Steven A. Olson · Farshid Guilak
Editors

Post-Traumatic Arthritis

Pathogenesis, Diagnosis and Management

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To Diane and Thomas—Without your love and support,
I could not do what I do
To Mom and Dad—Thank you for allowing me to pursue my interest in medicine
To My Mentors—Bill Allen, Mike Chapman,
and Joel Matta—your dedication to excellence inspires me in all I do
To Farsh Guilak—Who is a gifted researcher, an unselfish mentor, and a true friend

Thank you,
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To Lori, Dina, and Justin, the loves of my life.
To my dear parents, Hooshang and Nahid, whose love, support, and inspiration are unparalleled.
To the best lab group in the world, who make every day fun, challenging, and exciting.
To Steve Olson, an incredible surgeon, scientist, and friend.

Thank you,
Farshid Guilak, PhD

Foreword

If cancer is the emperor of all maladies, osteoarthritis might be considered to be the peasants and serfs that inhabit the countryside. The American Cancer Society (ACS) estimates that about 15 million people are living today in the USA who have, or have had, some form of cancer. Among this group nearly half are over the age of 70 and the majority were diagnosed over 5 years ago. The ACS also estimates that the total of all healthcare costs in the USA attributable to cancer in 2011 was $88.7 billion. To confront this emperor, the NIH invests over $5 billion yearly in cancer research. Resulting advances in genetics have transformed our understanding of this disease and opened a new era in its treatment. In the last 10 years, about 300 new cancer drugs have been approved for the treatment of cancer and another 800 drug candidates are presently being tested in 3,137 active trials.

Clearly, the emperor is receiving royal attention and respect—serfs and peasants not so much. The Arthritis Foundation estimates that over 27 million people in the USA suffer from osteoarthritis. Healthcare costs attributable to this disease in the USA were estimated to be $185.4 billion in 2009. In terms of drug treatments, more has been lost than gained over the last 10 years as toxicities of available inflammatory and analgesic agents have been recognized. No new disease modifying drugs have been introduced and few if any are in development. This slow progress is mirrored by comparatively low funding levels for a disease that is recognized as the leading cause of disability in the USA. In 2015 the projected NIH budget for arthritis research is $240 million, much of which is allocated to inflammatory diseases such as rheumatoid arthritis and lupus. The problem with the search for better therapeutics for OA is not so much a lack of drug targets or lead compounds as is the challenge of testing potential therapeutic agents in humans. It is difficult to identify patients with very early OA who are likely to progress to joint damage, typically the disease evolves slowly necessitating lengthy observation periods, and finally it has been recognized that the plain joint X-ray is largely impractical as an end point for human clinical trials. As a consequence, most therapeutic trials have been conducted in patients with relatively advanced disease, when chances for benefit are likely lowest, the costs of trials have been high, and signals of efficacy have been meager at best. Clearly a better approach to drug development for OA is needed. Fortunately

the NIH has recognized that a major hurdle is identification of better measures of disease outcome in OA and has launched the Osteoarthritis Initiative, a component of which is seeking new biomarkers that predict risk of progression of OA.

Clinically, joint space narrowing, osteophyte formation, and sclerosis of subchondral bone define OA. This radiologic picture represents the final outcome of diverse processes that alter the tissues of joints including genetic factors, mechanical forces, neurological alterations, metabolic disturbances, oxidative stress, inflammation, and cellular senescence. Most if not all of these processes are likely to be engaged to variable degrees in any individual with OA. Finding ways to optimize the care of patients with OA requires full understanding of each of these processes and how they interact to affect the musculoskeletal system. For example an ACL tear in a healthy adolescent is likely to be dominated almost exclusively by the mechanical alterations while the same injury in an older obese smoker with diabetes may superimpose this injury upon joint tissues that are already altered by metabolic and oxidative stress. As implied by results in the MOON study (reviewed in Chap. 20), evolution of OA in these two individuals proceeds at different rates and along different paths and is likely to require different treatment strategies. A classification scheme for OA based on the relative contribution of these different processes could potentially streamline clinical care and drug development for all patients who have or are in the process of evolving OA.

Post-Traumatic Arthritis: Pathogenesis, Diagnosis, and Management fills admirably a major need for a comprehensive and up-to-date assessment of the arthritis that follows joint injury. The authors use the terms post-traumatic arthritis and post-traumatic osteoarthritis interchangeably. Perhaps to put a fine distinction upon terminology, post-traumatic arthritis might apply when an overwhelming injury damages the joint tissues beyond recovery whereas post-traumatic osteoarthritis might apply when joint trauma sets in motion pathophysiological processes that over time render the joint dysfunctional. For example the long-term outcome of a fracture that tears cartilage apart so that healing is not possible might be categorized as post-traumatic arthritis whereas an ACL injury that initiates pathophysiological processes that lead to dysfunction in joint tissues that were not part of the initial injury might be categorized as post-traumatic osteoarthritis. This distinction becomes important because different interventions are likely to be required in these separate conditions. In this work, Guilak and Olson have worked with a distinguished group of authors to assemble the entire body of knowledge of how mechanical stress can promote joint dysfunction and how these processes can be studied to gain an even more refined picture in future studies. The work ranges across basic in vitro studies to animal models to human trials and includes analysis at both structural and biochemical levels.

This work will be of interest to basic investigators who are interested in gaining a better understanding of the tissues of joints and how they interact in health and disease, to clinical investigators who are seeking to translate basic

biological insights into an understanding of the disease mechanisms of OA in humans, to drug developers who are seeking strategies to improve tissue healing after overwhelming injury and new interventions that can halt OA progression, to clinical trialists as they contemplate testing of new therapeutic agents for OA, and to clinicians as they seek to optimize care for patients with OA. In summary this compilation of knowledge fills a major need that is expressed in rheumatology, Orthopedic surgery, bioengineering, physical therapy, and healthcare policy. I congratulate the editors and all of the authors for this enormously important contribution.

Bronx, NY, USA John A. Hardin, MD

Preface

This text is an outgrowth of the career-long interests of the editors. Over 12 years ago we had the good fortune to begin a collaboration that continues today. While we each had interests in the impact of arthritis on joint function, we approached this area in different ways earlier in our careers.

As a clinician (SAO) I recognized early in my career the adverse impact of post-traumatic arthritis (PTA) on my patients' lives. Understanding the role of articular fracture care in the development of PTA became a research interest of mine. As a basic investigator my early research focused on biomechanical assessment of the effects of articular fractures in the hip joint. We worked to develop a large animal model of an acetabular fracture. As a trauma surgeon my intent was to better understand the effects of varying accuracy of articular reduction.

As a bioengineer (FG) my early work focused on the role of biomechanical factors in joint health and disease. At the time, our studies of PTA were centered on soft tissue injury models of the knee, such as transection of the anterior cruciate ligament or meniscectomy models, which were generally regarded as models of "instability." As I began this collaboration with a trauma surgeon, I became fascinated by the complex and rapid PTA that he regularly observed clinically following articular fracture—this was a common, extremely debilitating condition, but we knew so little about the mechanisms that led to PTA following joint injury.

As our collaboration began we chose not to focus on reduction, but rather to observe the natural history of PTA development after an articular fracture. We decided to develop a new murine model in this regard so that we could then take advantage of the wide array of genetic models that could be used to study the disease process. More than any other, this one decision has set the stage for everything that followed in our collaboration. The work product of this collaboration from experimental model development to recent work is presented in Chap. 8.

When we began to collaborate, we recognized that very little work was published or funded on arthritis that develops after injury. This meant that the field of PTA was wide open; however it also meant that the impact of joint injury on PTA development was unappreciated. Over the past decade or more many investigators have contributed to our knowledge of joint injury and PTA development. Many of them have authored chapters for this text. A traditional approach of good scientific investigation is to study a complex

system such as an intra-articular fracture by focusing on an individual component of the injury while controlling for other factors. Yet clinically the multiple components of a joint injury that occur in the clinical setting do not happen in isolation. Another approach to studying such a complex system is to observe the mechanisms involved in the natural history of PTA development in a model of an articular fracture. In inviting authors to participate in this text we have tried to be as inclusive as possible regarding the approach to the problem of PTA development. We believe that all of these approaches are valuable. This text is an attempt to bring the current thinking on PTA into one document. It is intended to be a resource for those clinicians and investigators who are interested in working in the field of PTA. We hope that you find it valuable.

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Section I

The Problem of Post-Traumatic Arthritis

1 Arthritis That Develops After Joint Injury: Is It Post-Traumatic Arthritis or Post-Traumatic Osteoarthritis?

Steven A. Olson and Farshid Guilak

Arthritis is the nation's most common cause of disability, with one in three adults of working age (18–64 years) having arthritis-attributable work limitations [1]. An estimated 12 % of all patients seeking surgical intervention for symptomatic arthritis have an etiology post-traumatic arthritis (PTA) indicating that the development of arthritis followed a previous injury to the involved joint [2]. The overall burden of disease of PTA is explored in detail in Chaps. 2 and 3. It is sufficient here to indicate that this 12 % estimate likely understates the contribution of PTA to the overall burden of arthritis.

While the role of joint injury in the onset and progression of arthritis has long been recognized [3, 4], little direct research in the etiopathogenesis, disease mechanisms, or therapy was performed until the 1970s, when several animal models of PTA were popularized involving ligamentous or meniscal injury [5, 6]. Simultaneously, investigators began to study the effects of trauma on articular cartilage in vitro and in vivo [7, 8]. It is now apparent that many forms of joint injury may contribute to the development of arthritis, ranging from soft-tissue injuries, cartilage impact, and more severe injuries that involve articular fracture. The clinical data and basic science data in these areas are explored further in Chaps. 4–18.

One of the challenges of PTA is that frequently the clinicians that manage injured joints are surgeons, while in general the basic science of arthritis has been developed by biologists, bioengineers, and rheumatologists with specific interest in arthritis [9]. In traditional texts of arthritis it is common not to mention of trauma as a cause of arthritis, or only as a secondary cause of arthritis [10]. With the increased incidence of joint injuries due to greater sports activities as well as military injuries, this is changing. However as the knowledge in the field has grown, there is a void of pooled information on clinical and basic investigation into the various types of joint injury, the articular response to these types of injury, and the observed joint degeneration that develops after these various forms of joint injury. This text is an attempt to begin to fill this void.

The editors have collaborated in this area for some time and have traditionally referred to this condition as PTA [9, 11–14]. However, as we have worked with our contributing authors for this text, we have recognized that a variety of our colleagues prefer the term post-traumatic osteoarthritis (PTOA) when referring to similar conditions [15]. These differences in the semantics of this naming of this condition led us to

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think more deeply about the underlying pathophysiology and origins of joint degeneration that develops after joint injury. There is no recognized classification or diagnostic criteria specific to PTA, outside of standard osteoarthritis scoring methods. PTA is a clinical diagnosis made with a combination of symptomatic complaints and radiographic changes suggestive of articular degeneration after fracture or other joint injury [16–18]. Arthritis developing after joint injury has been referred to as both PTOA and PTA as we have in this text [19, 20]. Arthritis has been reported to occur following a variety of different joints, as well as varying types of joint injury. A workgroup from Osteoarthritis Research Society International (OARSI) tasked to define osteoarthritis (OA) did not provide guidance as to when osteoarthritis symptoms that develop after joint trauma should be considered post-traumatic in nature or simply standard OA per se [21].

In 2011, the OARSI workgroup published a broad definition of the disease state of OA [21]. The workgroup reported that "OA is usually a progressive disease of synovial joints that represents a failed repair of joint damage that results from stresses that may be initiated by an abnormality in any of the synovial joint tissues, including articular cartilage, subchondral bone, ligaments, menisci (when present), peri-articular muscles, peripheral nerves, or synovium. This ultimately results in the breakdown of cartilage and bone, leading to symptoms of pain, stiffness, and functional disability. Abnormal intra-articular stresses and failure of repair may arise as a result of biomechanical, biochemical, and/or genetic factors [21]." Such a broad definition appears to incorporate all potential aspects of sequelae of joint injury. However, when this definition is viewed in the context of discriminating OA from inflammatory arthropathy such as rheumatoid arthritis, some subtle distinctions emerge. In rheumatoid arthritis, the pathology is driven by the inflammatory synovitis that is part of the condition. However, to be distinct from osteoarthritis, this inflammatory state is not an isolated stress leading to joint damage as suggested in the definition of osteoarthritis. The inflammation in this situation is an ongoing process that when controlled leads to the restoration of homeostasis within the joint.

PTA can occur following a variety of types of joint injury. Not surprisingly, joint degeneration can occur rather rapidly after a severe joint injury such as an articular fracture [18, 22]. Articular fractures consist of a number of injurious elements to the joint. These include physical disruption of the articular cartilage, synovium, menisci, ligaments, or subchondral bone, as well as exposure of the joint to both necrotic and apoptotic cell death [9, 18]. In addition, there is an intra-articular response to injury that can include a substantial inflammatory component following an intra-articular fracture as well. Recent studies have reported that the inflammatory response after intra-articular fracture appeared to be strongly associated with the development of PTA in a closed articular fracture in a mouse knee [23]. This work is explored in more detail in Chaps. 8 and 25. In this sense, the intra-articular fracture has elements that appear within the definition of osteoarthritis as well as those elements that appear more consistent with the inflammatory arthropathy mechanism. Said in another way, it appears that the intra-articular fracture results in an "organ-level" response of the joint to injury [24]. This is consistent with the concept of osteoarthritis as a whole-joint disease that involves interactions among the articular cartilage, the synovial lining of the joint, the subchondral bone, and the bioactive substances present within the synovial fluid that generates the response within the joint after injury [23, 24]. In this way a severe injury such as an articular fracture can serve as the basis of an investigational model for multiple types of joint injury.

An organ-level response within the joint would necessarily consist of several levels: a cellular- and tissue-level response, a joint-level response, and a systemic-level response [23, 24]. The cellular and tissue level would include factors such as cell death and alteration in both local microstructural characteristics and physiologic state of joint tissues. These changes would serve as a stimulus to activate an innate immunity response to injury. The joint-level response would include the alteration in joint mechanics secondary to instability,

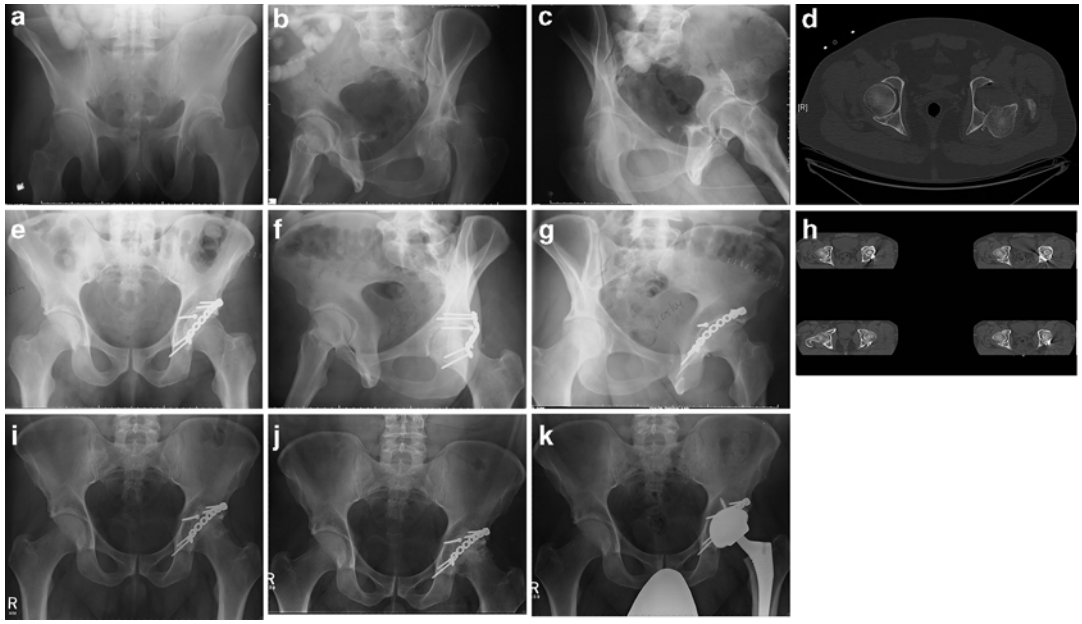

Fig. 1.1 A case presentation of a 40-year-old patient who sustains a posterior wall fracture of the acetabulum with subluxation of the femoral head. The patient reported no pre-injury hip symptoms. (**a**–**c**) AP, obturator oblique, and iliac oblique radiographs of a patient with a displaced posterior wall acetabular fracture. (**d**) CT scan demonstrating displacement of the posterior wall fracture and subluxation of the femoral head. (**e**–**g**) AP, obturator oblique, and iliac oblique radiographs of postoperative reduction of the acetabulum articular fragment and femoral head with fixation of the posterior wall. (**h**) CT scans after surgical reduction and fixation. Beginning from the *top left* the images proceed from *left to right*. Careful inspection of the image in the *top right* demonstrates a small gap in the articular surface representing focal cartilage and subchondral bone loss. The remaining images show an otherwise anatomic restoration of the joint surface. (**i**) AP radiograph taken at 1 year post-injury. There is evidence of joint space loss and sclerosis of the subchondral bone. This is early-onset post-traumatic arthritis (PTA). (**j**) AP radiograph taken at 4 years after the injury. Essentially complete loss of joint space is now apparent with severe PTA present. (**k**) AP radiograph taken after total hip replacement performed as treatment for PTA symptoms

focal articular defects, or fractures as well as alterations in the biologic environment such as an activation of an inflammatory response for a variable period of time after injury. The systemic response would include the shock associated with injury and systemic levels of released bioactive factors such as serum inflammatory cytokines or other signals available to the joint. All of these factors combined contribute the injury response within the joint that leads to degeneration and ultimately to PTA (Fig. 1.1).

Within this text, we have asked contributors to provide in-depth review of several potential mechanisms contributing to the development of arthritis after joint injury. These include cell death, reactive oxygen species available in the joint, direct mechanical overload, and inflammation within the joint after injury. There is some evidence for all of these mechanisms being present in various forms of arthritis that occur after joint injury. It is likely that some forms of arthritis after injury result in a similar clinical endpoint as primary (idiopathic) osteoarthritis. However, it also appears that there are situations in which the response to injury may be more suitable for a focal response such as an anti-inflammatory therapy or other targeted therapy that may not be generally applicable in osteoarthritis per se. In this sense, we believe that both terms, PTA and post-traumatic osteoarthritis (PTOA), are appropriate and they are interspersed throughout this text. These terms are used in an interchangeable

manner from chapter to chapter. We thank the many important contributions of the authors, and we hope that this text provides a basis for the next generation of studies on the pathogenesis, diagnosis, and management of this disease.

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Post-Traumatic Arthritis: Definitions and Burden of Disease

2

Joseph A. Buckwalter and David T. Felson

Introduction

Osteoarthritis is the most common form of arthritis. It accounts for more mobility disability than any other disease and is one of the leading causes of disability in the USA and worldwide [1]. Data from the National Arthritis Data Workgroup in the USA suggests that roughly 6 % of adults aged 30 and over have symptomatic OA of the knee and of those aged 60 and over, the prevalence rises to 12 % [2]. The prevalence of symptomatic hip OA is approximately half that of knee OA. The prevalence of knee OA in the UK is roughly similar to estimates in the USA [3]. Ankle OA, while often uniquely post-traumatic, is much less prevalent [4] than arthritis in the knee or hip. Roughly 7 % of older persons have symptomatic hand OA [5] and based on population surveys, hand OA more often has effects on pain and function than commonly acknowledged. These prevalence estimates are based on counting persons with a positive X-ray for OA and joint pain in the affected joint as having disease. Since it has become clear that radiographic changes of OA occur late in disease and many persons without X-ray OA may have joint pain with MRIs showing OA [6], the prevalence of OA is likely to be even higher than current prevalence estimates suggest.

The prevalence of knee OA in China appears to be at least as high as in the USA [7] but, like the USA, most of it is not associated with major prior joint injury. However, the high rates of knee OA in rural communities including in China suggest that joint injury, either acute or chronic, plays a major etiologic role in knee OA.

Knee OA prevalence is rising in the USA and there is a commensurate rise in the rates of knee replacement. While most of this increase in prevalence may be due to aging and increased ponderosity of the US population, other factors are also at play. For one, given the same severity of radiographic knee OA, persons currently seem to be more inclined to complain of knee pain and OA symptoms than their predecessors [8].

Major joint injury causes a large percentage of OA of the knee in the community and causes the majority of cases of OA in joints that are otherwise rarely affected by disease including ankle, wrist, and elbow. Also, post-traumatic knee OA secondary to sports-related injuries may be increasing.

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Post-Traumatic Osteoarthritis

Post-traumatic osteoarthritis (PTOA), the osteoarthritis that develops following joint injury, causes life-long pain and disability for millions of people [9–11]. Acute joint injury and post-traumatic residual joint abnormalities, primarily instability and articular surface incongruity, lead to progressive loss of articular cartilage, to bone remodeling, and to changes in the joint soft tissues, resulting in PTOA. Unfortunately, current treatments of joint injuries all too often fail to prevent PTOA [9, 10, 12].

PTOA is due to synovial joint degeneration initiated by mechanical joint injury followed by localized and whole-joint biologic responses, including release of inflammatory mediators, that contribute to progressive tissue destruction as well as repair responses [9, 13–17]. Such injuries include joint dislocations, joint ligament and capsular tears, meniscal injuries, intra-articular fractures, and articular surface blunt impact injuries and contusions [9, 13, 18–22]. A substantial fraction (approximately 12 %) of the overall burden of disease of OA in hips, knees, and ankles arises secondary to joint trauma [10, 23]. In addition, PTOA due to intra-articular fractures (IAF) is the most common cause of combat-related disability in US military service personnel [24].

Clinical and epidemiologic studies show that joint injuries dramatically increase the risk of OA [25, 26]. A study of 1,321 former medical students found that 13.9 % of those who had had a knee injury (including meniscal, ligamentous, or bone injuries) during adolescence or young adulthood developed knee OA, as compared with just 6 % of those who did not have a knee injury [26]. Other studies have shown that even with the best current treatment, as many as one in four patients develop OA after fractures of the acetabulum [27, 28], between 23 and 44 % of patients develop knee OA after intra-articular fractures of the knee [29–31], and more than 50 % of patients with fractures of the distal tibial articular surface develop OA [32–34]. A long-term follow-up study indicates that patients who suffer from ligamentous and meniscal injuries of the knee have a tenfold increased risk of OA, compared to patients who do not have a knee injury [35]. In the Framingham Study, a reported history of major knee injury (sufficient to require a cane or crutch) increased the risk of subsequent knee OA 3.5-fold in men and less so in women [36] and led to an estimate that roughly 10 % of knee OA was due to antecedent major knee trauma.

Since articular fractures and other joint injuries that lead people to seek medical attention occur at a rate estimated at 8.7 per 100 persons per year [37], the number of people at risk of PTOA is substantial. For these reasons, PTOA is almost certainly much more common than has been recognized [26]. A report from the University of Iowa supports this contention [10]. This study of patients presenting to the University of Iowa Department of Orthopedics and Rehabilitation with disabling hip, knee, and ankle OA showed that 1.6 % of patients with hip OA, 9.8 % of patients with knee OA, and 79.5 % of patients with ankle OA had a verified history of one or more joint injuries [4, 10]. Extrapolation from this patient population suggests that the total number of patients in the USA with disabling PTOA of hip, knee, or ankle approaches six million, and that PTOA accounts for approximately 12 % of societal expenditures for OA as a whole. In addition, unlike most other forms of OA, PTOA often affects younger adults for whom joint replacement is not a desirable treatment; in a study of patients with disabling hip, knee, and ankle OA, the patients with a history of joint trauma on average were more than 10 years younger at the time of presentation to the clinic than were patients without a history of joint trauma [4].

The time from injury to the onset of PTOA varies. Following severe joint injuries, including intra-articular fractures, PTOA may develop in less than a year; less severe injuries, including some articular surface fractures, joint dislocations, and ligamentous, meniscal, and joint capsular injuries, may not lead to PTOA for decades. With the best current care of significant joint injuries, the known lifetime risk of PTOA in those who have sustained major joint injuries ranges from about 20 % to more than 50 % [9]. And, despite the evolution of surgical interventions for the

treatment of joint injuries (in particular, articular fractures and anterior cruciate ligament tears), the risk of PTOA has not decreased appreciably in the last 25 years [9, 38].

Mechanisms Responsible for PTOA

Clinical experience and experimental data show that the mechanical causes of PTOA fall into two general categories: acute structural damage induced by the intense loads occurring at the instant of joint injury, and gradual-onset structural damage and cartilage compositional degradation due to chronic loading abnormalities of injured joints. In addition to structural damage, most acute joint injuries cause clinically apparent joint inflammation. In the specific case of articular surface impaction injuries, acute contusion of the cartilage may or may not be associated with clinically detectable articular surface fracture even though there may be significant cell death [39]. As regards articulation abnormalities responsible for gradual onset of progressive tissue damage and degradation after joint trauma, two common causes are joint instability and residual articular incongruity, both of which involve well-documented levels of chronic local contact stress elevation [9, 40–42].

Acute high-intensity joint injuries that initiate joint degeneration involve damage of the articular surface. In many instances, that damage includes macroscopic structural disruption of articular cartilage and subchondral bone: intra-articular fracture. Recent studies of human distal tibial articular surface joint fractures showed that the risk of PTOA following an acute articular surface injury is closely related to the mechanical energy absorbed at the instant of the joint injury: intra-articular fractures of the tibial plafond that involve absorbed energy levels exceeding a specific threshold predictably lead to OA within 2 years [43].

However, many acute joint injuries cause tissue damage even in the absence of visible disruption of the articular surface [9, 39, 44]. In these instances, the acute impact damage may be limited to alterations in matrix composition or microstructure, accompanied by localized cell death [38, 45–47]. As discussed above, evidence from in vitro studies shows that acute cartilage injuries initiate biologic responses that cause progressive cell death, extending from the site of the impact [9, 48]. In addition, cells that survive in damaged cartilage typically exhibit metabolic disturbances that tend to amplify the initial mechanically induced structural disruption, thus serving to further weaken the cartilage matrix and lower its tolerance for mechanical stress [9, 14, 48].

One of the most important recent advances in understanding of PTOA has been the recognition that while mechanical injury causes direct tissue damage, PTOA is not a direct or inevitable consequence of the initial mechanical damage. For example, an in vitro study of intra-articular fractures in human ankle joints showed that even high-energy joint impact kills relatively few chondrocytes, but the proportion of dead cells increases steadily over the 48 h following injury suggesting that mediators released from the damaged cartilage cause progressive cell death [49]. Other in vitro studies have shown that inhibiting or blocking reactive oxygen species and other mediators, including alarmins, that are released from damaged cartilage decreased injury-induced chondrocyte death [9, 14, 16, 17, 48, 50–52].

As suggested by the above studies of progressive cell death following cartilage injury, an increasing body of evidence shows that joint biologic responses to mechanical injury, including release of inflammatory mediators, play a key role in the onset and progression of cartilage loss following joint injury [9, 14, 48–51, 53–57]. This understanding, combined with in vitro identification of post-traumatic biologic mediators of progressive matrix degradation and chondrocyte dysfunction and death [9, 14, 48, 51, 58], in concert with improved understanding of how increased articular surface contact stress causes cartilage loss, creates the opportunity for development of new biologic and mechanical interventions to decrease the risk of PTOA [9].

The second major cause of PTOA is gradual structural deterioration caused by chronic loading abnormalities stemming mostly from effects of the acute injury. Based on clinical experience

surgeons have assumed that residual joint surface incongruity following an intra-articular fracture and joint instability following a ligamentous, meniscal, or joint capsular injury increases the risk of PTOA. A recent study confirmed the role of incongruity in causing PTOA and that articular cartilage is lost first in the areas of the highest cumulative contact stress [43]. Although clinical experience shows that joint instability due to ligamentous injury—for example, ACL tears—increases the risk of PTOA, quantifying joint mechanical instability in living humans and studying its relationship to OA are challenging. However, a study of human ankle joints in vitro, using a methodology (Tekscan) that measured instantaneous joint surface contact stress, showed that joint ligamentous instability increased peak contact stress by 20–25 %, and that it increased the magnitude of peak positive and peak negative contact stress time rates of change by 115 % and 170 %, respectively, in joints with a 2 mm step-off incongruity [40, 41, 59]. Investigation of varying degrees of knee joint instability in rabbits found that increased degrees of instability following partial versus complete ACL transections correlated directly with the development of histologically apparent articular cartilage damage [42]. These experimental studies support the clinical impression that joint instability increases joint contact stresses and stress rates of change, and that over time, increased contact stress leads to PTOA.

These experimental studies have suggested a role of injury-related joint instability as a cause of joint damage. However, they have not measured the degree of instability, or shown whether increased joint instability is associated with evidence of increased joint damage over time. To explore this important issue Tochigi and coinvestigators developed an in vivo model of variable instability in which joint stiffness could be measured, both for complete ACL transections and for graded partial ACL transections. That study demonstrated that increased joint instability is associated with increased cartilage degeneration, continuously over the range of instability increase [42].

Some PTOA patients have combinations of initial tissue damage due to intense acute injury and chronic post-injury joint abnormality, while others have primarily one or the other of these problems. For example, patients with comminuted intra-articular fractures have sustained not only a high-intensity joint injury, but also in many instances have residual joint incongruity. In contrast, mild (noncontact) ligament or capsule tears may not cause clinically apparent articular surface injury or joint inflammation, but nevertheless can lead to PTOA over a period of years, possibly due to increased joint instability.

Since the pathways through which the two general mechanical causes of PTOA (acute injury and chronic loading abnormality) that lead to joint degeneration are not well understood, and since it is usually not possible to separate their respective effects in studies of human joint injuries, it has been difficult to develop methods of evaluating an acute joint injury that will accurately predict which patients will progress to PTOA. This uncertainty obviously also hinders efforts to devise better treatments to forestall, mitigate, or prevent that progression.

Although overlap exists between the two general mechanical causes of PTOA, there is a substantial difference between the PTOA that develops primarily as a result of acute intense joint injury, versus the PTOA that develops chronically due primarily to instability or incongruity. Acute joint injuries are a single discrete event, causing immediate structural damage and cell death and triggering acute inflammatory and repair responses. By contrast, the PTOA arising primarily from residual instability and incongruity is the result of repeated smaller mechanical insults not involving significant fractional cell death or pronounced inflammatory responses, but instead involving gradual degradation of cell metabolic function, and reduced maintenance of matrix composition and structural integrity.

Evaluation of Joint Injuries and Risk of Post-Traumatic Osteoarthritis

Currently, physicians treating patients with joint injuries have limited ability to assess the severity of the injury. The patient's history of the injury and the physical examination of injured joint(s)

provide a general impression of the tissue damage, but do not reliably predict the risk of PTOA.

Commonly used methods of assessing a damaged articular surface include plain radiographs, CT scans, and MRI. Plain radiographic and CT scan studies of intra-articular fractures can demonstrate the disruption of the articular surface and the degree of displacement of the fracture fragments, and they therefore have been used to classify injury patterns. However, the reliability of current articular fracture classification systems is questionable [60, 61], and even articular fracture classifications based on three-dimensional CT reconstructions have disappointing reliability [62]. It is not surprising, therefore, that articular fracture classification systems have been characterized as useful in describing injuries, but not as being helpful in selecting a treatment [63].

MRI can demonstrate some types of articular cartilage disruption, but only recently have investigators started to define the relationships between MRI signal characteristics and changes in articular cartilage composition and mechanical properties [64–68]. And, as of yet, relationships between specific MRI changes following acute joint injury and development of PTOA have not been defined. Currently, therefore, there is limited understanding of the relationships between the severity of the structural injury to a joint, the biologic response to injury, and the onset and progression of PTOA.

Physicians currently base treatments intended to prevent PTOA on clinical impressions and accumulated experience. They have little basic scientific and bioengineering research to guide their clinical practice. Because the biologic response of the joint tissues to injury is not well understood, molecular- and cell-based treatments to minimize progressive joint damage are not a part of current injury management. Orthopedic surgeons routinely perform extensive surgical procedures in an effort to restore the alignment and congruity of articular surfaces following intra-articular fractures [69]. The purpose of these anatomic reconstruction procedures is to decrease residual joint incongruity, and thereby decrease focal elevations of contact stress presumed to be responsible for PTOA. Unfortunately, surgical exposure, reduction, and fixation of a fractured articular surface can lead to serious complications such as necrosis of bone fragments or soft tissues, infection, and nerve and blood vessel injuries. In some instances the complications of surgical treatment of fractured articular surfaces lead to disability and/or even to amputation. Surgeons also reconstruct torn ligaments, menisci, and joint capsules, partially to decrease instability and thereby lower the risk of PTOA.

The ability of surgeons to restore joint stability and articular surface congruity has improved dramatically in the last 25 years. However, a number of clinical follow-up studies show that between a fifth and over half of patients still develop OA following current surgical treatments of common articular surface and ligamentous injuries [28, 38, 70], an observation that suggests that the best current surgical restorations of joint stability and congruity alone neither prevent nor perhaps even significantly decrease the lifetime risk of PTOA for many patients. Surgical treatments of joint injuries will continue to improve, but better understanding of how mechanical injury leads to PTOA has the potential to lead to new methods of treating joint injuries that, combined with surgical treatment, decrease or prevent progressive loss of the articular surface.

Age and Post-Traumatic OA

After age 40, the incidence of OA rises dramatically with every passing decade [37, 71]. Articular cartilage normally undergoes significant structural, matrix compositional, and mechanical changes with age [72–77]. But, these changes differ in many respects from those seen in osteoarthritic joints, and therefore by themselves do not explain the association between increasing age and increasing incidence of OA [78].

In contrast with the extensive studies of aging changes in articular cartilage matrix composition, age-related differences in healing of articular cartilage injuries following joint trauma have not been well investigated. However, basic scientific studies show that articular cartilage chondrocytes undergo aging changes that could affect their ability to repair articular surface damage, or maintain undamaged articular cartilage following

a joint injury. These changes include declining response to IGF-I, decreased mitotic activity and cell senescence, and oxidative damage [58, 73, 74, 76, 79–81].

The available clinical studies indicate that the risk of developing post-traumatic OA following joint injury increases with age. The risk of OA following an intra-articular fracture of the knee increases as much as three- to fourfold after 50 years of age [29, 30, 82]. Other clinical studies demonstrate that age increases the risk, or decreases the time until development of OA following ligamentous and meniscal injuries [83, 84].

Increasing age and joint injury are the two most significant risk factors for PTOA. Taken together, the scientific and clinical observations reviewed above suggest that increased age significantly increases the risk of OA following joint injury, possibly as a result of an age-related decrease in the ability of chondrocytes, and possibly other cells, to restore and maintain the articular surface

Is Most OA Post-injury OA?

The current clinical definition of post-traumatic osteoarthritis is osteoarthritis that develops following a specific clinically apparent joint injury including ligamentous, capsular, and meniscal injuries and injuries to the articular surface. And based on evidence from epidemiologic studies in which subjects are queried about a history of joint injury and surgery, roughly 10 % of combined knee, hip, and ankle OA is due, in large part, to a memorable acute joint injury.

As noted earlier, joint changes with age including senescent changes in cartilage may make joint cartilage and other soft tissues within the joint more fragile and easily damaged. Recent evidence suggests that trivial joint injuries, often unappreciated when they occur, may account for a large percentage of OA, especially in the knees of older persons. Englund et al. [85] found that up to 50 % of persons in their 50s and 60s had meniscal tears, degenerative tears that were present without any recollection of knee injury and often without accompanying evidence of osteoarthritis. When followed in a longitudinal study in which subjects got repeated MRIs, these subjects had a threefold increased risk of developing radiographic OA of the knee when compared to others of the same age and sex who did not have tears [86]. Among those whose knees showed no cartilage defects, meniscal tears increased the risk of disease tenfold. While degenerative meniscal changes may indicate a more diffuse process of senescent change that also affects hyaline cartilage, Chang et al. [87] have shown that solitary degenerative tears create later cartilage defects at the site of the tear and not diffusely, suggesting that the tears caused disease. Given the high prevalence of meniscal tears in the knees of middle-aged and older men and women and the high attendant risk of OA conferred by these tears, up to 50 % of knee OA may be caused by them [88], far more than the proportion of disease caused by acute severe knee injuries. Ultimately, however, since these injuries are only occasionally recognized when they occur, it is unclear whether these injuries could be detected and, given the poor tissue substrate on which they occur, whether the progression to OA could be mitigated or prevented.

Implications

Recent clinical and experimental in vivo and in vitro studies of the relationship between injurious mechanical forces applied to synovial joints and articular surfaces and loss of articular cartilage have added considerably to the understanding of PTOA. Epidemiologic studies have confirmed a strong relationship between joint injury and PTOA. The magnitude of the acute and repetitive mechanical forces that cause PTOA in patients with tibial plafond fractures has been defined. Biologic mediators of cartilage destruction, including inflammatory mediators, triggered by mechanical forces have been identified, and a variety of agents that inhibit the actions of these mediators have shown promise as potential methods of decreasing articular cartilage degradation. These observations suggest that new surgical treatments of joint injuries to minimize

post-traumatic joint incongruity and new biologic treatments of joint injuries to inhibit the actions of biologic mediators of cartilage destruction have the potential to decrease the risk of PTOA following a wide range of joint injuries.

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Arthritis After Joint Injury: The Military Experience

3

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Abbreviations

ACL	Anterior cruciate ligament
IDEO	Intrepid dynamic exoskeletal orthosis
MSK	Musculoskeletal
OEF	Operation Enduring Freedom
OIF/OND	Operation Iraqi Freedom/Operation New Dawn

Osteoarthritis (OA) affects one in five Americans; and one in ten experiences significant activity limitations due to this disorder [1, 2]. The US military represents a special subset of our population and largely comprises young, healthy, and physically active individuals. The incidence of OA is more common among active duty persons compared to age-matched controls: 8 cases per 1,000 person-years for active duty versus 4.6 cases per 1,000 civilian person-years for ages 30–34; 14 cases per 1,000 person-years for active duty versus 7 cases per 1,000 civilian person-years for ages 35–39; and 27 cases per 1,000 person-years in active duty versus 12 cases per 1,000 civilian person-years [3]. Similarly, 33–43 % of US military veterans are affected by arthritis, a prevalence higher than in the general population [4]. As with most studies on osteoarthritis, it is difficult to discern in the military and veteran population differences in rates of degenerative osteoarthritis compared to post-traumatic arthritis (PTA). The rigorous physical demands of military service speculatively contribute to both disease entities by placing members at risk for both repetitive microtrauma and acute injuries.

That arthritis is a major cause of disability for military populations has been documented for nearly three decades [5–8] (Fig. 3.1). Disability, as defined by the Americans with Disabilities Act of 1990, is a physical or mental impairment that substantially limits one or more major life activities [10]. Disability is measured in the military in terms of how significantly an impairment impacts one's ability to perform active duty military tasks which include physical training and Military Occupation Specialty duties. The military health care system has mechanisms for determining if a service member is recovered sufficiently

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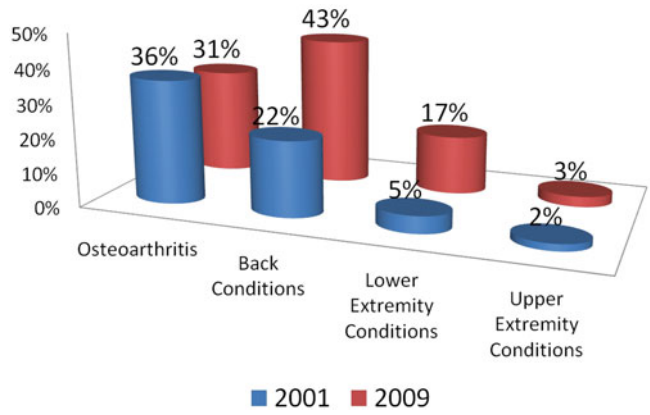

Fig. 3.1 Prior to and during OIF/OND and OEF, frequencies of medical discharge due to osteoarthritis have stayed nearly the same. However, back, upper, and lower extremity conditions have resulted in a higher proportion of medical discharges, possibly due to post-traumatic conditions [9] (Operation Iraqi Freedom/Operation New Dawn = OIF/OND; Operation Enduring Freedom = OEF, Musculoskeletal = MSK)

following an injury or illness to return to his or her active duty position. Returning to duty implies adequate recovery to perform all necessary physical tasks asked of general and occupation-specific military service. If one is unable to return to duty after a period of recovery that is considered adequate for optimal medical benefit, he or she is referred for examination by a physical evaluation board. The physical evaluation board, or service equivalent, comprises both medial and nonmedical military officers and is tasked with (1) determining if a service member is "unfit" for active duty service and should therefore be medically retired or separated from the military and (2) how significantly unfitting medical conditions contribute to his or her inability to remain on active duty which is expressed as a percentage of deficit.

Much of the broad information available on musculoskeletal disability in the military is derived from physical evaluation board data. In the mid-1990s musculoskeletal conditions resulted in 53 % of disabling conditions in the Army, 63 % in the Navy and Marines, and 22 % of conditions in the Air Force [8]. Of the top musculoskeletal disorders, joint derangements, most commonly of the knee, and back disorders resulted in the most common disabilities. While this particular study was unable to delineate specific injuries that may have resulted in musculoskeletal disability, the authors speculated that musculoskeletal related disability likely contributed to at least $450 million of military disability compensation costs per year. Another study from the same time period indicated that certain military occupations and female soldiers experience higher rates of musculoskeletal disabilities [6]. Among all disabilities demonstrated following work-related injury, the top five disabilities were all musculoskeletal in nature.

If disability causes in the military are largely musculoskeletal and arthritis is a substantial contributor to military disability, what subset of arthritis has a post-traumatic etiology? In examining the effect of post-traumatic arthritis in the military, one must consider the military experience in two ways. One avenue which exposes active duty personnel to trauma is combat-related injury which includes high-energy mechanisms often resulting in severe, multiply injured patients. While the extrapolation is imperfect, study cohorts of combat-wounded military are often compared to the cohorts in the civilian trauma literature; and military war medicine has contributed repeatedly and significantly to civilian trauma care [11, 12]. However, the military, with its rigorous physical demands, also places its members at risk for training, sport, and recreational injuries. Times of peace result in a military of a young, healthy population of athletes to which the civilian sports medicine literature more

aptly applies. Nonetheless, the burden of arthritis as a major cause of disability is present at both times of peace and war. The two most common conditions resulting in medical discharge from the Army prior to the most recent conflicts in our history and after 9 years of war remain osteoarthritis and back pain [9].

Combat Injury as a Source of Post-Traumatic Arthritis

Since 2001, our military overseas contingency operations (i.e., Iraq and Afghanistan) have resulted in 58,000 wounded and nearly 74,000 medical evacuations [13–15]. Several publications have elucidated the types of injuries incurred by those wounded in action indicating that musculoskeletal injuries are the most common and result in the greatest treatment resource utilization [16–18]. Over 50 % of injuries occur to the extremities and each injured service member sustains an average of four wounded body regions [17]. Seventy percentage of disability following combat injury is orthopedic in nature: 48 % of musculoskeletal injuries resulting in disabling conditions within the cohort were to the spine or appendicular joints [5]. As evidenced by this study, the burden of orthopedic injuries and resultant disability sustained in our recent conflicts is nearly half composed of injuries that place the joints at risk for permanent disability. This is consistent with reports from other historical conflicts where musculoskeletal injuries were common and intra-articular fractures comprised 71 % of combat-sustained fractures [19]. To further specifically define the impact of post-traumatic arthritis disability, Rivera et al. examined the same aforementioned cohort finding that 28 % of combat-wounded individuals were granted disability for post-traumatic arthritis conditions [20]. This is dramatically higher than the estimate in the civilian trauma population where the prevalence of post-traumatic arthritis among all arthritis is reported to be 12 % [21].

Rates of post-traumatic arthritis in the civilian trauma population are difficult to delineate: heterogeneous populations of patients with both post-traumatic and degenerative cause of arthritis are often studied together making conclusions about post-traumatic arthritis problematic. Furthermore, studies on how to define arthritis as an end point are variable, some studies using radiographic criteria, some using clinical symptoms, and others using arthroplasty rates as indicators of arthritis. However, the fracture literature for the lower extremity suggests that poor outcomes including post-traumatic arthritis are lower in the civilian trauma population compared to those in the military who are combat wounded. Following intra-articular fractures of the tibial plateau, rates of post-traumatic arthritis in the civilian trauma literature range from 23 to 44 % while 100 % of combat knee injury resulted in an arthritis-related disability [20, 22, 23]. Fractures of the tibial plafond result in post-traumatic arthritis in up to 74 % of civilian trauma patients while combat ankle injury leads to post-traumatic arthritis in 91 % of subjects [20, 24, 25]. Arthritis outcomes for fractures of the hip and acetabulum, however, appear to be similar in military and civilian populations, with reports of rates of 21 % and 24 %, respectively [20, 26] (Fig. 3.2, Table 3.1).

The differences seen in the rates of arthritis development may be contributed to the fact that mechanisms of combat injury are usually different from mechanisms experienced in the civilian trauma population. Reports of all injuries sustained in recent war indicated that explosions account for the vast majority of injuries, whereas over 90 % of civilian trauma is from blunt mechanisms [5, 12, 18]. Of joint injuries, 81 % are due to explosive mechanisms which can result in fractures, soft tissue injury, and/or penetrating injury from projectiles that result from the explosion [20]. Explosions cause multiple categories of injury including increases in stress and shear due to blast energy, fragmentary wounds, blunt and crush injury from the body being propelled by a blast, and injury from heat or other environmental exposure [27]. This inherent difference in injury mechanism may be the cause of differential outcomes following combat joint injury and post-traumatic arthritis outcomes reported in the civilian literature.

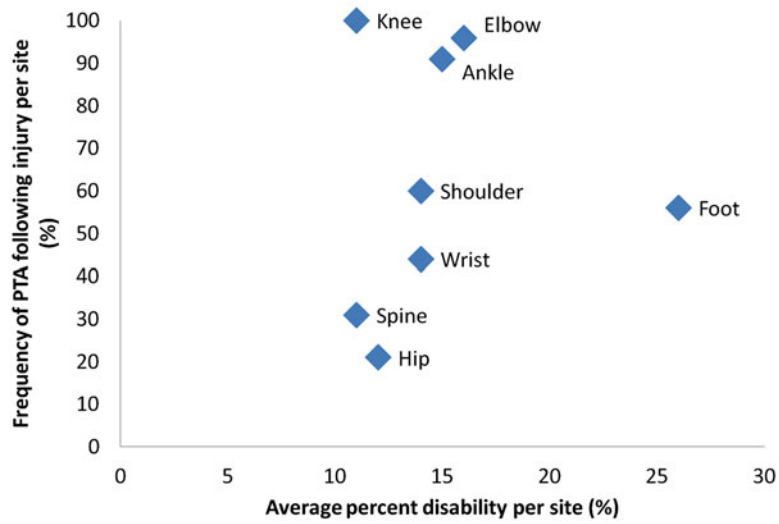

Fig. 3.2 Post-traumatic arthritis of the knee developed following 100 % of battle knee injuries. Foot injuries resulted in the highest average percent disability [20]

Table 3.1 Frequency of post-traumatic arthritis development after injury in military and civilian populations [20, 22–26]

	Military (%)	Civilian (%)
Acetabulum fractures	21	25
Intra-articular fractures of the knee	100	23–44
Intra-articular fractures of the ankle	91	50–74

Low back pain and spondylosis have historically been and continue to be major causes of medical discharge from the military [5, 9]. However, specific studies on arthritis of the spine in military populations are lacking. However, military service places individuals at risk for spinal disease as well. Spine injuries are more common in combat compared to non-battle injury [28]. Among spine injuries in medically evacuated battle-injured service members, 92 % are fractures [29]. In a cohort of 450 medically evacuated combat casualties who were eventually medically discharged, the rate of post-traumatic arthritis per spine injury was 31 % [20]. Only 25 % of these cases of spinal post-traumatic arthritis could be attributed to pre-deployment conditions.

The development of post-traumatic arthritis following combat appendicular joint injury is rapid and predictable. The military's disability system's disposition time indicating the presence of post-traumatic arthritis occurs on average 19 months following injury [20]. The sequelae of multiple injuries affect the demands placed on uninjured extremities as well. Biomechanical study of intact limb loading during transtibial and transfemoral prosthetic ambulation indicates that the intact limb experiences high mean and peak ground reaction forces, potentially placing the intact limb at risk of joint microtrauma [30]. Despite severe injuries, especially to the lower extremities, current reconstructive technology has allowed the successful limb salvage of multiple severely injured lower limbs [31, 32]. A salvaged limb, however, is also source of substantial disabilities to include deficits of nerve, volumetric muscle loss, and post-traumatic arthritis [33]. As the science supporting new and more advanced limb salvage options grows, additional research for these residual deficits is paramount to maximizing positive outcomes for retained severely injured limbs.

One success story for post-traumatic arthritis research includes an integrated rehabilitation protocol for patients with hind foot and ankle post-traumatic arthritis. While post-traumatic arthritis currently cannot be prevented, the physicians, therapists, and orthotists of the Center for the Intrepid's Return to Run Clinical Pathway at San Antonio Military Medical Center (Joint Base

San Antonio, Fort Sam Houston, TX) have improved functional outcomes despite foot and ankle post-traumatic arthritis. These outcomes are the result of a specific therapy regimen that begins early in the patient's course of limb salvage and the use in appropriate patients of the Intrepid Dynamic Exoskeletal Orthosis (IDEO), a custom carbon fiber, energy-storing ankle foot orthotic fabricated at the Center for the Intrepid [34, 35].

Compared to commonly used, off-the-shelf orthotics, the IDEO allowed significant improvements in agility, power, and speed in a cohort of 18 limb salvage patients [36]. In a small cohort of patients with tibiotalar or subtalar post-traumatic arthritis following lower extremity fractures, 81 % of patients were able to return to running activities, 69 % returned to agility sport, and 44 % continued active duty and were not medically discharged due to their injury [35, 37]. Up to 19 % of individuals who stay on active duty with assistance from the IDEO have been able to deploy with an orthotic, including those who have deployed with special operations forces [38]. The IDEO has allowed patients who desire an amputation to proceed with limb salvage because it often reduces pain and permits a higher level of activity. These results highlight the importance of how a rehabilitation pathway, improved orthotic technologies, and multidisciplinary treatments can improve outcomes for individuals with articular injuries despite post-traumatic arthritis.

Non-combat Injury as a Source of Post-Traumatic Arthritis

As previously mentioned, active duty service members are at risk from more than solely combat operations. The day-to-day activities of active duty individuals include physical training as well as typical sport and recreation. Even in a deployed setting, non-combat-related orthopedic injuries occur more frequently than battlefield injury [39, 40]. These injuries are classified as non-battle injury or illness. Medical evacuations from Operation Iraqi Freedom/Operation New Dawn from January 2003 to December 2011 included evacuations of over 50,000 service members for all causes, where 17.7 % were done so for battle injury, 16.3 % of medical evacuations, and over 8,000 service members were for non-battle musculoskeletal injury [15] (Fig. 3.3). Musculoskeletal non-battle injury compared to all other causes of medical evacuation was the second most common cause of evacuation for both male and female service members: second to battle injury in males and second to mental health disorders in females. A similar trend occurs in military operations in Operation Enduring Freedom between October 2001 and December 2012. In Afghanistan, 9.2 % of medical evacuations were for battle injury while non-battle musculoskeletal conditions resulted in 5.6 % [14]. As in Iraq, musculoskeletal non-battle injury was the second most common cause of evacuation for male service

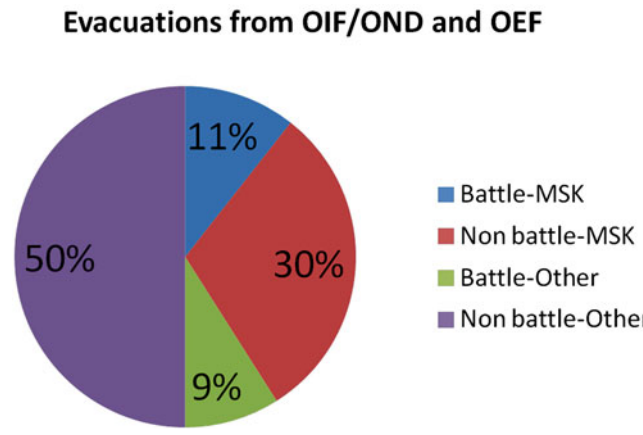

Fig. 3.3 Among all evacuations from OIF/OND and OEF, 30 % occur for non-battle musculoskeletal conditions and injuries. 41 % of evacuations, battle and non-battle, are due to musculoskeletal conditions [14–16]

Table 3.2 Incidence rates expressed as number per 1,000 person-years [46–50]

	Military	Civilian
ACL injuries	3.65	0.31–0.38
Meniscus injuries	6.02–7.08	0.33–0.61
Shoulder instability	28	Unknown
Shoulder dislocation	1.69–4.35	0.08–0.24
Ankle sprains	35–58.4	5.2–7.0

members following battle injury and the third most common cause for female service members following mental disorders and ill-defined conditions.

Following completion of full deployment, non-emergent orthopedic care is required in 19 % of service members returning after deployment [41]. A majority of these consultations are also the result of non-battle injury or exacerbations of conditions present prior to deployment. Among musculoskeletal non-battle injuries, fractures are the most common followed by inflammatory and overuse syndromes, sequelae of joint dislocations, sprains/strains, and other internal joint derangements [40]. The most common anatomical locations affected by non-battle injury are the back, knee, wrist, ankle, and shoulder. The most common mechanisms for these injuries are from sports and physical training.

Rupture of the anterior cruciate ligament (ACL) is among the most concerning sports injuries resulting in increased risk for post-traumatic arthritis [42–45]. Studies in military exclusive populations indicated that military personnel have a ten times higher prevalence of ACL injury compared to the general population: 3.65 per 1,000 person-years for acute injuries and 2.96 per 1,000 person-years for chronic injuries [46] (Table 3.2). Rates for the civilian population are generally reported to be 0.31–0.38 per 1,000 person-years. Among individuals hospitalized for sports or training injury, the knee was the most commonly injured body region and the ACL the most commonly injured structure [51]. As with civilian publications, ACL injuries in the military are associated with other knee pathology such as meniscus tears and chondral lesions [52]. Outcomes following ACL reconstruction are comparable in the military versus civilian cohorts with the exception that allograft graft selection may result in higher early failure rates compared to autograft in this young, active population [53]. The correlation between ACL injury specifically in military populations and post-traumatic arthritis has not been studied; however, the extrapolation from civilian research is likely valid and should be considered given the prevalence of ACL in injury in this young population.

Meniscus injury has also been associated with development of post-traumatic arthritis at rates of 50 % 10–20 years post-injury [44, 54]. Rates of meniscus injury in civilian, active populations range from 0.33 to 0.61 per 1,000 persons. Meniscus injuries in the military treated at both hospital and ambulatory settings however occur at a rate of 7.08 per 1,000 males and 6.02 per 1,000 females [48]. These rates increased with age from 2.99 per 1,000 persons in active duty individuals younger than 20–12.68 per 1,000 persons for those older than 40. Service in the Army or Marines and being of junior or senior enlisted rank are also risk factors for meniscus injury.

Rates of osteochondral lesions of the knee in military populations only are not available, though rates of chondral injury associated with other knee pathologies are likely comparable to civilian studies—incidental finding of articular cartilage lesions is 60 % at the time of arthroscopy [55, 56]. While evidence is available for return to sport outcomes following various cartilage repair surgical options, this data too is sparse for military populations where physical fitness is a requisite for continuing on active duty [57]. A single study demonstrates after osteochondral allograft transplantation that return to full duty rate is 29 % while return to pre-injury level of sport participation was only 5 %. Those in military occupation of combat arms were significantly less likely to return to duty [58, 59].

Ankle injuries are also higher in military populations compared to civilian counterparts. The risk of ankle sprains in civilian publications ranges from 5.2 to 7.0 per 1,000 person-years while studies in military individuals show a range from 35 to 58.4 per 1,000 person-years with higher rates in younger, cadet age groups [47]. Because osteochondral lesions of the talus are

associated with ankle instability, the high rate of ankle sprains in this population is important [60, 61]. Osteochondral lesions of the ankle occur at an incidence rate of 27 per 100,000 person-years for men and 31 per 100,000 person-years for military women [62]. As with other studies of joint injury, enlisted rank, service in the Army and Marines, and older age were risk factors associated with osteochondral lesion of the talus.

The upper extremity is also at risk in military personnel. Rate of shoulder instability among military cadets is 28 per 1,000 person-years and that of dislocation events ranges from 1.69 to 4.35 per 1,000 person-years [49, 50]. Furthermore, among patients with instability, MRI indicated a humeral head osteochondral lesion in 46 %; and for patients with frank dislocations, the rate of humeral head osteochondral lesion was 80 %. While rates and results from shoulder instability surgery and how instability and dislocation portend post-traumatic arthritis of the shoulder are unknown for the military, the civilian literature suggests that glenohumeral arthritis is more common in patients who have required a shoulder stabilization procedure [63].

Return to full military duty and performance of military-specific tasks are currently not available in the literature for these common sport-type injuries. Some extrapolations might be drawn from outcomes in athletes; however military-specific demands which often include significant burdens on the upper extremities, ability to carry and move heavy loads, and maneuver variable terrains may afford the military athlete greater challenges to return to pre-injury activity. Likewise, the natural history of these injuries and if they do predispose to or promote premature development of post-traumatic arthritis are not known. Again, what is known from civilian sports literature regarding the risk of arthritis may apply.

Results Following Arthroplasty

Data on outcomes following total joint arthroplasty are sparse for the military populace. Furthermore, the current arthroplasty literature in general lacks outcome information for arthroplasty specifically for post-traumatic arthritis. One report from the mid-1990s claims good outcomes in ten injured soldiers where joint replacement surgery was elected 9–42 months following their combat wounds [64]. This highlights that war wounds may in fact result in rapid development of arthritic joint impairment. Military personnel who do undergo total joint arthroplasty are not precluded from military service, though arthroplasty is not considered the best treatment option for young patients [65]. While the rates of retention on active duty remain modest, they are improving and individuals have been able to deploy after both total knee and total hip arthroplasty [66–69]. Improvements in return to duty rates may reflect actual improved outcomes or may indicate changes in disability perception; either way these reports do provide some information on return to military activities in patients who are most likely in part affected by post-traumatic arthritis.

Conclusion

Active duty service members and military veterans are at higher risk for arthritis-related disability of the upper extremity, lower extremity, and spine. This is not surprising because this is a very active population that is put in harm's way. Recent data from recent US conflicts indicate that 28 % of combat-wounded individuals experience permanent disabilities from post-traumatic arthritis and intra-articular fracture outcomes after combat injury appear to be worse than after civilian injury. It is believed that the higher rate of arthritis is due to the high-energy trauma that occurs on the battlefield. Combat trauma, however, comprises a fraction of the musculoskeletal injuries in the military. Common sports injuries including ACL, meniscus, ankle, and shoulder injuries have a higher incidence in the military compared to civilian counterparts. Among this growing body of incidence and prevalence data remains a lack of specific information on post-traumatic arthritis outcomes including the ability of those affected to return to pre-injury level of military-specific activities.

Much additional research is required to determine return to duty rates and to optimize joint recovery outcomes following severe combat and common sports injuries.

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Section II

Experimental Models of Joint Injury

In Vitro Cartilage Explant Injury Models

4

Christopher T. Chen and Peter A. Torzilli

Abbreviations

CC	Confined compression
COL	Collagen
DC	Displacement control
DT	Drop tower
ECM	Extracellular matrix
FN	Fibronectin
IDT	Indentation
kN	kiloNewton
LC	Load control
MCP-1	Monocyte chemoattractant protein 1
MMP-13	Matrix metalloproteinase 13
MMP-3	Matrix metalloproteinase 3
MPa	Megapascal
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OA	Osteoarthritis
OC	Osteochondral explant
PG	Proteoglycan
PGE2	Prostaglandin E 2
PTA	Post-traumatic arthritis
RL	Repetitive loads
SL	Single load
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
UCC	Unconfined compression

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Introduction

Impact and repetitive overload injury is a known risk factor for the onset of post-traumatic arthritis (PTA) [1–6]. In 1743, Hunter first noticed that patients with traumatic injury have a greater chance to develop chronic arthritis [7]. However, systematic studies did not begin until 1941 when Magnuson introduced an animal joint instability model by the division of the collateral and cruciate ligaments [3]. A number of animal studies in the 1970s used joint instability and traumatic impact models which reproduced typical osteoarthritic changes in articular cartilage, including a fibrillated articular surface, depleted proteoglycan (PG) content, and cloning of chondrocytes [8–12]. These in vivo studies demonstrated that either chronic repetitive overloads due to joint instability [3, 8] or a single impact or alone [10, 11] could induce PTA, although the biomechanical factors contributing to the degeneration of cartilage were not clear.

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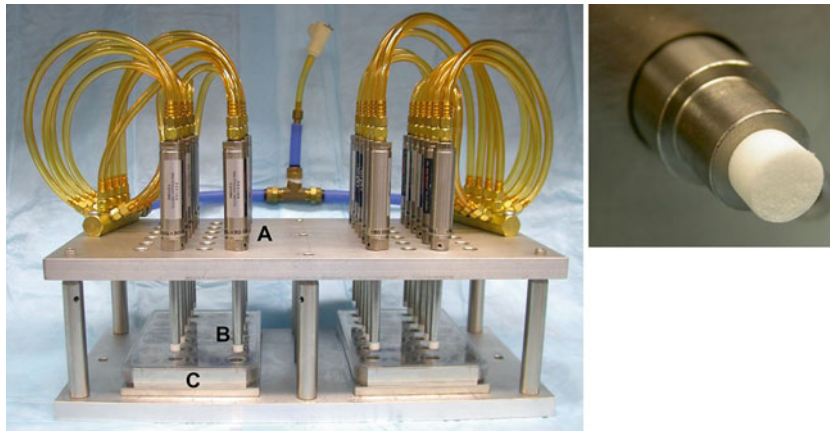

Fig. 4.1 A load-controlled test apparatus to apply single or cyclic loads to multiple cartilage specimens. (**A**) Pneumatic air pistons to load the explants. (**B**) Piston rod with flat-bottom, cylindrical, porous polyethylene load platen. (**C**) Twenty-four-well explant holding chamber. An expand view of the load platen is shown on the *right*

Several in vitro studies published at the same time demonstrated that the peak stress was an important factor to cartilage damage (i.e., repetitive overloads and a single impact) [13–17]. Weightman et al. [17] cyclically compressed articular cartilage for 250 h (90,000 cycles) at 0.1 Hz and found that a stress level of 2.0 MPa caused a fatigue failure at the cartilage surface. Repo and Finlay developed a drop-tower-type apparatus to impact cartilage explants from human knees [15, 18]. Using autoradiography, they found that a single impact at a stress level greater than 25 MPa fractured the articular surface of cartilage. They found chondrocyte death adjacent to the cracks induced at the articular surface [15]. This was the first reported use of a cartilage explant culture system to study cartilage injury. Together, these findings demonstrated that in vitro explant injury models are effective ways to study the role of biomechanical factors in different types of cartilage injury.

In the next three decades, scientists developed different loading systems to study the cell death and matrix damage in articular cartilage following acute injury. These loading systems often consisted of one or multiple autoclavable specimen-holding chambers and an incubator-compatible instrumentation which applied specific impact energies, loads, or displacements to cartilage or osteochondral explants, as shown in Figs. 4.1 and 4.2 [19–34]. These loading systems were designed based on similar biomechanical principles, even though they were used to study cartilage injury under different hypotheses and assumptions. We will introduce these loading systems in the following sections.

Biomechanically Controlled Systems to Injure Cartilage

Many mechanical factors can affect cartilage injury, including peak stress, peak strain, stress and strain rate, impact energy, and total loading time. In theory, it is better to examine all these parameters in order to accurately define the event during injury. However, in practice only three of these parameters can be independently controlled at one time. Thus, it is sometimes difficult to compare studies that use different control systems in which only selected parameters are reported. In this section, we describe three commonly used approaches: (1) load-controlled system, (2) displacement-controlled system, and (3) energy-controlled (drop-tower) system, and discuss the differences and advantages of each system.

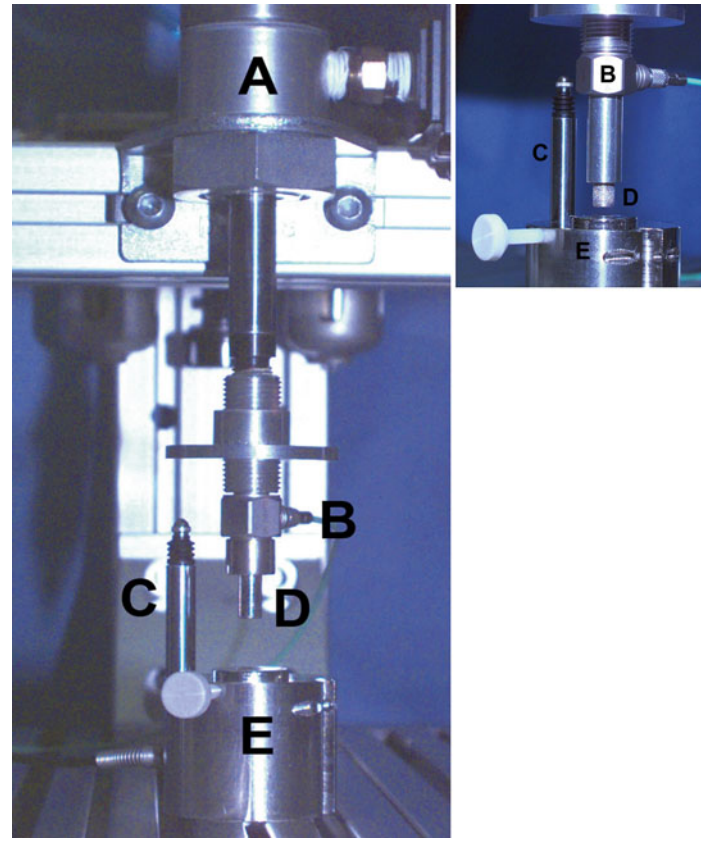

Fig. 4.2 A single-impact load-controlled test apparatus. (**A**) Pneumatic air piston to impact a single explant. (**B**) Piezoelectric load transducer. (**C**) Displacement transducer to measure explant deformation. (**D**) Piston rod with solid, cylindrical nonporous metal load platen. (**E**) Explant holding chamber. An expand view is shown on the *right* with a porous metal load platen

Load-Controlled System

In a load-controlled system, a solid or porous load platen is utilized to apply a uniaxial compression to the surface of the articular cartilage explant, as shown in Fig. 4.2. The compression can be applied at a fixed loading rate (or stress rate) until the load reaches the designated maximum load (or peak stress) [17, 20, 35, 36]. This system can compress a cartilage explant with either a single impact load or multiple times (cyclically) using a defined waveform, such as a ramp or sinusoidal compressive waveform or one determined to simulate a gait cycle [22, 35, 37, 38]. In this system, the stress level on the specimen can be controlled even when multiple explants with different thicknesses are simultaneously loaded. The independent mechanical parameters that can be set or controlled with this type of system are the maximum load (peak stress), loading rate (stress rate), number of repeats or loading cycles, loading waveform, dwell time (on-off period), and total loading time.

Displacement-Controlled System

The second type is displacement-controlled system. Like the load-controlled system, one can choose to apply a defined displacement to the surface of articular cartilage at a given displacement rate (or strain rate) through a solid or porous platen. This displacement can be applied once [30, 39, 40] or multiple times [28] to reach a defined peak stress [41] or final strain [39, 40, 42]. When performing repeated displacement-controlled compressions, an offset displacement is often implemented to prevent the loss of surface contact (liftoff of the platen) during the

unloading phase of the cycle due to the viscoelastic response of cartilage [43]. An electronic actuator with a spectrometer is commonly used to apply the prespecified displacement [43]. The system can easily load multiple samples at the same time with or without a lateral confinement. The independent mechanical parameters that can be controlled in this displacement-controlled system are the maximum deformation (peak strain), deformation rate (strain rate), number of repeats or deformation cycles, deformation waveform, dwell time (on-off period), and total deformation time.

Energy-Controlled (Drop-Tower) System

Probably the most commonly used method is the drop-tower type of apparatus. In this system, the specimen is impacted with a controlled amount of energy by dropping a known weight from a defined height above the surface of the specimen [15, 25, 44–48]. No separate controlled system (e.g., computer) is required, though an independent transducer and displacement transducers are often used to record the impact force and specimen deformation, respectively. This system can injure cartilage in a very short time interval, typically less than 5 ms [15, 49, 50] and at very high stress rates (5,000–80,000 MPa/s) and peak stresses (5–70 MPa), values close to those occurring in a joint injury from a motor vehicle accidents [15, 51, 52]. At these high stress and strain rates specialized accelerometers or force transducers, such as piezoelectric transducers, are required to record the injury event as shown in Fig. 4.3 [15, 46, 52]. In an impact energy-controlled system, the only controlled parameter is the impact energy (joule) or energy density (joule/cm^2), that is, the fixed weight and height.

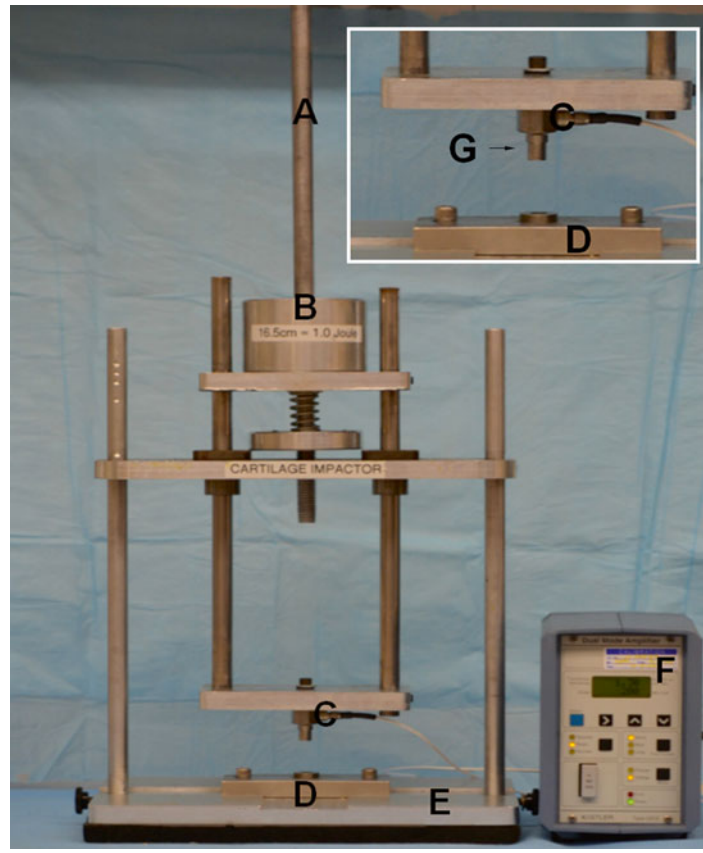

Fig. 4.3 A "drop-tower"-type test apparatus. (**A**) A cylindrical rod to guide and secure the mass during dropping. (**B**) Defined mass. (**C**) Piezoelectric load transducer. (**D**) Explant holding chamber. (**E**) Supporting frame and base. (**F**) Signal conditioner to amplify voltage generated by load transducer. (**G**) Nonporous metal load platen as shown in the *insert*

Confined Versus Unconfined Test Configurations

In normal and injured cartilage, interstitial fluid plays an essential role in resisting the applied load in a time-dependent fashion. The viscoelastic response of articular cartilage is well described by biphasic or poroelastic theories [53–55]. The flow of interstitial fluid through the extracellular matrix (ECM) and across the articular surface (exudation and imbibition) accounts for the viscous nature of the mechanical response of articular cartilage. This response is dependent on not only the elastic modulus of the solid matrix, but also the permeability of the solid matrix to resisting fluid movement though the solid matrix, which can occur in both the radial (depth) and transverse (tangential) directions. In confined compression, the cartilage specimen is usually cylindrical and placed into a tight-fitting, impervious cylindrical chamber such that a barrier to fluid flow is placed radially around the circumference of the specimen. When loaded in this configuration with a porous platen, the interstitial fluid is forced to flow in the axial direction passing through articular surface, and gives rise to a uniaxial compressive stress and strain in the ECM [29, 40, 52, 56]. In unconfined compression, the cartilage is compressed between two impermeable parallel platens, with the compression applied to the articular surface and either the underlying subchondral bone or the cut surface of the cartilage if the bone is removed. The sides of the cartilage are open to allow fluid transport, thus allowing the cartilage to bulge at the outer perimeter to exude and imbibe fluid in the radial direction [57, 58]. The decision to choose between confined and unconfined loading in a cartilage explant model depends on the hypothesis and assumptions in the experimental design [28–30, 34, 37, 40, 52, 59–61]. This is especially important for cartilage loading experiments performed in long-term culture where an adequate nutrient supply is needed [22, 34, 60]. In a single impact experiment, local interstitial fluid flow and ECM stress and strain are transient and if not laterally confined may result in nonuniform matrix and cellular damage or even cell death [13, 49, 62, 63].

Threshold Stress to Kill Chondrocytes

Using different loading systems, many studies reported the minimum stress or stain required to kill chondrocytes, one of the most important benchmarks for PTA. In the last 15 years, most researchers have chosen nonradioactive methods to measure cell death/viability, such as cell metabolic dyes or membrane-impermeable dyes [33, 37, 64–67]. One limitation of these methods is that after injury some chondrocytes are metabolically inactive with a leaky membrane and appear dead (false negative) while other cells undergoing apoptotic pathways may appear alive (false positive) for days. Delayed cell death or apoptosis is one of the major pathways for chondrocyte death in injured cartilage [23, 28, 68], and will be discussed in a greater detail later in this book.

Using a load-controlled system, Torzilli et al. loaded cartilage explant at a stress rate of 35 MPa/s and found that the threshold stress to kill chondrocytes at the time of impact (acute) was 15–20 MPa [36]. Matrix damage with apparent rupture of the collagen fiber matrix was also found at the time of impaction. The threshold stress of 15 MPa was also found to kill chondrocyte when cartilage explant was compressed by a single impact at 350 MPa/s [35]. Using a displacement-controlled system with a final strain of 50 %, Kurtz et al. [39] found no cell death when cartilage was loaded to 12 MPa at a stain rate of 0.01/s. However, dead cells with matrix damage were found when loaded to 18 MPa and 24 MPa with faster strain rates of 0.1/s and 1/s, respectively. D'Lima et al. [59] found that a static compression of 14 MPa applied over 0.5 s induced cell apoptosis which was inhibited by broad-spectrum caspase inhibitors.

The threshold stresses found for cell death in these studies are much lower than those reported using a drop tower [15, 26, 63]. Jeffrey et al. [26, 63]

found that a threshold stress of 50 MPa was needed to induce matrix damage and chondrocyte death when cartilage was impacted at strain rates of 1,600–2,300/s [26]. These findings highlight the differences in impact loading between a drop-tower and the other two controlled systems [51].

In repetitive overloading tests, the threshold stress to induce cell death and tissue damage was found at lower levels. Farquhar et al. [24] loaded canine cartilage explants cyclically at 100 MPa/s for 30 min and found subtle damage in most explants at 5–10 MPa compression. They also found that increased tissue swelling and fibronectin biosynthesis were associated with matrix damage. Loening et al. [28] found that a minimum stress of 4.5 MPa was needed to induce cell death and tissue swelling when immature bovine cartilage explant was compressed to a final strain of 30–50 %. They also found that chondrocyte apoptosis occurred at lower stresses than those required to stimulate cartilage matrix degradation and biomechanical changes. Chen et al. [22] loaded cartilage for 48 h and found chondrocyte death along with denatured collagen from a repetitive stress of 1.0 MPa. This was consistent with a study by Steinmeyer et al. [69] who found 7–14 % cell death after 6 days of repetitive loads of 1–5 MPa. This was only 8 % of the threshold stress found for a single impact [39, 56, 70].

In all loading systems, cell death is always found in articular cartilage adjacent to the cracks [15, 22, 26, 35, 67] where high shear stress is expected. When a full-thickness cartilage or an osteochondral explant is used, cell death usually appeared first in the superficial zone [15, 26, 56], the zone in articular cartilage with the lowest compressive modulus [71]. Together, these studies conclude that high compression and high shear in a single impact are important factors for acute cell death and matrix damage in articular cartilage, and that chondrocytes in the superficial zone are more vulnerable to mechanical injury. High compressive and shear forces and stresses are common in vivo events when joints are subjected to sudden and high-energy impact loads, such as with the rupture of anterior cruciate ligament during sports activities, falls from a height, and vehicle accidents. Based on in vitro studies, similar types of articular cartilage damage (cell death and ECM cracks) in the superficial and deeper zones would be expected to occur in vivo at the time of joint injury.

Loading-Rate-Dependent Cell Death and Matrix Damage

One of the important features in cartilage injury is that cell death and matrix damage are dependent on the loading rate and loading time [39, 56, 70, 72]. In a study by Ewers et al. [72], there was a greater amount of cell death in cartilage explants impacted with a stress of 40 MPa compression at 40 MPa/s compared to cartilage impacted at 900 MPa/s. In a systematical study of stain rate, Morel and Quinn [40] loaded cartilage explants in unconfined compression with peak stresses of 3.5–14 MPa at strain rates of 0.1–1,000 times of gel diffusion rate (stress relaxation time). They found that cells died throughout the full depth at the lowest strain rate, but only occurred near the superficial zone or near surface cracks when loaded at the highest strain rate [40]. Compressing cartilage more quickly resulted in the peak stress being reached more rapidly with less overall compression (strain), leading to a reduction in the death of chondrocytes [2, 40].

Cell death and matrix damage in cartilage are affected by loading rate and total loading time when subjected to repetitive overloads [22, 37, 69, 73]. Using a load-controlled system, Chen et al. [73] found that the stress rate and peak stress affected the amount of cartilage damage, as quantified by tissue swelling and denatured collagen using specific neoepitope [73]. Two further studies found that cell death in cartilage was increased with loading time when explants were loaded with a stress above the threshold level [22, 37]. These studies were consistent with the findings by Steinmeyer and Ackermann [38], who found a decrease in bovine cartilage fibronectin synthesis with increasing cyclic load duration and peak stress.

Subchondral Bone in Cartilage Injury

Subchondral bone provides mechanical support of articular cartilage during loading. Several studies have found subchondral bone stiffening and a reduction in ECM permeability in clinical osteoarthritis [10, 74]. Several studies have used osteochondral explants to study cartilage injury [15, 20, 26, 40, 75]. Finlay and Repo [75] found that the attachment of underlying bone reduced the splitting of cartilage under impact. This finding was confirmed by Jeffrey et al. [26] who also found the fracture of underlying bone at higher energy impacts. Using a load-controlled system, Borrelli et al. [20] later reported that the failure of cartilage and bone occurred at 50 MPa and 75 MPa, respectively. Flachsmann et al. [76] further reported that rupture occurred predominantly in the superficial zone when cartilage was attached to the underlying bone, and that cartilage was more resistant to rupture under dynamic loading than under static load. Together, these studies suggest that subchondral bone serves to prevent cartilage damage under a single impact or repetitive overloads, and that the threshold stress to kill chondrocytes in vivo could be much higher.

Synovial Fluid Interface in Cartilage Injury

Synovial fluid is important interface between opposing cartilage surfaces in movable joints. The synovial fluid provides almost frictionless lubrication and reduced cartilage wear, and supplies nutrients to the chondrocytes in the ECM. In most in vitro mechanobiological studies a metal platen was used to load the articular cartilage. In a recent study of loading interface, Heiner et al. [50] impacted an osteochondral explant at the articular surface with a metallic indenter or another osteochondral explant in the metal-on-cartilage or cartilage-on-cartilage settings, respectively. Using a drop tower with an impact energy of 3.09 J/cm^2, they found that cartilage-on-cartilage impacts resulted in about 50 % of the peak stress and 25 % of the peak stress rate measured in the metal-on-cartilage impacts, and also resulted in increased impact time and cell viability. This finding agrees with an earlier study by Milentijevic et al. [35] who paired two cartilage explants with their articular surfaces facing each other. They found no detectable chondrocyte death in either cartilage pair when impacted with 50 MPa at a rate of 350 MPa/s [35].

Two new models were recently developed to study the role of plowing in cartilage injury [77, 78]. Correro-Shahgaldian et al. [77] designed a rolling system to study the effect of shear plowing at different traction forces, i.e., compressive loads perpendicular to the articular surface and shear loads tangential to the articular surface. They compressed nasal cartilage explants with 50 or 100 N forces using solid cylinder at a sliding speed of 10 mm/s for 2 h. They found that the compressive load of 100 N produced at a higher traction force of 8.0 N and a 6.6-fold upregulation of type I collagen suggesting an injurious response in cartilage. Waller et al. [78, 79] developed a cartilage-on-cartilage bearing system to study the role of lubrication. They found that the addition of lubricin, as a lubricant, significantly lowered the static coefficient of friction and chondrocyte apoptosis in articular cartilage [79]. Together, these findings suggest that the synovial fluid interface, including surface contact, lubrication, and plowing, is an important factor for cell death and ECM damage in articular cartilage.

Summary and Implications in PTA

In this chapter, we reviewed (summarized in Table 4.1) the different explant injury models used to study the responses of articular cartilage after a single impact and repetitive overloads in vitro. Regardless of the preference in applying loading regimens [51], the findings from these studies clearly show that cell death and matrix damage in articular cartilage are associated with high compression (stress and strain) and prolonged loading time, which can be achieved by

Table 4.1 A summary of in vitro explant injury models used to study the response of articular cartilage following a single impact and repetitive overload injury

Leading author	Loading type	Loading regimen	Cell response	Matrix damage
Weightman [17]	RL	>2 MPa for 250 h	?	Fissure
Repo [15]	DT, SL, OC	<0.003 s, >25 MPa	Cell death	Fissure
Vener [6]	DC, SL, OC	1.7–2.4 kN, 64–88 kN/s	?	Crack
Jeffrey [26]	DT, SL, OC	0.05–2 J, 0.002 s	Cell death	Fissure
Farquhar [24]	LC, RL, IDT	30 min, <50 MPa, <100 MPa/s	↑ <FN>	Crack, swelling
Borrelli [20]	LC, SL, OC	55–75 MPa	↓ <PG>	Swelling, crack
Steinmeyer [69]	LC, RL, CC	0.1–1 MPa	Cell death, ↑ <FN>	?
Quinn [30]	DC, RL, UCC	50 % strain, 12 h	Cell death,↓ <PG>	Crack
Chen [73]	LC, RL, IDT	3.5–14 MPa, 0.3 Hz, 0–72 h	↑ <FN>, ↑ <protein>	Cleaved collagen
Loening [28]	DC, RL, CC	50 % strain, 6 cycles, 4.5–20 MPa	Apoptosis, nitrite	Damaged collagen
Torzilli [36]	LC, SL, IDT	0.5–65 MPa, 35 MPa	Cell death, ↓ <PG>	Swelling
Ewers [72]	DT, SL UCC	40 MPa, 40–900 MPa/s	Cell death	PG loss, fissure
Chen [68]	LC, RL, IDT	3.5–14 MPa, 0.3 Hz, 0–72 h	Necrosis, apoptosis	Swelling
Kurz [39]	DC, SL, CC	50 % strain, 0.01–1 Hz, 12–24 MPa	Apoptosis, ↓ <PG>, ↓ <protein>	Comp stiffness
Quinn [80]	DC, SL, CC	3–14 MPa	Cell death	Cracks
D'Lima [59]	LC, SL, UCC	14 MPa, 0.5 s	Apoptosis	PG loss
Chen [22]	LC, RL, CC	0.1–5 MPa, 0–72 h	Cell death	Cleaved collagen
Lewis [67]	LC, SI	53 MPa, 212 MPa/s, 0.25 s	Cell death	Crack
Milentijevic [35]	LC, SL, CC	10–60 MPa, 350 MPa/s	Cell death	Water loss
Waller [78]	Shear, RL, IDT	18 % strain, 12 cycles	Apoptosis	?
Patwari [70]	DC, SL, CC	50–65 % strain, 100 %/s, 11–23 MPa	Cell death, ↑ <MMP-3>	PG loss
Morel [40]	DC, SL, UCC	50–80 %, 0.1–1,000 gel diffusion	Cell death	Swelling, crack
Levin [37]	LC, RL, CC	1–5 MPa, 0.5–16 h	Cell death	Swelling, PG loss
Milentijevic [56]	LC, SL, CC	10–40 MPa, 25–1, 000 MPa/s	Cell death	Water loss
Jeffrey [25]	DT, SL, IDT	<0.003 s, 25 MPa	Cell death, apoptosis, ↑ <PGE2>	PG loss, crack
Chahine [81]	DC, SL, CC	65 % strain	Cell death	?
Ding [44]	DT, SL, IDT	14 J/cm^2	↑ <MMP-13>, ↑ <TNF-α>	PG loss
Correro-Shahgaldian [77]	Plow, RL, UCC	50–100 N/s, 10 mm/s	↑ <COL1>	?
Stolberg-Stolberg [33]	LC, SL, IDT, OC	70–90 % strain, 100 %/s	Apoptosis, ↑ <NF-κB>, ↑ <TLR>	Crack
Heiner [50]	DT, SL, IDT	3.09 J/cm^2	Cell death	PG loss, crack
Waters [82]	DL, SL, OC	100 mm/s, 0.25–1.25 mm	Cell death, ↑ <PGE2>, ↑ <MCP-1>	Swelling

DC displacement control, *LC* load control, *DT* drop tower, *SL* single load, *RL* repetitive loads, *CC* confined compression, *UCC* unconfined compression, *IDT* indentation, *OC* osteochondral explant, *kN* kiloNewton, *MPa* megapascal, *Hz* Hertz, ? not measured/reported, ↑ increase, ↓ decrease, <> synthesis or expression, *PG* proteoglycan, *FN* fibronectin, *MMP-3* matrix metalloproteinase 3, *MMP-13* matrix metalloproteinase 13, *COL* collagen, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *TLR* Toll-like receptors, *TNF-α* tumor necrosis factor alpha, *PGE2* prostaglandin E2, *MCP-1* monocyte chemoattractant protein-1

Table 4.2 A summary of major biomechanical, biological, and structural factors that influence cell death and matrix damage in articular cartilage

Factors influencing cell death and matrix damage in cartilage	
Increased by	Decreased by
Biomechanical factors	
Higher peak stress	Lower stress rate
Higher peak strain	Lower strain rate
Higher impact energy	Lower impact energy
Repeated loading	Lateral confinement
Increased loading time	
Biological and structural factors	
Tissue immaturity	Subchondral bone
Reactive oxygen species	Synovial fluid interface

Table 4.3 A summary of cartilage responses to acute impact and repetitive overload injury at structural and cellular levels that are similar to arthritic cartilage in the early stages of PTA

Cartilage response to acute impact and overload injury	
Increased in	Decreased in
At structural level	
Proteoglycan loss	Proteoglycan content
Damaged collagen	Compressive modulus
Cleaved collagen	Shear modulus
Tissue swelling	
Fissure or crack	
At cellular level	
Cell necrosis	Cell viability
Cell apoptosis	Proteoglycan synthesis
Fibronectin synthesis	Protein synthesis
Pro-inflammatory cytokines (TNF-α, NF-κB, TLR, PGE2)	
Collagen synthesis	
Degradative enzymes (MMP-3, MMP-13)	

increasing peak strain, impact energy, peak stress, and/or repetitions. These studies also found that subchondral bone, synovial fluid interface, and loading rate are major factors that protect articular cartilage from injury as summarized in Table 4.2. In the applications of these explant injury models, many studies found consistent cellular and structural changes that are typically seen in the early stages of PTA [8–12], such as cell death [22, 25, 28, 33, 50, 56, 59, 78], PG loss [37, 44, 59, 70], fissure [30, 33, 50], collagen breakdown [22, 28, 73, 83], tissue swelling [20, 24, 40, 73, 82], and decreased biomechanical properties [39, 48] as summarized in Table 4.3. These correlations are encouraging and provide incentives for us to proceed in our efforts to use these models to study the onset and progression of PTA.

Many studies have used these in vitro explant injury models to study the molecular mechanism of chondrocyte death [78, 79, 84, 85] and matrix degradation in injured cartilage [44, 47, 62, 70, 82, 83, 86, 87]. These studies indicate that acute injury in articular cartilage can induce the upregulation of reactive oxygen species (i.e., nitric oxide) and pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), Toll-like receptor (TLR), and prostaglandin E2 (PGE2) [33, 44, 47, 62, 82, 86]. These are important areas of research, since the prevention of cell death and the inhibition of matrix degrading enzymes in the injured joint are significant for the initiation of PTA [25, 59, 70, 88]. Furthermore, the production of these bioactive molecules is known to associate with cell apoptosis [28, 33, 59, 68] and increased production of degradative enzymes [44, 62, 70], such as matrix metalloproteinase (MMP) 3 and MMP-13. These changes are known to be detrimental for the biomechanical properties of injured cartilage [39, 48] and affect the functional ability of articular cartilage to withstand physiological load. These new findings touch on the important and yet intertwined relationships between cartilage injury, chronic joint inflammation, and pro-inflammatory cell signaling in the development of arthritis [88], which will be discussed further in the later chapters of this book. Together, we conclude that in vitro explant injury models are effective systems to study biomechanical and mechanobiological factors to initiate cartilage injury, biochemical factors associated with cell death and matrix degradation, and gene regulation that are critical for the advance of PTA.

