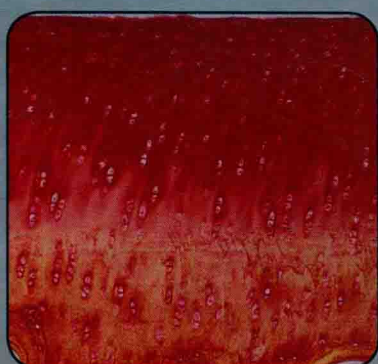
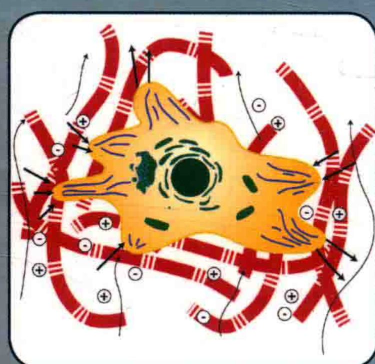
ARTICULAR CARTILAGE

SECOND EDITION

Kyriacos A. Athanasiou • Eric M. Darling
Grayson D. DuRaine • Jerry C. Hu
A. Hari Reddi

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ARTICULAR CARTILAGE

SECOND EDITION

To Thasos and Aristos, please remember that *pursuit of excellence* is the virtuous objective.
Αφιερωμένο στους Θάσο και Άριστο. Αίεν αριστεύειν. —KAA

To my past and present mentors, who have contributed to my professional success, and to my friends and family, who have contributed to my success in everything else. —EMD

To Irene, Demitri, and Donovan, who make this all worthwhile. —GDD

To my family, my friends, and our past and current students; I think of everyone on my treks, and wish that you could see what I see. —JCH

I dedicate this to Professor Kyriacos Athanasiou, a pioneer in articular cartilage biomechanics and tissue engineering. —AHR

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Foreword to Second Edition

In 1892, the American Poet Walt Whitman celebrated the remarkable design and function of synovial joints: "the narrowest hinge in my hand puts to scorn all machinery" ("Song of Myself," *Leaves of Grass*, Walt Whitman Complete Poetry and Collected Prose 1891-1892, New York, Library of America, 1982, p. 217). Over the last half century, dramatic advances in prosthetic joint replacement have made it possible to restore mobility and relieve pain for millions of people with advanced joint damage. Continuing translational research, technological and procedural advances have made the current practice of synthetic joint replacement one of the most noteworthy successes in the annals of surgery.

Yet, Whitman's observation stands unchallenged; no current artificial joint comes close to replicating the function and durability of synovial joints. These complex structures developed and progressively evolved over millions of years. Formed from multiple self-renewing tissues, including joint capsule, ligament, in some cases meniscus, subchondral bone, synovium, and hyaline articular cartilage, they provide stable pain-free movement with a level of friction less than that achieved by any artificial bearing surface. The tissue central to these extraordinary functional capabilities is hyaline articular cartilage. Although it varies in thickness, cell density, and to some extent composition and mechanical properties among joints and among mammalian species, all articular cartilages share the same general structure and perform the same functions. These include lubricating the joint surface and minimizing peak stresses on subchondral bone by distributing loads. Perhaps the most extraordinary property of articular cartilage is durability; for most people, it provides normal joint function for more than 80 years.

Deterioration of synovial joints due to multiple causes, most commonly osteoarthritis, is the leading cause of pain and impairment in middle-aged and older people. Osteoarthritis can occur in any synovial joint and develops in every human population. Although osteoarthritis increases with aging, it is not a direct or inevitable result of aging changes alone. In addition to increasing age, excessive cumulative joint loading and joint injury are universal risk factors for osteoarthritis in all joints and all populations; yet, despite the recognition of these risk factors, the pathogenesis of the joint destruction that leads to the clinical syndrome of osteoarthritis remains obscure. No current treatments have been shown to prevent the onset or progression of osteoarthritis, although recent findings suggest that interventions to decrease the risk of osteoarthritis following joint injury may be possible.

In a concise cogent fashion, this second edition of *Articular Cartilage* summarizes current understanding of articular cartilage structure, function, development, maintenance, and degradation. Furthermore, exciting new information included in this volume lays the foundation for fresh approaches to preventing loss of articular cartilage and even restoring lost or diseased cartilage.

