Articular Cartilage

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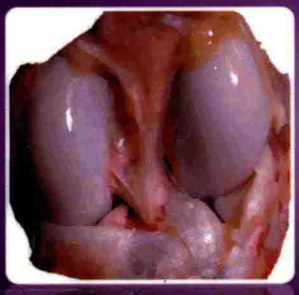

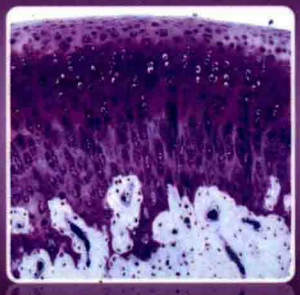

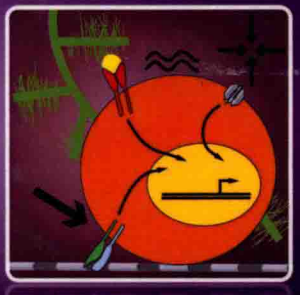

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Articular Cartilage

To Thasos and Aristos, please remember that pursuit of excellence is the virtuous objective.

Αφιερωμένο στους Θάσο και Άριστο. Αιέν 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, our past and current students, and my friends back in Houston; I think of everyone on my treks, and wish that you could see what I see.—JH

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

Abbreviations

Abbreviation	Name
2D	Two-dimensional
3D	Three-dimensional
AAOS	American Academy of Orthopaedic Surgeons
ACI	Autologous chondrocyte implantation
ACL	Anterior cruciate ligament
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADC	Apparent diffusion coefficient
AER	Apical ectodermal ridge
AERS	Adverse event reporting system
AFM	Atomic force microscopy
AGE	Advanced glycation end products
alphaGal	Gal alpha (1,3)gal antigen
Alx4	Aristaless-like homeobox
APC	Antigen-presenting cells
ASTM	American Society for Testing and Materials International
BCA	Bicinchoninic acid
BCP	Basic calcium phosphate hydroxyapatite
bFGF	Basic fibroblast growth factor
BLA	Biologics license application
BME	Beta-mercaptaethanol
BMP	Bone morphogenetic proteins
BSA	Bovine serum albumin
C-ABC	Chondroitinase-ABC
CACP	Camptodactyly-arthropathy-coxa vara-pericarditis
Cadherin	Calcium-dependent adhesion
CAIS	Cartilage autograft implantation system
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CDMPs	Cartilage-derived morphogenetic proteins
cDNA	Complementary DNA
CDRH	Center for Devices and Radiological Health
CFKH-1	Chicken winged–helix–loop/forkhead transcription factor 1
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
cGMP	Current good manufacturing practice
CILP	Cartilage intermediate layer protein
cmd	Cartilage matrix deficiency
COMP	Cartilage oligomeric matrix protein

COX2	Cyclooxygenase-2
CPM	Continuous passive motion
CPP	Calcium pyrophosphate
CS	Chondroitin sulfate
C_t	Cycle threshold
CT	X-ray computed tomography
Da	Dalton
DAB	3,3′-Diaminobenzidine
dGEMRIC	Delayed gadolinium-enhanced MRI of the cartilage
Dhh	Desert hedgehog
DLX	Distal-less homeobox
DMEM	Dulbecco's Modified Eagle's Medium
DMMB	1,9 Dimethyl methylene blue
DMOADS	Damage modifying osteoarthritis drugs
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSH	Disheveled
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethyleneglycoltetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ELP	Elastin-like polypeptide
ER	Endoplasmic reticulum
ERK	Extracellular-regulated kinase
ESCs	Embryonic stem cells
EULAR	European League Against Rheumatism
FACE	Fluorophore-assisted carbohydrate electrophoresis
FAOOS	Foot and Ankle Osteoarthritis Outcome Score
FBN1	Fibrillin 1 gene
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FGFs	Fibroblast growth factors
FRA2	FOS-like antigen
GAG	Glycosaminoglycan
GAIT	Glucosamine/chondroitin arthritis intervention trial
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GAPs	GTPASE activating proteins
GDF10	Growth/differentiation factor 10
GEFs	Guanine nucleotide exchange factors
GSK3	Glycogen synthase kinase 3
H&E	Hematoxylin and eosin
HA	Hyaluronan or hyaluronic acid
HCT/Ps	Human cells, tissues, and cellular- and tissue-based products
HDE	Humanitarian device exemption

HFB	Hydrodynamic focusing bioreactor
HGF	Hepatocyte growth factor
HHs	Hedgehog proteins
HIF1α	Hypoxia-inducible factor 1 alpha
HMG	High mobility group
HOOS	Hip osteoarthritis outcome score
Hox	Homeobox
HRP	Horseradish peroxidase
IACUC	Institutional Animal Care and Use Committee
IDE	Investigational device exemption
IFN	Interferon
IGF-1	Insulin-like growth factor
IHC	Immunohistochemistry
Ihh	Indian hedgehog
IKDC	International Knee Documentation Committee
IL	Interleukin
IND	Investigational new drug
iPSCs	Induced pluripotent stem cells
IRB	Institutional Review Board
IRS-1	Insulin receptor substrate
JNK aka SAPK	c-Jun N-terminal kinases
kDa	Kilodalton
KI	Knock-in
KO	Knockout
KOOS	Knee injury and osteoarthritis outcome score
kPa	Kilopascal
KS	Keratan sulfate
LCL	Lateral cruciate ligament
LEF/T	Lymphoid enhancer factor
LVDT	Linear variable differential transformer
MAPK	Mitogen-activated protein kinase
MCL	Medial cruciate ligament
MDa	Megadalton
MFH	Mesenchyme forkhead
MHC	Major histocompatibility complex
MMPs	Matrix metalloproteinases
MPa	Megapascal
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MSCs	Mesenchymal stem cells
MSU	Monosodium urate
MSX	Muscle segment homeobox
N-Cadherin	Neural-cadherin
N-CAM	Neural cell adhesion molecule
NDA	New drug application