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Animal Models of Meniscal Injury in Post-Traumatic Arthritis

5

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Introduction

In 2006, almost 500,000 arthroscopic procedures for medial or lateral meniscal tear were performed in the United States alone [1], a dramatic increase in numbers of procedure since the first clinical report of meniscal repair 130 years ago [2, 3]. As meniscal surgery has evolved, there has been increasing recognition of the importance of meniscal preservation and repair rather than meniscectomy [4, 5], to minimize the long-term development of post-traumatic OA (PTA) [5–7], and maintain the chondroprotective effects of the meniscus [4].

Animal models have been critical to improved understanding of not only the detrimental effects of meniscectomy and the role of meniscal tear in development of PTA, but also in evaluation of novel repair techniques and tissue engineering and biologic strategies. Meniscal tears not only initiate development of PTA following a traumatic event, but can also occur as degenerative lesions resulting from development of OA, most commonly in the posterior horn of the medial meniscus in human [8, 9]. In animal models, incidental meniscal tears occur as a result of ligamentous destabilization [10]. Therefore, it is recommended that the meniscus be evaluated routinely as an outcome measure after experimental induction of any form of PTA in animal models.

As with any animal model, selection of the most appropriate species, injury pattern and combination of outcome measures is crucial to the ultimate utility and translational relevance of the study. This chapter first outlines considerations for selection of a meniscal injury model, then describes the injury models that have been evaluated and then broadly discusses confounding variables and the outcome measures that should be used to evaluate structural and functional impairment that results from meniscal injury.

Selection of Meniscal Injury Model: Specific Considerations When Choosing an Animal Model

Anatomical Considerations

Each meniscus is divided into three main anatomic regions, the anterior horn, the mid-body or pars intermedia, and the posterior horn. Circumferentially from the meniscosynovial or meniscocapsular attachments are the peripheral, central or middle and inner zones. The relationship of the size and attachment of the meniscus to the

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tibial plateau may be important when evaluating repair technique and PTA lesion distribution after meniscal injury (Fig. 5.1). The medial meniscus of the goat and sheep is most similar anatomically to human [11], whereas the lateral meniscus of the sheep, goat and pig is most similar in size equivalence to the human, but the anatomy of the lateral meniscus is most similar to human in goat [11]. Therefore, when anatomic or size considerations are important, the goat may be the preferable model [11]. However, functional gait analyses in sheep following meniscal injury have been characterized and demonstrate many similar changes to humans with osteoarthritis (OA) [12]. The femorotibial joints of the dog, rabbit, sheep, goat, and primate are analogous to humans. Overall the dog, rabbit, sheep, goat, rat, and mouse have the most well characterized meniscal injury models, but species selection must take into account the equivalence of the animal meniscus to human for the outcomes under consideration [13].

Compositional Considerations

Cellular morphology of the normal meniscus is consistent between large animal species, with ovoid or round chondrocyte-like cells in the inner part of the meniscus and fibroblastic, stellate or fusiform cells on the outer surface [14]. However, in rodents (rats and mice) and rabbits, the meniscus is more cellular than that in human [13]. Proteoglycan content in human meniscus is lower than in rabbit and sheep, but across all species is identified mainly in the inner zone, with some species (rabbits and cows) demonstrating proteoglycan additionally in the middle zone. Type I collagen expression is highest in the peripheral zone, where it is organized as circumferential fibers, and lowest or absent in the inner zone. In contrast, Type II collagen is greatest in the inner zone, and is organized as radial fibers [14]. Rabbit menisci demonstrate significantly different vascularity, collagen orientation, and glycosaminoglycan content compared with human, limiting their usefulness in this respect [15].

Several species commonly undergo meniscal calcification, including the Dunkin Hartley guinea pig [16], rat, mouse and cat [17]; other species are affected sporadically (Fig. 5.2). In the Dunkin Hartley guinea pig, articular cartilage lesions are tightly correlated and co-localized to the site of development of OA, and inhibition of meniscal ossification significantly reduces OA [16]. In cats, meniscal mineralization is identified in the anterior horn of the medial meniscus and is associated with medial compartment OA [17]. In humans, meniscal calcification has been identified in patients undergoing total knee arthroplasty [18], and calcium deposition is commonly identified in OA menisci [9], but the role of meniscal calcification in pathogenesis of OA has yet to be determined in human. While the aforementioned species undergo increased calcification than is typical for human, the potential advantages of some of these species for studying meniscal injury outweigh this potential disadvantage in many respects.

Fig. 5.1 (continued) plateau in the dog knee (6A). The anterior attachment of the lateral meniscus courses between the anteromedial and posterolateral bundles of the ACL in the cow, sheep, and pig knees (2A, 3A, 5A). Column B represents the posterior aspects of the knees. In all knees the medial meniscus passes behind the PCL. In the human knees, the posterior meniscofemoral ligament inserts more inferiorly on the medial femoral condyle (1B). Note the posterior thickening of the menisco-tibial coronary ligament between the lateral meniscus and tibial plateau in the sheep, goat, dog, and rabbit knees (3B, 4B, 6B, 7B). Column C shows the different tibial attachments of the knees. Notice the splitting of the tibial ACL insertion by the anterior lateral meniscus in the cow, sheep, and pig knees (2C, 3C, 5C). The lateral meniscus attachments are located central to the medial meniscus attachments in the human knee (1C). Column D shows the morphology of the menisci with the medial meniscus on the left, the lateral meniscus on the right and the anterior horns facing down. In the human knee (1D) the posterior horn of the lateral meniscus attaches anteriorly to that of the medial meniscus, a feature not seen in any of the six animal species examined. (*ACL* anterior cruciate ligament, *ALM* anterior lateral meniscus; *AMM* anterior medial meniscus, *PCL* posterior cruciate ligament, *PLM* posterior lateral meniscus, *PMM* posterior medial meniscus). (Reproduced from Proffen et al. A comparative anatomical study of the human knee and six animal species. Knee. 2012 Aug;19(4):493–9)

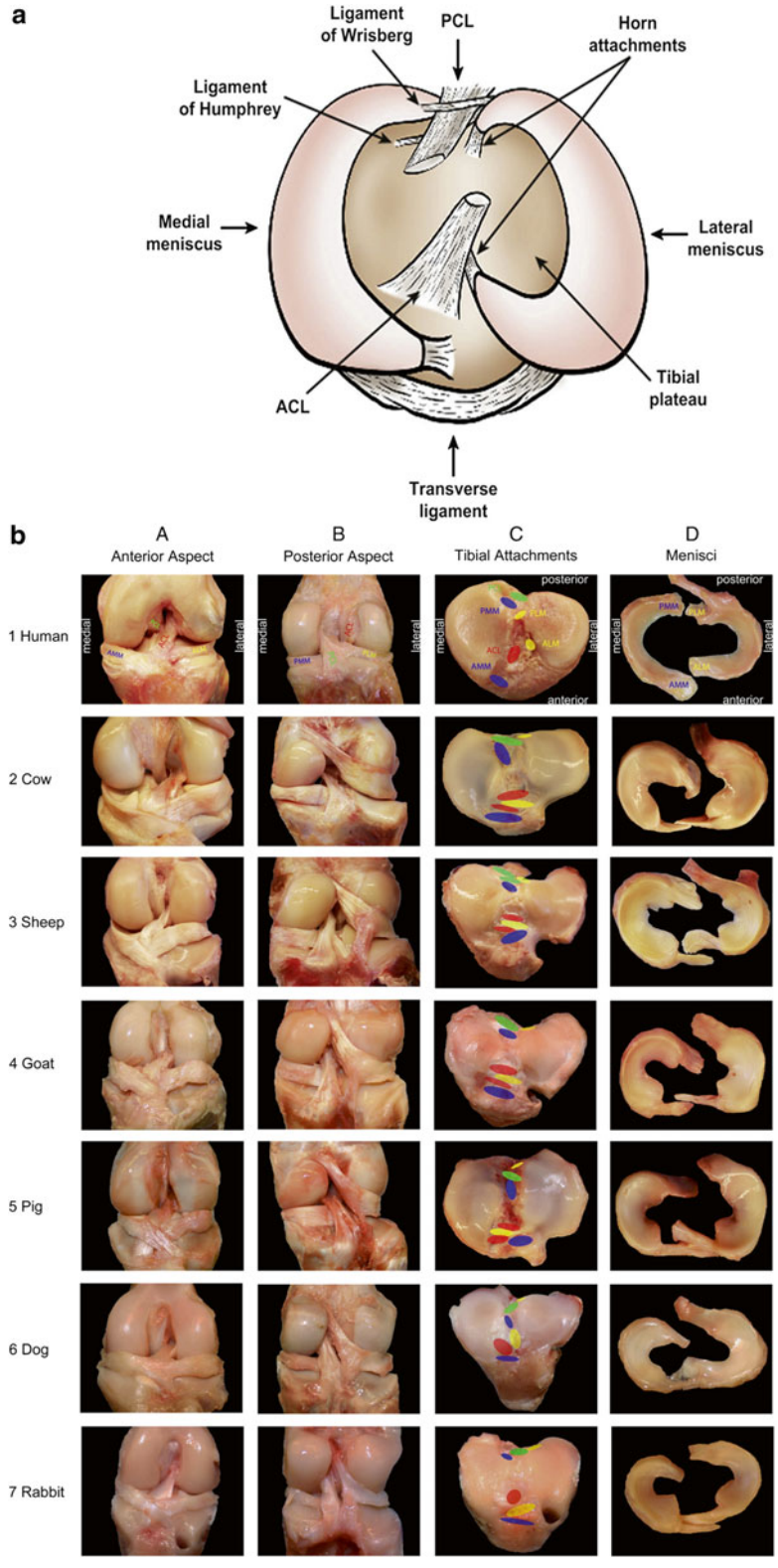

Fig. 5.1 (**a**) Illustration of anatomy of meniscus orientation on the tibial plateau in the human left knee. (Reproduced from Makris et al. The knee meniscus: Structure-function, pathophysiology, current repair techniques, and prospects for regeneration. Biomaterials. 2011; 32: 7411). (**b**) Different aspects of the knees of seven different species. Column A shows the anterior aspect of the knees with the medial side being on the left and the lateral side on the right. Noticeable is the small band attaching the intermeniscal ligaments to the tibial

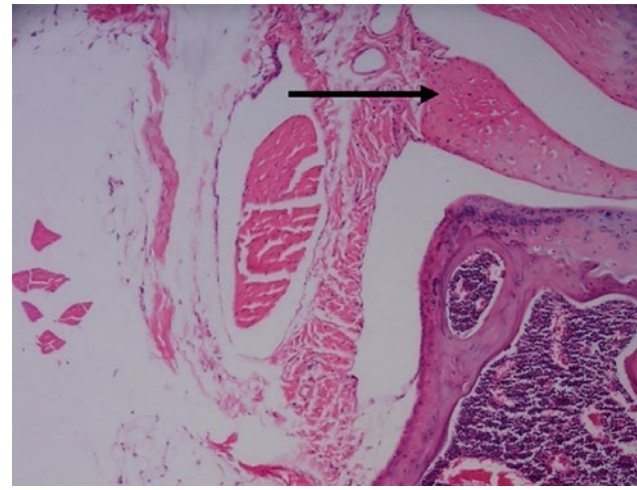

Fig. 5.2 Section of normal mouse knee joint with hematoxylin and eosin staining. The arrows points to an area of calcification within the meniscus 100×

Vascular Supply

At birth, almost the entire meniscus is vascularized, via the lateral and medial geniculate arteries, which form a perimeniscal capillary plexus with radial branches directed towards the inner zone of the menisci in human, cow, sheep, pig, dog, and rabbit [14, 19, 20]. During skeletal growth and development, radial meniscal vessels regress such that an avascular area initiates and develops from the inner zone to variable degrees in more peripheral locations. In the adult human, vascular penetration extends to only 10–30 % and 10–25 % of the meniscal width in the medial and lateral meniscus respectively [20]. This is comparable to the vascular penetration observed in sheep (6–17 % of meniscal length, compared to 9–19 % of meniscal length observed in human), but much greater than that observed in rabbit (1–3 % of meniscal length) [15]. Thus in the rabbit, the vascular region extends only 0.7–1 mm from the meniscosynovial junction, and 1.3 mm from this junction is considered to be in the avascular zone [21]. In the pig, the vascular pattern is similar to human [22], and in the dog, the peripheral 25 % of the meniscus is vascular [19]. Meniscal lesions in the vascularized zone have greater reparative ability than in the avascular zone, where lesions frequently fail to heal. Therefore, when considering the potential severity of subsequent PTA it is critical to know whether the induced lesion is in the avascular or vascular region, particularly if skeletally immature animals are used. If necessary, the vasculature should be mapped in pilot studies.

Tissues and Characteristics of Reparative Meniscal Surfaces

In humans, a small reflection of the synovium exists on both femoral and tibial surfaces of the peripheral meniscus and extends up to 3 mm from the peripheral margin. In other species, such as the rabbit, functional synovium is required to generate a robust fibrous reparative response following meniscectomy [23]. Reparative tissue is typically disorganized fibrous tissue which does not remodel to normal fibrocartilage within the time frame of any reported animal studies. This fibrous tissue may fill the gap formed after radial tear, or fill the site of the original meniscus following meniscectomy, or anterior or posterior horn resection. Fibrous reparative tissue is much less frequently encountered following longitudinal tears in the avascular zone, or following partial meniscectomy in a radial direction. Initiation of the reparative response requires extension of the injury into the vascular region, and reparative tissue generated from synovial origins attaches to the joint capsule and infrapatellar fat pad, but not the tibia. The reparative tissue is generally

narrower than the original meniscus with less organization of collagen structure and inferior mechanical properties when compared to the native meniscus. An unusual response occurs in sheep following lateral meniscectomy; the popliteal tendon may dislocate into the joint, undergo structural changes and function to protect the posterior tibial plateau [24].

Mechanical Properties

Under uniaxial confined compression, the deformation of the posterior medial and lateral meniscus in human, bovine, dog, and sheep is comparable, but the permeability of the sheep meniscus is most similar to human, while the aggregate modulus of both sheep and pig are similar to human [25]. When human was compared to baboon, bovine, dog, rabbit, and pig [26], the aggregate modulus of the anterior horn of human meniscus was greater than that of the pars intermedia and posterior horn; this gradient was also seen in rabbit. Overall the aggregate and shear moduli of human medial meniscus were most similar to bovine, but the permeability of dog, rabbit, and baboon were most similar to human. The Poisson's ratio of human, dog, bovine, and pig meniscus are similar. The pars intermedia of the medial meniscus of bovine, and pig are stiffer than human medial meniscus, whereas the stiffness of sheep meniscus was similar to human [27]. Overall, the mechanical properties of the sheep meniscus appear to more closely match those of the human than other species.

Severity of PTA

There is some species dependence with respect to the severity of OA that develops following meniscal injury. In general, the greater the degree of disruption of the meniscus, the more severe the resulting PTA, but there is some inter-species variation, and the degree of PTA may be different with lateral compared to medial injury. Compared to other models of PTA, rabbit histological lesions following meniscal injury alone are generally less severe than ACL transection models, or models in which meniscectomy is combined with ACL transection or other ligamentous injury [10]. In contrast, in the rat, medial meniscal transection may have a more rapid and severe disease course than either ACL transection or ACL transection and partial medial meniscectomy [28].

Animal Selection and Numbers

It is generally accepted that skeletally mature animals, for example rats more than 12 weeks of age [28], rabbits more than 7–8 months of age [10], and guinea pigs more than 3 months of age [29] should be used. There may be compositional differences between immature and mature animals [30], and differences in meniscal vascularity and reparative responses. For example, following medial meniscectomy in rabbits, the amount of reparative tissue formed was similar between immature and mature rabbits, but the rate of maturation was slower in older rabbits [31]. The experimental endpoint relative the rate of development of PTA is a major determinant of numbers of animals needed to detect a treatment effect; thus, pilot studies to permit adequate power analysis should be performed.

Animal Strain

With rodent species, a wide variety of genetically modified strains are commonly used and are helpful to investigate the pathogenesis of PTA. However, investigators should be aware of known differences in the propensity of "wild-type" strains to develop PTA. For example, rats commonly used in medial collateral ligament transection (MCLT) and medial meniscal tear (MMT) models are the Lewis, Sprague–Dawley, or Wistar [28]. These strains respond differently to MCLT/MMT; Lewis rats have more severe OA in the outer third of the tibial plateau with a gradient to less severe OA in middle and inner thirds, but Sprague–Dawley rats have the reverse gradient, with more severe lesions in middle third, and develop larger osteophytes. Sprague–Dawley rats

also develop spontaneous cartilage cysts with age, whereas Lewis rats do not [28]. Further, Lewis rats have a defective hypothalamic–pituitary–adrenal axis, thus may have increased pain after MMT/MCLT compared to Wistar rats [32].

Destabilization of the Medial Meniscus in Rodent Injury Models

Destabilization of the Medial Meniscus (DMM)/Cranial (Anterior) or Caudal (Posterior) Meniscal Pole Release

Destabilization of medial meniscus (DMM) was recently introduced in murine OA models using the 129/SvEv strain [33]. The surgical approach is to transect the medial meniscotibial ligament to induce PTA, while leaving the lateral meniscotibial ligament, medial collateral ligament and meniscus intact (Fig. 5.3). Mice following DMM surgery demonstrated greater stress on the posterior femur and central tibia of the medial side of the joint. DMM increases anterior–posterior range of motion, and results in bone and cartilage loss primarily on the posterior portion of the medial tibial plateau [34]. DMM induces mild-to-moderate knee PTA within 4 weeks, while moderate-to-severe PTA is observed at 8 weeks. The severity and location of lesions after DMM may be similar to lesions observed in naturally occurring OA in mice [33].

In rats following DMM, matrix loss occurs in the superficial and middle zones of cartilage by one week after surgery, and severe cartilage clefts are observed by four weeks [35, 36]. Similar to mice receiving DMM, rats also exhibit the most severe lesions in the central area of medial tibial plateau.

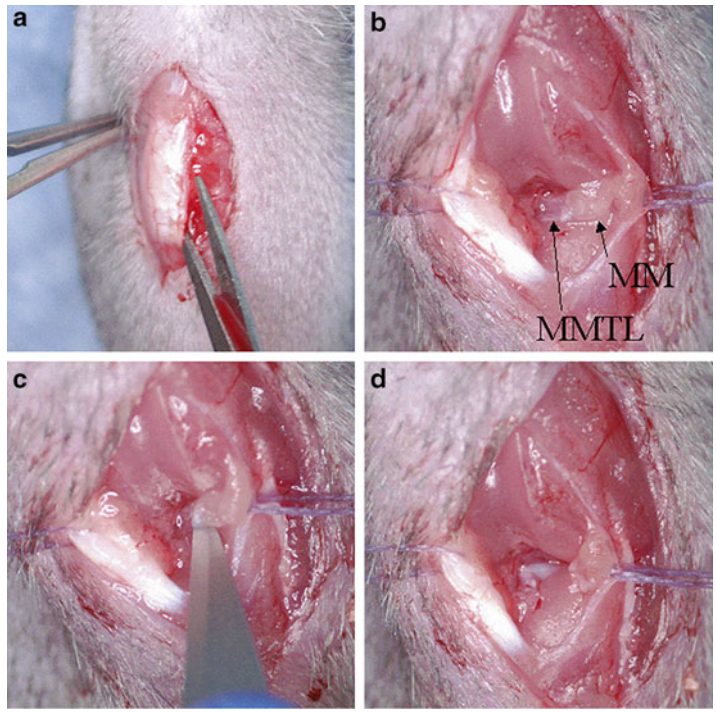

Fig. 5.3 (**a**) Surgical approach to the right mouse knee joint with a medial para-patellar ligament incision; (**b**) Following fat pad blunt dissection the medial meniscus (MM) and medial meniscotibial ligment (MMTL) are identified; (**c**) Transection of the MMTL; (**d**) Following transection, destabilization of the medial meniscus (DMM) is complete and the meniscus is free to displace medially. Note: Incisions are shown twice as long as usual in order to obtain good quality images

However, to date, the majority of DMM data are from the mouse, possible due to the increased opportunity for use of transgenic animals.

OA patients often present with joint inflammation, with increased inflammatory immune cells infiltrating into the synovium [37]. It is important to note that DMM only induces mild synovial inflammation in mice [38], while enzymatically induced-OA model such as intra-articular injection of collagenase causes prolonged joint inflammation [39]. Similar to OA in humans, wild type mice following DMM also show subchondral bone changes and osteophyte formation [33, 36, 40]. Due to its reproducibility, the murine DMM model has been widely used in various studies investigating OA pathogenesis and therapeutic treatments for OA. In the following sections, we will discuss several important parameters in applying the DMM model to mice.

Mouse DMM PTA Model and Aging

Through microarray analysis of joint tissue, 12-month old C57BL/6 mice demonstrated increased activity of matrix genes and matrix-degrading enzymes compared to 12-week old mice after DMM [41]. Therefore, the authors suggested that age of the mice should also be taken into consideration during mouse DMM PTA experimental design. Furthermore, although It has been implied that DMM surgery induces cartilage lesions similar to those observed in spontaneous OA in mice, we are not aware of any longitudinal comparisons of DMM-induced PTA compared to naturally occurring OA in mice [33]. Therefore, the relationship between DMM-induced OA and spontaneous OA in mouse models remains unknown.

Mouse DMM PTA Model is Sex-Dependent

Similar to humans, the severity of DMM-induced PTA in mice is sex dependent. In the 129S6/SvEv strain, male mice develop more severe PTA than females after DMM, and the same trend was observed in 129SvEv, FVB/N, and C57BL/6 mice [42]. These differences may be associated with sex hormones since ovariectomized female mice had less severe PTA in comparison to intact females following DMM. In humans, however, it is important to note that women have higher prevalence of OA than men after age of 50 [43].

Mouse DMM PTA Model and Obesity

Obesity is one of the primary factors in OA development; therefore, researchers have been seeking to understand the relationships between obesity and PTA using mouse DMM or other similar surgically induced meniscus-destabilization models [44]. Increased tissue adiposity predisposes to anesthesia-related complications and increases the difficulty of visualizing the meniscotibial ligament of the medial meniscus in obese mice due to increased size of the infrapatellar fat pad. The infrapatellar fat pad is located in the anterior of the knee joint, between the joint capsule and the synovium [45], and blunt dissection over the intercondylar region through the infrapatellar fat pad must be used to visualize the meniscotibial ligament of the medial meniscus [33]. The infrapatellar fat pad consists of mainly white adipocytes but also contains immune cells and stem cells that are capable of differentiating into mesenchymal lineages [46, 47]. The infrapatellar fat pad also plays a role in patellar blood supply [48]. High-fat diet increases the size and blood vessel network of the infrapatellar fat pad [49]. Therefore, DMM surgery in obese mice is also more challenging since increased angiogenesis in the infrapatellar fat pad may increase hemorrhage at surgery. Based on our observation, epinephrine frequently needs to be applied to the fat pad for hemostasis when performing DMM in obese mice. While some researchers prefer to completely remove the infrapatellar fat pad (personal communications in animal model study session, OARSI 2013), total removal of the infrapatellar fat pad may influence disease progression. The infrapatellar fat pad may play an active role and

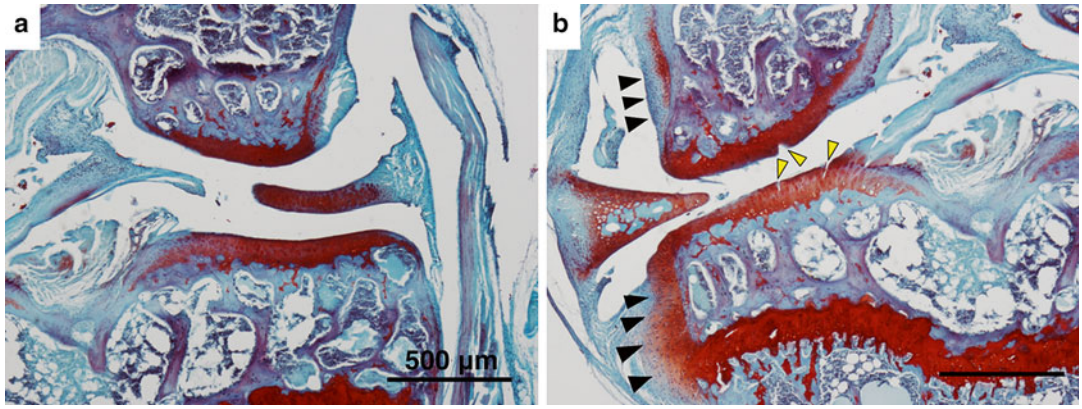

Fig. 5.4 Safranin O and fast green staining of knee joint of C57BL/6J obese mice (**a**) without DMM (**b**) with DMM surgery. The mice weighted around 35 g. Obese mice receiving DMM exhibited cleft and fissures in cartilage (*yellow arrowheads*) and osteophyte formation (*black arrowheads*) within 2 weeks post-surgery

stimulate development of PTA in human and animal models by secreting cytokines such as IL-6 [50–53], despite the fact that more clinical trials are required to elucidate the whether removal of IFP is beneficial or detrimental to development of OA in patients [54]. Therefore, care needs to be taken in interpretation of results if investigators remove the infrapatellar fat pad during DMM surgery. Here, we suggest that resection of the IFP should be restricted to the smallest amount possible to allow visualization of the medial meniscotibial ligament. Furthermore, clear description of the degree of resection of the infrapatellar fat pad in the manuscript will also help investigators compare PTA progression and severity in obese mice between different studies.

In addition, obesity accelerates DMM-induced PTA progression. The cartilage surface of obese mice after DMM exhibited deep fissures at 2 weeks (Fig. 5.4). The time needed to develop OA in obese mice is significantly shorter than in lean mice and subchondral bone erosion may occur in obese mice by 12 weeks post-surgery. Moreover, we found that metabolic factors such dietary fatty acids contribute a more critical role in DMM-induced PTA than do mechanical factors such as body weight in obese mice [55].

Mouse DMM PTA Model and Biomechanical and Neurobehavioral Activity Measurements

The DMM model has been used to elucidate the relationships between OA, pain and behavioral activities. Interestingly, several studies have shown that DMM-induced OA does not significantly affect spontaneous locomotion and rota-rod performance in mice at any time point post-surgery [56], while some have reported that DMM surgery decreased the distance mice traveled per hour [57]. The discrepancy between these studies may result from the length of time mice were assessed for spontaneous activity. However, when mice receive transection of both anterior and posterior cruciate ligament, they showed decreased rota-rod performance one week post-surgery but maintained the same spontaneous locomotor activity compared to the sham group [58], which may be related to development of more severe PTA in ACL transected mice than the mice receiving DMM surgery.

The DMM model has also been used to investigate PTA-related pain. Inglis et al. [59] demonstrated that pain behaviors develop several weeks after detectable histologic change of articular

cartilage post-DMM, and this delay could be associated with the inhibition by peripherally active endogenous opioid actions. It has been further reported that nerve growth factor (NGF) was highly upregulated in mice 3 days and 16 weeks post-DMM surgery but not at any interim time point. Treating mice with TrkAd5, a soluble NGF receptor, however, suppresses pain at these two time points, indicating that NGF could be a critical mediator in PTA-related pain. In addition, macrophages may be involved in OA pain resulting from DMM. Miller et al. [57] observed that macrophages massively infiltrate into dorsal root ganglia along with upregulated the monocyte chemoattractant protein-1 (MCP-1) and its receptor C-C motif receptor 2 (CCR2) at 8 weeks post-DMM surgery, correlating with the time mice start to exhibit pain behavior. Interestingly, the upregulation of MCP-1/CCR2 returned back to baseline levels at 16 weeks after DMM, while infiltrated macrophages still remained within the dorsal root ganglia [57]. The results of these studies imply that various mechanisms may regulate PTA pain at different time points following joint injury. Indeed, these mechanisms are further complicated by obesity and sex. For examples, in our previous studies, we found that C57BL/6 male obese mice preferentially affected spinal sites for hyperalgesia [55], while female C57BL/6 mice showed hyperalgesia primarily through supraspinal sites when fed a high-fat diet [60]. Thus, our findings and the results of others suggest that the effect of obesity on nociceptive responses of C57BL/6 mice may be sex dependent [61].

Mouse DMM OA Model and Cage Environment

In addition to age and obese status of the mice, mouse husbandry such as environmental enrichment devices may have a role in DMM-induced PTA. For example, Salvarrey-Strati et al. [62] reported that mice housed with heavy plastic tube (CPVC tube) and Tecniplast Mouse House exhibited higher OA score, while mice with Shepherd Shack had less cartilage degeneration. These authors also observed that the numbers of the mice housed in the cage had significant impact on OA severity as group-housed mice demonstrated higher OA score relative to individually housed mice. To establish a more reliable mouse DMM model, Kim et al. [63] suggest that using small cages (75 L×40 W×200 H mm^3) with limited movement may decrease individual variation and control OA severity; however, animal welfare considerations of limited housing space need to be considered.

Other Meniscal Injury Models

DMM in Non-rodent Species

DMM surgery in the rabbit has recently been described [64]. Peak contact stress in the medial compartment of operated joints was significantly elevated immediately following DMM surgery. In addition, both medial femur and medial tibia had more severe OA as compared to the lateral side of the operated joint 8 weeks post-surgery. These data suggest that DMM in the rabbit model has similar distribution of cartilage PTA lesions as murine DMM models. However, in contrast to the murine model, the posterior rather than the anterior horn of the medial meniscus is destabilized by transection of the posterior root of the medial meniscus. Posterior horn medial meniscal release in dogs results in lameness, joint effusion, radiographic evidence (effusion, subchondral bone sclerosis, osteophytosis) of OA, gross and sonographic evidence of meniscal pathology, and arthroscopic and gross evidence of articular cartilage pathology, primarily on both surfaces of the medial compartment. Reparative tissue was only evident across the third of the meniscus in the vascular zone closest to the meniscocapsular border [65]. In the sheep, cranial pole release of the medial meniscus caused a temporary unloading of the limb (approximately 85 % of baseline at 2.5 weeks postoperatively), and cartilage lesions and thinning at 12 weeks predominantly in the craniomedial tibial plateau and corresponding femoral contact areas. However, while location of the focal lesions was different from medial

meniscectomy and medial body transection groups, gross and microscopic OA scores were similar. The major pathological difference between medial meniscectomy, medial body transection, and cranial pole release was the degree of subchondral sclerosis in the medial femoral condyle [12]. Cake et al. also identified key molecular similarities between meniscectomy and cranial pole release, supporting the idea that maintenance of meniscal mechanical function and resistance to tensile hoop stresses is critical for prevention of cartilage pathology [12].

Medial Meniscal Tear/Medial Collateral Ligament Transection (MMT/MCLT)

In the rat, transection of the medial collateral ligament (MCL) distal to the attachment of the medial meniscus to the MCL, then transection of the medial meniscus at its narrowest point results in rapid cartilage degeneration and subchondral sclerosis by 3–6 weeks postoperatively, reduced weight-bearing by 3 weeks, and increased mechanical allodynia and modulation of motivational, reward-aversion and pain sensory circuitry in the brain at 3–5 weeks [28, 66–68]. However, increased thermal hyperalgesia or blunt pressure mechanical hyperalgesia are not features of this model [32].

PTA lesions are most severe on the outer third of the medial tibia adjacent to the synovium, and less pronounced in the middle third and inner third, adjacent to the ACL, although this is rat strain dependent [28, 67]. At 8 weeks, joint space narrowing is evident by μCT examination [69], and changes relating to chronic neuropathy and inflammatory pain are noted in dorsal root ganglia and spinal cord [69]. By 12 months, there is progression to eburnation of the medial tibial plateau [28]. Osteophytes progressively increase in size, and subchondral bone remodeling is also evident. The integrity of the MCL postoperatively is critical and influences the degree of capsular thickening observed, which alters joint stability and therefore development of OA [28]. Generally a 3-month time point is recommended to evaluate any local treatment effect [28], although longer time points may be needed for some outcome measures.

The additional effect of MMT compared to MCLT alone has been recently investigated in Lewis rats followed for 28 days postoperatively [70]. MMT/MCLT limbs had increased mechanical allodynia compared to unoperated in the same animal, but compared to control animals, there was only a trend to increased mechanical allodynia in MMT/MCLT groups, and no difference between surgical groups. High-speed videography was required to detect dynamic gait asymmetry that was identified in MMT/MCLT, but not MCLT groups. Peak vertical force and vertical impulse were reduced in MMT/MCLT, compared to control, and vertical impulse was reduced in MMT/MCLT, compared to MCLT alone. Propulsive forces were lower in MMT/MCLT animals than in controls, but this was only a trend in MCLT animals. Significant differences in serum cytokines were not identified between groups. Histological lesions were significantly worse in MMT/MCLT than MCLT [70]. Thus, MMT/MCLT produces very distinctive functional and histological deficiencies that MCLT alone does not. Together, these data demonstrate the importance of well-designed functional outcome measures, and the usefulness of MMT/MCLT in investigation of a range of joint-level to whole body measures of PTA.

Similar models in guinea pigs result in acute synovitis, loss of chondrocytes and proteoglycan, and collagen disruption in the superficial and middle zones of the tibial plateau as early as 3 days postoperatively. By 3 weeks, there is loss of one third of the medial tibial plateau cartilage and immature tibial and femoral osteophytes with synovial hyperplasia. By 12 weeks, cartilage loss extends into the deep zone of the tibial plateau and osteophytes are extensive. In contrast, in the guinea pig, lateral collateral ligament transection and meniscal tear results in inconsistent lesion development, suggesting that the degree and pattern of weight-bearing postoperatively is critical [29].

Radial Tears

In rabbits, healing ability following radial tear is similar to longitudinal lesions and is influenced primarily by the extent of the tear, and whether it incorporates the vascularized peripheral region. However, the radial margins of the tear retract, resulting in a greater volume of repair tissue in radial compared to longitudinal tear [71]. Radial tears in the pars intermedia of the rabbit heal rapidly with fibrocartilage by 7 weeks [72]. In contrast, complete radial tear of the medial meniscus in sheep between the anterior horn and pars intermedia separates and fills with disorganized fibrous tissue by 6–12 months. This lesion results in mild synovial effusion and hypertrophy, severe medial compartment OA and mild lateral compartment OA at 6 and 12 months, and a significant increase in Outerbridge grade of the retropatellar cartilage. Atypically, compared to other studies, at these extended time points, there was no difference in OA between tibial plateau and femoral condyle [73, 74].

Mid-body transection of the medial meniscus in sheep fills with reparative tissue and results in more persistent gait abnormalities, but less severe PTA than following either medial meniscectomy or cranial pole release. Medial tibial plateau lesions are centrally located, with opposing femoral lesions. Subchondral sclerosis of the medial femoral condyle also occurs [12]. It is possible that a less robust reparative response occurs in the dog and pig compared to the rabbit and sheep following radial tear. Wedge-shaped defects in the dog, with the base of the wedge in the peripheral zone, result in an incomplete fibrovascular healing response with little progression over 1-year. The resulting PTA is more prominent on the tibial plateau than on the femoral condyles [75]. In contrast, a radial incision of the medial meniscus formed a 1–2 mm gap, but by 2 weeks was filled with a fibrin clot, and 6 weeks the gap was filled with well integrated fibrovascular scar [19].

In pig, radial tears at the junction of the anterior horn and pars intermedia extending from the inner zone for two thirds of the width do not heal but development of PTA was not evaluated [22]. In general, radial tears heal with reparative tissue, but there is loss of the tensile hoop function of the meniscus which results in PTA. Healing response may be less robust in the pig and depends on minimal trauma to the synovium if a wedge-shaped defect is used.

Longitudinal Tears

The majority of longitudinal tears in the avascular zone of the pars intermedia of rabbit, dog, pig, and sheep fail to heal by 6 months, with no evidence of intrinsic repair. If the tear is extended to the meniscosynovial junction, then evidence of healing by extrinsic tissue is seen [76]. For example, peripheral lesions in the vascular region of the rabbit heal by 10 weeks and significantly better than lesions in the meniscal body and inner rim, which do not heal by 10 weeks [71, 77]. In rabbits, 3–6 mm longitudinal lesions in the avascular zone of the medial meniscus, between the pars intermedia and the anterior horn fail to heal and may lengthen by 11 months. PTA is identified by 3 months, with the most contribution to aggregate joint scores from the tibial plateau and no contribution from the lateral femur. By 9 months, OA is identified on all cartilage surfaces [78], and at 11 months, range of motion in extension may be limited by 10°–25° [79]. Likewise, in the lateral meniscus, 4 mm longitudinal lesions in the avascular zone of the pars intermedia fail to heal [80]. In the posterior horn longitudinal tears in the avascular zone only partially heal by 6–12 weeks, and the reparative tissue has tear load propagation resistance only 20–25 % of that of control tissue [81].

In large animal models of longitudinal tear, 15–20 mm lesions in the anterior horn are typical, also fail to heal and result in PTA. Longitudinal tears in the vascular region of the anterior horn of the medial meniscus in adult sheep result in a persistent hyperintense UTE T2* magnetic resonance imaging signal in the anterior horn and a hyperintense T2 MRI signal in the femoral and tibial cartilage at 4 months, even after immediate suture repair. On the tibial

plateau, the T2 signal was more hyperintense anteriorly than posteriorly, indicative of greater collagen disorganization in the anterior tibial plateau [82]. Sixteen weeks after 10 mm longitudinal tears were made in the anterior horn of the medial meniscus in adult sheep, 3 mm from the meniscocapsular junction, synovial effusion, synovial hyperplasia, and failure of the tear to heal with no evidence of intrinsic repair were noted, but cartilage was not evaluated [83].

In the dog, a longitudinal tear extending between the anterior and posterior horn, then "trapped" in the intercondylar notch by suture resulted in synovial effusion at 2 weeks and synovial hypertrophy at 4 weeks which resolved by 12 weeks, but meniscal atrophy and gross evidence of tibial and femoral PTA was evident [84]. In the central avascular portion of the medial meniscus in the dog, 10 mm longitudinal incisions fail to heal by 10 weeks [19]. Longitudinal incisions in the posterior horn of the canine medial meniscus, 2–3 mm from the meniscosynovial junction partially healed by 12 weeks after surgery but osteophytes were evident at the condylar margins, OA histological scores were increased, synovitis was observed and linear and toe region tensile moduli were reduced compared to control [85]. The majority of 15 mm longitudinal tears in the peripheral 25 % of the posterior horn-pars intermedia of the goat fail to heal within 6 months, but chondral injury was also not observed [86]. Similarly, bi-meniscal 10 mm longitudinal tears in sheep in the avascular zone result in synovial effusion, decrease in synovial fluid pH and total protein, and failure to heal at 1 month [87].

Partial Medial Meniscectomy

Several approaches to partial medial meniscectomy are reported: either radial resection of one- to two-thirds the width of the meniscus [88, 89], or resection of anterior or posterior horn. Further, wide variation in the degree of resection has been reported, ranging from 50 to 80 % in the dog [90]. The reparative response generated following partial medial meniscectomy depends on the plane of resection, and on whether the injury site includes the meniscosynovial junction. The reparative tissue is generally not protective against PTA, but may mitigate the development in disease in areas of the tibial plateau formerly covered by meniscus.

In the athymic rat, resection of the medial meniscus obliquely from the inner rim to two thirds of the width at the junction between the anterior horn and the pars intermedia fails to heal [91]. In contrast, in the Lewis rat, resection of the anterior half of the medial meniscus at the level of the medial collateral ligament results in reparative tissue filling 60–80 % of the tibial plateau surface area within 4 weeks, but the reparative response does not prevent a large area of PTA on the medial tibial plateau, which is evident from 4 weeks [92]. In the rabbit, the defect following removal of the anterior half of the medial meniscus may still be evident at 12 weeks, but there is some evidence of a reparative response by 12–16 weeks. Highly reproducible osteochondral lesions and sclerosis of the subchondral bone are observed and are worse on the medial tibial plateau than on the medial femoral condyle, and joint space narrowing is seen in simulated weight-bearing radiographs [93, 94]. Resection of the pars intermedia of the medial meniscus in immature rabbits results in formation of additional longitudinal tears in the posterior horn of the operated meniscus, and the original defect remains largely unfilled [95].

Formation of robust reparative tissue is variable following radial resection of one- to two-thirds of the meniscus in the dog, in contrast to the rabbit where repair tissue is prominent [88, 89, 96, 97]. In the dog following partial radial resection, the lateral compartment is grossly normal, but the appearance of the medial compartment is variable, from normal to synovitis and synovial hyperplasia with focal articular erosion, and moderate histological damage [88], but in general there is correlation between degree of meniscus resection and tibial plateau OA [89]. In contrast to partial radial resection, resection of the anterior 50 % of the medial meniscus in the dog results in

defect filling with large amounts of reparative tissue. Despite these reparative attempts, PTA is observed in both the tibial and femoral cartilages within 3 months. Medial compartment osteophytes are also observed, but lateral compartment or patellar groove osteophytes are rare [29]. Subchondral sclerosis is prominent at 3 months, but synovial pathology is mild [29]. Following 80 % subtotal medial meniscectomy, lameness increases after 180 days, and only 25 % of joints showed any reparative response of the medial meniscus [98].

Resection of the posterior horn of the medial meniscus in dogs results in obvious lameness and PTA at 12 months, with a persistent defect that is slow to fill and mature. Fibrous replacement tissue is disorganized, immature and shows poor integration even at 12 months. Osteoarthritic cartilage on the femoral condyles and tibia had reduced stiffness compared to normal [99]. Compared to longitudinal incisions in the posterior half of the medial meniscus, 2–3 mm from the meniscosynovial junction, partial posterior horn meniscectomy to within 2–3 mm of the meniscosynovial junction tends to have increased gross histological scores, but similar reductions in linear and toe region tensile moduli, compared to control, and histological scores correlate with linear region tensile moduli [85]. Ex vivo studies in the dog suggest that 30 % radial width partial posterior horn meniscectomy has no significant effect on contact mechanics, whereas 75 % radial width partial posterior horn meniscectomy and posterior horn meniscectomy increased contact pressures [100]. Though no direct comparison has been made, persistent lameness and reduced reparative response appear to be features of posterior horn compared to anterior horn resection in the dog. In general, compared to lateral meniscectomy models in rodent and rabbit, pathology develops more slowly in partial medial meniscectomy models in the dog [29]. In the sheep, resection of the anterior horn of the medial meniscus leads to filling of approximately 25 % of the defect with fibrous tissue, and evidence of PTA, particularly on the tibial plateau within 6 weeks of surgery [101].

Partial Lateral Meniscectomy

In rabbits, which preferentially load the lateral aspect of the knee, anterior horn lateral meniscectomy and transection of the fibular collateral ligament induces focal proteoglycan loss from the tibia by 3-days postoperatively, histological signs of PTA by 1 week, and gross evidence of osteophytes by 2 weeks postoperatively. Surgically induced synovitis resolves by 4 weeks, and consistent PTA lesions are evident within 6 weeks involving half of the lateral tibial plateau and femoral condyle and prominent osteophyte formation, fibrillation of retropatellar cartilage, synovial hyperplasia and subchondral sclerosis. In contrast, partial medial meniscectomy in rabbits without collateral ligament transection results in relatively mild to moderate PTA. Reparative tissue is common by 6 weeks following partial lateral meniscectomy [29, 102]. In sheep, resection of the mid-50 % of the lateral meniscus to within 1 mm of the capsule resulted in PTA of the lateral tibial plateau over the central weight-bearing area, with prolongation of T2 relaxation times and increased T1ρ values on MRI demonstrating disruption of collagen orientation and diminished proteoglycan content respectively. Newly formed tissue filled 30–50 % of the volume of these defects but did not consistently survive histological processing. Gross evidence of synovitis was not a consistent feature of this model by 6 and 12 months postoperatively [103].

Total Medial Meniscectomy

High repair potential following total medial meniscectomy has been shown in rabbits, dogs, primates, and humans [89]. However, in the rabbit, total medial meniscectomy results in more severe PTA than occurs with longitudinal tears [78], and is evident on all joint surfaces despite the formation of a 2–4 mm peripheral ring of reparative tissue forms in place of the meniscus [78]. The reparative tissue is wedge shaped and smaller than the normal meniscus, with some parallel collagen fiber organization

and mechanoreceptors present in the middle third [104]. Compared to DMM in rabbits, medial meniscectomy induced similar increases in contact area, lateral translocation of contact stress distribution, peak contract stress and Mankin scores in the medial joint compartment at 8 weeks postoperatively [64]. Following either medial meniscectomy or longitudinal tear in the rabbit, the sequence of OA development is the medial tibial plateau by 8 weeks, the posterior medial femoral condyle at 6–9 months, osteophyte development in 4–6 weeks. There is an initial increase in collagen biosynthesis occurs throughout the joint, but at 12 weeks is restricted to the medial compartment [105]. The synovium is hyperplastic and hypertrophic with infiltrating mononuclear cells at 2–4 weeks, and subsynovial fibrosis occurs by 12 weeks. Mature dendritic cells located peri-vascularly and in areas of lymphoid aggregation increase from 2 to 8 weeks [106]. Rarely, in some studies, PTA may not be observed by 6 months after medial meniscectomy even with reparative tissue filling the defect in only 30 % of rabbits [104].

Total medial meniscectomy in the dog results in more severe gross and histological damage than partial medial meniscectomy [88], but reparative tissue forms in 50–60 % of animals. However, there is conflicting evidence between large animal species as to whether the cartilage underneath this reparative tissue is protected from OA [88, 89]. By 12 weeks after meniscectomy in the dog, the tensile modulus of femoral cartilage decreases by 40 %, without change in the water and s-GAG content of the cartilage [107], but by 12–24 weeks, cartilage degeneration on the medial tibial plateau and medial femoral condyle is evident primarily in regions normally covered by meniscus, and disorganized fibrous tissue of low GAG content attached to the capsule fills up to 50 % of the size of the original meniscus [88, 108].

In the sheep, medial meniscectomy reduces contact area by approximately one half, and increases peak contact pressure by over 2.5-fold compared to control limbs [109]. Six months after medial meniscectomy in the sheep, PTA of both medial tibial plateau and femoral cartilage is seen, with thinning and fibrillation of cartilage in central load-bearing areas of the medial meniscus, and thickening of cartilage on the edge of the plateau formerly covered by the meniscus. In some studies, pannus formation was observed, particularly at the periphery of the tibial plateau as early as 6 weeks after surgery [101]. Joint effusion is mild, and synovial hyperplasia is most severe in the craniomedial joint compartment, and with some filling of the defect with reparative tissue [110]. PTA following total meniscectomy in the sheep is worse than after resection of the anterior horn [101]. Outcomes following medial meniscectomy in sheep are more similar to transection of the cranial pole of the medial meniscus than meniscal body transection, with similar degrees of gait deficits and gross and histopathological scores, but more widespread medial tibial plateau damage following medial meniscectomy compared to cranial pole transection. However, with the exception of increased subchondral sclerosis following medial meniscectomy compared to cranial pole release, molecular markers of OA were similar between cranial pole release and medial meniscectomy, suggesting that maintenance of the tensile hoop function of the medial meniscus is critical to prevention of OA [12].

In the goat, 4 months after medial meniscectomy contact area is reduced and more focal with abrupt boundaries compared to the normal broad irregular contact area. Contact pressures increase and this increase persists for at least 8 months after surgery, by which time contact area has again become more diffuse. These changes coincided with tibial subchondral sclerosis, and softening and fibrillation of articular cartilage. Contact pressures in the lateral compartment were unaffected. Interestingly, reparative tissue was not a feature in this goat model [111].

Total Lateral Meniscectomy

In immature rabbits, lateral meniscectomy results in a thinner and narrower reparative tissue than following medial meniscectomy, and more severe PTA in knees with a poor reparative response. Thus, OA is more severe after lateral meniscectomy,

and more severe on the tibial surface than on the femoral [112]. In rabbits, gross articular cartilage lesions are identified within 3 weeks of lateral meniscectomy and are more severe than following medial meniscectomy. Reparative tissue is evident within 1 week of surgery, and may replace the meniscus within 3 weeks of surgery, but fills the defect more slowly after lateral meniscectomy than after medial meniscectomy [113]. The more severe OA typically observed after lateral, rather than medial meniscectomy may be due to the increased coverage provided by the lateral meniscus over the lateral tibial plateau, even though contact stresses are generally lower [12].

At 3–6 months after lateral meniscectomy in the sheep, cartilage degradation is restricted to the lateral compartment, with most severe changes in the lateral femoral condyles and tibial plateau, especially in regions of the tibial plateau formerly covered by meniscus. Regions of focal articular cartilage degeneration in the lateral compartment have reduced shear modulus, collagen organization, proteoglycan loss, with thickening of articular cartilage, and increased s-GAG content but reduced shear modulus in regions previously covered by articular cartilage. In the medial compartment there is cartilage thickening, with increased s-GAG content, suggesting a global anabolic attempt at neutralizing increased contact pressures [114–116]. However, the anabolic response is not equal between lateral and medial joint compartments after meniscectomy in adult sheep. In the lateral compartment, there is higher loss of s-GAGs and a lower anabolic response from the articular cartilage following lateral meniscectomy, compared to the changes occurring in the medial compartment following medial meniscectomy [117]. In the dog, at 3–6 months after total lateral meniscectomy, no signs of synovial inflammation are evident, tibial OA lesions are diffuse and more severe than the femoral lesions which are localized to the posterior curvature of the condyle [118]. By 2 years postoperatively, a well-defined fibrous structure containing some degree of collagen organization, type II collagen and s-GAG deposition [119] is present in the original location of the meniscus, but is not protective against lateral compartment PTA which is worse and more diffuse on the tibia compared to the femur [120]. Thus, in the dog, PTA after lateral meniscectomy may progress more slowly than after medial meniscectomy.

Confounding Variables

Exercise and Immobilization

In the majority of reported studies, animals are not confined postoperatively. Return to normal gait patterns by subjective observation are typically reported by 2 weeks after surgery in large animal species, but lameness may be observed at later time points as PTA develops. Active exercise following medial meniscectomy in sheep increased synovial effusion, synovial hyperplasia, variable amounts of meniscal regrowth, marginal osteophytosis and cartilage hyperplasia. Consistent with these findings, active exercise stimulated chondrocyte proliferation, enhanced proteoglycan synthesis and maintained collagen levels for up to 6 months postoperatively [110]. Further, moderate consistent exercise may promote more severe and consistent OA lesions [29, 121]. Physical rehabilitation regimens have been well described for the dog and are potentially applicable to other species which are amenable to training; use of these may increase applicability for translation of findings to human [13], and controlled postoperative exercise may also standardize OA development [63].

Immobilization studies have produced conflicting results; some studies have suggested that immobilization does not substantially affect the rate of healing of longitudinal or radial tears of the medial meniscus in rabbit models [71]. Other studies have identified diminished or delayed healing following immobilization of 4 mm longitudinal tears in the vascular zone of rabbit medial meniscus [21]. In contrast, immobilization to reduce maximum load to less than 50 % of the uninjured level increased invasion of vascularized synovium into 5 mm longitudinal defects in the medial meniscus of immature sheep at 6 weeks after injury [122]. In dogs after medial meniscectomy, immobilization for 5 weeks [123]

did not influence the degree of new tissue formation at 6-months postoperatively, but reduced the organization of the new tissue and GAG content of medial compartment cartilage [123], and impaired collagen formation in sutured vascular region longitudinal tears within 2 mm of the mensicosynovial junction [124]. Further investigation is required to study the response to immobilization of the knee joint with external fixator compared other methods to unload the limb since this may influence the degree of continued limb loading, and thus the effects of partial weight-bearing in addition to immobilization.

Iatrogenic Injury and Surgical Challenges

Injury to the articular cartilage at the time of surgery may occur to the tibial plateau during creation of longitudinal defects in the meniscus, or to the femoral condyles in rats during medial meniscal tear surgery [28]. For lesions in the anterior horn, drawing the meniscus anteriorly was noted to minimize iatrogenic damage to tibial cartilage [79]. Failure to induce full thickness longitudinal tears from the femoral to tibial surface of the meniscus has been reported [82]; thus, appropriate instrumentation and technique are important to avoid iatrogenic cartilage injury while achieving full thickness lesions. Arthroscopic surgery has been helpful in this respect, where species size renders this possible. A further advantage of arthroscopic model induction is to minimize the biologic response to the surgery itself, which may enhance the healing response and functional outcome measures [13]. Minimizing surgical trauma results in faster recovery to weight-bearing and thus induction of OA [29]. The infrapatellar fat pad and medial aspect of the joint capsule are highly vascular; therefore, achieving hemostasis appropriate for the species involved is critical to surgical consistency and success. If meniscal injury is intended to be in the avascular zone, then this should be delineated if not previously reported before surgery, and confirmed at the time of surgery by lack of bleeding after meniscal incision. Equally, if reparative response is desired or expected, then viability of the synovium at the site of injury should be preserved.

The learning curve of the surgeon influences the severity of induced disease; therefore, adequate training and limiting the number of surgeons to minimize variability and surgical trauma is important. Evaluation of these factors as potential confounding variables should be performed through statistical evaluation for surgeon effect and adequate randomization across treatment groups.

Outcome Measures

A variety of structural, physiological and functional outcome measures can be used in all of the animal models described. Careful selection of the outcome measures to answer the specific research hypothesis will improve the applicability of the study to the human condition. Serum and synovial fluid can be harvested at various time-points before and after induction of meniscal injury to assess for known inflammatory and catabolic biomarkers of PTA.

Functional Outcome Measures: Gait analyses, activity monitoring, evaluation of pain pathways and imaging of both osteochondral and soft tissues (radiography, ultrasonography, MRI, μCT) are all extremely valuable in determining functional progression of PTA after meniscal injury in large animal species. The majority of these techniques are also applicable to small animal species (rabbit, mouse, rat, and guinea pig), although resolving cartilage in adequate detail may be problematic for some of these techniques. "Second-look" arthroscopy may be valuable in species where this technique is possible.

Macroscopic Evaluation: For small animal models (rats, and mice) preservation of the intact joint for histological evaluation may preclude macroscopic evaluation, but for larger animal models where synovium is harvested separately and size precludes evaluation of the intact joint (>rabbit) this should be performed and regional differences

evaluated. India ink or Evan's blue can be used to aid evaluation of gross lesions, and photography should be used to image the surfaces, and then mapped to total surface area.

Microscopic Evaluation: The first decision to be made is in which plane to section the joints—frontal, coronal, or sagittal. Frontal plane sectioning includes femoral condyles, MCL, LCL, menisci, tibial plateau, synovium, but not the patellar, trochlear ridges or femoral grooves. With sagittal plane sectioning, osteophytes are difficult to detect and quantify in medial compartment, and synovial tissue not as easy to evaluate [28]. Protocols for decalcification and staining should be established in similar tissue before use of valuable experimental tissues. Safranin O/fast green or toluidine blue staining is recommended for cartilage and meniscus and Hematoxylin and eosin for synovium and meniscus. It has been suggested that for early OA, as may occur with many models of meniscal injury, the modified Mankin scoring system may discriminate better than other scoring systems where there is relatively mild structural damage [12]. In induced meniscal disease models, cartilage lesions are frequently focal; therefore, consistent and representative sampling is critical between control and experimental animals. Further, evaluation of both the depth of lesion and the extent of the lesion across the plane of sectioning are critical. While the most severe osteoarthritic changes occur in the compartment (medial or lateral) of the joint corresponding to the meniscal lesion, changes in other regions of the joint have been reported, especially with extended experimental end-points. Therefore, evaluation of all joint regions should be considered for complete analysis, since abnormalities in long-term studies have been identified in retropatellar cartilage and the femoropatellar joint [73, 74]. When considering regional analysis on the tibial plateau, both regions previously protected by the meniscus, and those not covered by the meniscus and/or any reparative tissue should be evaluated [114].

Meniscal Pathology: Semiquantitative evaluation of meniscal pathology should be performed systematically across both femoral and tibial surfaces, at the inner border, and within the stroma [9]. Other semiquantitative grading systems have been described for the rabbit [10] and dog [125] meniscus. The meniscus should also be evaluated for tear propagation, for development of new tears, or for loss of additional meniscal tissue.

Osteophytes: Osteophytes should be graded macroscopically separately from other cartilage lesions, and microscopically may be graded from the same images as those used to assess cartilage degeneration [126]. Osteophytes may also be measured from their osteochondral base to the surface in frontal plane, and scored based on thickness [28].

Summary

Injuries to the meniscus are known to be a source of PTA in humans. While there are a variety of injury models available for the investigator interested in studying PTA, the murine DMM model is the one more widely reported and helpful for mechanistic studies. A careful analysis of the benefits and limitations of each model is presented in this chapter to help assist investigators in their study design.

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Anterior Cruciate Transection/Disruption Models of Post-Traumatic Arthritis

6

Kelly A. Kimmerling and Farshid Guilak

Introduction

The study of injuries of the anterior cruciate ligament (ACL) makes up a large body of research into the etiology of PTA in both an animal and clinical setting. Currently, animal models using ACL transection (ACL-T) include dogs, sheep, cats, rabbits, guinea pigs, rats, and mice [1–3]. The first reported ACL-T model was developed using a stab incision in a canine model by Pond and Nuki [4]. Subsequent studies in dogs and other animals have examined the effects of ACL-T on articular cartilage, synovium, gene expression, biomarkers, and pain, and have been used in a variety of settings to test various therapeutic interventions. This section focuses on the use of ACL-T animal models as a method for studying PTA, with the advantages, disadvantages, and relevant studies for each animal described below.

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Dog Model

The canine model has been used extensively for studying the effects of ACL-T, and there are a variety of advantages to using dogs for osteoarthritis (OA) research. For instance, they have a slow disease progression after injury, allowing for long-term observation of changes that occur as a result of PTA. Dogs also have thick articular cartilage, have larger joints, are trainable, and have well-documented outcomes to injury models, with a pathology that mimics naturally occurring arthritis. However, the high cost and public perception of using dogs are drawbacks to this model [1–3]. The first use of dogs for an ACL-T model was reported by Pond and Nuki in 1973, which utilized a stab incision into the knee joint to induce ACL-T [4]. Subsequent studies followed using the stab incision model, focusing on areas such as osteophyte formation [5], biochemical changes and gene expression [6–9], mechanical properties [10], and imaging techniques [11]. Therapeutic studies examined the effect of inhibiting nitric oxide (NO) [12] or delivering licofelone [9, 13] as a chondroprotective agent. A summary of the studies utilizing the stab incision model are given in Table 6.1.

After the introduction of the Pond-Nuki transection model (stab incision), other methods of ACL-T were studied. Brandt published a review validating the use of the canine ACL-T model for the study of arthritis [16], and open induction

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Table 6.1 Canine ACL-T models (closed induction)

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
Unknown	Stab incision	Unknown	1–26 weeks	OA changes in articular cartilage (fibrillation, loss of cells in superficial layer); synovitis subsides within 1 week	[4]
Varies	Stab incision	Adult	1–48 weeks	Periarticular osteophytes seen weeks 3–48 in marginal zone	[5]
Varies	Stab incision	2 years	3, 6, 9, 48 weeks	Thicker cartilage observed; chondrocytes synthesize GAGs with more chondroitin sulfate vs. keratin sulfate than normal	[8]
Foxhounds, Collies, Alsatians	Stab incision	2 years	1–48 weeks	Experimental, biochemical, morphological changes observed	[7]
Greyhounds	Stab incision	2–3 years	6, 12 weeks	Significant ↓ in tension, compression, shear; significant ↑ in hydraulic permeability, hydration of cartilage matrix	[10]
Mongrel dog	Stab incision	2–3 years	12 weeks	↑ chondrocyte apoptosis, caspase 3, and Bcl-2 in articular cartilage; L-NIL (NO inhibitor) group showed ↓ apoptosis, caspase 3 after ACL-T	[12]
Mongrel dog	Stab incision	2–3 years	12 weeks	↑ IL-1-converting enzyme (ICE), IL-18 in articular cartilage; ↓ PI-9; ICE not regulated by NO	[6]
Mongrel dog	Stab incision	2–3 years	8 weeks	↑ gene expression of MMP-13, cathepsin K, ADAMTS-4, ADAMTS-5, 5-lipoxygenase in OA; ↑ bone loss and osteoclast staining of MMP-13, cathepsin K; licofelone ↓ OA changes	[9, 13]
Mongrel dog	Stab incision	2–3 years	8, 12 weeks	↑ osteocalcin week 8; ↑ MMP, PGE_2 at week 12; ↓ NO levels in trabecular bone at week 12	[14]
Beagle	Stab incision	1 year	6, 12, 24, 48 weeks	Subchondral bone edema in tibia by week 6; articular cartilage erosion by week 12; menisci degeneration by week 24; osteophytes by week 48	[11]
Beagle	Stab incision	1–2 years	6, 12, 24, 48 weeks	Elevation of collagen I, II early; ↑ MMP-13 week 24; ↑ aggrecan, tenascin C week 48	[15]

models were implemented, where the ACL was visualized and transected either through an arthrotomy or arthroscopically. A wide range of studies followed, looking at aspects of open-induction ACL-T such as biochemical changes and gene expression [17–24], bone morphological changes [25–28], biomarkers [29–31], and imaging techniques [32, 33]. O'Connor and coworkers published two studies looking at the combined effect of nerve removal and ACL-T on the development of PTA [34, 35]. Two therapeutic studies used the open-induction ACL-T model, including a doxycycline therapy study [36] and an MMP inhibitor study [37]. Doom and coworkers published a review of immunopathological mechanisms that result from the ACL-T model, leading to PTA [38]. A summary of the studies using the open-induction canine ACL-T model are listed in Table 6.2 below.

Sheep Model

The use of sheep has not been widely utilized for the study of PTA; however, sheep may provide advantages because of their large joint size, which allows for the analysis of biochemical and biomechanical measures that may not be able to be performed in human subjects [39–41]. As with other large animals, sheep can readily undergo arthroscopic surgery and MRI observation, allowing for more direct translation of studies to the clinic. However, there are limited reagents and antibodies available, and until recently, a limited mapped genome for sheep, making it difficult for genetic studies [1–3]. Furthermore, their large size is a disadvantage in testing novel pharmacologic interventions. Most studies utilizing the ACL-T model in sheep have focused on radiographic tracking and kinematics of PTA. O'Brien and coworkers examined the effects of immediate reconstruction of the transected ACL on cartilage degeneration and osteophyte formation [41], while Atarod and coworkers examined the kinematic loads placed on soft tissue after ACL-T in the sheep [39]. A summary of the use of ovine ACL-T models is in Table 6.3 below.

Cat Model

Neuromuscular control has been extensively studied in cats, as well as muscle mechanics and locomotion [42]. Logically, cats would be well suited to study interventions towards musculoskeletal diseases, such as PTA that results from an ACL-T injury. Cats are advantageous to use because of their large size and known genome. However, like dogs, cats can be costly to house during experiments, and public perception and their role as companion animals discourage the use of cats for research [1–3].

Herzog and coworkers first studied the effect of ACL-T in cats on hindlimb loading and changes in articular cartilage [42]. Khalsa and coworkers studied the effect of severing the nerves associated with the joint capsule after ACL-T [43]. Herzog and coworkers monitored cats for a year, using force testing plates and radiographs to track kinematic and radiographic changes due to OA [44, 45]. Boyd and coworkers studied the changes to the periarticular bone as a result of ACL-T, while Clark and coworkers studied the adaptive response of cartilage after ACL-T [46, 47]. A summary of the studies utilizing feline ACL-T models follow in Table 6.4.

Rabbit Model

Rabbits have been a popular model for use with both ACL-T and meniscus injury models because of their low spontaneous joint degeneration, large joint size, and ease in use for testing new therapeutic agents. Rabbits preferentially load their lateral side, unlike rodents, and have the capability to spontaneous regenerate transected menisci with fibrous tissue, which can cause disadvantages for some studies. Similarly, rabbits have altered joint biomechanics, potentially resulting in a change in disease pathology compared to what may be expected in other animals. However, rabbits have been widely used as a model for OA because they form lesions similar to those seen in clinical OA [1–3].

The ACL-T model has been used in rabbits to study many aspects of PTA development. Studies

Table 6.2 Canine ACL-T model (open induction)

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
Varies	Arthrotomy	Adult	45, 54 months	Articular cartilage thicker at month 36, focal loss at month 45; osteophyte formation; ulceration of articular cartilage on medial side; fibrous thickening of capsule	[27, 28]
Varies	Not specified	Adult	Unknown	Neurectomy + ACL-T resulted in significantly higher OA scores than neurectomy without ACL-T, ACL-T	[35]
Varies	Not specified	Adult	72 weeks	Dorsal root ganglionectomy (DRG) + ACL-T after results in significantly severe OA compared to ACL-T, ACL-T + DRG, DRG	[34]
Beagles	Arthroscopic	Adult	16 weeks	Loss of tensile properties and remodeling of collagen network in surface zone of articular cartilage	[18]
Mixed breeds	Cranial transection	2–7 years	4, 10, 32 weeks	Aggrecan mRNA ↑ weeks 10, 32; collagen type II mRNA ↑ at all time points; transcription mechanisms must differ	[20]
Mongrel dog	Lateral arthrotomy	1–3 years	3, 12 weeks	Structural changes in trabeculae in cancellous bone at 3 weeks, more prominent at 12 weeks; ↓ anisotropy-accompanied changes	[25]
Mixed breeds	Lateral arthrotomy	2–7 years	3, 12 weeks	20–38-fold ↑ collagen type I, VI at 3 weeks; 11–19-fold ↑ at 12 weeks; higher concentrations in medial menisci versus lateral	[24]
Varies	Naturally occurring	1–13 years	N/A	MMP-3, TIMP-1 ↑ in SF of arthritic groups; KS ↑ in SF after ACL rupture	[29]
Mixed breeds	Lateral arthrotomy	Adult	3, 12 weeks	↑ aggrecan, collagen type II mRNA in cartilage; amount of collagen type II > aggrecan for OA	[21]
Foxhound	Medial arthrotomy	2 years	2 years	Cartilage changes of decorin, fibromodulin, aggrecan differ in models	[19]
Mongrel dog	Cranial transection	Adult	12 weeks	Significant changes from μMRI: depth of maximum T_2, ↓ SF zone thickness, ↑ total cartilage thickness; PLM confirmed	[32]
Mixed breeds	Cranial transection	Adult	3, 12 weeks	Collagen type II markers ↑ in SF; collagen type II, aggrecan markers ↑ in serum; collagen type II markers ↑ in urine	[30]
Mixed breed	Lateral arthrotomy	Adult	36, 72 weeks	Mechanical changes in ACL-T group at 36 weeks less noticeable in both ACL-T and ACL-T + Dox group; Dox therapy limited bone loss at week 72	[36]
Walker hounds	Arthroscopic	Adult	2, 10, 18 weeks	PGE_2 levels ↑ through study; correlated with gait, pain	[31]
Foxhound	Not specified	2–3 years	2 years	Minor/severe articular cartilage damage in medial compartment; joint space significantly ↑ for minor group, no change for severe group; minor group had 73 % of observed osteophytes; severity of damage of menisci and cartilage related	[33]
Varies	Naturally occurring	Adult	N/A	↑ cathepsin-K+ cells in CCL-ruptured group; TRAP+ cell levels correlate with inflammation	[17]
Varies	Naturally occurring	3–8 years	N/A	Treatment of CCL-explant cells with COL-3 (MMP inhibitor) led to ↓ collagen fragment generation	[37]
Varies	Naturally occurring	Adult	N/A	↑ Cathepsin-KMMP-9, TRAP in SF of OA group; TRAP ↑ OA versus other arthritis groups; matrix turnover/immune response genes ↑ in OA	[23]
Varies	Naturally occurring	Adult	N/A	CD4+, CD8+, CD3 + CD4-CD8-lymphocytes ↑ in CCL-ruptured dogs; CD3 + CD4-CD8-lymphocyte levels in SF inversely correlated to radiographic OA	[22]

Table 6.3 Ovine ACL-T models

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
Suffolk-cross	Arthrotomy + reconstruction	3–4 months	4, 20 weeks	ACL-R group had ↑ cartilage + osteophyte scores compared to controls; some OA development	[41]
Suffolk-cross	Arthroscopic	3 years	20 weeks	Load redistribution after ACL-T led to a significant ↓ in both PCL and LCL loads; no change in MCL loads	[39]

Table 6.4 Feline ACL-T models

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
Outbred	Anterior capsulotomy	1–3 years	4, 12, 35 weeks	↓ in muscle mass in ACL-T knee; ↑ in cell density, hexuronic acid in articular cartilage at weeks 12 and 35	[42]
Outbred	Lateral arthrotomy	Adult	0 days	Mechanoreceptor neurons in joint capsule are not affected by ACL-T	[43]
Outbred	Arthroscopic	Adult	16 weeks	Significant ↑ in articular cartilage thickness, significant ↓ in stiffness in ACL-T knee	[44]
Outbred	Arthroscopic	Adult	Ongoing (1 year)	↑ in knee instability, osteophyte formation, articular cartilage thickness, joint degeneration	[45]
Outbred	Anterior capsulotomy	Adult	16 weeks, 60 months	Significant ↓ in cancellous bone mass, subchondral bone thickness at 60 months; ACL-T intensified bone changes compared to control	[46]
Outbred	Anterior capsulotomy	Adult	16 weeks	↑ patellar articular cartilage, larger chondrocytes, more chondrocyte clusters, larger chondrocyte volume fraction; no femoral groove cartilage adaptation	[47]

have examined articular cartilage and meniscus properties [48–50], gene expression and surface receptors [51–53], osteophytes [54], bone properties [55, 56], and imaging techniques [57, 58]. The rabbit ACL-T model has also been used to test out therapeutics, such as HA therapy [59] and oral glucosamine supplements [60]. Furthermore, one study compared surgically induced ACL-T versus a blunt trauma ACL-T, which closely resembles clinical ACL-T in humans [61]. Studies using rabbit models of ACL-T are summarized in Table 6.5.

Guinea Pig Model

Guinea pigs have been used to study OA because the Hartley strain, among others, develops spontaneous OA beginning at 3 months of age [1, 62–65]. Other advantages of using guinea pigs for the study of PTA include the fact that their histopathology is very similar to humans and that they are easy to manage during long studies. Disadvantages for their use include the fact that they preferentially load the medial side of the

Table 6.5 Rabbit ACL-T models

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
New Zealand	Medial arthrotomy	8–12 months	9 weeks	[Articular cartilage] Significant ↓ modulus (18 %); ↓ in GAG density; significant ↑ water content	[50]
New Zealand	Medial arthrotomy	12 months	9 weeks	Menisci from ACL-T knees had degenerative changes; high # of apoptotic cells on medial side of menisci; ↑ nitrotyrosine reactivity	[48]
New Zealand	Medial arthrotomy	9–10 months	2, 4, 9 weeks	Rapid ↑in MMP-1, -3, -13 gene expression in articular cartilage; aggrecanase-1, -2 levels stable	[51]
New Zealand	Anterolateral capsulotomy	12 months	3, 8 weeks	Matrix deterioration; medial menisci showed cell-depleted areas, cell clusters, altered cell distribution; ↑ collagen type I, III staining lat/med; ↑ collagen type II staining on med side only	[49]
New Zealand	Anterolateral capsulotomy	12 months	3, 8 weeks	Significant ↑ RNA yield from med menisci only; significant ↓ DNA yield from med menisci week 8; significant ↑ collagen type I, TIMP-1; significant ↓ decorin, TNF-α, IGF-2; more mRNA changes by medial/lateral side	[52]
New Zealand	Medial arthrotomy	12 months	4, 9, 12 weeks	Osteophytes present in femur and tibia compartments by week 12; hypertrophic chondrocytes in osteophytes produce VEGF; NO production/ chondrocyte death during osteophyte formation	[54]
New Zealand	Medial arthrotomy	Adult	2, 8 weeks	Can detect synovial effusion, menisci/ligament lesions, and osteophytes accurately using MRI	[57]
New Zealand	Medial arthrotomy	2.5 years	4, 8, 12 weeks	Bone loss 4 and 8 weeks after, but returns to baseline by week 12; osteophyte volume significantly ↑ at weeks 8 and 12; damage to cartilage correlates to MRI values	[56]
New Zealand	Medial arthrotomy	2.5 years	4, 8, 12 weeks	MRI and μCT can be used to detect changes in articular cartilage, joint space, BMD, calcified tissue associated with OA	[55]
New Zealand	Medial arthrotomy	Adult	9 weeks	Apoptosis ↑ with ACL-T; treatment with HA ↓ apoptosis	[59]
New Zealand	Medial arthrotomy	Adult	3, 6, 12 weeks	Linear correlation between post-surgery time and OA scores; CD44v6 correlated with histology and macroscopic grades	[53]
New Zealand	Medial arthrotomy	9 months	11 weeks	Glucosamine group had significant ↓ in loss of GAG in lateral tibial plateau, ↓ in loss of GAG in lateral femoral condyle in ACL-T; glucosamine had a site-specific, partial disease-modifying effect	[60]
Flemish Giant	Blunt force OR medial arthrotomy	Adult	12 weeks	Blunt force caused tears in lateral menisci; both models had chronic degradation and meniscal tears, but blunt force was more severe	[61]
New Zealand	Medial arthrotomy	24 weeks	13 weeks	T2 values significantly ↑ in ACL-T; cartilage lesion levels significantly ↑ at 6 and 12 weeks in ACL-T; T2 correlated with histology grading	[58]

Table 6.6 Guinea pig ACL-T models

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
Hartley	Medial arthrotomy	40 days	1–8 months	Osteophytes visible at 3 months; Mankin score significant at 4–8 months compared to 1 month	[66]
Hartley	Lateral arthrotomy	3 months	3, 12 months	Coefficient of friction of cartilage significantly greater in ACL-T knees; lubricin levels significantly less in ACL-T knees	[67]
Hartley	Lateral arthrotomy	3 months	9 months	Lubricin significantly ↓; C2C, GAG, IL-1β, MMP-13, SDF-1 ↑ in ACL-T knees	[68]

knee joint, are mainly sedentary animals, and are too small to allow for use of arthroscopic techniques for injury induction and observation [1–3].

Recently, guinea pigs have been used to study the effects of PTA as well as spontaneous OA by looking at the effects of ACL-T such as osteophytes and histopathologic changes [66], coefficient of cartilage [67], levels of lubricin in the joint [67, 68], and levels of biomarkers in synovial fluid including C2C, GAG, IL-1β, MMP-13, and SDF-1 [68]. The use of guinea pig ACL-T models in PTA studies is summarized in Table 6.6.

Rat Model

Rats have been increasingly used for ACL-T studies due to their small size, rapid speed of OA symptom onset, ability for pharmacological testing, translational potential to human PTA, and low spontaneous degeneration of their knee joints [1, 3, 69–71]. Rats also have thick enough cartilage to allow for both partial and full cartilage defects, which allows for a low-cost defect model for OA research. Disadvantages include their small size for injury induction, and the rapid onset of disease [1–3].

Rats have been used to examine a variety of different PTA outcomes. One group of studies focused on the articular cartilage destruction, subchondral bone changes, and osteophyte production after ACL-T [71–73]. Another group introduced exercise as a therapy for reducing the symptoms of PTA after ACL-T [74]. Three other studies focused on the addition of supplements or inhibitors, including alendronate, which inhibits bone resorption, lubricin, hyaluronic acid (HA), and etanercept, an inhibitor of tumor necrosis factor alpha [75–77]. Finally, one group examined gene expression of different groups of OA progression markers, including matrix degradation, chondrocyte differentiation, and osteoclastic bone markers as a way to track disease progression [69]. Studies utilizing rat ACL-T models are summarized in Table 6.7 below.

Mouse Model

Mice provide a number of important advantages for studying OA and PTA. They are relatively inexpensive and easy to manage during studies, can incorporate genetic modifications, and are easy to use for pharmacological studies because of the low dosage required for efficacy [1–3]. However, relatively few murine models have been developed using ACL-T, likely due to small size and difficulty of the surgical approach. Mice also have fairly rapid onset of severe OA changes

Table 6.7 Rat ACL-T models

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
Wistar	Medial arthrotomy	10 weeks	2, 7, 14, 21, 28, 70 days	Cartilage destruction (margins) weeks 1–3; ↑ fibrillation of central cartilage weeks 3–4; ↑ denatured collagen type II staining present	[73]
Wistar	Medial arthrotomy	Unknown	2, 7, 14, 28, 70 days	Superficial zone cartilage changes (chondrocyte death/swelling, ↑ fibrillation); ↑ denatured collagen type II staining in fibrillated areas	[71]
Wistar	Medial arthrotomy	8 weeks	7, 14, 28 days	Mankin score lower for slight and moderate exercise at day 14; ↓ lesions in slight and moderate groups at day 28	[74]
Sprague-Dawley	Medial arthrotomy	20 weeks	2, 10 weeks	Alendronate (ALN) prevented ↑ bone formation, reduced area and instance of osteophytes, blocked osteoclast recruitment, ↓ local TGF-β release	[76]
Sprague-Dawley	Medial arthrotomy	10 weeks	1, 2, 4, 6, 10 weeks	Cartilage surface damage and GAG loss at week 1; subchondral bone loss weeks 2–10; osteophyte formation by week 10 in ACL-T	[72]
Lewis	Lateral arthrotomy	7–8 weeks	1, 4 weeks	Gene expression of lubricin ↓ in injured joints; ↑ TNF-α, IL-1β in synovial fluid of injured joints; TNF-α inhibition = ↑ of cartilage-bound lubricin, ↓ sGAG release	[75]
Lewis	Medial arthrotomy	3 months	6 weeks	Lubricin and lubricin + HA groups had ↓ in radiographic/histologic scores of cartilage damage; oral lubricin ↓ cartilage damage	[77]
Sprague-Dawley	Medial arthrotomy	10 weeks	1, 2, 4, 6, 10 weeks	↑ aggrecanase-1, MMP-13 weeks 1–10; ↑ collagen type IIA, Sox-9, VEGF, CD31 weeks 2–4 with ↓ later; ↑ cathepsin K, TRAP week 2; ↑ Runx-2, osterix weeks 4–6	[69]

after surgery [1, 78]. Mice also have thin articular cartilage, which has limited the use of certain techniques, such as MRI or gene expression studies, to study PTA.

One example of the use of surgical ACL-T for the study of PTA was in a study published by Glasson and coworkers. When they compared the effects of ACL-T and destabilization of the medial meniscus (DMM) on the development of OA, the DMM model resulted in a slower and less severe progression of OA. However, as an alternative to surgical ACL-T, recent studies have examined the effect of cyclic loading [79] or a single loading cycle [80–82] to induce ACL-T in mice. The use of murine ACL-T models in studies is summarized in Table 6.8. A more detailed description of the single loading cycle to create ACL transection in a mouse is presented in the next chapter.

Conclusions

In summary, transection or rupture of the ACL provides a reproducible model of PTA. This procedure can be performed surgically or noninvasively and has been demonstrated in a variety of different animals that range in size from the mouse to the sheep. The changes occurring in the joint appear to parallel the degenerative changes that occur in clinical PTA, and appear to affect all of the joint tissues including the cartilage, meniscus, bone, and synovium.

Table 6.8 Murine ACL-T models

Strain	ACL-T type	Surgery age	Exp. time	Results	Reference
129S6/SvEv	Medial arthrotomy	Unknown	4, 8 weeks	Severe OA compared to DMM model; subchondral bone erosion of the tibial plateau; chondrogenesis of joint capsule	[78]
C57BL/6N	Tibial compression	10 weeks	1, 3, 7, 14, 28, 56 days	Rapid trabecular bone loss by 7 days; mild OA detected by day 56	[80]
C57BL/6N	Tibial compression	10 weeks	0, 10 days; 12, 16 weeks	Loss of trabecular bone by 10 days; bone loss ↓ in ACL-T compared to avulsion fracture	[81]
FVB	Cyclic axial loading	3 months	1, 8 weeks	ACL-T had significant articular cartilage degeneration score; synovitis present at 1 week; osteophytes present at 8 weeks	[79]
C57BL/6	Tibial compression	8 weeks	5, 9, 14 days	Chondrocyte apoptosis, cartilage matrix degradation, disruption of cartilage collagen fibril arrangement, ↑ serum COMP, ↑ synovitis	[82]

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Closed Joint ACL Disruption Murine Model of PTA

7

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Introduction

Despite decades of effort and billions of dollars spent trying to cure established OA, the disease remains incurable. Because joint injuries and mechanical instability are widely thought to be the initiating events and underlying cause of many OA cases, current research has moved towards identifying intervention strategies after injury to prevent future OA. Given the powerful genetic information and relatively low expense of mice as experimental animals, there has been considerable effort to establish mouse models of OA that mimic aspects of the original joint injury and the subsequent degenerative changes observed in clinical OA progression. This chapter is focused on a recently developed OA model in mice consisting of nonsurgical ACL rupture and briefly summarizes other mouse OA models to provide an appropriate context [1–7].

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Historical Context

The earliest mouse models of induced OA used chemical forms of injury to the stifle (knee) joint, such as injection of iodoacetate or degradative enzymes such as collagenase. These approaches cause cell death and matrix degeneration through non-physiological means that clearly do not mimic the initiating events in clinical PTOA. However, once joint damage is established, there are aspects of the OA progression and further joint degeneration that correlate with what is seen clinically in PTOA. A review of ACL transection models of PTA is covered in Chap. 10.

The first noninvasive mouse model of induced post-traumatic osteoarthritis was described by Furman et al., in which they created an intra-articular fracture (IAF) of the proximal tibia in male mice [8]. The injury response included many aspects seen clinically with intra-articular fractures, including callus formation and subchondral bone thickening. Changes in the cartilage observed by histology indicated degeneration and progression toward terminal OA, including cartilage surface fibrillation, progressive proteoglycan loss, and finally exposure of subchondral bone at 50 weeks [8]. In this model, the extent of joint damage is somewhat adjustable by limiting

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the maximum displacement of the indenter used to create the injury, and increasing joint damage at the time of injury creates more severe OA. The model has been extensively characterized and used to study aspects of PTOA pathogenesis from obesity to MSCs to inflammation to measurement of OA biomarkers like such as Cartilage Oligomeric Matrix Protein (COMP) [9–14]. The model is generally considered especially relevant for clinical studies of very high impact joint injuries with accompanying bone fracture.

A more recent noninvasive model of joint injury uses a method similar to that used by bone biologists to study the bone adaptation mechanical loading. These models replicate the loading seen by bone during walking or running, and typically many cycles of loading are applied to the mouse tibia through the ankle and knee joints. The first description of this setup to study mouse PTOA was by Blandine Poulet et al. in 2011 [15]. In this study, episodes of multiple axial compressions to 9 N were applied repeatedly three times weekly for up to 5 weeks. Non-progressing articular cartilage lesions were observed histologically with a single loading episode, and multiple loading episodes induced progressive changes consistent with PTOA. Osteophytes were observed in over half the mice receiving 2 weeks of loading episodes, and more consistently in mice loaded for 5 weeks. Ko et al. used a similar cyclical tibial loading setup to study PTOA in young and adult mice [16]. In this setup, loading consisted of 1,200 cycles/day at 4 Hz and either 4.5 N or 9 N, applied 5 days/week, for between 1 and 6 weeks. At the higher loads, mice developed osteophytes, and mice loaded for the longer times and/or at the higher loads showed signs of histological OA including cartilage thinning, loss of proteoglycan, and changes in subchondral bone quantified by microCT. A repetitive loading model was used by Onur et al. in 2014, to load joints up to 12 N for up to 240 cycles or until ACL rupture occurred [17]. A similar repetitive loading model was also used by Wu et al. in 2014 [18], who applied 60 cycles of loading to 3, 6, or 9 N. All loading regimens induced chondrocyte apoptosis, cartilage matrix degradation, and increased serum levels of OA biomarker COMP. Higher loads initiated greater synovitis, and the highest load disrupted the ACL and initiated severe synovitis and ectopic cartilage formation. These models of joint injury are likely to be milder than the intra-articular fracture PTOA model described in the previous paragraph, although the relevance to the human clinical situation may not be as easy to interpret due to the multiple cycles of loading.

Initiation of ACL Rupture Injury by a Single Mechanical Overload

The first noninvasive mouse model of ACL rupture was characterized by our group in 2012 [19]. In this model, knee injury is created using a single cycle of tibial compression, also with instrumentation adapted from studies of bone adaptation to tibial compression. The compression system consists of two loading platens machined out of aluminum and fixed to an electromagnetic materials testing instrument (Bose ElectroForce 3200) (Fig. 7.1a). The top platen is designed to hold the ankle flexed at approximately 30° (Fig. 7.1b). The bottom platen is designed to hold the flexed knee in a shallow cup with an indentation to accommodate the femur and upper leg (Fig. 7.1c). The top and bottom platen are aligned vertically to transmit force through the long axis of the tibia. The setup further includes a Teflon platform, on which the anesthetized mouse rests, an inhalation tube for continued administration of isoflurane anesthetic, and a load cell underneath the bottom platen.

Mice are anesthetized using isoflurane and then placed onto the loading device with continued administration of isoflurane anesthesia, as shown in Fig. 7.1a. The right ankle and knee are placed into the upper and lower platens, and a pre-load of 0.5–1.5 N is applied to hold the leg in place. To create the ACL rupture injury, the upper platen is lowered to a target axial compressive load of 12 N, or a target displacement of 1.7 mm, depending on the type of ACL disruption desired (discussed in detail below). The axial loading

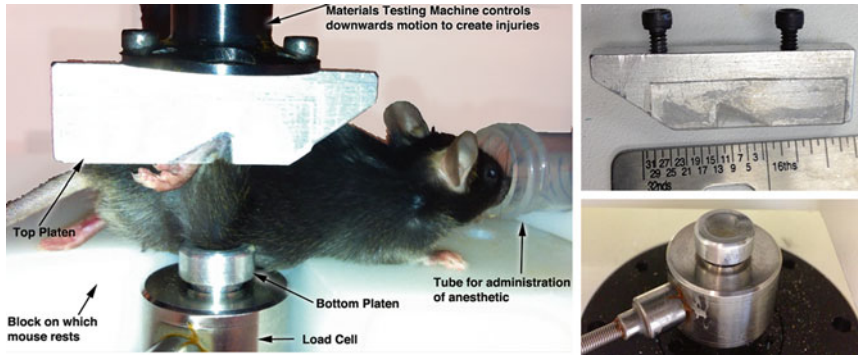

Fig. 7.1 *Left*: Setup to initiate ACL rupture injuries, consisting of custom platens designed to hold the knee and ankle joints, a load cell, and a tube for administering anesthesia, assembled on an electromagnetic materials testing instrument (Bose ElectroForce 3200). *Top right*: Close-up of the top platen showing a ruler calibrated in inches. *Bottom right*: Close-up of the bottom platen showing a shallow cup to hold the knee, with a groove on one side to accommodate the thigh

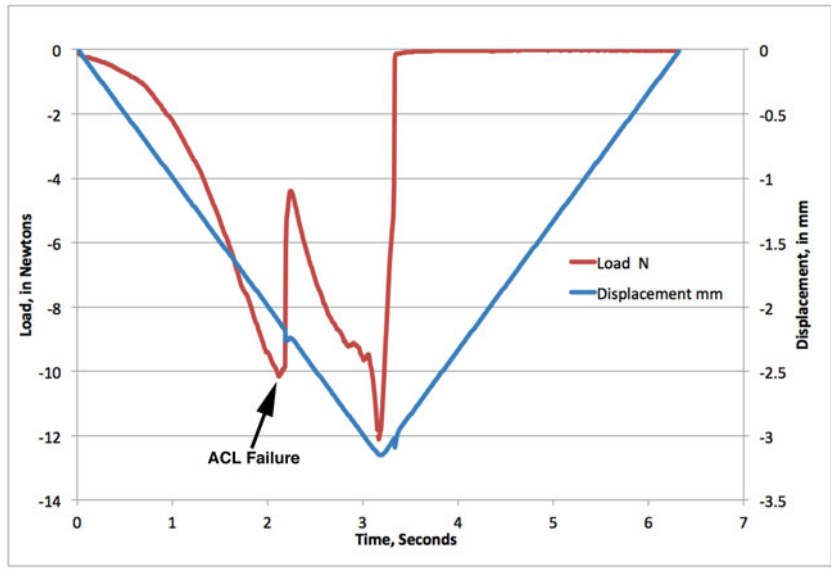

Fig. 7.2 Typical loading profile to initiate ACL rupture by avulsion fracture. The *blue line* indicates displacement of the top platen that holds the ankle, and shows a slow constant movement from 0 mm down to −3 mm, and back to 0 mm. The *red line* is the readout from the load cell under the knee. It shows that as the ankle is pushed downwards, there is a steady increase in load until just over 10 N, at which point the rapid release of compressive force (shown by the *arrow*) indicates disruption of the ACL

causes a transient anterior subluxation of the tibia relative to the distal femur, in effect moving the tibia in the direction that the anterior cruciate ligament stabilizes. A release of compressive force indicates failure of the ACL, which reproducibly occurs at 8–11 N compressive load for most mice (Fig. 7.2). After the target of 12 N or 1.7 mm is reached, the platen is returned to its initial position and the knee joint is restored to its native orientation. The entire procedure takes less than 5 min including anesthetization, with tibial compression loading requiring only about 6 s, with injury typically occurring after about 2 s (Fig. 7.2). Mice are removed from the setup gen-

tly and given a dose of analgesic while still anesthetized. To date, all animals have survived the injury with no fractures to the long bones, and they are typically mobile soon after recovering from anesthesia and show only very mild, if any, changes in apatite, behavior, or activity.

