluid flow and, effectively, 'explained' how mechanically induced electric fields *in vivo* may help regulate the transport of ions and interstitial fluid in charged, hydrated tissues. In the opinion of the authors of this book chapter, two additional key points among the many relevant contributions during 40 years of research are not only outstanding but truly relevant. Grodzinsky and colleagues have transformed our understanding of how complex structure-function relationships govern the tissue's behavior, define the tissue's response to injury, and can be utilized to overcome injury to the tissue by dynamic stimulatory loading. Moreover, his research has been instrumental in understanding the proinflammatory response to injury and in developing treatment strategies that are based on an in-depth understanding of the structure and function of articular cartilage.

Collectively, Alan Grodzinsky's work is not just highly impressive in content, quality, and significance, it also went full circle from uncovering groundbreaking electromechanobiological characteristics of articular cartilage to 'translating' them into a therapeutic strategy. As a prime example, the use of dexamethasone for preventing PTOA (ClinicalTrials.gov Identifier: NCT02318433) and linking dexamethasone to the small, highly cationic molecule avidin for full-thickness penetration and increased duration of stay. On a personal note, Alan's work ethics, quality of science, and motivational nature were instrumental in achieving these accomplishments and the authors are grateful for having played a small part in Alan's scientific success.

References

1. Lee RC, Frank EH, Grodzinsky AJ, Roylance DK (1981) Oscillatory compressional behavior of articular cartilage and its associated electromechanical properties. J Biomech Eng 103(4):280–292

2. Frank EH, Grodzinsky AJ (1987) Cartilage electromechanics–I. Electrokinetic transduction and the effects of electrolyte pH and ionic strength. J Biomech 20(6):615–627
3. Frank EH, Grodzinsky AJ (1987) Cartilage electromechanics—II. A continuum model of cartilage electrokinetics and correlation with experiments. J Biomech 20(6):629–639
4. Eisenberg SR, Grodzinsky AJ (1987) The kinetics of chemically induced nonequilibrium swelling of articular cartilage and corneal stroma. J Biomech Eng 109(1):79–89
5. Gray ML, Pizzanelli AM, Lee RC, Grodzinsky AJ, Swann DA (1989) Kinetics of the chondrocyte biosynthetic response to compressive load and release. Biochim Biophys Acta 991(3):415–425
6. Gray ML, Pizzanelli AM, Grodzinsky AJ, Lee RC (1988) Mechanical and physiochemical determinants of the chondrocyte biosynthetic response. J Orthop Res 6(6):777–792
7. Nia HT, Gauci SJ, Azadi M, Hung HH, Frank E, Fosang AJ, Ortiz C, Grodzinsky AJ (2015) High-bandwidth AFM-based rheology is a sensitive indicator of early cartilage aggrecan degradation relevant to mouse models of osteoarthritis. J Biomech 48(1):162–165
8. Sah RL, Kim YJ, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD (1989) Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 7(5):619–636
9. Sah RL, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD (1991) Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants. Arch Biochem Biophys 286(1):20–29
10. Quinn TM, Grodzinsky AJ, Hunziker EB, Sandy JD (1998) Effects of injurious compression on matrix turnover around individual cells in calf articular cartilage explants. J Orthop Res 16(4):490–499
11. Quinn TM, Maung AA, Grodzinsky AJ, Hunziker EB, Sandy JD (1999) Physical and biological regulation of proteoglycan turnover around chondrocytes in cartilage explants. Implications for tissue degradation and repair. Ann N Y Acad Sci 878:420–441
12. Frank EH, Jin M, Loening AM, Levenston ME, Grodzinsky AJ (2000) A versatile shear and compression apparatus for mechanical stimulation of tissue culture explants. J Biomech 33(11):1523–1527
13. Loening AM, James IE, Levenston ME, Badger AM, Frank EH, Kurz B, Nuttall ME, Hung HH, Blake SM, Grodzinsky AJ, Lark MW (2000) Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Arch Biochem Biophys 381(2):205–212
14. Patwari P, Gaschen V, James IE, Berger E, Blake SM, Lark MW, Grodzinsky AJ, Hunziker EB (2004) Ultrastructural quantification of cell death after injurious compression of bovine calf articular cartilage. Osteoarthr Cartil 12(3):245–252
15. Kurz B, Jin M, Patwari P, Cheng DM, Lark MW, Grodzinsky AJ (2001) Biosynthetic response and mechanical properties of articular cartilage after injurious compression. J Orthop Res 19(6):1140–1146
16. DiMicco MA, Patwari P, Siparsky PN, Kumar S, Pratta MA, Lark MW, Kim YJ, Grodzinsky AJ (2004) Mechanisms and kinetics of glycosaminoglycan release following in vitro cartilage injury. Arthritis Rheum 50(3):840–848
17. Patwari P, Cheng DM, Cole AA, Kuettner KE, Grodzinsky AJ (2007) Analysis of the relationship between peak stress and proteoglycan loss following injurious compression of human post-mortem knee and ankle cartilage. Biomech Model Mechanobiol 6(1–2):83–89
18. Pufe T, Lemke A, Kurz B, Petersen W, Tillmann B, Grodzinsky AJ, Mentlein R (2004) Mechanical overload induces VEGF in cartilage discs via hypoxia-inducible factor. Am J Pathol 164(1):185–192
19. Lee JH, Fitzgerald JB, Dimicco MA, Grodzinsky AJ (2005) Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression. Arthritis Rheum 52(8):2386–2395
20. Stevens AL, Wishnok JS, Chai DH, Grodzinsky AJ, Tannenbaum SR (2008) A sodium dodecyl sulfate-polyacrylamide gel electrophoresis-liquid chromatography tandem mass spectrometry analysis of bovine cartilage tissue response to mechanical compression injury and the inflammatory cytokines tumor necrosis factor alpha and interleukin-1beta. Arthritis Rheum 58(2):489–500
21. Stevens AL, Wishnok JS, White FM, Grodzinsky AJ, Tannenbaum SR (2009) Mechanical injury and cytokines cause loss of cartilage integrity and upregulate proteins associated with catabolism, immunity, inflammation, and repair. Mol Cell Proteomics 8(7):1475–1489
22. Wang Y, Li Y, Khabut A, Chubinskaya S, Grodzinsky AJ, Onnerfjord P (2017) Quantitative proteomics analysis of cartilage response to mechanical injury and cytokine treatment. Matrix Biol 63:11–22
23. Rolauffs B, Muehleman C, Li J, Kurz B, Kuettner KE, Frank E, Grodzinsky AJ (2010) Vulnerability of the superficial zone of immature articular cartilage to compressive injury. Arthritis Rheum 62(10):3016–3027
24. Imgenberg J, Rolauffs B, Grodzinsky AJ, Schunke M, Kurz B (2013) Estrogen reduces mechanical injury-related cell death and proteoglycan degradation in mature articular cartilage independent of the presence of the superficial zone tissue. Osteoarthr Cartil 21(11):1738–1745
25. Jones AR, Chen S, Chai DH, Stevens AL, Gleghorn JP, Bonassar LJ, Grodzinsky AJ, Flannery CR (2009) Modulation of lubricin biosynthesis and tissue surface properties following cartilage mechanical injury. Arthritis Rheum 60(1):133–142
26. Kurz B, Lemke A, Kehn M, Domm C, Patwari P, Frank EH, Grodzinsky AJ, Schunke M (2004) Influence of tissue maturation and antioxidants on the apoptotic response of articular cartilage after injurious compression. Arthritis Rheum 50(1):123–130

27. Kurz B, Lemke AK, Fay J, Pufe T, Grodzinsky AJ, Schunke M (2005) Pathomechanisms of cartilage destruction by mechanical injury. Ann Anat 187(5–6):473–485
28. Patwari P, Cook MN, DiMicco MA, Blake SM, James IE, Kumar S, Cole AA, Lark MW, Grodzinsky AJ (2003) Proteoglycan degradation after injurious compression of bovine and human articular cartilage in vitro: interaction with exogenous cytokines. Arthritis Rheum 48(5):1292–1301
29. Lee CM, Kisiday JD, McIlwraith CW, Grodzinsky AJ, Frisbie DD (2013) Development of an in vitro model of injury-induced osteoarthritis in cartilage explants from adult horses through application of single-impact compressive overload. Am J Vet Res 74(1):40–47
30. Lee JH, Fitzgerald JB, DiMicco MA, Cheng DM, Flannery CR, Sandy JD, Plaas AH, Grodzinsky AJ (2009) Co-culture of mechanically injured cartilage with joint capsule tissue alters chondrocyte expression patterns and increases ADAMTS5 production. Arch Biochem Biophys 489(1–2):118–126
31. Swärd P, Wang Y, Hansson M, Lohmander LS, Grodzinsky AJ, Struglics A (2017) Coculture of bovine cartilage with synovium and fibrous joint capsule increases aggrecanase and matrix metalloproteinase activity. Arthritis Res Ther 19(1):157
32. Kurz B, Steinhagen J, Schünke M (1999) Articular chondrocytes and synoviocytes in a co-culture system: influence on reactive oxygen species-induced cytotoxicity and lipid peroxidation. Cell Tissue Res 296(3):555–563
33. Rolauffs B, Kurz B, Felka T, Rothdiener M, Uynuk-Ool T, Aurich M, Frank E, Bahrs C, Badke A, Stockle U, Aicher WK, Grodzinsky AJ (2013) Stress-vs-time signals allow the prediction of structurally catastrophic events during fracturing of immature cartilage and predetermine the biomechanical, biochemical, and structural impairment. J Struct Biol 183(3):501–511
34. Orozco GA, Tanska P, Florea C, Grodzinsky AJ, Korhonen RK (2018) A novel mechanobiological model can predict how physiologically relevant dynamic loading causes proteoglycan loss in mechanically injured articular cartilage. Sci Rep 8(1):15599
35. Eskelinen ASA, Tanska P, Florea C, Orozco GA, Julkunen P, Grodzinsky AJ, Korhonen RK (2020) Mechanobiological model for simulation of injured cartilage degradation via pro-inflammatory cytokines and mechanical stimulus. PLoS Comput Biol 16(6):e1007998
36. Grodzinsky AJ, Wang Y, Kakar S, Vrahas MS, Evans CH (2017) Intra-articular dexamethasone to inhibit the development of post-traumatic osteoarthritis. J Orthop Res 35(3):406–411
37. Li Y, Wang Y, Chubinskaya S, Schoeberl B, Florine E, Kopesky P, Grodzinsky AJ (2015) Effects of insulin-like growth factor-1 and dexamethasone on cytokine-challenged cartilage: relevance to post-traumatic osteoarthritis. Osteoarthr Cartil 23(2):266–274
38. Lu YC, Evans CH, Grodzinsky AJ (2011) Effects of short-term glucocorticoid treatment on changes in cartilage matrix degradation and chondrocyte gene expression induced by mechanical injury and inflammatory cytokines. Arthritis Res Ther 13(5):R142
39. Bajpayee AG, Quadir MA, Hammond PT, Grodzinsky AJ (2016) Charge based intra-cartilage delivery of single dose dexamethasone using Avidin nanocarriers suppresses cytokine-induced catabolism long term. Osteoarthr Cartil 24(1):71–81
40. Bajpayee AG, Scheu M, Grodzinsky AJ, Porter RM (2014) Electrostatic interactions enable rapid penetration, enhanced uptake and retention of intra-articular injected avidin in rat knee joints. J Orthop Res 32(8):1044–1051
41. Bajpayee AG, Scheu M, Grodzinsky AJ, Porter RM (2015) A rabbit model demonstrates the influence of cartilage thickness on intra-articular drug delivery and retention within cartilage. J Orthop Res 33(5):660–667
42. Bajpayee AG, De la Vega RE, Scheu M, Varady NH, Yannatos IA, Brown LA, Krishnan Y, Fitzsimons TJ, Bhattacharya P, Frank EH, Grodzinsky AJ, Porter RM (2017) Sustained intra-cartilage delivery of low dose dexamethasone using a cationic carrier for treatment of post traumatic osteoarthritis. Eur Cell Mater 34:341–364
43. Geiger BC, Grodzinsky AJ, Hammond P (2018) Designing drug delivery systems for articular joints. Chem Eng Prog (5):46–51
44. Black RM, Wang Y, Struglics A, Lorenzo P, Tillgren V, Ryden M, Grodzinsky AJ, Onnerfjord P (2020) Proteomic analysis reveals dexamethasone rescues matrix breakdown but not anabolic dysregulation in a cartilage injury model. Osteoarthr Cartil Open 2(4):100099
45. Bajpayee AG, Grodzinsky AJ (2017) Cartilage-targeting drug delivery: can electrostatic interactions help? Nat Rev Rheumatol 13(3):183–193
46. Black R, Grodzinsky AJ (2019) Dexamethasone: chondroprotective corticosteroid or catabolic killer? Eur Cell Mater 38:246–263
47. Behrendt P, Preusse-Prange A, Kluter T, Haake M, Rolauffs B, Grodzinsky AJ, Lippross S, Kurz B (2016) IL-10 reduces apoptosis and extracellular matrix degradation after injurious compression of mature articular cartilage. Osteoarthr Cartil 24(11):1981–1988
48. Behrendt P, Feldheim M, Preusse-Prange A, Weitkamp JT, Haake M, Eglin D, Rolauffs B, Fay J, Seekamp A, Grodzinsky AJ, Kurz B (2018) Chondrogenic potential of IL-10 in mechanically injured cartilage and cellularized collagen ACI grafts. Osteoarthr Cartil 26(2):264–275
49. Weitkamp JT, Rolauffs B, Feldheim M, Bayer A, Lippross S, Weuster M, Smeets R, Naujokat H, Grodzinsky AJ, Kurz B, Behrendt P (2021) Regenerative potential of platelet concentrate lysate in mechanically injured cartilage and matrix-associated chondrocyte implantation in vitro. Int J Mol Sci 22(24):13179

50. Shah NJ, Geiger BC, Quadir MA, Hyder MN, Krishnan Y, Grodzinsky AJ, Hammond PT (2016) Synthetic nanoscale electrostatic particles as growth factor carriers for cartilage repair. Bioeng Transl Med 1(3):347–356
51. Geiger BC, Wang S, Padera RF Jr, Grodzinsky AJ, Hammond PT (2018) Cartilage-penetrating nanocarriers improve delivery and efficacy of growth factor treatment of osteoarthritis. Sci Transl Med 10(469):eaat8800
52. Byun S, Sinskey YL, Lu YC, Ort T, Kavalkovich K, Sivakumar P, Hunziker EB, Frank EH, Grodzinsky AJ (2013) Transport of anti-IL-6 antigen binding fragments into cartilage and the effects of injury. Arch Biochem Biophys 532(1):15–22
53. Sui Y, Lee JH, DiMicco MA, Vanderploeg EJ, Blake SM, Hung HH, Plaas AH, James IE, Song XY, Lark MW, Grodzinsky AJ (2009) Mechanical injury potentiates proteoglycan catabolism induced by interleukin-6 with soluble interleukin-6 receptor and tumor necrosis factor alpha in immature bovine and adult human articular cartilage. Arthritis Rheum 60(10):2985–2996
54. Li Y, Frank EH, Wang Y, Chubinskaya S, Huang HH, Grodzinsky AJ (2013) Moderate dynamic compression inhibits pro-catabolic response of cartilage to mechanical injury, tumor necrosis factor-alpha and interleukin-6, but accentuates degradation above a strain threshold. Osteoarthr Cartil 21(12):1933–1941
55. Grodzinsky AJ, Lipshitz H, Glimcher MJ (1978) Electromechanical properties of articular cartilage during compression and stress relaxation. Nature 275(5679):448–450

Hip Osteoarthritis: Bench to Bedside Perspective

9

Young-Jo Kim

Abstract

Osteoarthritis is a major source of pain, disability, and economic cost worldwide. For nearly a century, there has been a debate about the causes of hip osteoarthritis and the role that structural abnormalities may play as a causative factor. Recent advances in open and minimally invasive techniques such as the periacetabular osteotomy, surgical hip dislocation and arthroscopic approaches have allowed us safe access into the joint to not only improve the abnormal bony structure and repair damaged tissue but also to gain clinical insights into the cause of joint damage. At present, structural abnormalities such as acetabular dysplasia and CAM deformities of the proximal femur are thought to be a major factor causing premature hip OA. Over the past 30 years, our understanding of the function and biology of articular cartilage has evolved from a relatively acellular lubricating cushion to a metabolically active tissue that can modulate its tissue composition in response to mechanical loading. Using advanced biochemical MR imaging technique called delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC), it has been shown that alteration in the mechanical environment of the hip with a pelvic osteotomy in acetabular dysplasia can alter the articular cartilage composition. This further demonstrates the importance of mechanics in development of joint damage and the potential for surgical correction to prevent or slow down the progression of OA.