The book consists of seven chapters. Chapter 1 deals with the structure, composition, and function of articular cartilage, including lucid explanations of how the structure and organization of articular cartilage provide its unrivaled biomechanical properties, including lubrication of the joint surface. Chapter 2 covers articular cartilage embryogenesis, growth and maturation, and signaling pathways that have roles in these changes. Because articular cartilage lacks nerves and blood vessels, it was initially thought to be relatively inert; the identification of signaling pathways that control its formation, growth, and maintenance proves that this early impression was mistaken. Progress in understanding these pathways is likely to help explain the onset and progression of osteoarthritis and may lead to methods of detecting changes in tissue homeostasis before the cartilage begins to deteriorate. Chapter 3, "Articular Cartilage Pathology and Therapies," deals with the various forms of arthritis that lead to loss of articular cartilage and joint function, cartilage injuries and the response to injury, and contemporary and emerging methods for cartilage repair and restoration. Chapter 4 is devoted to tissue engineering of articular cartilage and explores the potential of *in vitro* tissue engineering for the restoration of articular surfaces. This chapter then goes on to summarize the sources of cells, biomaterials, and the use of scaffolds and bioreactors to promote formation of functional articular cartilage. As Chapter 4 clearly shows, tissue engineering has great promise for biologic restoration of synovial joints. In advancing understanding of the disorders of articular cartilage and their treatment or prevention, it is critical to have methods of evaluating articular cartilage quality. Chapter 5 discusses the imaging techniques and the quantitative assessment of cartilage components and methods of measuring mechanical properties to assess cartilage composition, structure, and function. It also includes a summary of the uses of large and small animal models to test the safety and efficacy of cartilage repair or restoration therapies. Breakthroughs in treatment of damaged or degenerated joints will depend on translating advances in basic cartilage research into clinical practice. Chapter 6 summarizes the challenges and opportunities in basic investigations of articular cartilage biology and regeneration and covers the business and regulatory aspects of potential methods of re-creating biologic articular surfaces. Chapter 7 presents detailed explanations of experimental protocols for generating and evaluating articular cartilage, including tissue and cell culture, tissue and matrix molecule analysis, RNA extraction, and testing mechanical properties, as well as animal protocols.

This well-organized, readable, and comprehensive second edition of *Articular Cartilage* is an important milestone in the understanding of one of nature's singular creations. It will serve as an essential resource for those who wish to contribute new insights into articular cartilage biology, as well as those who pursue clinically applicable technologies with the potential to reconstruct damaged or diseased joint surfaces. Although prosthetic joint replacement for people with advanced, essentially complete, destruction of hip, knee, and shoulder joints is effective, these procedures have limitations and in some instances devastating complications. Discovering ways to prevent the onset or progression of synovial joint destruction and to rebuild biologic articular surfaces would be among the most important developments in the history of medicine. This book gives encouragement and direction to those who seek to make these discoveries.

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Foreword to First Edition

The synovial joint is truly one of nature's marvels, providing our skeleton with a nearly frictionless bearing surface that can withstand forces of several times body weight for millions of loading cycles throughout life. To date, no man-made joint has been able to approach these capabilities. While the mammalian joint is clearly a highly complex biological and biomechanical organ that includes multiple structures, tissues, and cells, it is the articular cartilage—the tissue that lines the surfaces of synovial joints—that is fundamentally responsible for these unparalleled biomechanical properties.

Over the past century, our understanding of articular cartilage has grown exponentially. Building upon early studies that characterized the anatomy and histology of cartilage, scientists recognized its unique mechanical properties and function. By the mid-twentieth century, investigators had begun to develop new methods to quantify the elastic and tribological properties of the tissue. The 1960s and the 1970s were characterized by significant advances in the characterization of the biochemical composition of cartilage, primarily the proteoglycan and collagen components. With the development of the biphasic theory for modeling cartilage mechanics in 1980, the next two decades saw major breakthroughs in the understanding of the highly complex multiphasic, viscoelastic, anisotropic, inhomogeneous, and nonlinear properties of the tissue. Simultaneously, the study of cartilage development was revolutionized by the ongoing breakthroughs occurring in molecular biology and genetics in the 1990s. By the beginning of the twenty-first century, scientists and engineers had made tremendous strides in understanding how the incredibly complex composition and structure of cartilage were responsible for its load-bearing properties.

However, as with any other precision machine, even slight imbalances of the biological or biomechanical processes responsible for maintaining the tissue can lead to cumulative and progressive changes over decades of use, ultimately causing osteoarthritic failure of the joint. With the new depth of understanding of cartilage development, mechanics, and biology, the fields of tissue engineering and regenerative medicine have exploded in the effort to develop new therapies for preventing or treating cartilage damage by combining cells, biomaterials, bioactive molecules, and physical signals. While there are currently no disease-modifying therapies available for treating osteoarthritis, such tissue engineering approaches hold tremendous promise for the near future.

For the first time, the wealth of new knowledge in these areas is brought together in a single volume. *Articular Cartilage* represents the most comprehensive text to date focusing on this tissue and provides a unique and interdisciplinary approach that encompasses the breadth of basic science, bioengineering, translational science, and detailed methodologic approaches.