Immediate Assessment of Injury

Experienced orthopedic surgeons that were blinded to the injury status assessed the immediate effect of injury semiquantitatively. All injured knees were correctly identified as injured, but one (of 5) uninjured was also identified as injured. The most commonly observed indicators of joint injury were increased anterior/posterior translation and external rotation of the knee, with some minor swelling and hemarthrosis [19]. Based on the extent and the type of laxity observed, the damage to the joint was considered consistent with ACL rupture, but not indicative of damage to either the medial or collateral ligaments. Analysis by either standard or contrast-enhanced microCT imaging confirmed that ACL rupture was present in all injured knees that were imaged [19, 20]. Damage to other joint structures such as the PCL, meniscus, patella, collateral ligaments, was not obvious by high-resolution contrast-enhanced microCT [20].

The mode of ACL rupture depends on the rate of axial compression [20]. A relatively slow compression rate of 1 mm/s causes ACL disruption with an avulsion fracture, in which the ACL is disrupted at the site of insertion into bone, and pulls a segment of bone usually from the posterior femur into the joint cavity. Clinically, ACL injuries with an avulsion fracture are more common in children than in adults. A much faster loading rate of 500 mm/s causes a midsubstance disruption of the ligament with no evidence by microCT of an avulsion fracture. Clinically, midsubstance ACL tears are more common in adults. On a technical note, due to limitations in the software and data acquisition rates of the materials testing setup used in these studies, we used a target load of 12 N as a trigger to stop the injury only with the slow injury rate of 1 mm/s. In the fast injury rate (500 mm/s) it became necessary to use a displacement of 1.7 mm as a trigger to stop compression because of overshoot of the target force. In both cases, the loads required to induce injury were very similar, ranging from 8 N to 11 N in the majority of animals. Another technical limitation of our software and hardware setup is that the downward motion of the upper platen is not programmed to stop automatically when the ACL rupture injury occurs. Although we would ideally like to experimentally control this aspect of the ACL rupture injury in our mouse model, it is not necessary to do so in order to mimic clinically relevant human ACL injuries. In our system, the downward motion continues until a trigger point of either 12 N or 1.7 mm is reached. In general, for most of the assays with which we measured injury response, the fast injury midsubstance ACL tears induced very similar but somewhat milder injury responses than the slow injury avulsion fractures.

Serum Markers of OA Progression

Biomarkers of OA progression and bone remodeling can be measured from synovial fluid, blood, or urine, and are used as confirmatory measures of PTOA progression. Cartilage Oligomeric Matrix Protein is one of the more promising OA biomarkers, and serves as a marker of the earlier stages of OA in which cartilage is still present and cartilage turnover is elevated. We found that ACL rupture caused statistically significant increases in the serum levels of COMP within 1 day after injury, and COMP levels remained elevated at all time points until at least 8 weeks after injury (except the 4-week time point, which trended higher but not significantly) [19]. A serum marker of bone resorption (CTX-I) was significantly increased at 7 and 14, but not 56 days after ACL Rupture injury. At the same time points, a marker of bone formation (P1NP) remained unchanged [21]. Together these results suggest that in the earlier stages after joint injury, ACL rupture-induced changes in bone turnover

are primarily caused by changes in bone resorption rather than formation, and that elevated cartilage turnover occurs throughout the first 8 weeks after injury.

Histological Assessment of OA Progression

Histological assessment of joints injured by ACL rupture revealed deterioration of cartilage and osteoarthritic changes consistent with PTOA progression [19]. At the early time points, grading by a veterinary pathologist revealed that injury caused synovial hyperplasia, inflammation, and fibrosis. Cartilage damage was only mild at early time points (Fig. 7.3), but by 8 weeks significant loss of proteoglycan was observed. There was fissuring of the articular cartilage, frequent loss of the surface lamina and the flattened chondrocytes of the superficial zone, and atrophy of articular chondrocytes. Blinded histological grading of the 8-week sections revealed that injury caused significant increases in the OARSI score [22] at the medial tibia, medial femur, and lateral femur. No differences in OARSI scores were measured at the femoro-patellar joint or the underlying surface of the femur.

Radiographic Assessment of Injury Response

ACL rupture through avulsion fracture caused a rapid and substantial loss of subchondral trabecular bone volume very early after injury as measured by microCT. This trabecular bone loss became significant within 3 days after injury, and reached a maximum of approximately 40 % loss after 7 days compared to the contralateral uninjured knee. This initial remodeling phase was followed by a partial recovery of bone volume.

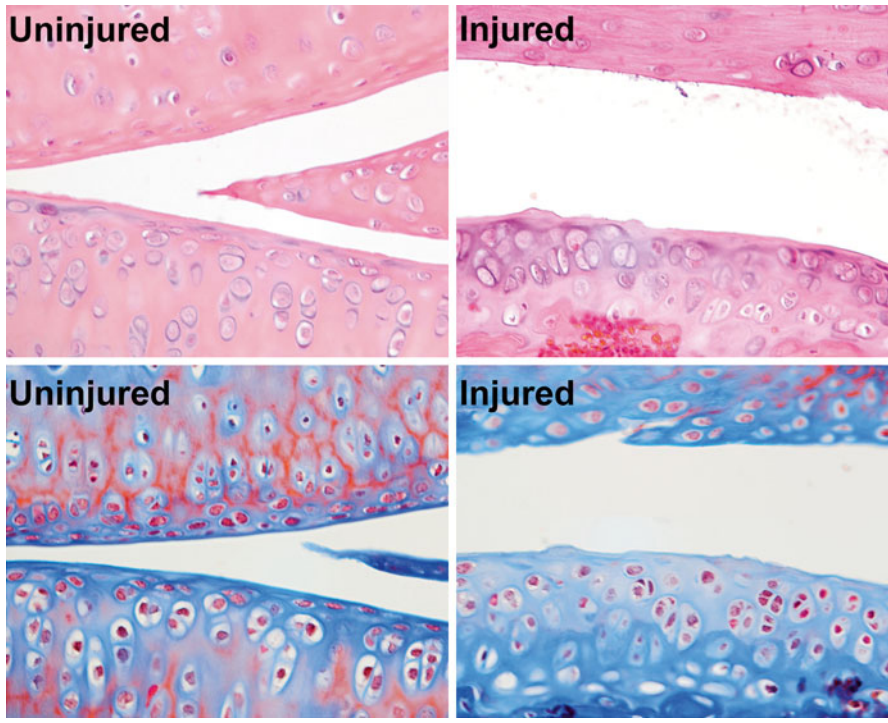

Fig. 7.3 Histological assessment of cartilage damage at 8 weeks after injury, showing injury-induced loss of surface lamina and flattened superficial chondrocytes, fibrillations in the cartilage, and cluster formation in the middle or deep layers

At the 4- and 8-week time points after injury, a new but lower steady-state bone volume was reached that was approximately 80 % of the day 1 volume [19]. This lower bone volume was maintained out to 12 and 16 weeks [20]. The loss of subchondral bone volume was somewhat less severe in the midsection ACL rupture injury than in the ACL avulsion fracture (−20 % loss versus −31 % at day 10). However, this difference in bone turnover between the modes of ACL disruption was only seen at the early time points. The lower steady state bone volume reached at later time points was not statistically different between injuries created by midsection ACL rupture or ACL avulsion fracture.

We were somewhat surprised by the rate and extent of the early bone remodeling after the ACL rupture injury. Locally, a 40 % loss of subchondral trabecular bone after 7 days is a significant biological event, which alone may have consequences for the health of the joint over time. Bisphosphonates such as alendronate are specific inhibitors of osteoclast-mediated bone resorption. Given the substantial bone remodeling induced by the ACL rupture injuries, we tested whether treatment with alendronate would attenuate this response, with a secondary hypothesis that this may protect against cartilage degradation and osteoarthritis progression. We found that high doses of alendronate did prevent the short-term loss of subchondral trabecular bone volume, but did not inhibit the osteophyte formation at the later time points, or the cartilage degeneration induced by the ACL rupture injury [21].

Osteophyte formation is a hallmark of clinical OA. Osteophytes are newly formed fibrocartilage and bone growths that are prevalent at the peripheral margins of joints, and at the interface between cartilage and periosteum [23]. The ACL rupture injury models described here cause substantial nonnative new bone formation that is readily detectable within 10 days after injury, perhaps even earlier [20]. Much of this injury-induced new bone formation appears to be osteophyte formation, and thus the model reproduces aspects of clinical PTOA. In addition, a portion of the new bone formation may be enthesophytes, or new bone forming at the insertion sites of ligaments to the bone, specifically around the collateral ligaments (personal communication, Chris Little). To date we have not rigorously characterized the nonnative bone to differentiate whether it is primarily osteophytes or enthesophytes, but we suspect that both occur in response to the ACL rupture injuries. Interestingly, the milder midsection ACL tear injury tends to produce slightly greater nonnative bone volume at the later time points than ACL avulsion fractures, which is in contrast to the generally milder response to the midsection tear. While the joint injuries caused an initial destabilization of the joint (as quantified [20] by anterior–posterior joint laxity), osteophyte formation appeared to correlate with a re-stabilization of the joint, albeit with a much reduced range of motion [20].

Sclerosis of the subchondral bone plate is also a hallmark of clinical OA. The sclerosis involves remodeling and hardening of the subchondral bone plate in early OA, often accompanied by an advancing tidemark of calcified cartilage, and decreased subchondral vascularity [23]. Analysis of the subchondral bone at the proximal tibia in our model of ACL rupture revealed that the injury induced a significant thickening of the subchondral bone plate [20], where we observed increases of 20–26 % in cortical thickness in both ACL midsection tear and avulsion fracture injuries at the 12- and 16-week time points. This is in contrast to the partial medial meniscectomy (PMM) surgical injury model, in which osteophyte formation and subchondral sclerosis was not seen by microCT scans until 20 weeks after surgery [24]. In our studies, all injured knees showed a similar extent of subchondral bone sclerosis, independent of whether the mode of ACL rupture was midsection tear or avulsion fracture.

In summary, the nonsurgical ACL rupture model includes many aspects of clinically relevant post-traumatic osteoarthritis. The immediate mechanical damage resulting from the ACL rupture injury is primarily limited to the ACL itself, which can occur through either midsec-

tion tear or avulsion fraction depending on the loading rate. The injury destabilizes the joint, and initiates a short-term biological response that includes mild inflammation, mild synovial hyperplasia, fibrosis, and a rapid remodeling of the subchondral trabecular bone. Longer-term outcomes include hallmarks of OA progression such as cartilage fibrillation, loss of superficial zone chondrocytes and cartilage proteoglycan content, subchondral bone sclerosis, and osteophyte formation. At the later time points joint stability is somewhat restored, perhaps because of the extensive osteophyte and ectopic bone formation, but the restored stability is at the expense of range of motion.

Additional Biological Response to ACL Rupture Injury

Having established that the ACL rupture injury model reproduces many aspects of clinical PTOA, we are pursuing additional characterization of the injury response. We placed specific emphasis on the very early time points, at which the native biological responses in other PTOA models may be masked by the injury method.

Gene Expression in Response to ACL Rupture Injury

Microarray gene expression analysis revealed that more than 500 genes are differentially regulated after 1 week in the injured knee compared to the uninjured contralateral knee of the same mouse. These genes included many with established roles in cartilage development, homeostasis or pathology, and a curated list is shown in Table 7.1. Analysis of molecular pathways induced by the ACL rupture injury was performed using Ingenuity Pathways Analysis. This revealed a significant involvement of pathways relevant to bone and cartilage turnover, as well as to osteoarthritis progression, shown in Table 7.2.

Table 7.1 Injury response genes at 7 days post-injury

Catabolic genes	Anabolic genes
ADAM12	Sox9
ADAMTS 1, 2, 3, 4, 12, 16	TGFß 2, 3
MMP 2, 3, 7, 8, 14, 19, 23	Inhibin b-A, BMP-1
Calpain 6	Aggrecan, Cartilage link protein
	Collagens type 2, 3, 4, 5, 9, 10, 11, 13

Curated list of the more than 500 genes with significantly different expression 1 week after ACL rupture injury. A total of 8 mice had the right legs injured, and the left legs served as uninjured contralateral controls. RNA was isolated after 1 week from all 16 joints, and cDNA from each joint was run on a separate Affymetrix microarray. Expression levels in the injured knee was normalized to the contralateral knee of the same mouse, and statistical comparisons were made to identify injury-induced genes that responded in all animals with a twofold cutoff and stringent filters for false-positives

Table 7.2 Pathway analysis of injury response genes

Pathway	*p*-value
Focal adhesion	1.26E-08
Endochondral ossification	1.27E-08
Adipogenesis	3.32E-07
Matrix metalloproteinases	3.51E-05
Senescence and autophagy	6.16E-04
Wnt signaling pathway	0.0015
Inflammatory response pathway	0.0065
Osteoblast signaling	0.0140

Pathway analysis of injury response genes at 1 week after ACL rupture injury. Ingenuity pathways analysis revealed significant involvement of several pathways with known or suspected roles in osteoarthritis progression

The noninvasive initiation of the ACL rupture injury means that the acute biological response to the injury probably follows a relatively natural progression. We therefore examined the expression of several injury-response genes and inflammatory genes at the very early time points, minutes or hours after injury. Using careful microdissection of the joint, our first analysis was limited primarily to the tissues that were mechanically affected by the injury. This included the subchondral bone, cartilage, meniscus, ACL/PCL, but did not include synovium,

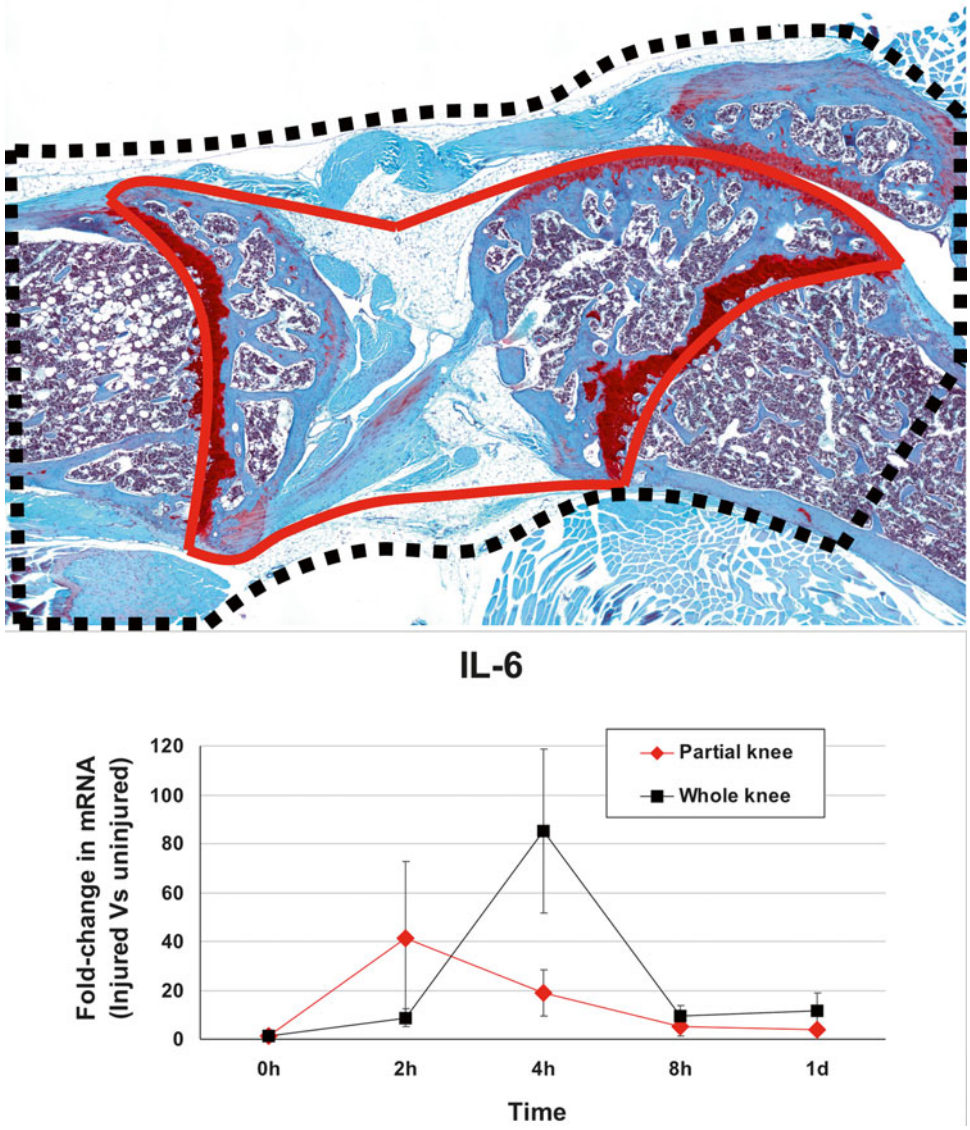

Fig. 7.4 Acute response to ACL rupture injury: mRNA expression of injury response gene IL-6 was measured in the partial knee (*outlined in red*) and whole knee (*outlined in black*). The partial knee included primarily those tissues that were directly mechanically affected by the injury, including the cartilage, ligaments, meniscus, and subchondral trabecular bone. The whole knee included the entire joint capsule, including the patella, patellar fat pad, and synovium. Gene expression analysis by real-time quantitative RT-PCR revealed that the partial knee responded within 2 h, and the whole knee responded with a delayed but much higher peak expression of IL-6 mRNA. The delayed response of the whole knee was larger in scope as well as magnitude (see text)

patella, fat pads, or any other surrounding tissues (Fig. 7.4). In these samples we found a significant transient elevation of IL-6 mRNA expression within 2 h after injury. The magnitude of the response was quite large, on average over 40-fold greater mRNA expression in the injured knee than in the uninjured contralateral knee of the same mouse. The induction of IL-6 was tran-

sient, and levels returned to baseline after 6 h. When the patella, synovium, fat pad and the entire joint capsule were included in the analysis, IL-6 induction at 2 h was no longer significant, but a much larger peak of IL-6 mRNA induction (over 80-fold) was observed at 4 h. The response of the entire joint capsule was not only larger in magnitude, but also in the number of responding genes. For example, significant increases in IL-1b and MMP13 were also measured in the entire joint capsule at 4 h, but not in the tissues immediately affected by the injury. In all cases, the elevated gene expression returned to baseline by 6–8 h, and remained at baseline at 24 h. From these observations, we are forming a model for understanding the biological response to joint injury, in which the tissues mechanically affected by the injury exhibit an acute response very early (peaking within 2 h), which then elicits a slightly delayed response from the entire joint capsule that is larger in magnitude and in scope [25].

Sex-Based Difference in Response to ACL Rupture Injury

Clinically, females account for almost two in three knee joint replacement surgeries. In the surgical DMM mouse model of joint injury, progression of OA is sex dependent, with differences in OA severity markedly greater in male mice as soon as 2 weeks after surgery [26]. In the DMM injury model, a protective role of female sex hormones was observed [26]. These observations in the DMM surgical model of PTOA are consistent with clinical findings of OA primarily in women, and of accelerated OA progression in postmenopausal women. To date we have not rigorously investigated the effect of sex on the injury response in our nonsurgical ACL rupture injury model. Using only a limited range of assays, we investigated sex-based differences in proteinase activity at early time points post-injury (using ProSense, MMPSense, and CatKSense in vivo imaging reagents), but while these assays demonstrated robust increases of in vivo proteinase activity upon injury in both sexes, and we found no differences between male and female mice. In the same study we examined terminal OA at 8 weeks by microCT and again found no significant differences in the injury-induced changes in bone between the sexes. Further investigation using histological analysis at intermediate time points needs to be performed to definitively establish whether there are sex-based differences in response to the nonsurgical ACL rupture injury model.

Conclusion

Significant progress was made during the last decade in the development of mouse models for post-traumatic osteoarthritis. The surgical DMM model is the currently the most widely cited PTOA model. The DMM model and closed articular fracture models have contributed to many of the recent advances in our understanding of OA pathogenesis and progression. Newer noninvasive models of PTOA represent another significant step forward, and are particularly useful for investigating the natural progression of biological processes at the very early time points after injury, which may be partially masked in more invasive models. Single impact noninvasive injury models such as the Intra-Articular Fracture model and our ACL rupture model may be more representative of clinically relevant human injuries, since the externally applied mechanical forces affect multiple joint tissues in a similar manner. The noninvasive single impact PTOA models have an additional advantage of being technically very simple to perform, and requiring much less time, than surgically induced PTOA. These new models approximate the human injuries more closely and may lead to novel insight into the biological and cellular responses to joint injury that are involved in OA initiation, which in turn holds promise for the development of more effective therapies and intervention strategies to reduce the tremendous burden of osteoarthritis.

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Whole Joint Models of Articular Injury and Articular Fracture

8

Bridgette D. Furman and Steven A. Olson

Introduction

The development of post-traumatic arthritis following an articular fracture is a complex clinical problem. In the case of an articular fracture the injury starts with a focal injury within the joint that consists of several injurious elements. These include focal loading of the articular surface, disruption of the subchondral bone and overlying articular cartilage, potential injury to ligament and/or synovium, and exposure of the joint to bone marrow and whole blood products. In this clinical setting it is known that disruption of the articular surface combined with potential residual misalignment may lead to focal areas of cartilage damage or loss at the site of joint injury. The mechanisms initiated by such an injury that lead to development of PTA globally in the injured joint are incompletely understood. During the development of PTA the articular degeneration extends throughout the joint from the focal site of articular fracture. Recent work suggests that attempts to understand the development of PTA are perhaps best addressed in an "organ system" model of joint trauma. Arthritic changes of the affected joint are characterized by progressive destruction of articular cartilage, but it also affects the entire joint, including the synovial membrane, joint capsule, ligaments, periarticular muscles and tendons, and subchondral bone [1].

Models of articular fracture and articular impact injury are crucial in advancing our understanding of the pathophysiology of the larger pool of post-traumatic arthritis that develops following articular injuries without an articular fracture. Joint injuries can occur with a spectrum of damage to intra-articular tissues without fractures. As early as 1931, researchers have used animal models of surgically induced articular incongruities or defects to assess healing in joint tissues. Key reported on a novel experiment that removed a 3×6 cm osteochondral segment from the trochlear groove of rabbits. There was a dichotomous response to this injury [2]. Either the trochlear defect sealed with cartilaginous like membrane and the joints were observed to be normal or the defects did not seal completely and in these cases the joints developed degenerative arthritis. Prolonged exposure of hematopoietic bone marrow elements to the intra-articular environment was sufficient to cause arthritis. In a review of the healing of cartilage defects Campbell [3] noted, "the injury to cartilage may precipitate a chain

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of complex pathophysiological and chemical reactions resulting in osteoarthritis."

The onset of PTA following articular fracture in an animal model, as in clinical practice, tends to be more rapid in injuries with an articular fracture than in joint injury models without an articular fracture. The rapidity of onset of PTA after an articular fracture makes these models ideal for study of mechanistic pathways. While the mechanisms by which joint trauma initiates arthritic changes are incompletely understood, three potential mechanisms have been implicated in the disease: These include chondrocyte death with or without elevated levels of reactive oxygen species, altered joint mechanics, and inflammation. Experimental models can vary from assessment of a single parameter such as contact stress in a cadaveric specimen to the observation of changes that follow a closed joint injury in vivo. Understanding the research question being addressed when the model was developed is important for investigators when selecting the most appropriate model system to study specific aspects of PTA following an articular fracture. We have summarized an array of studies using whole joints (not tissue explants) of human cadaver and animal models in an effort to highlight the benefits and limitations.

Human Cadaveric Models of Articular Fracture

Cadaveric models of cartilage impact and fracture have been used to assess altered contact area and pressure of the articular surface, acute structural damage to the cartilage, loss of chondrocyte viability, and release of catabolic and inflammatory mediators. Both whole joint and intact osteochondral specimens have been used in these studies. Cadaveric human joints are appropriate models for acutely studying various aspects of articular fracture but are limited in assessing long-term response to injury.

As early as 1968, Kennedy et al. experimentally created tibial plateau fractures in human cadaver knees using a custom loading device and characterized the resultant fractures [4]. The results provided information on the mechanism of tibial plateau fractures and the basis of a classification system of articular fractures for treatment planning.

Effect of Fracture Displacement on Contact Stress

Several studies have used cadaver models to evaluate contact pressures, stresses, and areas of the articular surface associated with articular fracture. These studies used pressure-sensitive film applied to the articular surface to measure resultant changes with disruption or average and peak values during impact loading. Brown et al. examined the effect of full-thickness osteochondral defect size on cartilage contact stress. In this work the authors ranged the defects from 1 to 7 mm in diameter and found that the greatest contact stress was an order of magnitude greater than the intact condyle, was located at the rim of the defect, and was maximal for the 2 mm defect [5].

Residual incongruity of the articular surface of the joint is common following fracture. There is variability concerning the maximum amount of articular offset, or step-off, which can be tolerated by the joint for an acceptable clinical outcome. Cadaver models have been used to evaluate the effect of the presence of a step-off in the articular surface on a variety of factors including surface contact areas and pressures. In all these studies, articular defects or step-offs are simulated by a surgically induced fracture via an osteotomy.

Cadaver studies have introduced fractures via osteotomies to assess altered contact areas and pressure distributions with residual surface incongruities or step-offs. Brown et al. demonstrated that moderately displaced tibial plateau fractures >1.5 mm in the human cadaver knee resulted in significantly greater contact pressures than the intact knee [6]. Under these passive loading conditions of 1,200 N, their findings suggest that fracture reductions within 1.5–2 mm are necessary to avoid exceeding 150 % of normal maximal pressure. Olson et al. used a single-leg stance loading model of the human cadaver pelvis and found that contact areas between the articular

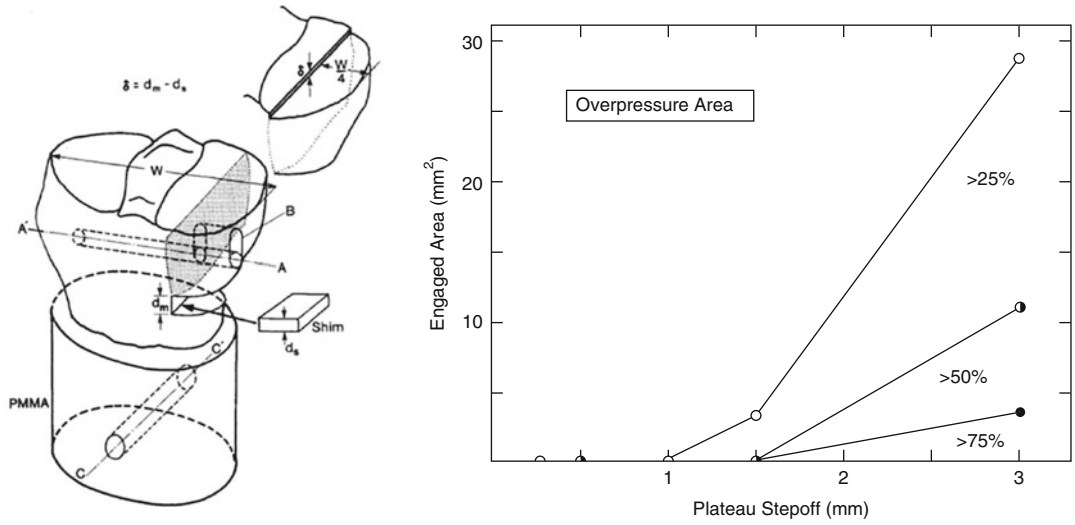

Fig. 8.1 *Left*: illustrates displacement of a lateral tibial plateau fracture model used to assess the effect of displacement on observed contact stresses. *Right*: shows that greater joint displacement leads to higher contact stresses affecting progressively smaller areas of articular cartilage. Reproduced from Brown, TD et al: Contact Stress Aberrations Following Imprecise Reduction of Simple Tibial Plateau Fractures. JOR. 1988; 6:851

surfaces of the hip were altered with the introduction of posterior wall defects, with the smallest defect producing the greatest alteration in contact area [7]. In a follow-up study using this model, Hak et al. found that both step and gap malreductions of transverse acetabular fractures altered the distribution of load within the hip joint as compared with both the intact and anatomically reduced conditions. [8] A step malreduction of a transtectal transverse fracture led to the greatest increase in peak contact stress. Bai et al. looked at knee joint alignment in addition to contact pressures in the human cadaver knee with simulated split fractures of the lateral tibial plateau created by osteotomies in combination with lateral meniscectomy [9]. With increasing step-offs from 0 to 6 mm of the lateral tibial plateau, both valgus angle and contact pressures in the knee joint progressively increased, and when combined with removal of the meniscus, increases in both valgus deformity and contact pressures were even greater. The clinical implication of these findings indicates that decreasing articular step-off heights in the treatment of lateral tibial plateau split fractures may be particularly important if meniscectomy is performed. All of these studies emphasize altered contact mechanics with residual incongruities of the articular surface. The paradox of these loading studies is while greater displacement of the articular surface leads to higher observed contact stresses, greater displacement also leads to a smaller area of cartilage seeing these higher loads (Fig. 8.1). Articular displacement leads to focal areas of abnormal loading. However, it is unclear how the focal areas of cartilage affected in these cadaver studies increase the whole joint risk clinically for the development of PTA.

Effect of Fracture on Chondrocyte Viability

Tochigi et al. impacted intact human cadaver ankles with a transarticular load of 50 J to generate tibial plafond fractures via a drop track [10]. Following impact, osteochondral fragments were sampled and cultured up to 2 days. Chondrocyte death was again reported to be higher along the fracture-edge compared to non-fracture regions in the joint. Unfortunately, there were no controls in these experiments to understand the effects of

specimen handling on chondrocyte death. To date there are no studies that have specifically quantified initial injury severity and then assessed the extent of arthritic changes in an articular fracture model.

Limitations of in vitro models with cadaver joints are that it is not possible to fully elucidate the mechanobiologic response of joint tissues to injury and potential healing over time. In vivo animal models allow for such investigations. Various species of animals have been used for cartilage impact models, effect of articular cartilage incongruity via osteotomy models, or impact with articular fracture models.

Animal Models of Joint Impact Without Fracture

Articular fractures mostly likely involve not only disruption of the articular surface but also impact to cartilage at supraphysiologic loads. Defining precise loading parameters which induce arthritic changes is a challenging problem. In vivo cartilage contact biomechanics have reported cartilage strains in the knee to be 20 % in normal knees and 29 % for ACL-deficient knees [11]. Additional quantification of physiologic loading of the knee joint, for example, has been best reported with instrumented total knee replacements. Peak axial loads ranged between 2.2 and 2.8 times body weight (BW) during walking and up to 3.0 times BW with stair climbing [12–15]. Radin et al. looked at factors that affected the peak coefficient of friction seen by native joint during impact loading. They determined that combined oscillations under load with additional intermittent impact loading of bovine phalangeal joints resulted in sequential wear of cartilage and bone with nearly doubling of the coefficient of friction after 196 hours [16]. The authors concluded that the bone spared the articular cartilage during supraphysiologic impact loading.

The connection between magnitude of load and/or strain to the articular cartilage and arthritis development has yet to be established clinically. However, there are a range of animal models which have examined the effect of impact loading to articular cartilage. The majority of studies investigating impact loading of cartilage, as it relates to joint trauma and post-traumatic arthritis development, can be divided into three categories: (1) animal models of repetitive impact loading on joint tissues; (2) animal models of a single-impact load to a weight-bearing joint; and (3) animal models of a single-impact load to a femoral condyle. The results of these investigations are summarized below.

Impact Without Fracture: Animal Models of Repetitive Impact Loading

Several early studies investigated the role of cyclic loading on the joint tissue, as would be experienced with repetitive loads, for example as experienced by drill operators. In 1972, Simon and Radin reported that repetitive impact loading to the guinea pig knee resulted in joint degeneration within 3 weeks, with degenerative changes in both the subchondral bone and articular cartilage [17]. Radin et al. then reported similar findings with cyclical loading of the rabbit hind leg for 1 h daily [16]. Stiffening of the subchondral bone along with trabecular microfractures was first reported between days 4 and 11, followed by synovial changes at day 16, and then cartilage degenerative changes starting at the surface at day 20 and progressing out to day 36. Serink et al. found that 1-h daily impact loading of the adult rabbit knee resulted in synovial thickening, cartilage degeneration, and subchondral bone changes which occurred concurrently in joints by 3 weeks [18].

Impact Without Fracture: Animal Models of a Single-Impact Load

Several experiments applied an external impact load across the closed patellofemoral joint at subfracture loads and evaluated the effect on cartilage and subchondral bone. Donohue et al. and Thompson et al. utilized a single impact to the adult canine patellofemoral joint, and found that

adult canine articular cartilage showed significant alterations in degenerative histological, structural, and biochemical changes without disruption of the articular surface at 4 and 6 weeks [19], and within 6 months, a single impact resulted in superficial disruption of the cartilage and subchondral changes that lead to arthritic-like degeneration of the cartilage [20]. Chrisman et al. used a subfracture impact load to canine femoral condyles and showed increased arachidonic acid in impacted cartilage [21]. This early study highlighted the association between mechanical injury and early biochemical changes of the arthritic process and the need for better understanding of the biochemical cascade to improve healing. Several others studies were used to characterize the effect of a single impact on joint tissue degeneration using a similar model of impact to the closed patellofemoral joint in skeletally mature rabbits [22–27]. Various factors associated with automobile dashboard-type injuries were investigated, including padding of the impactor, intensity of impact, and rate of impact. In general, a single impact resulted in acute surface fissures of the cartilage, progressive cartilage degeneration and softening by 12 months post-injury, and progressive subchondral bone thickening out to 12 months post-injury with high-intensity impact and no padding.

An important note from this series of investigations was the finding that nonimpact control animals were required to demonstrate statistical significance and that contralateral control limbs were not sufficient for comparisons. These studies model joint damage that may be observed following direct blunt trauma transmitted across articular surfaces without radiographic evidence of fracture. With transarticular loading models, it is difficult to determine the contact area between the articulating surfaces, and therefore difficult to determine the actual impact stress to the cartilage. Pressure films can be inserted between the articular surfaces, but this may interfere with joint congruity and introduce high variability.

Several studies performed assessments following subfracture impact loading of intact patellae to examine the effects of injury on viability of patella articular cartilage as a function of time. When the whole joint capsule is kept intact until testing, and the testing is done on the day of harvest, the articular cartilage is viable and useful to test the effect of injury on viability. Lewis et al. mounted normal bovine patellae and impacted the articular surface with a 6 mm indenter with rounded edges resulting in a subfracture impact of 53 MPa to study cell death as a function of spatial location and time [28]. This level of impact load created cracking in the articular cartilage surface that did not propagate into the underlying subchondral bone. They report that cell death occurred around cracks which were found only in zone 1 (surface layer) or zone 2 of the articular cartilage; with this model cell viability did not decrease with culturing of the cartilage from 18 h to 5 days post-injury, suggesting that in this study with rapid impact loading, the mechanism of cell death was necrosis. The authors reported that chondrocyte viability was affected predominately by cartilage tissue damage (Fig. 8.2). Ashwell et al. impacted porcine patellae between 40 and 50 MPa and examined gene expression immediately after impact and then after organ culture for 14 days [29, 30]. Immediately after impact, *mmp3* was upregulated on day 0, and *col1a1* was increased at day 14 which suggested that chondrocytes may be more fibroblast-like following impact.

Recently, Novakofski has demonstrated the feasibility of using high-resolution multiphoton microscopy (MPM) of live, intact cartilage following subfracture impact loading to 30 MPa of equine metacarpal and metatarsal joints [31]. They were able to identify damage to the cartilage and spatial distribution of chondrocyte death using this technique. As previously described by others, cell viability was significantly reduced along the cracks and within the first 100 μm of the superficial layer.

Impact Without Fracture: Animal Models of a Single-Impact Load Through an Arthrotomy

An alternative approach is to surgically open the joint and directly apply a load with an indenter of known surface area. Several studies have used

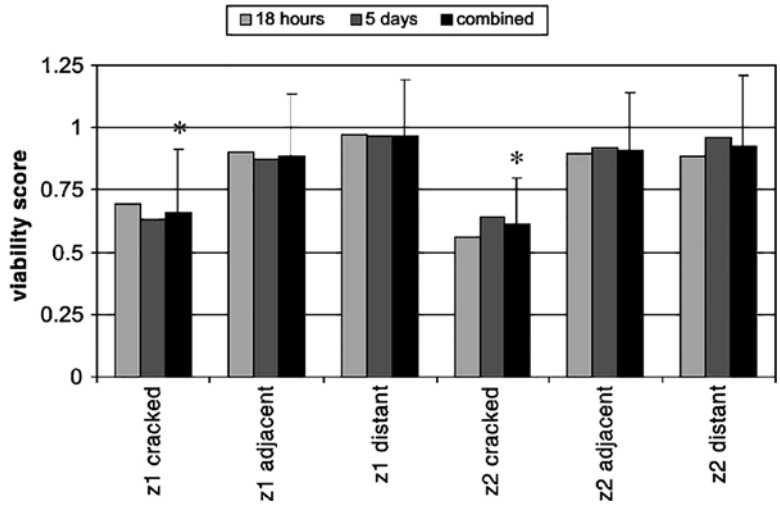

Fig. 8.2 Shows the relationship of chondrocyte viability and location relative to cartilage cracks involving zone 1 or zone II depth. *indicates significantly reduced chondrocyte viability $p<.05$. Reproduced from Lewis JL et al. Cell death after cartilage impact occurs around matrix cracks JOR 21:881 2003

this approach to apply a subfracture impact load to the femoral condyle in a rabbit knee to study the effect of a single traumatic load on cartilage matrix and viability. Milentijevic et al. impacted the lateral femoral condyle of the rabbit knee to 35 MPa and showed matrix damage, proteoglycan loss, and chondrocyte death at 3 weeks post-injury [32]. Borrelli et al. have reported on a range of impact loads (14–100 MPa) to the posterior aspect of the medial condyle in young rabbits at 3 months of age [33–36]. Higher impact loads resulted in acute superficial fissures in the articular cartilage with reported progressive loss of proteoglycan staining in the area of impaction over 6 months. Interestingly, Borrelli et al. reported that loads they had previously established from in vitro studies that resulted in damage in cartilage explants did not translate to in vivo animal model [37]. Higher impact loads were required with intact joints to produce similar levels of cartilage matrix damage and cell death.

Vrahas et al. used cadaver rabbit femoral condyles to develop a drop tower impact model capable of delivering predicable impacts and found that with varying impact energy and indenters, localized cartilage damage to the cartilage and bone was most frequent with a highly localized stress of >17 MPa delivered with a flat indenter [38]. Atkinson et al. found that with low energy impact of 2J, injury to the cartilage was observed, but at high-energy impacts of 22 J, deep injuries to the underlying bone were observed [39]. These studies suggested that the data was applicable to knee trauma occurring during motor vehicle accidents.

Recently, Brophy et al. have incorporated a radial transection of the medial meniscus in the rabbit knee along with the impact loading model developed by Borrelli et al. in young, 3-month-old rabbits [40]. At 3 months post-injury, they found that combined traumatic impact and meniscal transection was more damaging to articular cartilage than meniscal transection alone. Combined injuries to the articular cartilage and meniscus in the knee are common, and these results suggest that clinically combined meniscal and ligament knee injury with significant bone bruising may be more likely to lead to degenerative changes than an isolated meniscal tear.

Animal Models of Articular Fracture

Articular Facture Created with Impact Loading

Whole joint cadaver models of transarticular impact resulting in articular fracture have also been developed. Backus et al. impacted intact porcine knees on the day of joint harvest with a transarticular load via a drop track at high energy of 297 J [41]. With the same impact load, central alignment of the impact load resulted in no

fracture, whereas a laterally offset applied impact load resulted in a lateral tibial plateau fracture. As demonstrated by Kennedy, clinically lateral tibial plateau fracture is often created with a valgus stress coupled with an axial load. Osteochondral cores were obtained and cultured up to 5 days postimpact. Chondrocyte death, cartilage proteolytic enzyme activity, and S-GAG release were significantly upregulated in impacted joints that sustained an articular fracture. Increased levels of chondrocyte death were not observed in those joints that were impacted without creation of a fracture. Similar to data from Lewis et al., chondrocyte viability was not significantly affected by culture time post-trauma.

Tochigi et al. also impacted porcine hocks via a drop track (30 J) with introduction of a saw cut defect to the distal tibial cortex to mimic human ankle fractures, and cell viability of impacted fractured joints was compared to fractures created via an osteotomy [42]. Impacted fractured joints demonstrated ninefold greater chondrocyte death along the fracture edge compared to osteotomy-induced fractured joints. The authors concluded that the model development and data supported its translation to an in vivo study with survival animal surgeries.

Models of Articular Fracture Created with Osteotomy

A group of animal studies of intra-articular fracture (IAF) have mainly focused on cartilage healing with and without anatomic reduction of the articular surface. Mitchell and Shepard used an osteotomy model in a rabbit knee to study healing following a fracture-like disruption of the cartilage and subchondral bone. They observed hyaline-like cartilage healing following interfragmentary compression of the disrupted cartilage and bone [43]. Several other animal studies demonstrated, again with osteotomy models of articular fracture, that restoration of the subchondral plate and articular surface was possible even with incongruences of the articular cartilage [44–48]. However, healing was also associated with the development of osteophytes and other signs of degenerative arthritis. These studies indicate that some degree of articular surface remodeling is possible, but the progression of osteoarthritic changes with remodeling is unknown. Additionally, all of these studies utilized open surgical models that evacuate the hemarthrosis and do not incorporate blunt trauma to the articular surface and synovium. Clinically, these components of articular fracture cannot be isolated.

In Vivo Animal Models of Articular Fracture with Impact

Larger Animal Models

Animal models of articular fracture that incorporate both disruption of the articular cartilage and subchondral bone with articular impact may provide the most insight into the mechanisms of PTA development. The few in vivo articular models of articular fracture with impact are summarized here. In 1975, Farkas et al. created articular fractures in the femoral condyles of rabbits and reported that repair of the articular fracture was observed by 6 weeks postfracture with normal cartilage structure but a fibrous overgrowth of tissue that resembled a synovial pannus [49]. They concluded that no irreversible damage in cartilage composition was found with articular fracture in the rabbit knee at 8 weeks postfracture.

Another closed joint fracture model has been developed in the rat knee. A drop tower applied an impact to the closed patellofemoral joint of the rat knee to create intercondylar femoral fractures, again similar to a dashboard-type injury associated with automobile accidents. Chondrocyte viability was reported to drop from 68 % at day 0 to 46 % at 72 h following fracture. The level of cell death following fracture is similar to previous reports from cadaveric studies of impact with articular fracture.

Diestelmeirer et al. reported recently on the development of an instrumented pendulum impaction system to generate fractures of the distal tibia in mini-pigs to model human tibial pilon fractures [50]. As reported in the model development with

cadaver animals, fractures were generated with introduction of a saw cut defect to the distal tibial cortex. Although change to joint tissues was not yet reported, fractures exhibited the general clinical appearance of human tibial pilon fractures and energy absorbed during fracture was reported.

Olson et al. developed a dorsal wall fracture of the acetabulum in the goat hip [7]. In order to generate articular fractures, stress risers were surgically introduced in the posterior wall by predrilling and scoring the retroacetabular surface of posterior wall. The fractures were then generated with an impact (40–60 MPa) from a drop tower applied at the flexed knee in line with the femoral shaft resulting in a transarticular impact to the hip, similar to dashboard injury. Joints were then assessed with and without anatomic reduction at 90 days post-surgery. The residual defects were observed to be filled with fibrous tissue. Fractured joints demonstrated greater histologic scores of arthritis than control hips, and scores trended towards being greater in the displaced group. Such large animal models in mini-pigs or goats can provide a means to study the effects of treatment of displaced IAF.

The use of closed-joint articular fracture in a large animal model will allow investigation of joint injury treated with surgical realignment and fixation. Creating the fracture without arthrotomy, or opening of joint capsule, may allow for a more clinically relevant evaluation of the sequelae of joint degeneration following trauma to the cartilage and subchondral bone without the potentially confounding elements of surgical disruption of the capsule and synovium. Increasingly, survival models of articular fracture are leading to important contributions in the mechanisms of PTA development. However, use of a larger animal model in preliminary investigations attempting to understand basic mechanisms of joint injury is cost prohibitive.

Murine Model of Intra-articular Fracture

Murine models have the advantage of a relatively low cost of experimental animals; well-described

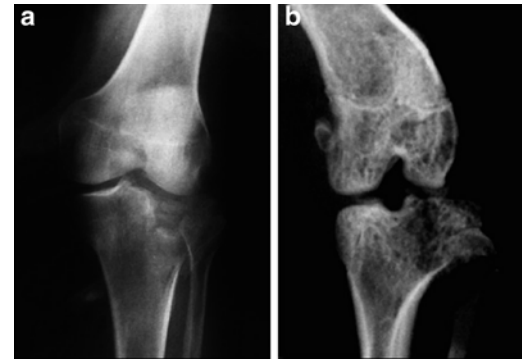

Fig. 8.3 Radiographs of a tibial plateau fracture in a human knee (**a**) and a mouse knee (**b**) (reproduced from Furman et al. Joint Degeneration following Closed Intra-articular Fracture in the Mouse Knee: A Model of Post-Traumatic Arthritis J Ortho Res 25:578, 2007)

analyses for serum and synovial biomarkers; and transgenic strains of mice are readily available. A murine model of IAF may provide insight into basic mechanisms of post-joint injury response that lead to PTA. Success in identifying the mechanisms involved in the progression of PTA after fracture could directly impact clinical practice in treating articular fractures.

One of the benefits of collaboration between clinicians and basic scientists is the opportunity for synergy in approach to research. The development of a murine model of closed IAF is the result of such synergy. The initial challenge was the lack of a validated model of an IAF that developed PTA. Surgeons interested in studying articular fractures often focus on the importance and effects of realigning or reduction of the articular surface as part of the treatment of the injury. Basic scientists seek to understand basic mechanisms in play leading to the outcome of interest. The collaboration of these two perspective results in the development of a novel murine model of a closed IAF that develops PTA to observe the natural history of development of PTA after an IAF.

Furman et al. described the mouse model of closed articular fracture of the tibial plateau for studying PTA (Fig. 8.3). The articular fractures are generated using a computer-controlled material testing system and custom-built indenter tip to apply a load to the anterior aspect of articular cartilage of tibial plateau. A detailed description

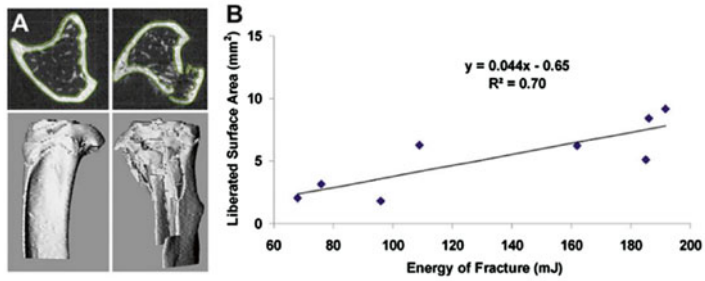

Fig. 8.4 (**a**) Illustrates the technique of peripheral surface measurement used to determine the exposed surface area for the intact and fractured limbs. The difference between the exposed surface area of the two sides gives the surface area liberated by the articular fracture. (**b**) The correlation between liberated surface area and measured energy of fracture is shown (reproduced from Lewis et al. Acute joint pathology and synovial inflammation is associated with increased intra-articular fracture severity in the mouse knee. OA & C 19:864, 2011)

is found in the original manuscript [51]. The indenter is applied in a load control mode that allows for creation of high-energy and low-energy articular fractures. The energy of injury is measured as the energy applied at the time of fracture creation. In order to validate this simple measure we performed an analysis determining the liberated area after an articular fracture using micro CT images of the fractured limb and the contralateral normal limb as described by Beardsly et al. There was a strong correlation between measured energy of fracture and liberated area ($R^2 = 0.70$) (Fig. 8.4).

In the proof of concept work, joint tissues were assessed in C57BL/6 mice at 2, 4, 8, and 50 weeks following fracture. Mice are allowed to be active without restriction of weight bearing immediately after injury within their cage. Effective anesthesia and postprocedure analgesia were provided at all times for experimental subjects involved in all studies using this experimental model following IACUC-approved anesthetic and analgesic regimens. Significant progressive arthritic changes were identified at 8 and 50 weeks postfracture with histology of the joint tissues and micro CT evaluations of bone morphology. The extent of PTA assessed with the modified Mankin score was similar at 8 weeks and 52 weeks. We have used the 8-week time point as a surrogate for end-stage disease in our subsequent investigations. A number of acute changes were also noted in periarticular bone mineral density following a closed articular fracture. These changes are too numerous to mention here, and are covered in detail in the original manuscripts.

A limitation of the mouse model is the small size of the tibial plateau which limits options for fixation. Therefore, the fracture in this model is not repaired. This is analogous to an ACL injury or meniscus injury model. However, the model has the benefit of having a relatively reproducible injury with a short time frame to develop end-stage disease, and has the benefit of allowing for investigation of the injury response in alternate genetic strains of mice. In addition, the model has provided an opportunity to study the molecular mechanisms involved in a "worst-case scenario" of PTA development following an unstabilized articular fracture [52–56]. In general, our approach has been to identify mechanisms that lead to therapies to influence this worst-case situation; such therapies will have a physiologic basis for action in the early time points after fracture creation.

The observation of extensive synovitis occurring within 7 days of fracture creation was an important insight into this line of investigation (Fig. 8.5). Physiologically active cells in the synovium provide another relevant source of bioactive agents to influence the post-injury response. Lewis et al. observed that the extent of intra-articular synovitis observed histologically were increased with increasing injury severity. However, loss of viability of chondrocytes was unchanged with variation in injury severity.

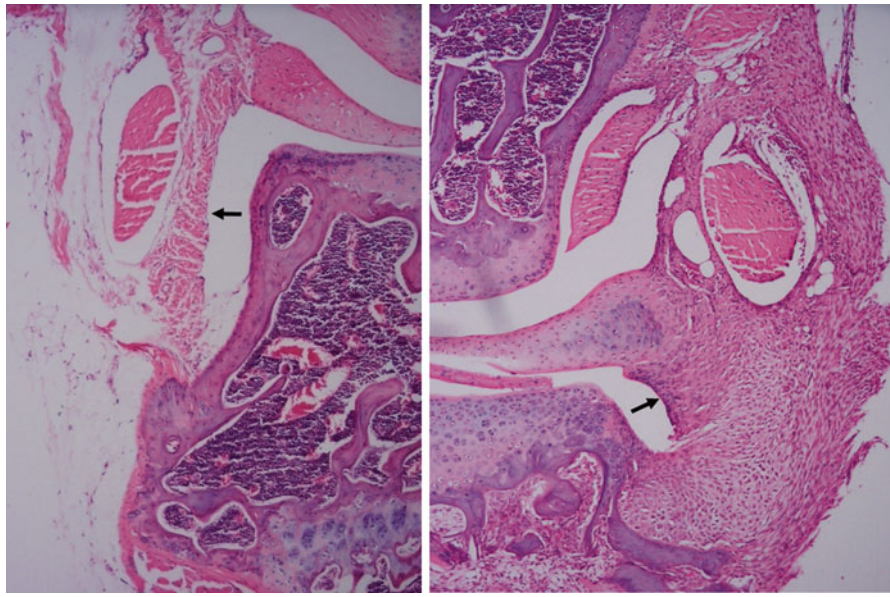

Fig. 8.5 100× view of H&E stain of knee joint taken 7 days after closed articular fracture creation. The *right-hand* figure is a non-fracture control mouse, and the *left* image is in a fractured joint. The *arrow* points to the synovial lining. Significant cellular infiltration in the synovial lining is present indicating an active intra-articular response to the injury

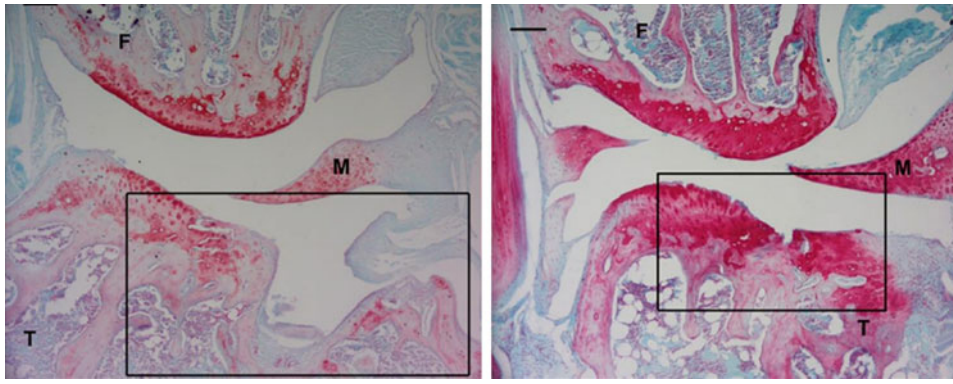

Fig. 8.6 Safranin-O staining of lateral tibial plateau 8 weeks after creation of a closed articular fracture. The *box* identifies the location of the articular fracture. The *left* image is in the C57BL/6 mouse with signs of PTA and the *right* image in the MRL/MpJ mouse with a near-normal staining (reproduced from Ward et al. Absence of Post-Traumatic Arthritis Following Intraarticular Fracture in the MRL/MpJ Mouse Arthritis and Rheumatism. 2008; 58:774)

Specifically, the higher the energy of injury the more extensive the synovitis was present throughout the entire joint, suggesting that the organ system response occurs in this injury.

Murine models offer the advantage of using unique genetic strains of mice to elucidate molecular and cellular mechanisms. The MRL/MpJ mouse offered such an opportunity. This strain of mice has the ability to spontaneously regenerate fibrocartilage following an ear punch. Ward observed that these mice did not develop degenerative changes of PTA following a closed IAF of the tibial plateau [52] (Fig. 8.6). Paradoxically following IAF the MRL/MpJ mice had no loss of staining for proteoglycan or other signs of PTA. The MRL/MpJ is a unique strain of mice

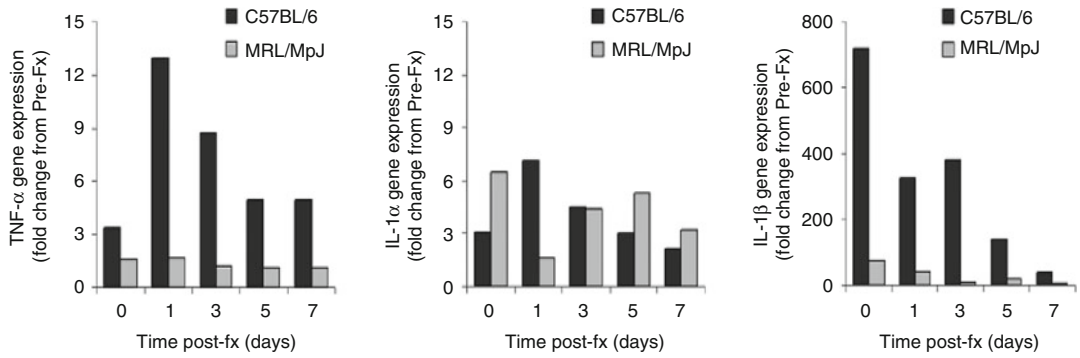

Fig. 8.7 Comparison between synovial gene expression immediately following articular fracture creation. The C57BL/6 mice have a greater proinflammatory gene expression for IL-1β and TNF-α (reproduced from Lewis et al. Genetic and Cellular Evidence of Decreased Inflammation Associated With Reduced Incidence of Post-Traumatic Arthritis in MRL/MpJ Mice. Arthritis and Rheumatism 65:660, 2013)

and not the result of a simple or known genetic mutation from another established genetic line of mice. As a result comparative studies are needed to understand the differences in post-injury response between the C57BL/6 and the MRL/MpJ strains.

In order to do analyses a technique to quantitatively assess levels of biomarkers and other bioactive molecules in the synovial fluid of mice was needed. Siefer et al. developed a novel technique of collecting synovial fluid from the knee of a mouse to allow for quantitative analysis of biomarkers and bioactive molecules. At the time of sacrifice, the knee joint is opened by elevating the extensor mechanism from proximal to distal without disrupting the remainder of the joint cavity. A known amount of calcium alginate is used to absorb all synovial fluid in the joint cavity. The calcium is then dissolved in a known amount of citrate buffer, usually 50 μl. Analysis can then be performed correcting for joint dilution. The analysis is limited by the total volume of sample.

Genetic analysis can be performed on the synovial tissue using a technique described by Van Meurs et al. At the time of sacrifice following synovial fluid collection a 3 mm punch biopsy is taken from the parapatellar synovium on each side of the joint, providing just over 9 mm^2 area of synovial tissue per joint. RNA was isolated from the tissue biopsies. Reverse transcription-polymerase chain reaction (RT-PCR) was run, in duplicate, and a commercially available RT Profiler PCR array was used to assess the synovium sample for messenger RNA. The specimens can be pooled in a treatment group or analyzed individually per test specimen as appropriate. Samples were pooled in the work reported by Lewis et al. [56].

Using this knowledge, a detailed comparison of the response to injury between the C57BL/6 and MRL/MpJ mice was undertaken [56]. This investigation showed that while both strains had an initial inflammatory response to injury, the MRL/MpJ mice were protected from a significantly prolonged proinflammatory injury response seen in the C57BL/6 mice. Significantly increased levels of IL-1α and IL-1β were observed after IAF, while only a minimal increase in TNF-α was not seen in this model (Fig. 8.7). IL-1α was observed to increase systemically after IAF with equal concentrations in both experimental and control joints. IL-1β was observed to increase initially in the injured joint and then increase systemically following the levels in the injured joint. Synovial RNA activation of IL-1β increased 720× within 4 h of injury in the C57BL/6 mice as compared with a 70× increase in the MRL/MpJ mice. Using immunohistochemistry techniques at both 7 and 28 days the synovium of the C57BL/6 had higher levels of macrophages as compared to the MRL/MpJ. The MRL/MpJ mice are protected from developing PTA after a severe joint injury

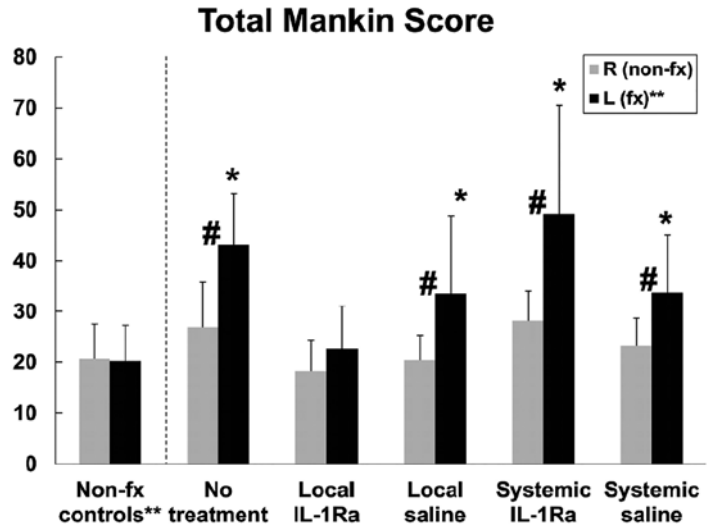

Fig. 8.8 Modified Mankin scores 8 weeks following creation of a closed tibial plateau fracture with treatment with local (intra-articular) IL-1Ra or saline and systemic IL-1Ra or saline. Only local IL-1Ra prevented changes of PTA. Significant difference between limbs, #significant differences between treatment groups (modified from Furman and Mangiapani et al. Targeting pro-inflammatory cytokines following joint injury: acute intra-articular inhibition of interleukin-1 following knee injury prevents post-traumatic arthritis. Arthritis Research & Therapy. 2014; 16 R-134)

involving an IAF. The MRL/MpJ strain is able to attenuate the duration and intensity of the post-injury inflammatory response.

Based on these observations a set of experiments were designed to assess the effect of therapies to attenuate the early stimulus of pro-inflammatory cytokines. We choose to investigate two methods of administering inhibitors to proinflammatory cytokines that were successful in preliminary studies of rheumatoid arthritis: prolonged systemic administration, and a single intra-articular injection. Furman et al. [57] reported the results of using either IL1-receptor antagonist (IL-1Ra) or soluble TNF receptor II using an osmotic pump for systemic delivery or via a single intra-articular injection immediately following fracture creation in C57BL/6 mice. IL-1Ra given via intra-articular injection reduced changes of PTA and did not alter bone healing following joint fracture (Fig. 8.8). However, neither systemic inhibition with IL-1Ra nor local or systemic administration of soluble TNF receptor II inhibited PTA development; conversely these interventions resulted in increased arthritic changes in the joint. Kimmerling et al. [58] reported similar results using elastin like polypeptide as a drug depo for sustained delivery of IL-1Ra intra-articulalry after IAF. These two studies provide proof of concept that acute inhibition of the post-injury response can reduce the development of PTA.

The ability to create a closed articular fracture provides many opportunities for investigating ways to prevent PTA. The collective body of work on this experimental model led to the finding that acute inhibition of IL-1 effects following joint injury is beneficial in limiting PTA after articular fracture. Similar beneficial effects in reducing the development of PTA were also observed by Diekman et al. with intra-articular administration of stem cells immediately following articular fracture in this same experimental model.

This body of work suggests that there may be a role for a pharmacologic as well as surgical treatment for displaced articular fractures. Well-done surgical reduction and fixation of articular fracture will always play an important role in the treatment of these injuries. This work provides evidence that a better understanding of the post-injury response in humans may lead to therapies to prevent PTA.

Future directions point to the role of modification of the intra-articular post-injury response that can be used in combination with surgical care as a means of preventing PTA.

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Instability: Dynamic Loading Models

9

Todd McKinley

Clinical Manifestations of Instability

Successful orthopedic management of articular injuries, primarily to prevent post-traumatic osteoarthritis (PTOA), is predicated on reestablishing a long-term mechanical environment in the joint that fosters healthy cartilage mechanotransduction. Clinically, it is emphasized that accurate restoration of joint surface congruity is necessary for that purpose [1–4]. However, the long-term effects of residual incongruity on joint outcomes are inconsistent, and there are many reports of cases or series where patients have done surprisingly well in the presence of substantial incongruity [5–8]. For example, while reduction accuracy plays an important role in outcomes in patients sustaining acetabular fractures [3], reduction accuracy has little effect on patients that sustain tibial plateau fractures [6, 8–10]. Clinical evidence linking incongruity to PTOA is inconclusive, in contrast to consistent observations that instability causes PTOA [11–13].

The rationale to study the mechanical and biologic effects of instability on injured joints is supported by clinical observations consistently relating instability to poor outcomes and PTOA. Patients sustaining acetabular fractures have uniformly poor outcomes in joints with residual instability [3]. Likewise, malaligned and unstable tibial plateau fractures fare poorly long term regardless of joint surface congruity [9, 11, 13]. Finally, patients with congruous but unstable ankles (residual mortise malreduction or chronic ankle sprains) have a high rate of PTOA [12, 14].

While clinicians often describe patients as having unstable joints, the definition of joint instability is nebulous. Clinical manifestations of instability are dominated by sudden catching or giving way of an affected joint. Patients will relate that their affected extremity gives out or that the joint feels loose. This typically occurs during walking and changing directions or during a twisting motion. Such changes in position of the joint surfaces can include excessive translational and rotational displacements. Mechanically, this likely translates into articulating surfaces sustaining sudden unphysiologic changes in position and motion resulting in abnormal loading magnitudes and rates of stress transfer; these loads can be significantly higher than seen in typical physiologic function. These alterations in loading result in abnormal contact patterns, with articular surface contact occurring in regions not habitually oriented to sustaining such stresses or even any contact. Repeated unstable loads accumulated over time develop changes in the affected joint that consistently leads to PTOA. In these patients, the original mechanical symptoms that typify instability evolve into more consistent pain

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that is characteristic of osteoarthritis. Therefore, understanding the macroscopic and microscopic mechanical effects of instability and how these effects are transduced into a degenerative biologic response of an affected joint is important in understanding PTOA.

The majority of tests investigating pathomechanical residual effects of intra-articular fractures on cartilage have focused on static testing of contact stresses as a function of articular surface displacement, as well as congruity-dependent perturbations in articular surface stress transfer [15–17]. Modeling instability is seemingly more difficult. Instability, by definition, cannot be reproduced in a static testing preparation. Recent improvements in analytical and computational techniques have made instability testing more feasible and meaningful. In this chapter, instability testing in cadaveric studies and computational models will be summarized highlighting articular surface and cartilage interstitial stress perturbations sustained in unstable joints. Subsequently, the effects of instability on cartilage biologic response in tissue-level and living organ-level models will be described.

Cartilage Material Properties and Physiology

The mechanical environment encountered by cartilage, in particular on the joint surface, is heterogenous in terms of a wide variety of stress magnitudes, stress rates, and directions of stress that are transmitted between the articulating structures. Articular cartilage transduces the heterogenous surface loading envelope into a relatively homogenous set of hydrostatic stresses and strains within the substance of the tissue and into the subchondral plate. Articular cartilage has highly evolved structure and complex material properties that optimize load transfer across articulating surfaces and through the interstitium of the tissue [18]. Articular cartilage transmits load by deformation of its solid matrix constituents and through hydrostatic pressurization of the interstitial fluid permeating the solid matrix. Cartilage material properties have been intensely studied in experiments ranging from molecular-level investigations to organ-level investigations. Because of its biphasic nature, stresses developed within cartilage are highly time dependent [18, 19]. Stresses and deformation in loaded cartilage are particularly sensitive to stress rates [18, 20]. The rate dependence on stress and deformation within cartilage that has been shown to affect tissue mechanics on a microscopic scale to the level chondrocyte deformation have been shown to be biologically relevant as biosynthetic responses at the chondrocyte level are primarily a function of loading rate. Likewise, tissue-level experiments have shown that biosynthesis of DNA, collagen, and proteoglycans is primarily affected by changes in stress rates [21, 22]. Similarly, mechanical damage of cartilage and biosynthetic response of cartilage to injurious impact loading have both been shown to be primarily load-rate dependent [23–25].

These studies highlight the importance of accurately reproducing physiologic and pathologic loading rates in cartilage investigations. Dynamic testing is especially pertinent in unstable joints because these joints will invariably include regions of cartilage with accentuated high stress rates and stress magnitude peaks. Therefore, simulating realistic stresses and stress rates during in vitro and in vivo preparations takes on increased importance in instability investigations. Recent improvements in real-time stress transducers have facilitated accurate physical testing of unstable and incongruous joints. In addition, computational methods that account for time-dependent cartilage structural and material properties and survey accurate motion encountered in physiologic joint duty cycles have provided new information on cartilage mechanics in normal and unstable conditions.

Macromechanical Tests of Instability

Contact Stress Rate Changes During Instability

Joint instability was simulated in a dynamic model using human cadaveric ankles. In this model, human cadaveric ankles were manipulated

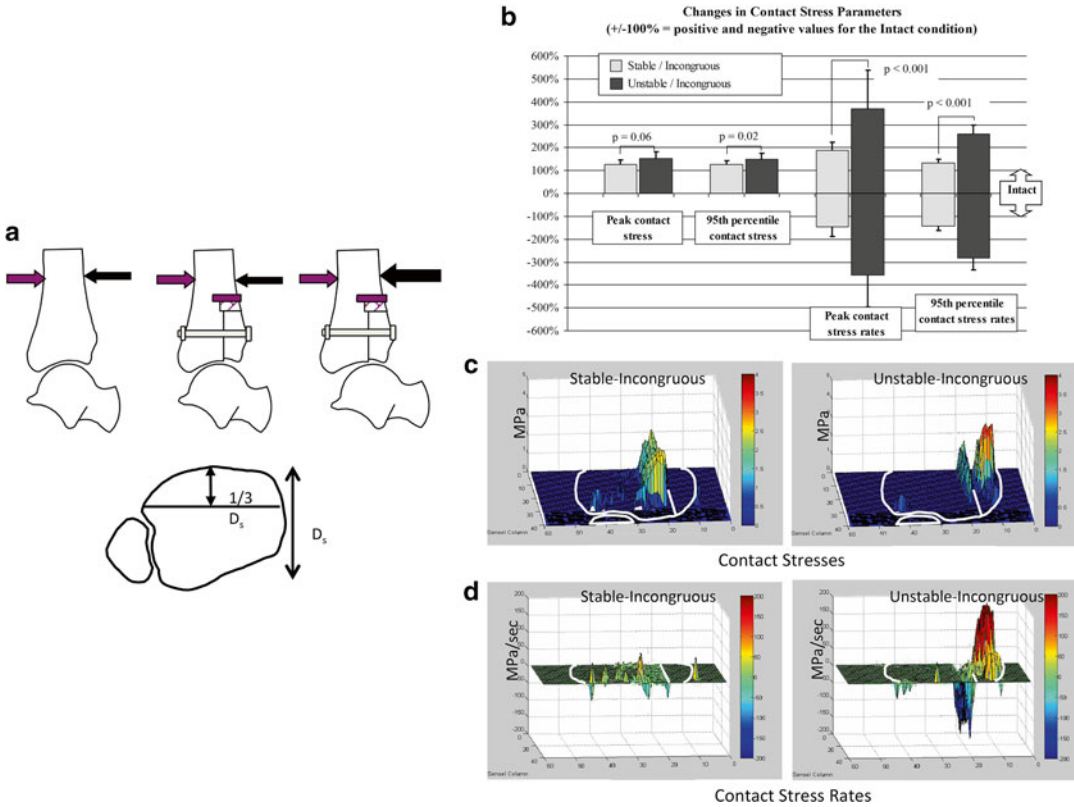

Fig. 9.1 Dynamic human cadaveric ankle loading model in which ankles were made incongruous with a coronally directed osteotomy of the anterior distal tibial surface displaced proximally 2.0 mm (**a**). Anterior and posterior forces were applied to the tibia through pneumatic cylinders to affect the stability of the joint. Incrementally increasing pulses were applied posteriorly to the tibia (**a**, *black arrow*) until subluxation occurred during loading. Changes in contact stress in stable-incongruous and unstable-incongruous specimens were modest compared to intact specimens (**b**). In contrast, contact stress rates increased dramatically in unstable-incongruous specimens compared to stable-incongruous specimens (**b**). Contour maps clearly depict relative equivalence in changes in contact stress peaks (**c**) and significant differences in contact stress rate peaks (**d**) that occurred during instability

to create a "metastable" articulation in which joint subluxation could be reproduced during normal joint motion with minimal changes in external loading conditions [26]. This allowed the investigators to sample contact stresses under incongruous conditions with nearly identical extrinsic loading in ankles that either remained stable or had an obvious instability event. The premise of the study was to simultaneously compare the effects of incongruity and instability on articular surface stress transfer.

In this model, the distal tibial surface was made incongruous by osteotomizing the anterior one-third of the distal tibia. The fragment was proximally displaced 2.0 mm and secured with internal fixation creating a coronally directed stepoff and a defect into which potential subluxation of the talus could occur (Fig. 9.1a). Subsequently, specimens were mounted into a custom fabricated dynamic ankle loading fixture that maintained physiologic motion of the ankle, hindfoot, and midfoot. The loading fixture was secured to a MTS machine that subjected specimens to physiologic loads and motion encountered during the stance phase of walking.

During testing, anteriorly and posteriorly directed forces were applied to the tibia to modulate the sagittal tibiotalar relationship and ultimately the stability of the ankle joint (Fig. 9.1a). Increasing incremental posteriorly directed

pulses were applied to the tibia during the gait cycle until the talus grossly subluxated anteriorly into the distal tibial defect during loading. Stresses were captured at a 132 Hz sampling frequency with a pliable piezoelectric real-time stress transducer that was inserted into the ankle joint. Raw data were used to calculate ankle joint contact stress and contact stress rates throughout the motion cycle.

Contact stresses and contact stress rates were compared under metastable conditions within each specimen. Attention was focused on comparing stress measurements between the last cycle in which the talus remained stable beneath the distal tibia (pre-instability cycle termed stable-incongruous) and the first cycle in which the talus subluxated into the distal tibial defect (instability cycle termed unstable-incongruous). Loading conditions were nearly identical between these two tests with identical motion and axial load, and only a 20 N increase in the posteriorly directed impulse applied to the tibia in the unstable-incongruous trial. Therefore, the effects of incongruity with or without instability could be measured.

Peak contact stresses were only 25 % higher in unstable-incongruous specimens compared to stable-incongruous specimens. In contrast, changes in contact stress rates increased 170 % in unstable-incongruous specimens compared to stable-incongruous specimens (Fig. 9.1b). These data clearly depicted the effects of instability. The rapid rise in contact stresses resulting in the increases in contact stress rates occurred over approximately 5 ms and the entire subluxation event lasted approximately 25 ms which was felt to be physiologically relevant. Real-time contact stress and contact stress rate contour plots demonstrate relative equivalence in peak contact stresses in incongruous ankles regardless of instability (Fig. 9.1c). However, the sharp peak in contact stress rate was clearly evident in unstable ankles (Fig. 9.1d). The instability event studied in this experiment was admittedly idiosyncratic. However, the experiment was designed to create conditions in which the talus would subluxate with minimal additional differences in extrinsic loading between stable and unstable conditions allowing the investigators to focus on the mechanical aberrations that occur primarily due to instability in already incongruous joints.

Contact Stress Directional Gradient Changes During Instability

Using an identical cadaveric ankle incongruity/instability model, the effects of incongruity and instability on contact stress directional gradients were determined under metastable conditions [27]. In this experiment, the researchers used regional contact stress measurements to calculate directional stress magnitude vectors (Fig. 9.2a). Peak transient directional gradients nearly doubled in unstable-incongruous specimens compared to intact specimens and increased 50 % in unstable-incongruous specimens compared to stable-incongruous specimens (Fig. 9.2b). Interestingly, under intact conditions, directional gradients were typically randomly oriented throughout the ankle motion cycle at any specific location and low magnitude (Fig. 9.2c left figure). When these individual vectors were summed over the entire motion cycle, the resultant vectors at each locus in intact ankles were close to zero. The authors concluded that small magnitude randomly oriented directional gradient vectors optimized interstitial stresses to maintain homogenous interstitial fluid distribution and healthy chondrocyte mechanotransduction. In contrast, directional gradients were higher in magnitude and preferentially oriented in incongruous specimens and the magnitudes increased significantly secondary to instability (Fig. 9.2c right two figures). Consistent high-magnitude directional gradients with a consistent orientation would increase shearing deformation of regional chondrocytes which has been shown to result in compromised mechanotransduction and cartilage degeneration. Additionally, this type of oriented stress distribution could lead to regions of interstitial fluid depletion.

In conclusion, human cadaveric testing using an ankle model has been used to develop a physiologically relevant macroscopic model of articular instability. Investigators have created an instability event and quantified resultant changes

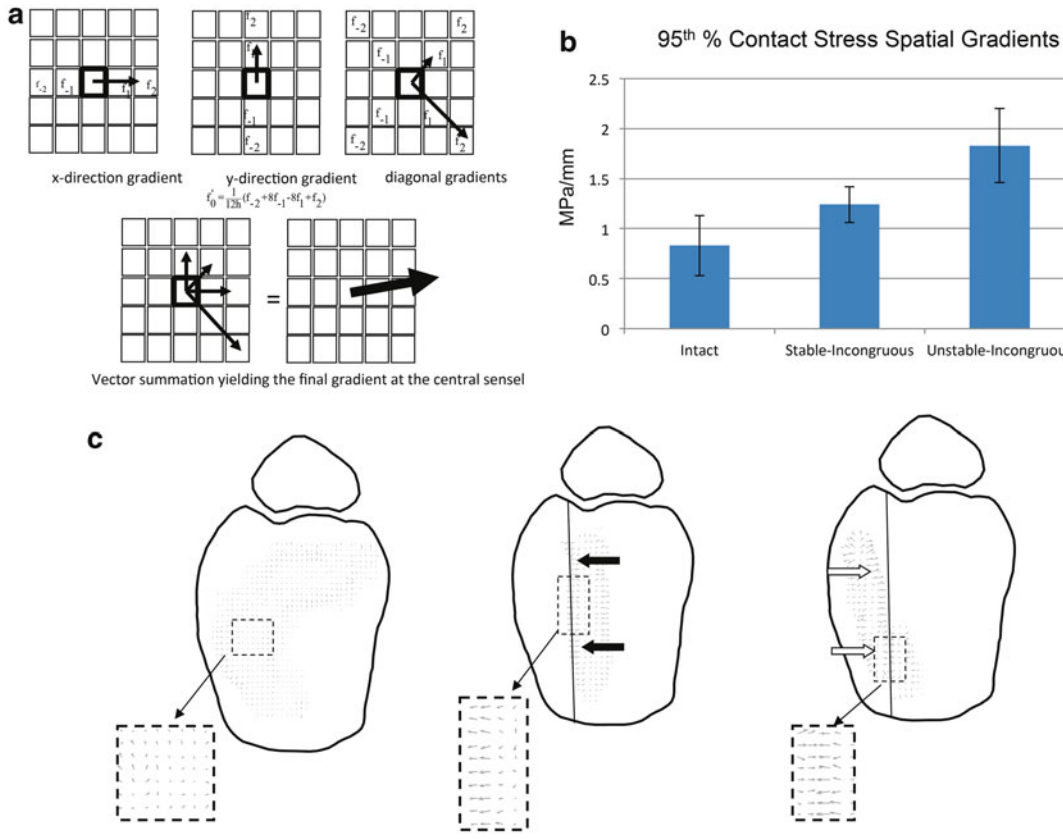

Fig. 9.2 Contact stress directional gradients were calculated by calculating a local derivative at each stress transducer sensel (f_0) based on values of neighboring sensels (**a**). Unstable-incongruous specimens had 100 and 50 % increases in 95th percentile spatial gradient values compared to intact and stable-incongruous conditions (**b**). *Vector plots* demonstrate low-magnitude randomly oriented spatial gradients in intact specimens in contrast to polarized higher magnitude vectors in stable-incongruous and unstable-incongruous specimens (**c**)

in articular surface stress transfer. Significant increases in contact stress rates and contact stress directional gradients were documented in incongruous specimens with instability compared to incongruous specimens that remained stable. Typical contact stress rates during instability under these conditions ranged between 100 MPa/s and 200 MPa/s.

Computational Models of Instability

Cadaveric macromechanical models quantify changes in surface stresses allowing measurements of pressure peaks and calculations of time and directional gradients. However, they do not quantify interstitial cartilage stresses and deformations resulting under both normal conditions and under pathologic conditions such as instability and incongruity. Currently, there are limited analyses that allow direct interstitial stress and strain measurements in loaded cartilage. Therefore, investigators have had to rely on computational methods to simulate such information. However, as outlined above, cartilage material properties are complex highlighting the difficulty and ultimately the importance of sophisticated computational methods to accurately simulate interstitial cartilage deformation and stress fields under load.

Goreham-Voss and colleagues developed a transversely isotropic poroelastic finite element model that could simulate unstable motion

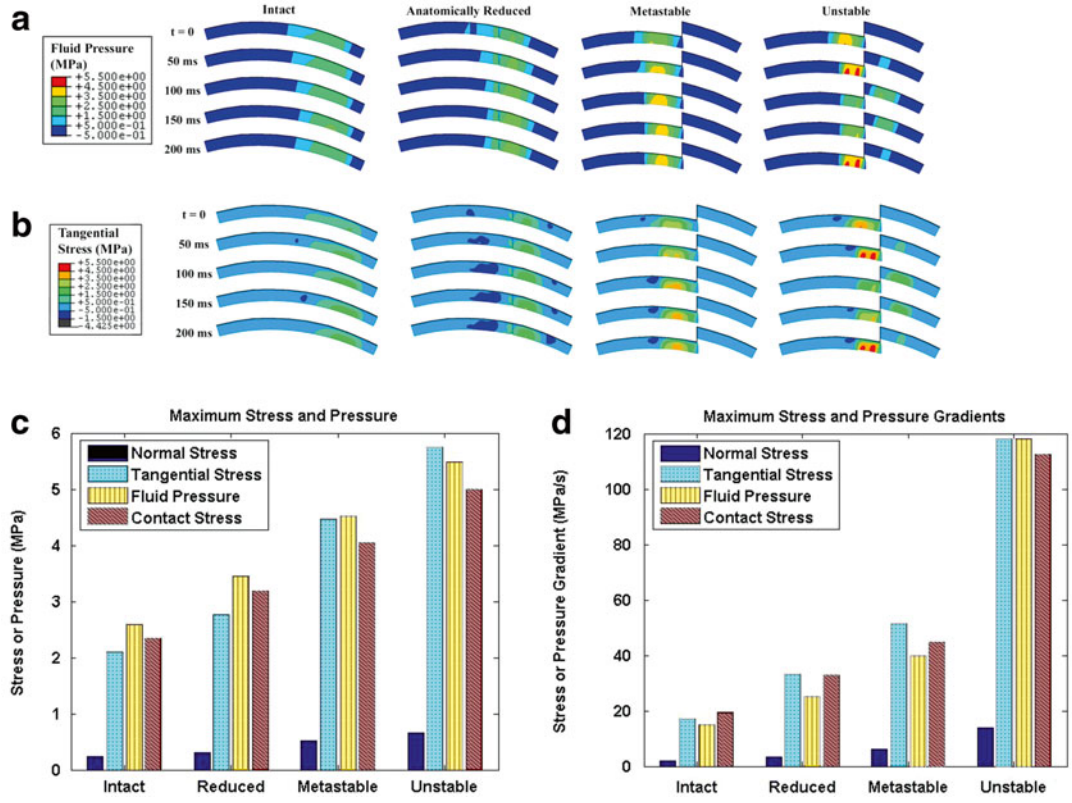

Fig. 9.3 Poroelastic two-dimensional finite element modeling of incongruous and unstable ankle motion demonstrated that both interstitial fluid pressure (**a**) and solid phase tangential stresses (**b**) significantly increased secondary to instability during the subluxation event ($t = 50$ ms) and when the talus reduced back under the distal tibia ($t = 200$ ms). Fluid pressure, normal stress, and shear (tangential) stress increased modestly in metastable and unstable specimens compared to intact and reduced specimens (**c**). In contrast, instability resulted in significant increases in temporal gradients of normal stress, shear stress, and fluid pressure compared to metastable, reduced, and intact conditions (**d**)

resulting from an incongruous articulation [28]. This was a two-dimensional model evaluating plane strain and two-dimensional stresses. Initially, the investigators validated the performance of the model by comparing results in congruous and stable ankles to finite element simulations. These comparisons included ankles that were entirely intact and in ankles that had an osteotomy that was anatomically reduced. Subsequently, they modeled a 2.0 mm coronal plane stepoff that simulated cadaveric ankle testing. Under these conditions, they simulated ankle flexion-extension in which the talus remained stable (metastable) under the tibia and conditions in which the talus subluxated anteriorly into the defect (unstable).

Interstitial fluid pressures and stresses increased in metastable and unstable conditions compared to reduced and intact conditions (Fig. 9.3a–c), but the increases were modest. In contrast, temporal gradients of fluid pressure, tangential (shear) stress, and normal stress all increased significantly in unstable conditions compared to metastable conditions. Solid-phase normal transient stress rates increased from approximately 4 MPa/s in metastable conditions to 14 MPa/s in unstable conditions (Fig. 9.3d). Likewise, temporal gradients of solid-phase tangential stresses and fluid pressures increased between 250 % and 300 % in unstable conditions compared to metastable conditions. Incongruity- and instability-related changes in stresses and stress rates were encountered throughout the entire thickness of the cartilage but were greater in the superficial and middle zones of cartilage.

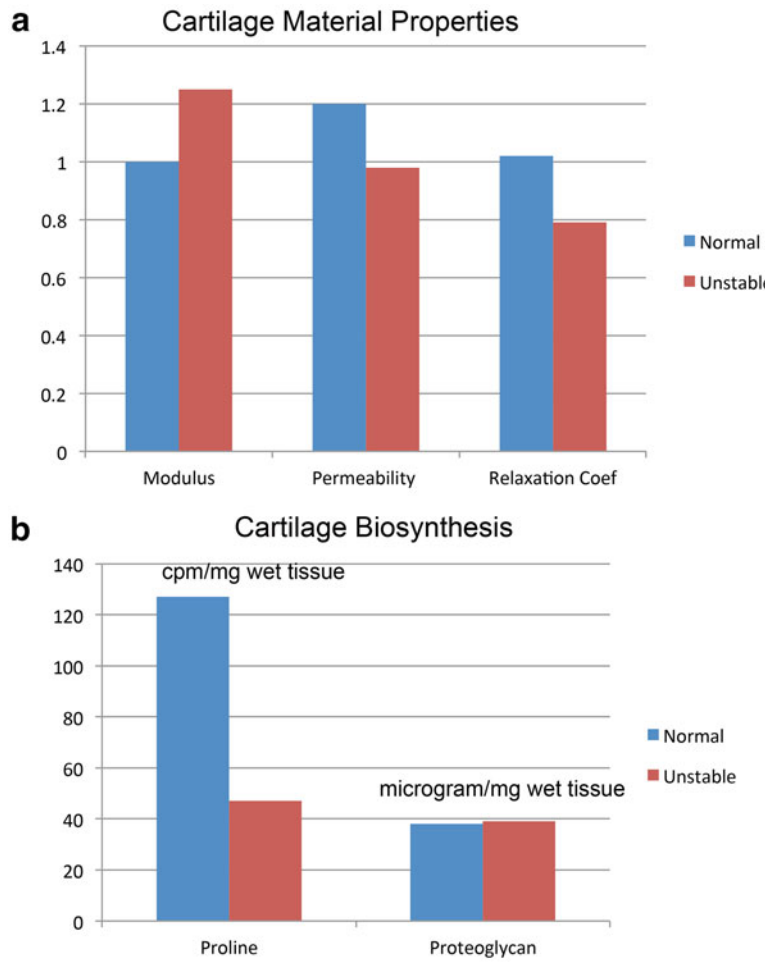

Fig. 9.4 Material property (**a**) and biochemical (**b**) changes in osteochondral specimens subjected to normal and unstable loads. Compared to preloading values, instability resulted in a modest rise in compressive modulus and decreases in permeability and relaxation coefficient compared to normally loaded specimens (**a**). These findings were corroborated in biochemical testing that demonstrated no changes in proteoglycan content but significant decreases in proline uptake consistent with disruption of the collagenous structure of cartilage in unstable specimens (**b**)

These findings demonstrated similar stress rate elevations compared to surface stress rates in cadaveric ankles. In addition, abnormal increases in interstitial stress rates dissipated throughout the thickness of the cartilage demonstrating how cartilage transduces abnormal surface loads to more uniform stresses in the deep layers of cartilage at the level of the subchondral plate.

Tissue-Level Models of Instability

Cartilage tissue is unique because it exists in vivo in relative anoxia and has no direct blood supply. This allows for explanted tissue-level investigations that can subject cartilage samples to prescribed mechanical perturbations and subsequently measure biosynthetic response under quasi-physiologic conditions.

In an ongoing series of experiments, investigators have subjected freshly explanted bovine tibial plateau osteochondral specimens to a series of repetitive loads. Explants were subjected to loading regimens representing normal loads (1.0 MPa applied at 10 MPa/s) and unstable loads (3 MPa applied at 100 MPa/s) [29]. Specimens were subjected to the prescribed loading regimen for 1,000 cycles at 1 Hz every other day for 2 weeks. In these specimens, materials testing demonstrated that unstable loads resulted in a 20 % increase in compressive modulus and a 20 % decrease in relaxation coefficient compared to normally loaded specimens (Fig. 9.4a). The investigators concluded that short-term exposure

to instability type stresses affected the collagenous matrix constituents of cartilage more so than the proteoglycan components. At the end of the testing period, cartilage biosynthesis as measured by proline uptake in specimens subjected to instability loading was 40 % of proline uptake in specimens subjected to normal loads (Fig. 9.4b). In contrast, proteoglycan content was unchanged between the two loading conditions.

In Vivo Models of Instability

In vitro cadaveric whole-joint mechanical tests yield information regarding changes articular surface stress transfer under pathologic conditions. However, these tests cannot determine tissue-level biologic response to changes in joint loading. Tissue-level testing in freshly harvested specimens has allowed investigators to determine how changes in loading parameters affect the biologic response of viable but isolated cartilage to various injurious loading regimens. However, these tests are reduced to evaluating isolated cartilage biologic responses to mechanical perturbations and cannot account for whole-joint biologic effects of injury. In vivo testing is necessary to truly investigate an organ-level response of a joint to injury or chronic changes in the prevailing loading environment. PTOA is an organ-level disease and ultimately the pathophysiology leading to PTOA needs to be described in survival organ-level investigations. Evidence continues to accumulate describing whole-joint responses to injury, highlighting contributions from cartilage and synovium to mechanical changes in loading.