Keywords

Osteoarthritis · Hip · Joint damage · Impingement

9.1 Introduction

Osteoarthritis is a major source of pain, disability, and economic cost worldwide. At present it is accepted that this disease is caused by multiple factors including genetic, biologic, and mechanical factors. Over the decades, the view of this condition has evolved from a wear and tear phenomena of the articular cartilage due to mechanical factors to that of a complex condition affecting the whole joint [1]. Similarly, the initial studies of articular cartilage focused on its material properties as it was thought to be relatively inactive biologically due to its acellular nature. However, it became increasingly clear that articular cartilage is not only biologically active but also mechanosensitive and is a mechanical/biological factor in the maintenance of the synovial joint and the

Y.-J. Kim (✉)
Harvard Medical School, Boston, MA, USA

Boston Children's Hospital, Boston, MA, USA
e-mail: Young-jo.kim@childrens.harvard.edu

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_9

disease process [2]. The surgical care of patients with hip osteoarthritis has undergone a similar evolution over time. Prior to arthroplasty, both femoral and pelvic osteotomies were performed to correct the underlying structural abnormalities. In addition, repositioning osteotomies were performed for osteoarthritic hips to relieve pain; however, due to the more predictable clinical outcomes with an arthroplasty, in most countries much of the osteotomy approaches were abandoned. The exception has been in the pediatric setting where arthroplasty is inappropriate and in countries such as Germany and Japan where they had a high incidence of acetabular dysplasia. The improved pelvic osteotomy [3] and hip surgical dislocation approach technique, and the understanding that subtle acetabular and femoral deformities are a causative factor in early joint damage, have renewed interests in hip preservation surgical techniques [4].

There are many parallels and interplay between the evolution in our understanding of osteoarthritis epidemiology, cartilage mechanics and physiology, and surgical care of our hip patients over the past 30 years. Clinical observations have informed our basic understanding and vice versa and I suspect will continue to do so in the future.

9.2 Osteoarthritis or Osteoarthrosis – What Is the Role of Biological vs Mechanical Factors in the Development and Progression of Hip OA?

Starting in the 1930s, clinical observations were made that hip deformity such as acetabular dysplasia [5] and then subsequently "tilt deformity" of the proximal femur [6] can lead to osteoarthritis. Murray used the term "tilt deformity" to describe an abnormal relationship between the femoral head and neck and in order to distinguish this asymptomatic development of hip deformity from a slipped capital femoral epiphysis, which often presents with a limp or an inability to walk. In fact, Murray hypothesized that perhaps abnormal stresses in adolescence may cause a minor degree of epiphysiolysis which can cause this deformity to develop which leads to damage to the joint. In 1975, Solomon [7] published a prospective study looking at the association between proximal femoral and acetabulum shape on radiographs and the pathological findings at time of joint replacement and made similar inferences as Murray. He even postulated that perhaps early intervention should be done to prevent further damage to the joint.

However, the hip joint is a dynamic organ that can remodel its shape in response to inflammation and injury. In the 1970s, Resnick made the observation that as part of the normal osteoarthritic process, the femoral head shape can remodel into a similarly abnormal shape as described by Murray and Solomon [8]. In fact, he specifically refuted that the observations made by Murray were simply due to the bony remodeling during osteoarthritis, i.e. a secondary effect, and not a cause of osteoarthritis [9].

Subsequent studies by Murphy et al. [10] and Harris [11] extended Wiberg and Murray's original hypotheses. The primary underlying mechanism by which joint damage occurs was thought to be mechanical in nature and the preferred term used to describe this condition was osteoarthrosis. Harris specifically made the argument that many of these femoral and acetabular deformities are seen prior to the onset of severe osteoarthritis. However, this was a period of rapid advancement in total hip arthroplasty. Much interest in understanding the etiology of hip osteoarthritis waned and we would have to wait for further advances in surgical technique to occur in the late 1990s that would allow additional clinical insights as well as improved ability to alter the underlying hip deformity.

For the hip, the importance of underlying mechanics as an initiator of joint damage was revitalized with the advent of advanced surgical techniques in the 1990s that allowed direct observations of damage within the joint even the early stages of joint damage. Traditionally, intraarticular joint damage was observed mostly in specimens at time of joint replacement, which by its very nature was in the advanced stage of disease. The safe surgical dislocation technique of the hip

was developed by Ganz and co-workers, which allowed complete exposure of the hip joint without the risk of avascular necrosis and with little or no morbidity [12]. This technique not only allowed direct observation of the joint damage pattern in pre-arthritic hips but also provided a surgical approach to repair damaged tissue as well as to address underlying structural abnormalities that may be the cause of the damage. Two distinct types of femoroacetabular impingement have been identified. The first type of impingement is caused by a direct contact between the acetabular rim and the femoral neck – pincer impingement. This is often caused by the acetabulum being retroverted or over covered. The second and more common type of impingement would occur due to a non-spherical extension of the femoral head into the acetabulum - the so-called cam impingement. The two types of impingements can be mixed but when they occur in isolation, they can cause distinctive patterns of intra-articular damage. The cam type impingement is most like the tilt deformity described by Murray but now there are multiple studies demonstrating that a subset of the cam deformity is developmental in nature with the formation occurring during the end of growth during adolescence [13, 14]. Furthermore, some of the cam deformity is caused by an extension of the femoral epiphysis (Fig. 9.1) and clearly not due to new appositional bone formation as described by Resnick [15].

Acetabular dysplasia (Fig. 9.2) has been a more accepted structural cause of hip OA. A more contemporary cohort study by Murphy [10] demonstrated the relationship between the extent of femoral head under coverage and the eventual development of osteoarthritis. With the use of advanced biochemical MRI techniques such as delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) [16], the relationship between the femoral head under coverage and the extent of chondral damage in the early stages of joint injury has been confirmed [17]. In addition, a recent systematic review and meta-analysis of prospective and cross-sectional studies have shown that cam deformity (alpha angle > 60 deg; OR = 2.52, 95% CI: 1.83–3.46) and acetabular dysplasia (LCEA < 25 deg; OR = 2.38, 95% CI: 1.84–3.07) are risk factors for the development of hip osteoarthritis [18].

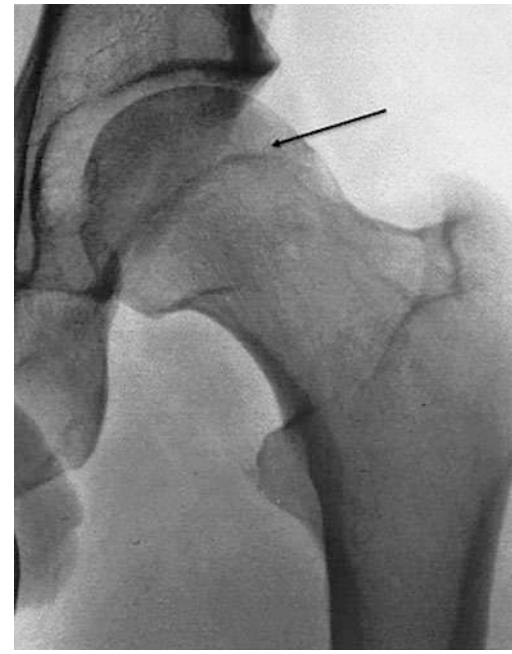


Fig. 9.1 Characteristic extension of the femoral epiphysis seen in hips with adolescent CAM impingement

It seems now evident, that hip mechanics plays an important role in the initiation of joint damage. However, we also know that this is not a simple wear and tear phenomenon. The cartilage is not simply being worn away. During the past 30 years when the role of mechanics in the development of osteoarthritis was become clearer, the role of mechanics and the modulation of articular cartilage metabolism and initiation of cartilage degradation was also being elucidated.

9.3 Articular Cartilage – Paradigm Shift from Inert Lubricating Cushion to Biologically Active and Mechanosensitive Tissue

Articular cartilage is a relatively acellular tissue that provides a near frictionless lubricating surface in synovial joints. It is also avascular and aneural; hence, initial concept of osteoarthritis

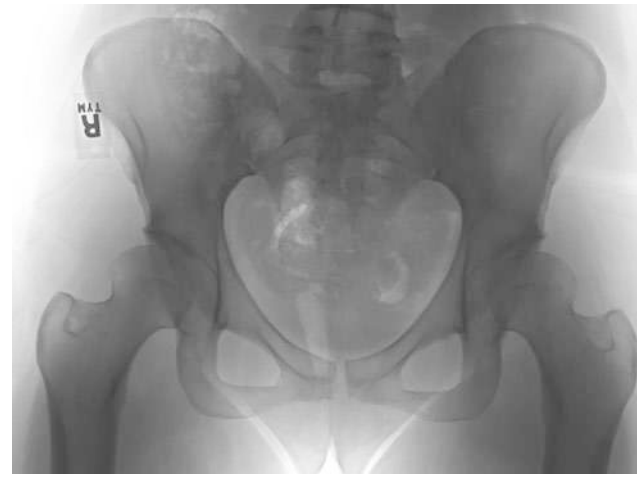


Fig. 9.2 Patient with bilateral acetabular dysplasia. The bony acetabulum does not cover the femoral head sufficiently

was that of a mechanically induced wear and tear process. However, the opposite is true where the articular cartilage tissue is often very active and reactive in the disease process [19, 20].

During the 1980s and 1990s, the interplay between the mechanical forces on cartilage and its metabolism and well as disease states was being elucidated. Sah et al.[21] demonstrated using calf cartilage explant system that static compression will inhibit glycosaminoglycan and protein synthesis (Fig. 9.3) while gentle dynamic compression will stimulate biosynthetic activity in a frequency dependent manner. Higher frequency (>0.001 Hz) small strain (1–5%) compression produced a stimulatory effect while lower frequency did not (Fig. 9.4). This study provided a framework for identifying the physical and biological mechanisms by which dynamic compression can modulate chondrocyte biosynthetic activity. Further studies using the radially unconfined compression explant system demonstrated that the increase in biosynthetic activity of glycosaminoglycans during dynamic small strain compression was confined to the radial periphery where there would be increased fluid flow. This only occurred in the high frequency range where the increased fluid flow was predicted to occur (Fig. 9.5) [22].

Compared to gentle dynamic compression, direct mechanical injury to the articular cartilage can not only disrupt the tissue structure but it can also induce matrix degradation mediated by chondrocytes via expression of matrix degrading enzymes (ADAM-TS5, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13). In addition, the biosynthetic activity will decrease and will also induce chondrocyte death by necrosis and apoptosis [2].

These and other studies have confirmed that articular cartilage is an active tissue that can modulate its composition in response to mechanical loads and when mechanically injured the tissue can degrade itself and will contribute to the development of osteoarthritis.

9.4 Bench to Bedside – Use of dGEMRIC in Understanding the Effect of Pelvic Osteotomy on Hip Articular Cartilage

Acetabular dysplasia is a natural model of mechanically induced cartilage damage that leads to osteoarthritis. The articular cartilage and labral damage start at the acetabular edge where the increased mechanical loads are predicted to occur. We have surgical interventions to correct the acetabular dysplasia, which would lead to normalization of the mechanical environment in the joint [23]. Furthermore, acetabular dysplasia is an important cause of premature osteoarthritis in young women.

A clinically important question in patients with acetabular dysplasia is what is happening to

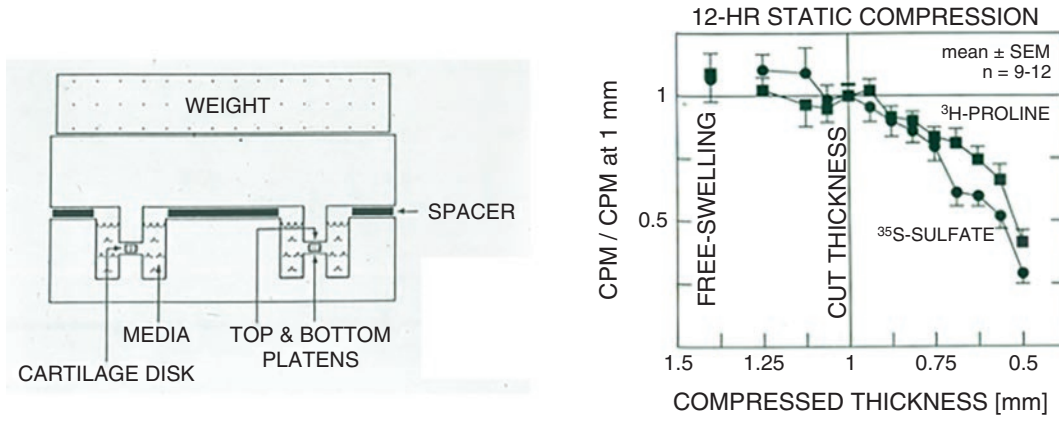


Fig. 9.3 Cartilage explants were statically compressed in a radially unconfined manner in a culture chamber. The glycosaminoglycan (sulfate) and protein (proline) synthesis decreased with increasing static compression. (Reprinted with permission from Sah et al. [21])

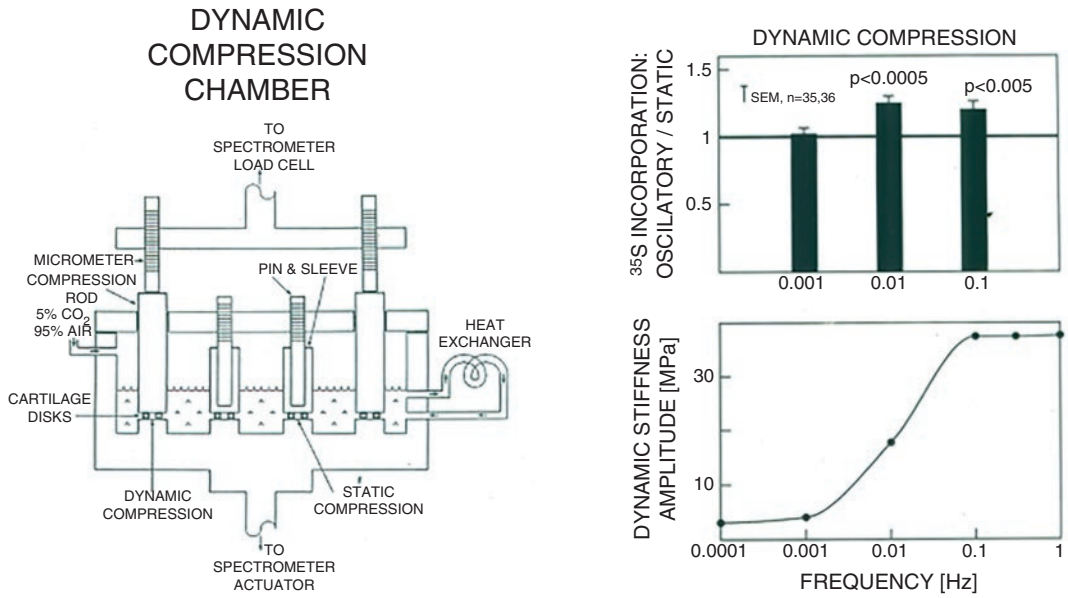


Fig. 9.4 Cartilage explants were dynamically compressed with small displacement sinusoidal manner with fixed frequencies. The glycosaminoglycan synthesis increased in a frequency dependent manner. No stimulation at very low frequencies of stimulation. (Reprinted with permission from Sah et al. [21]; Kim et al. [22])

the joint? Are we preventing or slowing down the inevitable onset of osteoarthritis? Traditionally, these patients would be followed for long term (~20–30 years) to be able to detect radiographically the onset of osteoarthritis. However, we have sought to see if we can gain insights sooner using a biochemical MR imaging technique call delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) [16], which estimates the charge density of the articular cartilage and hence the tissue composition and potential health of the joint.