Chapter 1 broadly reviews the current state of knowledge on the structure and composition of different types of cartilage as well as the chondrocytes. In addition to presenting the molecular components of the tissue, this chapter provides overviews of the biomechanical function and properties of cartilage, as well as the structure-function relationships of the primary constituents of the tissue and cells.

A critical step in understanding cartilage physiology, pathophysiology, and regeneration is an understanding of the fundamental processes involved in cartilage development, maturation, and aging. In Chapter 2, the current state of knowledge of cartilage development is summarized, including the sequences of growth and transcription factors necessary for proper cell-cell and cell-matrix interactions required during the formation of the limb bud and the subsequent formation of the synovial joint. This chapter also reviews the changes that occur in the extracellular matrix and chondrocytes with maturation and aging, under normal or pathologic conditions.

Chapter 3 focuses on the epidemiology, etiopathogenesis, and therapeutic approaches for the major arthritides that affect cartilage and the synovial joints, namely, cartilage injury, osteoarthritis, rheumatoid arthritis, and gout. While these represent distinct disease processes, they are all characterized by degeneration of the articular cartilage and, eventually, loss of joint function. In particular, significant emphasis is placed on the role of biomechanical factors in the onset and progression of osteoarthritis. Furthermore, a review of the (lack of) current therapeutic approaches for osteoarthritis or cartilage injury clearly reveals a substantial unmet need for disease-modifying approaches to diseases that affect articular cartilage.

With recent evidence suggesting that over 10% of osteoarthritis may arise due to joint injury, it is clear that the development of new tissue engineering approaches for cartilage repair or regeneration can have a significant impact on this disease. Chapter 4 provides an up-to-date overview of the field of tissue engineering as applied to articular cartilage repair. Different sections provide highlights of recent advances in the classical "three pillars" of tissue engineering: cell source, scaffold design, and external stimulation through the use of bioactive molecules and mechanical bioreactors. The chapter also includes important discussion of the relative advantages and potential limitations of different cell types, biomaterial scaffolds, bioactive molecules, and bioreactors.

One of the primary hindrances to the development of new therapies for joint disease has been the lack of surrogate measures that provide valid, reliable, and responsive readouts of disease severity or progression. Such biological markers, or "biomarkers," may include proteins, genes, noninvasive or invasive imaging, or even biomechanical measures that reflect certain events in the disease process. In other fields such as cardiology and infectious diseases, biomarkers such as cholesterol levels, blood pressure, or antibody levels have served critical diagnostic and therapeutic roles. Chapter 5 overviews a number of methods that are used to assess the structure, composition, biology, and biomechanical function of articular cartilage. In addition to novel imaging methods such as MRI, such assessments may include histologic or immunohistochemical measures of joint tissues, or direct measures of tissue function through biomechanical testing. Due to the highly complex nature of cartilage, the proper determination of tissue material-level properties often involves the use of mathematical modeling that simulates the precise testing condition in tension, compression, shear, or contact (i.e., tribological testing). Finally, this chapter also provides a summary of different animal models and scoring systems that are often used for modeling and assessing disease or repair processes, with a critical review of their relative advantages and disadvantages.

With these issues in mind, Chapter 6 provides important discussion and perspectives on many of the remaining challenges and opportunities in the development and translation of new approaches for treating diseases of articular cartilage. A variety of issues are discussed, including some of the intrinsic characteristics of cartilage that appear to make repair of cartilage insuperable. In this light, alternative factors are discussed that may influence the success of regenerative therapies for cartilage, such as potential immunogenic responses. The ultimate success of such cell-based or biologic therapies, however, is highly dependent on practical issues such as regulatory pathways, intellectual property concerns, the pathway to market, and potential reimbursement. This chapter provides an important snapshot of the ever-changing landscape of regulatory and commercial affairs for medical products for cartilage repair.

The final chapter of the text provides detailed working protocols for many of the methods used to study articular cartilage. Beginning with standard cell and tissue harvest and culture methods, the chapter also details several culture methods, such as the use of 3D gels, that are commonly used for chondrocyte culture or cartilage tissue engineering. Methods for cartilage assessment via histology and immunohistochemistry are also provided. Importantly, detailed methods are provided for protein and RNA extraction from cartilage, which is generally more complex than other cells due to the presence of significant amounts of extracellular matrix. Finally, detailed protocols for mechanical testing of cartilage are provided.

This thorough and comprehensive text seamlessly integrates concepts of basic science, bioengineering, translational medicine, and clinical care of articular cartilage. By revealing the wealth of knowledge we have accumulated in this area, as well as exposing the tremendous opportunities for advancement, *Articular Cartilage* provides a critical template for those seeking to study one of the most complex tissues of the human body. Only through this level of understanding will we eventually be able to develop new methods to diagnose, prevent, or treat diseases of articular cartilage.

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