There have been multiple models of ACL-transection instability used to study articular cartilage changes that result from ligament transaction [30]. Similar alterations of mechanical properties in articular cartilage that are observed in the human with osteoarthritis also occur in the canine after 12 weeks ACL transection [31]. However, rigorous measuring of post-transection instability was not done as a part of this type of investigation. Recently, an in vivo model of joint instability, in which instability was quantified, was developed to investigate the effect of increasing instability on cartilage degeneration and biologic response [32]. In this test, the investigators developed a model of graded instability in rabbit knees to determine the mechanical and biologic effect of instability on an intact joint. Rabbits were subjected to an anterior parapatellar arthrotomy, allowing the anterior cruciate ligament (ACL) to be isolated. Subsequently, graded portions of the ACL were transected. Immediately after transection, the anesthetized rabbits were subjected to sagittal plane translational stability testing using purpose-designed fixturing to quantify the instability resulting from ligament transection. Both the tibia and femur were secured with Steinmann pins which articulated with a custom mechanical actuator that applied a linear displacement to the tibia while the femur was statically stationed. Force/displacement curves were plotted to determine knee stiffness, allowing the investigators to quantify translational kinematics in transaction specimens compared to normal controls. The stiffness of the linear portion of the force displacement curve was a designated surrogate for instability. The laxity encompassing the neutral zone (the "toe" region in the force displacement curve representing normal laxity surrounding the resting position of the ligament around zero displacement) was also quantified. Both the linear stiffness and neutral zone laxity were found to be directly related to the percentage of ACL transaction.

Two experimental groups of rabbits were compared to sham-operated control specimens [33]. Experimental groups had either a complete or partial transaction of their ACL. Partial transection specimens had approximately 50 % of their ACL transected. Sham controls had an arthrotomy but no ligament transection. Complete transection of the ACL decreased translational stiffness by 70 % and doubled the neutral zone laxity compared to sham controls (Fig. 9.5a). Partial transection did not affect neutral zone laxity and decreased translational stiffness 30 % compared to sham controls (Fig. 9.5a). Eight weeks after surgery, Mankin cartilage scores correlated closely with post-transection stiffness demonstrating a linear relationship between joint

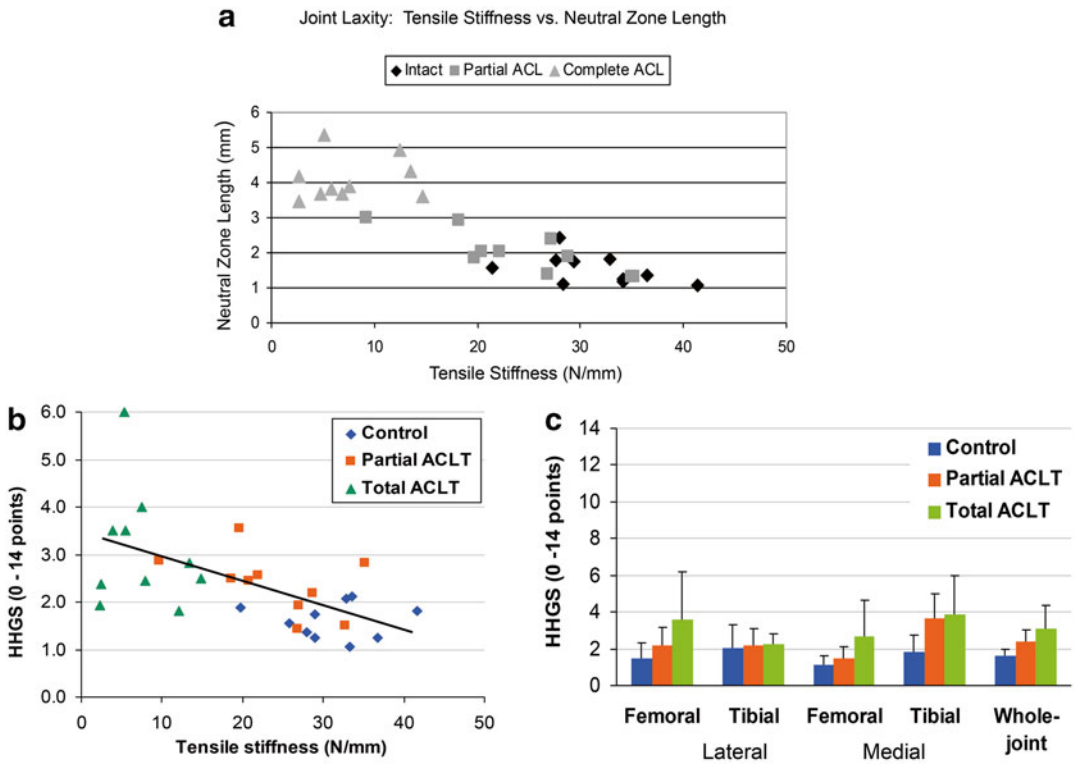

Fig. 9.5 Graded instability created in rabbit knees by progressive ACL sectioning decreased stiffness in both partial and completely transected specimens and increased neutral zone laxity in complete transaction specimens (**a**). PTOA correlated closely with resulting decreases in stiffness (**b**). Degenerative changes correlated were increased in partial and complete transaction specimens with significant differences measured between partial and complete transaction specimens (**c**)

stiffness and whole-joint degenerative changes (Fig. 9.5b). The greatest degenerative changes were encountered in the medial tibial plateau surface (Fig. 9.5c). This experiment clearly demonstrated that in vivo instability is directly related to cartilage degeneration.

A subsequent in vivo model of rabbit knee instability was developed to reduce the magnitude of experimentally induced trauma to the rabbit knee [34]. In this model a minimally invasive posterior arthrotomy was performed with rabbits positioned prone accessing the posterior knee joint. This allowed access to the posterior weight-bearing surface of the medial femoral condyle (the majority of load transfer in the rabbit knee occurs through the posterior part of the femoral condyle) and the posterior attachment of the medial meniscus. In this model, transection of the posterior horn of the medial meniscus resulted in nearly identical changes in medial knee load transfer compared to total medial meniscectomy (Fig. 9.6a, b). Animals were sacrificed at either 8 or 26 weeks after surgery. At 8 weeks, degenerative changes had occurred primarily in the medial tibial plateau (Fig. 9.6c). However, at 26 weeks, significant degeneration had progressed in the medial knee with increasing Mankin scores in both the medial tibial plateau and medial femoral condyle (Fig. 9.6c).

In a follow-up study, the investigators hypothesized that impact injury would potentiate instability-associated progression of articular surface degeneration [35]. In this study, the medial femoral condyle was impacted through the posterior arthrotomy at three different energy levels. The posterior horn medial meniscus destabilization technique was then applied to a group of experimental animals from each impact level.

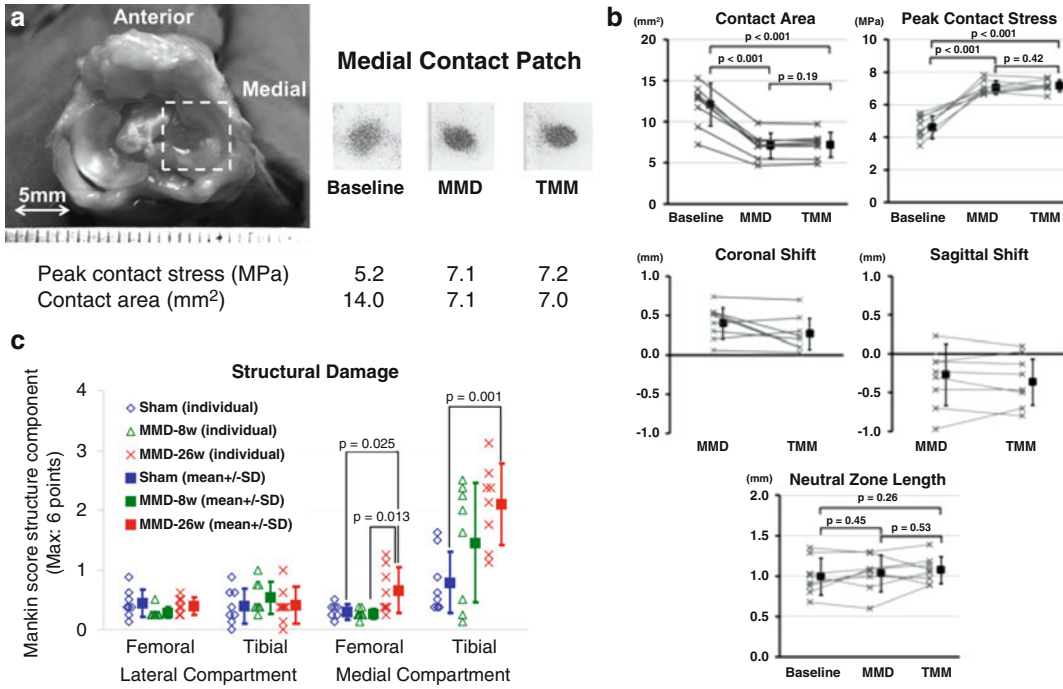

Fig. 9.6 A new in vivo model of instability can produce significant changes in loading area and peak stress with minimal joint invasion by releasing the posterior horn of the medial meniscus in the rabbit knee (**a**, **b**). This results in progressive degeneration, particularly concentrated in the medial knee compartment (**c**)

Histologic data confirmed that destabilizing the meniscus significantly exacerbated impact-related articular surface degeneration 8 weeks after injury. In addition, the degenerative changes progressed between 8 weeks and 26 weeks after injury.

Recently, gait analyses have been performed on both a rat model and sheep model of knee instability. Rats were subjected to division of their medial collateral ligament in concert with transaction of their medial meniscus. Gait analysis demonstrated significant shift of weight away from the affected limb which was progressive from days 9 to 24 after surgery. Follow-up analyses showed that joint degeneration and inflammatory cytokines were elevated in experimental joints [36]. Sheep knees were subjected to combination ACL/MCL transections. Twenty weeks after surgery, significant gait and knee kinematic abnormalities correlated closely with joint degeneration [37].

In summary, in vivo models of whole-joint degeneration resulting from instability have been created using small animal and large animal models. Preliminary data from these models have shown that instability reliably produces articular surface degeneration. The details of mechanotransduction of the abnormal joint mechanics secondary to instability into a physiologic and cellular response leading to PTA are unknown. In addition, therapies to mitigate hazardous mechanotransduction or improve the mechanics are untested. These models represent viable investigative tools to describe pathophysiologic cascades that link instability to PTOA and to survey treatments to mitigate PTOA in unstable joints.

Conclusions

Injuries resulting in chronically unstable joints predictably progress to PTOA, especially in the major weight-bearing joints. Therefore, it is important to understand the mechanical and biological effects of instability and to understand how instability results in pathologic mechanotransduction of unstable surface stresses into

degenerative biologic responses. Fortunately, recent improvements in analytical techniques have created new testing capabilities allowing investigators to model and investigate instability in a variety of settings. Accurate and validated real-time stress transducers have facilitated tests that have quantified relevant loads resulting from physiologically relevant whole-joint instability tests. These findings have highlighted the hazardous stress rates that occur during subluxation. Tissue explant experimentation has opened doors to quantify tissue-level biologic responses to a variety of loading environments including unstable loads. These experiments allow precise measurements of cartilage biologic response under unstable conditions but cannot account for living whole-joint responses to injury. Finally, investigators have developed techniques to quantify instability in survival models facilitating experiments that can account for living joint level biologic responses to unstable joints. Survival experiments have consistently de*m*onstrated instability-associated joint deterioration. These models will allow therapeutic interventions to be investigated.

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10 Survey of Animal Models in Post-Traumatic Arthritis: Choosing the Right Model to Answer the Right Question

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Selection of an animal model to investigate post-traumatic arthritis (PTA) is a challenging decision. The particular aspect of PTA development or intervention to be studied must be taken into account, along with practical issues of cost and technical expertise. The use of live animals for any scientific investigation must conform with guidelines for the humane treatment of each animal in every phase of the protocol, and the lowest species on the phylogenetic scale that is suitable for the proposed study must be selected [1]. A variety of animal models of PTA have been reported. Consideration of the benefits and limitations of a specific species should be considered when choosing an animal model. Small animal models of PTA include mouse, rats, and guinea pigs. Mid-sized animal models of PTA include rabbits and cats. Large animal models of PTA include dogs, sheep, goats, mini pigs, and pigs. Variations in outcomes for these animal models are reported in the literature. To highlight variations in the development of PTA, the chart below outlines various surgical and impact or trauma models for studying PTA for one representative species from each of these size categories of PTA models: mouse, rabbit, and dog (Fig. 10.1). Examples of considerations for each of these species include:

Mouse

Pros: low cost, availability of genetically modified models or inbred strains, and consistent genetic backgrounds, develop PTA in relatively short time frame.

Cons: Small size, limited quantities of tissue and biosample collection, surgical microscope and specialized instruments are often required, surgical variation may be higher between studies, some strains show different background levels of spontaneous OA; lack of consensus in the field as to whether osteoarthritis in the mouse is an appropriate representation of human OA.

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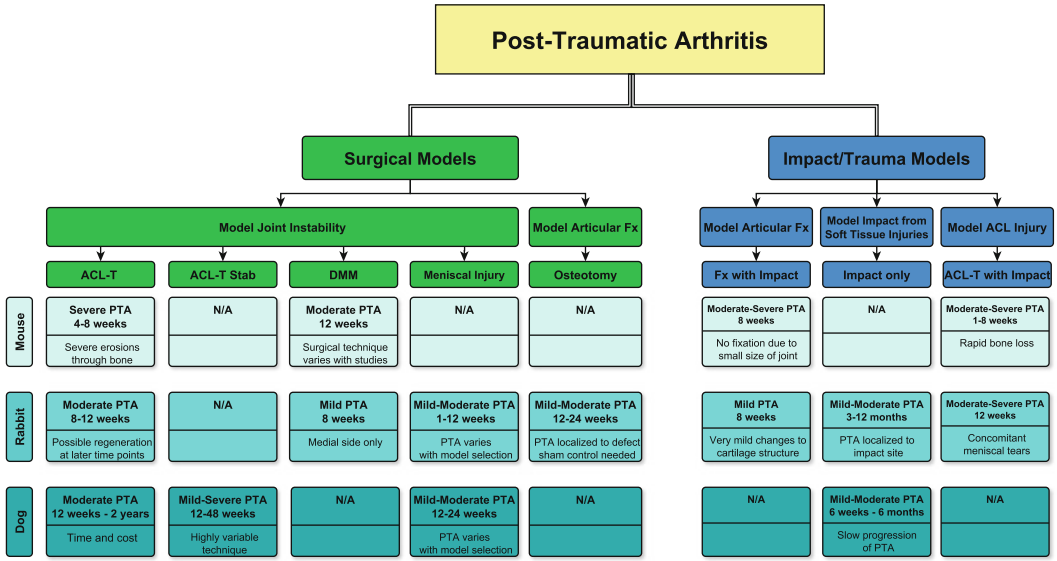

Fig. 10.1 Summary of outcomes for models of PTA for the mouse, rabbit, and dog. To demonstrate variability in the development of PTA for a small, mid-sized, and large animal model, the primary PTA pathology with the time to development is reported on *top* and limitations of the model are reported on *bottom* of outcome boxes. (*left*) Surgical (open joint) models of PTA can be characterized as joint instability models of arthritis development or articular fracture models that utilize an osteotomy to disrupt the articular surface. (*right*) Impact or trauma (closed joint) models of PTA can be divided into three categories: models of articular fracture which incorporate fracture induction with impact to the articular surface: models which only impact articular surface and mimic impact associated with soft tissue injuries, like ACL and meniscal tears; and impact, trauma models of ACL tears

Rabbit

Pros: relatively low cost, larger joint size, widely reported in literature, form lesions similar to clinically observed chondral defects.

Cons: Greater healing capacity relative to human joints, reach skeletal maturity at 10 months; unique gait biomechanics with preferential loading of lateral side of joint, not suitable for exercise studies.

Dog

Pros: suitable for longitudinal studies, large joint size, widely reported in literature, trainable for exercise studies, naturally occurring disease populations.

Cons: public perception, identified as companion animal, high cost, only limited reagents (e.g., antibodies) available, genetic variability between animals.

To highlight variations in the development of PTA, the specifics of the development of PTA in various joint tissues for the models outlined in the chart are detailed for the mouse, rabbit, and dog in the tables below (Tables 10.1, 10.2, and 10.3).

Table 10.1 Mouse

	Cartilage	Subchondral bone	Synovium
ACL-T [2, 3]	Cartilage lesions in posterior tibia	Subchondral bone erosions of the tibia through to the growth plate at 8 weeks	Increased cellularity and infiltration
ACL-T stab	N/A	N/A	N/A
DMM [3]	Degenerative changes in posterior femur and center of medial tibia; mild at 4 weeks and moderate to severe at 8–12 weeks	Sclerosis at 12 weeks	Moderate synovitis
Meniscal tear	N/A	N/A	N/A
Meniscectomy	N/A	N/A	N/A
Osteotomy	N/A	N/A	N/A
Fx with impact [4, 5]	Moderate to severe degenerative changes at fracture site and all articular surfaces	Sclerosis at 8 weeks	Severe acute synovitis with progression to mild synovitis
Impact only	N/A	N/A	N/A
ACL-T with impact [6]	Degenerative changes in both femoral condyles and medial tibia at 8 weeks	Rapid trabecular bone loss at 7–10 days	Acute synovitis at 7 days

Table 10.2 Dog

	Cartilage	Subchondral bone	Synovium
ACL-T [29–32]	Degeneration in medial compartment at 2 years; thickening at 3 years; focal cartilage loss at 45 months	Early trabecular bone changes at 3–12 weeks	Not reported
ACL-T stab [33–35]	Cartilage erosions at 12 weeks; cartilage thickening observed	Subchondral bone edema in tibia at 6 weeks	Acute synovial inflammation which resolved by 1 week
DMM	N/A	N/A	N/A
Meniscal tear [36–38]	Degenerative changes more prominent on tibial plateau at 1 year	Not reported	Synovitis at 4 weeks
Meniscectomy [39]	Medial side more severe. With partial resection, degenerative changes of tibial plateau at 3 months; with total resection, decrease in mechanical properties at 12 weeks; degenerative changes at 12–24 weeks at sites previously covered by meniscus	With partial resection, sclerosis at 3 months; not reported for full resection	With partial resection, synovial hyperplasia; with total resection of lateral meniscus, no synovitis reported at 3–6 months
Osteotomy	N/A	N/A	N/A
Fx with impact	N/A	N/A	N/A
Impact only (closed joint—patellofemoral) [39– 41]	Moderate loss of GAG staining at 6 weeks; deep clefts and loss of GAG at 6 months	No subchondral bone involvement at 6 weeks; new bone formation at 6 months	Not reported
ACL-T with impact	N/A	N/A	N/A

Table 10.3 Rabbit

	Cartilage	Subchondral bone	Synovium
ACL-T [7, 8]	Cartilage lesions at 6–12 weeks	Trabecular bone loss at 4–8 weeks; returned to baseline at 12 weeks	Not reported
ACL-T stab [9]	N/A	N/A	N/A
DMM [10]	Mild degenerative changes on medial tibia and medial femur at 8 weeks	Not reported	Not reported
Meniscal tear [11]	Degenerative changes in tibial plateau at 12 weeks and all surfaces at 9 months with longitudinal medial meniscus tear model	Not reported	Not reported
Meniscectomy [11–14]	Lateral side more severe and rapid degeneration with gross articular lesions at 3 weeks; medial side shows degeneration of tibial plateau at 8 weeks and posterior femoral condyles at 6–9 months	With partial resection, sclerosis of medial tibial plateau reported at 12 weeks; not reported for total resection	Not reported for partial resection; with total resection, synovitis at 2 weeks and fibrotic synovium at 12 weeks
Osteotomy [15–18]	Fibrocartilage and mild fibrillation with larger incongruity; successfully healed defects 2× cartilage thickness	Remodeled to adapt to surface incongruity	Synovial thickening
Fx with impact [19]	Early cartilage structural changes at 4 weeks; by 8 weeks cartilage appears normal with only mild changes	Fracture healed by 6 weeks	Pannus covering regions of articular cartilage
Impact only (closed joint—patellofemoral) [20–23]	Softening and swelling cartilage at impact site at 3 months; degenerative changes at 12 months	Sclerosis at 12 months	Not reported
Impact only (open joint—femoral condyle) [24–28]	Acute chondrocyte apoptosis; at impact site only, loss of GAG staining at 1 month and thinning of cartilage at 6 months	Increased bone formation at impact site at 1 month	Not reported
ACL-T with impact [9]	Severe degenerative changes	Not reported	Severe synovitis at 12 weeks

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Section III

Peri-articular Tissues Response to Joint Injury

The Response of Cartilage to Injury

11

Yang Wang and Alan J. Grodzinsky

Introduction

Traumatic joint injuries can result in damage to articular cartilage, subchondral bone, and nearby soft tissues including ligaments, tendons, menisci, the joint capsule, synovial membrane, and neural tissue. Such acute injuries are known to initiate a sequence of events that leads to a high rate of progression to post-traumatic osteoarthritis (PTOA) [1, 2]. In this chapter, we focus on cartilage injuries and their consequences: different levels of impact load can cause a variety of lesions to the articular surface and underlying cartilage tissue. Buckwalter [3] has classified cartilage injuries as including: (a) mechanical disruption of the articular surface and underlying subchondral bone (e.g., injuries associated with articular fractures [4]), (b) mechanical disruption of cartilage alone, and (c) chondral damage but with no visible disruption of the cartilage surface as assessed clinically by MRI or arthroscopy. Klocke et al. [5] reported that in many cases of knee injuries involving rupture of the ACL in the absence of overt chondral or osteochondral fracture, there is no visible evidence of cartilage injury at the time of knee arthroscopy. Nevertheless, biopsies of cartilage overlying MRI-detected bone bruises in ACL-injured knees have revealed important degradative changes including loss of proteoglycans and cell viability, even when there is no obvious macroscopic disruption of the collagen network (e.g., Fig. 11.1) [6]. It is not surprising, then, that animal studies and in vitro models have been critically important in elucidating the more subtle alterations in cartilage matrix and cell-mediated catabolic pathways that help to define the sequelae responsible for cartilage degradation associated with PTOA.

Macroscopically, four types of acute traumatic cartilage lesions were classified in a study of knee trauma [7] associated with shear or blunt compressive impact of cartilage. Tissue morphology ranged from stellate chondral fractures of cartilage to fissuring and milder fibrillation of the surface. Chondral lesions are common in the immature cartilage of children as well as in adolescents and adults and have been reported to be the most common injury to immature human knees [8].

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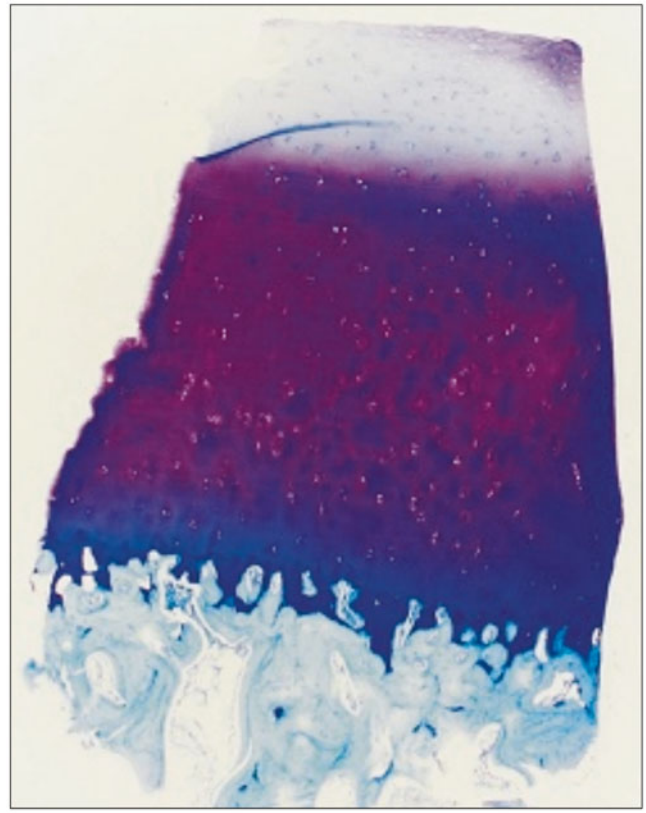

Fig. 11.1 Toluidine blue staining of a cartilage biopsy specimen overlying a bone bruise harvested from an ACL-injured patient before ACL reconstruction (from [6]). This specimen was histologically graded as an H-2 cartilage lesion (grading scale from H-0 (normal) to H-4 (moderate to severe fibrillation and complete loss to toluidine blue staining throughout the cartilage thickness)). Here, the articular surface is intact, but there is marked loss of matrix proteoglycans and noticeable cell death in the superficial zone

In a recent study of ACL rupture patients 18–35 years old [5] using quantitative MRI imaging at the time of surgical reconstruction, T1ρ relaxation measurements indicated an acute increase in water content of the matrix after blunt trauma, consistent with tissue swelling. In another study of ACL-injured patients with MRI taken within 8 weeks of injury just prior to reconstruction, the cartilage overlying a bone bruise in the lateral tibia showed T1ρ signal changes occurring immediately after injury and at 1-year follow-up [9]. Longitudinal MRI assessment of the central weight-bearing aspects of medial and lateral femoral cartilage via dGEMRIC [10] suggested that GAG content was lower in ACL-injured knees than in a normal healthy reference cohort, both at 3 weeks and 2.3 years after injury. At the same time, measurement of cartilage and bone markers in the synovial fluid in the acute phase of ACL injury with hemarthrosis showed clear local biochemical responses to trauma, including increases in proinflammatory cytokines IL-1β, IL-6, IL-8, and TNFα [11]. Increases in the levels of several of these cytokines were also reported in previous reports [12–14].

The timeline of degradative events to cartilage after traumatic joint injury has been very well summarized by Lotz and Kraus [1] (see Table 11.1). After the immediate consequences of injury, progressive degeneration of cartilage is hypothesized to be stimulated by a combination of biomechanical factors (e.g., kinematic gait changes [15] that may occur with or without reconstructive surgery [16]) as well as cell biological and inflammatory mediators [11, 17]. These processes involve further changes in cartilage extracellular matrix and concomitant alterations in tissue biomechanical and physicochemical properties. Taken together, these changes exacerbate the tissue's ability to withstand normal joint loading, and mechanobiological processes involving the remaining live chondrocytes cause a vicious cycle of loading-induced matrix catabolism and increased chondrocyte death within the hostile inflammatory

Table 11.1 Response of cartilage to injury (adapted from Lotz and Kraus 2010 [1])

Immediate (by 1–24 h)	Acute (1 day to weeks)	Chronic (years)
Cell necrosis	Apoptosis	Joint tissue remodeling
GAG loss	Inflammatory mediators	Inflammation
Collagen rupture	Leukocyte infiltration	Failed attempt at repair
Cartilage swelling	Continued GAG loss	Arthrofibrosis
Hemarthrosis	Matrix degradation	
	Deficient lubricants	

environment associated with injured synovium and other joint tissues. Several of these issues have been reviewed in detail previously [1, 2, 18, 19]. The following sections summarize recent literature and work in progress on studying matrix damage, altered biomechanical and transport properties of injured cartilage, chondrocyte viability, and the response of injured cartilage to inflammatory mediators and mechanical loading, which is relevant to rehabilitation post-injury.

Damage to Cartilage Extracellular Matrix

As described above, cartilage changes at the matrix level are most often thought to begin with the immediate release of aggrecan-containing GAG constituents, macromolecules that are directly related to both the compressive [20] and shear [21] moduli of cartilage. Rapid release of GAGs at low but significant levels from mechanically injured cartilage has been reported in many studies (as reviewed in [1, 22]). During the immediate time period following injury, this GAG release is associated with purely mechanical damage to the tissue, since inhibitors of aggrecanases and matrix metalloproteinases, as well as inhibitors of biosynthesis, do not abrogate this release [23]. Over subsequent days and months in vivo, however, the release of proteolytically generated aggrecan fragments becomes more significant; these fragments are found in synovial fluid samples from ACL-injured patients and therefore have the potential to become important molecular biomarkers of the progression of PTOA [24]. This process highlights the importance of the inflammatory component of the acute and follow-on stages of joint injury and has been simulated in studies in vitro involving coculture of mechanically injured cartilage with inflammatory cytokines [25] or coculture with injured joint capsule tissue [26] (highlighting both aggrecanase activity and transcription). The soft superficial zone of immature cartilage is particularly vulnerable to compressive injury, and loss of GAG occurs predominantly from the superficial zone [27]. Loss of GAG following a single compressive impact of adult bovine cartilage increases with the rate of loading [28].

Injury can cause immediate acute damage to the collagen network as well [28], including microdamage even in the absence of overt cracks or fractures. Collagen damage is thought to be an irreversible step in the progression to PTOA [29], and such deterioration is clearly seen in the loss of tensile behavior of the tissue [30]. In addition, Maroudas [31] showed that human OA cartilage having a fibrillated collagen network would swell significantly more than normal cartilage, even after loss of a substantial percentage of GAGs: the residual swelling pressure of the remaining GAGs could not be restrained by the fibrillated collagen network. Such swelling has been observed in vitro following injurious compression of explants [32, 33], and increased levels of denatured collagen neoepitopes [34, 35] are also seen, indicative of damage to the collagen network.

While matrix changes associated with loss of GAGs and damage to collagen have immediate effects on cartilage biomechanical properties, injury to cartilage can damage and/or release many other proteins as well. For example, quantitative mass spectrometry analyses [36] showed that cartilage explants subjected to a single injurious mechanical compression released over 500

proteins (intracellular and extracellular) to the medium. Such release could result from injury-induced increases in protein degradation or enhanced synthesis. Interestingly, many of the released extracellular proteins came from the pericellular matrix (PCM), and their release could be the result of higher turnover in the PCM or increased damage to the PCM with injury. Consistent with such findings in vitro, synovial fluid samples from patients after acute injury also reveal a variety of matrix proteins [11, 37]. The composition of lubricant molecules such as hyaluronan and proteoglycan 4 are also altered after injuries (see [38] for a review). The challenge in the search for biomarkers of cartilage injury remains to identify the tissue source of such fragments in order to understand whether they come from cartilage and/or other soft tissues or bone.

Altered Biomechanical, Physicochemical, and Transport Properties of Injured Cartilage

The effects of mechanical injury on the biomechanical and biophysical properties of cartilage tissue have been reviewed extensively in previous publications that summarize findings in animal models in vivo and in cartilage explant systems in vitro (e.g., [22, 39, 40]). Here, we focus on a few specific biomechanical and conceptual issues in the context of recent findings.

The response of cartilage to mechanical impact injury seen in animal studies is exemplified in the recent report of Borelli et al. [41], who used a pendulum device to deliver a *single* 3 mm-wide impact to the medial condyles of 3-month old rabbits, below the fracture threshold, but estimated to be at a high ~100 MPa peak stress. Creep indentation of condyle cartilage harvested immediately and at 1 and 6 months after impact revealed an immediate decrease in injured cartilage thickness (by ~40 %), a twofold increase in equilibrium creep strain, and a significantly impaired ability for the injured cartilage to recover its thickness after creep deformation [41]. Thus, a single high impact appeared to cause damage to the collagen network, thereby resulting in a dramatic loss of the tissue's poroelastic properties even in the absence of overt fracture. In a complementary study of the effects of *repetitive* abnormal injurious loading of glenoid cartilage in a rat model of rotator cuff tears, Reuther et al. [42] also reported a significant decrease in the thickness of anteroinferior region cartilage by 4 weeks. Using indentation, they performed stress relaxation tests and found a significant decrease in the equilibrium modulus calculated at 20 % indentation strain at several specified locations along the joint surface.

In general, studies in vivo can best mimic the effects of true joint anatomy and enable assessment of systemic processes, responses to injury-induced inflammatory mediators, and the effects of multi-tissue cross talk. However, variations associated with animal age, species, and geometry of loading can make it difficult to extrapolate certain findings to injury in humans [39]. Cartilage explant studies enable more precise control of injurious mechanical stimuli and the immediate cell biological environment, thereby enabling mechanistic studies of injury-induced matrix and cellular response pathways. In addition, it is possible to separate the direct effects of mechanical damage to cartilage, alone, from the combined effects of injurious loading and the subsequent presence of inflammatory mediators (simulated by adding cytokines and/or coculture with other joint tissues), which can synergistically degrade cartilage biomechanical properties with time in culture. Nevertheless, conclusions from in vitro studies must also be interpreted with caution, given the limitations of extrapolation to in vivo conditions.

For almost two decades, in vitro models have been developed to study the effects of acute mechanical trauma on articular cartilage. To help define the range of stresses, strains, and strain rates that initiate cartilage injury, Morel and Quinn [43] subjected adult bovine cartilage disks to unconfined compression at different strain rates (over 5 orders of magnitude) and peak stresses (between 3.5 and 14 MPa). The applied strain rates were above and below the tissue's intrinsic poroelastic relaxation time, $\tau \sim [\delta^2/(Hk)]$, for the tissue having equilibrium modulus H,

hydraulic permeability k, and characteristic tissue distance δ through which fluid flows. Strain rates below the relaxation time τ resulted in the highest final strains; cell viability was lost throughout the tissue depth but no cracks in the matrix occurred. In contrast, high strain rates resulted in impact-like surface cracking with cell death only near the tissue surface. Consistent with these findings, Kurz et al. found a dose-dependent decrease in compressive and shear stiffness and an increase in tissue swelling, with increasing strain rate [44].

It is well known that the biomechanical properties of articular cartilage vary substantially with age, species, and joint type [45, 46]; thus, it is not surprising that the tissue's resilience to mechanical impact injury also varies dramatically with these parameters. The soft superficial zone of immature bovine cartilage is especially vulnerable to compressive injury, causing superficial matrix disruption and extensive compaction which results in immediate and complete loss of biomechanical function compared to the deeper zone tissue of the same explants, which is much less affected [47]. In contrast, while the equilibrium modulus of the adult bovine tissue decreased in response to a "high"-impact injury, imparted by a drop tower to create grossly identifiable damage [48], the decreased stiffness was still ~55 % that of controls after 24 h, rather than the total loss of stiffness found in immature tissue [47]. In another drop tower-based blunt impact injury test using adult bovine osteochondral plugs, a decrease in creep strain in injured specimens versus control was observed by 7 days after injury, though no immediate decrease was found [49].

Micromechanical damage to cartilage matrix induced by injurious loading may also affect the transport of potential therapeutic drugs and diagnostic contrast agents into and at the surface of cartilage tissue. For example, Moeini et al. [50] observed that fluorescently tagged macromolecules showing potential for detection of surface injuries displayed decreased adsorption onto cracked surfaces of mechanically injured cartilage. These properties might aid in detecting microdamage or biochemical changes at the surface. Byun et al. found that 48 kDa anti-IL-6 Fab fragments (examined as a potential therapeutic) took over 3 days to diffuse into immature bovine and adult human femoropatellar groove cartilage explants; however, their uptake into these same explants increased significantly following compressive mechanical injury [51]. Bajpayee et al. used 66 kDa avidin protein as a model for charge-driven nanoparticle transport into cartilage and drug delivery for treating early stage post-traumatic osteoarthritis [52]. The high positive charge of avidin resulted in an uptake into normal young bovine cartilage that was 400-fold higher than that of its electrically neutral, same-sized counterpart, NeutrAvidin. Even after 40 % GAG depletion of explants to mimic early stages of PTOA cartilage degradation (i.e., similar to Fig. 11.1), avidin uptake ratio was still as high as 24 [52]. When injected intra-articularly in rats, avidin was able to penetrate the full thickness of articular cartilage within 6 h and was retained for 7 days, with a half-life of 29 h in rat cartilage [53]. The potential application of such approaches for new modes of intra-articular therapy is reviewed by Evans et al. [54].

Finally, there is still no routine, widely used approach for real-time measurement of the biomechanical properties of human cartilage during clinical examination post-injury. A recent pilot study using a handheld indentation instrument [55] was conducted to map the biomechanical properties of normal human cartilage in vivo. While the authors concluded that their approach would enable comparison measures of suspected degenerative cartilage, the instrument used could only measure force and displacement and, therefore, intrinsic material moduli could not be assessed. Kiviranta et al. [56] improved upon this instrument by incorporating ultrasound reflection measurements along with indentation; ultrasound enabled assessment of original tissue thickness from which stress, strain, and tissue dynamic modulus could be computed, as demonstrated using ex vivo human cadaver patellae.

Another recently developed instrument with the potential for post-injury arthroscopic assessment of cartilage is a streaming potential-based device [57] which was reported to be more sensitive to impact-related cartilage changes than direct

biomechanical measurement. A further evolution of the use of deformation-induced streaming potentials for cartilage assessment post-injury is a completely noninvasive approach [58] in which electrodes at the surface of the knee are shown to detect streaming potentials emanating from cartilage when patients shift body weight from one leg to the other in a controlled manner. Finally, ex vivo measurements of the speed of sound (SOS) in human and porcine cartilage specimens using MRI combined with ultrasound [59] show the potential of another new noninvasive diagnostic tool, since SOS can provide an index of tissue elasticity.

Cell Viability and Cartilage Injury

Impact injury of cartilage can cause immediate cell necrosis, especially in the softer superficial zone, followed over the next days and weeks by apoptosis of neighboring populations of chondrocytes, as reviewed previously [1, 2]. In vitro studies have enabled quantitation of the mechanical loading parameters [30, 33, 60–62] and the effects of tissue maturity [63, 64] that are associated with cell death. For example, the incubator-housed instrument shown in Fig. 11.2 can apply computer-controlled compressive loads or displacements to individual or multiple geometrically defined cartilage explant disks held in specially designed autoclavable loading chambers that are mounted within the instrument [65]. Using such instruments or related load frames, investigators discovered that mechanical injury to isolated cartilage explants could lead specifically to apoptotic cell death [33, 60], with cell viability further compromised by the presence of inflammatory cytokines such as TNFα or IL-1 [66] as occurs in joint injury in vivo.

Recent studies have probed various cell- and matrix-associated mechanisms by which injurious loading may cause chondrocyte death, including injury-induced reactive oxygen species and oxidative stress [63, 67] and mitochondrial transport as a source of oxidants [68]. Imgenberg et al. [69] found that estrogen reduces mechanical injury-induced apoptosis and GAG loss in adult bovine explants. Jang et al. [70] showed that loading-induced cell death could be initiated by strain on cell adhesion receptors involving integrin-cytoskeletal interactions. Caramés et al. studied the role of autophagy, a process for turnover of intracellular organelles and molecules that protects cells during stress response [71]. They discovered that mechanical injury can suppress autophagy regulators, and pharmacological activation of autophagy, e.g., using rapamycin, can prevent cell death and GAG loss in mechanically injured explants [66].

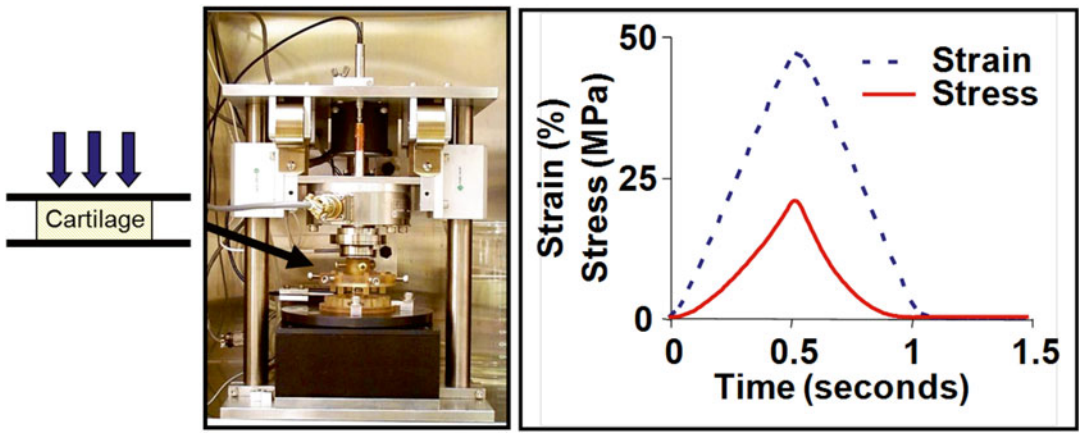

Fig. 11.2 An apparatus (from [65]) for applying injurious mechanical compression to cartilage explant disks. Graph shows a *triangle wave* of displacement applied to a cartilage explant, reaching 50 % compression in 0.5 s, resulting in a measured peak stress of ~20 MPa (from [80])

Clinical detection of intratissue cell death soon after traumatic joint injury could aid in treatment decisions but is technically challenging; however, new approaches are on the horizon. Using equine osteochondral blocks ex vivo, Novakofski et al. showed that quantitative multiphoton microscopy can detect cell death in situ in live tissue, with clinical potential for detection of early cartilage damage [72]. In addition, Rolauffs et al. [73, 74] identified a distinct spatial reorganization of superficial human chondrocytes associated with proliferative remodeling in response to early OA lesions; these cell patterns also have potential diagnostic utility. Both approaches above have the potential to be configured for arthroscopic examination.

Cartilage Changes Post-injury in an Inflammatory Environment

Cartilage degeneration in PTOA is driven by the entire synovial joint; however, chondrocytes can play a primary role when stimulated to increase local production of matrix-degrading proteases, downregulate the synthesis of ECM molecules, and produce inflammatory mediators. In response to joint injury or cytokine stimulation, latent or newly secreted aggrecanases produced by multiple joint tissues may rapidly degrade aggrecan, significantly altering the mechanical properties of the tissue. Irreversible protease-induced collagen degradation often occurs after aggrecan depletion, suggesting that aggrecan may protect collagen fibrils from proteolytic degradation [75]. The array of known matrix proteases can also degrade many other ECM macromolecules that are responsible for homeostatic assembly and remodeling of cartilage matrix [76].

Preclinical animal models are playing an increasingly important role in the study of mechanistic pathways that regulate cartilage degradation and attempts at repair in a post-injury inflammatory environment. Over 135 strains of genetically engineered mice are now available to explore various aspects of OA, and both surgical and mechanical methods for inducing joint injury in mice can be used [77]. Animal models can highlight the importance of inflammation and multi-tissue interactions within an injured joint. For example, a recent study of ACL transection in minipigs demonstrated that MMP-13 and ADAMTS-4 were highly upregulated in the synovium and ligament and could thereby affect cartilage matrix [78]. Similarly, in an ovine model of simulated ACL reconstructive surgery, changes associated with early PTOA were associated with acute post-injury synovial inflammation [79].

At the same time, quantitative cell biological and mechanobiological pathways can be difficult to delineate in animal models, and complementary in vitro studies can add powerful approaches to the understanding of these mechanisms. Injurious compression of cartilage explants alone, even in the absence of other joint tissues, significantly increases chondrocyte gene expression levels of MMP-1, MMP-3, MMP-13, and ADAMTS-5 within 24 h after injury [80]. Cartilage explantation also activates intracellular inflammatory signaling pathways (e.g., p38, JNK, and ERK) and induces mRNA expression of IL-1α and IL-1β [81]. Biologically active IL-1 proteins were also detected in cartilage lysates [81]. In a microarray analysis of cartilage explants subjected to recutting, strong upregulation of the Wnt-16 gene was detected [82]. Wnt pathways play an important role in chondrocyte differentiation [83] and dysregulation of Wnt pathways in adult tissues could contribute to chondrocyte hypertrophy seen in OA [84]. Taken together, these results suggest that impact injury of cartilage, alone, leads to dysregulation of chondrocyte metabolism and kick-starts catabolic and inflammatory processes in cartilage.

Anabolic activities are also upregulated in cartilage subjected to mechanical injury. Gene expression levels of TIMP-1 increased by 12-fold within 24 h of explant impact injury, suggesting attempts at early repair by inhibiting matrix proteinases; in addition, activation of BMP and FGF pathways was also shown in cartilage following mechanical injury [85, 86], which may play a role in reparative responses of cartilage to injury. These increases in anabolism appear to be attempts to offset the catabolic

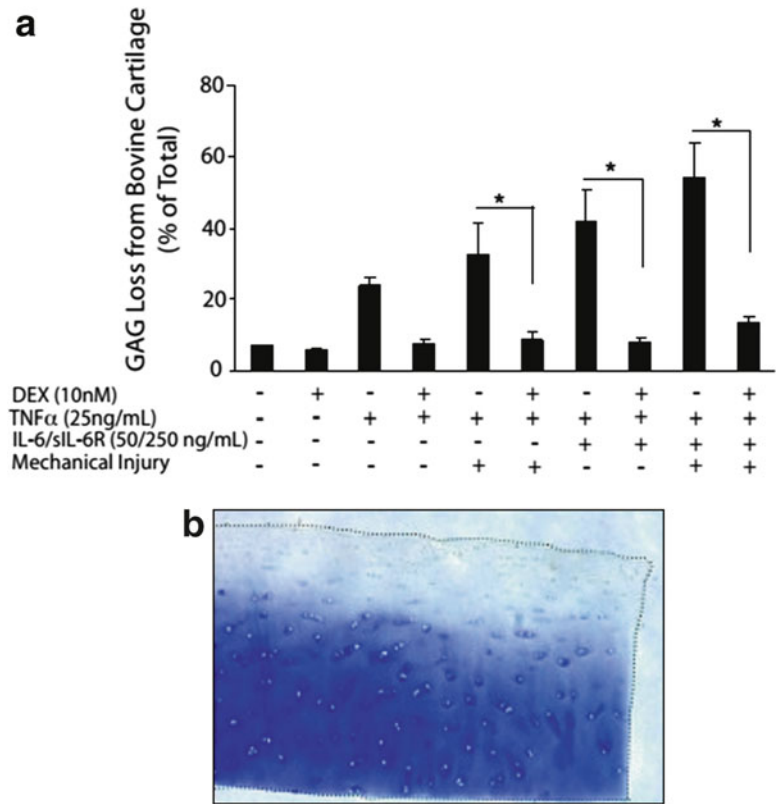

Fig. 11.3 (**a**) Effects of dexamethasone on sGAG loss in young bovine cartilage treated with combinations of mechanical injury, TNFα, and IL-6/sIL-6R for 6 days. The injury was applied using the waveform of Fig. 11.2. Dex abrogated sGAG loss caused by injury plus cytokine treatment in this system (from [95]). Similar trends were found with adult human knee cartilage explants. (**b**) Toluidine blue staining of 0.8-mm disks of human knee cartilage with intact superficial zone treated with injury plus TNFα plus IL-6/sIL-6R over 4 days (from [25]); the spatial profile of GAG loss and histological appearance of the tissue is similar to that of the human clinical biopsy specimen of Fig. 11.1

effects of MMPs, aggrecanases, and catabolic cytokines and chemokines. However, attempts to increase matrix synthesis are compromised by the presence of inflammatory responses in the synovial joint.

Traumatic joint injury rarely involves disruption of cartilage surface alone and may include bone bruises, rupture of ligaments and menisci, and lesions in the joint capsule and synovium. In vitro models have been developed to include aspects of these multi-tissue injuries with associated inflammation. These models are based on the knowledge that elevated levels of IL-1β, TNFα, IL-6, IL-8, and other inflammatory mediators are found in synovial fluid of patients with acute knee injuries [11], and concentrations of such mediators correlate with severity of damage [87]. Among the proinflammatory cytokines, IL-1 and TNFα are considered the major players. Several studies have shown that IL-1 and TNFα promote cartilage matrix degradation by inducing expression of extracellular matrix-degrading enzymes (MMP-1, MMP-3, MMP-13, ADAMTS-5) [88], inhibiting collagen and aggrecan synthesis [89], inhibiting anabolic activity of growth factors, and inducing production of IL-6 [90] and chemokines such as IL-8 [91]. The role of inflammatory cytokines in the pathogenesis of OA has been reviewed in more detail by Kapoor et al. [92].

Based on this knowledge, in vitro models have incorporated coculture of mechanically injured immature bovine and adult human cartilage explants with specific inflammatory cytokines such as IL-1 alone [93], TNFα alone [93], or the combination of TNFα, IL-6, and the IL-6 soluble receptor sIL-6R [25]. (The latter choice was motivated by the previous finding that IL-6 with its soluble receptor augments proteoglycan catabolism from cartilage [94].) These studies showed that mechanical injury potentiates aggrecan catabolism induced by inflammatory cytokines (Fig. 11.3). The value of such in vitro models for use in the discovery of potential therapeutics has

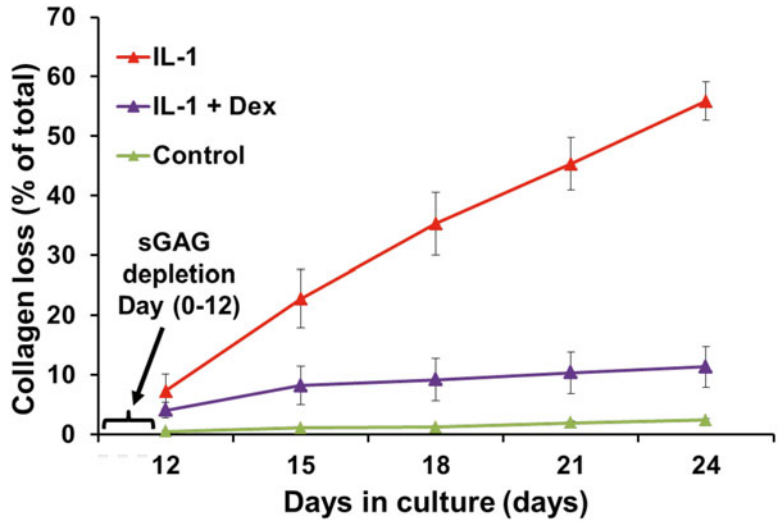

Fig. 11.4 Bovine cartilage explants were treated with 1 ng/ml IL-1 continuously for 24 days. After almost complete loss of sGAG by day 12, dexamethasone was added to the cultures, and collagen loss was measured. Dexamethasone was able to prevent proteolytic degradation and loss of collagen (from [96])

been demonstrated by the subsequent discovery that dexamethasone (Dex) essentially blocks the catabolic effects of injury plus cytokine treatment on GAG loss (Fig. 11.3a) [95] while preserving cell viability and metabolism. Even after incubation of explants with IL-1, which caused almost total GAG loss by 12 days, addition of Dex could completely inhibit collagen degradation (Fig. 11.4) [96].

In parallel complementary studies, coculture of normal or mechanically injured cartilage explants with injured (explanted) joint capsule specimens has been used to test the broader hypothesis that multiple factors from the synovium could induce catabolic pathways within neighboring cartilage. Patwari et al. [97] observed that coincubation of human joint capsule tissue with normal human knee cartilage explants inhibited chondrocyte biosynthesis through an IL-1-independent signaling pathway. Using this model, Lee et al. [26] studied the temporal evolution of 21 genes (by qPCR) in normal or injured cartilage cocultured with joint capsule explants; clustering analyses enabled identification of co-expression profiles for genes associated with injury alone, coculture alone, or injury plus coculture. While MMP-13 and ADAMTS-4 clustered with the effects of coculture, ADAMTS-5 expression and activity (by immunohistochemistry) clustered with injury and injury plus coculture.

Response of Injured Cartilage to Further Loading: Relevance to Rehabilitation

It is not well understood how loading of the joint after traumatic injury, with or without surgical reconstruction, affects chondrocyte metabolism and the anabolic-catabolic balance within the joint. In vitro explant models have been used to investigate the response of cartilage to intermittent dynamic loading after an injurious episode. In one study, it was shown that loading after injury decreased matrix biosynthesis in a strain rate-dependent manner [44]. In another study, it was postulated that a threshold dynamic strain amplitude exists above which loading becomes detrimental to cartilage [98]. Low strain amplitudes helped cartilage to recover from treatment with mechanical injury plus cytokines (TNFα, IL-6, and sIL-6R) by reversing sGAG loss, aggrecanase activity, and chondrocyte apoptosis. However, at dynamic strain amplitudes above a threshold, cell apoptosis increased and ADAMTS-5 and COX-2 gene expression were upregulated, suggesting a further catabolic response to high-amplitude loading. Taken together, these studies suggest that moderate dynamic compression can be an anabolic and potentially reparative stimulus for cartilage remodeling, while high dynamic compression has a further catabolic effect on cartilage post-injury [98].

Clinical studies utilizing combined dual fluoroscopic and MR imaging showed that ACL injury alters in vivo cartilage contact biomechanics by shifting the contact location to smaller regions of thinner cartilage and by increasing the magnitude of cartilage contact deformation [99]. In addition, while surgical reconstruction of ACL-injured knees restored some of the in vivo cartilage contact biomechanics, the increased cartilage contact deformation caused by initial ACL rupture was not reduced at lower flexion angles [16]. In an MRI study of ACL-reconstructed patients at 6 months after surgery, Van Ginckel and colleagues found a trend toward increased cartilage deformation and diminished cartilage function in patients returning to sports before 5 months after ACL reconstruction [100]. A review of longitudinal MRI studies to examine cartilage adaptation after ACL injury and reconstruction concluded that moderate evidences exist for persistent altered cartilage biomechanics to possibly influence the rate of cartilage change after ACL reconstruction [101]. Given these findings, the question still remains as to the optimal rehabilitation techniques in mediating cartilage remodeling after injury, and further research is warranted.

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The Response of the Subchondral Bone to Injury

12

Steven R. Goldring

Abbreviations

ACL	Anterior cruciate ligament
MRI	Magnetic resonance imaging
OA	Osteoarthritis
PTOA	Post-traumatic osteoarthritis
VEGF	Vascular endothelial cell growth factor

Structural Organization of the Periarticular Bone

Although this chapter will focus on the effects of injury on the subchondral bone, it is important to appreciate the complex structure and organization of the entire periarticular bone organ that provides structural stability and support to the other tissues of the joint and exhibits a remarkable capacity to remodel and adapt its structural and functional properties [1, 2]. The bone beneath the articular surface (Fig. 12.1) is organized into a plate-like structure comprised of compact cortical bone. Beneath the subchondral plate, the bone is organized into a cancellous network of trabecular bone. The bone at the joint margins, which is intimately associated with the synovium and adjacent entheseal structures where tendons and ligaments insert, is comprised of compact bone lined by the periosteum. The resident cells that remodel the cortical and trabecular bone are similar, but the rate and adaptive capacity of the cortical and trabecular bone exhibit differential capacities. For example, the remodeling of the subchondral cortical bone in physiologic and pathological states tends to occur more slowly than the adaptive changes in the underlying cancellous network of trabecular bone, which is surrounded by bone marrow cells and can rapidly remodel and change the shape and organization of the trabecular bone. A final structural component of the subchondral compartment is a zone of calcified cartilage that separates the overlying articular cartilage from the underlying subchondral bone. The interface between the articular and calcified cartilage can be identified by the so-called tidemark that can be distinguished based on its enhanced metachromatic staining pattern. As will be discussed in the subsequent section, the calcified cartilage is susceptible to mechanical injury and similar to the other mineralized subchondral tissues undergoes unique structural changes in response to injury.

Under physiological conditions, the subchondral bone adapts its structural and functional properties through a highly regulated cellular process involving distinct bone cell populations. The remodeling process involves the recruitment of myeloid lineage cells to the bone surface followed

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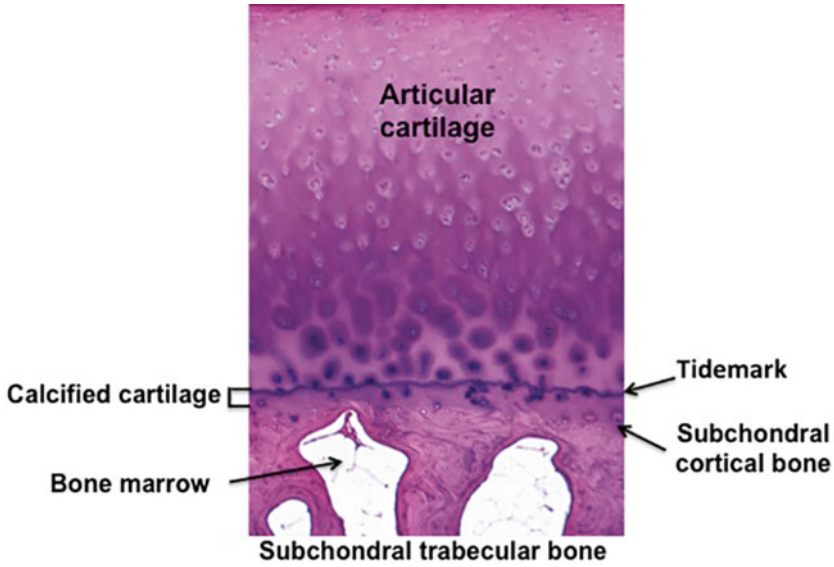

Fig. 12.1 Histologic cross section of a normal knee joint (Courtesy of Edward DiCarlo, MD, Hospital for Special Surgery, New York, NY)

by differentiation of these cells into osteoclasts that are uniquely adapted to removal of the mineralized bone matrix. Multiple lines of evidence have established that the osteoclast is required for resorption of the bone under physiological conditions and also is the principal cell type that mediates bone resorption in pathological conditions [3–5]. The phase of bone resorption is followed by a reversal phase in which the resorbed bone surface is populated by bone-forming osteoblasts that replace the resorbed bone. The remodeling process occurs throughout postnatal life and provides a cellular system for adapting the bone to biomechanical influences, repairing damage to the bone matrix, and, under certain conditions, releasing calcium for maintenance of mineral ion homeostasis [6]. In addition to the osteoclast and osteoblast, there is a third cell type in the bone, the osteocyte [7, 8]. Osteocytes are embedded within the bone matrix where they form an interconnected network with each other and with the cells on the bone surface. They play a critical role in regulating bone remodeling in response to local soluble mediators and systemic hormones and importantly are the principal regulator of the response of the bone to alterations in mechanical loading [9]. The unique capacity of the bone to adapt to its local mechanical environment is embodied in Wolff's hypothesis that states that the distribution and material properties of the bone are determined by the magnitude and direction of the applied load [10]. In this paradigm, the presence of increased bone volume is a reflection of increased load transfer and decreases in bone mass conversely reflect a relative decrease in the local loading history. The observed changes in the subchondral bone that accompany the adverse effects of excessive loading and injury will be described in the subsequent sections.

Bone Pathology in OA

Anatomic and histopathological studies have provided a comprehensive overview of the characteristic periarticular changes in human subjects with established OA. The changes include the presence of increased cortical plate thickness, decreased subchondral trabecular bone mass with localized regions of increased horizontal trabeculae, the presence of bone cysts, flattening and deformation of the subchondral articular contour, and osteophyte formation at the joint margins [1, 11–15]. The term "attrition"

has been used to describe the flattening and change in the subchondral articular contour and reflects the influence of local biomechanical influences on bone remodeling [16–18].

In addition to the role of mechanical factors, bone remodeling also may be initiated at sites of local bone damage, which results from mechanical loading that is sufficient to produce disruption of the integrity of the skeletal architecture at a microscopic level, so-called microdamage. Damage of this type may occur with a distinct episode of excessive loading associated with joint injury or may occur in the context of repetitive loading in an individual engaged in repetitive physical activity. This form of microdamage is associated with the appearance of microcracks in the bone architecture [1, 10, 19, 20] and is distinct from traumatic bone injuries associated with fractures that disrupt the gross bone architecture. The process of the so-called targeted remodeling provides a cellular mechanism for repairing the focal bone damage but under certain conditions may contribute to the formation of bone cysts that represent one of the radiographic and anatomic hallmarks of OA [2]. As will be discussed, targeted remodeling associated with bone microdamage likely accounts for the bone marrow lesions observed with MRI in patients with established OA.

Although not well visualized using standard radiographic techniques, the zone of calcified cartilage also undergoes marked alterations in its cellular composition and organization during the evolution of the osteoarthritic process. These changes include expansion of the calcified cartilage and advancement and replacement of the matrix of the overlying articular cartilage associated with duplication of the tidemark [1, 11, 19]. This expansion of the calcified cartilage is associated with the penetration by vascular elements that extend from the subchondral bone and adjacent marrow spaces recapitulating the vascular invasion of the growth plate that occurs during the development and growth of long bones [21–24]. Figure 12.2 depicts the histopathological features of the periarticular bone with expansion of the zone of calcified cartilage, tidemark duplication, and vascular invasion of the subchondral cortical bone.

The mechanical properties of the subchondral bone are influenced by the organization and composition of the organic bone matrix and the mineral content, which are highly dependent on rate of bone remodeling [25–27]. In physiologic remodeling, bone formation is initiated by the deposition of the organic bone matrix (osteoid), which undergoes rapid mineralization. After this initial phase there is a late phase of mineral accretion, which markedly influences the material properties of the bone matrix. In states of high bone turnover, the "late" phase of mineral accretion is attenuated by the rapid remodeling process, leading to a state of relative hypomineralization. This is associated with a reduction in the elasticity modulus of the bone that is more easily deformed under load. In contrast, in low bone turnover states, the continued deposition of mineral leads to an increase in the elastic modulus, and the bone becomes resistant to deformation and more "brittle." During the progression of OA, marked changes occur in the rate and extent of remodeling in the subchondral bone, and these changes in bone turnover affect the state of mineralization and modify the capacity of the bone to deform under load, which alters the susceptibility to both micro- and macrodamage [11, 25, 28].

Post-Traumatic OA: Response of the Bone to Injury

Epidemiological studies have helped to identify the role of joint injury in the pathogenesis of OA. Although it is challenging to make precise estimates, long-term studies from patients with knee ligament and meniscus injuries demonstrate a tenfold risk of OA compared to those without injuries, and the numbers are much higher for individuals who have sustained intra-articular fractures [29]. The study of individuals with OA associated with a specific joint injury has been informative in establishing the evolution of the sequential joint pathology. A retrospective analysis in patients with anterior cruciate ligament (ACL) rupture reveals that 60–90 % of the patients exhibit radiographic features of OA within 10–15 years, including both cartilage and

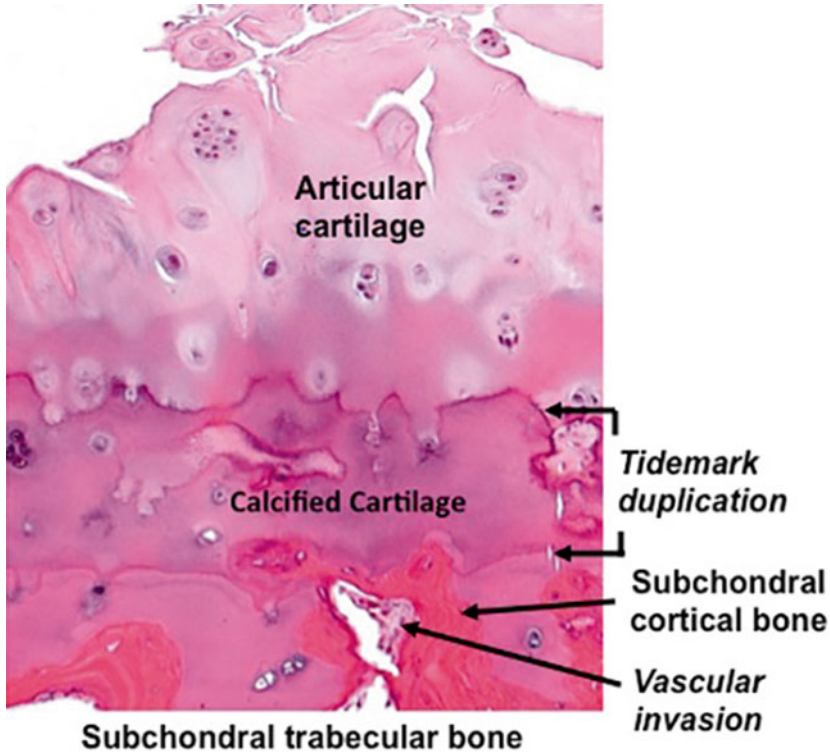

Fig. 12.2 Subchondral bone changes associated with advanced osteoarthritis. There is advancement of the calcified cartilage into the lower zones of the articular cartilage with duplication of the tidemark and vascular invasion of the subchondral cortical bone (Courtesy of Edward DiCarlo, MD, Hospital for Special Surgery, New York, NY)

bone pathologies [30–32]. When the ACL injury is accompanied by injuries to the collateral ligament and menisci, there is evidence of more rapid appearance and progression of the OA changes, indicating that the magnitude of the injury and the resulting alterations in joint mechanics play a contributory role to the more unfavorable natural history [31, 32]. Buckland-Wright and coworkers [33] performed a cross-sectional study of patients with anterior cruciate ligament rupture to define the sequence of periarticular bone changes. Their analysis revealed the development of progressive thickening of the subchondral horizontal trabeculae within three to four years after the injury. They detected osteophytes in approximately 50 % of the injured knees by the third year but did not observe a change in joint space width or cortical plate thickness. They noted that these findings contrast with the changes in the subchondral bone observed in patients with knee OA not associated with a discrete injury in which the increased thickness in the subchondral bone plate appears to antedate the alterations in the trabecular bone. Although they speculated that the differential patterns could be related to differences in the biomechanical and adaptive responses, they acknowledged that differences in the two groups could reflect the relatively short duration of the study in the patients with joint injury. It would be predicted that with the passage of time, the subchondral and periarticular bone changes in patients with or without traumatic joint injuries would exhibit similar features, since factors such as joint instability and mechanical overload are major contributory factors to OA progression in both conditions.

Further insights into the natural history of post-traumatic periarticular bone changes have been provided through the study of animal models in which it is possible to control the many

variables that impact on the evolution of the bone changes. Multiple species have been investigated in these studies and a variety of models exist based on the site and type of the tissue injury or disruption. However, the reported findings with respect to the subchondral bone are inconsistent and in some instances conflicting results have been obtained even among the studies using the same animal model. Nevertheless, these studies have been informative, and it is possible to draw several general conclusions from the results. Kuroki and Cook [34] utilized three different canine models of OA to define the evolution of the subchondral bone changes after joint injury. OA was initiated in one knee of mongrel dogs either by ACL transection, medial femoral grove creation, or medial meniscal release, and the knee joints were examined 12 weeks after surgery by histology and histomorphometry. A matching group of dogs underwent sham surgery and the non-operated knee in each of the groups served as an additional control. OA articular pathology assessed using the Mankin scores was present in all of the treatment groups. A significant decrease in subchondral bone plate thickness, trabecular thickness, and trabecular bone volume fraction compared to the sham-operated controls was observed in the ACL transection group. These findings were similar to those reported by others using this canine model [35–37]. Thinning of the subchondral plate was also observed in the groove model. In contrast, they observed significant thickening of the subchondral plate in the medial meniscus release group. A similar increase in the subchondral plate thickness after medial meniscectomy has been reported in a rabbit [38] and mouse model [39]. In the canine model study by Kuroki and Cook [34], the knee joints from the dogs in the meniscectomy group showed the most severe cartilage damage compared to the other groups, and the authors speculated that, although there may have been an early decrease in cortical plate thickness, as the OA progressed, the subchondral plate underwent a progressive adaptive increase in thickness. An important observation in these studies is that there was a good correlation between the changes in cortical plate thickness and the cartilage pathology in all of the models, which supports multiple other lines of evidence that both the bone and cartilage undergo cellular and morphological adaptation to local mechanical factors associated with the alterations in joint mechanics and loading induced by the initial injury.

A general feature of many of the animal models of PTOA is that they produce a significant injury to the joint tissues related to the surgical procedure. This may lead to alterations in the subsequent pattern of weight bearing and activity that could have a major influence on the subchondral bone adaptation. In the studies by Kuroki and Cook [34], the dogs that had undergone ACL transection still exhibited evidence of lameness, and they observed that the lameness scores correlated with the decreased bone volume and subchondral cortical bone thickness. In previous studies by the authors [40], they observed that lameness scores correlated with force plate patterns of loading, indicating the importance of the joint surgery on the subsequent pattern of physical activity and joint loading, which would be predicted to have significant effects on bone adaptation.

Lacourt et al. [41] utilized a model of repetitive impact-induced injury to examine the relationship between joint trauma and OA pathogenesis. They conducted a postmortem exam of the third carpal cuboidal bone in a group of fifteen racehorses that were exposed to repetitive trauma-induced OA at this skeletal site. Multiple analytic techniques revealed the presence of subchondral bone pits associated with articular cartilage damage manifested by fibrillation, fissuring, erosion, ulceration, and loss of proteoglycan. Histological analysis revealed extensive microcracks in the calcified cartilage and in localized regions of the subchondral bone plate. There were extensive areas of vertically oriented resorptive remodeling in the cancellous bone beneath many of the pits. A micro-CT analysis revealed reduced bone density, bone volume, and trabecular thickness with greater trabecular spacing in these regions. The authors speculated that the repetitive compressive forces and cumulative microdamage were responsible for the induction of remodeling changes that produced the alterations in bone architecture.

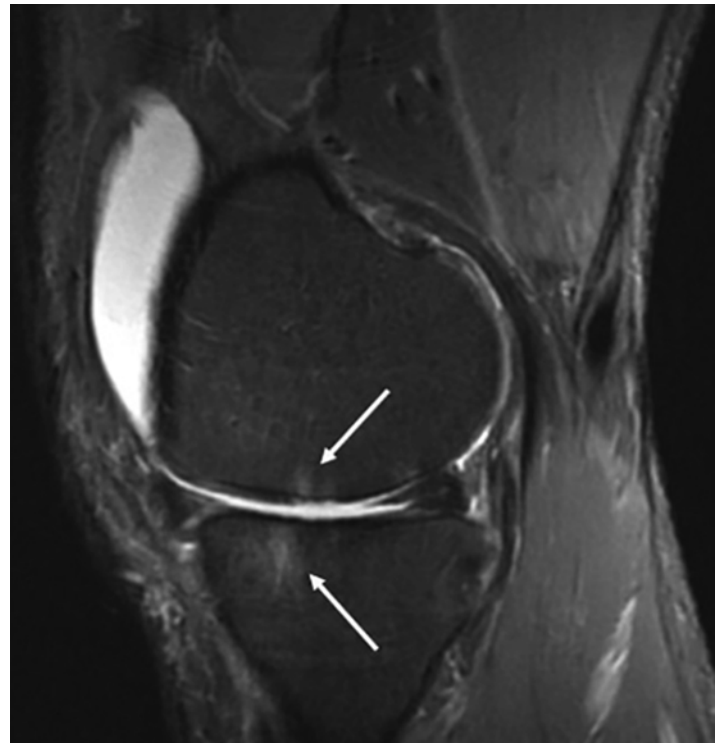

Fig. 12.3 MRI using fat-suppressed T2-weighted fast spin-echo (STIR) sequence demonstrating the presence of bone marrow lesions in the distal femur and proximal tibia in a patient with OA (Courtesy of Hollis Potter, MD, Hospital for Special Surgery, New York, NY)

The presence of active bone remodeling topographically associated with microdamage in the calcified cartilage has been described in both human and equine OA [42–45]. These changes are often accompanied by advancement of the zone of calcified cartilage into the overlying hyaline articular cartilage and duplication of the tidemark [19, 20, 46, 47] (Fig. 12.2). Histologic examination of this zone reveals the presence of penetration of the calcified cartilage by vascular elements and formation of new calcified cartilage and bone, recapitulating the morphological features of the growth plate. Walsh and coworkers [22–24, 48] have examined the osteochondral junction in patients undergoing arthroplasty for end-stage OA using immunostaining techniques and noted the presence of sensory nerve fibers expressing nerve growth factor in the vascular channels associated with osteochondral angiogenesis. They hypothesized that the sensory fibers in these regions could be a potential source of symptomatic pain. The regions of vascular invasion also were associated with localized replacement of the bone marrow by fibrovascular tissue with cells expressing vascular endothelial factor (VEGF). VEGF also was detected in chondrocytes that were in proximity to the new blood vessels, and the authors speculated that the VEGF could provide signals for recruitment of the vascular elements.

An additional feature of OA is the detection of so-called bone marrow edema in the subchondral bone most often associated with sites of OA cartilage pathology. The bone marrow edema signal is characterized by increased signal intensity using fluid-sensitive magnetic resonance sequences [49, 50] (Fig. 12.3). The histologic examination of the anatomic sites corresponding to regions of bone marrow lesions has revealed the presence of regions of localized marrow fibrosis and fat necrosis associated with microfractures of the trabecular bone. The presence of microfractures and localized bone remodeling is consistent with localized activation of bone repair

processes that accompany targeted bone remodeling [1, 20], and these findings indicate that the MRI signals are not generated by actual "edema" but rather by the replacement of the hematopoietic marrow with the reactive bone remodeling and repair process [51, 52]. Longitudinal studies indicate that bone marrow lesions may come and go, but importantly, their presence has been shown to correlate with the severity of joint pain and with progression of OA cartilage and bone pathology [50, 53–57]. The correspondence of the sites of bone marrow lesions with regions of bone and cartilage damage supports the concept that mechanical factors, including local traumatic bone injury, are responsible for the pathogenesis of the subchondral bone marrow changes. Further support for this concept is provided by the observation that bone cysts associated with OA frequently develop in the focal areas of bone damage and necrosis [58, 59].

An additional condition associated with the presence on MRI of bone marrow lesions has been described in patients with so-called bone bruises or contusions [60]. These lesions demonstrate similar characteristic to the bone marrow lesions detected in patients with OA but differ in their natural history and histological and pathological features. Characteristically, they are detected after an acute joint injury and have been described at multiple different skeletal sites, although the knee has been the most studied site. An analysis of the anatomic and histologic features of the tissue pathology associated with these lesions reveals the presence of bleeding, infraction, and edema related to true microscopic compression fractures of the cancellous bone. Mink and Deutsch [61] have classified bone bruises of the knee into four types: bone bruises, stress fractures, femoral and tibia fractures, and osteochondral fractures. According to the authors, the subtypes can be distinguished by their distinct patterns on MRI sequences and their anatomic localization. Bohndorf [62] proposed a classification of subchondral lesions in the knee based on the presence or absence of disrupted articular cartilage and separated these subtypes into "classic bone bruises" and subchondral impaction fractures. In general, the contusion patterns and location are a reflection of the mechanisms and site of the injury. According to this model, the *pivot shift injury* associated with anterior cruciate ligament disruption results in bone marrow lesions localized to the posterior aspect of the lateral tibial plateau and the midportion of the lateral femoral condyle [61], which correspond to the sites of acute impact loading during the injury. Roemer and Bohndorf [63] examined the long-term outcomes in a series of patients with acute traumatic knee injuries using MRI. They found a prevalence of bone bruising of 72 % in 176 patients, 25 of whom had isolated bone bruising in the absence of detectible subchondral fracture or overt cartilage disruption. 49 patients were evaluated with MRI after a minimum of 2 years, and they concluded that in the absence of a fracture, acute bone bruising vanishes in a majority of patients. The natural history in patients with subchondral fractures or osteochondral lesions may be much less favorable, and in these patients the presence of the bone bruise and the accompanying bone and cartilage pathology may be associated with a more rapid appearance and progression of periarticular and joint pathology.

Osteophytes are an additional characteristic radiographic feature of OA. Numerous lines of investigation implicate local biomechanical factors in their pathogenesis. Their location at the joint margins and their frequent association with the presence of joint instability has suggested that they may serve to stabilize the joint rather than contributing to joint dysfunction and OA progression [64, 65]. This conclusion is supported by the observations of Pottenger et al., who noted that removal of medial and lateral osteophytes from the knee joint increased joint instability [66]. Further evidence suggesting that osteophytes may not play a primary contributory role to OA progression has been provided by the studies of Felson and coworkers [67] who examined the relationship between osteophyte size and the risk for structural progression of knee OA. They found that the presence of large osteophytes did not appear to

affect the risk for OA structural progression and speculated that the relationship between osteophytes and OA progression was more likely attributable to the presence of malalignment that contributed to both the formation of osteophytes and the progression of OA.

Studies in animal models of OA have provided insights into the mechanisms involved in the formation of osteophytes. Histologic analysis suggests that osteophytes are initiated by the proliferation of periosteal cells at the joint margins. As the process proceeds, these cells undergo differentiation into chondrocytes, which hypertrophy and through the process of endochondral ossification create an enlarging skeletal outgrowth at the joint margin [65]. Growth factors, including transforming growth factor β and bone morphogenic protein-2, have been implicated in the formation and growth of the osteophytes [68, 69]. This conclusion is supported by the observations that intra-articular injection of these growth factors into joints in animal models induces the formation of osteophytes and, conversely, inhibition of their activity or interference with their signal pathways impairs osteophyte formation [70–74]. Although local mechanical factors are believed to be responsible for induction of the growth factors, the mechanisms involved in this process are not well understood.

In addition to the influence of mechanical factors on the initiation and progression of bone changes in OA, biological processes may also influence the remodeling and adaptation of the periarticular bone. These effects may be mediated by products derived from other joint tissues, including the synovium, menisci, adipose tissues, and cartilage. Recent studies have shown that soluble products can be directly exchanged between the subchondral bone and cartilage via a process of diffusion, providing a mechanism by which these two tissues can interact with each other to influence the activities of their resident cell populations [75–77]. The vascular invasion of the calcified cartilage and the advancement of the tidemark that are associated with OA represent biological processes that may result from these types of interactions [22–24, 48].

In summary, traumatic injury to the joint represents a major risk factor for the development of OA. The type and magnitude of the injuries may be highly variable and include a spectrum of alterations ranging from disruption of supporting ligamentous structures to overt intra-articular fractures. The progressive alterations in the structural and functional properties of the periarticular bone are dependent not only on the direct effects of the injury on the bone tissues but may result from the adverse effects of the initial injury on joint mechanics. This in turn produces alterations in the local biomechanical environment that modulate the activities and function of resident bone cells leading to progressive alterations in their synthetic and reparative activities (Fig. 12.4). Therapies to reduce the risk and incidence of PTOA must therefore be directed not only at targeting the bone cells but, importantly, at restoring physiological joint structure and function after the injury. This includes the institution of preventive programs that can reduce the risk of the initial injury. In the absence of gross anatomic disruption of the bone structure associated with fractures, the periarticular bone changes that occur after joint injury are not unique to PTOA and recapitulate the skeletal changes that are characteristic of other etiological forms of OA. An understanding of the pathophysiological processes associated with the periarticular changes that occur in OA provides a rationale framework for developing therapeutic interventions that have the potential to modify the natural history of the bone changes. Although therapies that specifically modify bone cell activity and remodeling have shown favorable effects on the progression of OA in animal models, these treatment interventions have not been successful in human trials. In part this lack of success can be attributed to the marked heterogeneity of the patient populations with OA and the complex processes associated with skeletal remodeling. Future studies will therefore require more rigorous classification and characterization of the stage of OA progression and the unique and specific etiologic mechanisms responsible for the bone and joint pathology.

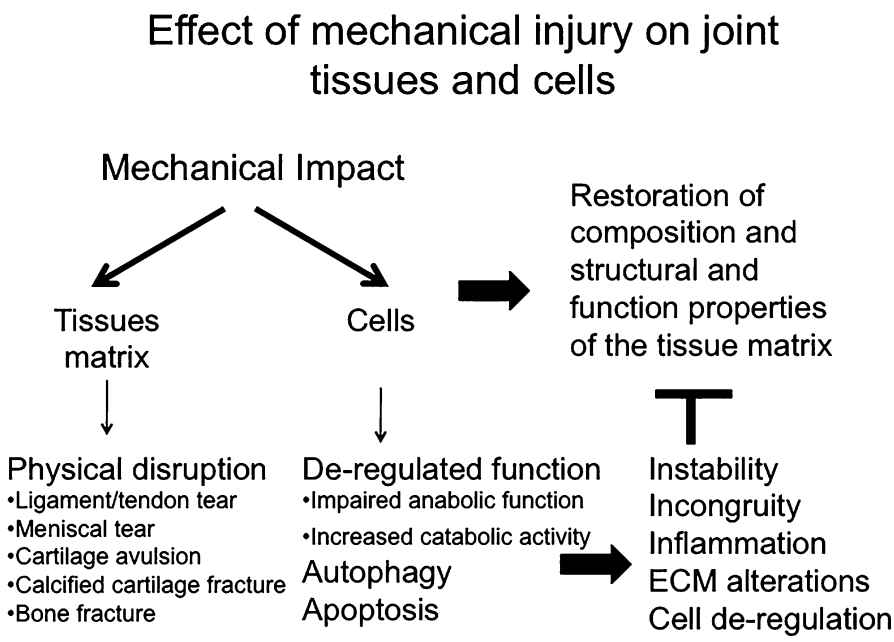

Fig. 12.4 Effects of acute traumatic mechanical injury to joint tissues. The magnitude of the energy of the impact determines the pattern and extent of the joint tissue injury and outcomes in terms of joint homeostasis. High-energy impact acutely disrupts the structural integrity of the joint tissues leading to irreversible alterations in joint stability and mechanics. Low-energy impact may not directly disrupt the integrity of the extracellular matrices of the joint tissues but may instead induce a cascade of events that modulate the activities and function of the resident cell types. This can initiate progressive alterations in their synthetic and reparative activities that modify the material properties of the tissues that they populate, leading to deterioration in the structural and functional properties of the tissues

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Genetic Variability in the Response to Injury

13

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Abbreviations

ACL	Anterior cruciate ligament
GWAS	Genome-wide association scan
OA	Osteoarthritis
PTOA	Post-traumatic osteoarthritis
QTL	Quantitative trait locus
SNP	Single nucleotide polymorphism

Introduction

Traumatic injuries are a major cause of mortality in developed nations and affect all age groups. Sports injuries on the other hand are less fatal and mostly affect people less than 40 years of age. It is estimated that 10–15 % of patients will develop osteoarthritis (OA) after a single traumatic injury and 100 % with repeated injury. Irrespective of injury type, it is hard for clinicians to predict the healing outcomes on the basis of clinical features. There could be several confounding factors that contribute to variation in healing outcomes such as age [1, 2], sex [3], obesity [4], environment, and extent and magnitude of the injury. The impact of these factors on the variation of wound healing following trauma has been reviewed by Guo and Dipietro [5]. In addition to those factors listed above, genetic background of the individuals is an important contributing factor in the variability in the outcome of injury. This is because subtle variations in genes that control healing response can impact the healing outcomes.

Traumatic injuries, especially those affecting the knees, are considered to be a major inducer of cartilage destruction and frequently lead to progressive joint degeneration and ultimately to post-traumatic OA (PTOA). PTOA is the leading cause of disability and pain in the community and results in high medical and social costs. In comparison to the frequency and extent of cartilage injuries, very little effort is directed toward predicting the outcome in patients. A great breakthrough in this context is the finding that OA runs in families and likely has a strong hereditary component to it [6–8], which accounts for 39–65 % of the variation in prevalence of OA due to genetics [7, 8].

In this chapter, we aim to cover the topics that relate to variability in the response of injury and include some examples both from cartilage repair and PTOA standpoints to give a clear and concise

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explanation why there is variability in the response to injury and how it is challenging at the bench and bedside. To this end, we present evidence from mouse studies that have shown genetic variation in the healing of ear wounds, regeneration of articular cartilage lesions, and susceptibility to develop experimental PTOA. Then, we summarize the studies in human population that have used genome-wide association scans (GWAS) to detect the gene variants that are thought to contribute to the susceptibility to OA. In addition, we discuss variation in the healing of human anterior cruciate ligament (ACL) injuries that may be genetically modulated. Lastly, we provide a few examples of genetic variation in the response to injury in non-musculoskeletal system. We are confident that this review will augment our understanding of genetic variation in the response to injury.

Response of the Ear-Wound Healing Is Genetically Modulated in Mice

Regeneration potential is phylogenetically dispersed among animal taxa and is a basal trait in vertebrates including fishes, amphibians, and mammalian fetal tissues [9–16]. Hydra and planaria can regenerate their entire bodies from their lost tissues [11, 17], and newts and salamanders can regenerate limbs or other structures after amputation [12, 13]. In contrast, the regenerative potential in adult mammals is extremely limited [9] and is usually confined to shedding and regrowth of antlers in deer [18] and moose [19].

The healing of ear wounds in mammals was first identified in rabbits in 1975 by Goss and Grimes [20]. These investigators also reported that similar ear holes in other mammals failed to regenerate and formed scar tissue instead of a blastema. Later at the end of the twentieth century, MRL mouse strain was recognized as a super-healer mouse strain because it has the ability to heal 2-mm circular wounds in its external ears. First discovered by Clark et al. [21], the healing ability in the MRL mouse strain for cartilaginous wound closure set an example in the field of tissue repair and regeneration in mice. These authors created through-and-through ear punches on the pinnae of external years of MRL and C57BL/6 mice. It was noted that MRL mice rapidly attain full closure of the 2-mm wound with normal tissue architecture reminiscent of regeneration seen in amphibians as opposed to scarring, as usually seen in mammals. They also undertook additional histological analyses to demonstrate cell growth and microanatomy in the healing ear wounds. In the same year, McBrearty and colleagues utilized inbred mouse strains to demonstrate that this healing potential is a heritable trait [22]. In addition, they identified genetic trait loci that controlled the healing process by performing genome-wide scan on (MRL/MpJFas*lpr* X C57BL/6) F2 and backcross populations. Several quantitative trait loci (QTLs) were identified, and it was found that all the alleles contributing to ear-wound healing were derived from the MRL/MpJFas*lpr* parents [22].

Our lab has also shown that the healing potential of ear wounds in recombinant inbred mouse strains is genetically driven [23]. Using several recombinant inbred strains generated from a LG/J by SM/J intercross, we have shown that the ear-wound healing phenotype is significantly heritable. Taken together, these studies strongly and convincingly suggest that the variability of healing in the response to ear wounds is due to genetic differences among the mouse strains.

Response of the Articular Cartilage to Healing Is Genetically Modulated in Mice

Articular cartilage is considered a simple tissue because it lacks blood and lymphatic vasculature as well as nerve supply and has only one cell type, i.e., chondrocytes [24, 25]. While on one hand this unique structure of the articular cartilage has an advantage for tissue engineering and stem cell therapeutic interventions, on the other hand, its structural features do not provide a robust repair capacity once injured. Injuries to articular cartilage occur due to either traumatic mechanical demolition or through a progressive degeneration throughout life. However, only

Table 13.1 Studies that provide genetic variability in the healing response of articular cartilage

First author	Year	Mouse strain	Phenotype	Outcome	Reference
Fitzgerald	2008	MRL/MpJ	Knee articular cartilage defects, ear wounds	Able to heal full-thickness knee articular cartilage defects, able to heal 2-mm ear wounds	[28]
Fitzgerald	2008	C57BL/6	Knee articular cartilage defects, ear wounds	Unable to heal full-thickness knee articular cartilage defects, failed to heal 2-mm ear wounds	[28]
Rai	2012	MRL/MpJ	Knee articular cartilage defects, ear wounds	Able to heal full-thickness knee articular cartilage defects, able to heal 2-mm ear wounds	[23]
Rai	2012	LG/J	Knee articular cartilage defects, ear wounds	Able to heal full-thickness knee articular cartilage defects, able to heal 2-mm ear wounds	[23]
Rai	2012	LGXSM-5	Knee articular cartilage defects, ear wounds	Had limited ability to heal full-thickness knee articular cartilage defects, able to heal 2-mm ear wounds up to some extent	[23]
Rai	2012	LGXSM-6	Knee articular cartilage defects, ear wounds	Able to heal full-thickness knee articular cartilage defects, able to heal 2-mm ear wounds	[23]
Rai	2012	LGXSM-33	Knee articular cartilage defects, ear wounds	Unable to heal full-thickness knee articular cartilage defects, failed to heal 2-mm ear wounds	[23]
Rai	2012	LGXSM-35	Knee articular cartilage defects, ear wounds	Had limited ability to heal full-thickness knee articular cartilage defects, able to heal 2-mm ear wounds up to some extent	[23]
Rai	2012	SM/J	Knee articular cartilage defects, ear wounds	Unable to heal full-thickness knee articular cartilage defects, failed to heal 2-mm ear wounds	[23]
Rai	2012	C57BL/6J	Knee articular cartilage defects, ear wounds	Unable to heal full-thickness knee articular cartilage defects, failed to heal 2-mm ear wounds	[23]
Rai	2012	DBA/1J	Knee articular cartilage defects, ear wounds	Unable to heal full-thickness knee articular cartilage defects, failed to heal 2-mm ear wounds	[23]
Rai	2012	DBA/2J	Knee articular cartilage defects, ear wounds	Unable to heal full-thickness knee articular cartilage defects, failed to heal 2-mm ear wounds	[23]
Eltawil	2009	C57BL/6	Knee articular cartilage defects	Unable to heal full-thickness knee articular cartilage defects	[29]
Eltawil	2009	DBA/1	Knee articular cartilage defects	Able to heal full-thickness knee articular cartilage defects	[29]

12 % of people get PTOA after injury [26], and by 5–11 years after injury, the incidence escalates to 74 % [27]. We hypothesized that individuals may vary in their capacity to heal articular cartilage.