This imaging technique was validated for use as a clinical imaging tool in the mature hip [17, 24, 25]. We performed a prospective cohort study of subjects [26] with acetabular dysplasia that

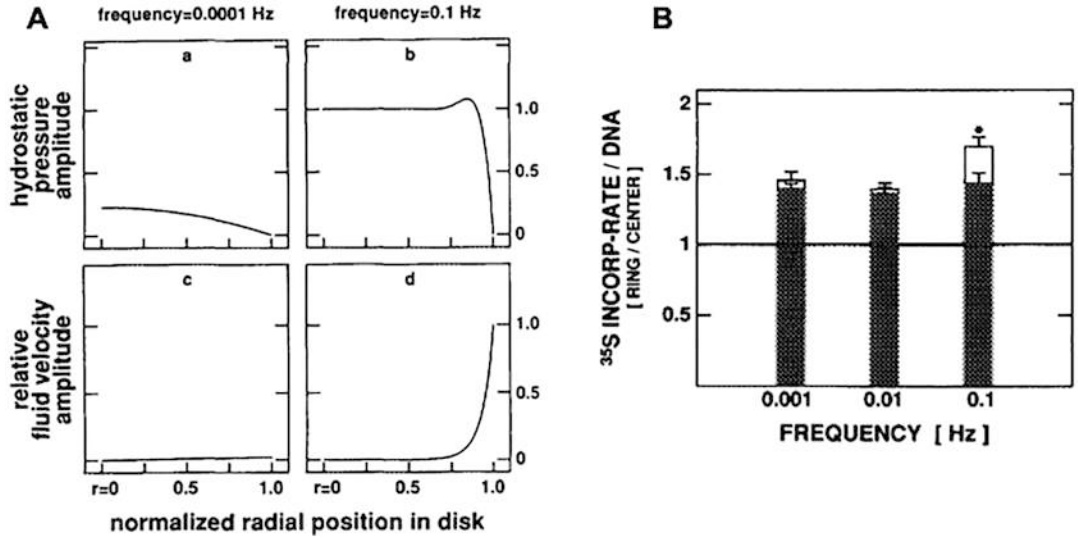


Fig. 9.5 The cartilage explants were separated from the radial (ring) vs center part of the disc and the biosynthetic rate assessed. The biosynthetic rate of glycosaminoglycans (**b**) only increased in the ring at high frequency stimulation (0.1 Hz) where the fluid flow is increased according to the poroelastic modeling data (**a**). (Reprinted with permission from Kim et al. [22])

were about to undergo surgical correction using the Bernese periacetabular osteotomy. Thirty-seven patients with no or minimal radiographic evidence of osteoarthritis were treated with a periacetabular osteotomy for symptomatic acetabular dysplasia. All patients had a pre-operative, one-year and two-year dGEMRIC scans. Standard radiographic and clinical follow-ups were performed at regular intervals.

As expected, we had clinical improvement in symptoms in this cohort and there were no major complications. Radiographically, we had good correction of the acetabular dysplasia and there were no significant radiographic progression of osteoarthritis. What we did find is a decrease in the dGEMRIC index from a preoperative measure of 561.6 msec to 515.2 msec at the 1 year postoperative scans and subsequently recovered to 529.2 msec at the two-year post-operative scan. dGEMRIC index is correlated with charge density; therefore, a decrease in the dGEMRIC index may suggest a worsening of the articular tissue quality.

However, upon closer inspection, it was noted that the most pronounced change in articular cartilage dGEMRIC index occurred at the superior aspect of the acetabulum which is where the increased mechanical load seen prior to surgery would decrease after correction of the acetabular dysplasia (Fig. 9.6). The MRI imaging data was acquired in the same scanner and using the same imaging protocol. However, due to the reorientation of the acetabulum, the 3D imaging dataset was reformatted in a rotating radial frame and realigned to the original orientation for comparison before and after periacetabular osteotomy (Fig. 9.7) [28]. The articular cartilage was manually segmented between the acetabular and femoral cartilages (Fig. 9.8) and when we looked at the change in dGEMRIC values in the acetabular cartilage at various locations around the joint, what we found is a decrease in the dGEMRIC values in the superior-anterior and superior part of the joint that sees increased mechanical load prior to the operation, and a decrease down to a range that is within normal range after osteotomy (Fig. 9.9). This suggests that in these pre-arthritic hips, the articular cartilage in the overloaded acetabulum is adapted to the increased mechanical load pre-operatively but is able to "normalize" its matrix composition after surgical correction. This suggests that a periacetabular osteotomy for

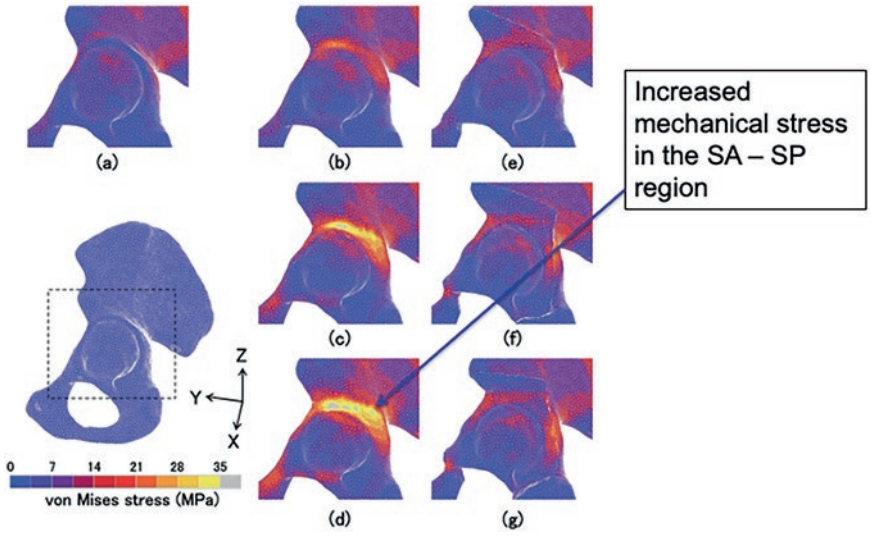


Fig. 9.6 The von Mises stress in hips with normal coverage (**a**) and varying severity of acetabular dysplasia were estimated using finite element modeling technique (**b**–**d**). After simulated pelvic osteotomy to normalize coverage, the mechanical stress in the joint decreased (**e**–**g**). (Reprinted with permission from Zhao et al. [27])

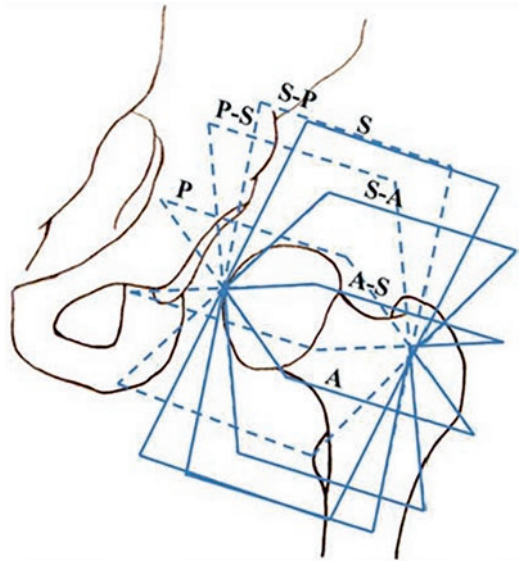


Fig. 9.7 The isotopically acquired 3D MRI data was reformatted in a rotating frame around the femoral neck axis. The dGEMRIC data was realigned back to the original acetabular position so same regions of interests could be compared before and after periacetabular osteotomy. (Reprinted with permission from Bittersohl et al. [28])

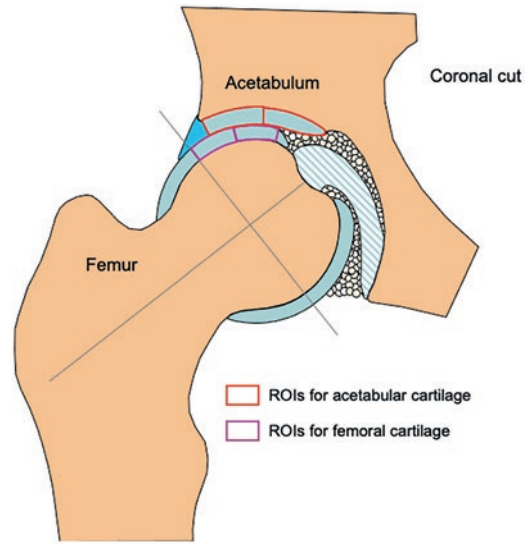


Fig. 9.8 The articular cartilages for the femur and acetabulum were manually segmented and average dGEMRIC index calculated

acetabular dysplasia will modulate the mechanical environment in the joint, which in turn affects the biosynthetic activity and the tissue composition of the articular cartilage.

9.5 Summary

Over the past decades, the role of mechanics in the initiation and progression of osteoarthritis, nature of articular cartilage, and surgical treatment paradigm for patients with joint pain and early joint damage have evolved greatly. Clinical

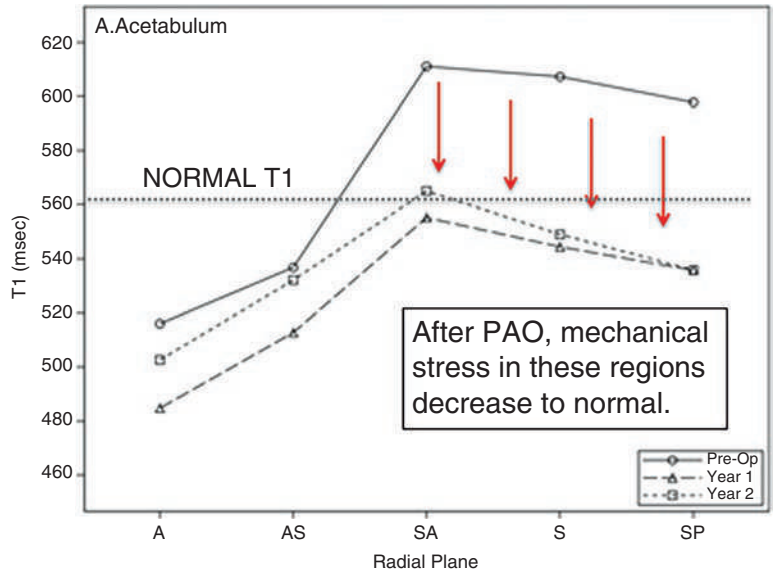


Fig. 9.9 The acetabular dGEMRIC value as a function of position within the joint. In the superior-anterior to superior-posterior regions the dGEMRIC values were high prior to periacetabular osteotomy. The values decrease down but not below the normal levels after osteotomy. (Modified graph based on data presented in Hingsammer et al. [26])

insights and basic science knowledge have informed each other and will continue to advance over time. It is our goal that in the near future, we will have the ability to prevent the development and progression of osteoarthritis with appropriate surgical interventions in the case of OA caused by structural abnormalities, the ability to replace or repair damaged tissue to prolong the function of the native joint in the case of joints with limited pre-existing damage, and finally continued advancements in joint replacement technology will allow us to restore hip joint function in the older population with predictable and long term outcomes.

References

1. Glyn-Jones S, Palmer AJR, Agricola R et al (2015) Osteoarthritis. Lancet 386(9991):376–387. https://doi.org/10.1016/S0140-6736(14)60802-3
2. Kurz B, Lemke AK, Fay J, Pufe T, Grodzinsky AJ, Schünke M (2005) Pathomechanisms of cartilage destruction by mechanical injury. Ann Anat Anat Anz Off Organ Anat Ges 187(5-6):473–485. https://doi.org/10.1016/j.aanat.2005.07.003
3. Ganz R, Klaue K, Vinh TS, Mast JW (1988) A new periacetabular osteotomy for the treatment of hip dysplasias. Technique and preliminary results. Clin Orthop 232:26–36
4. Leunig M, Ganz R (2014) The evolution and concepts of joint-preserving surgery of the hip. Bone Jt J 96-B(1):5–18. https://doi.org/10.1302/0301-620X.96B1.32823
5. Wiberg G (1939) Studies on Dysplasic Acetabula and Congenital Subluxation of the Hip Joint. Acata Chirgrgica Scand 83:5–131
6. Murray RO (1965) The aetiology of primary osteoarthritis of the hip. Br J Radiol 38(455):810–824. https://doi.org/10.1259/0007-1285-38-455-810
7. Solomon L (1975) Proceedings: osteoarthritis of the hip: pathological changes and their implications for treatment. J Bone Joint Surg (Br) 57(2):258
8. Resnick D (1975) Patterns of migration of the femoral head in osteoarthritis of the hip. Roentgenographic-pathologic correlation and comparison with rheumatoid arthritis. Am J Roentgenol Radium Ther Nucl Med 124(1):62–74. https://doi.org/10.2214/ajr.124.1.62
9. Resnick D (1976) The "tilt deformity" of the femoral head in osteoarthritis of the hip: a poor indicator of previous epiphysiolysis. Clin Radiol 27(3):355–363. https://doi.org/10.1016/s0009-9260(76)80089-x
10. Murphy SB, Ganz R, Müller ME (1995) The prognosis in untreated dysplasia of the hip. A study of radiographic factors that predict the outcome. J Bone Joint Surg Am 77(7):985–989. https://doi.org/10.2106/00004623-199507000-00002
11. Harris WH (1986) Etiology of osteoarthritis of the hip. Clin Orthop 213:20–33
12. Lavigne M, Parvizi J, Beck M, Siebenrock KA, Ganz R, Leunig M (2004) Anterior femoroacetabular impingement: part I. Techniques of joint preserving surgery. Clin Orthop (418):61–66

13. Bixby SD, Kienle KP, Nasreddine A, Zurakowski D, Kim YJ, Yen YM (2013) Reference values for Proximal Femoral anatomy in adolescents based on sex, physis, and imaging plane. Am J Sports Med 41(9):2074–2082. https://doi.org/10.1177/0363546513495346
14. Kienle KP, Keck J, Werlen S, Kim YJ, Siebenrock KA, Mamisch TC (2012) Femoral morphology and epiphyseal growth plate changes of the hip during maturation: MR assessments in a 1-year follow-up on a cross-sectional asymptomatic cohort in the age range of 9–17 years. Skelet Radiol 41(11):1381–1390. https://doi.org/10.1007/s00256-012-1358-9
15. Siebenrock KA, Wahab KHA, Werlen S, Kalhor M, Leunig M, Ganz R (2004) Abnormal extension of the femoral head epiphysis as a cause of cam impingement. Clin Orthop 418:54–60. https://doi.org/10.1097/00003086-200401000-00010
16. Burstein D, Velyvis J, Scott KT et al (2001) Protocol issues for delayed Gd(DTPA)(2-)-enhanced MRI (dGEMRIC) for clinical evaluation of articular cartilage. Magn Reson Med 6:36–41
17. Kim YJ, Jaramillo D, Millis MB, Gray ML, Burstein D (2003) Assessment of early osteoarthritis in hip dysplasia with delayed gadolinium-enhanced magnetic resonance imaging of cartilage. J Bone Jt Surg Am Vol 85(10):1987–1992. https://doi.org/10.2106/00004623-200310000-00019
18. Casartelli NC, Maffiuletti NA, Valenzuela PL et al (2021) Is hip morphology a risk factor for developing hip osteoarthritis? A systematic review with meta-analysis. Osteoarthr Cartil 29(9):1252–1264. https://doi.org/10.1016/j.joca.2021.06.007
19. Mankin HJ, Lippiello L (1970) Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. J Bone Joint Surg Am 52(3):424–434
20. Maroudas A (1975) Glycosaminoglycan turn-over in articular cartilage. Philos Trans R Soc Lond Ser B Biol Sci 271(912):293–313. https://doi.org/10.1098/rstb.1975.0054
21. Sah RLY, Kim YJ, Doong JYH, Grodzinsky AJ, Plass AHK, Sandy JD (1989) Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 7(5):619–636. https://doi.org/10.1002/jor.1100070502
22. Kim YJ, Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD (1994) Mechanical regulation of cartilage biosynthetic behavior: physical stimuli. Arch Biochem Biophys 311(1):1–12. https://doi.org/10.1006/abbi.1994.1201
23. Michaeli DA, Murphy SB, Hipp JA (1997) Comparison of predicted and measured contact pressures in normal and dysplastic hips. Med Eng Phys 19(2):180–186. https://doi.org/10.1016/s1350-4533(96)00051-3
24. Tiderius CJ, Jessel R, Kim YJ, Burstein D (2007) Hip dGEMRIC in asymptomatic volunteers and patients with early osteoarthritis: the influence of timing after contrast injection. Magn Reson Med 57(4):803–805. https://doi.org/10.1002/mrm.21190
25. Mamisch TC, Dudda M, Hughes T, Burstein D, Kim YJ (2008) Comparison of delayed gadolinium enhanced MRI of cartilage (dGEMRIC) using inversion recovery and fast T1 mapping sequences. Magn Reson Med 60(4):768–773. https://doi.org/10.1002/mrm.21726
26. Hingsammer AM, Kalish LA, Stelzeneder D et al (2015) Does Periacetabular Osteotomy for Hip Dysplasia modulate cartilage biochemistry? J Bone Jt Surg Am Vol 97(7):544–550. https://doi.org/10.2106/JBJS.M.01233
27. Zhao X, Chosa E, Totoribe K, Deng G (2010) Effect of periacetabular osteotomy for acetabular dysplasia clarified by three-dimensional finite element analysis. J Orthop Sci Off J Jpn Orthop Assoc 15(5):632–640. https://doi.org/10.1007/s00776-010-1511-z
28. Bittersohl B, Hosalkar HS, Werlen S, Trattnig S, Siebenrock KA, Mamisch TC (2011) dGEMRIC and subsequent T1 mapping of the hip at 1.5 Tesla: normative data on zonal and radial distribution in asymptomatic volunteers. J Magn Reson Imaging JMRI 34(1):101–106. https://doi.org/10.1002/jmri.22528

10 Harnessing Growth Factor Interactions to Optimize Articular Cartilage Repair

Stephen B. Trippel

Abstract

The failure of cartilage healing is a major impediment to recovery from joint disease or trauma. Growth factors play a central role in cell function and have been proposed as potential therapeutic agents to promote cartilage repair. Decades of investigation have identified many growth factors that promote the formation of cartilage *in vitro* and *in vivo*. However, very few of these have progressed to human trials. A growth factor that robustly augments articular cartilage healing remains elusive. This is not surprising. Articular cartilage repair involves multiple cellular processes and it is unlikely that any single agent will be able to optimally regulate all of them. It is more likely that multiple regulatory molecules may be required to optimize the maintenance and restoration of articular cartilage. If this is the case, then interactions among growth factors may be expected to play a key role in determining their therapeutic value. This review explores the hypothesis that growth factor interactions could help optimize articular cartilage healing.