NHIS	National Health Interview Survey
NSAID	Nonsteroidal anti-inflammatory drug
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
OOCHAS	Cartilage histopathology assessment system
OP-1	Osteogenic protein-1
PAI-1	Plasminogen activator inhibitor-1
PAX	Paired-box
PBS	Phosphate-buffered saline
PCL	Posterior cruciate ligament
PCL	Poly-caprolactone
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PDP	Product development protocol
PEG	Polyethylene glycol
PET	Positron emission tomography
PGA	Polyglycolic acid
PGE2	Prostaglandin E2
pH	Potential hydrogen
PI-3K	Phosphoinositide-3 kinase
Pitx	Paired-like homeodomain
pK_a	Acid dissociation constant
PLA	Polylactic acid
PLGA	Polylactic-co-glycolic acid
PMA	Premarket approval application
PRG4	Proteoglycan 4 (analogous to SZP)
PRX	Paired-box homeodomain
PSF	Penicillin–streptomycin–fungizone
PTC	Patched
qRT-PCR	Quantitative reverse-transcription PCR
QSR	Quality system regulation
RA	Rheumatoid arthritis
RAOS	Rheumatoid and arthritis outcome score
RGD	ARG–GLY–ASP
rhBMP	Recombinant human BMP
RNA	Ribonucleic acid
R-Smad	Receptor-activated Smads
RT-PCR	Reverse-transcription polymerase chain reaction
Runx2	Runt-domain transcription factor
SA-CAT	Stretch-activated cation channel
SCID	Severe combined immunodeficiency
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SHG	Second harmonic generation

SHH	Sonic hedgehog
SLRPS	Small leucine-rich repeat proteoglycans
SRY	Sex-determining region Y-box-9
SZP	Superficial zone protein (analogous to PRG4)
TBx	T-box
TEM	Transmission electron microscopy
TGF-β	Transforming growth factor-β
TIMPS	Tissue inhibitor of metalloproteinases
TnBP	Tributyl phosphate
TNFα	Tumor necrosis factor alpha
TNFβ	Tumor necrosis factor beta
TUNEL	Transferase dUTP nick end labeling
VEGF	Vascular endothelial growth factor
VSCC	L-type voltage sensitive calcium channel
Wnts	Wingless and interrelated proteins
ZPA	Zone of polarizing activity

Symbols	**Name**
E_R	Relaxed modulus
E_Y *or* E	Young's modulus
F_f	Friction force
G	Shear modulus
G^*	Complex shear modulus
H_A	Aggregate modulus
k	Permeability
N	Normal force
ε	Normal strain
γ	Shear strain
η	Viscosity coefficient
μ	Coefficient of friction
σ	Normal stress
τ	Shear stress
τ_ε	Relaxation time for constant strain
τ_σ	Relaxation time for constant stress

Foreword

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-20th 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 21st 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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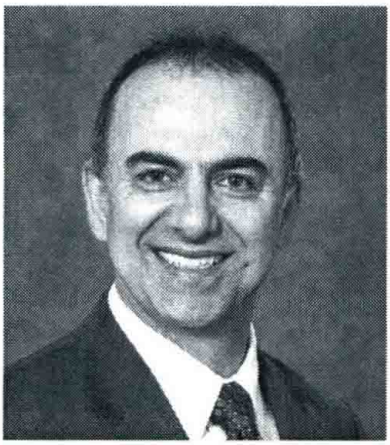

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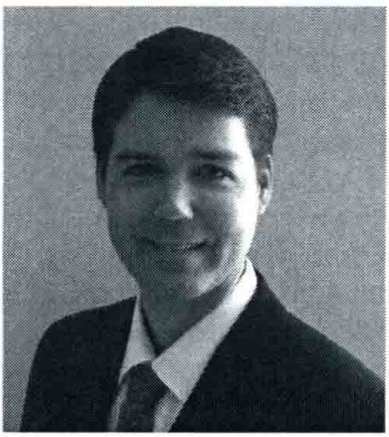

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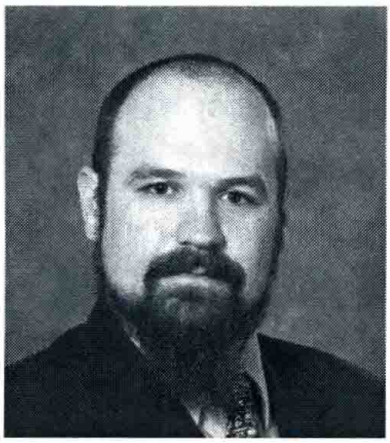

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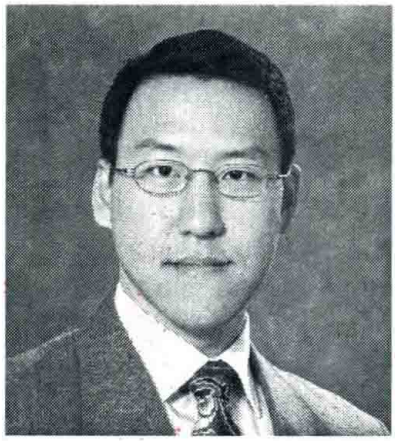

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