While articular cartilage repair phenotypes in human are not accessible, work on mouse models has generated a great deal of important data. So far, three laboratories [23, 28, 29] have looked at healing of articular cartilage using common inbred and recombinant inbred strains of mice (Table 13.1). First, Fitzgerald et al. [28] reported that MRL/MpJ could regenerate cartilage significantly better than C57BL/6 mice. These researchers created both full- and partial-thickness defects on the trochlear groove articular cartilage of

these mice. The healing outcomes were gauged at 6- and 12-week post-surgery through an established scoring criterion [30]. It was found that at both 6- and 12-week time points, there was a superior healing response in MRL/MpJ mice with abundant chondrocytes and an extracellular matrix rich in proteoglycan, collagen II, and collagen VI at the injury site. In contrast, the C57BL/6 mice utterly failed to heal articular cartilage lesions.

Second, in the laboratory of Francesco Dell'Accio [29], it was found that the C57BL/6 strain, like most mouse strains, does not heal articular cartilage, but the DBA/1 strain does. Longitudinal full-thickness articular cartilage injuries were generated in the patellar groove of these two mouse strains by the use of a custom-made device in which a glass bead was placed approximately 200 μm to the tip of a 26 G needle. The tip of the needle was placed anteriorly to the intercondylar notch and gently moved along the entire length of the patellar groove. The most significant findings were (1) 8-week old DBA/1 mice displayed consistent superior healing of the articular cartilage defect than C57BL/6, (2) DBA/1 mice showed a significantly less cell death and cell proliferation than C57BL/6, and (3) most importantly, the increase in articular cartilage repair was correlated with the development of OA; 8-month old DBA/1 mice failed to repair, but unlike age-matched C57BL/6, they had no signs of OA.

The above studies have used three genetically distinct mouse strains with the two strains (MRL/MpJ and DBA/1) having intrinsic repair ability compared to common C57BL/6 mouse strain. These differences in healing ability therefore provide a clue for the involvement of genetics in the ability of articular cartilage regeneration. Except for the phenotypic variation, no further insights were given for these studies from a genetic standpoint. Therefore, the need for further genetic exploration was necessary to provide a solid evidence of involvement of genetics in the response to injury. It is known that MRL/MpJ and LG/J mouse strains are closely related mouse strains since they share 75 % of their genomes identical by descent [17]. Like MRL/MpJ, LG/J mouse strain also displays complete healing of the ear wounds, including ear cartilage [31]. This raises the possibility that, similar to MRL/MpJ mice, articular cartilage may also heal in LG/J mice. To investigate this possibility, we examined the extent of cartilage regeneration in a set of common inbred mouse strains, including both healers and non-healers, and in a set of recombinant inbred lines formed from the intercross of the LG/J (healer) and SM/J (non-healer) inbred mouse strains [32]. The conceptual starting point for recombinant inbred strains is that any differential phenotype can be attributed to a restricted set of genes according to the way they have been inherited from parental strains. Therefore, we took the approach of investigating cartilage regeneration in genetic mouse models.

Third study has recently shown a variation in the magnitude and extent of cartilage healing ability among various recombinant inbred mouse strains following a full-thickness cartilage defect on the trochlear groove of distal femur. In this study, we found that some of the strains were able to heal their articular cartilage lesions superbly, others intermediately, and still others poorly. Since these mice were raised and maintained in an identical environment and subjected to same size cartilage injury (i.e., using 27 G needle) at the same age, i.e., 8 weeks, the differences in the repair response could only be attributed to the genetic diversity among the strains [23]. To this end, the broad-sense heritability estimates confirmed that variation in these mice to tissue healing is due to genetic differences among the mouse strains. There were significant differences among the inbred mouse strains in the type of cartilage formed at the site of injury (varied from typical hyaline articular cartilage to fibrous or no cartilage at all), staining intensity of proteoglycan contents (intense staining to no staining), surface regularity of injured area (smooth surface to extremely irregular), integration status of the injured area with the native health cartilage (both edges integrated to at all no integration), and finally thickness of the repair zone (in par with the native cartilage to no integration) (Fig. 13.1).

From these studies, it is obvious that healing capacity is dependent on mouse strains, and

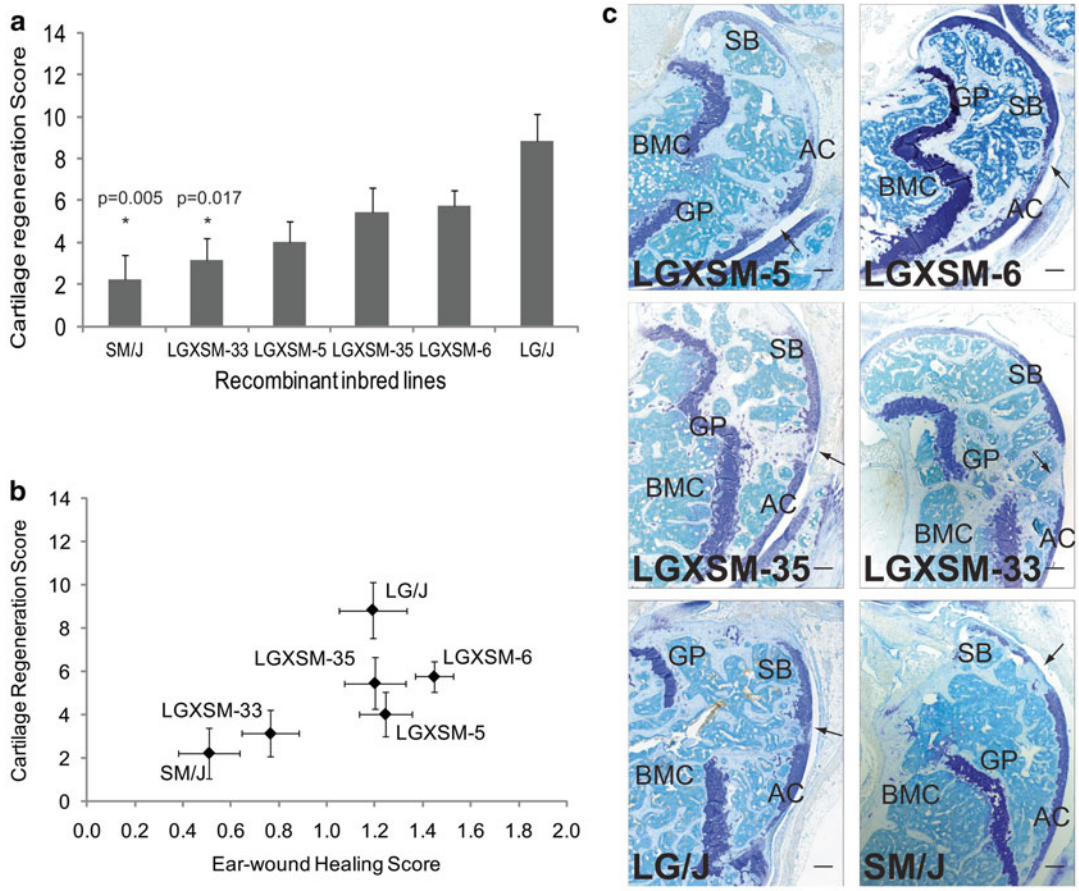

Fig. 13.1 Articular cartilage regeneration and ear-wound healing in recombinant inbred lines. (**a**) Articular cartilage regeneration in recombinant inbred lines showed no healing response in SM/J, LGXSM-5 and LGXSM-33 strains, while intermediate to good healing responses were observed in LGXSM-6, LGXSM-35, and LG/J strains. *Asterisk* with *p* given values indicates statistically significant difference from parental strain LG/J. (**b**) Correlation between knee articular cartilage regeneration and ear-wound healing showed that the two phenotypes are strongly correlated in the recombinant inbred lines. (**c**) Representative sagittal sections of full-thickness articular cartilage lesions from recombinant inbred lines LGXSM-5, LGXSM-6, LGXSM-33, and LGXSM-35 and parental strains LG/J and SM/J show that except for strains LGXSM-33 and SM/J, all other strains regenerated their articular cartilage and showed a deposition of proteoglycan indicating regenerative response. Arrow indicates site of defect; *AC* articular cartilage, *SB* subchondral bone, *GP* growth plate, *BMC* bone marrow cavity; bar (all panels) = 0.1 mm. Reprinted with permission from Rai et al. [23]

each mouse strain possesses a different genetic background/makeup. Having said that, it is tempting to believe that healing strength is genetically driven. The evidence presented by the above studies indicates that the ability to repair the articular cartilage is associated with the protection from OA. Therefore, the variation in cartilage injury response (i.e., the repair) and subsequent susceptibility to OA are both dependent on the genetics of the individuals.

Response of the Mouse Knee Joint to PTOA Is Genetically Modulated

So far, four studies have examined PTOA in mice from genetic standpoint. These mouse studies are described below and summarized in Table 13.2.

First, Ward et al. [33] in the laboratory of Steve Olson investigated the correlation between

Table 13.2 Studies conducted to get insights into PTOA in mice

First author	Year	Mouse strain	Site	Phenotype	Outcome	Reference
Ward	2008	MRL/MpJ	Knee	Articular cartilage, bone	Did not develop OA	[33]
Ward	2008	C57BL/6	Knee	Articular cartilage, bone	Developed OA	[33]
Eltawil	2009	C57BL/6	Knee	Articular cartilage	Developed OA	[29]
Eltawil	2009	DBA/1	Knee	Articular cartilage	Did not develop OA	[29]
Hashimoto	2012	LGXSM-6	Knee	Articular cartilage, bone	Did not develop OA	[35]
Hashimoto	2012	LGXSM-33	Knee	Articular cartilage, bone	Developed OA	[35]
Lewis	2013	MRL/MpJ	Knee, serum	Articular cartilage, bone, synovium	Did not develop OA	[34]
Lewis	2013	C57BL/6	Knee, serum	Articular cartilage, bone, synovium	Developed OA	[34]

intra-articular fracture healing and OA in C57BL/6 and MRL/MpJ mice. These authors experimentally created intra-articular fractures in the knees of these mice and analyzed the bone and cartilage changes by various techniques. It was found that the MRL/MpJ mouse is protected from PTOA after intra-articular fracture compared to C57BL/6. Second, Lewis and colleagues [34] again from the above research group have shown genetic and cellular evidence of decreased inflammation associated with reduced incidence of post-traumatic arthritis in MRL/MpJ mice compared to C57BL/6 mice undergoing tibial plateau fractures. These data further suggest a genetic association between joint tissue inflammations and the development and progression of PTOA in mice. Third, in the laboratory of Francesco Dell'Accio, Eltawil et al. [29] reported that C57BL/6 mice after experimental cartilage injury developed features of OA compared to DBA/1, which displayed no signs of OA. Fourth and lastly, Hashimoto et al. [35] in our laboratory used two distinct mouse strains (with the ability to heal or not to heal full-thickness cartilage lesions) to induce PTOA through destabilization of medial meniscus [36]. Changes in articular cartilage and bone showed that a mouse strain with healing ability (LGXSM-6) was protected from developing PTOA compared to the other mouse strain, which failed to heal its injured articular cartilage (LGXSM-33) and developed PTOA (Fig. 13.2). As stated above, these mouse strains are recombinant inbred strains of mice and have distinct genetic portfolio, therefore providing a direct evidence for the genetic variation in the development of PTOA in mice.

Genetics of OA as Evidenced by Genome-Wide Association Scan (GWAS) Studies

Since the start of the twenty-first century, several efforts have been focused on the search for QTLs that predispose to OA. Small- and large-scale GWAS, genome-wide linkage analysis, gene-based association studies, and candidate gene studies reflect the current status quo of the genetics of complex diseases such as OA. Several GWAS studies have been conducted to understand the involvement of genetic basis of deadly human diseases. Figure 13.3 shows the number of GWAS studies for common (complex) diseases. Note that numbers of GWAS studies for OA are more than other deadly diseases such as HIV, cardiovascular problems, and obesity and lag behind Alzheimer's disease, diabetes, and cancer.

The candidate gene association studies have been very successful since they are directed toward identifying susceptible loci for OA. While the scope of candidate gene association studies is broad, it would be injustice not to mention those studies, which have identified some of the important genes such as aspirin gene *ASPN* [37], *SMAD3* gene [38], *GDF5* gene [38–40], and *FRZB* gene (Table 13.3). These genes passed the strict criteria of replication in diverse Asian and European populations as well as the demonstration of functional significance. However, these findings are still debatable because of several shortcomings in the candidate gene association studies, such as lower sample size, the sparse

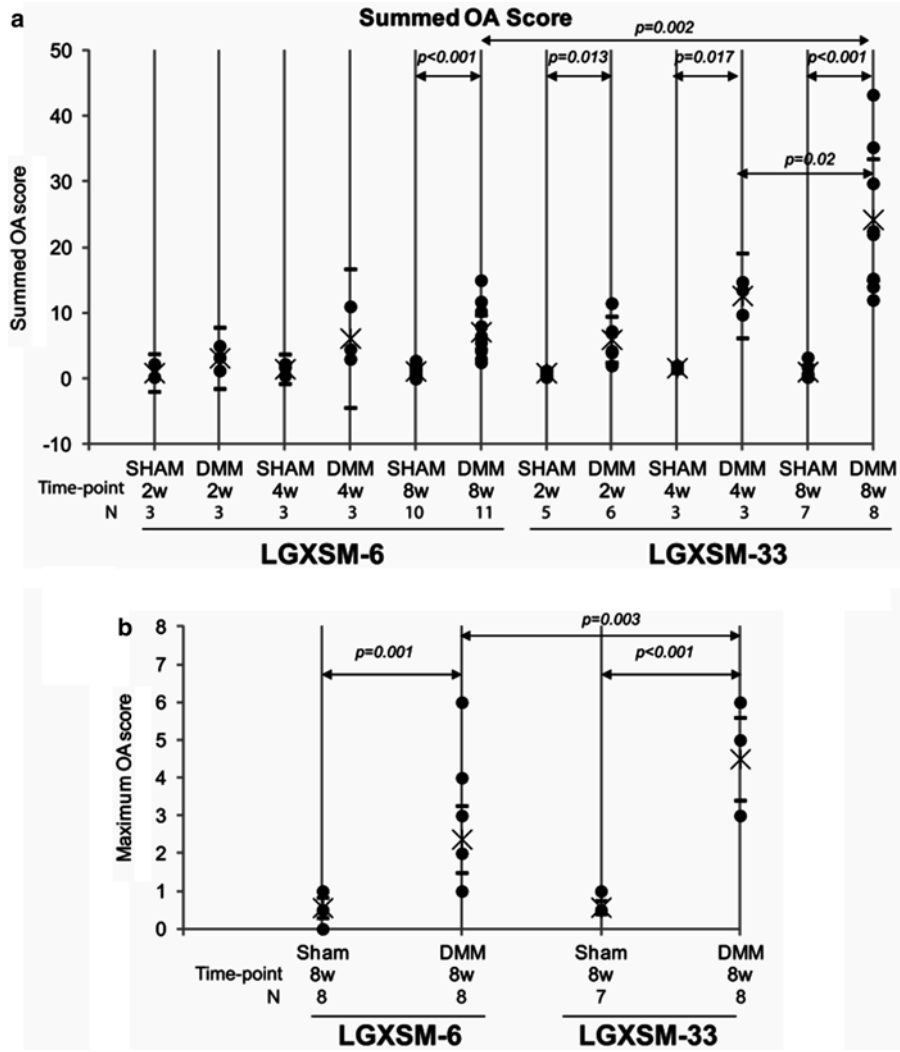

Fig. 13.2 OA score for LGXSM-6 and LGXSM-33. The sum OA score (**a**) from all the four quadrants and eight sections from each knee was based on cartilage damage and shows that LGXSM-33 strain overall develops more OA compared to LGXSM-6 after 8 weeks of surgery. A maximum score (**b**) representing highest score from medial compartment of LGXSM-6 and LGXSM-33 indicates that DMM knees had significant more OA score in both strains compared to sham knee. It also shows that LGXSM-33 has significantly higher grade of OA than LGXSM-6 at 8 weeks post-surgery. *Filled circles* individual data points, *Star* mean value, *hyphens* upper and lower limits of 95 % CI. Figure reprinted with permission from Hashimoto et al. [35]

coverage of markers employed by the relatively low-density microsatellite maps of linkage scans, and too many reports being false positive (mainly because of stringency of observed p values). These shortcomings have largely been removed by the GWAS recently (listed in Table 13.3). These GWAS studies fulfilled these criteria: genetic variants for which data was available from at least one replication cohort and *P* value adjusted for multiple comparisons.

In addition to the GWAS studies, heritability estimates from several sibling (twin) studies are summarized in Table 13.4 to further present evidence that OA is a genetic disease.

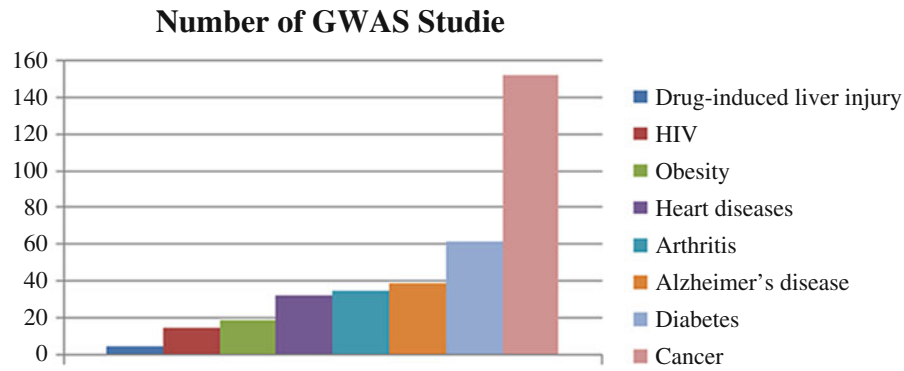

Fig. 13.3 Number of GWAS studies for the most common human diseases. These data have been taken from http://www.genome.gov and then graphed

Response of the ACL Repair Is Genetically Modulated

There are only a few case-control studies, which have attempted to gauge the possibility of familial predisposition toward tearing ACL [70, 71]. In the first study, Flynn et al. [70] used patients diagnosed with an ACL tear and age- and sex-matched controls to get questionnaire to determine the knee ligament injuries of the individuals' primary family members. There was at least twice higher incidence rate of ACL injury in the first-degree relatives of the ACL-injured group than that of the control group. In the second study, Harner et al. [71] also employed a matched case-control design, albeit with lesser sample size than the first study, and found a significant difference in the incidence rate of ACL injury in the family history of the experimental injured group compared with the control group, indicating a possible congenital aspect of ACL injury. Another team of researchers [72–75] has identified few genetic factors correlated with ACL injury (Table 13.5). These investigators employed unmatched case-control designs. In their first study in 2009 [73], the authors used surgically diagnosed ACL-injured Caucasian participants along with uninjured control subjects. The most intriguing finding was that a rare TT genotype of the *COL1A1* Sp1 (*COL1A1* gene encodes a protein chain within type I collagen, a major structural component of ligaments) binding site polymorphism was underrepresented in the injured ACL subjects compared to non-injured controls. In the same year, these authors claimed, for the first time, that there is a specific genetic risk factor associated with risk of ACL in female athletes [75]. Additionally, the authors showed that the CC genotype of a variant in the *COL5A1* gene (*COL5A1* gene codes for a protein chain in type V collagen, found in ligaments and tendon) has been associated with ACL tears in females. A year later, in 2010, these investigators investigate whether sequence variants within COL12A1 are associated with ACL ruptures in 139 individuals with ACL injury and 216 physically active participants. They found that the AA genotype of the *COL12A1* (*COL12A1* gene encodes for protein chains in type XII collagen, which is believed to regulate fibril diameter in ligaments) AluI polymorphism is overrepresented in female ACL-injured patients [74]. Recently, this same group of investigators has reported an association between the chromosomal region 11q22 and risk of ACL tear. Several matrix metalloproteinase genes, including those that are physiologic mediators of collagen cleavage and removal, are located on chromosome 11q22. In this group of ACL-injured patients, AG and GG genotypes of one matrix metalloproteinase variant were significantly underrepresented compared with uninjured patients [72]. The frequency of haplotypes of the variants within the gene was significantly different between injured and uninjured groups [72]. It is important that genetic variants be

Table 13.3 Candidate genes associated with OA in independent cohorts

First Author	Year	Population	SNP ID	Gene symbol	Gene name	Phenotype	*P* value	Reference
Miyamoto	2007	Asian	rs143383	*GDF5*	Growth differentiation factor 5	Hip OA	1.8×10^{-13}	[41]
Nakamura	2007	Asian	D14 allele	*ASPN*	Asporin	Knee OA	1.0×10^{-6}	[42]
Chapman	2008	European	rs143383	*GDF5*	Growth differentiation factor 5	Knee OA	1.0×10^{-7}	[40]
Mototani	2008	Asian	rs10980705	*EDG2*	Endothelial differentiation	Knee OA	2.6×10^{-5}	[43]
Meulenbelt	2008	European	rs225014, rs12885300	*DIO2*	Deiodinase, iodothyronine, type II	Hip OA	2.02×10^{-5}	[44]
Miyamoto	2008	Asian	rs11718863, rs7639618	*VWA*	Von Willebrand factor, intron A	Knee OA	7.0×10^{-11}	[45]
Valdes	2008	European	rs4140564	*PTGS2*	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	Knee OA	6.9×10^{-7}	[46]
Valdes	2008	European	rs4140564	*PLA2G4A*	Phospholipase A2, group IVA (cytosolic, calcium dependent)	Knee OA	6.9×10^{-7}	[46]
Valdes	2008	European	rs1207421	*PARD3B*	PAR-3 family cell polarity regulator beta	Knee OA	6.0×10^{-6}	[46]
Evangelou	2009	TREAT OA Consortium	rs143383	*GDF5*	Growth differentiation factor 5	Knee OA	9.4×10^{-7}	[47]
Evangelou	2009		rs288326	*FRZB*	Frizzled-related protein	Hip OA	0.019	
Valdes	2010	European	rs12901499	*SMAD3*	SMAD family member 3	Knee OA Hip OA	7.5×10^{-6} 4.0×10^{-4}	[38]
Attur	2010	Caucasian	rs419598, rs315952, and rs9005	*IL1RA*	Interleukin 1 receptor antagonist, type I	Knee OA	<0.0001	[48]
Nakajima	2010	Asian	rs7775228, rs10947262	*HLA class II/III*	Major histocompatibility complex class II	Knee OA	2.43×10^{-8}	[49]
Kerkhof	2010	Caucasian	rs3815148	*COG5*	Component of oligomeric Golgi complex 5	Knee OA, hand OA	8×10^{-8}	[50]
Kerkhof	2010	Caucasian	rs3757713	*GPR22*	G protein-coupled receptor 22	Knee OA, hand OA	4×10^{-12}	[50]
Nakajima	2010	Asian	rs1094726, rs7775228	*HLA class II/III*	Major histocompatibility complex class II		6.73×10^{-8} 2.43×10^{-8}	[49]
Valdes	2011	European	rs143383	*GDF5*	Growth differentiation factor 5	Knee OA		[39]
Kerkhof	2011	European	rs419598, rs315952 and rs9005	*IL1RA*	Interleukin 1 receptor antagonist, type I	Knee OA	0.006	[51]

(continued)

Table 13.3 (continued)

First Author	Year	Population	SNP ID	Gene symbol	Gene name	Phenotype	*P* value	Reference
Valdes	2011	European	rs8065080	*TRPV1*	Transient receptor potential cation channel, subfamily V, member 1	Knee OA	4.0×10^{-04}	[52]
Day-Williams	2011	European	rs11842874	*MCF2L*	MCF.2 cell line derived transforming sequence like	Knee OA	2.1×10^{-8}	[53]
Evangelou	2011	de-CODE, Rotterdam, Framingham, Twins UK	rs4730250	*PRKAR2B*	Protein kinase, cAMP-dependent, regulatory, type II, beta	Knee OA	9.2×10^{-9}	[54]
Evangelou	2011	de-CODE, Rotterdam, Framingham, Twins UK	rs4730250	*HPB1*	Polybromo 1	Knee OA	9.2×10^{-9}	[54]
Evangelou	2011	de-CODE, Rotterdam, Framingham, Twins UK	rs4730250	*COG5*	Component of oligomeric Golgi complex 5	Knee OA	9.2×10^{-9}	[54]
Evangelou	2011	de-CODE, Rotterdam, Framingham, Twins UK	rs4730250	*GPR22*	G protein-coupled receptor 22	Knee OA	9.2×10^{-9}	[54]
Evangelou	2011	de-CODE, Rotterdam, Framingham, Twins UK	rs4730250	*DUS4L*	Dihydrouridine synthase 4-like	Knee OA	9.2×10^{-9}	[54]
Evangelou	2011	de-CODE, Rotterdam, Framingham, Twins UK	rs4730250	*BCAP29*	B-cell receptor-associated protein 29	Knee OA	9.2×10^{-9}	[54]
Castaño Betancourt	2012	European	rs12982744	*DOT1L*	DOT1-like histone H3K79 methyltransferase	Hip OA	1.5×10^{-4}	[55]
Loughlin	2012	European	rs11177	*GNL3*	Guanine nucleotide binding protein-like 3	Knee OA, hip OA	2.12×10^{-10}	[56]
Loughlin	2012	European	rs11177	*GLT8D1*	Glycosyltransferase 8 domain containing 1	Knee OA, hip OA		[56]
Loughlin	2012	European	rs10948172	*SUPT3H*	Suppressor of Ty 3 homologue	Knee OA, hip OA	5.02×10^{-06}	[56]
Loughlin	2012	European	rs10948172	*CDC5L*	Cell division cycle 5-like	Knee OA, hip OA		[56]
Loughlin	2012	European	rs8044769	*FTO*	Fat mass and obesity associated	Knee OA, hip OA	5.98×10^{-08}	[56]

Table 13.4 Heritability estimates of human OA

First author	Year	Phenotype	Subjects	Age (years)	Heritability	Design	Reference
Spector	1996	Hand OA, knee OA	130 monozygotic, 120 dizygotic	48–70	–	Twin	[57]
Chitnavis	1997	Knee OA, hip OA	1,171 siblings, 376 spouses	38–95	0.27	Sibling, cross-sectional	[58]
MacGregor	2000	Hip OA	135 monozygotic, 277 dizygotic	>50	0.58	Cross-sectional, twin	[59]
Stankorich	2002	Hand OA	456	24–92	0.28–0.35	–	[60]
Demissie	2002	Hand OA	684 original cohort, 793 offspring	62	0.28–0.34	Framingham heart	[61]
Manek	2003	Knee OA	261 monozygotic, 524 dizygotic	24–79	0.504	Twin	[62]
Kirk	2003	Knee OA, hip OA	1,242 twin pairs	>50	0.3–0.46	Twin	[63]
Page	2003	Hip OA	6,419 twin pairs	86–96	0.61	Twin	[64]
Neame	2004	Knee OA	490 knee OA, 737 sibling, 1,729 community subjects	>40	0.62	Sibling	[65]
Zhai	2004	Knee OA	128	45	0.61	Sibling	[66]
Livshits	2007	Hand OA	538 monozygotic, 1,256 dizygotic	>55	0.48–0.67	Twin	[67]
Zhai	2007	Knee OA	114 monozygotic, 195 dizygotic	>40	0.69–0.80	Twin	[68]
Ishimuri	2010	Hand OA	136 probands, 150 sibling	65.5	0.35–0.63	Twin	[69]

assessed in different populations at risk of suffering ACL injury and that their associated phenotypes be identified. Taken together, these twin studies provide genetic basis for the involvement of genetics in the variation to ACL tears.

Examples of Genetic Variation in Non-musculoskeletal Systems

Recently associations between cytokine gene polymorphisms and clinical outcomes following head injury has been identified [77]. In this study, the authors used huge cohort of patients to assess clinical outcomes of head injuries. An initial screen of 11 cytokine gene SNPs (single nucleotide polymorphisms) previously associated with disease susceptibility or outcome was identified as having a likely association. Further evaluation of a selected cytokine showed a significant association with 39 % of allele 2 carriers having an unfavorable outcome compared with 31 % of noncarriers. This raises the possibility that although any single cytokine SNP has a small effect, possession of different combinations of alleles across the range of cytokine genes may have an additive larger effect. Genetic variability in cytokine genes can impact the magnitude and duration of an individual's neuroinflammatory response to head injury, affecting long-term recovery response.

Thermal injury induces immune dysfunction and alters numerous physiological parameters. Studies have proposed that genetics influences the outcome after traumatic injury and/or sepsis; however, the contribution of genetics to the immune-inflammatory response postburn was not been investigated until Schwacha et al. [78] studied genetic variability in the immune-inflammatory response after major burn injury. In this study, mice of three distinct genetic backgrounds (C57BL/6NCrlBR, BALB/cAnNCrlBR, and 129S6/SvEvTac) were subjected to thermal injury or a sham procedure, and 3 days later, splenocytes and macrophages were isolated for analysis. Splenocytes from the C57BL/6NCrlBR strain

Table 13.5 Genetic risk factors for ACL tear

First author	Year	Design	Trait	Total subjects	No. of injured subjects	No. of control subjects	Odd ratio (95 % CI)	Important risk factors	Reference
Harner	1994	Matched case-control	ACL tear	54	31	23	–	Familial disposition toward tearing ACL, possible congenital aspects	[71]
Flynn	2005	Matched case-control	ACL tear	342	171	171	2.00 (1.19–3.33)	Familial disposition toward tearing ACL	[70]
Posthumus	2009	Case-control	ACL tear	345	129	216	6.6 (1.5–29)	Underrepresentation of *COL5A1* sequence variants in injured females	[75]
Posthumus	2009	Case-control	ACL tear	247	117	130	0.08 (0.01–1.46)	Overrepresentation of *COL1A1* sequence variants in injured females	[73]
Posthumus	2010	Case-control	ACL tear	345	129	216	2.40 (1.0–5.5)	*COL12A1* polymorphism	[74]
Posthumus	2012	Case-control	ACL tear	345	129	216	–	MMPs gene variants on the 11q22 chromosome	[72]
Raleigh	2013	Case-control	ACL tear	340	126	214	–	Failed to determine the sequence variants of *GDF5*	[76]

displayed suppressed splenic T-cell proliferation post-injury, whereas the other strains were unaffected. Burn injury also induced a shift toward a Th2-type T-cell response (suppressed IFN-gamma production) in the C57BL/6NCrlBR strain, but not in the other strains. Macrophages from C57BL/6NCrlBR and 129S6/SvEvTac mice were highly proinflammatory with elevated productive capacity for TNF-alpha and nitric oxide, whereas no such changes were observed in macrophages for BALB/cNCrlBR mice. C57BL/6NCRLBR macrophages produced increased IL-10 levels postburn, and BALB/cNCrlBR macrophages had suppressed IL-10 production post-injury. No differences in fasting blood glucose and insulin were observed after thermal injury. However, significant postburn weight loss was observed in the BALB/cNCrlBR and 129S6/SvEvTac strains but not in the C57BL/6NCrlBR strain. In summary, these findings support the concept that the immune-inflammatory response postburn is influenced by genetic makeup. Further elucidation of the influence of genetics under such conditions is likely to contribute to the improvement in existing, and the development of new, therapeutic regimes for burn patients.

The genetic determinants influencing the response to injury, inflammation, and sepsis have also been studied [79]. The genetic background has recently been recognized as an important element in the response to injury, contributing to the variability in the clinical outcome of critically ill patients. The traditional approach to studying the genetic contribution requires the availability of families with multiple members who have experienced similar disease conditions, a situation that is nearly impossible to find in the case of trauma. Association studies looking at unrelated individuals across populations require large economic and labor-intensive efforts. Thus, a candidate gene approach has been the sole methodology used to correlate genetic variability with clinical outcome. However, this approach cannot provide a comprehensive description of a multigenic condition. Animal models are an alternative for studying the genetic contributions to variability in the response to injury. A murine model is ideal because a large set of inbred strains is available; congenic, consomic, transgenic, and recombinant strains can also be used. Employing this paradigm, we have demonstrated that the response to several stressors, such as injection of *Escherichia coli* lipopolysaccharide and polymicrobial sepsis induced by cecal ligation and puncture, is modified by the genetic background. The inflammatory response in mice has also been shown to be affected by sex, age, and other nongenetic components such as diet. Studies have exploited the differences in response among various inbred mouse strains to map loci contributing to the inflammatory response. Fine-mapping strategies allow the refinement of sets of candidate genes, which can be identified by positional cloning. Detection of genetic variation affecting the inflammatory response in murine models provides a basis for determining whether polymorphisms in orthologous human genes correlate with particular clinical outcomes from injury. Thus, discovery of these genes could impact patient care by acting as markers of a specific predisposition in humans.

Conclusion

Defining genetic variation in the response to injury may lead to the identification of patients who are likely to have different responses to therapeutic interventions. Results from various mouse and human studies support our central thesis that the response to tissue repair and PTOA is modulated by the genetic background. Our own studies have demonstrated the feasibility of using genetic mouse strains to study the variation in the response to three injury phenotypes: ear-wound healing, articular cartilage regeneration, and susceptibility to PTOA. These studies, combined with the studies from other laboratories on similar phenotypes have further demonstrated genetic contributions in the response to injury. From this information, combined with the information coming from the GWAS for primary OA, a picture of the genes necessary for protection and susceptibility to OA will emerge. In the future, there will be targets for treatment of OA at stages before the joint is destroyed, and surgery is the only answer.

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Aging and Post-Traumatic Arthritis

14

Richard F. Loeser, Cristin M. Ferguson, and Martin K. Lotz

Abbreviations

ACL	Anterior cruciate ligament
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
AGEs	Advanced glycation end products
CHOP	C/EBP homologous protein
DMM	Destabilized medial meniscus
ECM	Extracellular matrix
ER	Endoplasmic reticulum
IGF-1	Insulin-like growth factor 1
IL	Interleukin
MMP	Matrix metalloproteinase
OA	Osteoarthritis
PTOA	Post-traumatic OA
RAGEs	Receptor for advanced glycation end products
ROS	Reactive oxygen species
SASP	Senescence-associated secretory phenotype
SZP	Superficial zone protein
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor

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Introduction

Aging is established as a major contributor to the development of osteoarthritis (OA) [1, 2]. Increasing age is a risk factor for hand, hip, and knee OA [3]. The incidence and prevalence of radiographic knee OA increases with age in both men and women with a gradual slow increase starting at around age 40 years and a more steep increase noted after about age 50–55 [3–5]. As expected, symptomatic knee OA also increases with age starting at around age 35 years with the highest estimated incidence occurring between 55 and 64 years of age [6]. Because post-traumatic OA (PTOA) is most common in the knee and because involvement of the knee joint is the major contributor to pain and disability, this chapter will focus mostly on the relationship between aging and PTOA of the knee with selected references to other joints where sufficient literature exists.

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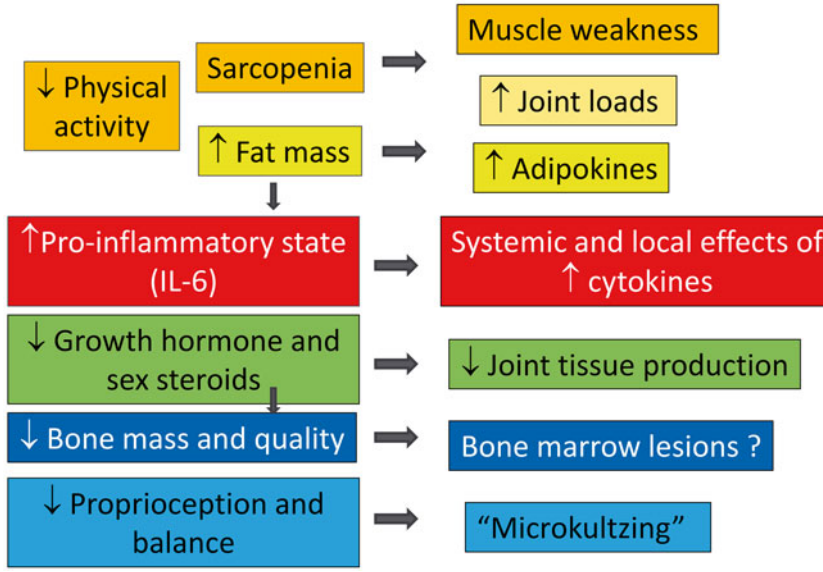

Fig. 14.1 Systemic aging changes that could contribute to the development of osteoarthritis, including post-traumatic OA. Reprinted with permission from HSS Journal 2012; 8:19

Aging-related changes in specific joint tissues, including the articular cartilage, meniscus, and ligaments, that could make the joint more susceptible to the development of OA have been described and will be further discussed below. These include matrix changes, such as the accumulation of advanced glycation end products (AGEs), and cellular changes such as cell senescence and the development of the senescence-associated secretory phenotype (SASP). Systemic or nonarticular changes associated with aging could also be contributing factors to aging-related OA. These include muscle weakness related to sarcopenia, increased fat mass, increased bone remodeling, reduced proprioception, and a low-grade chronic inflammatory state (Fig. 14.1). What has not been as well established is the specific role that joint tissue changes and systemic factors related to aging play in the development of post-traumatic OA. However, given the aging of our population and the increasing number of adults who have sustained joint injuries earlier in life as well as in their later years, the relationship between aging and PTOA is an important topic to consider and an area of active investigation.

Relationship Between Age and the Development of PTOA

OA is a slowly progressive chronic disease that develops over years. Therefore, the classic OA pathological and radiological findings (cartilage destruction, subchondral bone thickening, and osteophytes) are not seen immediately after a joint injury but rather take time to develop. The influence of time on the development of OA allows for aging to contribute to the development of PTOA. In a longitudinal study of former medical students who experienced a knee injury at a mean age of 22 years, the cumulative incidence of clinically diagnosed knee OA by 65 years of age was 13.9 % compared to 6.0 % who did not report an injury [7]. In that study, which followed a young cohort, the average time to symptomatic OA after the knee injury was 22 ± 13 years with the majority of the subjects not having OA diagnosed until after 45–50 years of age.

The time frame between injury and the diagnosis of OA appears to vary from person to person due to a number of factors that include the

age at which the individual experienced the joint injury as well as the type of injury. Risk factors for noninjury-related OA, such as lower extremity malalignment, obesity, female gender, joint overuse, and certain genetic polymorphisms, are also likely to accelerate the onset of PTOA and account for the variability in this patient population. In a retrospective cross-sectional study of adults with injuries to the anterior cruciate ligament (ACL) or the meniscus, Roos et al. [8] reported that radiographic OA appeared on average at 10 years after the injury. However, the time frame varied by patient age such that those who had an isolated meniscus injury between the ages of 17 and 30 took about 15 years to develop radiographic OA, while patients over the age of 30 when the injury occurred took only about 5 years.

Attempting to study the relationship between aging and PTOA in cross-sectional studies is complicated by the length of time it takes for OA to develop after an injury. Because of this, older adults may not recall an injury that occurred earlier in life or when it occurred. MRI studies of older adults with symptomatic OA commonly find evidence of ACL tears and/or meniscal tears in people who do not recall having a joint injury [9–11]. These tears may have resulted from relatively minor injuries or from aging changes in joint tissues that predispose to nontraumatic or "degenerative" tears. In women over the age of 40 with knee OA, 73 % were found to have a meniscus tear by MRI [11], and in a group of male and female subjects with an average age of 67 years, 22.8 % had a complete ACL rupture [10]. Even in a Korean cohort with an average age of 71 years randomly chosen regardless of knee OA, meniscal and ligament damage was present in 49.75 and 8 % of men and 71.2 and 26.9 % of women, respectively [9].

Studies have begun to investigate mechanisms for the relationship between meniscal injury and the development of OA. The meniscus is a structure vital to the normally functioning knee joint that contributes to shock absorption, joint stability, articular congruity, and proprioception [12]. Loss of meniscus function has detrimental effects on the knee joint [13–16]. Total meniscectomy reduces knee joint contact area by 50 % and greatly increases the load on the knee with subsequent damage and degeneration of articular cartilage and development of OA [17]. Even partial meniscectomy significantly increases contact pressures and leads to increased joint wear [13].

Meniscus tears in non-osteoarthritis subjects are felt to be an early event in the OA disease process and may be a risk factor for subsequent articular cartilage degeneration [18]. Meniscus tears or degeneration noted by MRI was found to be significant predictors for the development of OA 30 months later in middle-aged and elderly individuals (between 50 and 79 years of age) with an odds ratio of 5.7. Meniscus extrusion on MRI, which is consistent with diminished hoop mechanical function of the meniscus, has been shown to be associated with loss of cartilage volume over 2 years mediated by subchondral bone changes (bone expansion and cartilage loss) [18]. Furthermore, body mass index, tibial bone area, joint alignment, past knee injury, and presence of osteophytes may be causally related to the development of meniscus extrusion [18, 19].

In addition to altered joint mechanics, the injured meniscus has also been suggested to play a biologic role in the development of knee OA [20–23]. A study of gene expression in human meniscus tissue, removed at the time of partial meniscectomy after a meniscal injury with or without an ACL injury, compared individuals younger or older than 40 years [20]. The younger individuals appeared to have a greater response to the injury with higher expression levels of interleukin (IL)-1β and several matrix-degrading enzymes including a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-5; matrix metalloproteinase (MMP)-1, MMP-9, and MMP-13; as well as NFκB2 [20]. The same group also reported results of a microarray study, again using samples from partial meniscectomy, and correlated the results with age and degree of cartilage damage [23]. Using a cutoff difference of ≥1.5-fold, 866 genes were found to be differentially regulated in individuals equal to or younger vs. older than 40 years.

Studies in a mouse model of PTOA have shown an influence of age on the severity of OA.

The destabilized medial meniscus (DMM) model is a commonly used model for OA in mice. Histological features of OA at 8 weeks after surgery were found to be twice as severe when DMM surgery was performed on male C57BL/6 mice at 12 months of age compared to 12 weeks of age [24]. In that study, age-associated differences in gene expression were evaluated using microarrays which revealed an age-related decline in expression of matrix genes that included aggrecan and types II and IX collagen in the sham control joints accompanied by an increase in immune and defense response genes. In the DMM joints, more genes (251 vs. 52) were significantly upregulated in the older mice compared to the younger. Genes expressed at higher levels in the older adult mice included ADAMTS-5, asporin, and the chemokine receptors CXCR2 and CXCR7 as well as type III collagen, among others. Younger mice had a greater increase in genes related to the immune response. These results, albeit in a mouse model, demonstrate that the response to joint injury differs significantly between younger and older individuals. The aging changes in joint tissues that might be responsible for these differences will be discussed next.

Aging Changes in Joint Tissues and the Development of PTOA

Cartilage

Among the different joint tissues, cartilage has been most closely examined for changes that occur during the aging process. These changes include alterations in cartilage cells and extracellular matrix. The overall pattern can be described as "chondropenia" and features a reduction in cartilage ECM and cartilage cells [25–35].

Conceptually, loss of chondrocytes could lead to decreased production and maintenance of ECM. Conversely, loss of ECM could expose chondrocytes to abnormal biomechanical stress, leading to changes in their biosynthetic function and cell death. Consequently, age-related changes in the cell and the ECM are closely linked and may potentiate each other. As these changes progress with advancing age, an acute superimposed joint injury can be expected to lead to symptomatic and structurally overt OA more rapidly in older individuals.

Changes in Cartilage ECM

Age-related changes in cartilage ECM include increased cross-linking between collagen fibers and glycosaminoglycans [36, 37] and changes in size and sulfation of aggrecan [38–41]. With aging, there is also an accumulation of advanced glycation end products (AGEs) that are formed by nonenzymatic glycation of cartilage proteins, which are prone to this modification due to their slow turnover [42]. Consequences of AGE accumulation are increased stiffness and increased susceptibility to fatigue failure [43, 44]. AGEs also stimulate chondrocytes via binding to the receptor for advanced glycation end products (RAGEs) to produce inflammatory cytokines and MMPs [45–47]. The deposition of calcium pyrophosphate and basic calcium phosphate crystals is very common in aging cartilage [48], and these crystals promote inflammation and degradation of cartilage ECM [49]. Collectively, these ECM changes lead to deterioration in cartilage biomechanical properties [40, 50, 51]. The confined compression modulus of full-thickness cartilage from the patella decreases by ~33 % with age and degeneration grade [52]. Cartilage tensile properties change dramatically with aging in the human knee joint. With increasing age, tensile modulus and strength of human articular cartilage from the distal femur are reduced by about fourfold [53].

Lubrication at the cartilage surface zone is an essential mechanism that protects the tissue from damage caused by friction, heat, and wear. The cells in the superficial zone produce lubricin also called superficial zone protein (SZP) [54], a highly glycosylated protein that functions as a boundary lubricant [55]. With aging, there is a reduction in cellularity in the superficial zone [56] and consequently in the cell sources of lubricin. Low SZP may be one mechanism that accounts for the earliest occurrence of cartilage structural lesions at the cartilage surface.

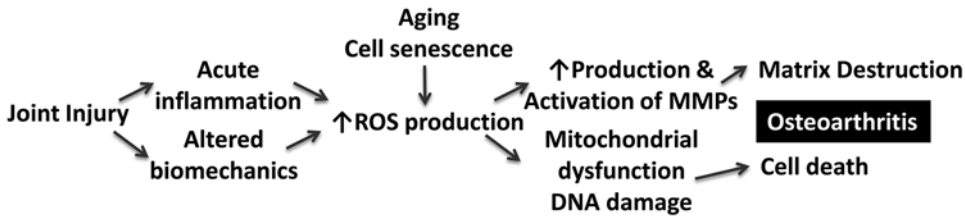

Fig. 14.2 Hypothetical mechanism for an interaction between joint injury and aging leading to osteoarthritis due to excessive production of reactive oxygen species (ROS)

Changes in Cartilage Cells

Age-related changes in cartilage cells (chondrocytes) include increased cell death and abnormal biosynthetic function. Analyses of human and animal tissues have shown increased levels of apoptosis in aged cartilage compared with mature cartilage, suggesting that apoptosis, or programmed cell death, may be responsible for chondrocyte hypocellularity during the aging process [57–60]. A controlled study in rabbits showed that age predisposes articular cartilage to a reduction in viable cell density and increased expression levels of proapoptotic genes, and this was observed in cartilage that showed no prior signs of OA [58]. Aged rabbits with macroscopically normal cartilage have higher rates of expression of apoptotic genes, including Fas, FasL, and caspase 8, compared with mature rabbits with a similar grade of cartilage [61]. Additionally, MMP-1 was increased in normal aged cartilage compared with normal mature cartilage [61]. These changes may contribute to the observations in both humans and animals, that aging results in a loss of proteoglycans within the ECM and thinning of the articular cartilage [33, 34, 62, 63]. Overall, these results suggest that although aged cartilage may appear grossly normal, microscopic and molecular changes have occurred, including increased expression of apoptotic and matrix-degrading genes with loss of viable chondrocytes and ECM, which may predispose older individuals to more rapid development of OA following joint injury.

In chondrocytes, aging also leads to a decreased sensitivity to critical anabolic factors, such as insulin-like growth factor (IGF-1). A key function of IGF-1 is as a potent inhibitor of apoptosis in many cell types, including chondrocytes [64] as well as a stimulus of ECM synthesis and antioxidants. In OA, there is an increased expression of TRB3, an Akt inhibitor that inhibits the capacity of IGF-1 to promote proteoglycan synthesis and viability [65]. Aging of chondrocytes is also associated with increased production of reactive oxygen species (ROS) [66, 67], decreased ECM synthesis [68], and increased secretion of matrix-degrading enzymes [69]. Elevated intracellular ROS have been linked to apoptosis and catabolic activity in chondrocytes [70–73]. Because both joint injury and aging can contribute to increased production of ROS, oxidative stress may be an important mechanism by which aging contributes to the development of PTOA (Fig. 14.2).

Mechanisms of Cartilage Cell Aging

Several mechanisms that contribute to aging-associated cartilage cell dysfunction and death have been identified. There is evidence for senescence in at least certain subsets of cartilage cells [74], and this is associated with an abnormal biosynthetic activity termed senescence-associated secretory phenotype (SASP) [75]. This includes IL-1, IL-6, IL-7, IL-8, GROα, MCP-2, and MMP-3, which are also produced by OA chondrocytes. In human OA cartilage, RNA levels of MMP-1, MMP-8, and MMP-13 and tissue inhibitor of metallproteinase-1 were altered in cells isolated from lesion sites, where telomere shortening and the senescence marker senescence-associated beta galactosidase were observed, but also in sites distal to the lesions where a lower number of cells exhibited the senescence-like changes [76].

Mechanisms that protect against aging-associated cell damage include autophagy and the unfolded protein response (UPR). Autophagy is a process that is responsible for the removal of damaged cellular organelles such as damaged mitochondria and macromolecules [77]. Conceptually, autophagy in normal adult articular cartilage is an important mechanism for cellular homeostasis. Cells in the normal cartilage superficial zone show stronger expression of autophagy proteins, such as beclin-1, Atg5, and LC3 [78], as compared to cells in the mid and deep zone, and their expression also differs between cartilage regions that are exposed to high versus low levels of mechanical load. Under normal conditions, there is a clearly detectable level of autophagy activation in healthy mouse joints. The highest levels were observed in the chondrocytes present in the superficial and upper middle zone of the articular cartilage in knee joints [79]. In contrast, only few cells in the deep zone contained detectable levels of autophagosomes. As with other tissues, starvation increased the number of autophagosomes in chondrocytes [79].

During the aging process in mouse and human knee joints, there is a decrease in Ulk1, LC3, and beclin-1 protein expression in articular cartilage. The reduction of these key regulators of autophagy is accompanied by increased apoptosis [78]. In a rapidly progressing experimental OA model in mice induced by surgical knee destabilization (DMM surgery plus medial collateral ligament transection), which can be considered as a model of PTOA, there is also a time-dependent reduction in these autophagy proteins [78]. Since this was observed in relatively young mice, it is apparently not a consequence of aging-related mechanisms but related to excessive mechanical load. However, for both surgical OA and mechanically injured cartilage, the increased cell death suggests that dysfunctional autophagy may contribute to cell death.

When full-thickness cartilage explants are exposed to single high impact mechanical compression, there are immediate matrix changes and a low level of cell death. It was observed that there was a short and transient increase in the levels of LC3-II, followed by a marked reduction in the levels of several autophagy proteins, including Ulk1, LC3, and beclin-1 [80]. Thus, during the development of OA, autophagy may increase as an adaptive response to protect cells from various stresses, and failure of the adaptation may lead to further progression of degeneration. The reduction in autophagy protein levels and autophagy activation supports the hypothesis that the basal autophagic activity decreases with the age, thus contributing to the accumulation of damaged organelles and macromolecules and susceptibility to aging-related diseases [81]. A series of prior studies demonstrated mitochondrial dysfunction in various OA models and in human OA [82]. In addition, mitochondrial DNA mutations are increased in OA chondrocytes [83]. Damaged mitochondria, producing high levels of ROS, have an important role in pro-inflammatory signaling, as they initiate formation of inflammasomes and activation of other inflammatory pathways [84].

Autophagy is also involved in regulating differentiation and gene expression. In human knee chondrocytes, IL-1 or nitric oxide increased expression of LC3 and beclin-1 and activated autophagy [85]. Further, autophagy activation prevented IL-1-mediated suppression of cartilage matrix degradation and reduced levels of MMP-13, ADAMTS5, and ROS. Given that one of the cytoprotective functions of autophagy is the removal of damaged mitochondria [83], the IL-1-induced OA-like gene expression changes might possibly occur through reduction of the intracellular ROS level via elimination of damaged mitochondria.

The UPR is normally a repair response that sends simultaneous survival, death, and inflammatory signals and culminates in coordinate transcriptional and translational reprogramming to modulate protein folding, inflammation, and cell fate [86]. Both autophagy and UPR are compromised in aging cartilage, and this contributes to increase oxygen radical production, DNA damage, and cell death. In OA cartilage, increased XBP1 activation and expression of GRP78 and C/EBP homologous protein (CHOP) are evidence of heightened endoplasmic reticulum (ER) stress in situ [87, 88]. Cultured OA

chondrocytes demonstrate evidence of PERK module activation [89, 90]. This includes expression of TRB3, an Akt inhibitor that inhibits the capacity of IGF-I to promote PG synthesis and viability. Excess UPR activation can promote chondrocyte hypertrophy or apoptosis, thereby potentially accelerating OA [89, 90]. Excess CHOP expression is one feature of UPR and ER stress. UPR activation was increased in human knee OA cartilage in situ and in biomechanically injured cultured chondrocytes in vitro [91]. In normal human chondrocytes, CHOP "gain of function" sensitized chondrocytes to IL-1β-induced nitric oxide and MMP-3 release without inducing these responses by itself. Excess *CHOP* expression, by itself, induced superoxide production and apoptosis.

Aging-Related Differences in Cartilage Response to Injury in Experimental Models

A limited number of in vitro and animal model studies have directly addressed age-related changes in cartilage tissue and the cell response to injury. Injurious loading elevates secretion of matrix-degrading enzymes, inflammatory cytokines, and oxygen radicals and causes chondrocyte death [92]. In the ACL transection model, aged rabbits showed faster and more severe cartilage degradation than mature rabbits. Aged rabbits with macroscopically normal cartilage had higher rates of expression of apoptotic genes, including *Fas*, *FasL*, *caspase 8*, and *p53* compared with mature rabbits, and this correlated with decreased chondrocyte numbers [58, 61]. As detailed above, experimental OA is also more severe in older as compared to young mice [24]. Gene expression analysis of entire knee joints showed that there were significant age-related differences in gene expression in the sham-operated knees, which included reduced ECM genes, *Sox9*, and *TGFβ2* and increased inflammatory cytokine genes. Following knee destabilization, a significantly larger number of genes were upregulated in the older mice, potentially indicative of a more active disease process [24].

These studies in animal models show that experimental OA that is induced by chronic mechanical injury is more severe in older animals. Preexisting changes in gene expression and a different response of the older tissues to injury appear to determine increased tissue damage.

Meniscus

Age-related changes in the meniscus that contribute to OA are likely due to the complex interplay of changes in cellularity, vascularity, and the extracellular matrix in addition to changes in gene expression and senescence (Fig. 14.3). The meniscus is a fibrocartilaginous tissue with a heterogeneous cell composition. The fibrochondrocyte, the cell type populating the meniscus, has two main phenotypic forms: elongated fibroblast-type cells found in the outer meniscus and more rounded chondrocyte-like cells found in the inner meniscus [93–95]. Research involving meniscus explant cultures has identified regional differences between the inner and outer zone cell populations in response to growth factors such as platelet-derived growth factor which correlate with the greater healing potential in the outer zone which is the vascular region of the meniscus [96, 97]. Interestingly, regional differences in healing between inner and outer zone cell populations are maintained in organ culture models, without the active presence of a peripheral vascular supply, suggesting some component of intrinsic healing present in outer zone meniscus cells that is separate from the variable of blood supply [96].

Changes in Meniscus Cells

Age has been further shown in meniscus fibrochondrocyte culture studies to be an independent factor contributing to tissue repair. In an inherently avascular system, cultured fetal and juvenile fibrochondrocytes have been shown to have superior repair capacity to adult fibrochondrocytes [98]. Additional cell culture studies have shown that although total collagen production between inner

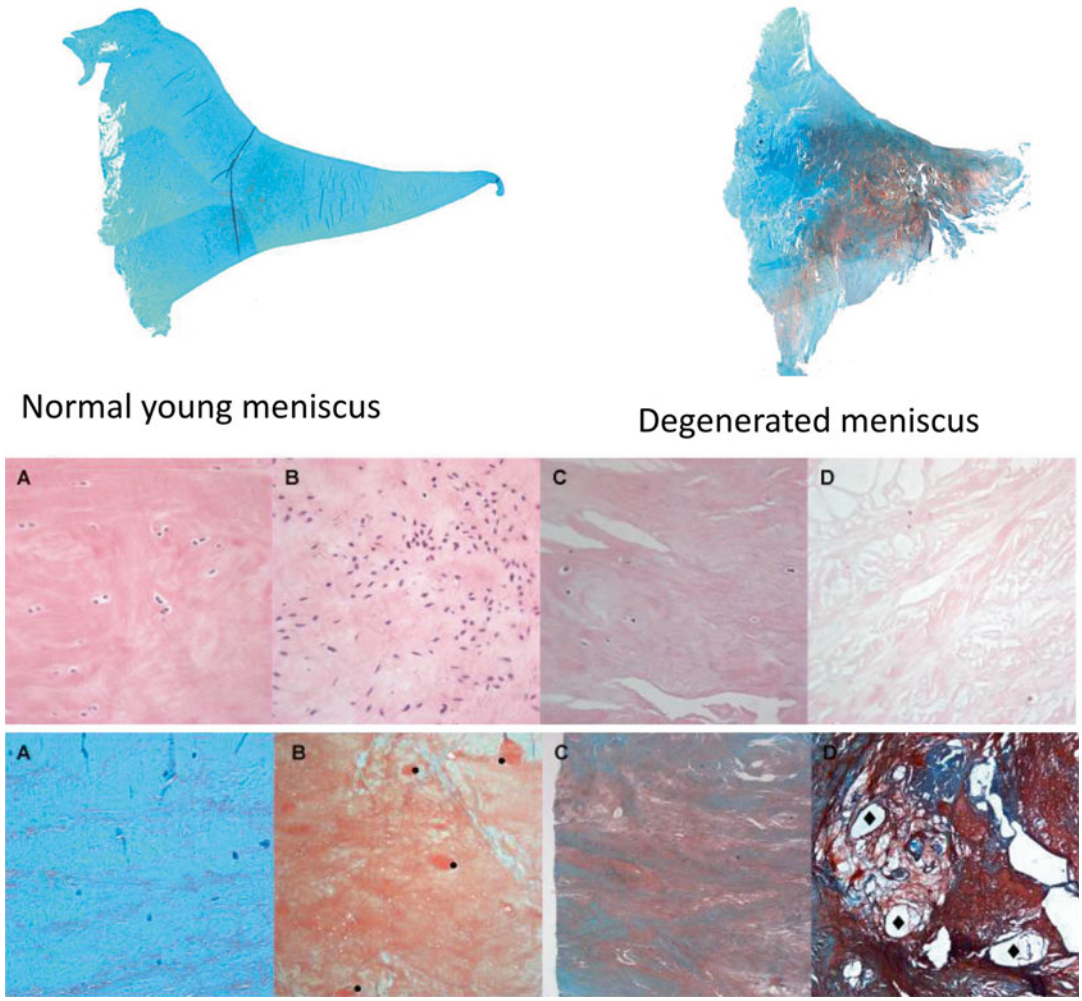

Fig. 14.3 Aging-associated changes in meniscus. Menisci were collected from human knees at autopsy. Representative samples were selected to illustrate different stages and severities of aging-associated changes. *Top panel* shows cross sections of human medial meniscus stained with safranin O. *Middle panels* show hematoxylin- and eosin-stained sections of human meniscus at varying degrees of degeneration to illustrate changes in cell density and organization: (**a**) normal, (**b**) diffuse hypercellularity, (**c**) diffuse hypo-/acellular regions, (d) hypocellularity, empty lacunae, and pycnotic cells. *Bottom panels* show safranin O-stained sections of human meniscus at varying degrees of degeneration to illustrate changes ECM organization. (**a**) Normal meniscus, collagen fibers organized, homogenous eosinophilic staining of ECM; (**b**) collagen fibers organized, diffuse foci of hyaline or mucinous degeneration; (**c**) collagen fibers unorganized, confluent foci or bands of hyaline or mucinous degeneration, fraying; (**d**) collagen fibers unorganized, fibrocartilaginous separation (edema, cyst formation), severe fraying and tears

and outer zone populations remains similar, inner zone populations produce significantly higher levels of proteoglycan and exhibit a more chondrogenic phenotype than outer zone cells [99]. Diminished cell density with acellular regions has been noted with age, most dominant in the inner regions of the meniscus [100]. Diminished cellularity is present in tears of meniscus tissue in patients >40 years making that tissue potentially more vulnerable to degeneration and retear following repair compared with younger tissue [101].

Changes in Meniscus Vascularity

Changes in vascularity with age represent an intriguing aspect of meniscus tissue homeostasis and repair capacity that could contribute to an influence of age on PTOA. The main blood supply to the meniscus originates from branches of the geniculate arteries that ultimately penetrate the meniscus through its peripheral attachment to the surrounding capsular and synovial tissues. Branches of the geniculate arteries give rise to a perimeniscal capillary plexus that supplies the periphery, ranging from 10 to 30 % of the total meniscus width in humans [102]. The remaining central portion of the meniscus receives its nutrition by diffusion. The meniscus is fully vascular at birth, but during the second year of life, an avascular area develops in the inner region. By the second decade, the lateral third of the meniscus is vascularized followed by a decline to the outer quarter by age 50 years [103, 104]. Tears in the vascular (red zone) and vascular-avascular junction (red-white zone) can be repaired, while avascular tears (white zone) are typically resected [105]. Some clinicians have reported repairs in the avascular zone and have shown good outcomes in terms of initial improvement in symptoms, but higher rates of incomplete and failed healing present in these tears with age [106, 107].

Animal studies of meniscus healing have shown the expression of vascular endothelial growth factor (VEGF) at higher levels in the avascular region compared with the vascular region, and externally applied VEGF could not augment healing in avascular regions, suggesting that failure of healing in the avascular zone is not due to cellular inability to generate angiogenesis signals [108, 109], but rather due to other possible issues of intrinsic healing potential, vascular and cell repair supply, and growth or inhibitory factors.

Changes in Meniscus ECM

The function of the meniscus is reflected in the biologic and architectural composition of the extracellular matrix. Histologically, the meniscus is a fibrocartilaginous tissue populated primarily by fibrochondrocytes and type I collagen. Other extracellular matrix proteins include collagens II, III, V, and VI as well as elastin, proteoglycans, and glycoproteins [110, 111]. The extracellular matrix components are arranged to accommodate compressive, shear, and hoop stresses within the environment of the knee [112, 113]. It has been shown that meniscus tissue composition varies with the aging process, particularly in collagen fiber organization (Fig. 14.3).

Specific matrix proteins have been shown to be altered by aging in the meniscus. Perlecan, a large multidomain heparin sulfate- or chondroitin sulfate-substituted proteoglycan, declines with age relative to aggrecan and type I, II, and IV collagen [114]. Perlecan is present in the middle and inner meniscal zones and is expressed by cells of oval or rounded morphology [114]. In contrast to the other components visualized by this study which dropped marginally or remained relatively constant with age, perlecan was strongly cell associated, and its levels steadily declined with the onset of age and a loss of viable cells in the meniscus [114]. As an ECM component with roles in ECM organization, stabilization, cell attachment, and migration, and with binding capacity for fibroblast growth factors and connective tissue growth factor, the loss of perlecan with age has potential degenerative effects on meniscus ECM.

Meniscus total proteoglycan synthesis rates have been shown to be lower in older tissues (20–62 years) than in younger tissues (<20 years) with a higher proportion of decorin in younger tissues [115]. With age, there is an increase in aggrecan synthesis and mRNA expression as the major biosynthetic product of mature fibrochondrocytes. In contrast to lower synthesis rates with age, mRNA expression levels for decorin have been shown to increase with age [115]. Decorin is a small pericellular matrix proteoglycan that has the ability to bind type I collagen fibers and control fibril diameter thus contributing to its role in stabilizing and organizing the ECM of the meniscus [115]. Decorin also binds and sequesters the anabolic factor TGF-β which has a putative role in mediating intrinsic repair [115].

As a major structural component of the meniscus (60–70 % dry weight), the network of collagen fibers within the meniscus represents a major functional unit of the meniscus. Collagen cross-links occur via posttranslational modification in newly synthesized collagen, and intermolecular cross-links may play an important role in the pathologic progression of OA and aging in the meniscus. Pyridinoline and deoxypyridinoline are considered physiologic cross-links that maintain the structure of the collagen fibril and contribute to normal collagen function. The development of pathologic cross-links with age results in loss of elasticity of collagen and decreased proteolytic susceptibility upon accumulation of AGEs, such as pentosidine, which has been shown to increase with age [116, 117]. Analyses of OA and normal meniscus across a spectrum of ages have shown decreased pyridinoline and deoxypyridinoline cross-links with OA. In contrast, these cross-links did not change with age; rather, there was an exponential increase in the pathologic cross-link, pentosidine, with age consistent with susceptibility to age-associated degeneration of the meniscus. Both inner and outer regions of the meniscus demonstrated these findings [118].

Amyloid deposits can accumulate in certain tissues with age, and the meniscus seems to be particularly susceptible [119]. A study which examined the structure and chemical nature of amyloid in the meniscus determined that it was formed from deposits of apolipoprotein A-I made by meniscal cells [120]. Although the effects of amyloid deposits on the meniscus have not been determined, they could certainly interfere with normal meniscus function and cause cytotoxicity.

The biologic findings of reduced cellularity, diminished vascularity, and declining ECM properties are reflected in clinical findings. Although the available epidemiologic data may not be fully accurate, it is very uncommon for patients <10 years (high vascularity, cellularity, and healthy ECM) to present with acute meniscus tears unless they have a predisposing congenital abnormality such as a discoid meniscus [121]. With an extended vascular zone in such patients, attempted repair is almost always recommended regardless of tear morphology. In the adult and older population, healing is decreased in complex and degenerative tears as well as avascular tears [122, 123]. With age (>40 years), more tears have complex and horizontal morphology compared with bucket-handle, longitudinal, and radial tears being dominant in young as well as more tears occurring in the medial compartment in old versus young [122]. Age alone is not a contraindication to repair, but repair is less likely to be pursued due to degenerative tissue quality and tear complexity rather than exclusion with age alone [122, 123].

While these studies collectively provide important insight into the role that the meniscus plays in the biology of age-associated PTOA, they are merely a starting point for truly understanding this process. With the incidence of degenerative meniscus tears increasing with advancing age, gaining a better understanding of the mechanisms of meniscus degeneration could lead to better therapeutic strategies and interventions for meniscus disorders and OA prevention.

Ligaments

Joint injury is often associated with damage to ligaments, and in particular in the knee joint, ACL rupture is a major determinant of the risk for the development of PTOA [124, 125]. The ACL is essential for knee kinematics especially in rotation and functions as an anterior/posterior stabilizer [126, 127]. In the setting of ACL deficiency, the articular cartilage, as well as the menisci, in the medial tibiofemoral compartment is more susceptible to arthritic change than the lateral compartment [128]. A large number of OA patients without prior history of ligament injury have ACL deficiency at the time of total knee arthroplasty [10, 129], and a correlation between the radiologic OA grade and the histological grade of ACL degeneration has been reported in end-stage OA [130]. In addition, ACL rupture is more common among patients with symptomatic knee OA. As noted above, between 22 and 35 % of these patients have incidental

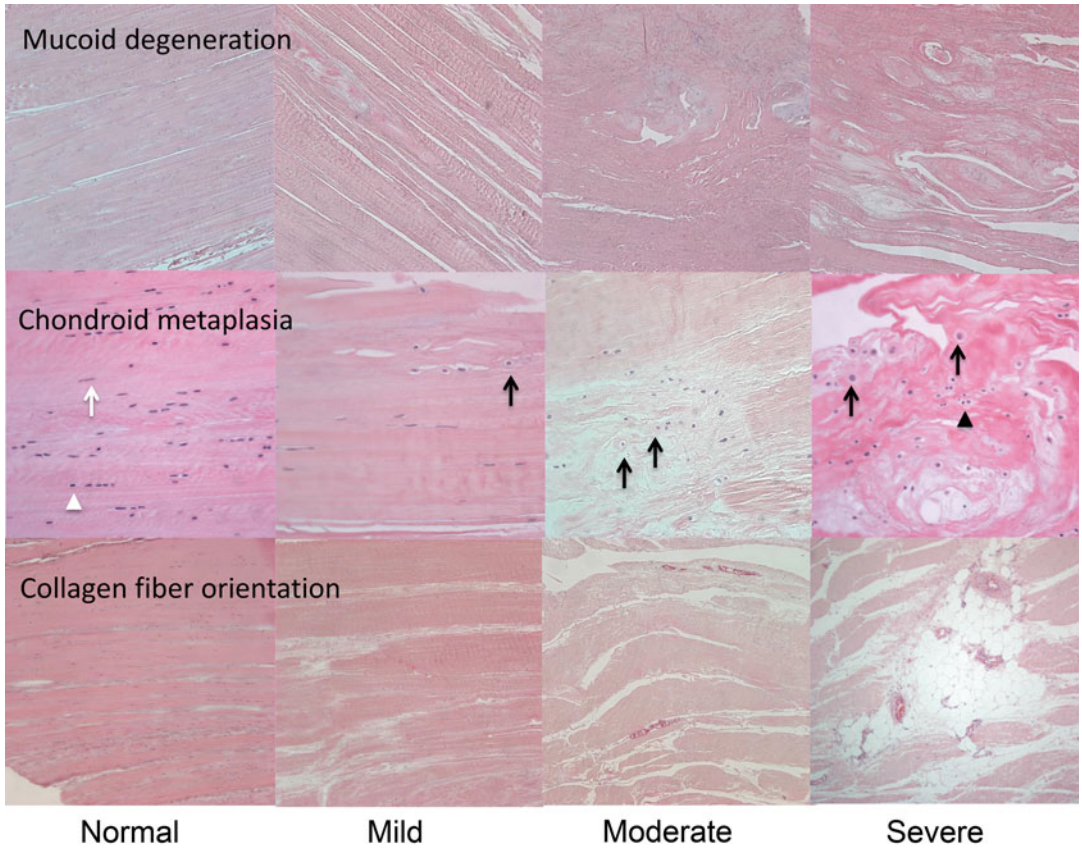

Fig. 14.4 Aging-associated changes in ACL. ACLs were collected from human knees at autopsy. Representative samples were selected to illustrate different types and severities of aging-associated changes

complete ACL tears identified by MRI [10, 131, 132]. It has been reported that fewer than half of subjects with ACL rupture recall a knee injury, suggesting that this risk factor for knee OA is under recognized [10].

Histological abnormalities in ACL are highly prevalent in knees with severe OA and include cystic changes, disorientation of collagen fibers, and mucoid degeneration [130]. However, histological changes are present at early stages and can precede cartilage histopathology. An analysis of the ACL in a large number of human knee joints across the entire adult age spectrum addressed aging-related changes in the ACL and their relationship with changes in cartilage [133]. Degenerated ACL was found in knees without cartilage degeneration. Also, knees with minimal cartilage degeneration can have moderate to severe ACL damage. These findings suggest that ACL degeneration can be initiated before or progresses more rapidly than cartilage degeneration, at least in a subpopulation of individuals [133].

Changes in Ligament ECM

The earliest and most prevalent age-related change in the ACL extracellular matrix is disorganization of collagen fibers, which can be seen in ACL from young donors without cartilage degeneration or inflammation (Fig. 14.4) [133]. An aging-related decrease in the diameter of the collagen fibrils and a corresponding increase in the concentration of small fibrils have been described for the human ACL [134]. Mucoid degeneration of ligaments refers to the presence

of discontinuous and disorganized collagen fibers and the presence of stainable mucoid matrix (Fig. 14.4) [135, 136]. Histologically, mucoid degeneration is highly prevalent and can be observed in ACL from young donors with normal cartilage [133]. Cystic changes represent areas within fascicles that are devoid of extracellular matrix and are a relatively late event in ACL degeneration [130, 137, 138]. Calcium deposition within ACL is evident as slightly basophilic material, compatible with calcium pyrophosphate dihydrate crystals [139]. Crystal deposition is reported to occur with a frequency of 0.1 % of adult persons and to increase with age and is typically seen in older donors (age >70 years) that also have degenerated cartilage [133].

MMP-3 is a key enzyme involved in extracellular matrix degeneration [140]. Synovial fluid MMP-3 concentrations were markedly higher in knees with ruptured ACL than in normal knees [141]. MMP-3 may originate from synovial and inflammatory cells but also from cells within the ACL itself. Even normal human ACL expresses MMPs [142]. The average percentage of MMP-3 positive cells in ACL from normal knees decreased with aging, but increased in ACL from knees with severe cartilage degeneration. Cells expressing MMP-3 in degenerated ACL were predominantly cell aggregates of chondrocyte-like cells but not fibroblast-like cells. These results suggest that the decrease of MMP-3 positive cells and total cell number density with aging may reflect a reduced capacity to remodel and maintain the tissue, while increased MMP-3 positive cells in ACL from knees with severe cartilage degeneration may be caused of phenotypic changes and contribute to degeneration.

ACL Cellular Changes

Major changes in cell density, organization, and phenotype occur during ACL aging and in OA-affected joints. First, there appears to be a general reduction in cell density with age in joints that do not have cartilage destruction. Aging-related changes in ligament cell response to growth factors may contribute to cell loss [143]. This cell loss is seen uniformly in all regions of the ACL and within all tissue layers. The reduction in cellularity may compromise the ability of the ACL to maintain homeostasis and respond to injury [144]. At a certain point following cell depletion or damage to the ECM, focal areas with increased cell density emerge. This occurs in two distinct patterns, one where fibroblastoid cells are found concentrated around blood vessels and a second consisting of patches with high numbers of chondrocyte-like cells. This local increase in cell numbers may be the result of cell proliferation, and in the perivascular areas, it is possible that fibroblast-like cells are infiltrating from the circulation.

Chondroid metaplasia is a well-known feature of degenerated ACL [130, 137] and was observed in knees with cartilage degeneration but not in knees with normal cartilage. Chondroid metaplasia represents an ACL intrinsic process that is associated with mucoid degeneration and is independent of inflammation. Mechanisms mediating the change in ligament cell phenotype may include mechanical and biochemical stimuli. Compression of tendons over bone can increase proteoglycan content and the proportion of round cells [145]. Biochemical mediators, such as cytokines and growth and differentiation factors, that are part of the abnormal synovial fluid composition in arthritic joints may also lead to the alteration in ligament cell phenotype. The expression of the tendon and ligament-specific transcription factor Mohawk is reduced in human ACL from aged and OA-affected knees, and IL-1 inhibits Mohawk expression in cultured ligament cells [146].

Ligament Biomechanics

These aging-related changes in ECM and cells in the human ACL can be expected to lead to altered biomechanical properties. Analyses of the human femur-ACL-tibia complex revealed an inverse relationship between mechanical strength and donor age [147, 148]. Such age-related deterioration was not observed in the patellar tendon [149, 150]. This suggests that the severity of

aging-related biomechanical changes differs among specific ligaments. This also applies to histological changes which are much more severe in the ACL as compared to the posterior cruciate ligament in the same knees [151].

Conclusions

Aging-related changes in joint tissues have been described which would make joints more susceptible to developing PTOA (summarized in Table 14.1). Most of the work to date has been on articular cartilage, meniscus, and ligaments. Changes in both the cells and ECM in these tissues are highly prevalent and closely associated with joint tissue degeneration suggesting common mechanisms. Prominent among these are loss of cellularity, senescence of the remaining cells, and increased cross-linking of matrix proteins, especially collagens, due to advanced glycation end products. Such changes compromise the biomechanical properties of the individual tissues, leading to abnormal mechanical load on the others. In the setting of knee injury, older individuals are at increased risk for meniscal tears and/or ACL rupture, which further increases the risk for the development of PTOA.

Studies of PTOA in animal models can be informative but must include the age of the animal as an important variable. Many studies have been performed in very young animals which do not reflect the equivalent human ages where PTOA is most likely to develop. Differences in gene expression after joint injury, combined with age-related differences in the cells and matrix, are important considerations. What has been learned from gene knockout or gene overexpression in very young mice may or may not apply to the development of OA in older adult humans.

Future studies testing the efficacy of a therapeutic intervention applied after joint injury will need to consider age as well. Something that works in young animals may not work in older adult humans. This appears to be particularly important in considering growth factor therapies due to a reduced response of joint tissues to growth factor stimulation with age. Although it may be more costly and time consuming to perform preclinical studies in older animals, the information gained may be well worth the investment.

Table 14.1 Changes related to aging in joint tissues that could contribute to the development of post-traumatic OA

Joint tissue	Cell changes	Matrix changes
Articular cartilage	↓ Cell numbers, ↓ autophagy, ↑ oxidative stress and damage, ↑ senescence-associated secretory phenotype, ↓ response to growth factors	↑ AGEs, ↑cross-linking, ↓ aggrecan size, ↓ hydration, ↑ collagen cleavage, ↑ calcification
Meniscus	↓Cell numbers (inner region), ↓ repair capacity	Disorganization of collagen fibers, ↑ AGEs, ↑ cross-linking, ↓ vascularity, ↓ perlecan, ↑ amyloid, ↑ calcification
Ligaments	↓ Cell numbers, ↓ response to growth factors, ↓ Mohawk expression	Disorganization of collagen fibers, ↑ mucoid degeneration, ↑ calcification

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Potential Mechanisms of PTA: Cell Death

15

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Introduction

Post-traumatic osteoarthritis (PTOA) is the development of secondary osteoarthritis (OA) after severe traumatic joint injury, especially one that involves an intra-articular fracture. The initial acute injury causes structural damage to the matrix of articular cartilage. This acute damage is accompanied by cellular responses ranging from the upregulation of matrix degradative and synthetic pathways, release of oxidants and inflammatory cytokines, and cell death. In the chronic phase, residual alterations in joint biomechanics such as instability secondary to chronic joint laxity (following ligament rupture) or abnormality in articular surface geometry (such as malreduction associated with intra-articular fractures) compound the initial injury and can lead to ongoing matrix degradation with concomitant cell death.

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The significance and impact of cellular and tissue damage and the relative contributions of each component are not clear and are under active investigation. Uncovering precise contributions is further complicated by the presence of major interactions between cells and matrix. These interactions range from the biomechanical in which structural damage to the matrix increases the risk for cell damage to the biochemical in which catabolic and inflammatory responses from injured and dying cells reduce the structural integrity of the matrix [1, 2]. It seems intuitive that reduction in cellularity reduces the capacity for post-injury repair or regeneration. However, sublethal injury and responses of surviving cells can also play a significant role in the progression of damage. Finally, there are temporal shifts in the balance between anabolic and cell survival factors on the one hand and catabolic and cell death factors on the other. This overall ongoing balance presumably determines the rate of repair, degeneration, and inflammation which eventually dictates the onset, progression, and severity of PTOA.

Cartilage homeostasis is mediated by resident chondrocytes, and loss of cells due to death leads to the characteristic features of OA tissue including loss of cartilage extracellular matrix (ECM) and abnormal tissue remodeling, the latter most likely an attempt of the remaining cells to repair degenerating tissue [3, 4]. Parallel changes in other joint tissues are also evident, such as subchondral bone remodeling and synovial

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inflammation [5–9]. Substantial evidence has linked post-traumatic cell death to PTOA [10–15]. Strategies aimed at preventing or rescuing cells from cell death or mitigating the effects of cell death are very attractive for controlling the progression of PTOA. These approaches are conceptually similar to the current treatment for myocardial infarction and cerebrovascular stroke where targeting critical cellular and tissue response has been credited with substantial reduction in morbidity and mortality.

This chapter compares the various types of cell death that have been documented after mechanical injury, reviews the potential mechanisms and pathways leading to post-traumatic cell death, discusses possibilities for therapy to prevent cell death as a means of reducing ongoing degeneration, and suggests directions for future research that are necessary to advance our understanding of cellular death and therapeutic implications.

Types of Cell Death

Traditionally, cell death has been classified into three major modes: apoptosis (type I), autophagy-associated cell death (type II), and necrosis (type III). The active process of apoptosis is identified by specific biochemical events including caspase activation, cytochrome *c* release, internucleosomal DNA fragmentation, and the formation of membrane-enclosed apoptotic bodies that are typically cleared by phagocytosis. Morphological features such as double-membrane vacuole formation are characteristic of autophagy, while necrosis is distinguished by the absence of these events. In the absence of phagocytosis, in tissues such as cartilage, accurately defining necrosis can be difficult since apoptotic cells will eventually appear as secondary necrotic cells sharing the morphological features of primary necrosis [16]. In 2009, the Nomenclature Committee on Cell Death (NCCD) outlined definitions of distinct cell death morphologies and cell death-related terminology series [17]. However, in 2012, NCDD reduced the focus on morphological features and instead recommended molecular definitions of cell death modalities due to advances in biochemical and genetic analyses of cell death [18]. Applicable to both in vitro and in vivo cell death environments, the committee now describes the functional classification of cell death to include (1) extrinsic apoptosis, (2) caspase-dependent or caspase-independent intrinsic apoptosis, (3) regulated necrosis, (4) autophagic cell death, and (5) mitotic catastrophe. Under these classifications, cell death subroutines are now defined by a series of precise, measurable biochemical features, each of which is briefly expanded on below (see Figs. 15.1 and 15.2 for an overview of pathways).

Extrinsic Apoptosis

Cell death is mediated by extracellular stress signals that are detected and propagated by specific transmembrane death receptors (Fig. 15.1). Examples of extrinsic binding of lethal ligands to death receptors include (1) tumor necrosis factor α (TNF-α) by TNF-α receptor 1 (TNFR1), (2) FAS ligand by CD95 ligand (FASL/CD95L), and TNF (ligand) superfamily, member 10 (TNFSF10), or TNF-related apoptosis-inducing ligand (TRAIL) by TRAIL receptors (TRAILR) 1–2 [19–21]. In contrast, the so-called dependence receptors can signal an extrinsic proapoptotic signal, including netrin receptors, which can exert lethal functions when specific ligand concentrations fall below a critical concentration [21].

Ligand and death receptor interaction activates three major lethal signaling cascades leading to caspase activation including (1) activation of the caspase-8 (or caspase-10)-caspase-3 cascade; (2) activation of the caspase-8-tBID-mitochondrial outer membrane permeabilization (MOMP)-caspase-9-caspase-3 pathway; or (3) ligand deprivation-induced dependence receptor signaling followed by (direct or MOMP-dependent) activation of the caspase-9-caspase-3 cascade [18] (Fig. 15.1). Due to a common dependence on caspase activity, these death pathway cascades can be suppressed by the overexpression of viral inhibitors of caspases like cytokine response modifier A (CrmA) [22] or by pan-caspase chemical inhibitors such as N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-fmk) [11, 23].

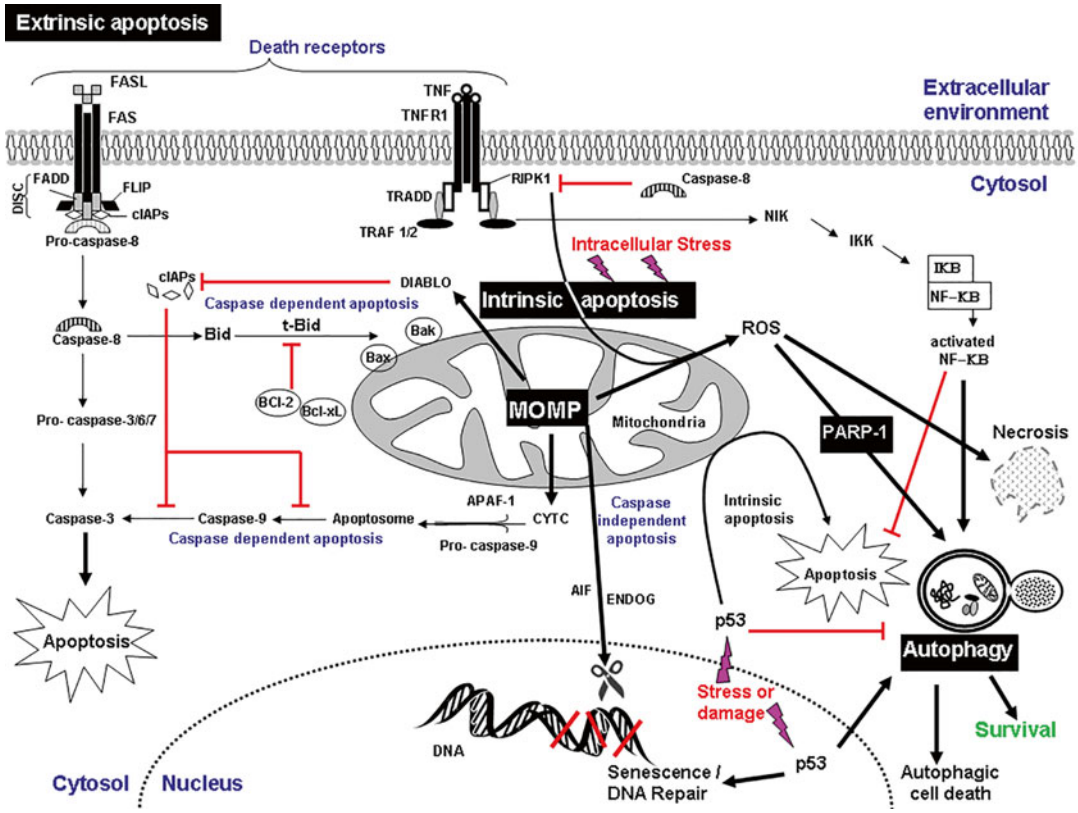

Fig. 15.1 Overview of cell death-related pathways. Extrinsic apoptosis involving death receptors and extracellular ligands leads to the activation of caspases. Inhibition of caspases is mediated by various molecules including cellular inhibitor of apoptosis proteins (cIAPs), BCl-2, and Bcl-xL. Intrinsic apoptosis is mediated by a largely mitochondria-centric manner that involves either caspase- or non-caspase-directed cell death. Mitochondrial outer membrane permeabilization (MOMP) leads to mitochondrial transmembrane potential dissipation and to subsequent release of reactive oxygen species (ROS) and an assortment of proteins such as cytochrome *c* (CYTC), which interacts with other cytoplasmic proteins to form the apoptosome, a multiprotein complex that triggers the proteolytic cascade including caspase 9 and the executioner caspase 3. MOMP also leads to the release of direct IAP-binding protein with low pI (DIABLO), which inhibits cIAPs activity. Other molecules such as apoptosis-inducing factor (AIF) and endonuclease G (ENDOG) act independently from caspase activity and translocate to the nucleus to induce large-scale DNA fragmentation. The release of ROS can lead to necrotic cell death or can trigger survival pathways including autophagy [149] (see Fig. 15.2). Autophagy can also be activated by extrinsic TNF signaling via NF-κβ activation. The location of p53 can initiate different pathways. Following cellular stress or damage, nuclear p53 can directly induce autophagy, while cytoplasmic p53 induces apoptosis (Figure adapted from [18, 150]

Intrinsic Apoptosis

Intrinsic induction of apoptosis involves bioenergetic and metabolic catastrophe together with multiple active executioner mechanisms that may or may not include caspase activity (Fig. 15.1). Overall, intrinsic apoptosis is mediated by mitochondrial outer membrane permeabilization (MOMP) and is always accompanied by dissipation of mitochondrial transmembrane potential, a release of mitochondrial intermembrane space (IMS) proteins into the cytosol, and inhibition of the respiratory chain. Caspase activity involvement in intrinsic apoptosis involves MOMP, cytosolic cytochrome *c* interaction with APAF1 and dATP to form the apoptosome, and triggering of the caspase-9-caspase-3 proteolytic cascade [24]. Independent of caspases, apoptosis-inducing factor (AIF) and endonuclease G (ENDOG) relocate from the mitochondria to the nucleus to mediate DNA fragmentation [25–27] (Fig. 15.1).