Keywords

Articular cartilage · Growth factors · Interaction

10.1 Introduction

Articular cartilage provides the gliding surface that enables pain-free joint motion. Articular cartilage damage due to disease or trauma is among the most disabling conditions affecting American adults [1]. This tissue has a particularly poor intrinsic repair capacity compared to most other tissues and damage tends to be progressive over time. There is currently no disease modifying agent that prevents, arrests or reverses cartilage damage.

Growth factors are essential regulators of cell behavior. Numerous growth factors have been shown to augment the repair capacity of articular chondrocytes in *in vitro* and *in vivo* models [2, 3]. Among these are insulin-like growth factor 1 (IGF-1), [4, 5] Fibroblast growth factor 2

Dedication: This review is dedicated to Prof. Alan J. Grodzinsky on the occasion of his 75th birthday.

S. B. Trippel (✉)
Cell Biology & Physiology, Indiana University School of Medicine, Indianapolis, IN, USA

Department of Biomedical Engineering, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA
e-mail: strippel@iupui.edu

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_10

(FGF-2), [6–11] Fibroblast growth factor 18 (FGF-18), [12–15] bone morphogenetic proteins 2 and 7 (BMP-2, BMP-7), [16–20] and transforming growth factor beta (TGF-β) [21, 22]. These growth factors regulate critical chondrocyte reparative functions by distinct and overlapping signal transduction pathways.

The sheer number of chondrogenic growth factors poses the challenge of determining which among them is the best one to pursue in translational studies toward clinical application. To date, few growth factors have progressed through clinical trials to test their potential as therapeutic agents. One of these is TGF-β1. A phase III study delivered non-transformed and retrovirally transduced juvenile human chondrocytes carrying the TGF-β1 gene to subjects with moderate (Kellgren-Lawrence grade III) osteoarthritis of the knee. The effect on articular cartilage was a trend toward greater cartilage thickness at one year [23]. A second example is FGF-18. A phase II study delivered a series of recombinant FGF-18 injections to knees of subjects with mild or moderate (Kellgren-Lawrence grade II-III) osteoarthritis. The results showed a statistically significant increase of 0.05 mm in mean tibiofemoral cartilage thickness compared to placebo at two years that persisted to 5 years [24]. These studies reflect substantial progress in the search for disease-modifying growth factors. Although encouraging, these results also suggest that further improvement could be achievable.

The regulation of the multiple, distinct chondrocyte functions that are involved in chondrogenesis is sufficiently complex that a single regulatory factor is unlikely to optimally promote articular cartilage healing. Indeed, an extensive literature has shown that two or more growth factors can improve chondrocyte biosynthesis compared to just one growth factor [7, 25–29]. These data suggest that, instead of attempting to identify the best growth factor for cartilage healing, perhaps it would be better to attempt to identify the best growth factor combination for cartilage healing. Central to such an approach is understanding how growth factors interact with each other in regulating articular chondrocyte reparative functions.

10.2 Multiple Growth Factors

To test the hypothesis that growth factors interact in chondrocyte regulation, a study delivered the genes encoding IGF-1, FGF-2, BMP-2, BMP-7 and TGF-β by transfection using an adeno-associated virus-based vector individually, or in combination, to primary adult bovine articular chondrocytes in culture, and measured their effect on chondrocyte aggrecan, type II collagen and type I collagen gene expression. The results showed that the growth factor transgenes differentially regulated the magnitude and time course of expression of all three chondrocyte matrix protein genes. The data further demonstrated interactions among the growth factors that ranged from inhibitory to synergistic. Maximum stimulation of type II collagen gene expression (35 fold) and also of aggrecan gene expression (16-fold) was by the combination of IGF-1, BMP-2 and BMP-7 transgenes. Interestingly, the FGF-2 transgene, individually and in combination with other growth factor transgenes, tended to stimulate aggrecan gene expression, but nearly abolished the expression of both type I and type II collagen gene expression [30].

A subsequent study sought to determine whether these growth factors interact to modulate articular chondrocyte proliferation and the production of cartilage matrix. As in the prior study, the genes encoding IGF-1, FGF-2, TGF-β1, BMP-2 and BMP-7, individually and in various combinations, were delivered to primary adult bovine articular chondrocytes in culture. Dependent variables included changes in DNA content, an index of chondrocyte proliferation, and changes in glycosaminoglycan (GAG) and collagen content, indices of cartilage matrix synthesis. Glycosaminoglycan that was released into the culture medium or retained in the cell layer were measured separately [31]. This distinction is important because retained matrix molecules contribute to the formation of new cartilage, while released molecules do not provide structural benefit.

The results showed that, in concert, the growth factors interacted to generate widely divergent effects on both chondrocyte proliferation and

matrix synthesis. As was seen for the regulation of gene expression, these interactions ranged from inhibitory to synergistic. The IGF-1 transgene synergistically stimulated proliferation when combined with any of the other growth factor transgenes, and synergistic stimulation by the combination of IGF-1 and FGF-2 transgenes maximized cell proliferation (8.5 fold). Synergistic stimulation by the combination of the IGF-1, BMP-2 and BMP-7 transgenes maximized matrix production (14.9 fold), and also maximized the proportion of GAG retained in the cell layer. Similar results were obtained for collagen, the other major component of articular cartilage matrix. In contrast to the other growth factor transgenes, the FGF-2 transgene, when combined with any of the other transgenes, increased the proportion of collagen that was lost into the medium such that the majority of the newly synthesized collagen did not contribute to matrix formation. Further, when added to the combination of the IGF-1 transgene and either of the BMP transgenes, the FGF-2 transgene abolished their synergistic stimulation of both cell-associated GAG and collagen [31].

These and other studies reveal imitations to the use of growth factor combinations for articular cartilage repair. First, some growth factor combinations inhibited chondrocyte biosynthesis [32, 33]. Second, the optimal combination for proliferation was poor at augmenting matrix production (IGF-I plus FGF-2), and the optimal combination for matrix production was only a mediocre mitogen (IGF-I plus BMP-2 plus BMP-7). Thus, no combination of growth factors was found that optimized both of these key chondrocyte reparative functions.

10.3 Multiple Combinations of Growth Factors

Taken together, the foregoing results suggest that instead of attempting to identify the best growth factor combination for cartilage healing, perhaps it would be better to attempt to identify the best combination of growth factor combinations for cartilage healing. Using data from the above studies, one example would be to select the combination of IGF-1 and FGF-2 to increase the number of chondrocytes and use the combination of IGF-1, BMP-2 and BMP-7 to increase matrix production.

This approach fits the conceptual framework of chondrogenesis as a four-dimensional process. During cartilage development and repair, cell functions change over time. These changes are effected, in part, by changes in the signaling factors that regulate those functions. As a result, interventions that deliver different agents in sequence may be superior to those that deliver different agents simultaneously, or to the same agent delivered repeatedly. However, treatments that deliver combinations of growth factors simultaneously take advantage of their synergistic interactions, a benefit that would likely be lost when multiple growth factors are delivered sequentially. These are not mutually exclusive options. Multiple combinations of sequentially delivered growth factor combinations offer the dual advantages of both synergistically activating distinct sets of signal transduction pathways to optimize cellular responses, and of doing so at times appropriate to distinct phases of cartilage repair.

In its simplest form, this approach would involve a combination of just two distinct combinations of regulatory factors. If the first combination of growth factors optimized cell proliferation and the second combination optimized matrix production, the combination of two combinations of factors would first increase the number of cells and then stimulate that enlarged population of cells to generate matrix. In such cases, the order of delivery of the combinations would be important (Fig. 10.1). To address more than two cell-regulatory phases during repair or regeneration, the model would require additional combinations and sequences of delivery over the course of treatment.

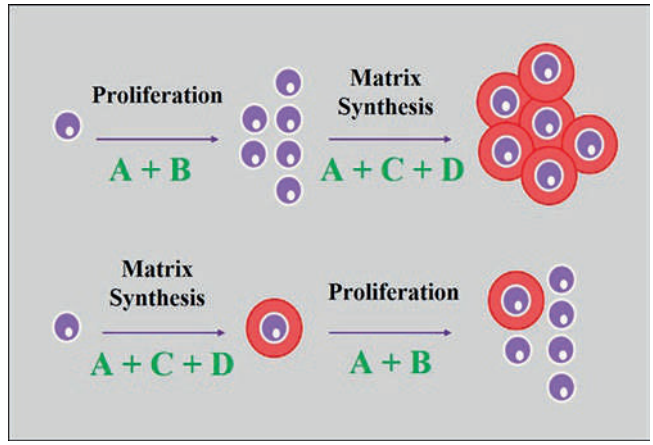


Fig. 10.1 Delivery sequences of two combinations of growth factor combinations. Delivery of a two-growth factor combination that increases the number of chondrocytes, which are then stimulated to produce matrix by a three-growth factor combination (upper sequence) would likely generate more robust neocartilage than the delivery of the same two growth factor combinations in the reverse (lower) sequence. The reverse sequence would be expected to produce a comparatively matrix-deficient tissue

10.4 Multifunctional Growth Factors

The application of growth factors to cartilage repair will also require a more complete understanding of their actions on chondrocytes. Many growth factors are pleiotropic and regulate multiple chondrocyte functions. As illustrated by FGF-2, some of these functions may mitigate against chondrogenesis. Although FGF-2 is an asset as a potent mitogen for articular chondrocytes, and has been shown to promote chondrogenesis, it is also a potential liability as a potent stimulus of cartilage matrix catabolism, [34–37] an effect that is mediated, at least in part, by MMP-13 [38].

A recent study tested the catabolic effect of IGF-1, FGF-2, BMP-2, BMP-7 and TGF-β by transferring individual or combinations of the genes encoding these factors to primary bovine articular chondrocytes, and measuring the expression of A disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS)-4, ADAMTS-5, matrix metalloproteinase-3 (MMP)-3, MMP-13, and interleukin 6 (IL-6). Unexpectedly, the growth factor transgenes generally increased the expression of these catabolic genes. Further, interactions among these growth factors transgenes produced a wide range of synergistic and inhibitory effects on these genes. The regulation of IL-6 and MMP-13 are illustrative. Individually, IGF-1 and FGF-2 increased IL-6 gene expression to 3.0-fold and 10.8-fold respectively. In combination, they synergistically increased IL-6 expression to 40-fold. In the case of MMP-13, IGF-1 initially reduced MMP-13 expression and then increased it to 2.3-fold, while FGF-2 progressively increased MMP-13 expression to 71-fold. In concert, the addition of IGF-1 to FGF-2 brought the stimulation by FGF-2 down to 5.4-fold. Thus, the interaction between IGF-1 and FGF-2 was opposite for the two catabolic genes: synergistic for IL-6 and inhibitory for MMP-13. Conversely, the different growth factor transgenes all tended to produce similar effects on ADAMTS-4 and ADAMTS-5 gene expression, but in opposite directions. They upregulated ADAMTS-4 and down-regulated ADAMTS-5 [39].

Taken together, available evidence indicates that growth factor interactions are remarkably diverse with respect the direction, magnitude, time course and specific genes that they regulate, including degradative functions. This diversity

extends beyond the previously noted inhibitory-to-synergistic range of interactive effects on reparative functions. The competing actions of growth factors on reparative and degradative chondrocyte behaviors add an additional level of complexity to the development of these regulatory molecules as therapeutic agents for restoring articular cartilage homeostasis or promoting repair. The diversity of these actions could also offer a potential benefit. It provides the opportunity to select specific growth factor combinations, and specific phases of cartilage repair that can be tailored to produce specific outcomes.

10.5 Opportunities for Progress

A major obstacle to identifying growth factor combinations for translational studies is the current lack of ability to predict the actions of a combination of growth factors based on their individual actions. This problem reflects a deficient understanding of the mechanisms underlying growth factor interactions. While the mechanisms of action of individual growth factors have been fairly well established, the mechanisms of interaction among the networks formed by these pathways are only beginning to be elucidated. One approach to this problem is to identify the sites of cross-talk in the growth factor signal transduction networks that mediate their interactions. An omics approach to understanding of the specific determinants of growth factor interaction will be enhanced by the application of advanced machine learning, high-throughput combinatorial experimental methods, and bioinformatic analytics. For example, characterization of the articular chondrocyte interactome could facilitate the development of therapeutic agents designed to elicit specific chondrocyte behaviors.

Another approach to identifying these mechanisms is to better understand the interactions between growth factors and the subcellular anatomic structures that contribute to their function. A recent study employed a novel approach to elucidating structure-function relationships among intracellular proteins and their environment [40]. The authors combined imaging and biophysical data on the intracellular location of several hundred proteins. They employed neural networks to relate the proteins to each other and to subcellular structures. The study identified multiple previously unknown subcellular functional systems, including cross talk between them [40]. Such information obtained for growth factor networks might lend insight into the mechanisms underlying the interactions in their regulation of chondrocytes.

10.6 Biochemical and Biophysical Factor Combinations

Growth factor actions are not determined just by interactions with each other. They are also determined by interactions with a variety of other cell-regulatory stimuli. Prominent among these are mechanical forces. Bonassar et al. tested the hypothesis that the mechanical regulator, static compression, and the biochemical regulator, IGF-1, modulate each other's effects on articular chondrocyte biosynthesis. Bovine articular cartilage explants were treated with IGF-1 (0–300 ng/ml), static compression (0–50%), or the combination of both, and the incorporation of [35S]sulfate and [3H]proline into the cartilage matrix was measured. As expected, [41] IGF-1 increased, and static compression decreased, both [35S]sulfate and [3H]proline incorporation in a dose-dependent fashion. When delivered together, static compression progressively inhibited the stimulatory effect of IGF-1 and 50% compression nearly eliminated the effect of IGF-1. The time course of action of the two stimuli differed; IGF-1 stimulation plateaued at 24 hours while static compression reached a steady state by 4 hours. Static compression also reduced the concentration of IGF-I in the tissue at equilibrium [42].

Expanding on the study of static compression, Bonassar et al. tested the hypothesis that dynamic compression and IGF-1 modulate each other's actions on articular chondrocyte biosynthesis. Bovine articular cartilage explants were treated with IGF-1 (0–300 ng/ml), dynamic compression (2% strain, 0.1 Hz), or both, and [35S]sulfate and

[3H]proline incorporation were measured. IGF-1 and dynamic compression each increased both [35S]sulfate and [3H]proline incorporation. When given together, the stimulation was greater than the maximum stimulation by either IGF-1 or dynamic compression alone. Further, the time constant of stimulation for IGF-1 and dynamic compression was 12.2 hours and 2.9 hours respectively, and 5.6 hours for the combination. Dynamic compression also increased the rate of diffusion of IGF-1 into the cartilage matrix [43].