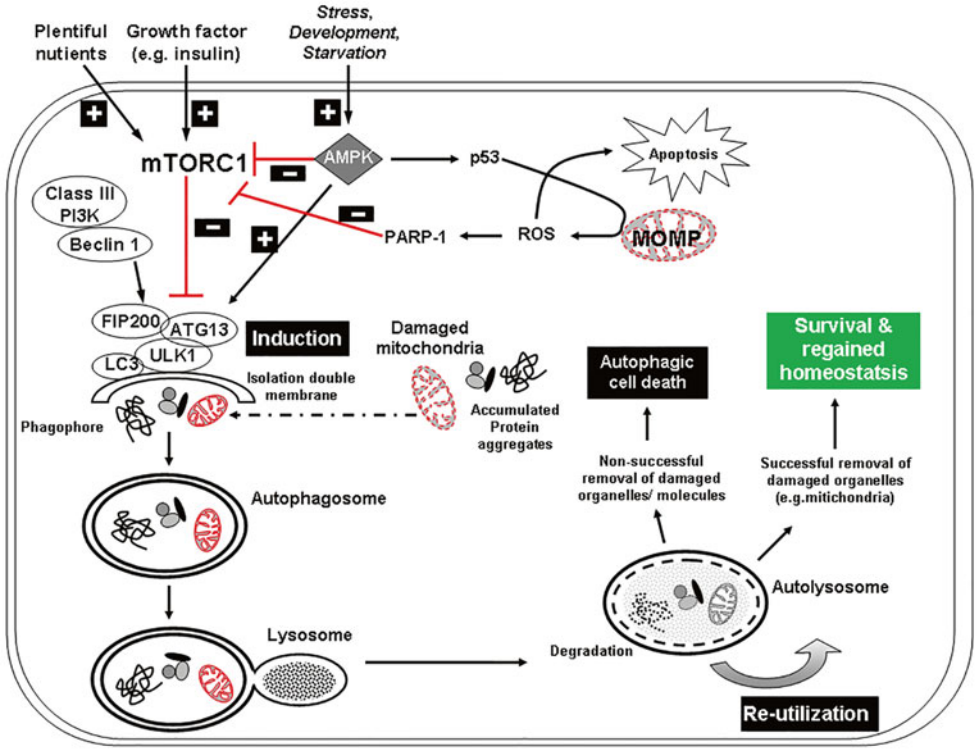

Fig. 15.2 Autophagy pathway in homeostasis and response to stress. As a major degradation pathway in eukaryotic cells, autophagy is essential for the removal of damaged organelles and protein aggregates from the cytoplasm that can lead to cellular dysfunction [151, 152]. Three central kinases are pivotal for controlling cell homeostasis including AMP kinase (AMPK), the mammalian target of rapamycin complex 1 (mTORC1), and Unc-51-like kinase 1 (ULK1). mTORC1 inhibits autophagy by preventing the induction of the double-membrane phagophore and subsequent autophagosome formation by phosphorylating autophagy-related protein 13 (Atg 13) and ULK1, thus preventing the formation of a kinase complex [153–155]. mTORC1 can simultaneously inhibit autophagy and stimulate protein synthesis and cell growth, which can lead to an accumulation of damaged proteins and organelles and hence contribute to damage at the cellular level [156]. Following external or internal stressors, such as injury, starvation, energy depletion, and during development, AMPK can initiate apoptosis via p53 or can inhibit mTORC1's capacity to inhibit autophagy and activate ULK1, thus initiating higher autophagic activity [27, 157]. As well, ROS can induce PARP-1 activity to reduce mTOR signaling and activate autophagy [149]. The cytoprotective response that autophagy provides is a means for the cell to recover following stress and restore homeostasis. However, when the autophagic response is not sufficient, imbalanced, or excessive, this may lead to cell death [47, 158, 159]

Many intracellular stress conditions can trigger intrinsic apoptotic demise, but are principally controlled by or coordinated with the mitochondria. Documented stressors include oxidative stress, DNA damage, cytosolic Ca2+ overload, mild excitotoxicity, and accumulation of unfolded proteins in the endoplasmic reticulum (ER), among others [28].

Regulated Necrosis

While cell death in the form of necrosis can result from a traumatic event, as defined by the absence of apoptotic or autophagic features [17, 29], it is now recognized that necrosis can also occur in a regulated manner [30–34] in many physiological and pathological contexts [35].

Under specific circumstances, the induction of regulated necrosis or "necroptosis" can be induced by several ways including ligation of death receptors (e.g., TNFR1), excitotoxicity, and alkylating DNA damage, involving receptor-interacting protein kinase (RIP1) RIP3 and caspase-8 [30–34, 36, 37].

Autophagic Cell Death

Autophagy is a controlled cellular catabolic process that is critical for cell survival, differentiation, development, and homeostasis that involves the formation of double-membrane vesicles, called autophagosomes [38, 39] (Fig. 15.2). Autophagosomes deliver cytoplasmic contents (typically unwanted organelles or protein aggregates) to lysosomal machinery for degradation and recycling. In addition to homeostasis, the autophagic pathway also has a cytoprotective role as it is stimulated to counter various cellular or subcellular stresses for cell survival including reactive oxygen species (ROS), DNA damage, dysfunctional organelles, hypoxia, protein aggregates, or intracellular pathogens [40] (Fig. 15.2). In skeletal tissues, the autophagic system enables osteoblasts, osteoclasts, and chondrocytes to survive within the local hypoxic, and even hypertonic environment, and to overcome the presence of stressors and nutrient deficiencies. However, under situations of chronic stress or certain physiologic and pathologic states, cell death by autophagy can occur [17, 39, 41–44] and in most cases due to an inhibition or reduced activity of this process [45]. Dysregulation of this process has been associated with a variety of human pathophysiological processes, such as aging, cancer, cardiovascular diseases, neurodegenerative disorders, and OA [46, 47].

Mitotic Catastrophe

Although mitotic catastrophe has been thought to be a separate mode of cell death, more recently it has become evident that mitotic catastrophe does not execute cell death in the pure sense, but rather it represents an oncosuppressive mechanism that leads to either apoptosis, necrosis, or cell senescence to eliminate mitosis-deficient and genomically unstable cells [48].

Anoikis or Oncosis

Another distinct form of cell death is principally regulated by changes or loss in adhesion between integrins and the epidermal growth factor receptor (EGFR) and the extracellular matrix [49, 50]. Anoikis displays some features associated with necrosis, including increased membrane permeability, cell and organelle swelling, and absence of internucleosomal DNA fragmentation [51, 52]. However, the inhibition of extracellular signal-regulated kinase 1 (ERK1) signaling and an overexpression of the BCL-2 family member BIM [50, 53] can be used to identify this phenomenon. It is important to note that downstream of anoikis-initiated cell death, the same molecular machinery for intrinsic apoptosis is utilized (Fig. 15.1). Cell death resembling anoikis has been observed in ischemic heart disease [54], in atherosclerotic lesions [55], and in both cartilage and bone [56].

Chondroptosis

In cartilage tissue, Roach et al. used the term "chondroptosis" to indicate a specific form of chondrocyte apoptosis [57] that involves altered protein synthesis as observed by an increased number of endoplasmic reticulum (ER) and Golgi apparatus, which appears distinct from typical receptor-mediated or mitochondrial pathways. The ER membranes appear to segment the cytoplasm to produce autophagic vacuoles in the cytoplasm where organelles are digested and disposed into the lacunae. This divergent cell death process is consistent with the avascular nature of cartilage, where chondrocytes are isolated within their lacunae and cannot rely on the phagocytic removal or apoptotic cell remnants typical of other tissues [58].

Mechanisms of Post-Traumatic Cell Death

Mechanical Stress

Mechanical injury is known to trigger cell death, and the resultant cartilage matrix degradation has been reported in animal [11, 59–63] and human cartilage [11, 64–66]. Acute impact or more chronic repetitive injury can significantly reduce cell viability. In human cartilage explants, a single episode of mechanical injury increased the number of apoptotic cells in a time-dependent pattern, which was inhibited by incubating the impacted explants with the pan-caspase inhibitor z-VAD-fmk [11]. Repetitive trauma can also induce apoptosis in vitro [67]. In vivo, the cartilage degeneration induced by repetitive injury (anterior cruciate ligament transection in rabbit knees) was significantly reduced with caspase inhibition supporting a potential therapeutic role [23]. Other studies also demonstrated the potential therapeutic role of caspase inhibitors [11, 66], BMP7 [68], FGF-18 [59], P188 surfactant [65], and inhibitors of focal adhesion kinase (FAK) and Src family kinase (SFK) [69] for chondroprotection after impact trauma or tissue injury as a result of a surgical procedure [70].

Early cell death was reduced by a nonionic surfactant (P188), which suggested the loss of cell membrane integrity as one mechanism by which blunt trauma can affect cell viability [71]. P188 has also been shown to affect stress-related p38 signaling and the inhibition of GSK3 and IL-6 suggesting other pathways leading to cell death [72].

Autophagy

Autophagy involves recycling of long-lived protein organelles and is a critical process for cellular homeostasis and cytoprotection. Cell stress stimulates autophagy, for example, autophagy is upregulated in response to ischemia/reperfusion and pressure overload in the heart [73, 74]. Therefore, autophagy may be similarly affected in mechanoresponsive tissues such as cartilage. Recent observations indicate that there is a basal level of autophagy in normal cartilage and this is increased in response to nutrient deprivation [75]. In aged and OA-affected human articular cartilage and in animal models of OA, such as surgically induced joint instability, there is a marked reduction in the expression of important proteins that regulate the autophagy pathway including ULK1, Beclin1, and LC3, which was accompanied by increased apoptosis [76] (Fig. 15.2) and may also play a role in programmed cell death implying complex interactions.

Several instances of crosstalk are known between members of autophagy and apoptosis pathways [77, 78]. The autophagy protein, Atg5, induces mitochondria-based apoptosis, while Bcl-2 overexpression protects against Atg5-mediated mitochondrial dysfunction. Beclin 1, an essential autophagy protein, is regulated by the Bcl-2 proteins and in normal conditions. Bcl-2 and Bcl-XL suppress autophagy by associating with Beclin 1 [79]. Reduced Beclin 1 heterozygous mice (Beclin 1+/–) have reduced autophagy and apoptosis and heart infarct size after I/R injury [80], suggesting that Beclin 1 might be actively involved in mediating apoptosis.

LC3-II and Beclin-1 are both expressed by chondrocytes in the maturing region of the growth plate. In the hypertrophic zone, there is reorganization of these proteins into punctate granules that are characteristic of the autophagolysosome, while TEM studies indicated the presence of double-membrane autophagosomes. Once mineralization has begun, autophagic changes in hypertrophic chondrocytes are seen followed by the initiation of programmed cell death [81]. Further research into the relationship between mechanical injury, apoptosis, and autophagy may reveal additional targets to preserve articular cartilage viability.

Oxidative Damage

Mechanical injury can release reactive oxygen species (ROS). ROS are formed during metabolism of oxygen and play important roles in cell

signaling. ROS activity is dynamically balanced by enzymatic and nonenzymatic antioxidants, which act by inhibiting oxidative enzymes or via scavenging free radicals [82]. Increased levels of ROS (oxidative stress) can be damaging and impact cell survival. Mitochondria are a source of post-injury ROS, and chondrocytes express enzymes (such as NADPH oxidase and iNOS) that generate superoxides. Superoxide release can occur within five minutes of impact injury [83]. Decreased mitochondrial superoxide dismutase within OA chondrocytes affects chondrocyte intracellular metabolism [84], and superoxide dismutase mimetic increased cell viability after injury [85]. Further indirect evidence of the oxidative cell death is provided by reports of antioxidant treatment that enhanced cell viability after injury [83, 85]. The antioxidant resveratrol also protected against IL-1beta-induced catabolic effects and prevented IL-1 induced chondrocyte apoptosis via its inhibition of mitochondrial membrane depolarization and ATP depletion [86]. Finally, oxidative stress can have implications beyond post-traumatic cell death. Antioxidative capacity diminishes in degenerating regions of OA cartilage, and the resulting oxidative stress induces replicative senescence and telomere genomic instability [87]. Inadequate control of ROS may be one of the central factors in OA pathophysiology.

Extracellular Matrix

Chondrocytes secrete and maintain a spectrum of proteins serving multiple functions in addition to structural support, such as providing a reservoir for cytokines and growth factors that regulate cell behavior, proliferation, and differentiation, all providing cues that are critical for cell survival [88–90]. Loss of glycosaminoglycan can indirectly predispose cells to necrosis or programmed cell death following mechanical injury [91]. Significant changes in the structure and composition of the perichondrocytic environment during the progress of PTOA can alter the transmission of physical forces to the cell as well as biochemical stimuli that regulate cell response [92]. Ongoing degeneration, deterioration, disintegration, and erosion of matrix lead to an expanding zone of cell death that further intensifies tissue degeneration. Profound changes in adhesion molecules and ECM signaling via receptors can directly activate apoptotic pathways (e.g., FAS and TNF-αR), induce anoikis through loss of cell adhesion, or secondarily induce apoptosis, for example, via cytoskeletal changes [93–96].

Anoikis is a variant of apoptotic cell death as a result of lost, reduced, or inappropriate cell adhesion (see review by [92]). Despite the initiation via changes in the ECM, the downstream apoptotic process is mediated through pathways that converge to activate caspases. Several ECM proteins are essential for cell survival; among them collagen type II is the most critical in maintaining chondrocyte viability and preventing apoptosis [97]. Integrin binding to ECM components is also important for cell survival. Proteins important for binding to integrin receptors are laminin, fibronectin, and collagen types II and IV [88, 98]. Several studies directly implicate integrin-ligand interactions in chondrocyte death. For example, blocking antibodies against the integrin α5 subunit (CD49e) induced death in human chondrocytes [99]; RGD peptides reduced cell viability in cultured chicken chondrocytes [100] and induced apoptosis in cultured chondrocytes and in cartilage explants [101]; and type X collagen deposition and chondrocyte survival in chicken sterna are dependent on CD49b and CD49c integrin subunits [93].

Although ECM proteins are important for cell survival, it is well known that ECM protein fragments have deleterious effects: the more common response being inflammatory and catabolic. For example, the 29 kDa fragment of fibronectin induces inflammatory responses [102] and catabolic proteases such as matrix metalloproteinase-13 (MMP-13) [103]; fragments of hyaluronan generate nitric oxide [104] and can increase MMP-13 production [105]. However, a synthetic peptide fragment of type II collagen (residues 195–218) (CB12-II) without the RGD sequence induced apoptosis in bovine cartilage explants in a manner related to chondrocyte hypertrophy [106, 107].

Mitochondrial Damage

The evidence for mitochondrial involvement in cell death is overwhelming (see reviews [108–111]). Mitochondrial dysfunction has been linked to apoptosis, aging, and OA [82, 112–115]. Although IL-1 is not generally considered a proapoptotic signal, IL-1 can induce apoptosis in chondrocytes by inducing mitochondrial dysfunction and depleting cellular energy stores [114]. In addition to the mitochondrial involvement in NO-induced apoptosis [116], oxidative stress and mitochondrial dysregulation play an important role in the initiation and progression of OA [117, 118]. Mitochondria are involved in epiphyseal chondrocyte death during bone development and are associated with maturation-dependent reduction of oxidative phosphorylation [119] and changes in Bcl-2 protein levels [120]. The causal relationship between phosphate ions, NO production, and mitochondrial dysfunction in growth plate chondrocytes suggests a similar mechanism in PTOA [121, 122].

p53 and c-Myc

p53 is commonly involved in increased cell death in aging cartilage. In arthritic cartilage, DNA fragmentation positively correlated with p53 [123]. p53 increased in articular cartilage with aging and was associated with decreased viable cell density in rabbits [124]. p53 is also implicated in nitric oxide-induced cell death via p38 MAP kinase and NF-κβ supporting an active role in chondrocyte survival [125]. In cultured human chondrocytes, hydrostatic pressure-induced apoptosis was associated with increased p53 expression [126].

c-Myc is associated with hypertrophic differentiation of chondrocytes [127, 128] and frequently colocalized with cells containing breaks in DNA strands. c-Myc levels increased in areas of cartilage erosions in canine models of OA [129] and colocalized with apoptotic cells in human arthritic cartilage [123]. c-Myc has also been implicated in apoptosis induced by hydrostatic loading [126]. These findings suggest that, in addition to developmental cell death, p53 and c-Myc may also regulate injury- and OA-related chondrocyte death.

Therapeutic Targets

Despite the common etiology of joint trauma, the multifaceted nature of development and progression of post-traumatic arthritis indicates that effective therapy will likely involve multiple approaches. Matrix homeostasis relies on a balance between net anabolic and catabolic activities, which are directly influenced by the number of available chondrocytes. The weight of existing evidence offers chondrocyte death as an excellent target for therapeutic intervention in OA. To achieve prophylactic and therapeutic success, further research into chondrocyte death, cartilage degeneration, and arthritic progression is required. Several potential treatments are being actively translated to clinical application.

Inhibition of Apoptosis

Caspase inhibitors and other chondroprotective agents such as BMP7 [68] are being translated for clinical use. Caspase inhibition is being pursued in several diseases, including acute and chronic neurodegenerative diseases, myocardial infarction, and liver apoptosis [130–132]. Application of these clinically relevant inhibitors may facilitate translation into the treatment of acute post-traumatic joint injuries. A proof of principle for the efficacy of caspase inhibitors in attenuating cartilage damage has been provided in vitro and in animal models [11, 23, 66]. Mechanical injury to chondrocytes and cartilage explants is associated with caspase activation, and caspase inhibitors reduce ECM damage and protect against cell death. Studies with cartilage explants showed that the cartilage superficial zone is where the highest level of cell death occurs. Reducing damage in this zone that is critical for cartilage integrity by the use of caspase inhibitors would be expected to reduce the severity of PTOA, and this has been demonstrated in

animal models. A more precise identification of the intrinsic and extrinsic mechanisms leading to chondrocyte death is also ongoing to broaden the spectrum of antiapoptotic targets for therapeutic interventions.

Antioxidant Treatment

ROS can lead to accelerated ECM degradation and cell death. Therefore, the use of antioxidants or enhancement of superoxide dismutase to combat excess ROS has promise [133, 134]. A superoxide dismutase mimetic increased cell viability after injury in cartilage explants [85], and *N*-acetylcysteine reduced postimpact chondrocyte death in osteochondral explants [12]. Rotenone, an electron transport chain inhibitor, reduced superoxide released by mitochondria after injury and enhanced cell survival [83]. Resveratrol is a naturally occurring polystilbene (to which the health benefits of red wine have been attributed). Resveratrol has an antiapoptotic effect in osteoarthritic chondrocytes stimulated with IL-1, mediated presumably via inhibition of the induction of PGE_2 [86].

Autophagy Targets

Reduced expression of autophagy proteins has not only been observed in the knee cartilage of mice with joint instability but also in cartilage explants that were subjected to single-impact mechanical injury [76]. In such models, a time-dependent increase in cell death and ECM damage has been documented. The autophagy pathway is controlled through diverse mechanisms and signaling pathways, and a large number of pharmacological activators and inhibitors of autophagy have been identified [135]. The kinase mammalian target of rapamycin (mTOR) is a key regulator of autophagy and integrates signals from various extracellular stimuli. Cartilage-specific ablation of mTOR enhanced autophagy and protected against OA development in mice [136]. Rapamycin, an inhibitor of mTOR and an activator of autophagy, reduced the severity of degenerative changes in a mouse model that is associated with increased chronic mechanical load [137]. In the cartilage explant model, rapamycin also protected against injury-induced cell death and ECM damage. These observations suggest that deficits in autophagy contribute to cell death and ECM damage after joint injury. The availability of autophagy activators in clinical use, such as rapamycin, renders autophagy as a promising therapeutic target in PTOA.

Cell Membrane-Stabilizing Surfactants

Poloxamer surfactants are water-soluble copolymers with hydrophobic and hydrophilic chains that insert directly into and repair damaged cell membranes [71]. Poloxamer 188 (P188) increased the percentage of cell viability in vitro and in vivo within four days of injury, increasing cell density in vivo after 6 weeks [71, 138, 139]. In addition to increasing cell viability in injured cartilage, P188 also prevented secondary cell death in the uninjured tissue adjacent to the site of injury [65].

Cartilage Progenitor Cells

Despite the lack of vascularity, articular cartilage contains distinct populations of progenitor cells [140–148], most of which are found in the superficial zone [141, 142, 148]. Mechanical injury commonly induces cell death that is predominantly concentrated in the superficial zone. Losing the majority of progenitors as a consequence of injury may impact tissue homeostasis, since these cells are likely to be more suited to repair injured or degenerated tissue than the terminally differentiated chondrocyte. Preserving the progenitor subpopulation or utilizing these cells in cell therapy or tissue engineering could expand our therapeutic toolset for preventing post-traumatic OA.

Summary/Conclusion

The occurrence of cartilage cell death following joint injury has been documented in human tissue samples, in animal and in vitro models. There is also a close relationship between cell death and ECM damage, which appear to potentiate each other and thus generating a key mechanism that mediates the chronic process that ultimately manifests as PTOA. Although incomplete, there is a growing understanding of causes, mechanisms, and types of cell death following injury, and this has led to the identification of several promising therapeutic targets which have been validated in vitro and in animal models. The link between chondrocyte death and PTOA remains to be conclusively proven. The development of sustained release formulations of drugs such as caspase inhibitors or autophagy activators for intra-articular application holds great promise to reduce the effects of injury, thereby potentially reducing the risk for PTOA in the patient with joint injury.

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Potential Mechanisms of PTOA: Inflammation

16

Mary B. Goldring

Introduction

Post-traumatic osteoarthritis (PTOA) is a subset of osteoarthritis (OA) that results in similar end-stage pathology, but the initiating factors and how the disease progresses are quite distinct, thereby impacting on design of therapeutic approaches [1, 2]. OA is whole joint disorder with cartilage destruction as its hallmark, but involving changes in all joint tissues including ligaments, tendons, menisci, subchondral bone, and synovial membrane and capsule and current therapies involve symptomatic relief of pain, physiotherapy, and ultimately total joint replacement after joint failure [3, 4]. In PTOA, severe cartilage damage may occur months or years following the initial injury. The early loss of proteoglycans may not be easily detectable, except by MRI, and since the component glycosaminoglycans are renewable up to the pivotal point when more severe damage of the aggrecan core protein and disruption of the collagen network occurs, targeting early events are critical for halting the irreversible progression of OA disease.

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Following the joint trauma accompanied by damage of ligaments, tendons, or menisci, the objectives of arthroscopic surgery are not only to repair these tissues but also to correct the biomechanics of the affected joint. However, orthopedic surgeons performing these procedures are aware that the presence and extent of inflammation in the joint may affect their outcomes. Thus, surgeries are often delayed until the inflammation, which is characterized by joint swelling and synovial effusion, subsides usually with treatment with anti-inflammatory drugs. Although most investigations have focused on the cartilage as the target in the subsequent development of OA and cartilage may be one source of early diagnostic biomarkers in the synovial fluids of PTOA patients, it may not be the critical target for anti-inflammatory therapy immediately following trauma. In addition to synovial inflammation and effects of inflammatory mediators on cartilage, other tissues, including tendons, ligaments, and menisci, have important roles in maintaining joint biomechanics and contain cellular targets for inflammatory and mechanical signals [5].

A large number of studies have shown that there are different etiologies and time courses that result in the initiation and development of OA in patients that undergo operative procedures. Epidemiologic studies have established that there is a strong relationship between ACL disruption and the risk for subsequent development of OA [6–10]. Injuries to the ACL frequently occur in young patients, especially in athletes, leading to

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pain and functional impairment in young or middle-aged adults. The reported radiographic rates of OA after an ACL injury vary between 10 and 90 % at 10–20 years after the ACL injury [11, 12]. Studies of populations with meniscal injury have also been useful for identifying risk factors for PTOA [13]. Meniscal injuries are commonly seen in association with ACL injury [14, 15]. These subsets of PTOA are represented within a continuum of early, progressive and end-stage OA and include (1) anterior cruciate ligament (ACL) injury (<35 years of age); (2) acute meniscal injury (26–40 years of age); (3) degenerative meniscus (40–65 years of age); and (4) total joint replacement (>50 years of age).

Role of Inflammation in Cartilage Damage in PTOA

Injury to the ACL or meniscus alters the biomechanical dynamics of the joint, often in a young and otherwise healthy individual, and affects joint kinematics [15]. In this environment, the altered distribution of forces applied to the articular cartilage articular cartilage leads to altered mechanotransduction in the chondrocytes, activation of catabolic and inflammatory genes, deregulated matrix synthesis, and decreased repair capacity [2, 9]. While loss of articular cartilage inevitably occurs in PTOA, the cartilage responses are substantially different depending upon whether the trauma results in acute injury or chronic loading abnormalities. Traumatic injuries that result in intraarticular fracture are associated with focal loss of chondrocyte viability, due to either necrosis or apoptosis, and altered cartilage structure. In acute ACL injuries, cartilage lesions can appear macroscopically as chondral softening, chondral fractures, impaction lesions, creases, crack, or flaps and occur in the lateral compartment of the knee [16]. In chronic ACL deficiency, the joint kinematics may alter the tibiofemoral biomechanics and the normal pattern of loading on the articular surface of the knee, leading to recurrent episodes of instability. The severity of the cartilage defect graded by the Outerbridge score is correlated with age, BMI, and the chronicity of the ACL rupture [17]. While clinical outcomes are closely linked to the severity of the trauma and the subsequent cartilage degeneration, it is likely that collective damage to all joint structures and their cellular responses contribute to joint pathology and that cartilage damage may not progress rapidly without inflammation in the synovial compartment. For example, when cartilage explants undergo mechanical injury in vitro, the presence of synovial capsule shifts the chondrocyte responses to pro-catabolic pathways [18].

Although injury and traumatic joint loading are considered initiating factors in events that lead to PTOA, they are accompanied by the release of inflammatory mediators from the synovium and other joint structures [19]. The signaling events activated in chondrocytes and other resident cells are common to both mechanotransduction and cytokine stimulation [20]. A large number of studies have addressed interleukin-1 (IL-1) as a major inflammatory cytokine that drives the progression of the disease (see for review [21, 22]). Chondrocytes in OA cartilage, especially those in clonal clusters, have cell surface receptors that can respond to cytokines and chemokines produced in the synovium and other periarticular joint tissues and detected in OA synovial fluid. Although long assumed that IL1B mRNA induced in chondrocytes could result in secretion of IL-1β, the inflammasome complex consisting of NALP-3 and the IL-1β activator caspase-1 in OA cartilage, does not participate in activating the pro-IL-1β so that it can be secreted and act in an autocrine manner [23]. Many studies have shown that inflammatory cytokines stimulate expression of MMP-3, -9, and -13, which co-localize with type II collagen cleavage epitopes in regions of matrix depletion in OA cartilage and species-specific up-regulation of ADAMTS-4 and -5 in chondrocytes by inflammatory stimuli has been reported in OA cartilage [24–26].

Early studies showed that IL-1 is a potent inducer of the catabolic program, promoting cartilage matrix loss even at picomolar levels. Both in vitro and in vivo studies have demonstrated its ability to induce proteolytic enzyme synthesis in

chondrocytes, driving enhanced production of matrix metalloproteinases (MMPs) and aggrecanases that degrade the cartilaginous matrix. IL-1 is also a primary mediator of the inflammatory cascade by stimulating articular cells to produce numerous downstream effector molecules, including nitric oxide (NO), phospholipase A2, prostaglandin E2 (PGE2), as well as other cytokines such as TNFα, IL-6,-8,-15,-18 and chemokines, including IL-8, CXC-2,-5,-10, and CCL-3,-5,-7. Release of these agents further stimulates cartilage matrix degradation, bone erosion, synovitis, fibrosis, and pain sensitivity.

A critical role of IL-1 in the development of OA has been demonstrated in vivo in several animal models of PTOA, in which transection of the ACL induces IL-1 synthesis by the synovium and articular chondrocytes and the knee joints predictably develop pathologies of OA [27, 28]. Administration of IL-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of IL-1 signaling, is soon after ACL transection by repeated intra-articular injections can effectively suppress early degenerative changes in the articular cartilage of the tibial plateau and femoral condyles, block MMP synthesis, and reduce the number and size of osteophytes [29, 30]. However, it has not been possible to achieve and maintain sufficiently high intraarticular concentrations of recombinant IL-1Ra (Anakinra), which is required at 50-fold higher concentrations than IL-1. Thus, in vivo gene delivery of IL-1Ra, using a safe and effective form of adeno-associated virus, is currently under investigation in a large mammalian preclinical model, equine PTOA, as a proof-of-principle for clinical translation to human PTOA [31, 32].

Lessons from Mouse Models

We have learned much about the molecular mechanisms driving cartilage destruction from studies of gene and protein expression in clinical material and in culture models derived from human tissues. However, it is not possible to study the time course of the disease in vivo in humans. Studies in animal models, in which the ACL or other knee ligaments are transected or injured, generally model aspects of PTOA that may be translated to humans. Although the focus has been on the biomechanical impact on cartilage damage [33], the availability of transgenic and knockout mice has permitted the examination of roles of inflammatory genes and associated pathways in promoting the initiation and development of OA. Among the models of PTOA in mice [34, 35], the surgical model of destabilization of the medial meniscus (DMM) has become the gold standard for studying the time course of cartilage destruction during disease development and progression. Notably, the DMM model has been used to demonstrate the importance of the key aggrecan- and collagen-degrading enzymes in cartilage destruction, including ADAMTS5 [36, 37] and MMP13 [38], but also the consequences of knockout of key inflammatory genes such as IL-1 [34]. However, depending upon the surgical technique, the contribution of the fat pad, which may or may not be left in the joint, the nature of the gene knockout or knock-in, and the time of tissue sampling, there is generally less synovial inflammation in this model than we would expect in PTOA in humans [39]. The dysregulation of genes in the acute phase response signaling category in both wild type and Adamts5Δcat mice at 1 week following DMM surgery suggests an early inflammatory reaction that is independent of aggrecanase activity [40].

Alternative to the surgically induced PTOA models are noninvasive loading models in which repetitive in vivo cyclic compression causes cartilage degeneration depending upon the peak load level [41, 42]. Also notable are the rapid subchondral bone changes [41], although whether synovial inflammation is a feature has not yet been thoroughly evaluated. However, whether the model has an inflammatory component may depend upon the genetic background, since the strain least susceptible to DMM-induced cartilage loss, DBA/1, is highly susceptible to autoimmune or inflammatory arthritis [34]. The C57BL/6 strain, used most frequently for generation of knockout and transgenic mice either alone or on a mixed background with 129/SvDv strains, has

intermediate susceptibility, indicating its utility whether the genetic modification is expected to enhance or attenuate cartilage loss following challenge. Vincent and Saklatvala [43] pointed out the prominence of repair response genes in late OA in both surgical PTOA models human cartilage, which is reflected in ex vivo cartilage explant culture models [44], in contrast to the noninvasive cartilage injury models, which exhibit more obvious bone phenotypes. More severe mouse models result from intra-articular fracture, in which the joint pathology involves synovial inflammation, bone morphological changes, and increased circulating inflammatory cytokines and biomarkers, as well as chondrocyte death and cartilage degeneration within 7 days post-fracture [45].

Gene profiling studies of cartilage or whole joints from experimental PTOA models have begun to identify pathways that may be targeted for therapeutic development [46–49]. Age-related responses in joint tissues are accelerated in PTOA mouse models [49], as well as in spontaneous OA models such as the Str/ort mouse [50], and involve upregulation of genes of the senescence-associated secretory phenotype, including inflammatory cytokines (IL-6, IL-33, etc.), chemokines, MMPs, and immune and defense response genes [39, 51]. The molecular phenotypes resemble those reported in profiling studies of human cartilage [52, 53], in which both catabolic and anabolic gene signatures have been identified. Together, the findings to date suggest that the OA signature may be specific to the disease and unrelated to aging and lend credence to the possibility of identifying gene signatures in at-risk populations, including those susceptible to PTOA, prior to the onset of overt disease.

The prominence of the NF-κB pathway as a common thread among the different gene signatures has been emphasized in these preclinical PTOA models, suggesting a key regulatory role for stress and inflammatory signaling via canonical NF-κB signaling in human OA (see for review [22, 54]). The findings that mechanical stimuli modulate NF-κB signaling [20] provide an explanation for why NF-κB-related gene signatures may be upregulated in mouse models of PTOA in the absence of overt signs of inflammation such as synovitis and immune cell infiltration. Such profiling studies have revealed that inflammatory signatures are present before the appearance of overt OA and, in some models, are associated with increased numbers of activated T- and B-lymphocytes in the spleens of the mice destined to develop OA [50].

Contributions of Synovial Inflammation to PTOA

As highlighted in Chap. 12, synovitis is frequently observed at the time of arthroscopy, as well as at various times following joint injury, and may be a major prognostic indicator of the rate of development of PTOA. MRI findings correlate with microscopic and macroscopic evaluations of synovitis and suggest that synovitis is often present soon after a traumatic event [55]. ACL rupture by itself may not have high impact, but the synovitis is often more severe, if it is accompanied or followed by meniscal injury, collateral ligament tear, cartilage damage, and bone contusion. The acute symptoms following joint injury include joint pain and swelling due to intraarticular bleeding, synovial effusion and inflammation [2, 9]. The low-grade synovitis following injury is associated with the infiltration of mononuclear cells, including activated B cells and T lymphocytes, and production of inflammatory cytokines [19, 56, 57]. Overall, the presence of synovitis correlates with more rapid progression to structural deterioration, suggesting a role for cross talk between cartilage and synovial tissues [58–64].

In cases of ACL injury accompanied by cartilage and meniscal damage or of traumatic meniscal injury with no radiographic evidence of OA, the synovium retrieved during arthroscopy is frequently inflamed and synovitis is most commonly observed in the suprapatellar region remote from the sites of injury [65]. Inflammation scores are associated with increased pain and dysfunction, and inflammatory

infiltrates, including cells of the innate (macrophages) and adaptive (T and B cells) immune systems, can be observed within the synovial membrane. Importantly, microarray analyses show unique cytokine and chemokine profiles [66, 67], including increased IL-15 mRNA in synovial membranes of patients with degenerative menisci [66] and increased expression of genes encoding the chemokines, IL-8, CCL19, CCL21, and CCL5 and the chemokine receptor, CCR7, associated with synovial inflammation in patients undergoing arthroscopy for treatment of acute meniscal injury [67]. Generally, it is believed that the synovial changes represent a generalized early response to the injury and the presence of synovial inflammation increases the likelihood of cartilage degeneration and progression to PTOA. The damaged meniscus is an additional source of inflammatory cytokines, chemokines, and reactive oxygen species that could promote expression and activation of proteolytic enzymes and adversely affect cell survival and synthetic activity of chondrocytes and other joint tissues [68, 69].

Inflammatory Biomarkers in PTOA

Investigations of the biomarkers in synovial fluids, blood, and urine have attempted to map the disease process in OA patients in order to develop methodologies for diagnosis or response to therapy [70]. Distinct categories of biomarkers have been defined based on tissue origin and/or pathological process and include inflammatory mediators such as cytokines and chemokines, acute phase proteins, wound repair markers, extracellular matrix degradation products, and proteinases [9, 71–75]. Studies evaluating synovial fluids aspirated from the injured knees of patients with acute, subacute, and chronic ACL deficiency have shown an association between inflammation and increased concentrations of several cytokines. Following the acute ACL rupture, the initial burst of production of cytokines, such as tumor necrosis factor (TNF)α, IL-6, IL-8, IL-10, interleukin (IL)-1β, and IL-1ra, is similar to that observed in wound healing. The inflammation subsides but does not completely resolve, with persistence of a cytokine imbalance involving dramatically decreased concentrations of IL-IRa in patients with chronic ACL deficiency, suggesting insufficient levels of this protective cytokine to neutralize IL-1β [72, 76, 77]. Higher serum C-reactive protein (CRP), an acute phase reactant found in patients with cardiovascular disease and inflammatory arthritis, is present in patients undergoing arthroscopy in association with synovial inflammation [57, 71].

Other candidate biomarkers released in association with joint trauma include the damage associated molecular patterns (DAMPs), also known as alarmins, which can interact with the Toll-like receptors (TLR)-2 and -4 and receptor advanced glycation end-products (RAGE). DAMPs include extracellular matrix products and other ligands such as high mobility group box-1 (HMGB1), S100A8 (MRP8, calgranulin A) and S100A9 (MRP14, calgranulin B), and serum amyloid A (SAA). They can induce cellular inflammatory responses via NF-κB and are indicators of innate immunity that may drive synovitis in OA [78]. Other candidate biomarkers that may also be effectors of the disease include the complement proteins [71, 79] and uric acid, which is a danger signal associated with inflammasome activation [80]. Correlation of the transcriptomes of synovium and cartilage with synovial fluid proteomes from different OA phenotypes and with clinical and outcomes data has provided the opportunity to identify potential biochemical markers for monitoring the effects of joint injury on the clinical course and, importantly, for gaining insights into the mechanisms associated with development and progression of disease [81].

The observations of persistent and evolving disturbances in the cytokine profiles suggest that, in addition to the effects of the adverse biomechanical environment, biological processes also contribute to the development of OA changes after ACL or meniscus injury and indicate a potential role of synovitis in the pathogenesis PTOA. Understanding the mechanisms involved in the

initiation and perpetuation of this inflammatory process in PTOA could provide more sensitive and specific tools for monitoring patients after ACL or meniscus repair and also lead to the development of more specific and effective therapeutic approaches to improve the outcomes. Early targeted therapies against events associated with mechanical and inflammatory stress and injury include antioxidants, which prevent release of reactive oxygen species due to cell death, mitochondrial dysfunction, and cytoskeletal disruption, mitogen-activated protein kinase inhibitors, activators of ATP-AKT pathway signaling, and blockade of alarmins/DAMPs [82].

Significance

Joint trauma affects all joint tissues to some degree, but once cartilage is degraded, it is particularly refractory to repair by resident cells. While regenerative medicine techniques are not sufficiently advanced to substitute for end-stage joint replacement, the joint damage that occurs following injury is amenable to early intervention to prevent subsequent development of PTOA. Arthroscopic procedures are routinely employed in young individuals following ACL or meniscal damage, and this population could provide a window of opportunity for therapy targeted at preventing synovial inflammation and cartilage damage during the days and weeks after injury. There is a critical need for rigorous clinical and laboratory studies to define the factors that are responsible for joint deterioration associated with injury of the meniscus or ACL. Animal models of acute or chronic joint injury have been informative for defining the factors involved in the initiation and perpetuation of PTOA and could lead to the identification of new therapeutic targets. Inflammatory and catabolic biomarkers associated with synovitis and cartilage damage may have diagnostic or predictive value for defining outcomes pre- and post-arthroscopy and long-term risk for the development of PTOA. They could also help to identify patient cohorts for evaluating new therapies targeted at inflammation and the associated tissue damage.

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Potential Mechanisms of PTA: Oxidative Stress

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Oxidative Stress and OA

Post-traumatic osteoarthritis (PTA) arises from a broad range of articular injuries that cause direct physical damage to cartilage and other joint tissues. Chronic factors such as joint surface incongruity and laxity that cause excessive mechanical stresses are known to increase the risk for PTA, and excessive stress from joint overuse predisposes otherwise normal joints to osteoarthritis (OA). A growing body of evidence indicates that oxidative stress is a common element in all of these pathogenic factors. Acute synovial inflammation associated with joint injuries leads to oxidative damage to articular cartilage chondrocytes and matrix via the secretion of superoxide ($O_2^{\bullet-}$) by monocyte myeloperoxidases [1, 2]. Moreover, lethal or damaging amounts of reactive oxygen species (ROS) are produced intracellularly by chondrocytes in mechanically traumatized or overloaded cartilage [3–6]. As for most other cell types, chondrocytes react to high levels of ROS by undergoing programmed cell death or necrosis. Chondrocytes that survive ROS overexposure may senesce prematurely or exhibit other irreversible phenotypic derangements [7–9]. In addition to directly damaging cellular proteins, lipids, and nucleic acids, ROS synergize with pro-inflammatory cytokines and nitric oxide to promote catabolic gene expression via activation of the mitogen-activated kinases ERK1 and ERK2 and JNK [10–12]. On the other hand, treatment of chondrocytes with low doses of hydrogen peroxide suppresses interleukin-1- and lipopolysaccharide-induced increases in the expression of pro-inflammatory mediators such as nitric oxide synthase [13]. Furthermore, cartilage explants conditioned by repeated peroxide treatment were protected from apoptosis and other harmful effects of mechanical compression through the upregulation of catalase gene expression and downregulation of matrix metalloprotease-3 [14]. Together these observations suggest a complex relationship between oxidants and cartilage homeostasis that poses a challenge to the development of antioxidant therapies for PTA, as treatment effects are likely to be highly dose and time dependent.

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History of Antioxidant Therapies for OA

The therapeutic utility of antioxidants, or lack thereof, will ultimately determine whether oxidative stress is relevant to the treatment of OA. This utility has been in question at least since 1985 when RA Greenwald articulated the common problems with the earliest clinical studies: discrepancies between expectations and results, poor experimental design, and poor choice of control groups [15]. Many of these problems play a theme through a controversial body of literature, but past and recent data suggest that a role for antioxidants exists in the treatment of arthritis. Unfortunately, as described below, there is a need for improved experimental design and more rigorous development of treatment protocols with regard to specific targets and sources of oxidative stress.

The example of orgotein, an enzymatic superoxide scavenger, serves as a microcosm of clinical investigation of antioxidants for OA treatment. In 1980, a paper was published showing that injection of orgotein did not alter the development of OA in a rabbit model [16]. Nine years later, a paper published by a group from DDI Pharmaceuticals, Inc., showed that a 3-week regimen of orgotein injections was effective at reducing OA symptoms in people up to three months later [17]. These may not be mutually exclusive results, as pain and functional scores do not necessarily reflect the cartilage status in patients. Further, the question of whether the drug is effectively being delivered into the cartilage where it can be of use is not addressed in either case. Similarly, a paper published in 1990 demonstrated that dietary selenium and the vitamins A, C, and E had no impact upon patient reported outcomes, and studies published in 2001 and 2002 showed similar negative results with vitamin E alone [18–20]. Meanwhile, studies published in 1978 showed that administration of dietary vitamin E or tocopherol has an analgesic effect after only 10 days [21]. Studies from several groups noted improvements following tocopherol administration in functional scores and the lipid oxidation marker malondialdehyde (MDA) in serum, an end point that certainly indicates increased peroxidation and may indicate the presence of oxidative stress in OA patients [22]. Because tocopherol acts primarily as a lipid peroxidation chain breaker, decreases in MDA are encouraging, particularly with a correlative increase in function. A more recent example of the controversial nature of clinical data regarding antioxidants in OA treatment is the published commentary on a recent study of Phytalgic, a cocktail of fish oil, Urtica dioica, zinc, and vitamin E [23, 24]. This study showed a startlingly positive effect on OA symptoms with this particular cocktail and serves as an excellent example of the literature on clinical antioxidant treatment. Because this cocktail is developed privately and data published may in part contradict past publications, the data earned commentary by the publisher because of a clear potential for bias. Each of these studies determined a different result, or in the case of Phytalgic a somewhat incredible result, from different populations receiving different dosing regimens in different settings. The lack of a clear consensus from these trials suggests that a more refined approach to antioxidant delivery and oxidative stress measurement in conjunction with disease state in vivo is required.

More recent results with flavonoid compounds and flavonoid-containing mixtures risk comparison to vitamin E or orgotein studies even though data appear positive. Flavonoids are attractive because they are known to have both anti-inflammatory and antioxidant effects. In a recent comparison to naproxen, flavocoxid, a potent anti-inflammatory and antioxidant, improved clinical symptoms in both 6- and 12-week studies of pain and function in knee OA patients [25]. A flavonoid-containing purple passion fruit peel extract shows similar promise [26]. A recent Japanese study has shown modest improvements in pain and functional scores as well as trends toward decreases in cartilage degradation with a cocktail of three different dietary antioxidants, vitamin D, methylsulfonylmethane, and guava leaf extract, combined with glucosamine [27]. A second study by this group has demonstrated similar effects on clinical symptoms

with a cocktail containing flavonoid compounds called quercetin glycosides or glucosides [28]. These studies recognize that the effects of antioxidants are controversial in the treatment of osteoarthritis; despite that, they demonstrate statistical significance in modest data that suggests a role of antioxidants in the treatment of osteoarthritis. However, the equivocal nature of the vitamin E and orgotein literature suggests that more refined approaches to investigating and ameliorating oxidative stress are required. For example, one recent study used an anti-inflammatory/antioxidant compound called Pycnogenol and showed positive results, specifically, lowering both C-reactive protein and peroxide formation in serum, supporting a correlation between inflammation and oxidative stress that can be prevented with flavonoids [29]. These increases in peroxidation echo previous findings of correlations with increased MDA, a peroxidation end point, in OA. This outcome and those detailed above underscore the need, and recent push, for easily detectable biomarkers that clearly indicate disease state, as well as joint oxidative stress and oxidation levels. There is also increasing recognition that early failures and controversial results from dietary and the so-called "nutriceutical" approaches to relief of oxidative stress may be too weak to intervene once OA has advanced to presentation, a thought process commonly recognized in literature reviews [30, 31]. Intra-articular administration of a targeted antioxidant capable of entering the cartilage itself may be necessary for efficacy.

Chondrocyte Mechanotransduction

Aside from trauma, one of the strongest risk factors for OA is a history of joint overuse: numerous epidemiologic studies show that elite athletes and people in occupations involving heavy repetitive loading of their joints are at greater risk for OA than the general population [32–36]. This has been regarded as an inevitable effect of mechanical wear and tear on joints, but a growing number of laboratory studies indicate that the biologic response to overloading shares much in common with the mechanical injuries associated with joint trauma [6, 37, 38].

Mechanical deformation of cartilage induces oxidant production in chondrocytes and the amount of oxidants produced is proportional to the magnitude of deformation [4]. Deformation and oxidant production under physiologic loads are modest and beneficial, but self-inflicted oxidative damage occurs when cartilage is deformed by super-physiologic loads. Extreme forms of overloading including high-energy impacts induce lethal oxidant overproduction [6]. The response to modest overloading, such as that which occurs in people who habitually overuse their joints or when contact stresses at cartilage surfaces are chronically elevated by joint incongruities, is more muted: while chondrocytes typically survive such overloads, they do so with compromised energy metabolism, an effect that may limit non-vital protein synthesis including ECM production (Fig. 17.1). Oxidative damage accruing slowly via this mechanism is a plausible basis for the gradual progression of OA in such cases [8, 9].

Oxidants produced by chondrocytes in response to cartilage deformation are mitochondrial in origin [5, 39]. Metabolic inhibition experiments revealed that $O_2^{\bullet-}$ radicals are released from the mitochondrial electron transport chain in a strain-dependent fashion. When cartilage in the center of osteochondral explants (15 mm × 15 mm) is compressed with varying stresses (0.1–1 MPa) (imparted by an 8 mm diameter nonporous steel platen), the number of chondrocytes producing detectable $O_2^{\bullet-}$ increased linearly from 10 to 60 % strain ($r^2 = 0.87$) (Fig. 17.2) [4]. Strains below 40 % were well tolerated (<15 % death), but the death rate increased sharply as strains rose above 40 %. $O_2^{\bullet-}$ release and cell death were suppressed by rotenone, an electron transport inhibitor, and by cytochalasin B and nocodazole, which inhibit the polymerization of actin and tubulin respectively [40]. Additional work revealed $O_2^{\bullet-}$ overproduction and a chondrocyte mortality rate of ~60 % in explant cartilage subjected to a single blunt impact injury [6]. It is noteworthy that death in this model occurred mainly near matrix cracks

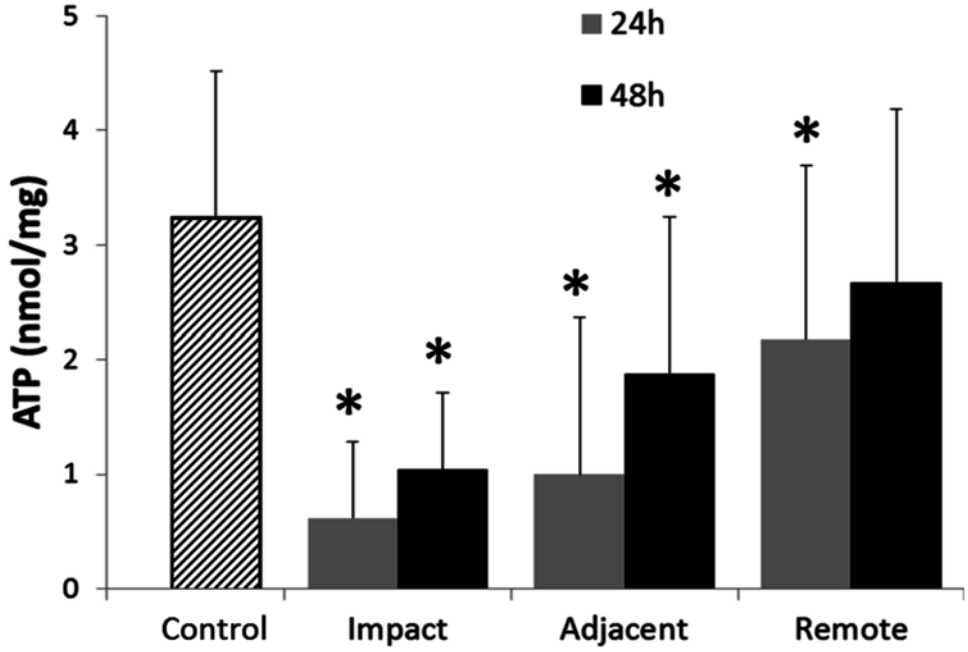

Fig. 17.1 Blunt impact suppresses cartilage ATP production. Explant cartilage was harvested for ATP assay 24 or 48 h postimpact (7 J/cm^2). Cartilage was harvested from control (non-impacted) explants and from impacted explants from the impact site itself, from a site immediately adjacent to the impact, or from a remote site several mm distant from the impact. One-way analysis of variance indicated that at 24 and 48 h postimpact, ATP in the impacted and adjacent cartilage was significantly lower than in cartilage from control explants. Remote cartilage ATP content was significantly lower than control at 24 h, but not at 48 h postimpact ($*p < 0.001$). *Columns* and *error bars* indicate means and standard deviations ($n = 4$)

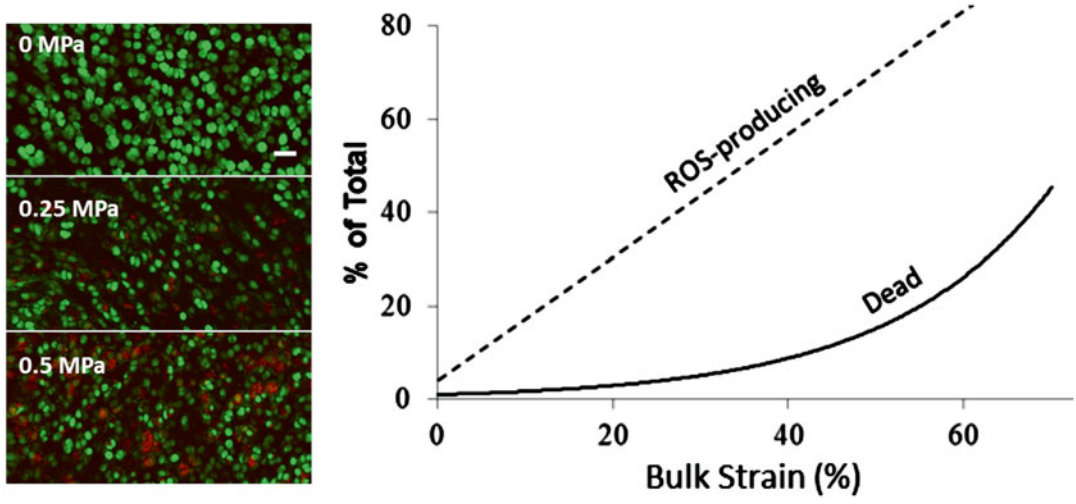

Fig. 17.2 Strain-related ROS and death. *Left*: representative confocal images show oxidant-producing cells (*red*) and live cells (*green*) after compression with the indicated stress magnitudes. The number of red cells increases from 0 to 0.5 MPa. The *bar* indicates 40 μm. *Right*: plot summarizing strain effects on ROS production and cell death under bulk tissue strains ranging from 0 to >60 %. The linear correlation coefficient (r^2) for ROS versus strain was 0.87

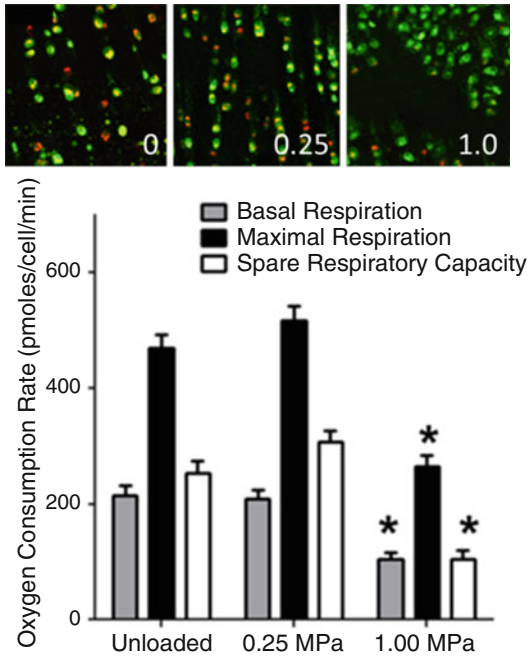

Fig. 17.3 Overloading leads to mitochondrial dysfunction. The *top panel* shows representative confocal micrographs taken of JC-1-stained chondrocytes after 1 week of loading with the indicated stresses (0, 0.25, 1.0 MPa). The *orange* and *yellow* staining indicating mitochondrial polarization was diminished in the 1.0 MPa-loaded explant. The graph in the *lower panel* shows loading effects on mitochondrial respiration in chondrocytes as measured by oxygen consumption rate. Basal and maximal respiration and spare respiratory capacity were all significantly reduced in the 1.0 MPa group versus the unloaded and 0.25 MPa-loaded groups

where the greatest matrix strains occurred during the impact load. This effect was also mitigated by a variety of drugs targeting strain-induced $O_2^{\bullet -}$ production including antioxidants, rotenone, inhibitors of cytoskeleton polymerization, and inhibitors of cell-ECM adhesion [41].

Explant experiments involving repeated cyclic loading over time frames of 1 week showed potentially pathogenic overloading-related changes in mitochondria. Seven days of repeated high-amplitude loading to strains of 40 % or more reduced mitochondrial potential and respiratory activity as measured by oxygen consumption rate (Fig. 17.3). In contrast, mitochondrial performance was unaffected after 7 days of low-amplitude loads that produced strains of less than 25 %. Gavriilidis and Young recently published results showing that OA chondrocytes exhibit the same constellation of mitochondrial abnormalities including depressed mitochondrial membrane potential and diminished respiratory capacity [42]. The authors also showed substantially lower expression of SOD2 in OA chondrocytes, which may expose mitochondria to oxidative damage. This interpretation was supported by additional findings showing increased proton leakage, which is thought to reflect increased lipid peroxidation in mitochondrial membranes. Interestingly, work in a mouse joint injury model showed that mitophagy, a major mechanism for mitochondrial turnover and repair, is suppressed in overloaded cartilage and treatment with rapamycin, which stimulates mitophagy by blocking the mammalian target of rapamycin, showed chondroprotective effects [43, 44]. These findings suggest that oxidant overproduction from chronic overloading together with deficiencies in SOD activity lead to the mitochondrial derangements present in OA chondrocytes.

The mechanoresponsive oxidant production pathway outlined above seems maladaptive to the extent that it is responsible for oxidative insults to cartilage. However, there is evidence to suggest that the pathway performs a crucial physiologic function by regulating glycolytic ATP synthesis in response to normal loads. Although mitochondria contribute only a small fraction of cellular ATP in chondrocytes, it was found that oxidants formed as a by-product of mitochondrial electron transport are required for glycolytic activity [45]. Cyclic loading of explant cartilage that yielded low- to medium-amplitude strains increased ATP by fourfold over resting controls [39]. Remarkably, this stimulatory effect was abolished by the same antioxidants and transduction inhibitors that suppressed trauma- and overload-induced damage and death. Thus, it appears that the very process that wreaks havoc in overloaded cartilage maintains redox balance and modulates energy production in response to normal loading (Fig. 17.4). These findings also suggest that impairment of ATP production is one of the main consequences of mitochondrial dysfunction of the kind seen in overloaded and osteoarthritic cartilage.

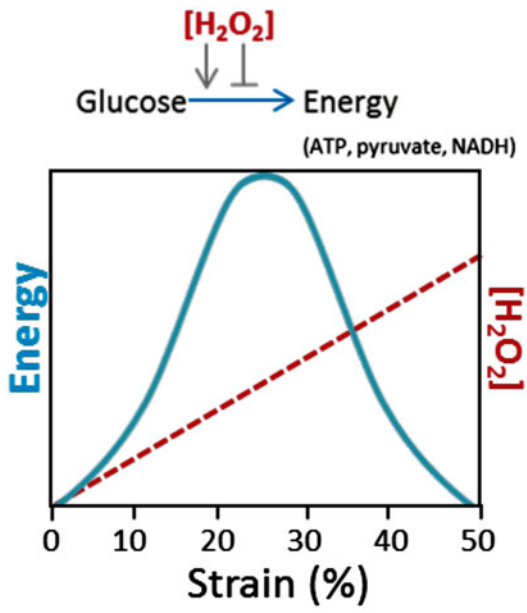

Fig. 17.4 Effect of strain-induced peroxide on energy production. The diagram illustrates the concept that H_2O_2 resulting from superoxide dismutation has concentration-dependent effects in regulating energy production via glycolysis. Energy production in the form of ATP, pyruvate, and NADH is stimulated at low concentrations produced under moderate strains (10–25 %). As strain increases to 30 % or more, H_2O_2 formation increases to levels that inhibit glycolysis, resulting in progressive depletion of energy stores

Prospects for New Therapies

The fact that oxidative damage to chondrocytes occurs through a defined mechanotransduction pathway suggests there are diverse opportunities for intervention that include, but are not limited to, antioxidants. The central role of mitochondria in mechanotransduction makes them an obvious candidate for drug development (Fig. 17.5). Indeed, amobarbital, a drug that suppresses $O_2^{\bullet-}$ production by blocking electron transport, has shown some promise in a rabbit cartilage injury model [46], where intra-articular injection improved chondrocyte viability and ATP content (Fig. 17.6). Treating with nocodazole or cytochalasin B, which reduces $O_2^{\bullet-}$ by blocking mechanotransduction at the level of the cytoskeleton, also improved viability and ATP content. Oxidant scavengers also remain on the list of potential therapeutics to moderate overloading effects. *N*-acetylcysteine (NAC), for example, spared chondrocytes from oxidative death in the rabbit and in a variety of other overloading circumstances. While the low cost and safety of NAC and other common oxidant scavengers add to their appeal as therapeutic agents, antioxidant activity is lost after only one reaction. In contrast,

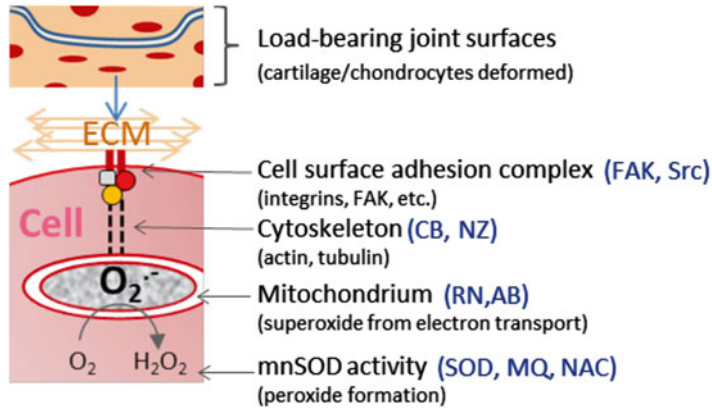

Fig. 17.5 Intervening in chondrocyte mechanotransduction. The diagram illustrates known components of the mechanotransduction pathway and inhibitors that have been shown to block the pathway at various steps. These include FAK and Src inhibitors targeting ECM adhesion, cytochalasin B (CB) and nocodazole (NZ) that block cytoskeleton formation, rotenone and (RN) and amobarbital (AB) that block mitochondrial electron transport, and superoxide dismutase mimetic (SOD), mitoquinone (MQ), and *N*-acetylcysteine (NAC), which participate in superoxide and peroxide detoxification

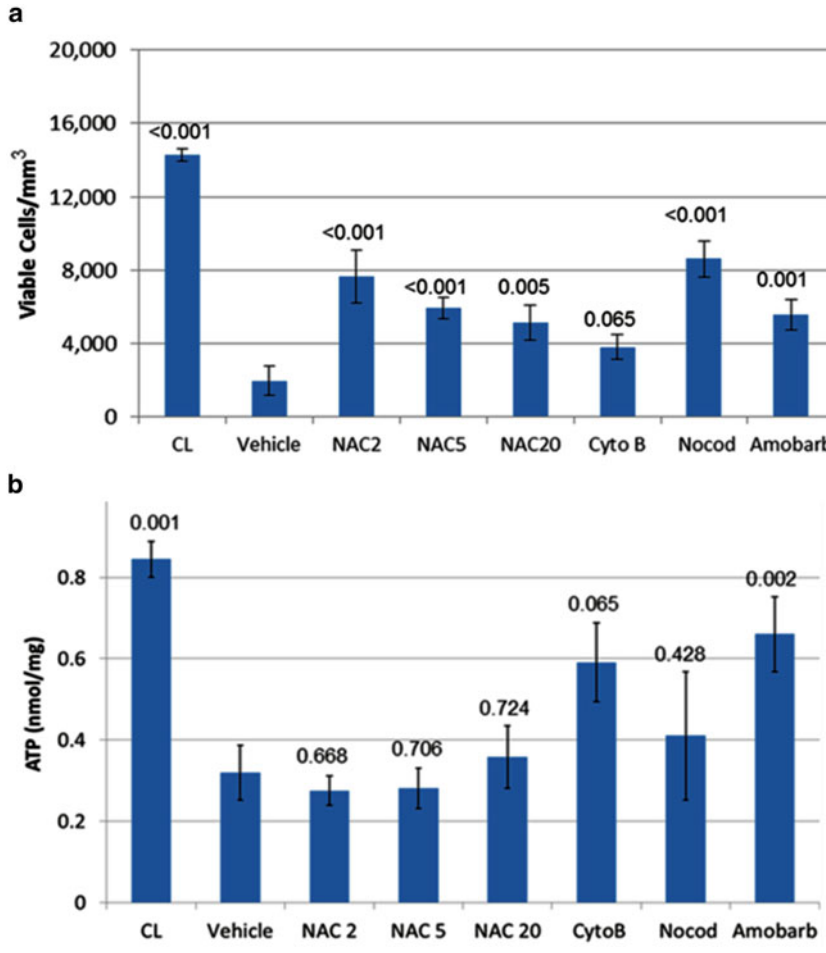

Fig. 17.6 Effects of treatment on chondrocyte viability and ATP content in injured cartilage. Rabbits with chondral damage caused by blunt impact were treated after injury. One-week post-op injured cartilage was imaged by confocal microscopy to determine viable cell density (**a**) and then assayed for ATP content (**b**). Cartilage from contralateral limbs (CL) was used as uninjured controls. Injured joints were untreated (no Rx) or treated with NAC at 2, 5, or 20 mM (NAC2, NAC5, NAC 20), cytochalasin B (CytoB), nocodazole (Nocod), or amobarbital (Amobarb). *Columns* and *error bars* indicate means and standard errors. The numbers above the columns show *p*-values for treated versus vehicle (one-way ANOVA)

antioxidant enzymes such as superoxide dismutase (SOD) are not consumed by oxidant catalysis and may have more lasting effects. Recent increases in the availability of small and stable SOD mimetics offer promising options as oxidant scavengers [47]. Combinations of drugs such as SOD mimetics and NAC may be used to ensure that the rapid dismutation of $O_2^{\bullet-}$ does not overwhelm the cells' intrinsic ability to detoxify H_2O_2 and other secondary oxidants.

That damaging oxidants can be produced intracellularly by chondrocytes buried in the dense, avascular cartilage matrix presents substantial obstacles to the bioavailability of compounds administered systemically or even intra-articularly. Indeed, negative clinical trial results for some antioxidant therapies may be attributable to poor drug availability in cartilage. Agents that diffuse through the cartilage ECM must also either pass through cellular membranes

or be actively taken up by cells to finally reach their targeted site of action. Importantly, the antioxidants and mechanotransduction inhibitors mentioned above penetrate the cartilage ECM and pass readily through cell membranes.

As a note of caution, recent gains in our understanding of chondrocyte mechanobiology make it apparent that over-suppressing ROS can disrupt normal responses to loading that are critical for cartilage homeostasis. Thus, the high levels of antioxidants used to combat oxidative damage in other tissues may not be tolerated in cartilage. This potential for harm underscores the need to carefully optimize dosages and treatment duration in animal models before contemplating testing in people.

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Potential Mechanism of PTA: Alterations in Joint Loading

18

Timothy M. Wright and Suzanne A. Maher

Introduction

Mechanical loads are an important factor in osteoarthritis (OA) and certainly in post-traumatic arthritis (PTA), where trauma implies that the mechanical burden on the joint exceeded that which could be withstood by one or more of the tissues that comprise the joint. OA is now recognized as a joint disease [1], and as such, the biomechanical factors that affect its etiology and progression must be understood by considering the mechanical function of the whole joint. The same is true for PTA, since trauma is seldom isolated to one component of the joint. To that end, understanding the influence of joint loading on PTA requires understanding the impact of those loads on the whole joint as well as on each of the tissue components (cartilage, bone, meniscus, synovium, ligaments) that contribute to joint function.

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The Load Transfer Problem

The challenge becomes one of understanding joint mechanics in both normal and traumatized joints. A major mechanical role of diarthrodial joints is to transfer large loads across the joint surface, through cartilage, subchondral bone, trabecular bone, and finally out to the cortex. Two important considerations for this load transfer function are as follows: how the load is distributed on the joint surface and how that load distribution interacts with the joint's structural components to cause distortion and hence strains in the tissues that comprise those components [2].

Load distribution is complicated by the fact that the load is received in the mid-region of the structure, even for bicondylar joints like the knee (Fig. 18.1), and must be transferred across the joint surfaces into the underlying cancellous bone bed. Load distribution and hence the contact stresses on the articular surface of the joint are determined by the shape of the contacting surfaces and the compliance of the contacting materials [3]. For the case of thin structures, such as the articular cartilage that covers the joint surface, the thickness of the cartilage itself also influences the load distribution at the contacting surface. The hip joint, with its conforming ball-in-socket shape, generates large contact areas, and therefore contact stresses and strains vary as a function of geometry alone. But the knee, with

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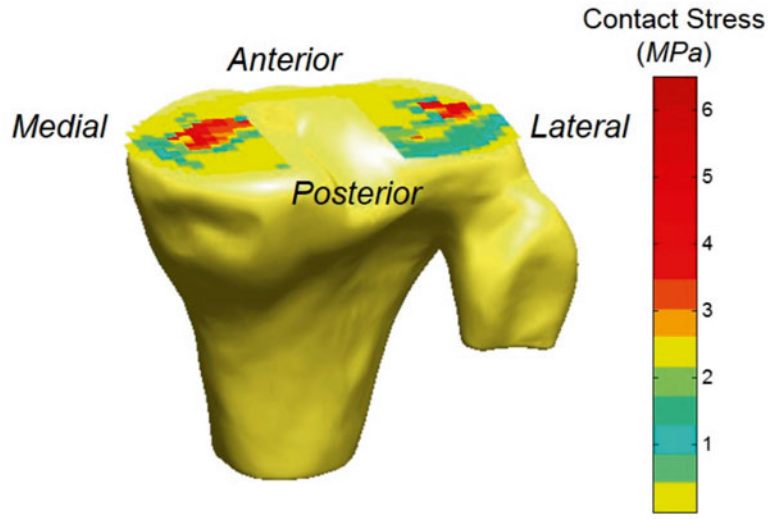

Fig. 18.1 Contact stress patterns across the surface of the proximal tibia at 14 % of the gait cycle show that load is distributed across both tibial plateaus but varies as to magnitude and location [Artwork courtesy of Hongsheng Wang, Ph.D.]

less conforming joint surfaces, requires additional meniscal structures to help in load distribution.

Consider the knee joint. Its bicondylar geometry, combined with the anisotropic, inhomogeneous properties of the articular cartilage and the menisci, and the variation in properties that occur among the normal patient population [4, 5] and even within a specific knee over time make understanding load transfer a complex endeavor. Forces perpendicular to the joint surface can be measured experimentally [6], but to understand how those forces are transferred through the articular cartilage to the underlying bone and out to the cortex requires the use of computational finite element models. Elegant models have been developed that capture the biphasic response of the soft tissues of the knee [7] and the inhomogeneity of the natural knee [8], but few efforts have focused on how alterations in tissue geometry and material properties affect load transfer. Segal et al. [9] explored the association between knee joint contact stress magnitude in patients identified as being "at risk" for the development of OA. They found that elevated contact stress was a predictor of subsequent cartilage damage and bone-marrow lesions, but a direct connection between the stresses in the tissues and subsequent tissue damage was never made. As more research focuses on how knee injuries affect surface contact stresses, additional efforts will be required to make the connection between how the resulting changes in load transfer influence the underlying tissues [10]. Without such an analysis, a direct connection with the pathogenesis of post-traumatic arthritis is purely speculative.

The Influence of Joint Loads

The magnitudes of the loads that are transferred across joints in the skeleton are high, even during normal activities of daily living. Direct measurements of joint loads have been performed in the knee and hip joints through the use of instrumented total joint replacements. For the knee joint, in vivo loads exceed 2–2.5 times the body weight for activities like walking, climbing stairs, or swinging a golf club [11]. Similar results were found for the hip for these same activities, though loads reached as high as 8.7 times the body weight during an unanticipated stumble [12].

In one respect, alterations in joint loading as they pertain to PTA can be considered on the basis of changes in load magnitude alone. Such an approach, understanding how increased magnitude affects the propensity for arthritis to develop in the joint, is fruitful because the mechanical factor, load magnitude, can be easily controlled in experimental models. Consequently, numerous animal models have been developed to characterize the mechanical effects of blunt trauma as an extreme alteration in load magnitude. In these models, trauma is induced by directly impacting the articulating surface of the joint with

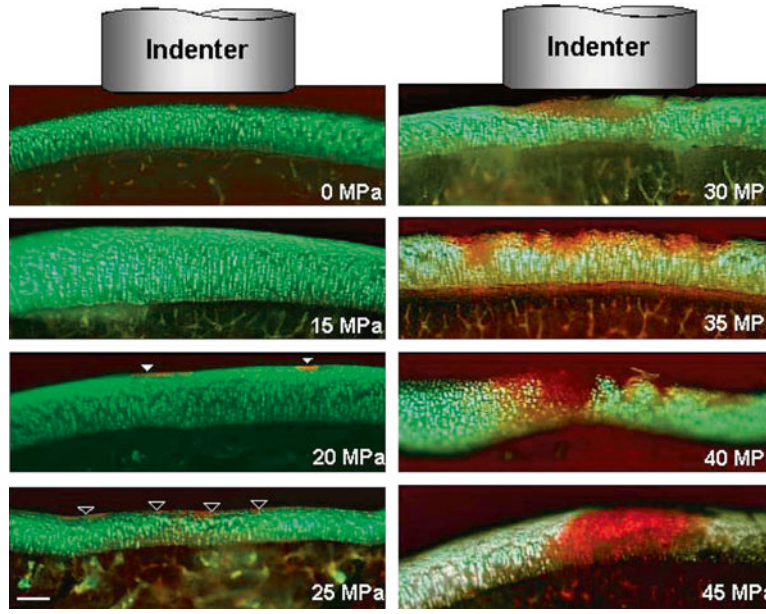

Fig. 18.2 Microscopic images of cross sections of the articular cartilage in the impacted and surrounding regions [taken from reference 12]. Shown are the full thickness of the articular cartilage and a thin layer of subchondral bone. *Green stain* indicates viable and *red stain* indicates dead cells. Cell death initiated at the surface in locations that corresponded to the edges of the indenter (*solid arrows*). The cell death was evenly distributed on the surface (superficial region) in the impacted region at ~25 MPa (*open arrows*). Scale bar is 250 microns

a rigid indenter. Altering the radius of curvature of the indenter (e.g., flat versus curved) can be used to vary the load distribution and hence the applied stresses for the same magnitude of applied traumatic load. Tissue alterations as a result of the blunt trauma can then be assessed as a function of the time after impact.

As expected, studies with blunt trauma models show that a relationship exists between matrix damage and the magnitude of the contact force (Fig. 18.2) [13] and that the extent of cell death in articular cartilage is significantly affected by the magnitude of applied contact stress [14]. Perhaps more importantly, subsequent consequences of alterations in load magnitude and distribution are revealed through mechanically driven adaptations in tissue quality (composition and structure). These include decreases in cartilage modulus and subchondral bone thickening that begin immediately after impact and persist out to 36 months after the trauma [15, 16].

The location at which the traumatic load is applied on the articular surfaces is another important factor affecting the subsequent response of joint structures and tissues. Loads are transferred at many points within the joint's range of motion in response to the mechanical demands that arise during daily activities of living, and in a traumatic event, the magnitude of the applied load can be abnormally high compared to what is normally transferred at a given point in that range. For example, in a study with paired human cadaveric knee joints, Atkinson and Haut [17] applied both fracture and subfracture loads across the patellofemoral joint with the knee positioned in different flexion angles. For the subfracture loads, 45 % of the impact energy required to fracture the contralateral knee was applied. But even at this lower load, microfractures of the subchondral and trabecular bone and fissures of the articular surface occurred at every flexion angle that was studied; damage varied with flexion angle but always coincided with the patellofemoral contact region.

Most blunt impact models utilize an open arthrotomy to expose the joint surface, enabling direct application of the indenter in a known location. Recently, Furman et al. [18] developed a closed-joint mouse model of intra-articular fracture in which a cradle holds the animal's knee while a wedge-shaped indenter mounted to the actuator of a material testing machine applies the necessary fracture load. The complexity of the fracture is correlated with the energy imparted to the joint. Eight weeks after fracture, proteoglycan loss occurs in the articular cartilage and the subchondral plate thickens. Developing such

mouse models allows not only for impact loads to be applied in a more physiological manner but also provides the opportunity to study genetic factors associated with PTA [19].

Joint Stability

While much has been learned from models of blunt trauma that vary load magnitude and location in a static or quasistatic fashion, an appreciation of how alterations in joint loading affects PTA requires consideration of joint kinematics. The musculoskeletal system functions to provide controlled motions, so factors that affect joint motion critically affect skeletal performance. Controlled motion implies joint stability, in which the joint maintains an appropriate functional position throughout its range of motion [2]. Thus, a stable joint can move through a normal range of motion, transferring the functional loads without pain and producing normal intensity stresses and strains in the joint tissues.

Stable joints have one position of joint equilibrium for any particular functional loading situation. Neither small additional increments of load nor small changes in the direction of the functional load produce rapid, large changes in the contact position. For example, if a knee joint is supporting a flexion moment, the application of a small tibial torque should not produce a sudden, large angular displacement. Stable joints also maintain contact between surfaces covered by cartilage, an important consideration in PTA; edge loading at the periphery of the joint surfaces does not occur in normal joints but can occur during a traumatic event.

Maintaining joint stability is a complex interplay among compression between the contacting joint surfaces, ligamentous constraints, and muscle forces. Compression between the joint surfaces is the most direct mechanism to produce a joint reaction force; however, providing sufficient force in an appropriate direction is limited by the shape of the joint. Given the low coefficient of friction between cartilage surfaces, compressive force can only be generated perpendicular to the contacting surfaces. Thus, joint reaction force can only be produced within a limited arc of motion (Fig. 18.3).

The occurrence of an intra-articular fracture disrupts the curvature of the joint, creating instability at the fracture site. The total arc of motion over which a joint reaction force can be maintained is not altered by the fracture; however, if the joint reaction force is required to move across the fracture site during a functional motion, the joint surfaces will undergo a sudden motion as the contact surfaces jump from one curvature to the other (Fig. 18.4). Such a small change in the joint surface associated with a surface discontinuity produces an unstable joint, which can significantly alter contact stresses [20]. Thus, in the intra-articular fracture model of Furman et al.

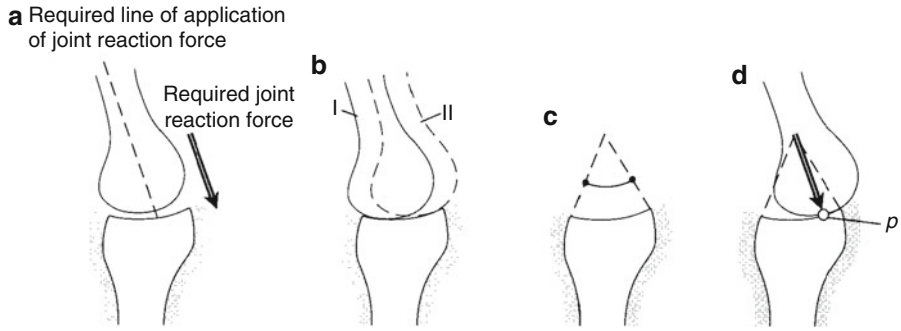

Fig. 18.3 (**a**) Assume that a joint must produce the joint reaction force shown in the figure, while the joint position remains constant. (**b**) For the required angle, the joint can assume any stable position between locations I and II. (**c**) The range of orientation of the joint reaction force that can be produced by joint contact alone is shown. (**d**) The joint will seek the contact position where the perpendicular to the contacting surfaces at the contact point (P) is in the required direction [Taken from 2]

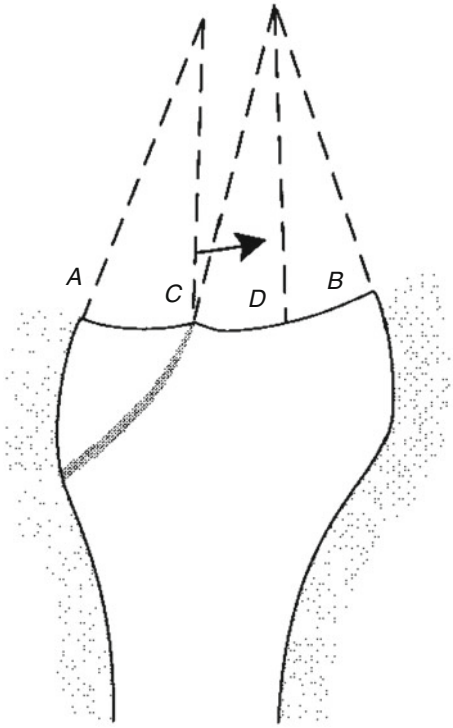

Fig. 18.4 A joint has sustained an intra-articular fracture at point *C*, causing a discontinuity in the joint surface. The range of orientation of the joint reaction force is bounded by lines *A* and *B*. If the joint contact at position *C* is altered slightly, a large motion of the contacting surface from *C* to *D* will occur. The orientation at position *D* is only slightly different from the orientation at position *C* [Taken from 2]

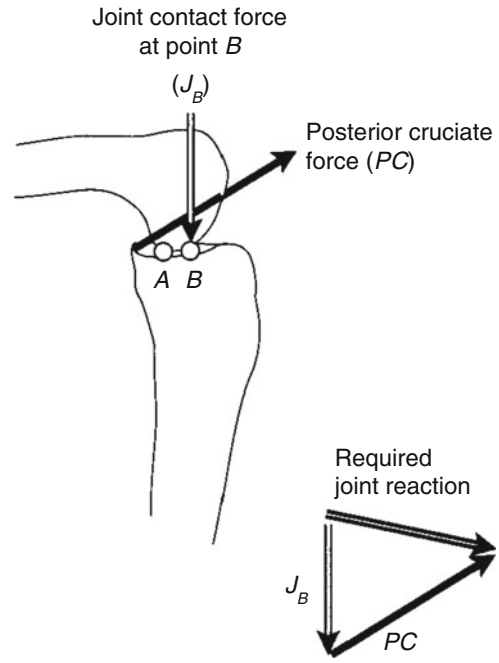

Fig. 18.5 At the contact position shown (point A), the joint contact force cannot provide the required joint reaction. When the femur moves to contact at point B, the posterior cruciate ligament will stretch and pull anteriorly and proximally on the tibia. When these two forces (JB and PC) are added, they produce the required joint reaction force [Taken from 2]

[18], the subsequent alterations in cartilage may result as much from alterations in joint stability after the fracture as from the mechanical damage that accompanied the original blunt trauma.

Forces created by tensioning of the passive ligaments that connect the bones across the joint also serve to stabilize the joint. For example, during normal knee flexion, functional loads are often required that cannot be generated by the joint reaction force alone, even as the femur translates anteriorly (Fig. 18.5). But as that translation occurs, the posterior cruciate ligament is stretched, thereby creating tension within the ligament. This tensile force provides the additional joint reaction force to maintain joint stability. Depending on where the joint is in its range of motion, the ligament may be loaded or lax. For the contact point to move enough along the joint curvature to create joint compression force, the ligament must allow relative joint motion without producing tension. Thus, ligaments possess a neutral zone, in which the joint can find a stable position based upon joint curvature and the required direction of the joint reaction force. If an equilibrium position does not lie within this zone, additional joint translation induces ligament tension that contributes directly to joint stability.

Ligament injuries impact the mechanical burden placed on the joint in important ways. The velocity and distances through which the articular surfaces move relative to one another in the presence of a stretched or torn ligament increase dramatically. For example, a torn anterior cruciate ligament (ACL) in the knee can produce a threefold increase in anterior translation of the tibia relative to the femur [21]. Similarly, a tear in the meniscus also detracts from its stabilizing

function, not only allowing larger sliding distances (and hence larger surface velocities) between the tibial and femoral surfaces but also markedly increasing the contact stresses in the underlying cartilage [19].

Muscle forces actively provide joint motion, but they also provide a stabilizing effect by combining two common features of a joint. The first is that the joint must undergo relative motion to change the point of application and hence the direction of the joint contact force. The second is that such small changes in relative position of the joint surfaces provide meaningful changes in orientation and moment arm of the muscle force. If necessary, additional stability can be achieved through abnormal co-contraction of muscles that span opposite sides of the joint. For example, with an ACL injury, co-contraction of the quads and hamstrings across the knee provides additional joint reaction force to help stabilize the joint [22]. Of course, the disadvantage of this additional mechanism is that it creates increased burden on the cartilage and underlying bone because the additional co-contraction force adds to the magnitude of the joint reaction force across the joint surfaces.

Lessons from Total Joint Arthroplasty

The role of kinematics in creating damaging conditions to joint surfaces has been the focus of efforts to improve the wear performance of bearing surfaces in total joint arthroplasty for more than 40 years. Central to those efforts has been an understanding of the wear mechanisms and the mechanical and material conditions that control them. For example, polyethylene wear is dominated by abrasive wear in which the harder opposing metallic surface cuts through the surface of the softer polyethylene, and fatigue wear occurs as a result of the formation and growth of cracks in and below the polyethylene-bearing surface [3, 23]. The amount of abrasive wear is directly proportional to the product of the load across the contacting surfaces and the distance or velocity that the surfaces slide under load. Increased load or large, rapid translations make the polyethylene surface more susceptible to wear. Applying the same principle to articular cartilage, any situation that increases the combination of load times sliding distance or velocity could increase abrasive damage to the cartilage surface.

Fatigue wear is exacerbated in nonconforming metal-on-polyethylene joint replacements where the local stresses are higher due to smaller polyethylene contact areas compared to a more conforming joint [3, 23]. The stress distribution is complex as the contact region moves across the surfaces (Fig. 18.6), with several types of deformation occurring in the polyethylene. Under the contact region, the polyethylene is compressed perpendicular to the surface with the greatest compressive stress occurring at the surface. But because the surrounding material below the surface constrains the compressed material from spreading tangentially, compressive stresses are also created parallel to the surface, the result being a state of hydrostatic compression. At the edge of the contact area, however, the polyethylene is stretched tangent to the surface, resulting in tensile stresses. Finally, the material is also distorted under the contact area, with shear stresses that vary throughout the region and, for nonconforming surfaces, can be greatest below the surface of the polyethylene.

Can these relationships between mechanisms of abrasive and fatigue wear and joint loads in polyethylene joint replacements help explain the role of biomechanics in a normal joint experiencing the onset and progression of PTA? Recent evidence suggests that they can. For example, in a recent experimental study, Tochigi and colleagues [24] used a rabbit model, in which they subjected animals to either complete or partial ACL transaction as a means of altering joint stability (and, hence, sliding distance and velocity). Joint stability was measured in vivo using an anterior drawer test, from which anterior drawer stiffness and neutral zone length were reported; a lower stiffness and a larger neutral zone were indicative of a more unstable joint. After both 8 and 16 weeks, cartilage degeneration as determined histologically increased with the degree of instability (Fig. 18.7).

Similar results were found in a larger animal model. Frank et al. [26] subjected three groups of sheep (normal, sham operated, and ACL/MCL

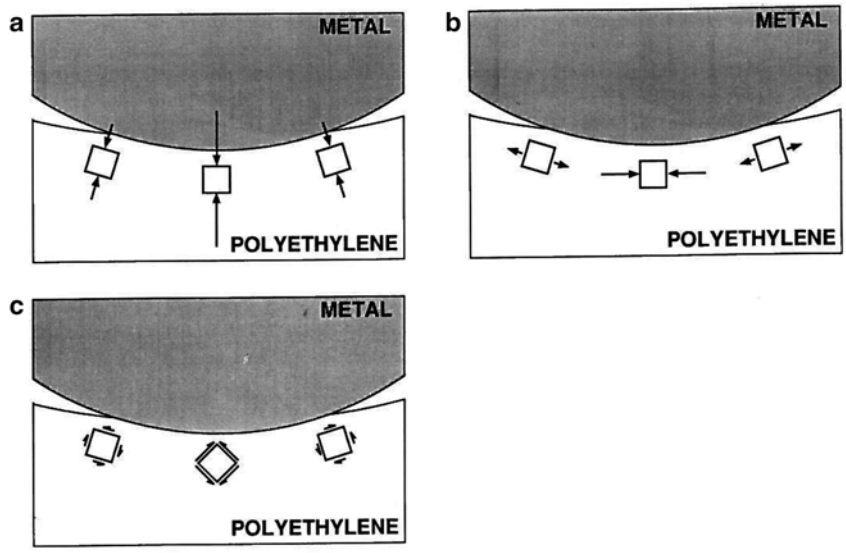

Fig. 18.6 When the metallic femoral component indents the polyethylene, complex stress distributions result, including (**a**) compressive contact stress perpendicular to the surface, (**b**) compressive and tensile stresses tangent to the surface, and (**c**) shear stresses

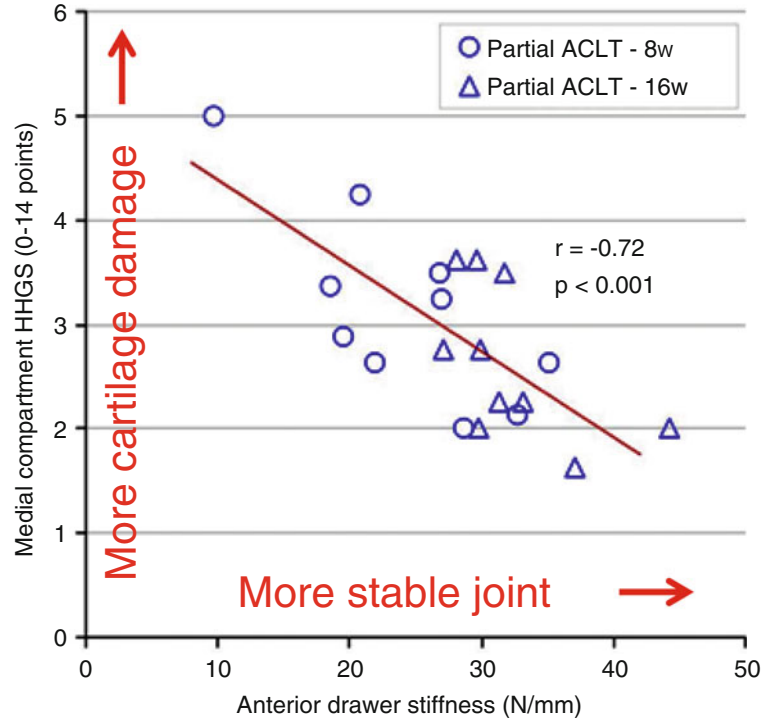

Fig. 18.7 In a rabbit model of partial ACL transection to effect variations in joint stability, the less stable the joint, the greater was the subsequent degree of cartilage degeneration as measured by a histological histochemical grading scale (HHGS) [Adapted from 25]

transected) to gait assessment at 4 and 20 weeks postoperatively and subsequently assessed the cartilage and bone after euthanizing the animals 20 weeks later. The ACL/MCL-transected animals had significantly more internal-external tibial rotation and anterior-posterior and medial-lateral tibial translations during gait. Though the largest magnitude and most consistent change

after ACL/MCL transection occurred in the anterior direction, greater joint degradation was directly related to increased medial translation.

Subtle changes in joint stability can occur even when the ACL is transected and then immediately reattached, with profound consequences for joint health. O'Brien et al. [27] transected and then immediately reattached the ACL in sheep knees. Though anteroposterior stability improved with repair of the ACL compared to that of the knees that remained ACL deficient, significant differences occurred, specifically increases in tibial abduction and internal rotation relative to sham controls. Similar to the rabbit study of Tochigi et al. [24], considerable variability was found in morphology and kinematic data among the animals. But all ACL-reconstructed knees showed cartilage changes consistent with OA, and many had developed osteophytes.

The impact of joint stability on progression of PTA has also been demonstrated in humans. For example, Potter and colleagues [28] conducted a prospective, observational analysis of 42 knees in 40 patients with acute, isolated ACL injury. One third of the knees had undergone surgical reconstruction to restabilize the knee. MRI examinations were performed yearly for up to 11 years. All knees showed chondral damage and bone-marrow edema as a result of the initial injury, and all knees showed increased chondral degeneration over time. But the odds of cartilage degeneration were more than four times higher in patients who had not undergone surgical reconstruction, most likely due to the continued joint instability associated with the original (untreated) ACL tear.

Joint Loads Revisited

Besides the appreciation that stability can vary greatly in animal models of arthritis, an additional limitation of these models is that they do not include a means for measuring or controlling the alterations in joint load that accompany the alterations in stability. Thus, the role of biomechanics cannot be completely appreciated. For example, transection of the ACL no doubt leads to joint instability and larger sliding distances, causing cartilage regions not normally loaded during routine activities by the animal to experience contact loads. But the animal may co-contract muscles across the joint in an effort to add stability in the same way that humans suffering an ACL tear co-contract their hamstrings and quadriceps [22]. Thus, not only will sliding distance (and velocity) increase in an ACL transection model but joint loads as well, but in an unpredictable manner that cannot be measured experimentally.