To extend these studies from static and dynamic compression to shear deformation, Jin et al. employed a similar model as above but applied shear strains (0–6.0%) rather than compression. IGF-1 and dynamic shear each increased both [35S]sulfate and [3H]proline incorporation. When given together, the stimulation was greater than the maximum stimulation by either IGF-1 or dynamic shear alone. Unlike static compression, shear did not change the concentration of IGF-1 in the cartilage tissue and unlike dynamic compression, it did not change the transport of IGF-1 into the tissue [44].

Taken together, the results of all three of the above studies indicate that IGF-1 and mechanical stimuli regulate the same articular chondrocyte reparative functions and that these two classes of stimuli act through distinct signal transduction pathways.

A recent illustration of growth factor interaction with mechanosensors is the observation by Trompeter et al. that IGF-1 regulates the mechanosensitivity of chondrocyte-like ATDC5 cells by modulating TRPV4 (transient receptor potential vanilloid 4) ion channel. TRPV4 is central to chondrocyte mechanotransduction and may play a role in osteoarthritis. This study demonstrated that IGF-1 suppressed hypotonic-induced TRPV4 currents and intracellular calcium flux by increasing apparent cell stiffness associated with actin stress fiber formation. IGF-1 also abrogated the release of ATP that is mediated by TRPV4 in response to mechanical stimulation [45]. This study demonstrates a direct connection between a growth factor and a mechanotransduction pathway in chondrocyte-like cells. A second illustration of interaction between growth factor and mechanotransduction pathways is provided by the chondrocyte primary cilium, an organelle that serves, in part, as an interface between extracellular forces and intracellular growth factor signaling [46].

10.7 Growth Factor – Matrix Interaction: Role in Growth Factor Delivery

The clinical application of growth factors requires an effective delivery system. Methods will be needed to deliver the desired growth factor combinations in the desired sequence over the desired time periods to the desired locations. They must be retained at the desired site of action long enough to produce their effect and focal enough to avoid off-target effects. This will involve another class of growth factor interactions: those between the growth factor(s), the delivery vehicle and the site of cartilage damage to be treated. Free growth factors generally have a relatively short residence time ($t_{½}$ = hours to ±1 day) when delivered by intra-articular injection [47, 48] and, for better or for worse, have at least as good access to synovial cells as to articular chondrocytes. Articular chondrocytes reside in a dense, highly anionic matrix. For growth factors such as FGF-18 that have a high isoelectric point (pI~10), this can facilitate binding to the negatively charged proteoglycan sulfate groups in cartilage matrix, a property that may account for the articular cartilage localization of intra-articular FGF-18 when delivered to rat knees [48].

To augment the delivery of growth factors to articular chondrocytes embedded in an anionic matrix, Geiger et al. engineered a charged PEGylated dendrimer and conjugated it to IGF-1 [49]. The authors demonstrated that this penetrated the full thickness of 1 mm thick bovine articular cartilage explants. Further, when injected into rat knees, the dendrimer-IGF-1 prolonged the residence time in the joint from a half-life of 0.41 days for unconjugated IGF-1 to 4.21 days. In a rat model of surgically induced knee osteoar-

thritis, the dendrimer-IGF-1 decreased the area of medial tibial degeneration to 8.4% from the 19.7% observed with unconjugated IGF-1 [49].

An alternative method to improve the delivery of growth factors to sites of articular cartilage damage is to create a fusion protein composed of a growth factor and a specific binding domain, and deliver it in a hydrogel functionalized with the binding domain target sequence. Zanotto et al. used a heparin-binding IGF-1 and delivered it in a self-assembling KLD hydrogel to articular cartilage defects treated by microfracture in equine femoropatellar joints [50]. The treatment also included preparation of the site with trypsin and delivery of platelet derived growth factor with the heparin-binding IGF-1. The results showed improvement in multiple histological parameters and overall quality of the repair tissue compared to microfracture alone [50].

10.8 Conclusions

Available evidence suggests that growth factor interactions have the potential to promote articular cartilage healing. Harnessing these interactions to help create effective therapies for damaged cartilage will require new research and development technologies that are able to elucidate and then take advantage of the complex mechanisms underlying those interactions. This enterprise will, in turn, require new interactions among representatives of various, currently under-connected disciplines.

In the meantime, sufficient information exists to suggest certain growth factor combinations for further study. One example is FGF-18 and IGF-1, with or without a member of the TGF-β family. A review of growth factors not referenced in this limited review is likely to suggest additional options.

This enterprise, if pursued, would not be without challenges. One is a prolonged time frame. Articular cartilage damage usually progresses slowly and clinical trials may require many years to generate useful results. Another is the regulatory challenge of gaining approval for multiple simultaneously and sequentially delivered agents, particularly if combined with other tissue engineered constructs.

It is important to put the field of biologic approaches to cartilage repair in context. There exists the possibility that progress in the fields of skeletal reconstruction, joint replacement, or other interventions will outpace those of biologics. Although such alternative solutions to preventing and/or treating articular cartilage damage would potentially reduce the need for biologic agents, growth factor-based interventions may well augment the benefits of the other forms of treatment. Further, different approaches would likely prove to be most useful for different conditions, or different stages of disease. Ideally, the elucidation of the causes of cartilage-damaging joint disease would enable the prevention of at least some of the conditions altogether. None of these options are mutually exclusive and all of them hold promise.

The field of cartilage repair, including the role of growth factors, owes an enormous debt of gratitude to Professor Alan Grodzinsky for his decades of innovative research, education of new researchers and collaboration with fellow investigators.

Acknowledgements Supported by the Department of Orthopaedic Surgery, Indiana University School of Medicine. The author thanks Sue Samson for assistance with manuscript preparation.

References

1. Centers for Disease C, Prevention (2010) Prevalence of doctor-diagnosed arthritis and arthritis-attributable activity limitation—United States, 2007–2009. MMWR Morb Mortal Wkly Rep 59(39):1261–1265
2. Trippel SB, Coutts RD, Einhorn TA, Mundy GR, Rosenfeld RD (1996) Growth factors as therapeutic agents. J Bone Joint Surg (Am Vol) 78:1272–1286
3. Kuo CK, Li W-J, Mauck RL, Tuan RS (2006) Cartilage tissue engineering: its potential and uses. Curr Opin Rheumatol 18(1):64–73
4. Trippel SB, Corvol MT, Dumontier MF, Rappaport R, Hung HH, Mankin HJ (1989) Effect of somatomedin-C/insulin-like growth factor I and growth hormone on cultured growth plate and articular chondrocytes. Pediatr Res 25(1):76–82
5. Fortier LA, Mohammed HO, Lust G, Nixon AJ (2002) Insulin-like growth factor-I enhances cell-

based repair of articular cartilage. J Bone Joint Surg 84(2):276–288
6. Jentzsch KD, Wellmitz G, Heder G, Petzold E, Buntrock P, Oehme P (1980) A bovine brain fraction with fibroblast growth factor activity inducing articular cartilage regeneration in vivo. Acta Biol Med Ger 39(8–9):967–971
7. Osborn KD, Trippel SB, Mankin HJ (1989) Growth factor stimulation of adult articular cartilage. J Orthop Res 7(1):35–42
8. Henson FM, Bowe EA, Davies ME (2005) Promotion of the intrinsic damage-repair response in articular cartilage by fibroblastic growth factor-2. Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society. 13(6):537–544
9. Cucchiarini M, Madry H, Ma C, Thurn T, Zurakowski D, Menger MD et al (2005) Improved tissue repair in articular cartilage defects in vivo by rAAV-mediated overexpression of human fibroblast growth factor 2. Mol Ther 12(2):229–238
10. Yokoo N, Saito T, Uesugi M, Kobayashi N, Xin KQ, Okuda K et al (2005) Repair of articular cartilage defect by autologous transplantation of basic fibroblast growth factor gene-transduced chondrocytes with adeno-associated virus vector. Arthritis Rheum 52(1):164–170
11. Sawaji Y, Hynes J, Vincent T, Saklatvala J (2008) Fibroblast growth factor 2 inhibits induction of aggrecanase activity in human articular cartilage. Arthritis Rheum 58(11):3498–3509
12. Ellsworth JL, Berry J, Bukowski T, Claus J, Feldhaus A, Holderman S et al (2002) Fibroblast growth factor-18 is a trophic factor for mature chondrocytes and their progenitors. [Erratum appears in Osteoarthritis Cartilage 2002 Oct;10(10):826]. Osteoarthr Cartil 10(4):308–320
13. Davidson D, Blanc A, Filion D, Wang H, Plut P, Pfeffer G et al (2005) Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. J Biol Chem 280(21):20509–20515
14. Moore EE, Bendele AM, Thompson DL, Littau A, Waggie KS, Reardon B et al (2005) Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. Osteoarthr Cartil 13(7):623–631
15. Lohmander LS, Hellot S, Dreher D, Krantz EF, Kruger DS, Guermazi A et al (2014) Intra-articular Sprifermin (Recombinant Human Fibroblast Growth Factor 18) in Knee Osteoarthritis: randomized, double-blind, placebo-controlled trial. Arthritis & rheumatology (Hoboken, NJ) 66:1820–1831
16. Cook SD, Rueger DC (1996) Osteogenic protein-1: biology and applications. Clin Orthop Relat Res 324:29–38
17. Sailor LZ, Hewick RM, Morris EA (1996) Recombinant human bone morphogenetic protein-2 maintains the articular chondrocyte phenotype in long-term culture. J Orthop Res 14(6):937–945
18. Frenkel SR, Saadeh PB, Mehrara BJ, Chin GS, Steinbrech DS, Brent B et al (2000) Transforming growth factor beta superfamily members: role in cartilage modeling. Plast Reconstr Surg 105(3):980–990
19. van der Kraan PM, Vitters EL, van Beuningen HM, van de Loo FA, van den Berg WB (2000) Role of nitric oxide in the inhibition of BMP-2-mediated stimulation of proteoglycan synthesis in articular cartilage. Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society. 8(2):82–86
20. Chubinskaya S, Kuettner KE (2003) Regulation of osteogenic proteins by chondrocytes. Int J Biochem Cell Biol 35(9):1323–1340
21. Morales TI, Roberts AB (1988) Transforming growth factor beta regulates the metabolism of proteoglycans in bovine cartilage organ cultures. J Biol Chem 263(26):12828–12831
22. Rosier RN, O'Keefe RJ, Crabb ID, Puzas JE (1989) Transforming growth factor beta: an autocrine regulator of chondrocytes. Connect Tissue Res 20(1–4):295–301
23. Kim MK, Ha CW, In Y, Cho SD, Choi ES, Ha JK et al (2018) A multicenter, double-blind, Phase III clinical trial to evaluate the efficacy and safety of a cell and gene therapy in knee Osteoarthritis patients. Hum Gene Ther Clin Dev 29(1):48–59
24. Hochberg MC, Guermazi A, Guehring H, Aydemir A, Wax S, Fleuranceau-Morel P et al (2019) Effect of intra-articular sprifermin vs placebo on femorotibial joint cartilage thickness in patients with osteoarthritis: the FORWARD randomized clinical trial. JAMA 322(14):1360–1370
25. Inoue H, Kato Y, Iwamoto M, Hiraki Y, Sakuda M, Suzuki F (1989) Stimulation of cartilage-matrix proteoglycan synthesis by morphologically transformed chondrocytes grown in the presence of fibroblast growth factor and transforming growth factor-beta. J Cell Physiol 138(2):329–337
26. Tsukazaki T, Usa T, Matsumoto T, Enomoto H, Ohtsuru A, Namba H et al (1994) Effect of transforming growth factor-beta on the insulin-like growth factor-I autocrine/paracrine axis in cultured rat articular chondrocytes. Exp Cell Res 215(1):9–16
27. Chopra R, Anastassiades T (1998) Specificity and synergism of polypeptide growth factors in stimulating the synthesis of proteoglycans and a novel high molecular weight anionic glycoprotein by articular chondrocyte cultures. J Rheumatol 25(8):1578–1584
28. Loeser RF, Pacione CA, Chubinskaya S (2003) The combination of insulin-like growth factor 1 and osteogenic protein 1 promotes increased survival of and matrix synthesis by normal and osteoarthritic human articular chondrocytes. Arthritis Rheum 48(8):2188–2196
29. Leipzig ND, Eleswarapu SV, Athanasiou KA (2006) The effects of TGF-beta1 and IGF-I on the biomechanics and cytoskeleton of single chondrocytes. Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society 14(12):1227–1236
30. Shi S, Mercer S, Eckert GJ, Trippel SB (2012) Regulation of articular chondrocyte aggrecan and collagen gene expression by multiple growth factor gene transfer. J Orthop Res 30(7):1026–1031

31. Shi S, Mercer S, Eckert GJ, Trippel SB (2013) Growth factor transgenes interactively regulate articular chondrocytes. J Cell Biochem 114(4):908–919
32. Sonal D (2001) Prevention of IGF-1 and TGFbeta stimulated type II collagen and decorin expression by bFGF and identification of IGF-1 mRNA transcripts in articular chondrocytes. Matrix Biol 20(4):233–242
33. Loeser RF, Chubinskaya S, Pacione C, Im HJ (2005) Basic fibroblast growth factor inhibits the anabolic activity of insulin-like growth factor 1 and osteogenic protein 1 in adult human articular chondrocytes. Arthritis Rheum 52(12):3910–3917
34. Sah RL, Chen AC, Grodzinsky AJ, Trippel SB (1994) Differential effects of bFGF and IGF-I on matrix metabolism in calf and adult bovine cartilage explants. Arch Biochem Biophys 308(1):137–147
35. Yan D, Chen D, Cool SM, van Wijnen AJ, Mikecz K, Murphy G et al (2011) Fibroblast growth factor receptor 1 is principally responsible for fibroblast growth factor 2-induced catabolic activities in human articular chondrocytes. Arthritis Res Ther 13(4):R130
36. Ellman MB, Yan D, Ahmadinia K, Chen D, An HS, Im HJ (2013) Fibroblast growth factor control of cartilage homeostasis. J Cell Biochem 114(4):735–742
37. Sah RL, Trippel SB, Grodzinsky AJ (1996) Differential effects of serum, insulin-like growth factor-I, and fibroblast growth factor-2 on the maintenance of cartilage physical properties during long-term culture. J Orthop Res 14(1):44–52
38. Im H-J, Muddasani P, Natarajan V, Schmid TM, Block JA, Davis F et al (2007) Basic fibroblast growth factor stimulates matrix metalloproteinase-13 via the molecular cross-talk between the mitogen-activated protein kinases and protein kinase Cdelta pathways in human adult articular chondrocytes. J Biol Chem 282(15):11110–11121
39. Shi S, Mercer S, Eckert GJ, Trippel SB (2019) Regulation of articular chondrocyte catabolic genes by growth factor interaction. J Cell Biochem 120:11127
40. Qin Y, Huttlin EL, Winsnes CF, Gosztyla ML, Wacheul L, Kelly MR et al (2021) A multi-scale map of cell structure fusing protein images and interactions. Nature 600(7889):536–542
41. Kim YJ, Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD (1994) Mechanical regulation of cartilage biosynthetic behavior: physical stimuli. Arch Biochem Biophys 311(1):1–12
42. Bonassar LJ, Grodzinsky AJ, Srinivasan A, Davila SG, Trippel SB (2000) Mechanical and physicochemical regulation of the action of insulin-like growth factor-I on articular cartilage. Arch Biochem Biophys 379(1):57–63
43. Bonassar LJ, Grodzinsky AJ, Frank EH, Davila SG, Bhaktav NR, Trippel SB (2001) The effect of dynamic compression on the response of articular cartilage to insulin-like growth factor-I. J Orthop Res 19(1):11–17
44. Jin M, Emkey GR, Siparsky P, Trippel SB, Grodzinsky AJ (2003) Combined effects of dynamic tissue shear deformation and insulin-like growth factor I on chondrocyte biosynthesis in cartilage explants. Arch Biochem Biophys 414(2):223–231
45. Trompeter N, Gardinier JD, DeBarros V, Boggs M, Gangadharan V, Cain WJ et al (2021) Insulin-like growth factor-1 regulates the mechanosensitivity of chondrocytes by modulating TRPV4. Cell Calcium 99:102467
46. Thompson CL, Plant JC, Wann AK, Bishop CL, Novak P, Mitchison HM et al (2017) Chondrocyte expansion is associated with loss of primary cilia and disrupted hedgehog signalling. Eur Cell Mater 34:128–141
47. Caldwell J (2000) A safety tolerability and pharmacokinetic study of intra-articular recombinant human insulin-like growth factor (rhIFG-I) in patients with severe osteoarthritis (OA) of the Knee. America College of Rheumatology 64th Annual Scientific Meeting; 2000:Abstract 941, Abstract Supplement 2000, p S233
48. Ladel CH, Barbero L, Riva S, Guehring H (2020) Tissue distribution of sprifermin (recombinant human fibroblast growth factor 18) in the rat following intravenous and intra-articular injection. Osteoarthritis and Cartilage Open 2:100068
49. Geiger BC, Wang S, Padera RF Jr, Grodzinsky AJ, Hammond PT (2018) Cartilage-penetrating nanocarriers improve delivery and efficacy of growth factor treatment of osteoarthritis. Sci Transl Med 10(469)
50. Zanotto G, Liebesny P, Barrett M, Zlotnick H, Grodzinsky A, Frisbie D (2019) Trypsin pre-treatment combined with growth factor functionalized self-assembling peptide hydrogel improves cartilage repair in rabbit model. J Orthop Res 37(11):2307–2315

Part II Personal Tributes

A Toast to Al

Susan Chubinskaya
Departments of Pediatrics, Orthopaedic Surgery, and Medicine, Rush University, Chicago, IL, USA

It is my distinct pleasure to be part of Al's close circle and celebrate his 75th birthday. I am the lucky one who met Alan almost three decades ago and have the honor of calling myself Al's collaborator. The first picture (Fig. 1a) is one I found in Klaus's collection, my first chairman and a huge admirer of Al. It was taken probably sometimes in late eighties, close enough to the time we met. My first meeting with Al took place at dinner in a famous and one of Klaus's favorite restaurants "Casbah", when Al and Gail came to Chicago and Rush for an advisory committee meeting of the Biochem SCOR grant. I was amazed with how intelligent, approachable and fun to talk with he was. He acted as he was one of us, Biochem team, rather than one of them, an opposite camp of giants who evaluated us. If I am not mistaken, the relationship between Al and Rush began at that time.