Thus, only an empirical link has been established between the extent of instability and the degree of arthritis with the underlying premise that an unstable joint alters the contact mechanics on the articular cartilage relative to that experienced in a stable knee joint. To reach a more comprehensive, mechanistic understanding requires knowledge of the changes in stresses and strains that accompany changes in knee stability. A team from Duke University Medical Center and Harvard Medical School has started to tackle this challenge by quantifying changes in knee kinematics of patients immediately after ACL reconstruction [29] and again 6 months later [30] using dual fluoroscopy of a lunge activity combined with MRI analysis. At both time points, a posterior and lateral shift in cartilage contact to smaller regions of thinner cartilage was found relative to contact in normal knees, despite a restoration of anterior knee stability at 6 months. Thus, a change in contact forces alone, even without changes in knee stability, may be detrimental to the health of the joint.

More recently, Bedi et al. [31] explored the effect of ACL injury on joint mechanics by directly measuring contact stresses in human cadaveric knees during simulated walking. Significant variability in the response of the knees to ACL rupture was found, emphasized by variable changes in contact stress in the anterior portion of the tibia. But a consistent, significant increase in contact stresses was measured in the posterior aspect of the medial tibial plateau. Decreased tibial medial concavity, increased tibial slope, and smaller changes in the location of the center of rotation through the gait cycle were predictive of higher stresses in the anterocentral region of the tibial plateau, suggesting that the bony geometry of the joint may predispose knees to more destructive changes in contact mechanics.

Other animal models of arthritis also do more to alter load distribution (and, therefore, stresses in the cartilage and underlying bone) than does joint stability. For example, surgical destabilization of the medial meniscus (DMM), a popular mouse model for arthritis, has a large impact on tissue stresses as the cushioning role of the meniscus is lost. The effect is to concentrate the joint reaction force over smaller contact areas. Indeed, comparisons of the location and extent of osteoarthritic histological changes between the ACL transection and DMM model reflect differences consistent with these differences in altered biomechanics. Glasson and colleagues [32] compared an ACL transection model to the surgical DMM model in the same mouse strain. By 4 weeks after surgery, the ACL transection model produced severe OA, chondrogenesis of the joint capsule, and, in some cases, severe subchondral erosion of the posterior tibial plateau, while the DMM model produced less severe changes. The cartilage lesions with the DMM model were primarily on the central weight-bearing region of the medial tibial plateau and medial femoral condyles, consistent with greater localized stresses in these load-bearing areas than changes elsewhere on the cartilage surface as occurred in the destabilizing ACL transection model.

Tissue Adaptation and Alterations in Joint Mechanics

Any understanding of the role of alterations in loading and kinematics that accompany joint trauma is complicated by the ability of the joint tissues to adapt to changes in the mechanical burden that they must withstand. Mechanical adaptation is a natural phenomenon in musculoskeletal tissues, though the signals that trigger adaptation and the mechanisms by which tissues adapt remain poorly understood. The additional burden that accompanies trauma, both at the time of injury and subsequently, can result in localized regions experiencing stresses that exceed their strength. But failure strengths for joint tissues, especially under complex states of stress such as those caused within and near the moving contact regions in the joint, are unknown, as are the alterations in adaptation that accompany failure.

Bone adaptation in response to increased loads across the joint has long been an underlying hypothesis for explaining the etiology of osteoarthritis [33]. The postulate is that stiffening of subchondral bone is an intuiting event that results directly to increased stresses in the cartilage, resulting in its inevitable destruction. Little experimental support exists for this concept. Indeed, later work by Day and colleagues [34] showed that while the density of subchondral bone in OA was indeed higher, at the bone tissue level, the elastic modulus was only about half that of normal; the combination of these two effects (more tissue but of lower elastic modulus) results in little difference in the structural stiffness of the subchondral bone supporting the cartilage layer.

However, this does not imply that bone adaptation is not an important component of the progression of arthritis. Recently, for example, Weinans and colleagues used a mouse destabilization model in which intra-articular knee ligaments are selectively damaged through injection of a chemical into the joint. Within 2 weeks, subchondral bone had appreciably thinned, and the subchondral plate itself was perforated as a consequence of increased osteoclastic activity [35, 36]. Though the plate subsequently thickened at later time points, the perforations persisted, and damage to the calcified cartilage increased (Fig. 18.8).

Other recent efforts have centered on using models intended to control changes in load and joint stability without surgery or chemically induced tissue degradation. Ko and colleagues [37] and Poulet and colleagues [38] have used similar mouse models in which axial loads are applied across the flexed knee joint. These noninvasive loading models provide a means to dissect temporal and regional changes in joint tissues. They allow the application of bouts of controlled loading across the joint above the loading applied by the mouse during daily activities. Ko et al. assessed the influence of load magnitude and the duration of loading on the adaptive responses of the cartilage and bone. Loads that produced physiological levels of strain in the midshaft of the tibia nonetheless induced cartilage matrix damage, epiphyseal bone adaptation, and osteophyte formation in both young and old mice, recapitulating the morphologic and anatomic features of OA

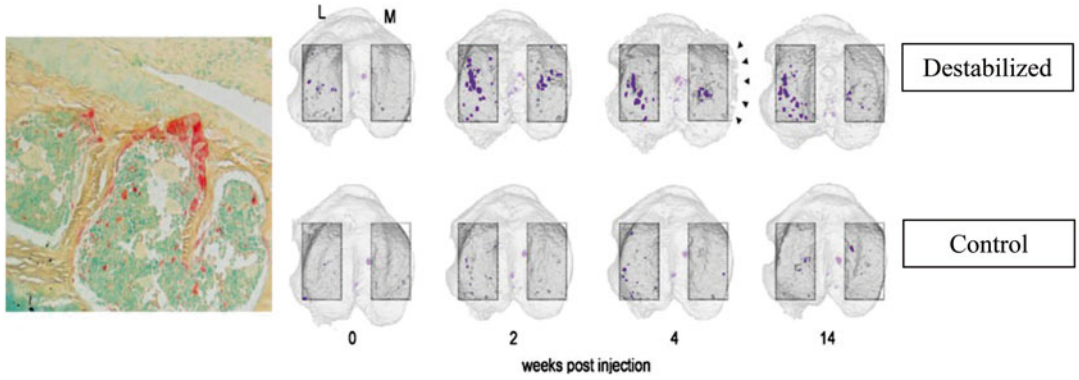

Fig. 18.8 Perforations of the subchondral plate and calcified cartilage through increased osteoclast activity. Histology with specific osteoclast staining (*left*) and counted perforations at different time points measured with in vivo micro-CT (*right*) [Taken from 32]

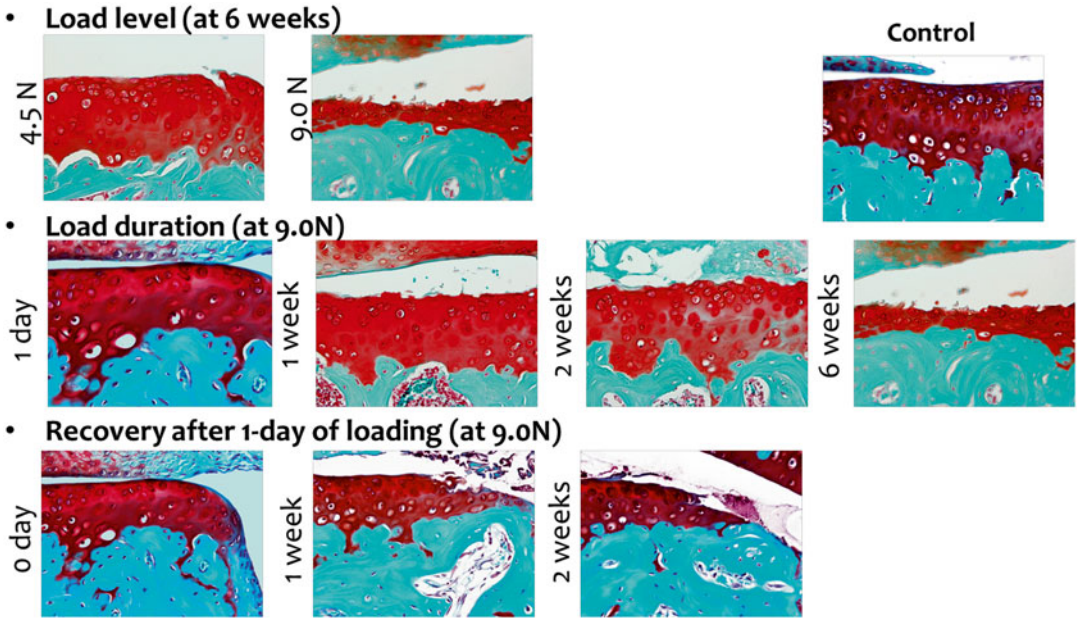

Fig. 18.9 Cartilage matrix changes in the tibia after cyclic compressive loading of the knee joint. *Top left* shows the proximal tibia of an unloaded control knee. The *top row* shows the effect of increased load magnitude (over 6 weeks of loading). The *middle row* shows the effects of repetitive loading duration (at 9 N of daily loading). The *bottom row* shows changes created by a single bout of loading followed out for 2 weeks

(Fig. 18.9). Since the load magnitudes were not excessive, joint stability alterations that occurred when loading the flexed knee presumably contributed to the adaptive and destructive changes to the tissues. This theory is supported by the finding that more severe changes were found in the posterolateral aspects of the knee, consistent with increased sliding of the femur on the tibia in that direction as a result of the applied load.

More recently, these same investigators applied a single nondestructive loading bout to mice knees to differentiate between the contributions of traumatic tissue damage versus cell-mediated processes to OA pathology in the bone and cartilage [39]. While no change was evident in the cartilage or subchondral bone immediately after loading, cartilage thinning and proteoglycan loss occurred as early as 1 week later (Fig. 18.9)

with decreased expression of the autophagy marker LC3 in chondrocytes. Cancellous bone loss was evident by that time as well and was associated with increased osteoclast number. The cell-mediated processes that were initiated by this single loading session may relate to changes occurring after an abnormal loading event in human knee joints, even though the event itself was not overtly traumatic.

Poulet and colleagues [38] used the same model of tibial loading across the knee in Str/ort mice that develop tibial arthritic lesions spontaneously at 20 weeks of age. The genetic susceptibility to arthritis was not apparently linked to greater likelihood of mechanical damage to the cartilage, possibly due to the thick cartilage layer in this mouse strain. Nonetheless, as with the work of Ko et al., load application accelerated the arthritic changes in the knee.

Summary

The complex interaction between biomechanics and biology in the etiology and progression of arthritis remains largely unknown. In some respects, the role of biomechanics in arthritis research has been ignored at worst or poorly controlled at best. Animal models have been used to create a mechanical environment that would induce arthritic changes, but the exact nature of the mechanical alterations and the individual impact of load magnitude and kinematics have not been well documented. Lessons learned from tribology of man-made joints underscore the importance of the interaction between joint force and stability among these models to begin to unravel how these mechanical factors affect the disease [3, 23] (Fig. 18.10). However, consider-

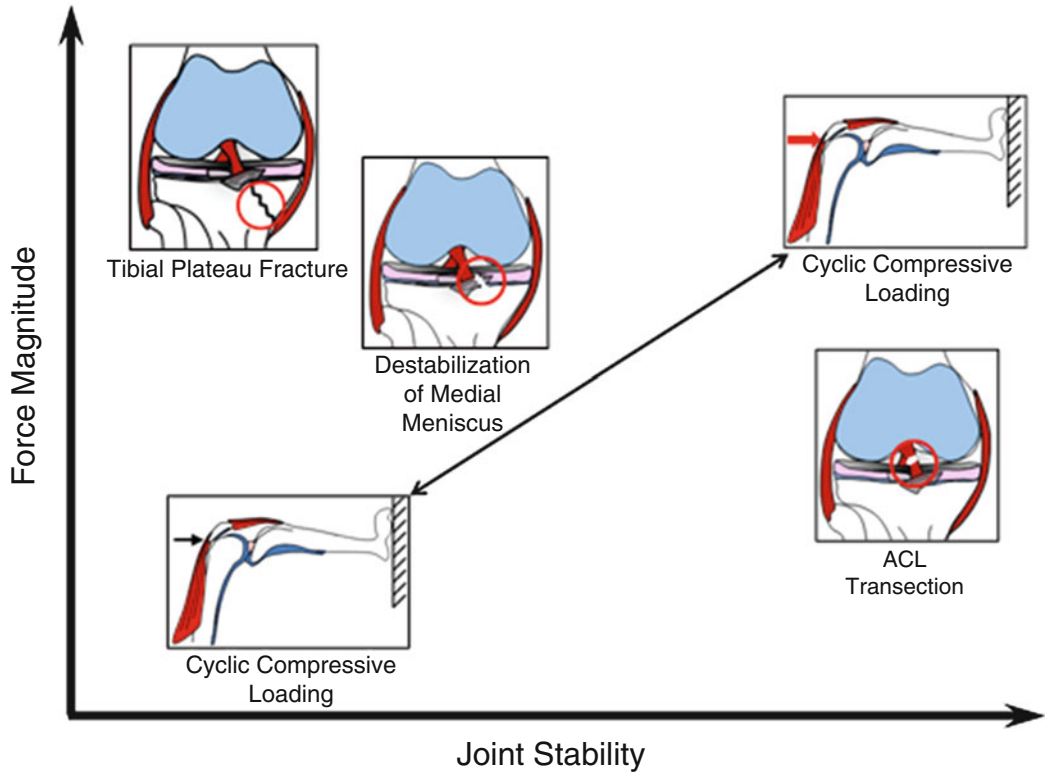

Fig. 18.10 Animal models for arthritis research vary in how they alter the magnitude of the force that crosses the joint and the stability of the joint, i.e., the relative directions and distances that the joint surfaces slide under the influence of the applied loads. Most models are poorly characterized as to the changes in these important mechanical variables and are not robust in their ability to control the variables from one animal to the next. The cyclic compressive loading model has the advantage of controlling variations in the applied load, which will be coupled in some way to alterations in joint stability [Artwork courtesy of Hongsheng Wang, PhD]

able progress is underway, supported by increases in computational tools and computing power, more robust imaging techniques such as single-photon emission computed tomography [35], and the availability of mouse models that can incorporate genetic alterations in specific joint tissues.

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Section IV

Clinical Assessment and Treatment of Patients with Joint Injury

Imaging Specific to Cartilage Injury

19

Nathaniel E. Calixto, Aditi Guha, and Sharmila Majumdar

Introduction

Osteoarthritis (OA) is a complex heterogeneous disease affecting a large number of individuals, manifested by changes in joint cartilage, meniscus, bone and other underlying tissues, leading to progressive damage and degeneration. One of the risk factors for OA is injury to the joint, which could include osteochondral injury, ligament or meniscus injury, and cartilage damage. Following joint trauma, a host of biochemical responses are activated; some of these responses can last transiently, while others may persist for years following the injury [1]. Anterior cruciate ligament injuries and tibial plateau fractures often lead to the development of OA despite clinically assessed successful reconstruction and surgical interventions. Imaging the connective and hard tissues of the joint, plays an important role in monitoring the development of OA, and magnetic resonance imaging (MRI) has become a vital technique in imaging of joint abnormalities, especially OA. MRI is a noninvasive, nonionizing technique which due to its excellent soft tissue contrast images is capable of depicting articular cartilage structure, lesions and accurately evaluating cartilage repair, in addition to providing information with regards to the meniscus, bone, bone marrow, and ligaments post-injury [2–4].

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Morphological Assessment of Cartilage Post-injury

Two-dimensional fast spin-echo (2D FSE) imaging is the method most commonly utilized in clinical settings for imaging of the knee joint and knee cartilage. A combination of proton density and T_2 contrast results in higher cartilage contrast than would be seen on a purely T_2-weighted image. 2D FSE images have good signal-to-noise ratio (SNR), contrast between tissues, visibility of cartilage lesions, and visibility of menisci, bone marrow, and ligaments [5, 6] (Figs. 19.1 and 19.2). Anisotropic voxels produced by the 2D FSE pose an obstacle to image resolution, and often requires scanning in multiple planes in order to gain high-resolution coverage of the full joint.

A 3D version of the FSE sequence has recently been developed, featuring high contrast and isotropic spatial resolution; these developments have resulted in increased accuracy of cartilage imaging. The resulting image data can be reformatted for evaluation of the joint in various planes, and is comparable with multi-planar 2D FSE regarding the evaluation of cartilage, menisci, and ligament. One disadvantage of 3D

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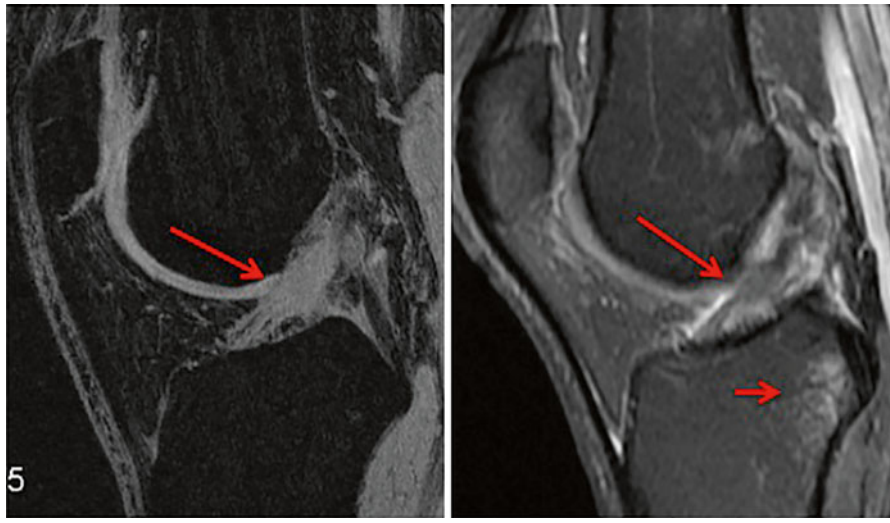

Fig. 19.1 This cut-through demonstrates a tear within the ACL (*long arrow*) on a 3D SPGR image (*left*) and a 2D Fast Spin Echo (FSE) T2 weighted image (*right*). The marrow is better visualized in the FSE image. Figure courtesy of Dr. Lorenzo Nardo, MD, UCSF

FSE is that while it has aided the accuracy of cartilage imaging, imaging of the adjacent bone has not similarly improved [7, 8]. A variation of the 3D FSE sequence is 3D FSE SPACE, which varies the flip-angle of the applied pulses and provides high T_2-weighted tissue contrast. The technique features better SNR and SNR efficiency (SNR normalized by time) compared to other sequences but is not as effective at delineating cartilage lesions as 2D FSE, has poor contrast between cartilage and fluid as well as between cartilage and surrounding tissues, and requires lengthy imaging times [9–11].

Three-dimensional spoiled-gradient-recalled acquisition in steady state (3D SPGR) features higher sensitivity than 2D techniques and is comparable to arthroscopy in the depiction of cartilage defects [12, 13]. The sequence elevates the signal intensity of cartilage versus other tissues and features nearly isotropic spatial resolution. However, the elevated cartilage signal results in poor cartilage-to-fluid contrast, so small defects and edema can be overlooked [11]. In addition, 3D SPGR is unreliable for assessment of joint anatomy aside from cartilage, and long acquisition times are necessary (Figs. 19.1 and 19.2). These sequences have been primarily used for cartilage volume and thickness quantification [14].

Like 3D SPGR, 3D dual-echo steady-state (DESS) imaging is a gradient-recalled echo (GRE) sequence with acquisition in a steady state. In some regards, DESS is superior to SPGR, as DESS features a higher SNR as well as greater cartilage-to-fluid distinction than does SPGR. The quality of DESS in cartilage evaluation is comparable to that of other GRE sequences; 3D DESS showed comparable diagnostic accuracy and precision, and can assess cartilage thickness, volume, and longitudinal change in cartilage thickness similarly to other GRE sequences (Fig. 19.3) [15, 16].

Balanced steady-state free precession (3D bSSFP) imaging also provides good cartilage-to-fluid contrast; this is achieved by selectively increasing the signal of fluid without altering the cartilage signal. For diagnosing cartilage morphology, 3D bSSFP is comparable to 2D sequences and other 3D GRE sequences; however, 3D bSSFP can also effectively image other anatomical features in the knee such as ligaments and menisci [17–19].

Like 3D bSSFP, 3D driven-equilibrium Fourier transform (DEFT) imaging achieves cartilage-to-fluid distinction by selectively elevating the fluid signal. The 3D DEFT sequence does so with an applied 90° pulse,

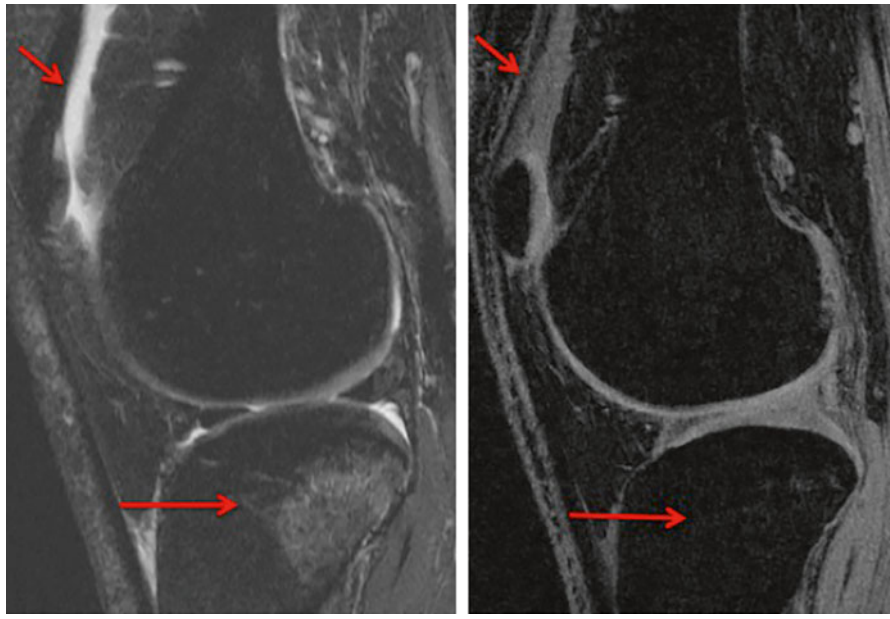

Fig. 19.2 Findings such as joint effusion and bone marrow edema are very common in acute injury and are noted in these images. On this cut-through of the lateral compartment of the knee, bone marrow edema (*long arrow*) and effusion in the joint (*short arrow*) are better seen on the 2D FSE T2-weighted sequence (*on the left*) than on 3D SPGR (*on the right*). However, on the SPGR image, the definition between bone and cartilage is better demonstrated. Figure courtesy of Dr. Lorenzo Nardo, MD, UCSF

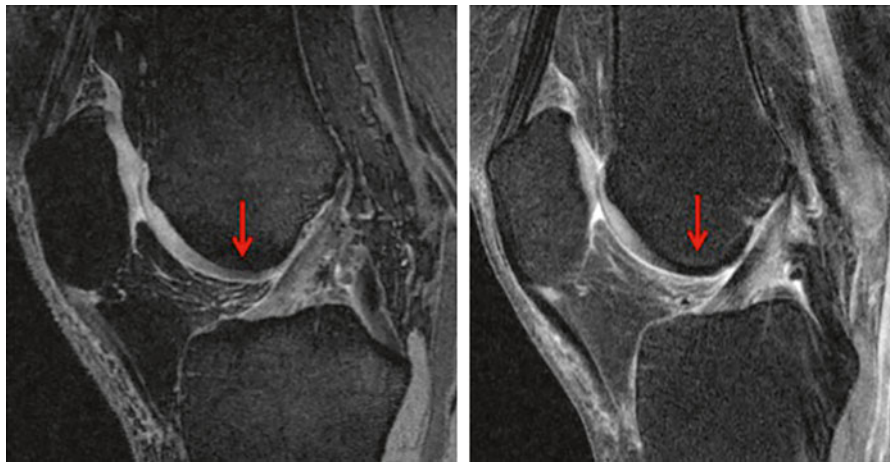

Fig. 19.3 Comparison between 3D sagittal dual-echo steady-state (DESS, *left*) and T2-weighted (*right*) images at the level of the ACL. The cartilage is well-demonstrated on the DESS image, while on the T2-weighted image, the presence of chemical shift artifacts makes the correct visualization of the cartilage difficult, especially on the femoral aspect (*arrow*). Figure courtesy of Dr. Lorenzo Nardo, MD, UCSF

which results in a higher signal from anatomical features with long T_1 relaxation time, such as fluid. The effectiveness of 3D DEFT in the diagnosis of cartilage lesions has been shown to be similar to 2D techniques as well as SPGR [11, 20]. The disadvantages of the 3D DEFT sequence include long acquisition time, low sensitivity to bone marrow abnormalities, and elevated fat signal due to inadequate lipid suppression.

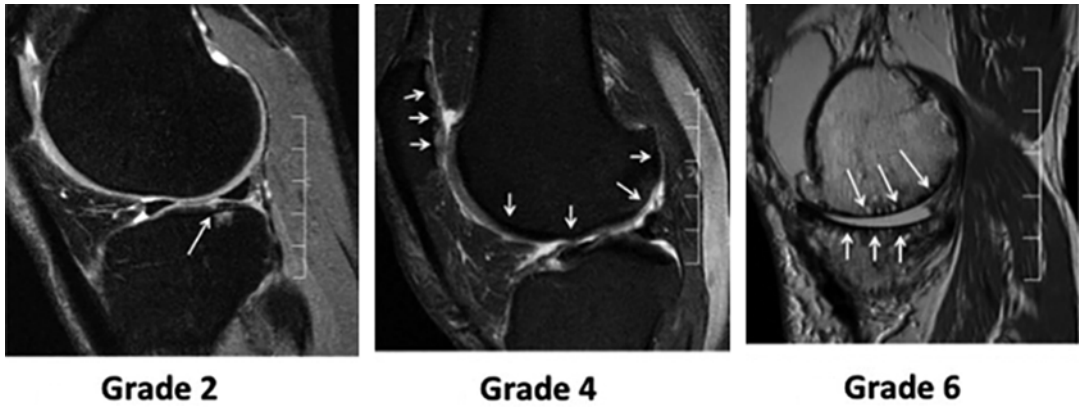

Fig. 19.4 Representative MR images with different stages of cartilage lesions and corresponding WORMS scores

The longest-established rubric for osteoarthritis (OA) image grading is the Kellgren–Lawrence (KL) scale, which assigns a score based on the severity of degeneration as seen on an X-ray image [21]. The KL grading system provides a simple, low-cost assessment of structural change based on joint space narrowing and osteophytes, both readily identifiable OA hallmarks. A number of studies have also used KL grading to evaluate longitudinal OA progression [22, 23].

By the early 1990s, as MRI revealed its potential to provide insight into OA, an alternate MRI-based approach to OA assessment and diagnosis emerged alongside the X-ray image and KL grading. Spector and Cooper commented on inconsistencies in the descriptions of radiographic features by Kellgren and Lawrence themselves as well as inconsistencies between grading at different joints; they also raised concerns about the associations of osteophyte formation with low KL grade and joint space narrowing (JSN) with high grade [24]. Since osteophyte formation and JSN are caused by independent processes, the KL scale provides a skewed depiction of OA progression [25]. The KL system does not evaluate the patellofemoral joint, nor can it assess tissues not visible on radiographs such as cartilage, ligaments, menisci, or the joint capsule [26–28].

While the KL system assigned a single score to the whole joint, the alternative approach favored separate assessment of bone and soft tissue [29]. The emerging technique initially borrowed grading scales used in arthroscopy [30]. Since then, four compartment-based, semiquantitative systems have been formulated to evaluate MR images of cartilage: the Whole-Organ Magnetic Resonance Imaging Score (WORMS) system was the first. WORMS grading assigns separate scores not only to the various knee cartilage compartments (Fig. 19.4), but also bone, menisci, and ligaments. The system also assesses joint effusion, loose bodies, and periarticular cysts [31].

Like WORMS, the Knee Osteoarthritis Scoring System (KOSS) evaluates cartilage, bone, and menisci, and records the presence and extent of effusion, synovitis, and cysts. KOSS also demonstrated both high inter-observer and intra-observer reproducibility; WORMS made no mention of intra-observer reproducibility [32]. The Boston-Leeds Osteoarthritis Knee Score (BLOKS), assesses the same features as WORMS and KOSS, but describes bone marrow edema-like lesions (BMEL) in further detail [33]. The MRI Osteoarthritis Knee Score (MOAKS) system further refines the rubrics of previous scoring instruments, particularly BLOKS. MOAKS features an altered BMEL scoring method, adds scoring for cartilage subregions, and incorporates additional categories of meniscus pathology [34].

The Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) system was specifically intended to evaluate repair cartilage following surgical interventions such as microfracture, chondrocyte transplantation, or osteochondral

transplantation [35]. MOCART showed a strong, significant correlation with clinical outcome [36], and the MOCART system remained effective for both 2D and 3D imaging techniques [37]. The greatest limitation of MOCART is that it only evaluates the repair site, ignoring the remainder of the joint. To simultaneously assess change in cartilage repair sites and the surrounding native anatomy, the Cartilage Repair Osteoarthritis Knee Score (CROAKS) combined variables from MOCART and MOAKS in a comprehensive system suitable for whole-joint grading [38].

Quantitative morphological measures of other features such as bone marrow edema like lesions, meniscal injuries and fractures after anterior cruciate ligament injury have also been quantified, and have been associated with long term evolution into OA. Frobell et al. [39] found fractures in 60 % of ACL injured knees. Meniscal tears were found in 36 % subjects in one compartment, in 20 % of subjects extended to two compartments. BMEL are a common manifestation (98 % of subjects), especially in the lateral compartment (97 %). These authors also demonstrated that 1 year after injury joint fluid and BMEL volume decrease gradually; however, BMEL still persisted and cartilage volume showed increases in certain femoral compartments and decreases in others [40].

Quantitative Imaging of Cartilage Post-injury

Imaging T_2 Relaxation Time

The basic premise of MRI is the excitation of protons and their subsequent relaxation back to an equilibrium state; the T_2 MRI sequence evaluates the excitation–relaxation phenomenon of water protons with regard to the surrounding proteins. T_2 refers to the spin–spin relaxation time, related to the rate at which nuclei lose phase coherence following excitation. Nuclei in phase coherence after excitation result in transverse magnetization and a strong MR signal. When nuclei lose phase coherence, the signal diminishes.

Cartilage is primarily composed of water and proteins such as type-II collagen and proteoglycans (PG). Water protons surrounded by the cartilage matrix undergo interactions with the various macromolecules, which cause faster magnetization decay and a shorter T_2. Free water, however, experiences fewer of these interactions, lengthening T_2. Differences in T_2 are thus sensitive to variations in the free water content of cartilage [11, 41–44]. Studies have demonstrated that cartilage T_2 is correlated with water content [45] but poorly with PG content [46]. Xia et al. showed using microscopic resolutions, that spatial variation in T_2 is dominated by the ultrastructure of collagen fibrils, and thus angular dependency of T_2 with respect to the external magnetic field can provide specific information about the collagen structure [30]. This angular dependency of T_2, however, also results in the "magic angle" effect and commonly seen laminar appearance in cartilage imaging [47, 48]. Using clinically relevant resolutions, T_2 studies revealed three laminae in cartilage - a deep layer adjacent to the bone, a superficial layer on the articular surface, and a transitional layer in between [49]. T_2 generally increases across cartilage from the bone layer to the articular layer [50, 51]. Histologic experiments related regional T_2 variation to differences in collagen orientation and distribution from one layer to the next.

Thus, collagen degradation as seen in osteoarthritis allows increased movement of free water and T_2 has been shown to be elevated in patients with osteoarthritis [48, 52, 53] (Fig. 19.5). Studies using grey level co-occurrence matrix (GLCM) texture analysis of cartilage have shown that T_2 is also more heterogeneous in osteoarthritic cartilage than in controls [53]. Though some studies have found associations between T_2 and osteoarthritis in patellar cartilage [54], tibiofemoral cartilage has been the more noteworthy region for osteoarthritic change [48]; the vast majority of studies investigating T_2 and osteoarthritis report significant findings in tibiofemoral cartilage. This is most likely due to the weight-bearing role that the tibiofemoral compartment plays in normal daily function and movement. T_2 has shown a strong, significant

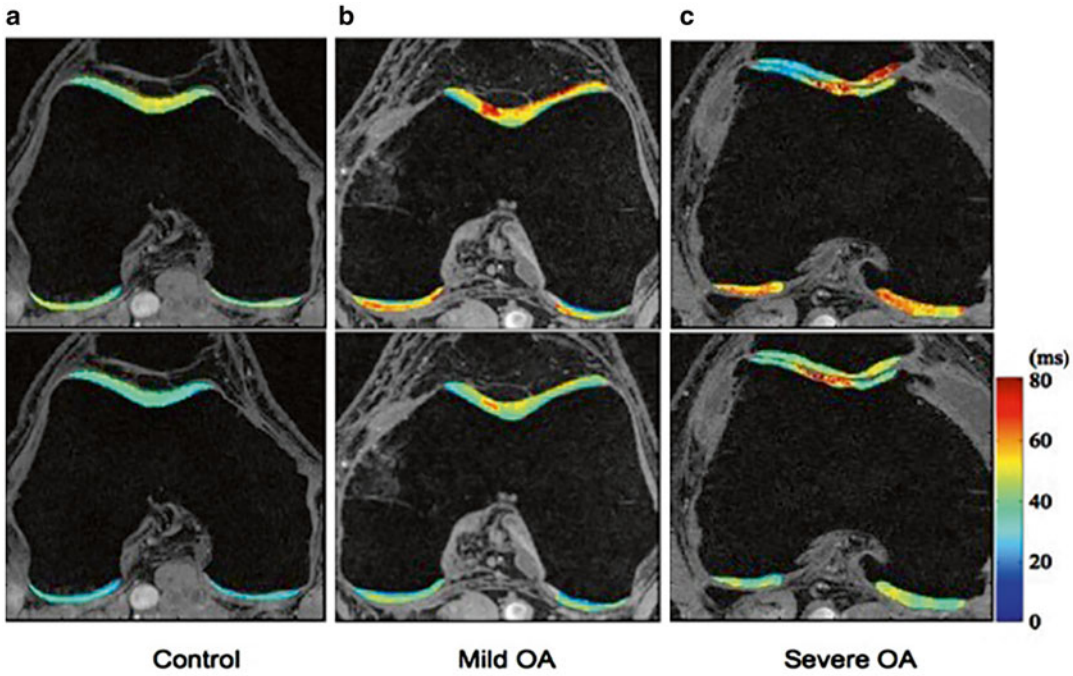

Fig. 19.5 $T_1\rho$ and T_2 maps of a healthy control (**a**), a subject with mild OA (**b**) and a subject with severe OA (**c**). Significant elevation of $T_1\rho$ and T_2 values were observed in subjects with OA. $T_1\rho$ and T_2 elevation had different spatial distribution and may provide complementary information associated with the cartilage degeneration

correlation with a collagen degradation serum biomarker and a significant negative correlation with concentration of glycosaminoglycans (GAG), the component chains of proteoglycans [55], albeit with considerably small sample size.

T_2 elevation has also been associated with trabecular bone loss [56] and BMEL [54], a common occurrence in ACL injury and traumatic injury, and T_2 GLCM heterogeneity has been associated with both BMEL and meniscal lesions [53]. T_2 also has some degree of predictive power regarding osteoarthritis. In a cohort with KL and WOMAC pain scores of 0, those determined "at risk" for osteoarthritis had significantly elevated and heterogeneous cartilage T_2 [57]. In addition, T_2 at baseline is associated with progression of osteoarthritis and cartilage defects 2–3 years later [54, 58]. In subjects with ACL injury, 1 and 2 years post-reconstruction, T_2 values in cartilage of the central aspect of the medial femoral condyle were significantly elevated compared with control knees, indicating a potential change in cartilage biochemistry akin to OA [59].

Finally, T_2 has been used to distinguish repair cartilage from normal cartilage; in an equine model, control cartilage showed the expected trend of T_2 spatial distribution, but sites of cartilage autograft harvest as well as microfracture sites did not [60]. A multimodal approach using T_2, diffusion weighted imaging and grading of MR images has been used for assessing postoperative cartilage. While grading did not show differences between the two repair techniques, T_2-mapping showed lower T_2 values after microfracture, and diffusion weighted imaging between healthy cartilage and cartilage repair tissue in both procedures [61]. Mamisch et al. [62] prospectively used T_2 cartilage maps to study the effect of unloading during the MR scan in the postoperative follow-up of patients after matrix-associated autologous chondrocyte transplantation (MACT) of the knee joint. They demonstrated that T_2 values change with the time of unloading during the MR scan, and this difference was more pronounced in repair tissue. The difference between the repair and control tissue was also

greater after longer unloading times, implying that assessment of cartilage repair is affected by the timing of the image acquisition relative to unloading the joint.

Articular cartilage has very limited intrinsic regenerative capacity, making cell-based therapy a possible approach for cartilage repair. Tissue engineered collagen matrix seeded with autogenous chondrocytes designed for the repair of hyaline articular cartilage have also been proposed and early studies combining MR grading and quantitative T_2 mapping have been used to assess the impact of such repair [63]. In addition to fill efficacy the layered appearance or partial stratification of T_2 as a result of collagen orientation was detected in this study for two patients at 12 months and four patients at 24 months.

Imaging $T_1\rho_{(rho)}$ Relaxation Time

A similar sequence that has recently gained widespread use is $T_1\rho$ (T1 rho) or spin-lattice relaxation in the rotating frame. The sequence employs a constant, low-power radiofrequency (RF) pulse known as a "spin-lock" in the transverse plane [11, 41, 43, 44], which eliminates T_2 relaxation. As is the case for T_2, $T_1\rho$ relaxation is affected when water interacts with large macromolecules. In vitro studies have showed that the elevation of $T_1\rho$ relaxation time was correlated with PG loss in both bovine [46, 64] and human cartilage [65], and with histological grading [65, 66]. In $T_1\rho$ quantification experiments, the spin-lock techniques reduce dipolar interactions and therefore reduce the dependence of the relaxation time constant on collagen fiber orientation [67]. This enables more sensitive and specific detection of changes in PG content using $T_1\rho$ quantification, although $T_1\rho$ changes in cartilage may be affected by hydration and collagen structure as well. Early experiments with the $T_1\rho$ sequence found a similar but not identical spatial distribution to that of T_2, with a trend of increase across the cartilage from bone layer to articular layer [68, 69]. Increases in mean $T_1\rho$ and $T_1\rho$ GLCM heterogeneity are associated with OA [66, 70, 71]. Studies have also shown significant $T_1\rho$ increase in more severe OA as compared to milder OA (Fig. 19.5), controls, or both [72, 73]. Cartilage $T_1\rho$ elevation has also been associated with trabecular bone loss [56], presence and location of BMEL [74], and higher WOMAC scores [75, 76]. In addition, elevated baseline $T_1\rho$ has been shown to predict OA progression at 2-year follow-up [58].

While T_2 changes have been associated with collagen concentration and arrangement, biochemical assays suggest that $T_1\rho$ is more sensitive to proteoglycan content than to collagen [65, 77] and show that elevated $T_1\rho$ is associated with proteoglycan loss [64, 65, 78, 79]. Comparisons between $T_1\rho$ and T_2 have found that $T_1\rho$ features superior delineation of cartilage lesions [80], signal-to-noise ratio [80], larger range [70], higher effect size [70], and greater percentage change with increasing severity of osteoarthritis [66] as compared to T_2.

In patients with acute ACL tears, significantly increased $T_1\rho$ values were found at baseline (after injury but prior to ACL reconstruction) in cartilage overlying BMEL when compared with surrounding cartilage at the lateral tibia [56, 81]. In the posterolateral tibial cartilage, $T_1\rho$ values were not fully recovered 2 after ACL reconstruction. $T_1\rho$ values of medial tibiofemoral cartilage in ACL-injured knees increased over the 2-year study and were significantly elevated compared to that of the control knees (Fig. 19.6). Concomitant meniscal injury also reflected changes in articular cartilage, patients with lesions in the posterior horn of the medial meniscus exhibited significantly higher $T_1\rho$ values in weight-bearing regions of the tibiofemoral cartilage than that of control subjects over the 2-year period, whereas patients without medial meniscal tears did not [59].

Using arthroscopy as a gold standard, Nishioka et al. have shown that in anterior cruciate ligament injured knees, authors have demonstrated that $T_1\rho$ has a sensitivity and specificity of 91.2 and 89.5 % respectively for detecting grade 1 cartilage lesions (as assessed by the ICRS grading system). On the other hand, the sensitivity and specificity for T_2 were 76.5 and 81.6 %, respectively [82]. The cutoff values for determining the presence of a cartilage injury were determined using ROC curves to be 41.6 and 41.2 for $T_1\rho$ and T_2 respectively.

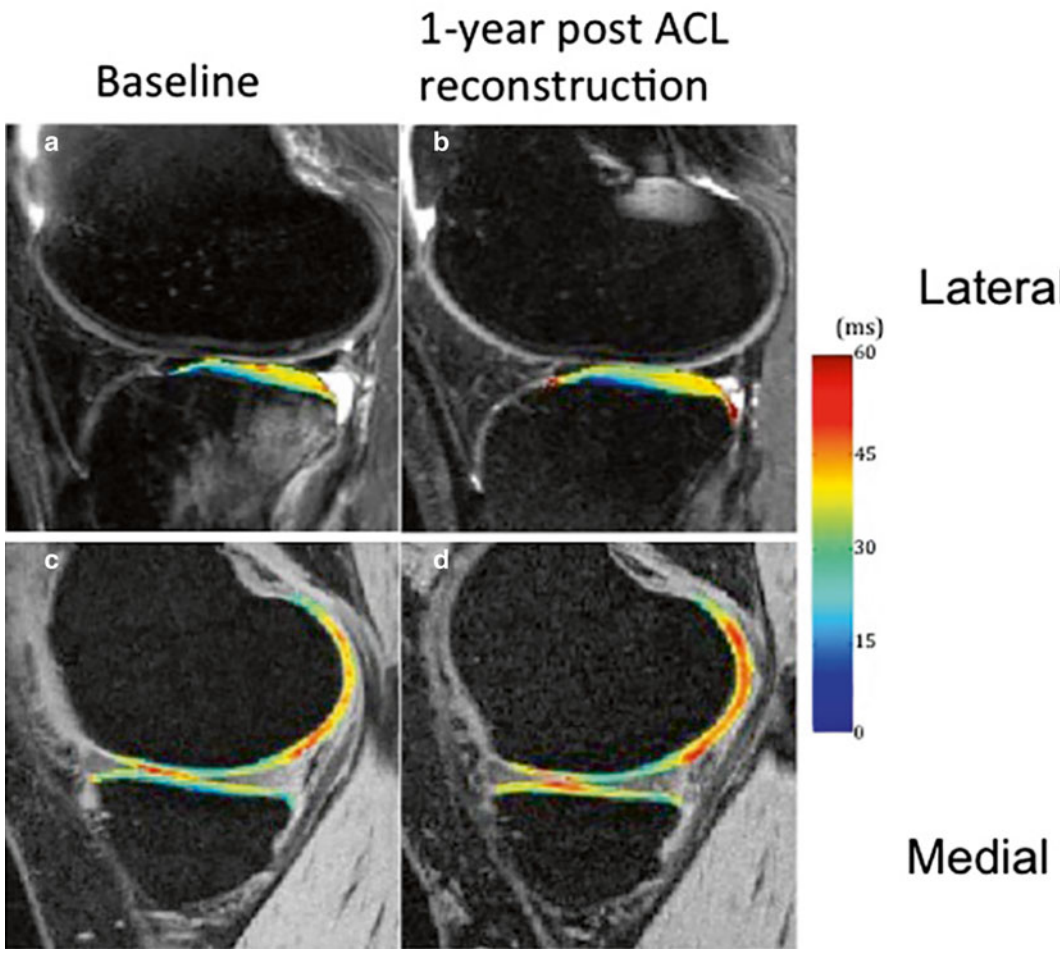

Fig. 19.6 $T_1\rho$ Maps of ACL-injured knees

A combined $T_1\rho$ and T_2 study examining repaired and the surrounding cartilage has demonstrated differences in cartilage after microfracture and mosaicplasty 3–6 months and after a year [83] (Fig. 19.7). In subjects who had microfracture for focal cartilage defects, primarily in the medial femoral condyle, Theologis et al. found that while the average surface area of the lesions did not differ significantly overtime, at 3–6 months, repaired tissue had significantly higher full thickness $T_1\rho$ and T_2 values relative to surrounding cartilage [84]. After 1 year, this significant difference was only observed for $T_1\rho$ values. Analysis of the different laminae of cartilage also showed different trends, with the superficial layer having significantly higher $T_1\rho$ value after 12 months, while the T_2 values had reached the levels of the normal cartilage. Thus, these methods may be used to probe the level of integration of the repair tissue over time [83].

Delayed Gadolinium-Enhanced MRI of Cartilage (dGEMRIC)

The main premise of dGEMRIC is derived from variations in fixed-charge density (FCD) of cartilage [11, 41–44, 85]. Proteoglycan content is thought to influence cartilage FCD, as proteoglycans have glycosaminoglycan (GAG) side chains rich with negatively charged carboxyl and sulfate groups. Ions in the cartilage matrix will distribute

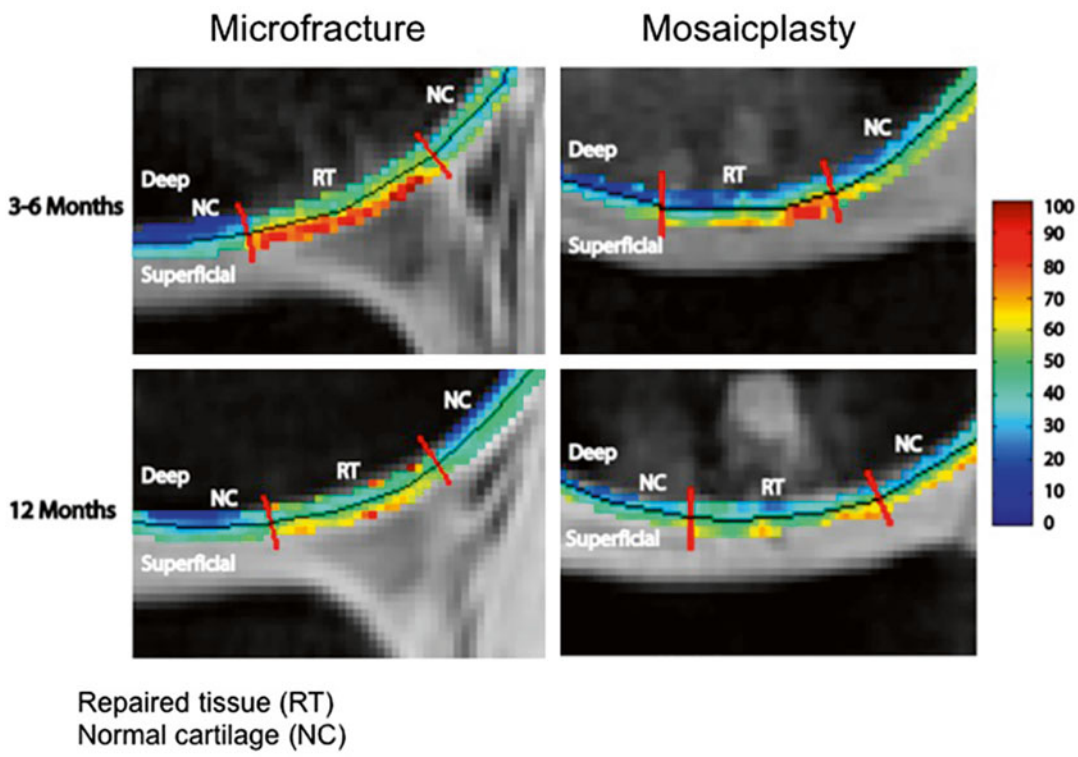

Fig. 19.7 Representative $T_1\rho$ maps of repaired tissue (RT) and normal cartilage (NC) divided into deep and superficial layers (*black line*) 3–6 months and 12 months after microfracture (*left*) and mosaicplasty (*right*) surgeries. The superficial and deep layers of RT 3–6 months after microfracture have elevated $T_1\rho$ values compared to NC. After 1 year, the RT is more homogeneous. The superficial layer of RT 3–6 months after mosaicplasty has elevated $T_1\rho$ values relative to NC. The deep layer is similar to NC. After 1 year, deep and superficial layers of RT resemble those of NC

accordingly in relation to the FCD and approximate the GAG concentration and distribution. Cations will pool in areas of high FCD; anions, in areas of low FCD. The ionic compound gadopentetate dimeglumine, or $Gd(DTPA)^{2-}$, is an effective and FDA-approved contrast medium for use in human MRI. The highly paramagnetic $Gd(DTPA)^{2-}$ reduce the T_1 relaxation time of surrounding tissue, such that areas of high $Gd(DTPA)^{2-}$ concentration will result in reduced relaxation time, and areas of low $Gd(DTPA)^{2-}$ will have elevated relaxation time. Since $Gd(DTPA)^{2-}$ is an anion, it will accumulate in cartilage regions with low FCD and, by extension, low GAG concentration.

Cartilage lesions in specimen studies were first revealed by signal intensity differences between cartilage and contrast medium [86]; since then, lesions been revealed quantitatively by lowered $T_1 + Gd(DTPA)^{2-}$ values [87, 88], even focal lesions surrounded by largely intact cartilage [68]. Biochemical studies have found a positive correlation between $T_1 + Gd(DTPA)^{2-}$ and GAG concentration [88, 89], and between in vitro and in vivo $T_1 + Gd(DTPA)^{2-}$ values [89]. In imaging studies, $T_1 + Gd(DTPA)^{2-}$ has been shown to be significantly elevated in controls versus osteoarthritic individuals, and in moderate osteoarthritis versus severe cases [90, 91]. Finally, dGEMRIC has shown a strong correlation with WOMAC pain scores [92].

Fleming et al. have shown a significant difference (13 %) in the mean dGEMRIC indices of the medial compartment between ACL injured and uninjured knees ($P < 0.007$) [93]. Despite its strengths, dGEMRIC is an invasive and

time-consuming procedure. The quickest delivery of $Gd(DTPA)^{2-}$ into cartilage is intravenous administration [87], and the joint of interest must be moved for 10 min afterward to distribute the contrast medium [94]. Data acquisition also begins 90 min post-injection [95].

Sodium Imaging

Sodium MRI imaging provides a noninvasive protocol specific to proteoglycan assessment. Sodium-23 is an ideal MRI contrast agent, occurring naturally in the body and possessing a nuclear spin momentum [11] due to its odd number of protons. Cations like Na^+ will pool in areas of high negative FCD; since negatively charged proteoglycans influence FCD, Na^+ concentration will be elevated with high proteoglycan content. Reduction in Na^+ MRI signal has been correlated to proteoglycan depletion through FCD mapping [79] and trypsinization assays [96, 97]. Na^+ MRI has shown a significant increase of Na^+ T_1 and Na^+ T_2 in proteoglycan-depleted cartilage [98]; Na^+ relaxation times follow the same trend as proton relaxation times in compromised cartilage. Finally, the SNR of Na^+ MRI is significantly higher for native cartilage than for repair tissue [99, 100]. There are a number of difficulties with Na^+ MRI; Na^+ ions exist in the body at lower concentrations than do H^+ ions, and Na^+ features a lower resonant frequency and shorter T_2 relaxation time. These factors necessitate high magnetic field strength, special equipment, and lengthy imaging to attain a proper SNR [11, 44].

Glycosaminoglycan Chemical Exchange-Dependent Saturation Transfer (gagCEST)

Sodium MRI and dGEMRIC both use FCD to indirectly measure proteoglycan content in cartilage. Glycosaminoglycan chemical exchange-dependent saturation transfer (gagCEST) aims to directly measure proteoglycans by observing the behavior of ^-OH protons in GAG [101]. An off-resonance pulse targets restricted protons such as those bound to a macromolecule; the pulse excites and saturates these protons in the process [41, 43]. The excited, saturated protons then exchange magnetization with surrounding free water molecules. Free water protons lose magnetization more slowly than do restricted protons; however, since restricted proton magnetization is more rapidly dephased, water molecules receiving magnetization from restricted protons experience faster dephasing and lower signal. gagCEST involves the transfer of the excited ^-OH protons themselves, present throughout cartilage GAG. The contrast achieved is quantified as CEST effect or CEST asymmetry. In regions of low GAG content, low transfer occurs, and lower signal is observed.

CEST signal decreases with increased ex vivo proteoglycan depletion by trypsinization, as well as cartilage lesions in vivo [101]. gagCEST has shown useful results when evaluating repair cartilage following microfracture and chondrocyte transplantation [99, 100]. Asymmetry is significantly higher in native versus repair cartilage, and locations of gagCEST signal reduction agreed with those found with Na^- MRI. The ratio of native to repair cartilage determined by gagCEST also negatively correlated with MOCART score [99].

Image quality is adversely affected by variations of the principal magnetic field (B_0) within cartilage, low signal, and interference due to magnetization transfer from water and other macromolecules. The gagCEST signal has shown improvement with B_0 correction [102], imaging at 7T instead of 3T [102], and a uniform magnetization transfer (uMT) technique that has effectively eliminated extraneous magnetization transfer effects [103].

Diffusion-Weighted Imaging (DWI)

Diffusion-weighted imaging (DWI) measures the motion of free water in cartilage. In DWI, two diffusion-sensitizing gradient pulses are applied; the first dephases the spins of molecules in the tissue, and the second rephases the spins. Only the spins of stationary molecules will be fully

rephased by the second pulse; the spins of mobile molecules such as free water will not refocus and thus result in MR signal loss [11, 42, 43].

With DWI, one can measure the apparent diffusion coefficient (ADC) of free water, with a higher ADC indicating increased diffusion capability. Low ADC is indicative of slower diffusion and healthy cartilage, as the protein matrix serves as a barrier to free water movement [47]. An early study of diffusion MRI revealed that the diffusivity of several ions and water decreased in cartilage as compared to free solution [104]. Water diffusivity is elevated in proteoglycan-depleted cartilage [104, 105]. In addition, the diffusion coefficient is higher in repair cartilage following chondrocyte transplantation versus controls at early and late follow-up time points; DWI can still distinguish between repair and native cartilage 4–5 years post-transplantation [37, 106]. Additional results suggest that DWI has the capability to monitor the maturity of repair cartilage [37], and that it detects increased heterogeneity in repair versus native cartilage [106].

Periarticular Implants and Imaging

Unfortunately, post traumatic arthritis cannot be prevented and there is no effective treatment currently available. Low impact exercises, strengthening muscle around the joint and pain management can improve the quality of life of patients significantly. These measures help in alleviating the condition but cannot cure the arthritis. In some advanced cases, metallic implants may be used to surgically reconstruct the whole joint or a part of it. According to the Center for Disease Control and Prevention (CDC), there have been 719,000 total knee and 332,000 hip replacements in the USA in 2010 [107]. Joint replacement metallic parts are normally made of titanium, cobalt-chromium, or MR-safe stainless steel (screws), which are non-ferromagnetic and are convenient for MR imaging.

Standard MR sequences are used post operatively to look at the success of implantation, identify potential complications and to look at cartilage healing status. Although any imaging technique discussed in the first part of this chapter may be used to investigate the post operative changes, the presence of these implanted metal objects cause problems and produce artifacts near the implants interfering with the clinical quality of the MR images (Fig. 19.8). Although implants made of ceramic with even lower magnetic properties, longer lasting and higher biocompatibility (http://www.hss.edu/newsroom_11290.asp) compared to the ones currently being used have been developed, they have not been accepted widely by the orthopedic surgeons [108]. This part of the chapter discusses the artifacts, their causation factors, pulse sequences that are used to minimize these artifacts and emerging artifact reduction techniques.

Artifacts near metal implants can be broadly categorized into in-plane and through-slice artifacts. The most common in-plane artifacts related to metal implants occur in the readout direction and cause signal voids due to dephasing, failure in fat suppression techniques, signal pile-up, or geometric distortions near the implants. Through-slice artifacts are commonly seen in the form of slice distortion in the excited slice [109, 110].

Signal voids occur due to the fact that there is no MRI signal from metal. Other artifacts like geometric distortions and signal pile-up occur because of metal induced magnetic field variations that result in a phenomenon known as "susceptibility" variations between the metal and the surrounding tissue. Magnetic susceptibility ("χ") is defined by the magnitude of a material's response to a magnetic field. When an object is placed in a magnet with homogenous magnetic field, the object depending on its susceptibility interferes with the imaging gradient field [111]. The material's magnetization is equal to the dimensionless susceptibility "χ" multiplied by the applied magnetic field strength (B_0). As seen in Table 19.1, the susceptibility of metals used in implants is much higher than the surrounding tissue. The tissue–air interface by itself is also capable of producing susceptibility artifacts that are noticeable on MR images. These susceptibility differences give rise to inhomogeneities in the local magnetic field. These field variations are affected by various factors as seen in Table 19.2,

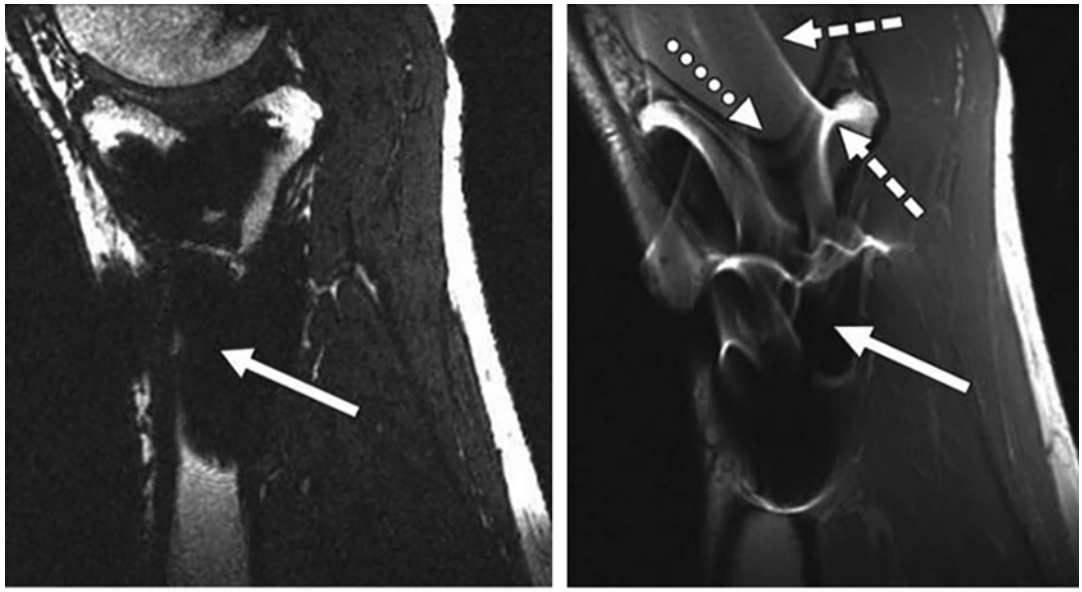

Fig. 19.8 Examples of artifacts observed in MR images due to presence of stainless steel screws in healthy 37-year-old man. *Left*: In gradient-echo image with ± 62.5 kHz receive bandwidth. Right: spin-echo image with ± 16 kHz receive bandwidth. *Solid arrows* show signal loss that can be due to dephasing or from signal being shifted away from region. *Dotted arrow* in B shows geometric distortion of femoral condyle, and *dashed arrows* show signal pile-up, which can be combination of in-plane and through-slice displacement of signal from multiple locations to one location. Reprinted with Permission from ARRS

Table 19.1 Magnetic susceptibilities of human tissue and metals commonly used in implants [133]

Material	Susceptibility (χ, ppm)
Human tissue	−10
Air	0.36
Titanium	178
Cobalt-chromium	900
Stainless steel (MR safe)	3,000–7,000

which describes general factors affecting metal implant artifacts.

Apart from the signal loss and geometric distortions an important impediment to imaging near metal are the artifacts caused by failure of fat suppression and signal pile up. The suppression of fat signal is very useful to improve the soft tissue contrast in anatomical MR images. The bright (hyper-intense) appearance of fat causes problems in contrast enhanced bright lesions in T_1 weighted images. Likewise, in T_2 weighted images, the bright fat tissue signal intensity is

Table 19.2 Table showing general factors that impact metal artifacts [105, 107, 108, 113]

Factor	Effect
Metallic composition of the implant	Non-ferromagnetic implants (titanium alloy) produce less artifacts than ferromagnetic (stainless steel)
Implant size	Smaller implants produce less artifacts than larger
Orientation of the implant	Artifact size increases and shape varies with increase in angle between the implant and the direction of the main magnetic field
Magnetic field strength	Lower field strength produce less artifacts than higher field strength magnets
Pulse sequence	Signal loss due to implants in Spin echo (SE) based sequences less compared to Gradient echo (GRE) sequences
Pulse sequence parameters	Smaller voxel size, high resolution matrix, longer echo train length, shorter TEs and thinner slices produce less artifacts

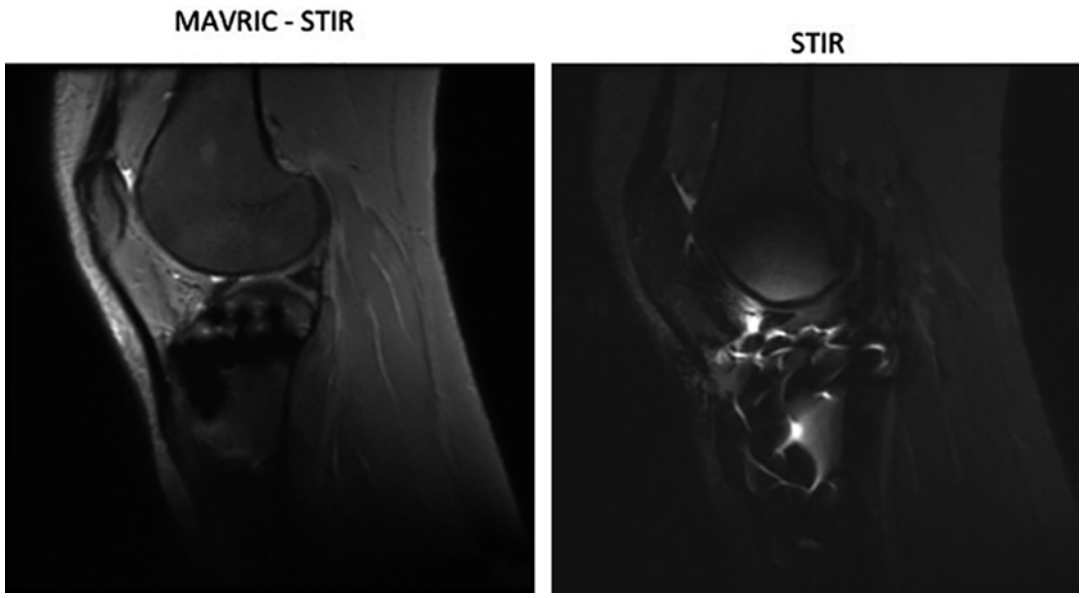

Fig. 19.9 MAVRIC STIR and standard STIR images obtained at 3 T in a patient with internal fixation of tibial plateau fracture with multiple screws and plate. Note visualization of tibial plateau cartilage and reduced signal pile up (*bright areas*) in MAVRIC STIR but not in the standard STIR image. Figure courtesy of Dr. Thomas Link, MD, UCSF

confused with fluid or lesions exhibiting similar signal intensity. Also, being the second most abundant after water protons in the human body, fat protons are the major contributor of chemical shift artifacts. The most common technique used to avoid these artifacts is chemically selective fat suppression, also called fat saturation. This technique selectively excites fat instead of water molecules taking advantage of the fact that the resonant frequency of fat is ~220 Hz below water at 1.5T. However, the frequency difference (in the 3–80 kHz range) near metallic implants are much greater than the chemical shift frequency, which causes the fat saturation pulse to miss the resonant frequency of fat near metal implants altogether [108, 112]. Currently, the standard methods used for fat suppression in imaging near metal are STIR (Short T1 Inversion Recovery) [113] and Dixon method [114]. The T_1 relaxation time of fat at 1.5T is ~230 ms, which is shorter than most of the other tissues in the body. This property is exploited in STIR, by using a short inversion time to null the signal from fat while maintaining the signal from water and soft tissue (Fig. 19.9). A 180° RF pulse is applied that inverts the magnetization followed by a 90° RF pulse which brings the residual longitudinal magnetization in the transverse plane where it is read by the RF coils. The delay between the 180 and 90° pulses is called inversion time (TI). In simple terms, in DIXON (2-point) method, two images are acquired, one when the water and fat is in-phase and the second in which water and fat is out of phase. During reconstruction a water only image can be calculated. The "point" refers to the number of images acquired [115].

Several techniques have been proposed and used for reducing metal artifacts. One of the first techniques used was pre-polarized MRI (PMRI), where the main magnetic field is created using a polarizing magnet and another magnet, which is typically of lower strength (~0.05T) is used for readout. Susceptibility related artifacts are almost negligible due to the low strength of the readout field. Also, PMRI systems are usually cheaper than the typical MR systems. However, its implementation requires specialized hardware and it cannot be applied to whole body imaging as there are concerns about heating of the polarizing magnet [116–118].

In single point imaging (SPI) only one k-space point is sampled for each excitation pulse. Since it is a pure phase encoding method the slice selective and readout encoding gradients are not used and distortions due to susceptibility are eliminated. SPI is also known as constant time imaging, because the phase encode time (t_p) is constant throughout the sequence. The long acquisition time is a major drawback of this method. A modification of SPI, called the *single point ramped imaging with T1 enhancement* (*SPRITE*) technique was introduced by Balcom et al. where the gradients are not switched on and off for each acquisition like in the SPI technique. Instead, they are kept on and ramped in discrete steps, enabling sampling of a single data point at each gradient step before the next TR period. The use of ramped gradient reduces acquisition time and wear on gradients compared to SPI [108, 119, 120]. But there are major drawbacks in this technique due to which its clinical application has been challenging. Sufficient acceleration cannot be achieved using the existing technique, which impacts resolution and volumetric coverage of the desired region of interest. Also artifacts have been reported in tissues with longer T_2 when using SPRITE [120]. A number of tissues observed in clinical orthopedic imaging fall in this category. Several other versions of the SPRITE sequence have been proposed to address the acquisition time issue, like the spiral, conical [121], and diagonal [119] SPRITE referring to the shape of the k-space trajectories used in each method but these have largely been proof of concept studies.

View Angle Tilting (VAT) proposed by Cho et al. [122] is a technique in which an additional gradient along the slice selection direction is applied along with the readout direction in a conventional spin echo sequence. This extra gradient is of the same magnitude as the slice selection gradient and is played simultaneously during readout, thus producing a "tilting view angle." This results in displacement along slice cancelling out the displacements along the image in-plane. The in-plane distortions of the image are taken care of but this technique introduces blurring in the images if the readout duration does not match the duration of the excitation pulse [123]. To reduce blurring in MR images, conventionally the bandwidth of RF pulse is reduced but doing this introduces slice warping or "potato chip" or curved slice artifact in the presence of magnetic field in homogeneities. A second alternative is to increase the readout bandwidth that results in loss of signal-to-noise ratio (SNR). Butts et al. suggested using a quadratic phase pulse or multiple readout method in which each readout is shorter than the excitation pulse. But this causes loss of SNR and performing multiple readouts will result in increased acquisition time [123]. Another technique where VAT is used in conjunction with increased slice selection and readout gradient strength referred to as "*metal artifact reduction sequence* (*MARS*)" was introduced by Olsen et al. They also suggested increasing RF bandwidth and a narrow slice thickness (3–4 mm). However, the method does not eliminate blurring and results in low SNR in images [124]. Additionally, all the VAT sequences suffer from through-slice artifacts (Fig. 19.10). Some exceptions are the sequence proposed by Butts et al. using field map and post-processing correction [125]; Koch et al. using frequency offsets in transmission and receive frequency [126] and a combination of VAT and SEPI (slice excitation profile imaging) proposed by Lu et al. [127] which includes an additional phase encoding along the slice select encoding direction. But these sequences work at the expense of SNR efficiency and require longer scan time, and residual artifacts near the implants are observed.

Through-slice artifacts manifest as slice distortions, signal voids (dark areas) or signal pile up (bright areas) in the excited slice profile. These artifacts can be avoided by increasing readout bandwidth but at a cost of increased RF power deposition resulting in increased specific absorption rate (SAR) to the patient and heating of the implant. Due to SAR issues, through slice artifacts pose a challenge when imaging near metal implants. Decreasing slice thickness may help at the cost of increased scan time because sufficient number of slices have to be acquired to image the desired region of interest and reduced SNR because of reduced voxel size. One method to correct through slice distortion was shown by Butts et al. using VAT sequence, acquiring thin slices along with a field map.

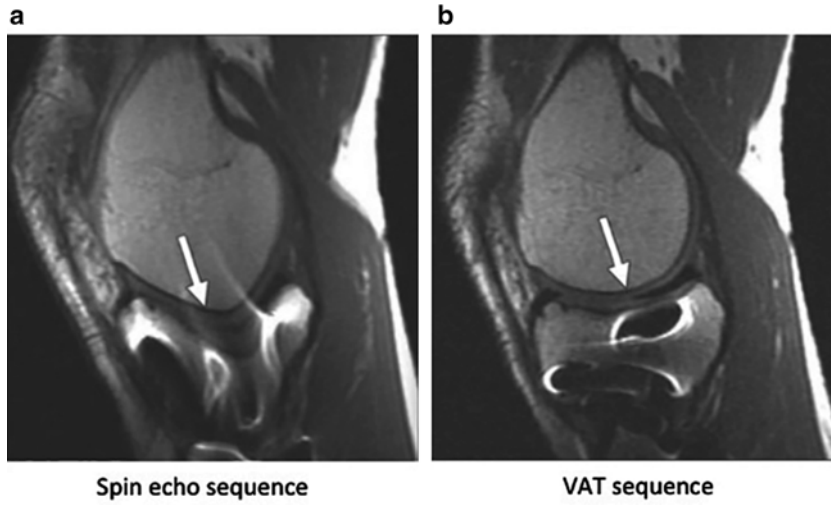

Fig. 19.10 Representative MR images of a 37-year-old man with stainless steel screws in knee tibia using (**a**) Conventional Spin echo and (**b**) View angle titling (VAT) pulse sequences with identical parameters. Note that the geometric distortion (shown with *arrows*) seen in spin echo (SE) image is completely corrected in the VAT image but through-slice signal loss and signal pile up artifacts still exist (*bright areas* in the tibia). Reprinted with Permission from ARRS

They used the field map to correct for small shift and intensity variations in the slice profile. The method has been shown to work reasonably for some metallic implants with reduced SNR efficiency [104, 120]. Another technique called the gradient echo slice excitation profile imaging (GESEPI) uses incremental gradient offset in the slice encoding direction and an additional Fourier transform to recover signal losses [123]. 3D z-shimming method acquires extra k-space lines in the slice encoding direction to make up for the magnetic field variations near the metallic implants [124]. In the past few years, more robust and advanced methods have been introduced by researchers to address both in-plane and through-slice distortion issues, which will be discussed in the next section.

The multi-acquisition variable-resonance image combination (MAVRIC) proposed by Koch et al. is based on 3D FSE (fast spin echo) sequence [125]. The MAVRIC sequence does not use any slice encoding gradient so surface coils which restrict signal in the *z*-axis have to be used to prevent through-slice distortions, but in-plane artifacts still exist. As mentioned previously, conventionally used 3D FSE images show signal voids near the implants due to the phase of the spins being outside the frequency (off resonant spins) of the RF pulses applied during the sequence. In MAVRIC, multiple 3D FSE images are acquired at different transmission and reception frequencies instead of exciting a whole slice or slab. Any other frequency outside of this range is not acquired. These individual images or "bins" are individually reconstructed using either maximum intensity projection (MIP) or sum of squares (SOS) computation. Then each unique subimage acquired within a single TR period is interleaved to obtain the entire composite image of the knee including the implant. The number of interleaved images acquired during each TR period is determined by the echo train length (ETL) of the FSE sequence. Using MAVRIC, clinically viable images can be obtained (Fig. 19.9) but the major challenge encountered is the long acquisition time. However, partial k-space filling, zero filling, compressed sensing, and parallel imaging techniques have been used to reduce acquisition time [125, 126]. The only artifact (in-plane) that is seen near the metallic implants in the images while using a MAVRIC sequence is when the local field inhomogeneity gradient exceeds the magnitude of the readout gradient causing signal from multiple pixels to accumulate in a single pixel causing bright "signal pile-up." [109, 125, 126, 128]

The "Slice encoding for metal artifact correction" (SEMAC) sequence combines two metal imaging techniques using 2D spin echo along with VAT [113, 127]. SEMAC selectively excites 2D slices but reconstruction of each slice is done in 3D to deal with the through-slice distortions. In-plane artifacts are resolved by using VAT-SE (view angle tilting-spin echo) sequence with additional phase encoding along the slice select (z) axis [118, 127]. Specifically, as described previously, the spin echo sequence prevents signal losses due to dephasing (with the use of the 180° refocusing pulse) and the VAT compensation gradient together address in-plane distortion issues [129]. The additional phase encoding allows for a distortion free reconstruction. It is used to obtain subimages with additional phase advances from each slice that are registered to one another and added in the end (using either linear summation or sum of squares) to get a final artifact free image. The number of subimages acquired for a particular acquisition will depend on the extent of distortion expected from the metallic implant and it is important that sufficient number of subimages be acquired to cover the entire volume of the region of interest to be imaged.

To summarize, SEMAC uses a 2D multi-slice excitation, whereas in MAVRIC a series of receive and transmit bandwidth is used to produce volumetric images. Both use standard 3D spin echo readout to limit through-slice distortions. SEMAC also uses additional phase encoding in slice select gradient and VAT for in-plane distortions. While these work excellently to resolve near metal implant artifacts, both require significantly longer scan time. However, accelerated acquisition techniques like parallel imaging including data-driven methods such as ARC (Autocalibrating Reconstruction for Cartesian imaging) and GRAPPA (Generalized Autocalibrating Partial Parallel Acquisition) and physically driven methods like SENSE (Sensitivity encoding), partial Fourier reconstruction or compressed sensing can be used with both SEMAC and MAVRIC to bring down the acquisition time within reasonable limits [129, 130].

A new set of advanced imaging methods collectively termed as "3D multispectral imaging" (3D-MSI) based on the principles of MAVRIC and SEMAC imaging sequences has been proposed by various researchers and has shown promise yielding better clinical grade images with lesser artifacts near metal implants. These methods mainly include the MAVRIC-SL, MSVAT-SPACE, UTE-MAVRIC, SEMAC-VAT, and MAVRIC-SEMAC hybrid that have been shown to have the potential to be combined to obtain images with better diagnostic value in reduced scan time compared to the existing MAVRIC and SEMAC sequences individually. Other non-spin echo based accelerated sequences such as RUFIS (rotating ultra fast imaging sequence) [131], WASPI (Water and fat Suppressed Projection MR Imaging) [132] and SWIFT (Swept Imaging with Fourier Transform) [131] can be explored in combination with MAVRIC and is currently the topic of interest among researchers [104, 109, 130, 132–134].

In the context of post-traumatic osteoarthritis occurring as a result of tibial or plafond fractures, imaging the articular cartilage is a challenge due to the presence of metal implants, screws and other hardware. Future work is warranted to try and minimize metal artifacts in $T_1\rho$, T_2, diffusion and other sequences characterizing cartilage biochemistry. Furthermore, there is a great need for standardization of these methods, and dissemination of these techniques so that they may be translated to the clinic.

In summary, the potential for MR imaging in post-traumatic OA is immense; however, orthopedic surgeons, radiologists, other musculoskeletal clinicians should urge the vendors of MR equipment to make this a priority in order to accomplish the much needed translation to the clinic.

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Outcomes of ACL Injury: The MOON Consortium

20

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Abbreviations

ACL	Anterior cruciate ligament
ACLR	Anterior cruciate ligament reconstruction
ADL	Activities of daily living (a subscale of the KOOS)
autoBTB	autograft bone–(patella)tendon–bone
autoHAM	autograft hamstrings (quadruple)
BMI	Body mass index
IKDC	International Knee Documentation Committee subjective knee questionnaire
IQR	Interquartile range
KOOS	Knee injury and osteoarthritis outcome score
MARS	Multicenter ACL revision study
MOON	Multicenter orthopedic outcomes network
NCAA	National Collegiate Athletic Association
OR	Odds ratio
QoL	Quality of life (a subscale of the KOOS)
SF-36	Short-form 36

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Introduction

In this chapter we review a spectrum of clinically relevant outcomes of Anterior Cruciate Ligament ("ACL") Reconstruction ("ACLR"). Validated patient reported outcomes and reoperation risks are summarized from the Multicenter Orthopedic Outcomes Network ("MOON") longitudinal prospective cohort study of 2,340 ACLRs. The MOON design identifies both modifiable (e.g., body mass index, activity level, graft choice) and non-modifiable (e.g., age, sex, concurrent injury) factors that predict these outcomes. The rationale, benefits, and limitations of a longitudinal prospective cohort study design are reviewed; however, the reader is referred to a recent publication for more in-depth detail [1]. MOON data represents the highest available level of evidence in the literature for outcomes in ACLR, specifically related to prognostic factors, and can be utilized to guide clinical decision making, individual patient expectation/prognosis and to design high level comparative studies in areas identified for potential improvement.

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The Epidemiology of ACL Injury and ACLR

The incidence of ACL injury has been estimated in the general population between 8.1 and 36.9 per 100,000 person-years [2, 3]. Although females are thought to be at higher risk of ACL tear, there is a paucity of high quality prospective injury surveillance data examining the incidence of ACL tear by sex or sport. In a systematic review of level IV studies [4] the comparative incidence of tears in females was approximately 3:1 for basketball and soccer. Similarly, incidence of ACL tear reported in NCAA athletes over a 15-year period [5] was 0.28 and 0.32 per 1,000 athlete exposures in women's basketball and soccer, respectively, compared to 0.03 and 0.13 in men's. Thus, it would appear that females, especially those involved with basketball or soccer, are at highest risk for an ACL tear.

Whether treated operatively or nonoperatively, the goal of initial management in a patient with a torn ACL is to reduce knee pain and symptoms. Typically, this is achieved with non-prescription oral analgesics and the initiation of rehabilitation including a range of motion exercises, icing, and sport restriction. MOON data ($n=525$) has been utilized to identify prognostic factors for increased pain and knee symptomatology at the time of ACLR after undergoing the standard-of-care preoperative rehabilitation [6, 7]. Pain was defined from Knee Injury and Osteoarthritis Outcome Score ("KOOS") and Short Form-36 ("SF-36") subscales, with the following predictors found to be associated with higher pain scores: body mass index ("BMI"), female sex, lateral collateral ligament injury and older age. The same factors, with the exception of age, were also associated with a lower KOOS knee function score. Only for lateral collateral ligament injury in isolation was the influence on pain considered clinically significant. The presence of or number of bone bruise(s) on MRI (which was found in 80 % of patients on the posterior lateral tibial plateau and lateral femoral condyle) were not associated with the aforementioned patient reported outcomes. Bone bruises were, however, associated with younger age and a non-jumping mechanism of injury; the latter finding has been previously corroborated in prospective data collection [8].

ACLR is the standard of care for persons who subsequently experience recurrent giving way, and who participate in high-demand activities. This has resulted in an estimated >175,000 ACLR performed in the USA each year [9]. The outcomes following primary ACLR are discussed in subsequent sections.

Concurrent Injuries: Meniscus and Articular Cartilage

A torn ACL often occurs with injury to the articular cartilage or meniscus. The reported rates of concomitant injuries vary widely in the literature, which may reflect factors such as chronicity from injury to surgery, preexisting conditions, and the mechanism of injury. In MOON, concurrent meniscal tears were identified in 65 % of patients, and 46 % of patients had an articular cartilage injury, including 19 % with a lesion classified as grade III or IV by modified Outerbridge.

The MOON cohort was compared to a National registry of knee ligament reconstruction in Norway [10], to identify similarities and differences between baseline patient characteristics at surgery and outcomes of ACLR. The MOON cohort in that analysis consisted of 713 patients undergoing isolated ACLR, compared to 5,329 patients in Norway. In both cohorts almost 90 % of ACL injuries were associated with sports; however, cultural and geographical differences likely contributed to a difference in the most common type of sport involved between MOON in the USA (basketball—20 %, soccer—17 %, American football—14 %) and Norway (soccer—42 %, handball—16 %, downhill skiing—10 %). At the time of surgery, MOON patients had higher rates of concurrent injuries including meniscal tears (65 % vs. 48 %) and articular cartilage (46 % vs. 26 %) including Outerbridge grade III or IV lesions (19 % versus 7 %). One plausible hypothesis is a higher activity level and younger age seen in MOON patients.

MOON patients have also been compared [11] to a cohort of patients from a similarly designed longitudinal prospective cohort study of revision

ACLR (Multicenter ACL Revision Study—"MARS"). Concurrent meniscal and chondral injuries were compared at the time of primary and revision ACLR in patients prospectively collected between January 2007 and November 2008 using identical grading systems with established high inter-observer reliability [12, 13]. In total 789 patients were identified, including 508 who underwent primary ACLR and 281 who underwent revision ACLR. There were no demographic differences between the two groups. Patients presenting for revision ACLR had a decreased odds of a new lateral meniscus tear, while the odds of having an Outerbridge grade III or IV lesion on the lateral femoral condyle or in the patella-trochlear compartment was higher. Furthermore, the odds of chondral damage was higher in both the medial and lateral compartments with prior meniscectomy, regardless of status as either primary or revision ACLR.

ACL Injury Management

In practice, the current management of a torn ACL is based upon a combination of experience-based and evidence-based information. Most surgeons would recommend early reconstruction for younger persons who play organized or high-level sports such as soccer, basketball, and American football, once range of motion of the knee has been restored [9]. For most other patients, the decision between surgical and nonsurgical reconstruction is less clear, and probably dependent on many factors including activity level, age, and patient and surgeon belief and expectations.

A recent level-1 trial compared structured rehabilitation with early reconstruction and structured rehabilitation with optional late reconstruction in persons aged 18–35 years with an acute ACL tear [14]. Using a total $KOOS_4$ score, there was no difference with intention to treat analysis between groups 2-years after injury. In patients with the option of delayed reconstruction, 23/59 (39 %) underwent subsequent ACLR, and a total of 61 knee surgeries were undertaken (the majority meniscal) from baseline to final follow-up. The high crossover renders the study a comparison of early vs. delayed ACLR not rehabilitation vs. surgery. Although equivalent 2 year outcomes were reported with each strategy, the critical question of how to match an individual patient to the optimal strategy remains unknown.

When a decision for surgical reconstruction is undertaken, there are numerous techniques and graft choices available. In meta-analysis of randomized trials, there are few reported differences with one technique over another, or when selecting either of the two main types of autograft—patellar tendon and quadruple hamstrings [15].

As a multicenter study, MOON surgeons have employed a variety of these techniques and graft choices, allowing them to be modeled as covariates in a multivariate regression analysis predicting patient-reported outcomes, return to sport and reoperation after ACLR. Graft types in MOON have included autograft bone–patella tendon–bone ("autoBTB"), autograft quadruple hamstrings ("autoHAM"), and allograft. MOON surgeons also utilized transtibial, anteromedial, and two-incision femoral tunnel drilling techniques for graft placement. In a recent observational study using CT analysis in 78 ACLR MOON cohort patients [16], the inter- and intra-surgeon variability of tunnel placement was high. Notably, 91 % of the femoral tunnel axes were within the footprint. Tibial tunnel placement varied only 4 % in the medial lateral direction, and 16 % in the anteroposterior direction between surgeons.

Short-Term (2-Year) Outcomes Following ACL Reconstruction

This section reviews published MOON outcomes data concerning return to play, patient reported outcomes, and reoperation at between 1 and 2 years after primary ACLR.

Patient Reported Outcomes (KOOS, IKDC)

The scoring systems used in MOON include the KOOS and the International Knee Documentation Committee Subjective Knee Questionnaire ("IKDC"). All subsets of the KOOS were

Table 20.1 Mean 2-year patient reported outcomes ($n=393$)

	Baseline	2-year	Difference
IKDC	53 (40, 65)	84 (74, 92)	+31
KOOS ADL	88 (72, 97)	98.5 (92.6, 100)	+10.5
KOOS QoL	38 (19, 50)	75 (62, 88)	+37
KOOS Sport/Rec	50 (25, 75)	85 (70, 95)	+35
KOOS symptoms	68 (57, 82)	86 (75, 93)	+18
KOOS pain	78 (61, 89)	92 (83, 97)	+14

Adapted from Table 4 (Dunnand and Spindler 2010 [6, 7]). Lower and upper quartile in parentheses

considered, including pain, symptoms, function in daily living ("ADL"), knee-related quality of life ("QoL"), and function in sports and recreation ("Sport/Rec").

Each KOOS subset and the IKDC achieved a statistically and clinically significant improvement from baseline to 2-year post-ACLR [6, 7]. Clinically significant improvements have been defined as 11.5 points in the IKDC [17] and 8 points in the KOOS [18]. No differences were found between males and females with respect to IKDC or KOOS, a finding which is supported in the literature. See Table 20.1.

The 2002–2004 cohort was similarly analyzed [19]. Results were similar between 2- and 6-year outcomes and so will be discussed in that section.

Return to Play (Soccer, Football) and Marx Activity Level

The goal of knee stabilization through ACLR is to restore knee function and optimize patient activity level. The MOON cohort was used to evaluate activity level 2-years after ACLR and to model predictive factors for a higher activity level. In addition, return to play for the two most common sports associated with a torn ACL [10]—soccer and American football—were evaluated at between 1 and 2-years after ACLR. The reviewed studies represent the highest level of evidence for activity level and return to sports after ACLR, which is useful information for surgeons and patients.

The Marx Activity Scale was chosen as the primary outcome measure for activity level in the MOON cohort. This validated scale was designed as a self-reported measure of specific functions that are potentially challenging for ACL deficient persons. Other advantages of the Marx scale over other measures (e.g., IKDC and Tegner) include a lack of ceiling effect and the incorporation of activity frequency. Nonetheless, our data demonstrated a high correlation (Spearman $p=0.63$; $P<0.001$) between reported activity level from IKDC and the Marx scale at 2-years [6, 7] in MOON patients.

At 2-years after ACLR [6, 7] in the original MOON 2002 cohort ($n=393$, 88 % follow-up) there was a significant decrease in Marx activity level from a baseline score of 12 (range 8–16) to 9 (range 3–13). Only 45 % of patients achieved the same or a higher level of activity 2-years after ACLR by this score. When controlling for age, marital status, student status, sport, competition level, associated articular/meniscal injuries and the status of the contralateral knee, only two factors were associated with a higher activity level at 2-years: a high baseline activity score and lower body mass index. In contrast, female sex, smoking and an index ACLR which was a revision (8 % of this cohort subset) all contributed to a decrease odds of maintained activity level. It is important to note that despite common belief, concurrent injuries (meniscus, cartilage) did not influence activity level at 2-years. The MOON group hypothesized that psychosocial factors, including a fear of re-injury or a change in circumstance (e.g., graduate from school, loss of interest) may also play a considerable role in activity level post-ACLR.

McCullough et al. [20] examined return to American football in 147 MOON cohort patients from 2002–2003. This included 68 high school and 26 collegiate level players. Overall return to football was 70 %, including 43 % who felt they returned to play at the pre-injury level. Among those who did not return to play, two thirds cited "other interests" as a significant contributor and half cited "fear of re-injury or further damage." Furthermore, at 2-years after ACLR clinically and statistically significant differences were seen in favor of those who returned to football for KOOS knee-related QoL and Marx Activity Scale.

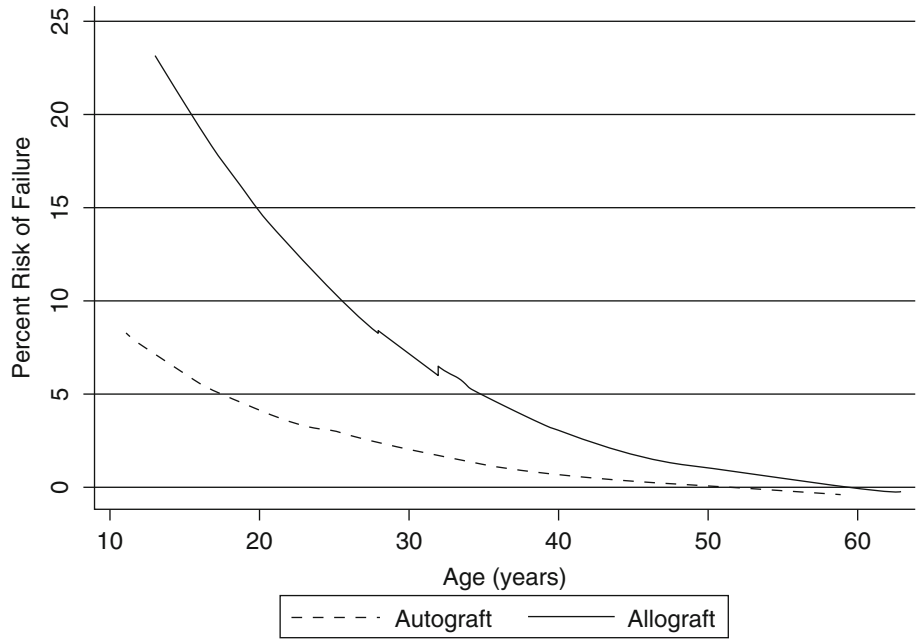

Fig. 20.1 Probability of retear (in percentages on vertical axis) for autograft versus allograft by age for the combined consortium cohort (reprinted with permission from Kaeding et al. 2011 [26]; Copyright Sage Publications)

No differences were seen by player position. Athletes who primarily play soccer can expect similar return to play results [21]. At a mean of 12 months, 72 % returned to soccer including 85 % at the pre-injury level or higher.