As many of you know, Alan is a phenomenal host if you don't ask for shrimp at Legal Seafood and survive his driving through traffic lights. My first trip to MIT took place in 1998. In addition to being treated as a VIP, I had the pleasure of observing Al's interactions with his students, lab members, clinical personnel, and other MIT staff. No matter who you are, he treated everyone gently with genuine interest and enormous respect. Alan's ability to make you feel smart, his support and his generosity are priceless and I have experienced them many times. Since that May, I have many memories to share. The 1998 Gordon conference is one of them (Fig. 1b).

Another one, for example, is the trip in 1999 to the Pan-Pacific Connective Tissue Workshop organized by Tony Poole in Queenstown, New Zealand. During this trip we witnessed the biggest flood in the history of the city, when people used boats to get around the town since the water level was up to the middle of the doors. During this trip, we took one of the organized tours in the countryside and suddenly experienced a snow fall and almost inability to return to the hotel. How many of you can imagine Alan wondering through small boutique clothing shops? I can bet no one. However, since there was absolutely nothing else to do and we were forced to wait for roads to clear, he immersed himself in the process. As passionate as he always is with his research, he was passionately looking for a coat for Gail. I had to be a model – I tried a bunch of coats on until he found the right one. I hope it wasn't too big.

The third photo (Fig. 1c) must be taken around 2000, when all of us celebrated one of Klaus's retirement parties. Don't get me wrong. Alan is not retiring. We are only here today to mark another big milestone in his life. What about Davos conferences with their multiple social

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5

Fig. 1 Alan Grodzinsky at various conferences and meetings through the world over the years

activities and hikes, or proteoglycan dinners during the ORS meetings organized by Klaus's event planner, Mr. Petro? I can't say we really loved these dinners, but with Al being among participants, we always had lots of fun and laughs.

Another great memory is the 2005 Cartilage Gordon Conference (Fig. 1d) in the mountains of Italy, in Il Ciocco, where in addition to science we had tons of social interactions during our hikes or our trip to Lucca, an amazing medieval town. I don't know whether this was a middle-age crisis or Al simply decided to look as young as his students, but here he is without his beard. I think he stopped wearing it for good around that time. The best part of social interactions with Al is that Gail often accompanied him and made

Fig. 1 (continued)

them even more fun and enjoyable. I guess she is behind many of Alan's successes and accomplishments.

Coming back to Alan and Rush, this is more than collaboration; I would almost call it love or life affair. As I mentioned, this collaboration began more than three decades ago and is continuing now (Fig. 2). Key projects of this collaboration include the biomechanical characterization of human cartilage, comparison of knee and ankle cartilage responses to biochemical and biomechanics stimuli, biomechanics of different joints, morphological and topographical assessment of cartilage superficial layer, growth factors in cartilage regeneration after injurious compression, post-traumatic osteoarthritis, and finally the Chips-in-Space project. Miraculously, regardless of the project we would work on, Al always found the niche for biomechanical/bioengineering questions. I can't even tell you the number of Al's students and postdocs that have benefited from our human tissue collaboration. I am the last victim of Al's charm, captivation, and appeal. Based on the amount of collaborative scholarly work I am surprised that Al still does not have a faculty appointment at Rush.

Al's latest passion is the NIH-NASA Chip Consortium project that I mentioned earlier. If Alan had only known what he was getting himself in to… I don't know if this funding is a blessing or a curse. But with his sense of humor, Al navigates this ship with dignity and excitement. The best role one could ask for in our field is to be Al's collaborator. You feel that your wings are

Fig. 2 (left to right) Susan Chubinskaya, Ph.D., Joel Block, M.D., Alan Grodzinsky, Sc.D., Anne-Marie Malfait, M.D., Ph.D., and Rachel Miller, Ph.D

spread, you are smarter and more accomplished in your own eyes than in reality, and you constantly hear that without your support, Al would not be where he is. However, all of us know that there is a huge distance between us and Al. He is a planet that we can only look up to and thrive to reach. What about his ability to explain the most complex issues in a very simple and clear way? Listening to Al's presentations could be compared only to a great concert – same joy and pleasure.

And finally, Al's biggest honor is to be nominated by his trainees for the ORS 2018 Mentoring Award (Fig. 3). Al is the biggest influencer, teacher with a capital "T", mentor, and a giant in so many ways. Al, I want to propose a toast for you, for your legacy, for generations of those you touched, for many more healthy, exciting and productive years, and of course, for your family. All the best – Cheers!

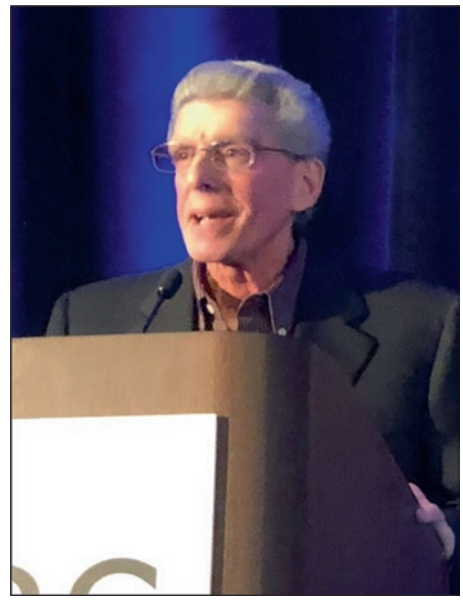


Fig. 3 Alan Grodzinsky accepts 2018 ORS Mentoring Award

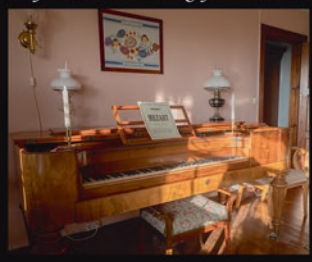
Cheers to you Alan,
for another creative 10, 20, or more!
/Stefan Lohmander

Celebrating Midsummer Night in 2011, near Vadstena, Sweden

Alan reflecting on refraction

sustainable engineering, this church door's been hanging there for 1000 years

and the old piano is waiting for another tuning



A Letter to Alan

Dear Alan,

I am pleased to have the opportunity to join many others in wishing you a very happy and memorable 75th birthday.

Over the many years we've known each other your scientific expertise and accomplishments have inspired me; I have admired your skill as a teacher and valued your friendship and advice.

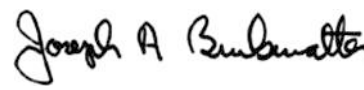
Your groundbreaking work on the mechanobiology of articular cartilage has been invaluable to all of us at the University of Iowa with an interest in post-traumatic osteoarthritis. You have dramatically advanced and deepened our understanding of the responses of articular cartilage injury and how these responses result in osteoarthritis. All of us look to you as a leader in this field of research.

For many years the event I've enjoyed most at the American Academy of Orthopaedic Surgeons (AAOS) Meeting is teaching the basic science synovial joint course with you. For the first several years you and Henry Mankin and I taught the course together, and wrote an article based on the course. After Henry retired you and I have carried on. It has pleased and surprised me that year after year we draw a committed audience for a basic science course at the AAOS meeting. Some of the course registrants have attended our course every year for more than a decade.

You have a masterful way of explaining the complexities of articular cartilage mechanobiology and how the responses of articular cartilage to injury lead to the clinical syndrome of osteoarthritis. You have a great sense of humor, and you present your work in a clear and entertaining fashion. The audience always enjoys your presentations and your remarks during the question-and-answer period.

It has been a great pleasure to work with you and learn from you. I've benefited immensely from your wise counsel and from your insightful observations on mechanobiological processes in the musculoskeletal system. I am so happy that you are being recognized for your many fundamental contributions. I wish you the very best on your 75th birthday and I wish you many more happy birthdays.

Sincerely,



Joseph A. Buckwalter, MS, MD
Professor and Steindler Chair
University of Iowa Department of Orthopedics and Rehabilitation

Tribute to Al from Finland

Rami K. Korhonen · Atte S. A. Eskelinen · Amir Esrafilian · Cristina Florea · Petri Tanska
Department of Applied Physics, University of Eastern Finland, Kuopio, Finland

Gustavo A. Orozco
Department of Applied Physics, University of Eastern Finland, Kuopio, Finland
Department of Biomedical Engineering, Lund University, Lund, Sweden

Al has left an indelible impact in the northeastern Europe. Specifically, the University of Eastern Finland and Kuopio Campus have benefited from Al's wisdom. The collaboration with Biophysics of Bone and Cartilage -research group led by Professor Rami K. Korhonen, Ph.D., has concentrated on computational models of osteoarthritis progression enhanced with experimental biomechanical testing of articular cartilage.

The foundations of the fruitful collaboration were laid already in ORS conferences in the early 2000s, during Al's visit to Finland when he served as the opponent of a doctoral thesis defense in 2013, and when Cristina Florea, Ph.D., was granted a highly anticipated researcher exchange into Al's lab via Marié Skłodowska-Curie Actions. Moreover, in 2020, Al was chosen as the Honorary Doctor of the University of Eastern Finland, underlining how the local research community holds Al and his world-renowned contributions to mechanobiology and fight against osteoarthritis in high esteem.

Although not all of us authors have had the tremendous opportunity to visit and intimately work with Al thus far, as the vanguard of the science of soft tissue mechanobiology Al has made a profound impact on our scientific life and attitude of hard work. We feel honored for the chance to contribute to this book and want to express our deepest gratitude to Al for the collaboration, scientific discussions, Continuum Dinners at ORS conferences, and research visits to the Grodzinsky Lab. Next, we phrase a few individual words of tribute to Al, enriched with stories and memories.

Rami "Trusted Astronaut" Korhonen

I got a chance to join your fabulous, friendly, and funny Continuum Dinner at ORS first time sometime around 2005, by the invitation of Mike "timantti" Buschmann. Since that time, I have felt that we have been like friends and our age difference does not matter at all in our communication and humor (Fig. 4). I highly appreciate your open and easy-to-approach attitude and being "not so serious".

A kickoff to our scientific collaboration started in 2013 when you visited Finland in the PhD thesis defense of Siru Turunen. Following that, we have received some grants and worked in several projects together. Maybe I never get a chance to be your astronaut in some of the space flights with cartilage, but that does not diminish my appreciation to your long-term dedication to mechanobiology and osteoarthritis research, especially in those studies we have worked together. I wish you delightful and enjoyable times for the future and hope to see you later in Finland for the ceremony to confer you an Honorary Doctorate by the University of Eastern Finland.

Cristina "Chief AFM Sailor" Florea

My first interaction with Al took place in June 2013 during his visit in Finland at the University of Eastern Finland, while he acted as PhD thesis opponent. During the post-doctoral party, after having a relaxed and inspiring conversation about cell and cartilage research done in his lab in MIT, I immediately saw in Al all I wanted for a mentor and scientific supervisor. That evening was the moment that I knew that I need to find a way to visit Al's lab and develop further my seedling research ideas on cell and cartilage mechanobiology.

In the following years, Al's constant encouragement and support helped me immensely to succeed in securing funding through a Marie Skłodowska-Curie Global Fellowship, among Europe's most competitive and prestigious awards which allowed us to strengthen the research bridge between our labs.

Fig. 4 In 2018, Al hosted a dinner where (from left to right) Cristina Florea, Petri Tanska, Rami Korhonen, and Gustavo Orozco took part. Scientific discussions and unofficial, friendly chatter were accompanied with pieces of lobster flying in the busy Cambridge night

What I truly admire about Al is his ability to make you believe that you can achieve great things and he is genuinely committed to supporting you in pursuing your dreams. By aiming at the Sun, you may end up on the Moon, or a bit closer on ISS. I am deeply grateful to have had the incredible opportunity to spend two and a half years in the Grodzinsky Lab. This was an extremely eye-opening and transformational experience. The research environment that Al creates in the lab is truly enriching, supportive, and inclusive. Words cannot express my appreciation to Al and the whole gang for the memorable time spent together (in Area Four, our favorite 3-in-1 restaurant/bakery/bar, having Friday Margaritas, scientific meetings with delicious pizza, celebrating birthdays, published articles, and unsatisfactory experiments; Fig. 5). Al has taught me plenty during my time in MIT about what it means to be an academic, a teacher, a cheerleader, a mentor, a psychologist, an advisor, an advocate, and a leader. But what Al has taught me most, simply through his actions, is what it means to inspire and empower generations of students and colleagues to dream big and to have fun during this journey. Thank you so much, Al for your continuous availability to me, your empathy, guidance and patience. You are one of the most remarkable and humble people I have ever met. It has been such a joy and privilege to know you. Cheers to many more years and continued and fruitful collaborations! I'm looking forward to being your guide in your grandfather's birthplace in Romania!

Petri "Paulaner Brother-in-Arms" Tanska

You know that we Finns are not persons with many words, so I keep it brief (maybe Atte is an exception to the rule, see below). I got the chance to join your terrific Continuum Dinner at ORS in 2011 in Long Beach. After that, I have had the privilege to join several Continuum Dinners with the rest of the "Finnish Mob". After working with you and Rami through several projects, I got opportunity to visit your lab a few times (Fig. 6). Those visits were a true pleasure. The atmosphere in your lab was always warm and welcoming, and your way of doing science has been quite inspirational for me, a delicate mix of professionalism and humor. Furthermore, let's not forget that one "special" draft in Area Four;

Fig. 5 Celebrating Al's birthday in the best place on earth – Area Four, Cambridge, MA (December, 2019)

Fig. 6 The Finns (from left, Petri Tanska, Atte Eskelinen) visited Al's lab in August–December 2019. By that time, Cristina Florea had already accustomed to the life in Cambridge during the 16 months of her stay. As a close mentee of Al, Cristina could already tell the newbie boys where to find some great food, as well as some refreshing beverages to the Nordic liking. "Uh oh, seems that the Finns are heading to the Area Four again," whispered Al in the corridors, locking his office door and shutting the window blinds, while the visitors prowled around the lab looking for more companion to join them for yet another "PhD student-and-supervisor meeting"

it is one of my favorites too. It seems the "European Duffman" likes it too (see Fig. 7), which I noticed during my second visit. Hopefully, we all can still have a few pints of that golden nectar in the future, maybe even in Finland! I wish you and your wife enjoyable days for the future!

Atte "Duffman" Eskelinen

I was extremely thrilled for the opportunity to join Al's lab as part of my doctoral studies for five months in 2019. I felt honored, as well as nervous, due to my background not being in biochemistry or biological engineering. But that nervousness vanished quickly when Al introduced me to the

Fig. 7 Al kindly guided Duffman into local restaurants during Halloween 2019. After long days filled with biomechanical loading of cartilage and inflammatory cytokine cocktails, Al's invitation for some Paulaners downstairs warmed the heart of Duffman. "Check out the glass", Al supervised the young student flexing his fake musculoskeletal appearance. "It is the small details that make an experience feel great and complete. As you can taste, this official Paulaner glass creates the feeling of wholeness to the drinking experience. The same philosophy of detailed work goes for writing great scientific papers, which leave a feeling of wholeness to the reader". Cheers!

rest of the wonderful group during a weekly group meeting, where we had pizza. Oh, Area Four pizza! I really miss that now! After that first meeting, I thought that the atmosphere Al had created there was something extraordinary. As a short-term visitor, I really felt welcome which naturally bumped up the motivation. Well, I already knew that Al is a welcoming, friendly, supporting, and ambitious person, and not this faceless, emotionless, skyrocketing h-index robot from MIT, since I already met him during the Continuum Dinner of ORS 2019 in Austin. That was my first time in the US, and we talked with Al about his affection for Belgian- and German-style wit-/weissbiers. Naturally, I shared the interest with him, and Al recommended me to try Blue Moon. Boom, love at first sight with that beverage!

On the following year at ORS 2020 in Phoenix, I somehow ended up in a sumo ring fight with Rami and won it fair-and-square 2–1. Despite Rami's praises about his last-round textbook case of a "perfect" bodyslam, I really appreciated Al stating that this time the student, the underdog from Mikkeli, Finland, claimed the win. Later on, I came to appreciate on Al's tips on writing manuscripts, as well as the open door policy: whenever I had trouble with my work, I could try to ask for an audience with him. Despite Al's super busy schedule, these meetings could always be squeezed in.

So, Al, happy birthday, I hope you enjoy this book and the tributes written here! I know I'm not the only one, but you and your magnificent career has left an enormous impression at least into this young "rookie in the lab" that likes to call himself scientist. In the eyes of a European Duffman-copy (Fig. 7), you are an exemplary man, a paragon of science, the Bossman driving a black Tesla Model S (maybe a Plaid-version is already in order?), rocking a long leather jacket, putting the Autopilot on while going to highfive with Elon, after successful space mission of sending cartilage to space. Just wow, what science fiction is that! Lifegoals, if you ask me. May you and your wife lead a healthy and osteoarthritis-free life in the future, filled with viola playing. We are waiting for you to come visit Finland again!

On Behalf of the Undergraduate Trainees

Hannah M. Zlotnick
Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA

Al, thank you for being the best UROP mentor, instructor, and supporter.

Mentor

Thank you for creating and cultivating a lab environment inclusive to undergraduate researchers. Eager but unskilled, I joined the Grodzinsky Lab early in my time at MIT with no prior research experience. You welcomed me just as you welcomed new graduate students, post-docs, or other visiting engineers. Beyond this initial welcome, you offered me opportunities to develop as an independent scientist. Undergraduates were encouraged to present in lab meeting, and could always meet directly with you to discuss their data. I learned a lot from our conversations about my project in the context of historical literature. Later in my time at MIT, you supported my interest in applying to and attending the ORS (Fig. 8) and ICRS meetings. While conference funding for undergraduates is limited, you encouraged me to seek out funding sources on campus—a skill which I take with myself today. Thank you for seeing the potential of all of the undergraduates who entered the lab, and giving us the guidance and resources to fulfill such potential.

Thank you for being equally as excited about my latest sports (or other extracurricular) achievements as lab progress. During my time in the Grodzinsky Lab, I was not alone in being a student-athlete UROP. We had representation from the baseball, football, soccer, and track and field teams. At least for me, this direct connection to athletics and sports injuries fueled my interest in orthopaedics. I always knew that when I walked into lab you would ask about our latest game and season record. This meant a lot to me that you cared about my life inside and out of the lab.

Thank you for sponsoring gatherings to celebrate life with us. Some of my fondest Grodzinsky Lab memories are from the lab thanksgivings (Fig. 9a), holiday parties, and birthday celebrations (Fig. 9b, c). At my first lab thanksgiving I was amazed with the assortment of side dishes, giant turkey, and comprehensive wine list. It was fun to share this meal with not only the current members of the lab, but lab alumni from the area as well. This was when I first realized how large the Continuum family is. Outside of our planned parties, I would like to thank you for the spur of the moment happy hours, pizzas, and lunches around tech square. These smaller impromptu events were what made lab seem like a second home and family to me.

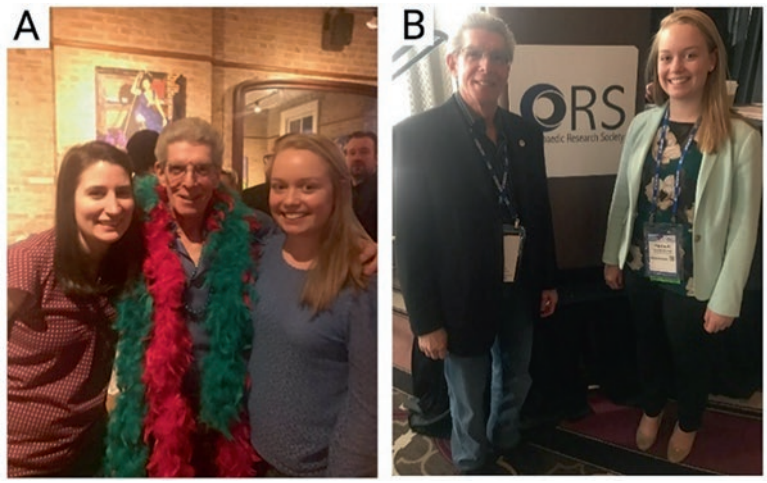


Fig. 8 Orthopaedic Research Society (ORS) Annual Meeting 2018, New Orleans. (**a**) Continuum dinner featuring feather boas, ORS 2018. (**b**) My first ORS talk, ORS 2018

Fig. 9 Grodzinsky lab celebrations. (**a**) Three-course thanksgiving wine list, 2014. (**b**) December birthdays, 2015. (**c**) April birthday, 2017

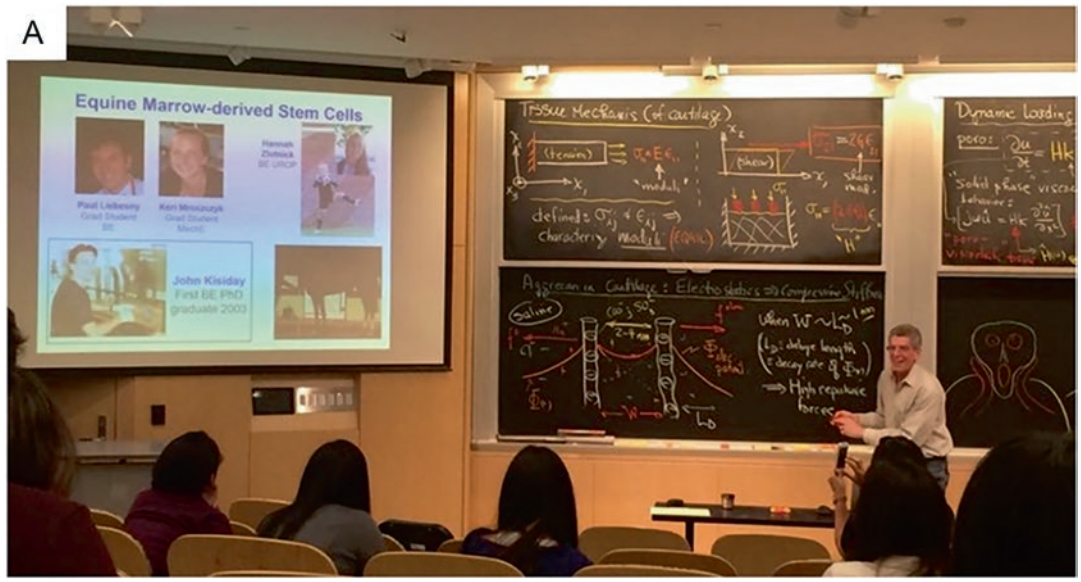


Fig. 10 Al's board work and excitement in class. (**a**) Board notes—with famous red, orange, yellow, and white chalk, describing cartilage tissue mechanics and the role of aggrecan. Scream face (bottom right board) revealed at the end of the lecture. (**b**) Al performing rat tail tendon collagen extraction (live in-class) for 20.310. (Photos taken in Spring 2015)

Instructor

Thank you for your dedication to teaching. I remember early after I joined the lab someone told me, "If Al's office door is closed, that means he is preparing for class, or at class." At that time and even more so today, I was and am amazed with your continued commitment to teaching. Sure, 20.310 was entitled, "Molecular, Cellular, and Tissue Biomechanics," but it was also a masterclass in how to engage students. I learned that the secret to a giving a great lecture is 1 part preparation, 1 part colored chalk (Fig. 10a), and 1 part coffee/enthusiasm with the occasional live demo (Fig. 10b). This preparation involved spatially writing out the board notes on sheets of paper and even annotating the chalk colors on these notes. I later implemented this preparation strategy when I taught my first lecture for the MIT Women's Technology Program. Looking back, in many ways your teaching style reflects your energy and instruction in the lab. In both venues, you have a true talent for distilling complex topics into clear language, equations, and illustrations.

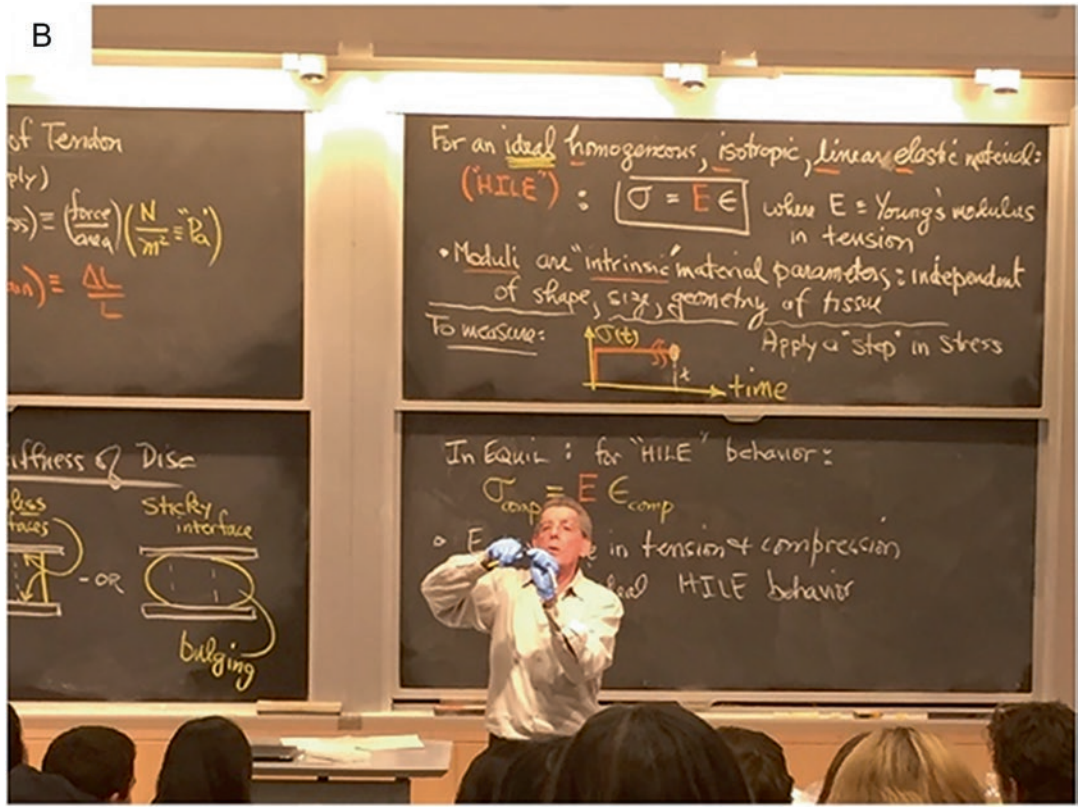


Fig. 10 (continued)

Supporter

Thank you for encouraging and supporting me, and your numerous other undergraduate research students and advisees. I remember coming to you with an interest in completing an undergraduate research thesis, which did not exist at the time in Course 20. You were immediately on-board with this idea and helped garner support from the greater BE department. This was not something that I could have jumpstarted on my own, and I thank you for pushing the undergraduate thesis program forward for not only me, but for future BE students as well. I additionally am appreciative of your support throughout my graduate school application process. I remember meeting with you to discuss my list of places to apply. Thank you for acting as a sounding board throughout that process, and universally throughout my time at MIT. While MIT is a wonderful place, it is an undoubtedly challenging environment. Thank you for providing perspective (and coffee and pizza) to help me get through these challenges (Fig. 11).

Fig. 11 Area 4 meal around graduation time, 2017

To conclude, I would like to thank you for continuing to have your door (and email inbox) open. Just recently, we celebrated your 75th birthday. The day before the celebration I came into lab and knocked on your office door to say hi. Naturally we ended up at Area 4, which I thoroughly missed over the past few years. Thank you for your words of encouragement there as I am navigating my next career steps. I truly value having you as a lifelong mentor and look forward to many more Paulaners in the years to come.
Cheers, Al!

Congratulations, Al

Han-Hwa Hung
Department of Biological Engineering,
Massachusetts Institute of Technology,
Cambridge, MA, USA

Congratulations on your 75th Birthday and symposium! It was a great tribute!

I have been so fortunate to have the opportunity to work in your lab for the past 25 years. I love coming to work every day and am so grateful to be able to participate in the challenging research. Moreover, you have not only been a wonderful boss, but have also become an invaluable mentor and friend. Thank you for being so supportive not only at work but also during difficult times at home.

Working with you and students has been such a rewarding experience and I will treasure all of the memories in the lab as well as at all the wonderful parties that you and Gail hosted over the years.

Thank you from the bottom of my heart.

Ode to Al: A Tribute from the Boston Osteoarthritis Researchers (BOAR)

Sandra L. Shefelbine
Departments of Bioengineering and Mechanical Engineering, Northeastern University, Boston, MA, USA

A professor as such there never was.
It would be pages to name all that he does.
He has received so many honors, given so many talks,
Inspired so many students, written so many docs.
The keywords from these docs as a sum
Can be found in Fig. 12.
He sits in departments of which there are three,
Electrical Engineering, Bioengineering, and MechE.
That means he thinks in both frequency and time space.
Buildings 3, 38 and 55 are his home space.
Now he sits atop Technology Square
With all of those biotech start-ups direct in his lair.
He investigates the properties of cartilage the tissue
It is very complex and that is the issue.
The composition of cartilage is water and organics
And it exhibits some very complicated mechanics.
Proteins and water and sugars compose the matrix
allowing cartilage the ability to play-tricks
Load it quick and it is strong, indeed, quite robust
Load it slow and it squishes (a technical term we trust)
If you apply load to a cartilage plug
The cells inside, they feel the tug.
The cartilage cells respond by pumping out GAG
Al's shown this with a radioactive tag.
Loading can also cause the activation of genes,
Resulting in production of more and more proteins.
And then there's that story with TGF-beta,

Fig. 12 Word cloud created from Alan Grodzinsky's publications

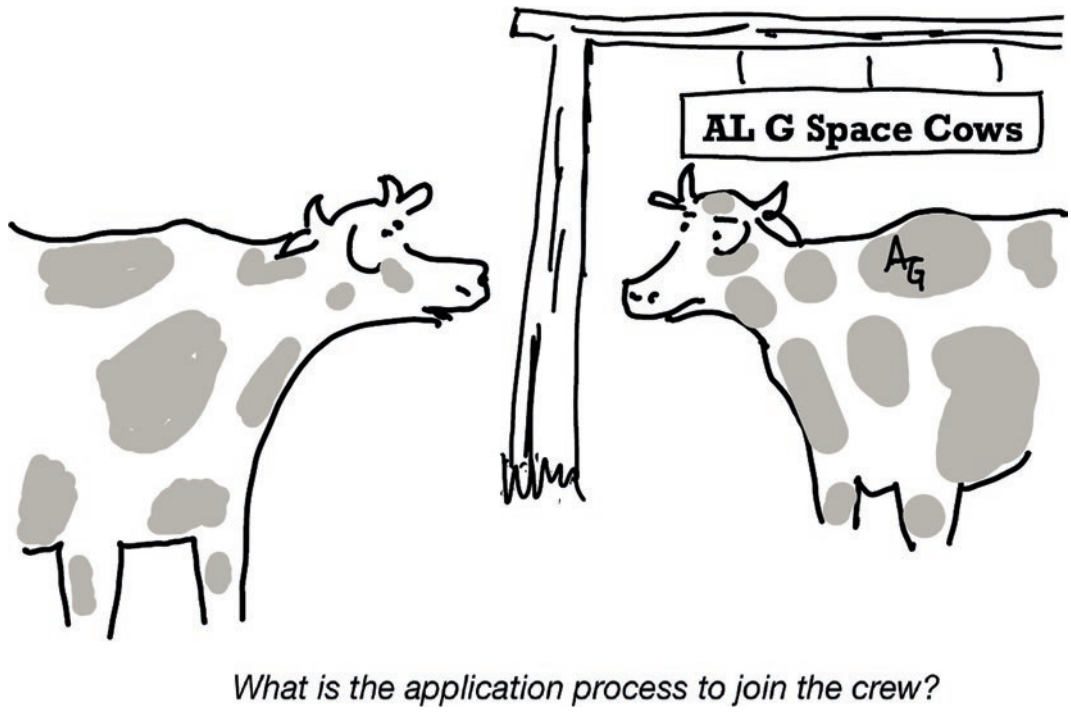


Fig. 13 Cartoon depicting Alan Grodzinsky's latest project to send bovine cartilage to space

Now talk about complex – just go look at the data.
Al likes to play with cartilage's electricity
And that is not because of its simplicity
Using the negative charge, he can pull the drugs in
And to treat arthritis that will be a win.
Al's shown if you treat cartilage with dex
And then give a drug, things get quite complex
Particularly when the drug is charged positive,
it seems to protect – a result that is causative.
You see, the cartilage within the joint of your knee,
Will eventually disappear both for you and for me.
Al's work tries to keep it there as long as can be
So that we can all walk around completely pain free.
So if he can trick cartilage with this positive drug thesis
We may save ourselves from getting a prosthesis.
Recently he has been sending cartilage plugs into space
The work for this must happen at quite a pace.
When the rocket is ready, the cells must be too,
Preparing and shipping to launch is quick (see Fig. 13).
With cartilage plugs in space he's hoping to find
The effect of microgravity on the knees of mankind.
Years ago he started a group, unfittingly called BOAR;
Neither pigs nor yawns tell you what is in store.
Really, the name could be BOAST[1]
We meet around Boston with a rotating host
And Boston-based researchers in cartilage domains
Come together monthly to share research gains.
We are so lucky to have such a high concentration
Of cartilage researchers with high acclamation.

[1] Boston OsteoArthritis Summative Talent.

Al shares his wisdom and thoughts when we meet
His curiosity and passion would be hard to beat.
He brings to the table that distinct Al G. smile,
Probing questions, intriguing ideas – that is his style.
His insightful comments and inquisitive remarks,
Set the tone for the meetings, indeed the benchmarks.
Al is thoughtful, supportive, respectful, and kind,
And asks the tough questions, but we don't really mind.
You see Al brings us all up to the next research level,
To discuss, critique, and praise and not to bedevil.
We sincerely thank Al for being our guide.
In Boston cartilage researcher pride!

Grateful

Linda D. Bragman
Department of Biological Engineering,
Massachusetts Institute of Technology,
Cambridge, MA, USA

Al,

I am grateful to be part of your journey for the last 37 years. You have enriched not only my work life, but also my personal life as a dear friend. Thank you for being there for me in good times and sad times as well. Hope this 75th birthday brings you much joy and happiness. You deserve it!

Much love,
Linda D. Bragman

Reflections from a Graduate Student

Rebecca Black
Department of Biological Engineering,
Massachusetts Institute of Technology,
Cambridge, MA, USA

When I joined the MIT Biological Engineering graduate program in 2017, I had one criterion for joining a lab: a good mentor. I had a vague idea of what kind of research I wanted to do, but I knew that my relationship with my PI was my most important, non-negotiable priority. I was pointed in the direction of Al Grodzinsky's lab not for scientific reasons, but for coffee recommendations – I had asked another professor where the best coffee in the area was, and he responded that Al would be the one to know. I looked further into his research and saw there was overlap with my interests in tissue engineering, and decided to reach out about a formal rotation in his lab, as well as coffee advice.

I was nervous approaching the meeting, trying to cram in as much background research into the lab's papers as I could in the days leading up to it. But when I stepped into his office, I was immediately at ease: Al jumped up with a smile to greet me and we chatted as much about the excellent espresso at the café two floors down as we did the research being done in the lab. A couple months later and I had joined the lab officially, beginning my nearly daily trips to that same café with Al and my fellow labmates. In my time with the lab, Al's mentorship has gone above and beyond what I could have expected, and I have stepped into every meeting to be greeted with that same excited smile.

It's no question that Al Grodzinsky has produced an incredible body of research that has shaped the field of orthopedics and our understanding of cartilage biology. Looking back at this impressive history of scientific discoveries, it's easy to wonder what the key to doing such transformative research could be. I think the key question to ask here isn't about what we can learn from the research itself, but what we can learn from Al and the kind of mentor he is. I strongly believe the success of the lab and the work it has produced lies in Al's commitment to putting the people in his lab over the research.

As I reflect back on the last five years as a graduate student in Al's lab, two things stand out to me as anomalies working for Al compared to other labs I've seen: his ability to seek out and attract scientists who are good people while cultivating a social, supportive lab, and his method of guidance that doesn't rely on negative criticism, but nurtures growth in students with in the most subtle ways. Both of these traits shaped my experience in the lab from the first day I met with Al to discuss a rotation all the way through putting together my final Ph.D. thesis.

Al was always at the center of the social heart of the lab, coordinating birthday celebrations, lab lunches, and the famous monthly Margarita Fridays that brought everyone together to relax and chat about scientific and non-scientific topics in equal parts. This culture permeated our work too, creating a collaborative atmosphere where you could ask anyone for help at any time, and in turn supported those around you in any way you could. We lifted each other up through successes and failures, and did better research because of it.

The second part of Al's mentorship that cultivated such a successful lab is his method of giving guidance and criticism in a way that allows for personal growth and isn't centered in negativity. In my first year in the lab, I asked Al for input on a guiding document of best mentorship practices that a student group was developing with department leadership. Al's suggestion has stuck with me all these years: "student success ≠ project success". This encapsulates perfectly for me what I experienced in my time in the lab. I call this an anomaly because too many times in academia we see examples of graduate students belittled and berated by their PIs for not meeting expectations, and breakdowns in communication that leave graduate students feeling frustrated and inadequate. But with Al, we as people always came first – long days were offset by invitations to take a break for coffee or pizza, and project failures were never causes for disappointment and negativity but springboards towards the next hypotheses to pursue.

Being given the space to make mistakes and grow shaped me and every student that came through this lab. Some of the (truly awful) first drafts of proposals or papers that I put before him weren't torn apart, but carefully critiqued with the right kinds of questions: Al always re-framed what I had said and guided me to seeing where the holes were and what needed to change. Without explicitly saying any parts were bad, he helped me see where I needed to be clearer, where my aims were far too specific, where certain analyses weren't accomplishing what we wanted to answer. This environment where failure is okay and mistakes are improved by guiding the student towards figuring out themselves what to improve is something I believe every PI should strive towards. It creates independent, confident students that take every mistake in stride and do better research for it, and it starts with a PI who values the students and their growth over the research output.

When I've talked to colleagues and other students in the department, I always say that I'm "lucky" to have Al – a PI with so much compassion and empathy in his mentorship, who has always valued those in his lab as people first and researchers second. With such an incredible quantity of research that he has produced, there is much to learn about how to ask research questions and tenaciously follow those questions with careful experiments. At the same time, I think it's just as important to not lose sight of the man behind it and how his approach to leadership created the perfect environment to do world-class research.

So from me, from every graduate student you have shaped and developed along the way – thank you Al! I wouldn't be the scientist and person I am now without your every guidance and support. My most exciting presentations were elevated with congratulatory emails in all capital letters from you, and in some of my most disappointing moments I would walk out of your office with a smile on my face reinvigorated to pursue the next path forward. It's an honor to have a mentor like you, and I hope to take the lessons you've taught me to heart as I start on my next path forward in my career.

Thanks to Alan

David W. Smith
Department of Engineering, Computing and Mathematics, The University of Western Australia, Perth, Australia

A key element to Alan's success in life are his personal attributes. Anyone who has known Alan for any length of time will testify to his very generous welcome that he extends to everyone, his openness to listening while conversing, and his extraordinary interpersonal skills. Alan has employed this to good effect for those around him, providing a safe environment where a vast number of people can grow (and in some cases grow up). Such a beautiful nurturing environment, an oasis if you like, in an otherwise highly competitive and in many ways intimidating and rather unforgiving environment, develops human capital—that most precious of all capital—enabling it to take root, to establish, to thrive and ultimately to blossom. This alone is a huge achievement.

It is apparent once you have quite a few years behind you that this is almost axiomatic—you cannot give of yourself in such a generous way without first knowing oneself. Alan's ease with himself no doubt is grounded in the kind of family he grew up in, in the enjoyment he and his family found while playing music, in the success he found in academic achievements, and in his own family with Gail and Michael. And with such secure foundations, one can begin to contemplate a career being unusually generous in the rough and not so humble world of academia. And Alan knows a thing or two about discreetly navigating those academic minefields. To give but one example, moving from the electrical engineering building on Vasser St to Tech Square was plainly a masterstroke—close enough to the action but just sufficiently removed to avoid the shrapnel, the geographical balance being perfect, if you know what I mean. And recognizing his extraordinary interpersonal skills, the university administration leaned on these skills to help manage more than one situation that called for being able to listen and read another person extraordinarily well. In a sentence, they relied on his reputation and his judgement.

Alan and Gail have been perfect hosts to my wife and I. They entertained us at home, at the Gardiner Museum of Fine Arts, on the freedom trial and a nearby ice-creamery. And then we checked out the lobsters in Maine and compared them to the lobsters in Newport. And by the bye, after sipping some of that malty-bready Trappist beer, Alan and I had some fun getting to know the ins and outs of that new-fangled Tesla motor car, which was something very new way back when. Our first meeting with the Tesla was surprises all the way. First issue—we couldn't find it. It was clearly marked on the map, so we did a drive by past many the car-yards and all the familiar brands, and turning up nothing, doubled-back to them check again. Nothing. How can we be missing it, when it is so clearly pinned right here on Google maps—it should be right here? A mystery. Then we realized the pin was actually at the back of the said car-yards, somewhere in that darn shopping centre right over yonder. That was a first for both of us—shopping for a car with a Starbucks right across a promenade full of grocery trollies. And check out that Tesla cut-away—the floor is completely covered in batteries—and they look like torch batteries for goodness-sake. And that monster touch screen seemed to dominate the space and to control the whole car—and the seats were pretty comfy too. I sat in the back while the sale person explained that touch screen. I noticed the sound system wasn't half-bad, to use an Australian expression. Apparently, software updates occurred while you slept, which seemed pretty remarkable at that time, as we didn't know cars got software updates at all. We sat down and sipped on our Starbuck's coffee and tried to take in all this new information, as well as how you had to book for a test drive and they brought one around. Says Alan, "You know I think I'll book for a test drive". And so right there and then, a new direction began in Alan's life. He is very fond of his car, which is about as American as baseball and apple pie (and I might add, a very warm American welcome). Thank you, Alan, thanks from everyone.

A Thank You Note to Alan J. Grodzinsky from Germany

As a tribute to Alan Grodzinsky as a researcher, mentor, friend, and long-term role model, we would like to express our thoughts and gratitude from the perspective of two Germans who spent their postdoctoral research fellows in Alan's lab, which followed with Alan being a collaborator and long-term advisor and friend since many years.

Writing the book chapter "Mechanical Articular Cartilage Injury Models and Their Relevance in Advancing Therapeutic Strategies" had us go through a list of Alan's publications. Although we knew many of them, it was the chronological order that made us realize their overarching connectedness and how the topics developed over time – like a story arc – from basic science to therapeutic translational medicine and it has never stopped being unique and interesting. This really showed us Alan's enormous dedication to 'his' topics over time, which he spread to the people he worked with. However, Alan not only spread scientific dedication but also high work ethics, quality, motivation, and fun. Alan's lab was a fun place to be. We remember vaguely that M.I.T. had a message in its welcome movie for newcomers that advertised work regardless of the day but it was Alan's lab that put this statement into reality because one liked to always be there and to do interesting work. At least that is how we felt.

Having had our first visits in 1998–1999 (BK) and 2003–2005 (BR) we still think about the great impressions and the guidance that we received during our time in Alan Grodzinsky's lab, and it is good to know that nobody can take that from us. After moving back to our own country, Alan had us equipped with our own injury machines and we took this proudly as a sort of award. Looking back at how Alan ran his lab and dealt with complicated situations, it is no shame to say that we often remember and try similar approaches, almost like a small scientific outpost of Alan's lab. Importantly, Alan always had and has time for us and continues to stay in touch. We are deeply grateful for everything we have learned and experienced and we wish Alan many more successful and happy years to come!

Bernd Rolauffs
G.E.R.N. Research Center
Department of Orthopedics and Trauma Surgery
Albert-Ludwigs-University of Freiburg
Breisgau, Germany

Bodo Kurz
Department of Anatomy
Christian-Albrechts-University
Kiel, Germany

A Tribute to Al

Ambika Bajpayee
Department of Bioengineering, Northeastern University, Boston, MA, USA

Rachel E. Miller
Department of Internal Medicine, Rush University, Chicago, IL, USA

As scientists we are honored to have trained under someone with the stature of Alan Grodzinsky, of the field of biomechanics, a true giant, in academic parlance. And as humans we are lucky to have worked with someone as kind and fun as Al, who has become, to many of us, a lifelong friend. There are few names in the field that shine as bright as Al's professionally and of those there are even fewer who are loved as widely as Al personally.

Al's scientific contributions and achievements need no repetition here as this book itself is a product of those. A compilation of the research output and contributions of many of his students, this volume is extensive in its depth, breadth, and impact. During his long career as a professor at MIT, Al has mentored more than 50 doctoral candidates and 25 post-docs. This track record of training several generations of researchers many of whom have gone to be leaders in the field, is Al's invaluable gift to posterity.

For Rachel, working in Al's lab spurred her fascination with biomechanics in general and osteoarthritis and cartilage in particular. Working with Al was a primary reason why she enjoyed research and stayed in academia. Al's lab provided an ideal scientific environment for maturing as a scientist – we had the freedom to pursue our own ideas yet the support to work through problems when projects did not go as planned. This spirit of mentorship is something that she hopes to continue in her own lab.

Ambika remembers Al as a kind and caring mentor, always encouraging, and explaining his own work in the most unassuming way – an approach that cultivated true passion for research, which is an essential foundation for long term success. The most successful scientists are those that love research to such an extent that they don't notice the passage of time. Al enabled an environment where this was true. Additionally, Al is remembered as one of the absolute best and most memorable teachers at MIT. Many students across disciplines and not just those in his lab or department will agree that the first class they took with Al is still clear in their memory.

Both of us are faculty members today at Rush University and Northeastern University, respectively, and are extending what we learned under Al for the advancement of science and society, and like many alumni of the Grodzinsky lab, actively collaborating with each other.

We are confident that we speak for many former and current members of the Grodzinsky lab in saying that we enjoyed coming to work every day as Al made sure to foster a fun environment and sense of community in the lab. We learned from Al that it is the people that make science fun, that a collegial lab is a productive lab, and that we must celebrate all wins, no matter how small, including making it to Friday or winning the annual Margarita Chair.

Well beyond the years spent in his lab, Al continues to support the careers of all his trainees, which is no small contribution to their life-long progress. Many of us continue to hear from Al every time he sees our name in the news or on a new paper. We hope that we can emulate Al's success while staying as kind and humble as he's always been. We know that when our students and their students look at their academic family trees, they will be proud to find Alan Grodzinsky's name there.

Index

B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5