Repeat Surgery (Failure, Contralateral ACLR, Meniscal Repair Failure)

A MOON subset, consisting of 235 patients with a normal EUA and IKDC score of the contralateral knee and without history of prior contralateral surgery, were followed for 2 years to examine rates of revision and contralateral primary ACLR [22]. A rate of 3.0 % ipsilateral revision, and 3.0 % contralateral primary ACLR were found. With the expansion of this cohort to include all patients registered in MOON from 2002–2003 ($N=980$), the 2-year ipsilateral reoperation rates were 4.8 % revision ACLR, 5.0 % repeat meniscal surgery, and 1.8 % subsequent chondral procedure [23].

Hettrich et al. [23] also reported on complications of ACLR that required reoperation at 2-years. Among 980 patients, 4.1 % returned to the operating room for anterior debridement, manipulation under anesthesia or synovectomy after a diagnosis of arthrofibrosis. Hardware removal from the tibia was less common at 0.6 %. Five deep infections were reported (0.5 %), including two which occurred 3 weeks. These data included 91 % primary ACLR and 9 % revision ACLR index events; however, revision was not found to be a significant predictor of reoperation in regression modeling. In multivariate analysis, only increasing age reduced the odds of reoperation (34-year-old vs. 17-year-old; OR = 0.47), while the use of allograft (OR = 2.33) significantly increased the odds.

The success of meniscal repair is higher when performed concurrently with ACLR [24]. Among the original MOON cohort, the rate of successful repair using a variety of surgical techniques and implants was 96 % for 82 meniscal repairs performed concurrently with ACLR [25]. Only three patients (3.7 %) underwent reoperation for failed repair at 2-years.

The relationship between graft choice, specifically autograft and allograft, and the risk of revision was elucidated in MOON by Kaeding et al. [26]—Fig. 20.1. The highest percentage of failures occurred in the age 10–19 year old category, and for those who underwent allograft (8.9 %) compared to

autograft (3.5 %) reconstruction. When controlling for age in the model, allograft was still a significant risk factor for revision (Odds ratio=4). From this data a clinical prediction algorithm for age and graft choice was developed, the results of which were illustrated by two clinical scenarios. In a 14-year-old the risk of ACL re-tear is 22.0 % for allograft and 6.6 % for autograft ACLR. In a 40-year old the risk of ACL re-tear is 2.6 % for allograft and 0.6 % for autograft ACLR. A surgeon and older patient may together decide that a 2.6 % risk of revision is acceptable and thus proceed with allograft ACLR, but clearly a 22 % risk of failure in a young patient is not tolerable (Fig. 20.1).

Intermediate-Term Outcomes Following ACL Reconstruction

This section will review the available MOON data concerning return to play, patient reported outcomes, and reoperation at between 3 and 8 years after primary ACLR. In MOON, most commonly the 6-year follow-up point was chosen for the investigation of intermediate outcomes.

Patient Reported Outcomes (IKDC, KOOS)

Intermediate outcomes from MOON have been reported in two separate publications. The original 2002 cohort of 448 patients was examined 6-years after surgery and reported in 2011 [27], followed by the larger cohort from 2002–2004 in 2013 [19], with the latter including 86 % (1,307/1,512) follow-up. The larger cohort allowed a more in depth examination and expanded number of predictive factors, and will be the focus of this review.

The 2002–2004 cohort was comprised of 56 % males, with a median age of 23 years (interquartile range 17–25 years), and of whom 91 % ($n=1,278$) underwent primary ACLR. At 2-years from surgery (see section "Patient reported outcomes (KOOS, IKDC)"), there was a significant improvement in patient reported outcomes from baseline to 6-years; however, there was little difference between 2- and 6-years. All increases in IKDC and KOOS subset scores were clinically significant (see Table 20.2).

Table 20.2 Median (interquartile range) patient reported outcomes at 6-years ($n=1,307$)

	Baseline	2-year	6-year	Difference
IKDC	53 (41, 64)	85 (74, 92)	86 (74, 93)	+33
KOOS ADL	88 (74, 97)	99 (93, 100)	99 (94, 100)	+11
KOOS QoL	38 (25, 50)	75 (56, 88)	75 (63, 94)	+37
KOOS Sport/Rec	55 (30, 80)	85 (70, 95)	85 (70, 100)	+30
KOOS symptoms	71 (57, 82)	86 (75, 93)	89 (75, 96)	+18
KOOS pain	78 (61, 89)	92 (83, 97)		+14

Multivariate analysis to identify factors that predicted patient reported outcomes in the original 2002 cohort identified revision compared to primary ACLR, smoking status, higher BMI, operated lateral meniscal tears and the use of allograft, as predictors of poorer outcomes [27]. These results were largely corroborated in the expanded cohort. Among meniscus and articular cartilage variables, lower IKDC scores and lower scores in all KOOS subsets were associated with medial meniscal repair (compared to no tear), and lateral meniscal tears left untreated. Articular cartilage damage on the lateral femoral condyle (grade III or IV) predicted poorer KOOS symptoms subset scores and IKDC scores. Medial femoral condyle damage (grade IV) predicted poorer IKDC and KOOS pain and knee related QoL scores at 6-years [19].

Numerous patient factors also predicted worse IKDC and KOOS (all subsets) scores, including higher BMI (28 kg/m^2 vs. 23 kg/m^2), current smoking and lower education level. Those undergoing revision ACLR also had consistently poorer outcomes at 6-years compared to after primary ACLR. Interestingly, while IKDC scores were lower for female sex (compared to male), there was no gender influence on any KOOS subset score.

Only small differences in patient reported outcomes were noted for choice of graft when auto-BTB was compared to autoHAM. Patients who underwent autoHAM ACLR had a higher odds of worse outcome for KOOS sports/rec (OR 1.28 of poorer score) but better odds for KOOS symptoms (OR 0.71). The impact of autograft choice overall was minimal.

In summary, modifiable risk factors were identified, including smoking, BMI, and treatment decisions in lateral meniscal tears. Measures to improve outcomes in patients with non-modifiable risk factors should come from improvements in technique, rehabilitation and injury prevention.

Return to Play (Soccer, Football) and Activity Level

The Marx activity scale (rated from 0 to 16) was found to be significantly lower at 6-years after surgery, and even declined between 2- and 6-years post ACLR from a baseline score of 12, to 9 at 2-years and 7 at 6-years in the original MOON 2002 cohort [27]. Updated findings from the 2002–2004 cohort ($n=1{,}307$; [19]) corroborate these findings with an identical pattern of lower Marx activity score over time. Activity level is not maintained from baseline in ACLR patients.

The original cohort [27] identified only revision ACLR and female patients as independent risk factors for a clinically relevant drop in the Marx score (defined as ≥2 points). Cox et al. [19] examined the expanded cohort from 2002–2004 to identify factors that predicted Marx activity score. Among meniscal (e.g., tears) and articular cartilage variables in multivariate analysis, only the presence of a grade IV lesion on the medial femoral condyle was associated with a worse outcome (comparison of normal or grade 1 to grade 4: odds ratio 0.47 (range 0.24–0.92), $p=0.01$). However, numerous patient factors were also identified to prognosticate a larger decrease in activity score and these included older age, female sex, higher BMI, smoking, and non-competitive athletes. Revision ACLR was also a risk factor for lower activity score compared to primary ACLR.

The lower Marx activity score seen in ACLR patients 6 years after surgery was supported among a subset of MOON soccer players [21]. By 7-years, only 36 % of soccer players were still playing, and the risk factors for not returning to play in these athletes included older age and female sex in multivariate analysis.

Repeat Surgery (Failure, Contralateral ACLR, Meniscal Repair Failure)

As with 2-year outcomes, Hettrich et al. [23] reported reoperation after ACLR in 980 MOON patients 6 years after their index procedure. The overall revision ACLR rate was 7.7 %, but only 37 % of these were performed between 2 and 6 years. The rate of contralateral primary ACLR was 6.3 %; however, 60 % of these were performed between 2 and 6 years. As with revision ACLR, the majority of repeat procedures for a diagnosis of arthrofibrosis (which included anterior debridement, MUA or synovectomy), took place within the first 2 years and by 6 years only an additional 13 patients (1.3 % of total) had undergone such a procedure. Meniscal and cartilage surgery were common in the ipsilateral knee—the rate of medial meniscectomy was 5.7 %, lateral meniscectomy 3.7 %, and cartilage procedures 3.9 %. With the exception of lateral meniscectomy (36 %), for which fewer procedures took place between years 2 and 6 compared to within the first 2 years, approximately half of these additional procedures took place between years 2 and 6. Overall, contralateral meniscal or cartilage procedures were less common (medial meniscus 4 %, lateral meniscus 2.7 %, cartilage 2.1 %), with between 50 and 60 % of them occurring between years 2 and 6.

In multivariate analysis, age was the strongest predictor of subsequent surgery. In comparing patients aged 34 years to those aged 17 years, the odds of reoperation was 0.47 (range 0.32–0.71; $p=0.0001$). The use of allograft also increased the odds of reoperation at 6 years (OR = 2.33; range 1.14–4.78; $p=0.02$). In contrast to findings for activity level (see section "Return to play (soccer, football) and activity level"), BMI and revision ACLR did not influence the odds of reoperation; neither did technical considerations such as the method of drilling the femoral tunnel or the choice of fixation implant.

Brophy et al. [21] reported reoperation after a mean of 7 years in MOON soccer players. They noted a high rate of reoperation overall, including a 20 % rate of subsequent ACL surgery (revision or contralateral primary) in females compared to 5.5 % rate in males.

Reoperation rates reported to date in MOON are similar to previously published level-1 and level-2 data. In a systematic review of 6 level-1 and level-2 studies with a minimum 5 years follow-up, Wright et al. in a meta-analysis [28]

determined that the rate of graft rupture was 5.8 %. The rate of contralateral primary ACLR was, however, higher than in the published MOON data at 11.8 %.

The Incidence of Osteoarthritis After ACLR

The extent to which post-traumatic osteoarthritis (OA) develops in the ACL reconstructed knee at intermediate to long-term follow-up has been explored by only a few high quality studies. The definition of OA in this context has also focused primarily on radiographic criteria. Follow-up radiographs at minimum of 2 years after ACLR in MOON patients have been captured within a nested cohort returning onsite, but data analysis is ongoing including joint space changes on standing radiograph.

A systematic review of studies evaluating radiographic OA between 5- and 10-years after ACLR [29] was performed by MOON investigators, and identified an association between meniscus status and greater prevalence of OA. Patients in reviewed studies had undergone primary ACLR with an autograft, with those undergoing concurrent meniscectomy at higher risk of radiographic OA. Wide variation in the classification systems used to define radiographic OA, however, have limited data interpretation and cohesion.

Since that time only three studies level II or higher have investigated the incidence of OA post-ACLR. Song et al. [30] published the follow-up of a randomized trial comparing double to single bundle ACLR and found a progression in radiographic Kellgren–Lawrence arthritic change in approximately 10 % of all patients at a mean follow-up of between 5.3 and 5.7 years. Frobell et al. [31] also published follow-up results of the KANON randomized trial and noted that 16 and 24 % of acutely reconstructed patients ($n=58$) had tibiofemoral and patellafemoral radiographic arthritis, respectively, at 5-years. Finally, one prospective cohort study [32] followed 56 ACLR patients for 6 years and found a 48 % rate of K-L OA in radiographs which was grade 1 or grade 2.

Long-Term Outcomes Following ACL Reconstruction

Unfortunately, there is a paucity of high-quality published data on the long-term (10+ years) outcomes following ACL reconstruction. In the literature, loss-to-follow-up remains one of the most significant limiting factors, including for registry data. A unique strength of the MOON cohort is the achievement of 83 % 10-year follow-up from the first 2002 enrollment year, with similar efforts planned or underway for the 2003–2004 cohort at the present time. The maintenance of a high follow-up rate out to 10 years is ideal to examine long-term outcomes, and in the coming years will be the standard of best evidence long-term outcomes of ACLR.

Only a few high quality studies have examined long-term ACLR outcomes. Holm et al. [33] reviewed a 10-year follow-up of a randomized trial and noted Kellgren–Lawrence grade 2 or higher radiographic osteoarthritis in 55 and 64 % of hamstrings and bone–tendon ACLR, respectively. In total, this included 57 patients. In contrast, prevalence of K-L grade 2 or higher OA on radiographs was 28 and 22 % for the same groups on the uninvolved knee. No statistically significant differences were found between groups in measured functional outcomes or clinical scores (measured laxity, Cincinnati knee score, single-leg hop test).

In a recent systematic review of a minimum 10-year follow-up of patients with ACL injury, Oiestad et al. [34] identified only seven prospective studies, and considerable variation in the reported rate of radiographic osteoarthritis (0–100 %). These studies included a total of 714 patients treated with a mix of operative and nonoperative (e.g., various rehabilitation and activity modification protocols) methods, including those treated operatively with historical techniques such as ACL direct repair and synthetic ligaments.

Long-term outcomes, including the development of OA, patient-reported outcome scores and activity levels following ACLR remains an important goal of future study.

Gaps in Knowledge

Although significant progress has been made in understanding the outcomes of ACLR, there are many questions to be answered with regard to prevention, rehabilitation, and the long-term risk of post-traumatic OA.

- The high rate of contralateral primary ACLR and ipsilateral revision ACLR in young patients suggests a heightened role for advances in rehabilitation and prevention.
- Successful treatment of some patients with a strategy of early structured rehabilitation only, such has been demonstrated by the KANON study, suggests a need for further defining the "functional coper."
- Consistently found in MOON results is a trend over time towards decreased activity level. Although modern activity scores, such as the Marx scale, have improved there is still a need to define external factors (i.e., not knee-related) that influence a patient's activity level after ACLR such as graduating from college or starting a family, in addition to psychological factors such as trusting the injured knee. The predominance of patient factors over concomitant cartilage injuries as predictors of lower activity score further support this notion (see section "Return to play (soccer, football) and activity level")
- A better understanding is needed of the intra-articular cellular, molecular, and genetic events that occur with an ACL injury, how these physiologic changes are modified by surgical repair, and ultimately how these events impact clinical outcome.

In time, MOON data will help answer some of the biggest questions, including the true incidence of and prognostic factors for development of post-traumatic OA in a nested cohort of patients (<33 years old) within MOON who have undergone a unilateral ACLR and have a "normal" contralateral knee. The need to identify modifiable risk factors that delay or lessen the odds of developing post-traumatic OA and improve functional outcomes is paramount.

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Current Treatment and Outcomes of Intra-articular Fractures

21

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Assumptions abound about the outcome of intra-articular fractures and the likelihood of post-traumatic arthrosis (PTA). Ostrum stated that there was much evidence to suggest that PTA was not guaranteed after an articular fracture [1]. Surgical tactics for intra-articular fractures assume intra-articular fractures are analogous to broken teacups and need to be put together like puzzles for good outcomes. Nonetheless, modern fracture care has not extinguished PTA after articular fracture.

Residual articular step-off and the quality of the fracture reduction appear to be important for the outcome of some, but not all, articular fractures. In many instances, a perfect articular reduction does not guarantee a good result. PTA that occurs following an intra-articular fracture often has a rapid onset. There is heterogeneity among various intra-articular fractures in terms of the risk of PTA and the factors and variables that may contribute to its onset.

What are the non-articular step-off (i.e., not related to the quality of the fracture reduction) variables and factors associated with clinical outcomes after intra-articular fractures and the development of PTA? Examples include patient demographics, cartilage thickness, concomitant cartilage degeneration, mechanical axis deviations and malalignment, timing of articular reduction, and invasiveness of the fracture surgery.

In this chapter, we discuss important variables and factors that contribute to the clinical outcome of treatment of three articular fractures: acetabulum, tibial plateau, and distal radius. Specifically, we examine the role of intra-articular step-off (quality of reduction) in clinical outcomes and the development of PTA. In addition, we also examine other (non-articular step-off) variables and factors that contribute to clinical outcome and the development of PTA. Lastly, we discuss factors associated with the need for total joint replacement after each of three intra-articular fractures: acetabular fractures, tibial plateau fractures, and distal radius fractures.

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Acetabular Fractures

Articular Step-Off

Bircher and Rickman noted that "a mismanaged acetabular fracture results in osteoarthritis" [2]. Bhandari and Matta stated that 25.5 % of anatomically reduced acetabular fractures will develop arthritic changes [3]. The quality of articular reduction after acetabular fracture surgery (i.e., residual step-off) has been reported to correlate with poorer patient outcomes assessed by the Merle d'Aubigné–Postel score (MDA) [4–8].

Lichte et al. noted a significantly higher MDA in those with an anatomic reduction (step-off <1 mm measured radiographically) than those with an imperfect reduction (>1 mm), 17.5 vs. 12, respectively [5]. Zha et al. reviewed the outcomes of 86 elderly patients (>60 years of age) noting the average MDA with an anatomic reduction of 16.8, whereas a poor reduction (>3 mm) was associated with a score of 11.3 [8].

Anatomic reduction is reported to be achieved in 75 % of 816 acetabular fractures and associated with simple fracture types, acute treatment, and age <40 years [7]. Both column fractures had a higher incidence of poor reduction, whereas posterior column and posterior wall fracture patterns were associated with a significantly higher rate of anatomic reduction [7].

Giannoudis et al. in a review of isolated acetabular fractures, noted the EQ-5D (quality of life outcome score) was closer to the population norm in those with excellent radiographic results, and significantly lower with poor radiographic results [9]. Giannoudis et al. also reviewed ten studies (one biomechanical and nine retrospective) based on pre-specified criteria [4]. These investigators noted that restoring the weight-bearing dome of the acetabulum to its pre-injury morphology improved outcomes and decreased the incidence of PTA [4].

Bircher and Rickman noted the "key anatomical structure with regard to the outcome is the roof or dome of the acetabulum" [2]. These authors also stated that damage to the medial part of the anterior column, as in a low anterior column fracture, would not lead to long-term arthrosis of the hip [2]. They noted "one factor that is important for a good and lasting clinical outcome" following an acetabular fracture is a femoral head that is centered, parallel, and stable beneath an anatomically reduced acetabular roof [2].

Many studies have relied on plain radiographic analysis of postoperative films to determine anatomic reduction. CT scans are more sensitive than plain radiographs for gaps and step-offs. Nonetheless, plain radiographs appear to be able to detect nonanatomic reductions that will likely have a poor outcome. In the older patient with an acetabular fracture, plain radiographs may be enough to determine the variables associated with early conversion to total hip arthroplasty.

Moed used CT scans after ORIF of posterior wall fractures and reported gaps or step-offs in 85 % of cases [50]. These authors were unable to correlate clinical outcomes with the quality of the reduction [50]. These findings underline the strong possibility that factors other than the quality of reduction or articular step-off determine clinical outcome.

Older literature reiterates the importance of articular step-off and patient clinical and radiographic outcomes. Letournel and Judet noted a good clinical outcome in 86 % of 492 patients following an anatomically reduced and surgically repaired acetabular fracture [11]. Matta reported on 262 fractures treated within 3 weeks from injury with a mean follow-up of 6 years noting worsening clinical results (assessed by MDA score) with increased articular step-off, associated femoral head injuries, and older patient age [12]. A strong association between final radiographic outcome and clinical outcome was demonstrated [12]. Moed et al. published their results on 100 surgically treated posterior wall fractures with a mean follow up of 5 years noting an excellent clinical outcome in 55 % and excellent radiographic results in 81 % [10]. Factors contributing to a poorer outcome were older age (>55 years of age), delayed hip reduction >12 h, intra-articular comminution, and osteonecrosis [10].

Tannast et al. noted in a survivorship study the relationship of age to anatomic reduction: 59 % anatomical reduction in patients >65 years old

compared to a 74 % rate for patients 40–65, and an 82 % rate for patients <40 years old [7].

Degenerative changes after acetabular fracture are reported to range from 10.9 to 31 % [5, 6, 13]. Lichte et al. reviewed 115 both column fractures and reported 10.4 % (12) were found to have degenerative changes on radiographic follow-up of 2 years, and 9.6 % (11) had undergone total hip arthroplasty (THA) [5]. Tannast et al. retrospectively reviewed 816 surgically treated acetabular fractures with a 2-year minimum follow-up and noted that 120 hips (14.7 %) were converted to a THA and four to a hip arthrodesis (0.5 %) at an average of 4.5 years after the initial procedure [7]. Native hip survivorship was 88 % at 5 years, 85 % at 10 years, and 79 % at 20 years [7].

The Matta radiologic follow-up criterion has been identified as the best predictor for return to sports [9]. Giannoudis et al. investigated 52 patients with isolated acetabular fractures noting 42 % were able to return to their prior level of activities, and 35 % were able to participate in sporting activities at some level within 36 months postoperatively [9].

In summary, restoration of anatomy (less articular step-off and better anatomic reduction) after acetabular fractures appears necessary but not sufficient to ensure good clinical outcomes. What other variables might be important?

Variables Other than Articular Step-Off

Many variables have been correlated with poorer clinical outcomes. Giannoudis et al. also noted that involvement of the posterior wall seemed to be an adverse prognostic sign which may be independent of the articular reduction [4]. Lichte et al. noted an initial fracture displacement <10 mm developed degenerative changes in 10.9 % of 115 patients; yet, if >10 mm of displacement, the rate of degenerative change was seen to be 27.5 % [5]. These authors noted intra-articular fragments as factors associated with a worse clinical outcome; however, the presence of associated injuries and dislocation were not significant.

These findings contrast with those of Meena et al. who, after retrospective review of 118 patients with 4-year follow-up, found no association with femoral head impaction, acetabular joint surface impaction, or dislocation. However, if multiple factors were present, an increase in degenerative changes was observed [6].

Bircher and Rickman stated that an operatively treated T-type fracture with a posterior wall component often has a poor outcome despite anatomical reduction "due to the extensive chondral damage that occurs at the time of the injury." [2].

Zha et al. identified poorer clinical outcomes with a comminuted posterior wall fracture, a radiographic gull sign, and femoral head injury in patients >60 years of age [8]. Marginal impaction and fracture comminution were associated with a significantly poorer radiographic outcome [13].

Tannast et al. identified the following as significant negative predictors on native hip survivorship: nonanatomic reduction, age >40 years, anterior hip dislocation, acetabular roof incongruence, posterior wall involvement, acetabular impaction, femoral head cartilage lesion, initial displacement >20 mm, and extended iliofemoral approach [7]. Hip survivorship was significantly greater in both column fractures and lower in anterior column fractures, despite the increased rate of malreduction in both column fractures [7].

Survivorship of the native, operatively treated acetabular fracture that has not gone on to conversion to a total hip replacement is a unique way to look at the outcomes of operative treatment of acetabular fracture in relationship to PTA. However, there are at least two patient cohorts to look at which are defined by an age cutoff (older or younger than 55/60 years old). The role of total hip replacement for PTA in older patients with acetabular fracture is getting increased attention.

Carroll et al. studied operatively treated acetabular fractures in an older population (patients ≥55 years old) with a mean age of 67 and reported a 30.95 % rate of total hip replacement [13]. They reported three variables associated with delayed hip arthroplasty: nonanatomic fracture reduction, development of avascular necrosis, and previous contralateral total hip arthroplasty [13].

Table 21.1 Variables and factors that influence patient outcomes following acetabular fracture fixation

Positively	Negatively
Anatomic reduction	Posterior wall involvement
Congruent joint reduction	Articular step-off >2 mm
Age <40 years	Femoral head involvement/ dislocation
	Age >55–60 years
	Intra-articular fracture fragmentation
	Osteonecrosis
	Fracture displacement >10–20 mm
	Acetabular impaction
	Extended iliofemoral approach

Many variables and factors associated with the initial injury and surgical intervention affect the clinical outcome after acetabular fracture as it relates to the development of PTA. We have summarized the variables and factors that influence patient outcomes following acetabular fixation into two categories: those which influence outcomes positively, and those that influence outcomes negatively (Table 21.1). Although many of these variables are out of the surgeon's control, poorer clinical outcomes are associated with malreduction. What are the variables and risk factors associated with the need for arthroplasty after acetabular fracture?

Arthroplasty After Acetabular Fracture

Prediction of PTA after acetabular fracture remains difficult. Meena et al. reported that 20 % of anatomically reduced acetabular fractures develop PTA [6]. Lichte et al. observed radiologic joint degeneration in 20.4 % of anatomically reduced fractures, and 11.1 % required total hip arthroplasty [5]. These authors confirmed Letournel and Judet's results with 19 % of 492 patients developing radiographic arthritic changes in anatomically reduced fractures [11]. Giannoudis et al. reiterated the importance of anatomic reduction, especially the weight-bearing dome of the acetabulum, in addition to the impact of injury severity with patient outcome, clinically and radiographically [4].

Three peaks of total hip replacement after acetabular fracture have been reported [14]. The first is acute total hip replacement (at time of initial surgery or within 6 weeks of injury), the second peak is at 2–4 years (usually due to either sepsis or fixation failure in osteoporotic bone), and the third peak is at the 10+ year mark (likely significantly displaced fractures that involve the weight-bearing roof or dome) [2]. In the 10+ group, the need for THA is thought to be the "amount of comminution and damage at the time of injury, and the accuracy of reduction at the first operation" [2].

They further discuss that chondral damage at the time of injury will lead to an increased incidence of arthritis which presents beyond 5 years, and most commonly between 10 and 20 years after injury [2]. The total hip replacements performed at the 1- to 2-year time interval after acetabular fracture surgery are usually performed for fixation failure, chondrolysis, avascular necrosis, and infection [2].

Carroll et al. retrospectively identified 29 of 93 patients (31 %) >55 years of age who required a conversion to THA at an average of 28 months postoperatively. In addition, a nonanatomic reduction was identified as a significant predictor for subsequent THA ($p < 0.02$) [13]. Hayes et al., in a 5 % Medicare database sample, noted that 37 of 359 fractures (10.3 %) were converted to THA within 1 year postoperatively [15]. According to Meena et al., 10 of 118 (8.5 %) patients were treated with a THA between 2 and 5.3 years postoperatively for secondary arthritis [6]. Matta reported that 6.5 % of 262 patients required THA for post-traumatic arthritis [12].

Tibial Plateau Fractures

Articular Step-Off

There continues to be debate about whether intra-articular fractures of the tibial plateau doom patients to poor outcomes and PTA. Ostrum stated that many lateral tibial plateau fractures do not progress to severe PTA or total knee arthroplasty [1].

Ostrum stated that the "literature does not support the belief that an intra-articular tibial plateau fracture will progress to arthritis" [1]. What are the factors that contribute to clinical outcomes, PTA, and the need for TKA after intra-articular tibial plateau fracture? We first discuss the role of articular step-off (articular reduction).

Dirschl et al. stated that articular incongruity was well-tolerated after tibial plateau fractures [16]. He noted that there is "little support in the literature for the assertion that accurate reduction of tibial plateau fractures, particularly to tolerances <2 mm, is critical for a good clinical outcome" [16].

Giannoudis et al. noted that articular incongruities were well-tolerated for tibial plateau fractures [4]. These authors noted that other factors only partially related to articular reduction (e.g., joint stability, retention of the meniscus, and coronal plane alignment) were more important in determining outcome than articular step-off alone [4]. Of the 11 studies reviewed, 5 showed no effect on the outcome comparing articular step-off and no step-off [17–20]. Of the other six studies, one showed acceptable results with step-off <10 mm [21] and another study showed inferior results with step-off >10 mm [22].

Lower tolerances for articular step-off were reported in the two other studies: one showed satisfactory results for <4 mm displacement with conservative treatment [23], and another showed that increased articular step-off heights progressively increased valgus angulation and maximum contact pressure was apparent at more than 4 mm step-off [24]. Another study noted that operative stabilization should be based on knee stability in full extension and not on roentgenographic criteria [25]. Blokker et al. noted that the adequacy of reduction is the most important factor in predicting outcomes of operative treatment with a residual step-off more than 5 mm was associated with an unsatisfactory result [26]. Wilde stated that preserving the normal alignment of the knee was critical to the end result of the treatment of tibial plateau fractures [27]. He further noted, "Joint depression, per se, if not associated with malalignment, does not necessarily cause poor results" [27]. In terms of the effect of joint stability, he noted that joint depression in a stable knee was not necessarily associated with a poor result, but depression more than 4 mm did have an effect on outcome [27].

Articular step-off after intra-articular fractures of the tibial plateau is less of a determinant of outcome, particularly when the step-offs are small (≤4 mm) and the fracture involves mainly the lateral tibial plateau.

Variables Other than Articular Step-Off

Dirschl noted that the tibial plateau has thicker articular cartilage than many other joints [16], and that the effect of factors other than articular reduction such as knee instability, malalignment, and meniscectomy were more important to the outcome [16]. Ostrum also noted that certain plateau fractures (e.g., medial tibial plateau fractures and those having had an excision of the meniscus) have a much poorer prognosis [1].

Rademakers et al. reported a 31 % incidence of radiographic arthritis after operatively treated tibial plateau fractures at 14 years, but most were asymptomatic [28]. However, results were much worse with malalignment >5°. Twenty-seven percent of patients reported moderate to severe symptoms [28].

Multiple studies demonstrate poorer clinical, radiographic, and functional outcome scores with increasing fracture classification number using the Schatzker classification [29–31]. Prasad et al. reviewed 40 Schatzker type V and VI tibial plateau fractures treated with dual plating with 4 years follow-up [32]. All patients had final radiographic articular step-off <2 mm, good coronal and sagittal plane alignment, and mean condylar width was <5 mm. Final clinical outcome was assessed by the Oxford Knee Score with 32 of 40 with a final score >30 (excellent) and only eight patients with a score between 20 and 29 good.

The AO Classification of tibial plateau fractures did not correlate well with outcome. According to Jansen et al. 30.4 % of 23 type C (AO/OTA classification) fractures demonstrated no signs of PTA and 39.1 % had prominent

arthritic changes at 67 months [33]. At the final follow up, 90.9 % (20 patients) achieved a good to excellent range of motion (full extension and flexion >110°) with mean flexion of 124.9°. The average Lysholm score was 66.2 points (out of 100). A varus/valgus malalignment >3° resulted in significantly lower outcome scores; however, there was no correlation with the onset of post-traumatic arthritis (Kellgren score).

Ehlinger et al. reviewed 13 patients who were surgically treated for a Schatzker IV–VI tibial plateau fracture after a mean follow-up of 39.1 months [34]. The average Lysholm score was 94.1, mean HSS score was 93.6, and all patients previously employed returned to work after 4.5 months [34]. Five patients were noted to have an articular step-off >2 mm, yet all 13 patients demonstrated no radiographic evidence of osteoarthritis at final evaluation [34].

The magnitude of the energy at the time of injury may be important in determining outcomes after intra-articular tibial plateau fractures. When reviewing the treatment of open high-energy tibial plateau fractures with significant soft tissue injury treated with modified hybrid external fixator, Ariffin et al. found 15 of 33 patients (48 %) to have an excellent Rasmussen knee functional score at 12 months postoperatively, 13 (42 %) patients had a good score (20–26) and 3 (10 %) had a fair score (10–19) [35]. The mean Lysholm score following higher energy fracture patterns varied from 66.2 to 94.1. Malalignment and articular step-off >2 mm may contribute significantly to the development of post-traumatic arthritis and poorer clinical outcomes after tibial plateau fracture.

Various surgical approaches to tibial plateau fractures appear to have a relationship with clinical outcome and the onset of PTA. Solomon et al. retrospectively reviewed 17 patients assessing fracture reduction and maintenance following direct posterolateral transfibular approach (9 patients) or an indirect anterolateral approach (8 patients) for unicondylar posterolateral tibial plateau fractures with a 2-year follow-up [36]. Radiographically, all nine patients who underwent the direct posterolateral transfibular approach were reduced anatomically (i.e., step-off <2 mm, condylar widening <5 mm, and a medial proximal tibial angle (MPTA) within normal range). In contrast, the anterolateral approach leads to an average step-off of 5.5 mm postoperatively, which progressed to an average of 6.0 mm in six of eight patients at the 2-year follow-up. At 2-year follow-up, the Lysholm scores were significantly higher in those treated through a direct approach when compared to the indirect anterolateral approach. Again, this data supports the idea that malreduction and increased articular step-off results in poorer clinical outcome.

Dall'Oca et al. reviewed 100 patients, and compared arthroscopically assisted reduction and internal fixation (group A) with open reduction and internal fixation (group B) [29]. One patient developed lateral compartment arthritis with residual valgus at 1 year resulting in a unilateral knee prosthesis. In addition, only two patients in group B (ORIF) developed symptomatic arthritic changes resulting in significant post-traumatic valgus deformity treated with TKA. Clinical outcomes assessed by Rasmussen and HSS scores were 27.62 and 76.36, respectively, for those treated with arthroscopically assisted fixation. With regard to the classic ORIF treatment group, the scores were 26.81 and 73.12, respectively. No significant difference was identified between the clinical outcome scores for arthroscopically assisted versus ORIF. Arthroscopically assisted internal fixation is a valid treatment option.

Siegler et al. examined the clinical outcomes of 27 arthroscopically assisted percutaneous fixation for Schatzker I–III fractures with a mean follow-up of 59.5 months noting a mean Lysholm score of 86 and a mean Rasmussen clinical score of 25.5 (maximum score of 30) [37]. On radiographic evaluation, 47.6 % presented with early arthritic changes.

Malakasi et al. investigated 60 tibial plateau fractures with either ORIF (30 patients) or hybrid external fixation (30 patients) for 12 months noting no significant differences with regard to functional or radiographic outcomes [31]. Poorer clinical and radiographic outcomes correlated with increasing Schatzker classification.

Biggi et al. reported no radiographic evidence of arthritic changes in 41 of 47 patients (87 %) after a mean 18 months following a

minimally invasive percutaneous osteosynthesis (MIPO) technique of internal fixation for a tibial plateau fracture [38]. The Rasmussen functional score was 27 at 1 year postoperatively. Chan et al. noted a mean clinical Rasmussen score of 28.4 and 19 % (10 of 54) demonstrated post-traumatic arthritis radiographically when reviewing arthroscopically assisted fixation of tibial plateau fractures at a mean follow-up of 87 months [39]. Bicondylar fractures reported poorer clinical outcomes; yet the numbers were not statistically significant in comparison to unicondylar fracture patterns. The rate of arthritic changes ranges from 19 to 47.6 % with an average good to excellent Rasmussen clinical outcome scores and Knee Society Scores.

Loibl et al. studied the rate of return to sporting activities, an excellent indicator of functional outcome, following internal fixation of a tibial plateau fracture with the responses of 103 patients after a mean of 7.8 years [40]. Eight-eight percent of patients were participating in sports at the time of the survey with no change in the frequency or duration of activity; however, an increase in low-impact activities was noted (i.e., walking, fitness/weight training, water aerobics). More severe fracture patterns, specifically B3 and C3 fractures, were associated with poorer clinical functional scores and decreased rates of return to sports.

According to Kraus et al., 73 % of 89 patients were participating in sporting activities at an average of 52.8 months postoperatively following a tibial plateau fracture, with 88.8 % participating in sports at the time of injury, a 15.8 % reduction [30]. Of the 11 highly competitive athletes, only two returned to the same level of competition at the time of the survey. These authors noted a significant decline in the number of sporting activities and the frequency of activity per week [30]. The Lysholm score averaged 76.6. Higher-energy fracture patterns (i.e., Schatzker IV–VI) reported significantly poorer clinical outcome scores.

Because articular step-offs of the tibial plateau are well-tolerated, non-articular step-off fractures seem to be more important factors in determining outcome for the fractured tibial plateau.

Table 21.2 Variables and factors that influence patient outcome following tibial plateau fracture fixation

Positively	Negatively
Stable knee joint	Medial plateau involvement
Retention of meniscus	Articular step-off >4–10 mm
Anatomic coronal alignment	Higher numerical Schatzker classification type

We have summarized the factors that influence patient outcomes positively and those that influence outcomes negatively (Table 21.2). Finally, what are the variables and risk factors associated with the need for arthroplasty after tibial plateau fractures?

Arthroplasty After Tibial Plateau Fracture

The need for total knee arthroplasty after tibial plateau fracture may be low; but when performed, the complication rate is high [1, 41, 42].

Risk factors for total knee arthroplasty (TKA) after plateau fracture have been reported to be age over 48, bicondylar fractures, and comorbidities [43].

Prior ORIF for a tibial plateau fracture significantly changes the clinical outcome of TKA. Saleh et al. analyzed the outcome of 15 patients who underwent TKA after ORIF of a tibial plateau fracture [41]. They found a high rate of infection (three patients had one), patella tendon disruption (two patients), and postoperative secondary procedures (three patients who required closed manipulation). They concluded that TKA after ORIF of a tibial plateau fracture decreased pain and improved function, but is technically demanding and is associated with a high failure rate (5 of 15) [41].

Weiss et al. also reported a high postoperative complication rate (26 %) and a high reoperation rate (21 %) associated with TKA after tibial plateau fracture [42]. Wasserstein et al. reported that 10 years after tibial plateau fracture surgery, 7.3 % of patients had undergone a TKA [43]. They noted that this was a 5.3 times increased likelihood compared to the general population [43]. They also noted that older age, higher comorbidity, and

bicondylar fractures were all associated with an increased risk of future TKA [43].

The rate of post-traumatic arthritic changes following tibial plateau changes varies widely from 19 to 48 % at a mean of 2 years postoperatively; 10-year Kaplan–Meier survivorship is 96 % (i.e., 96 % of patients will not undergo a reconstructive procedure for post-traumatic arthritis at 10 years after plateau fracture). In summary, it appears that radiographic arthritic changes may not correlate with the need for TKA after a tibial plateau fracture. Furthermore, TKA after tibial plateau fracture is associated with both higher complication rates and higher failure rates.

Distal Radius

Articular Step-Off

Historic literature reported a high rate of PTA on radiographic evaluation after an intra-articular distal radius fracture. Articular incongruity >2 mm significantly increased the rate of arthritic development (50–100 %) [44–48]. Knirk and Jupiter observed 65 % of 43 fractures developed radiographic PTA with the following breakdown various treatments: casting, 21 of 43 had PTA; percutaneous pinning, 17 of 43 had PTA; external fixation, 2 of 43 had PTA; and ORIF, 3 of 43 had PTA. Articular incongruity was the most critical factor in the development of arthrosis [47]. Ninety-one percent of the patients who healed with an articular step-off developed arthritis, but only 11 % in those with a congruous joint developed arthrosis. Bradway et al. further reiterated the impact of step-off on the development of arthritic changes radiographically, noting 100 % (4 of 4) developed PTA in patients with a step-off >2 mm after a mean follow up of 4.8 years, and only 25 % (3 of 12) in those with incongruity <2 mm [44]. Biologically, this radiographic development is confirmed with significantly increased intra-articular contact pressures in the lunate fossa with scaphoid fossa depressions as small as 1 mm in all loading positions of the wrist [49].

The clinical impact of arthritic changes following articular incongruity of 1–2 mm remains unclear. Strange-Vognsen identified 42 patients who sustained an intra-articular fracture and reexamined them after an average of 16 years (2–26) noting >50 % demonstrated radiographic changes consistent with arthrosis [48]. Although subjective patient outcomes correlated with deformity and arthrosis, they did not correlate with intra-articular step-off [48]. Forward et al. reevaluated 106 intra-articular fractures after an average of 38 years (33–42) observing 68 % of the patients had developed radiographic arthritic changes; yet the DASH scores were unchanged from population norms, and patient function as assessed by Patient Evaluation Measure (PEM) was impaired by <10 % [46]. Further, Catalano et al. reported a 76 % rate of arthritic radiographic changes in 21 patients after an average of 7.1 years [45]. The authors also noted a significant correlation with residual displacement of articular fragments and the development of arthrosis; however, functional and clinical outcomes did not correlate with radiographic findings, specifically gap formation or articular incongruity. All patients reported a good to excellent functional outcome irrespective of radiographic evaluation [45].

Giannoudis et al. analyzed the effect of articular step-off on the outcome of intra-articular fractures of the distal radius in ten studies (two biomechanical studies and eight clinical studies) [4]. Two studies used a 1 mm step-off with one of these studies noting acceptable results with a step-off of <1 mm [50] and the other reporting no radiographic evidence of PTA in fractures that healed with a step-off of up to 1 mm [51]. Six studies utilized a 2 mm threshold which was associated with the best outcomes [45, 52–56]. In two studies, the best outcomes were noted when the reduction step-off was within 2 mm [44, 47]. In two other studies, worse outcomes occurred when the step-off exceeded 2 mm [54, 56]. PTA was noted with a step-off/articular incongruity of more than 2 mm in two additional studies [45, 55]. Two more studies reported a 3 mm tolerance with two biomechanical studies reporting increased radiocarpal stresses with a step-off of 3 mm [52, 53].

Dirschl et al. stated that radiographic changes consistent with PTA of the radiocarpal joint after

fracture "may be well tolerated clinically, causing few symptoms and little impairment, at least during the first several years after injury" [17]. These authors further questioned "whether clinical results will deteriorate at longer follow-up and whether deterioration is correlated with greater step-off or gap deformities at the time of union." [17]

Mignemi et al. assessed the ability of volar locked plating to achieve normal radiographic parameters in 185 distal radius fractures [57]. Volar locked plating achieved an articular step-off <2 mm in most fractures, but only restored normal measurements for volar tilt, radial inclination, and ulnar variance in 50 % of the patients [57]. In addition, these authors noted that the ability of volar locked plating to restore and maintain ulnar variance and volar tilt decreased with more complex intra-articular types [57].

Imperfect reductions may not result in symptomatic long-term arthritis. The clinical implications of PTA following an intra-articular distal radius fracture remain unclear. Further long-term studies investigating the impact of radiographic arthrosis on functional and clinical outcomes are needed.

Variables Other than Articular Step-Off

Variables other than articular step-off and their effect on outcome are important. Amorosa et al. studied the subjective functional outcomes of patients who were at least 70 years of age who had sustained distal radius fractures [58]. They used the DASH and SF-8 surveys and examined radiographic parameters such as articular step-off, dorsal tilt, ulnar variance, and the presence/absence of an ulnar styloid fracture. They found that the only radiographic parameter that affected functional outcome was an associated ulnar styloid fracture [58]. Females had worse outcomes than males [58]. Paksima et al. assessed 335 patients to evaluate the association of patient education level on pain and disability after distal radius fracture [59]. They found that each increase in the level of education (as in from high school to college) corresponded to a 2 to 1 rate of improvement over time [59].

How do various treatment options, including closed reduction and casting/immobilization with or without percutaneous pinning, external fixation with or without percutaneous pinning, and internal fixation, influence patient outcomes? Williksen et al. performed a randomized study of 107 unstable distal radius fractures treated with external fixation with adjuvant pins versus volar locking plate fixation and followed for 1 year [60]. The volar plate group demonstrated a statistically significantly higher Mayo score (90 vs. 85, measured out of 100), better supination (89 vs. 85 degree), and less radial shortening (+1.4 vs. +2.2 mm). For complete articular fractures, volar plating demonstrated statistically significant improvements in supination (90 vs. 76) and less radial shortening (+1.1 vs. +2.8 mm). Of note, the QuickDASH score was not significantly different between the groups.

Karantana et al. piloted a randomized controlled trial focusing on outcomes of distal radius fractures treated with closed reduction and percutaneous fixation (with or without a bridging external fixator) versus volar locking plate in 130 patients followed for 1-year postoperatively [61]. Patients who were treated with volar locking plates had significantly better Patient Evaluation Measure (PEM) scores, QuickDASH scores, and range of motion at 6 weeks; however, no significant differences were identified at 12 weeks or 1 year. Multiple meta-analyses conclude that volar locking plates are significantly better with regards to DASH scores, volar tilt, and fewer complications (mainly infection) [62–66].

Walenkamp et al. found patients treated with volar locking plate were found to have significantly lower DASH scores at 3, 6, and 12 months when compared to external fixation [63]. No differences were found between the groups with regard to complications, grip strength, range of motion, or radiographic measures (ulnar variance, radial length, radial inclination). Xie et al. noted internal fixation had significantly better DASH scores at 12 months, fewer surgical complication, and better restoration of volar tilt and radial inclination [66]. Radial length was superior with

external fixation at 12 months postoperatively. Wang et al. noticed statistically significant DASH scores at 3, 6, and 12 months; volar tilt at 12 months; and range of motion at 3 months [64]. There was an increased rate of infection with external fixation, and no difference in malunions. Esposito et al. observed significantly lower DASH scores with plate fixation, ulnar variance (better radial height restoration), and lower rates of infection [62]. No significant differences were seen with range of motion, grip strength, volar tilt, or radial inclination. Wei et al. identified better functional outcomes (DASH scores), supination, and restoration of volar tilt with ORIF; however, external fixation had better grip strength and wrist flexion [65]. In conclusion, it appears patient outcomes following volar plating result in lower DASH scores; however, statistical significance does not necessarily indicate clinical significance.

Fracture types (e.g., intra-articular versus extra-articular fractures, and open versus closed fractures) have also been investigated. Brogren et al. retrospectively reviewed 123 distal radius fractures treated with external fixation versus nonoperative casting focusing on the impact of malunion and functional outcome at 2 years postoperatively [67]. DASH scores of patients with >10° of dorsal tilt and/or ulnar variance >1 mm were statistically worse than those with no evidence of radiographic malunion, average difference 13 points. Interestingly, no difference was observed when comparing extra-articular versus intra-articular fracture patterns or intra-articular step-off >1 mm. Beumer et al. identified posttraumatic positive ulnar variance >2 mm as the single factor that correlated with a poorer functional outcome [68].

Bolmers et al. evaluated 46 patients following an intra-articular distal radius fracture with an average follow-up of 20 years [69]. No difference was observed between AO type B and C fracture patterns with regard to DASH scores, motion and grip strength, arthrosis, and pain. Open fractures were associated with significantly lower DASH scores. MacKay et al. investigated the impact of an open versus closed distal radius fracture upon outcome measures in 36 patients followed for 12 months [70]. At final follow up, no significant differences were identified with regards to pain, range of motion, grip strength, and DASH scores.

Patient characteristics such as age play a role in patient outcomes. Amorosa et al. examined 58 patients >70 years of age for an average of 33 months [58]. The average DASH score was 22.3 with the SF-8 score 31.5. Factors noted to be associated with statistically worse DASH scores included ulnar styloid fractures (12.9 vs. 26.2) and female gender (24.4 vs. 6.9). In addition, Egol et al. recognized a loss of wrist motion and grip strength in patients >65 years of age treated nonoperatively for distal radius fractures; however, that did not correlate with a poorer functional outcome [71]. No difference in DASH scores or pain scores was observed at 3, 6, or 12 months; yet radiographic parameters were superior for those who underwent operative intervention at each follow-up.

Diaz-Garcia et al. conducted a systematic review of distal radius fractures in patients >60 years of age identifying 21 articles [72]. Worse radiographic parameters were observed with cast immobilization; however, that did not correlate with poorer functional outcomes, confirming the findings of Egol et al. [71]. Further, Arora et al. performed a prospective randomized trial comparing nonoperative treatment with volar locking plate fixation for distal radius fractures in 73 patients >65 years of age followed for 12 months [73]. Those treated operatively had improved DASH and PRWE (Patient-Rated Wrist Evaluation) scores at 3 months, but no difference at 6 and 12 months. The operative group also had significantly better radiographic parameters, grip strength, and a higher rate of complications. Anatomic reduction did not guarantee improved functional outcomes.

Other patient demographics and characteristics have been reported to affect patient outcomes. Walsh et al. noted ethnic disparities in recovery after distal radius fractures. There was poorer physical function and greater pain in African American and Hispanics than in Caucasians [74]. Paksima et al. documented an increase in education level doubled an improvement of pain, range of motion, grip strength, and DASH score after the review of 227 patients [59].

Wilson et al. identified improved functional outcome scores following distal radius fractures in patients without diabetes, hypertension, depression, and nonsmokers [75]. In addition, full-duty work status was significantly related to improved pain and perceived disability. Overall, patient outcomes following a distal radius fracture are impacted by a multitude of factors including fracture pattern, age, education, and patient comorbidities; however, the average DASH score remains within the good to excellent range despite all these factors.

Minimizing articular step-off (and optimizing reduction quality) is neither necessary nor sufficient to ensure an excellent clinical outcome after distal radius fracture. Articular step-off and reduction quality, however, are associated with the development of radiographic PTA. Radiographic PTA after distal radius fracture is not well-correlated with clinical outcome, and therefore functional outcome. We have summarized factors that influence patient outcomes positively and negatively after distal radius fracture (Table 21.3). Furthermore, we will discuss the ole of total joint arthroplasty and arthrodesis after distal radius fractures.

Table 21.3 Variables and factors that influence patient outcome following distal radius fracture fixation

Positively	Equivocally	Negatively
Male sex	Articular step-off	Complex intra-articular fracture pattern
Increased educational level	Treatment method	Ulnar styloid fracture
Caucasian race		Positive ulnar variance >2 mm
Nonsmoker		Medical comorbidities

Arthroplasty and Arthrodesis After Distal Radius Fracture

Although traditionally associated with the treatment of rheumatoid arthritis, wrist arthrodesis and wrist arthroplasty are an option in the treatment of PTA after distal radius fractures. Nagy noted prosthetic replacement in non-rheumatoid with PTA "merits serious consideration" [76]. He also stated that prior to wrist arthrodesis for PTA that patients have a trial of immobilization [76]. Nagy further stated that patients "without pain relief from test anesthesia, trial immobilization, and no apparent distal radioulnar joint pathology" were poor candidates for arthrodesis [76].

Boecstyns et al. reported the results of a multicenter study of total wrist arthroplasty as a salvage procedure for wrists with severe PTA [77]. At an average follow-up of 39 months, pain had improved significantly, mobility was unchanged, and the total revision rate was 3.7 % [77]. These authors concluded that "total wrist arthroplasty can be an alternative procedure and gives results that are comparable to those obtained in rheumatoid cases" [77].

In addition to wrist arthrodesis and total wrist arthroplasty for PTA after distal radius fracture, other options include primary wrist arthrodesis for fractures that are not repairable, and limited (partial) fusions such as radio-scapho-lunate arthrodesis. These procedures, however, are outside of the scope of this chapter. For now, the indications, risk factors, and roles for total wrist arthroplasty and wrist arthrodesis (after PTA from intra-articular fracture of the distal radius) are not supported by available evidence. Therefore, the usefulness of total wrist arthroplasty and wrist arthrodesis in PTA after distal radius fracture remains undefined.

Conclusion

The development of PTA following operative and nonoperative treatment of intra-articular fractures appears to be complex and multifactorial. Restoration of normal joint anatomy does not guarantee the prevention of PTA in all joints and in all individuals. Many fractures with comminution have less satisfactory clinical outcomes, regardless of the accuracy of the articular reduction.

There are differences among joints with regard to morphological, mechanical, and biological properties of the articular surface. Moreover, the propensity for joint surface remodeling depends on the age of the patient and any underlying degree of instability causing abnormal loading of

the articular cartilage. Basic science work has demonstrated that remodeling after articular fractures occurs [78]. Whether interventions can enhance or limit remodeling after an articular fracture remains to be seen.

Sensitivity to step-offs appears to be inversely correlated with cartilage thickness. Variation in articular cartilage thickness may be the reason why different joints appear to have different tolerance for fracture and subsequent step-off and subsequent risk for developing PTA. Comorbidities such as diabetes, obesity, hypermobility syndrome may also affect a patient's capacity to repair the articular surface after injury. Thompson et al. demonstrated that increasing severity of injury in an articular fracture animal model lead to increased post-injury inflammatory responses within the joint [79]. Little is known about the injury response of intra-articular structures of human beings after fracture.

The three intra-articular fractures we discussed are all unique with regard to articular step-off. Distal radius fractures healed with an incongruous step-off >2 mm are associated with early arthrosis; however, this observed arthrosis does not correlate with poor clinical outcomes. For tibial plateau fractures, the acceptable range of intra-articular step-off is 2–10 mm, with other factors (malalignment, joint stability, and meniscal pathology) contributing to patient outcomes and the development of PTA more significantly than articular step-off.

The accuracy of acetabular fracture reduction relates directly to clinical outcomes and the development of PTA. Anatomic or near anatomic reduction of acetabular fractures appear essential to good clinical outcomes and forestalling the development of PTA.

Many anatomic, biomechanical, demographic, biochemical, and genetic factors contribute to the pathophysiology of PTA after intra-articular fracture of the acetabulum, tibial plateau, and distal radius. PTA is ripe for future investigation. Research with these fractures should focus on defining clinical opportunities for early intervention and treatment to minimize the development of PTA.

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Non-arthroplasty Treatments for PTA in the Lower Extremity

22

Brandi R. Hartley and Craig S. Roberts

Post-traumatic arthrosis (PTA) after intra-articular fracture of the lower extremity has not been erased by modern fracture care. Total joint replacements are best for the treatment of severe or end-stage PTA of the hip, knee, and ankle. Total joint arthroplasty for PTA, however, has a higher risk of complications and poor outcomes. Prior to the consideration of total joint replacement, non-arthroplasty (non-total joint replacement) options have an important role in the treatment of PTA. This chapter will discuss the non-arthroplasty nonoperative and operative options for PTA of the hip, knee, and ankle.

PTA Hip: Nonoperative

The treatment of PTA begins with nonoperative measures: nonsteroidal anti-inflammatory medicine, physical therapy focusing on gait training, and musculature strengthening. Hando et al. investigated a standardized manual therapy and therapeutic exercise intervention protocol on 15 subjects suffering from hip osteoarthritis [1]. Harris Hip Scores, Numerical Pain Rating Scale, and hip range of motion measures were statistically significant and remained significant at repeat evaluations at 8 weeks and 29 weeks [1]. Clearly, there is a role for the initial treatment of hip PTA with physical therapy and nonsteroidal anti-inflammatory medicine.

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PTA Hip: Operative

Hip Arthrodesis

Hip arthrodesis has historically been an option for young laborers with severe arthrosis. Early degenerative changes may manifest as signs and symptoms of femoral acetabular impingement (FAI). The treatment of post-traumatic FAI is a technique to prevent the development of PTA in specific circumstances and is beyond the scope of this text. Once severe PTA has been diagnosed in the hip, arthrodesis is one of the mainstays of non-arthroplasty treatment. Although hip arthrodesis is effective for relieving hip pain, patients develop ipsilateral knee pain and low back pain in the long term. Schafroth et al. followed 47 hip arthrodesis patients at 18.9 years follow-up and found the walking tolerance averaged 115 min [2]. Many patients reported difficulties with putting on shoes or socks [2]. The contralateral hip demonstrated decreased hip motion with no substantial decrease in ipsilateral or contralateral knee motion. Three of 30 ipsilateral knees demonstrated radiographic evidence of severe osteoarthritis. The average visual analysis score (VAS)

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for pain with regard to the lower back was 3.6. Degenerative changes of the lumbar spine were seen in 12 of 30 patients with 10 patients suffering from lumbar scoliosis. Ultimately, 7 patients were converted to total hip arthroplasty (THA) with "good" results over 18 years.

In a retrospective review of 53 young patients with hip arthrosis, Sponseller et al. reported all were capable of work; yet, 57 % reported low back pain and 45 % reported ipsilateral knee pain at 38-year follow-up [3]. Callaghan et al. noted in a series of 28 patients that ipsilateral knee and low back pain had an average time to onset of 23 years and 25 years, respectively [4]. Seventy-five percent of the patients reported they could walk greater than one mile and could sit comfortably for greater than 2 h.

Jain and Giannoudis published a systematic review of hip arthrodesis and identified 8 studies of 249 hips (all level III or IV evidence), which revealed variable union rates (37.5–100 %) and patient satisfaction (69–100 %) [5]. Adjacent joint pain was reported in the low back (75 %) and ipsilateral knee (57 %). Return to work also varied from 50 to 100 %.

Many patients with a hip arthrodesis are now electing to undergo conversion to THA. THA gives variable pain relief with back pain more reliably relieved than ipsilateral knee pain [6]. Jain and Giannoudis reported low back pain relief ranged from 49 to 86 % and ipsilateral knee pain ranged from 22 to 86 % in a systemic review of arthrodesis conversion to THA [5]. A decrease in normal range of motion is expected postoperatively; however, restoration of motion does not correlate with patient satisfaction. Overall, patient satisfaction utilizing the Mayo hip score is 50 % good to excellent results. Improvements in Harris Hip Scores are more variable. Morsi noted that continued functional improvement occurs up to 3 years following conversion to THA [7]. Kilgus et al. reported improvement in abductor function up to 2 years [8].

Complication rates are much higher with an arthrodesis conversion than a primary THA or a revision THA. Postoperative infection rates also vary from 0.2 to 2 % with an increased risk if indication for arthrodesis involved an infective process. Dislocation rates vary from 0.02 to 0.2 %, and nerve palsies range from 0.08 to 0.18 %. Aderinto et al. reported outcomes on 18 hip fusion conversions to THA with a mean follow-up of 5 years [9]. Complications were reported in 11 of the 18 and included peroneal nerve injury, need for revision surgery, and heterotopic ossification (one resulted in ankylosis).

Table 22.1 Non-arthroplasty options for hip PTA

Nonoperative
NSAIDs
Physical therapy
Aquatic therapy
Operative
Hip arthrodesis

Risk factors for failure of conversion include multiple previous surgical procedures, age <50 years at the time of conversion, arthrodesis indication related to previous fracture, and >30 year duration of arthrodesis [10]. Richards and Duncan reported that 10-year survivorship was 74.2 %, with a complication rate of 54 %, after a review of 17 patients with an average follow-up of 9 years [11]. When comparing arthrodesis conversion THA patients with cohorts of primary THA and revision THA, standardized outcome questionnaires demonstrated statistically significant outcome scores when compared to primary THA, but not revision THA patients. The authors argued that although quality of life outcome scores are not statistically significant when compared to revision THA, they are clinically significant. Revision THA and primary THA mean satisfaction scores were 91 and 92, respectively, in comparison with 75 for the hip fusion conversion cohort.

Jain and Giannoudis performed a systematic review of hip arthrodesis conversion to THA and found inconsistent pain relief and a high complication rate [5]. Patient satisfaction rates ranged from 63 to 100 %. Revision rate reported was 4.4 to 27 % with indications for deep infection, aseptic loosening, and dislocation.

We have summarized the non-arthroplasty options for PTA of the hip (Table 22.1). These are divided into nonoperative and operative options.

PTA Knee: Nonoperative

Let us assume for a moment that the treatment of osteoarthritis of the knee can be translated to treatment of PTA of the knee. The American Academy of Orthopedic Surgeons (AAOS) Evidence-Based Guidelines for osteoarthritis of the knee recommended strengthening, low-impact aerobic exercise, neuromuscular education, and weight loss [12]. A systematic review of 60 randomized control trials of 8,218 patients on exercise interventions for knee osteoarthritis noted the best clinical improvements were observed with an exercise program which integrated strengthening, flexibility, and aerobic activities [13].

Many orthopedic surgeons use intra-articular corticosteroid injections. A Cochrane review performed by Bellamy et al. evaluating the efficacy of intra-articular corticosteroids identified 28 trials involving 1,973 patients ([14], Art. No. CD005328). Intra-articular corticosteroids at 1 week after injection were found to be more effective than placebo for pain reduction, but the results after 4 weeks and 24 weeks after injection were much less favorable.

Intra-articular hyaluronic acid (HA) injections (viscosupplementation) are an option when intra-articular injections of corticosteroids fail. Adverse side effects associated with HA injections include benign local skin/soft tissue reactions. A Cochrane review performed by Bellamy et al. evaluated the efficacy of viscosupplementation for knee osteoarthritis ([15], Art. No. CD005321). Seventy-six trials were analyzed supporting the use of viscosupplementation over placebo with improvements in pain from 28 to 54 % at 5–13 weeks postinjection and 9 to 32 % for function. In comparison with corticosteroids, longer-term benefits were observed with viscosupplementation ([15], Art. No. CD005321). Housman et al. investigated the efficacy of intra-articular hylastan compared to intra-articular corticosteroids via a double-blind, randomized, multicenter trial with follow-up of 6 months [16]. Both hylastan and corticosteroids significantly reduced pain scores. All secondary outcomes were similar including responder rates, global assessments, and walking pain. In addition, Cheng et al. performed a literature review which supported the use of intra-articular corticosteroids and noted significant pain relief and improved function up to 1 year postinjection [17]. Nonetheless, intra-articular HA injections may provide longer pain relief than intra-articular corticosteroid injections.

Platelet-rich plasma (PRP) is another injectable option. The clinical outcomes of PRP intra-articular knee injections with 6 months follow-up were investigated by Raeissadat et al. and demonstrated significant improvements in both physical and mental domains of the SF-36 and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaires [18]. Khoshbin et al. performed a systematic review of six level I and II studies investigating PRP in knee osteoarthritis consisting of 577 patients identifying PRP as significantly better than HA or normal saline injections with pooled results using the WOMAC Index Scale [19]. No significant difference was seen for visual analog scale score or overall patient satisfaction. Of note, a higher incidence of nonspecific adverse events were witnessed with PRP over HA or placebo. This data suggests that PRP injections may be an alternative treatment; however, there is a need for more evidence to better define clinical guidelines.

PTA Knee: Operative

Non-arthroplasty surgical interventions for PTA of the knee with good clinical outcomes include knee arthroscopy, osteochondral allograft transplantation for localized defects, and distal femoral or proximal tibia osteotomies for deformity correction or single-compartment disease. The AAOS developed appropriate use criteria (AUC) on the non-arthroplasty treatment of knee osteoarthrosis and four "appropriate" treatment recommendations: self-management, prescribed physical therapy, nonsteroidal anti-inflammatory drugs, and acetaminophen [20]. There were 3 "maybe appropriate" recommendations: arthroscopic partial meniscectomy or loose body removal,

hinged knee brace and/or unloading brace, and intra-articular steroids [20]. Although osteoarthrosis is not the same entity as PTA, these recommendations are compelling.

Knee Arthroscopy

Knee arthroscopy for osteoarthrosis has fallen out of favor. Moseley et al. reported the results of a controlled trial involving patients with osteoarthritis of the knee and noted that the outcomes after arthroscopic lavage or arthroscopic debridement were no better than a placebo (sham) procedure [21]. Katz et al. conducted a multicenter, randomized, controlled trial to determine whether arthroscopic partial meniscectomy for symptomatic patients with a meniscal tear and osteoarthrosis resulted in better functional outcome than nonoperative therapy [22]. They found that there was no difference between the two study groups (arthroscopy with postoperative physical therapy versus physical therapy alone). However, 30 % of the patients who were assigned to physical therapy alone underwent surgery within 6 months [22]. They found that arthroscopic surgery for knee osteoarthritis provides no additional benefit compared to "optimized physician and medical therapy" [23]. Nonetheless, osteoarthritis of the knee may not be completely analogous to PTA of the knee. It is possible that knee arthroscopy for PTA may prove to be more efficacious than arthroscopy for knee osteoarthritis.

Osteochondral Autograft Transplantation

Patients who suffer from localized pain due to an identifiable cartilaginous defect within a single compartment of the knee have successfully responded to an Osteochondral Autograft Transfer System (OATS) procedure, where a lower weight-bearing portion of normal autologous cartilage and bone are inserted into the defect following debridement. A systematic review of 19 studies of 644 knees with a mean follow-up of 58 months reported an overall satisfaction rate of 86 % and an overall failure rate of 18 % based on varied definitions of failure [24]. Sixty-five percent had little or no radiographic arthritic changes on final follow-up. Favorable outcomes were associated with those patients who had shorter symptom duration, traumatic etiologies, and young patients with focal unipolar defects. A short-term complication rate of 2.3 % was reported. The most common complications included removal of hardware, repeat arthroscopy, and infection [24].

Osteotomy

Residual deformity is common after fractures around the knee joint. Patients with early to moderate PTA, and changes in the mechanical axis and knee orientation, may benefit from osteotomies for deformity correction. An osteotomy may provide years of improved quality of life and delay the need for a total knee arthroplasty. Nonetheless, the AAOS AUC on non-arthroplasty treatment of osteoarthritis of the knee (which we believe is analogous to PTA for the sake of the discussion here) noted that "realignment osteotomy is rarely appropriate" [20]. This statement should be interpreted in the context that deformity is less common with osteoarthritis and more common with PTA, where osteotomy clearly has a role.

A retrospective review analyzed 28 patients who underwent an osteotomy for PTA due to an intra-articular or extra-articular malunion with a mean 3.8-year follow-up [25]. On average, the trauma occurred 17.3 years prior to the surgical intervention. Two patients with intra-articular malunions went on to require total knee arthroplasty (TKA) for continued pain. Four patients required repeat surgery for infection, stiffness, and pseudoarthrosis. At final follow-up, pain scores were significantly improved. Corrective osteotomies around the knee with post-traumatic coronal plane deformity can relieve pain and improve function. We have summarized the non-arthroplasty options for PTA of the knee in Table 22.2.

Table 22.2 Non-arthroplasty options for knee PTA

Nonoperative
Low-impact exercise
Aquatic therapy
Intra-articular steroid injection
Viscosupplementation
Platelet-rich plasma injection
Operative
Knee arthroscopy
Osteochondral autograft transplantation
Osteotomy

PTA Ankle: Nonoperative

Initial treatments of PTA of the ankle are nonsteroidal anti-inflammatory medications, physical therapy, shoe-wear modifications, orthotics, intra-articular injections of corticosteroids, and viscosupplementation. Glazebrook noted that the current literature that supports most nonsurgical treatments for ankle arthritis use lesser quality, level IV studies [26]. He stated that a systematic review was necessary in the future to determine the level of evidence available to guide the recommendations of nonsurgical options for treating ankle arthritis [26].

A series of three HA viscosupplementation injections were performed under fluoroscopic guidance and evaluated at 4 and 12 months postinjection with the American Orthopedic Foot and Ankle Society (AOFAS) score [27]. AOFAS scores were statistically significant at 4 and 12 months, with 73 % of patients reporting satisfaction at an average follow-up of close to 4 years. Five patients required surgical intervention an average of 27 months.

DeGroot et al. performed a randomized, double-blind, placebo-controlled trial of a single intra-articular HA injection versus an injection of normal saline for knee osteoarthritis [28]. Sixty-four patients were assessed at 6 weeks and 12 weeks postinjection with AOFAS scores. Changes from baseline in both groups were significant, yet the analysis between groups demonstrated no significant differences. This demonstrates the variability present in the literature concerning HA injections as treatment for arthrosis of the ankle. Currently, more clinical trials investigating both corticosteroid and viscosupplementation efficacies are necessary in order to fully support ankle injections for posttraumatic arthrosis.

Mousopoulos et al. noted that corticosteroids or HA joint injections for PTA offer "temporary pain relief with hardly any mid- or long-term benefit" [29]. Johnson et al. noted that evidence-based guidelines for the use of injectable corticosteroids were lacking [30]. These investigators noted that younger orthopedic surgeons (less than 5 years in practice) performed fewer injections than those in practice 6–10 years [30].

Wexler et al. noted that corticosteroid injections are not typically done for ankle arthritis, are generally of limited duration, but can provide excellent temporary pain relief in patients with end-stage disease [31].

PTA Ankle: Operative

Ankle arthrosis after trauma can be treated with a myriad of surgical options. These include arthroscopic debridement, allograft resurfacing, osteotomy, distraction arthroplasty, and tibiotalar arthrodesis.

Arthroscopic Debridement

Arthroscopic debridement is selected for patients with large osteophytes which limit motion. Patients who report pain with extremes of motion or certain activities (i.e., stair climbing, patients with anterior impingement, etc.) are ideal candidates for arthroscopic osteophyte resection and debridement. Relative contraindications include patients who report pain at rest, complete loss of articular joint space, and advanced arthrosis. Rasmussen and Jensen performed arthroscopic ankle debridement for ankle impingement on 105 patients and reported complete pain relief for 65 patients, whereas 28 patients reported a reduction in pain [32]. Complications included four deep infections and one synovial fistula, all of which responded well to repeat arthroscopy and antibiotics [32].

Allograft Resurfacing

Allograft resurfacing with fresh tissue allografts can be used to replace damaged articular surfaces. These allografts are indicated for young, active patients with focal unipolar defects within either the plafond or talar dome and are contraindicated in patients with vascular disease, malalignment greater than 10°, ankle instability, and obesity. Raikin published a prospective review of 15 patients who underwent osteochondral allograft resurfacing with a minimum of 2-year follow-up and score improvements in AOFAS of 45 points with 11 patients reporting good to excellent outcomes [33]. Bipolar fresh osteochondral allografts after 14 months are associated with a significant improvement in AOFAS score and improved ankle range of motion in the frontal plane identified by gait analysis [34].

Osteotomy

Patients with early to moderate ankle arthrosis and concomitant deformity with tibiotalar malalignment and reasonable ankle motion are ideal candidates for a supramalleolar osteotomy. Giannini et al. reported on 22 patients with ankle PTA who were treated with corrective osteotomies [35]. After an average follow-up of 5 years, 15 patients denied pain, had no gait abnormalities, and had no limitation in daily activities. Two patients reported persistent pain limiting their activities and a prominent limp; radiographically, these patients also had a loss of correction and went on to require an ankle arthrodesis. Fifty-seven malunited ankle fractures treated with reconstructive osteotomy were retrospectively reviewed by Reidsma et al. with an average follow-up of 15.5 years [36]. Forty-one patients (85 %) had a subjective good to excellent result, and an objective (radiographic, ankle, and subtalar motion) good to excellent result was seen in 42 patients (88 %) [36]. A poor objective result was correlated with increased duration from the time of the initial injury to the time of reconstructive surgery, and arthritic changes present prior to surgery.

Cheng et al. reviewed 18 cases of supramalleolar osteotomy with an average follow-up of 47.7 months [37]. Dramatic improvements were seen with pain relief, function, and ankle range of motion. Complications included a single late infection treated with wound debridement and hardware removal and two delayed unions treated with revision osteotomy. All 18 patients had good to excellent results. Overall, there is the potential for satisfactory results and a delay of future ankle arthrodesis or arthroplasty when a corrective osteotomy is performed for properly selected patients.

Distraction Arthroplasty

Distraction arthroplasty with a temporary external fixator across the tibiotalar joint may be indicated in patients with a congruent joint (no deformity) and early painful arthrosis recalcitrant to conservative treatment. Intema et al. demonstrated that joint distraction allows for subchondral bone remodeling verified by CT imaging after 2 years following 5 mm joint distraction for 3 months [38]. Pain and disability scores were significantly improved. Correlation with resolution of subchondral cystic lesions and pain was observed.

van Valburg et al. reviewed 11 patients after joint distraction for an average of 15 weeks noting a decrease in pain in all patients, 55 % improved range of motion, and joint-space widening in 50 % after 20-month follow-up [39]. Ankle joint distraction may contribute to changes in mechanical stresses within the joint which encourage cartilage repair and bone remodeling; however, the actual mechanism of pain relief remains unclear.

Tibiotalar Arthrodesis

Arthrodesis remains the most popular treatment for tibiotalar arthrosis and is ideal for severe PTA associated with infection, instability, avascular necrosis, deformity, and stiffness. Tibiotalar arthrodesis also remains the main salvage procedure for ankle surgery. Complications include

injury to the lateral plantar and superficial peroneal nerves, nonunion (10 %), and development of adjacent joint arthrosis. Fuchs et al. retrospectively reviewed 17 patients with 18 ankle arthrodesis over 20+ years (average follow-up, 23 years), in which 50 % of patients reported no limitation or slight limitation in activities of daily living [40]. A greater tendency for arthritic changes in the subtalar joint than in the midtarsal joints was observed. The development of radiographic arthritic changes in the subtalar joint alone correlated with a poorer subjective clinical outcome. A retrospective review of 23 patients who underwent an isolated ankle arthrodesis for PTA with an average follow-up of 22 years (range, 12–44 years) noted significant development of subtalar, talonavicular, calcaneocuboid, naviculocuneiform, and tarsometatarsal joint degeneration [41]. The degeneration of adjacent joints was independent of operative technique and alignment. Yet, most patients were subjectively satisfied with their overall outcome and would even recommend it to another patient. Buchner and Sabo reported 73 % good to excellent results based on the AOFAS scores, with an average of 34.2 score improvement at 9.3-year follow-up [42]. Fusion in greater than 5° of plantar flexion was correlated with a less successful outcome. Subtalar motion was decreased by 54 % on average when compared to the contralateral side with moderate to severe arthritis in 47 % of patients. Subtalar arthrosis correlated with worse clinical outcome. Forty-four of 48 patients reported that they would choose to perform the surgery again given the same situation and would recommend it to another patient [42]. Although there is increasing interest in total ankle replacement, tibiotalar arthrodesis remains a very powerful treatment option for PTA of the ankle. Finally, we have summarized the non-arthroplasty options for PTA of the ankle (Table 22.3).

Table 22.3 Non-arthroplasty options for ankle PTA

Nonoperative
NSAIDs
Physical therapy
Shoe-wear modifications
Orthotics
Intra-articular corticosteroid injections
Viscosupplementation
Operative
Arthroscopy
Allograft resurfacing
Osteotomy
Distraction arthroplasty
Tibiotalar arthrodesis

Summary

Non-arthroplasty (non-total joint arthroplasty) options are an important part of the treatment of PTA of the hip, knee, and ankle. Non-arthroplasty options include both nonoperative and operative measures. These options ought to be considered prior to total joint replacement. There remains the need for additional evidence-based medicine and systematic reviews to further clarify the efficacy of these non-arthroplasty options and their future role in the treatment of PTA of the hip, knee, and ankle.

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Results of Arthroplasty in Post-Traumatic Arthritis

23

Antonia F. Chen and Javad Parvizi

Introduction

Post-traumatic arthritis (PTA) of the hip comprises 10–15 % of symptomatic osteoarthritis (OA) that are more likely to develop secondary to articular incongruity, damage to the articular cartilage, or femoral head avascular necrosis [1]. Patients who develop PTA are often younger, and these patients are often surgically treated with total hip arthroplasty (THA). While the results of THA in patients with PTA are good, there are specific concerns that must be addressed when performing THA, such as addressing abnormal anatomy from previous surgeries, dealing with existing hardware, evaluating fracture nonunion, working with bony deficiency, and addressing possible scarring of the sciatic nerve. The purpose of this chapter is to describe the preoperative considerations, concerns, and surgical techniques when performing delayed THA for PTA. The outcomes of arthroplasty in hip, knee, ankle, shoulder, and elbow patients with PTA are also discussed.

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Surgical Approach

The surgical approach may be performed based on whether the patient was previously treated operatively or nonoperatively. If nonoperative management was utilized and no other pelvic surgeries have been performed, then the surgical approach is the surgeon's choice. If there is a posterior defect that requires augments or grafting, then a posterior approach to the hip may be utilized. Approaches that provide adequate visualization of the acetabulum are important, as scar tissue must be mobilized and extensive soft tissue dissection might necessary. Minimally invasive approaches may not be ideal for performing delayed THA for PTA.

If a patient underwent open reduction and internal fixation (ORIF) for treatment of an acetabular fracture, the previous incision may be used in its entirety, partially incorporated into a new incision, or a new incision performed. A posterior approach may be used if a previous Kocher-Langenbeck approach was used for fracture fixation, so that hardware may be removed. However, tissue scarring may be present after the previous ORIF including scarring around the sciatic nerve. If the old incision is in a reasonable place, part of the previous incision may be used. The new incision should diverge from the old incision by 60° to avoid skin necrosis between the incisions.

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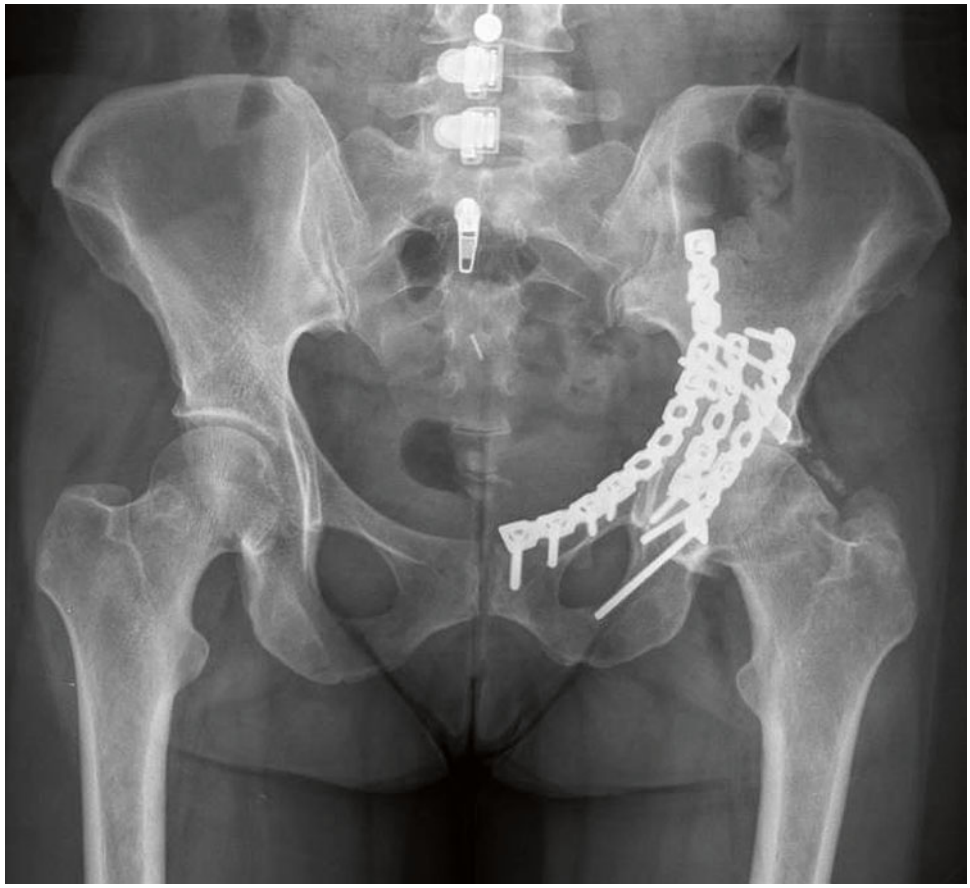

Fig. 23.1 PTA after previous both column plating of an acetabular fracture

Other surgeons choose to use the approach that they are most comfortable with and make a new incision. Performing a THA through native tissue may decrease some of the complications of working through established scar tissue after acetabular ORIF, but heterotopic bone and scar can still occur after the trauma to the hip.

Hardware Removal

When hardware impedes with the insertion of the acetabular or femoral components, they may need to be removed. For femoral components, screws may be present if a previous trochanteric osteotomy was performed for access to the hip [2]. Posterior wall and posterior column plates and screws for acetabular fracture fixation are the most common hardware found in the acetabulum during surgery, but hardware may also be found in both column fractures (Fig. 23.1). Acetabular hardware can be removed in one stage if the fracture had adequately healed, or in two stages if additional fixation is necessary or if there is the question of infection. For one-stage THA, if screws are present, a high-speed burr can be used to remove intraarticular metal hardware. If it is impossible to remove the intraarticular screws to achieve adequate fixation, then an acetabular component may be cemented into the acetabulum. Two-stage THA may need to be performed in light of acetabular nonunion or malunion, where hardware is removed, bone graft is applied, and a revision acetabular ORIF can be performed in the first stage, and then the patient undergoes subsequent THA during the second stage.

Bone Stock

Patients with PTA from acetabular fractures treated nonoperatively may have altered bony anatomy secondary to remodeling and may have deficient bone stock that requires bone graft [3]. For preoperative planning, a computed tomography (CT) of the hip can be performed to define bony morphology, and acetabular bony deficiencies can be assessed using the Paprosky classification [4]. This classification system is diagnostic and prognostic based on the location and size of the bony defects. Type I defects have a supportive bony rim with no bone lysis or migration and can often be treated by primary THA. Type II defects have intact bony columns but have distorted acetabular sockets with <2 cm of migration. These defects may be treated with autograft, allograft, or metal augments. Type III defects have severe ischial and medial osteolysis with more than 2 cm of superior migration, and these defects may require more extensive acetabular reconstruction using structural grafts, mesh, jumbo cups, or cages.

To reconstruct these defects, the goal is to create a concentric bone bed for acetabular cup placement, and it is best to place the cup in contact with native healthy bone to provide bony ingrowth. It is important to ensure that the operating room has enough bone allograft available, including demineralized bone matrix, cancellous chips, and larger structural allograft. For a primary THA, the femoral head can be used as autograft. If the defect is contained, bone graft can be placed in the defect and reversed reamed or impaction grafting can be used [5]. If there is evidence of protrusion, wire mesh or structural grafting using allograft or autograft can be fixed to the pelvis and used to add support to the acetabular cup [6]. Finally, if bony support is inadequate, trabecular metal augments of various shapes and sizes can be used to fill structural defects [7]. These augments are fixed to the pelvis with screws and cemented to the acetabular cup, as demonstrated in Fig. 23.2.

Acetabular Fixation

In the past, acetabular cups were commonly cemented in if performed after acetabular fractures. Cups that were cemented in the acetabulum after impaction grafting when performing THA

Fig. 23.2 Trabecular metal augments are used to fill bony defects and provide structural support to the acetabular cup (Printed with permission from Zimmer, Warsaw, IN)

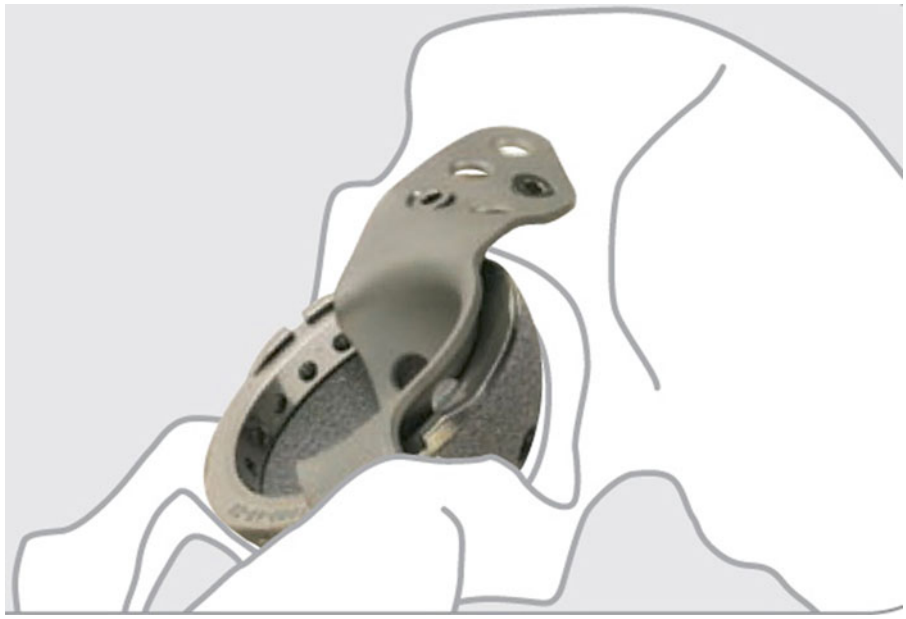

Fig. 23.3 A cup-cage construct may be used to provide fixation in inadequate bone stock by allowing the porous cup to ingrow into bone graft and native bone (Printed with permission from Zimmer, Warsaw, IN)

after acetabular fractures demonstrated 100 % survival at 10 years and 80 % survival at 15 years [8]. Currently, uncemented acetabular cups have become more popular as the metal surfaces of implants have improved. Titanium porous-coated sockets have shown good 10–16-year survival when performed in patients who developed PTA from previous acetabular fractures [9], and patients with trabecular metal acetabular components were found to have adequate fixation in native bone with less than 50 % contact at a minimum of 2-year follow-up [10].

When using uncemented components, there are multiple options for fixation. Multihole cups provide more screw options in the dome for acetabular cup fixation, which can provide greater support if press fit fixation cannot be achieved in the acetabulum. Screw fixation in the acetabular rim of components may provide additional fixation, but these thicker shells may reduce head size and liner thickness by 6 mm for each corresponding cup size.

If it is difficult to achieve adequate acetabular bone fixation, a gap cup can be used if there is pelvic discontinuity. Bone graft can be placed into the acetabulum and a gap cup is temporarily applied to allow for bone graft healing with a cemented liner until definitive THA is performed with an improved bone bed. Gap cups have plate extensions that allow for fixation into the ischium, teardrop, or obturator foramen with screws, hooks, or a blade plate. However, studies have demonstrated high rates of fatigue and catastrophic failure ranging from 37 to 42 % [11, 12].

Another option is to use a cup-cage construct, where a second-generation porous titanium cup is impacted into bone (Fig. 23.3). If there is inadequate bony fixation, bone graft can be applied and an acetabular cage can be placed on top of the cup and fixed to the ischium and ilium with screws [13]. A polyethylene liner is then cemented into the cup-cage construct, and the acetabular cup has been shown to achieve osseointegration in 88.5 % of patients with no signs of loosening at 44.6 months [14].

Intraoperative Complications

It is important to obtain adequate exposure intraoperatively to remove existing implants, place new implants, and remove HO. Despite careful

surgical technique, delayed THA for PTA may have greater intraoperative complications compared to THA for OA. Heterotopic ossification (HO) is more likely to develop in patients who undergo ORIF for previous acetabular fractures, and extensive Brooker grade 3 ossification may make it more difficult to mobilize the femur. Brooker grade 4 HO, or autofusion of the hip, may require aggressive removal of HO prior to performing THA. Additionally, cutting the femoral neck in situ and performing extensive capsular release may need to be done to facilitate dislocation.

In addition to HO, patients with PTA may have significant limb length discrepancies. Scar tissue may develop after previous ORIF and may result in increased traction on the sciatic nerve. Thus, careful dissection of scar tissue is important, and using somatosensory evoked potentials (SSEPs) may be helpful for monitoring potential nerve damage, especially with leg length discrepancies greater than 2 cm.

Postoperative Management

PTA patients who undergo THA may require different postoperative management, although most patients can be managed similarly to patients with OA. If extensive bone grafting is used, then weight bearing status might need to be altered to be partial or touchdown weight bearing to reduce the forces across the hip. Weight bearing restrictions may need to be implemented for 6–8 weeks to allow for adequate healing of the acetabular implants to native and graft bone.

Additionally, patients who receive THA with PTA may have increased dislocation risk and hip precautions may need to be instituted. Avoidance of excessive flexion, adduction, and internal rotation in patients with a posterior approach to the hip may reduce dislocations. Finally, patients with previous excessive HO may require irradiation or treatment with indomethacin to prevent recurrence of HO, which can limit motion and function after THA.

Outcomes

The average time that PTA patients undergo THA after sustaining acetabular fractures ranges from 36 months to 15 years [15–19]. THA performed for PTA have generally fair outcomes, which depend on minimizing complications, such as infection and nonanatomic restoration of the hip center, as well as achieving adequate bony fixation of THA components [18, 20, 21]. A study conducted in 1978 by Boardman and Charnley found that patients that received a cemented Charnley THA after injury to the hip demonstrated good to excellent results after 15-year follow-up [16], but did not compare it to a cohort of patients without hip injury. Uncemented acetabular components have also demonstrated good outcomes, as patients had improved Harris Hip Scores (HHS) with a 5-year survival of 79 %, which increased to 97 % if survival for aseptic acetabular loosening was only evaluated [18]. While other studies have also documented improvement in HHS, these same studies have also demonstrated that performing THA after ORIF of acetabular fractures, especially those with complex fracture patterns, has increased surgical duration, blood loss, transfusion rates, sciatic nerve injuries, heterotopic ossification, and greater instability that may require treatment with an elevated acetabular liner [17, 22]. Performing a THA conversion from previous cephalomedullary nail or sliding hip screw fixation has higher complication rates, greater blood loss, and longer operative time than primary THA [23, 24]. When comparing the outcomes of THA for patients with PTA to those with OA, one study found that the patient populations had similar rates of femoral component loosing and revision, but there was four to five times greater acetabular component revision and loosening in patients undergoing THA for PTA [19].

Besides THA, outcomes of arthroplasty performed for PTA are generally poorer than arthroplasty performed for other forms of arthritis. For total knee arthroplasty (TKA) performed for PTA, there are higher complications such as extensor

mechanism avulsions, increased infections, and wound breakdowns as well as poorer outcomes such as stiffness and greater instability when compared to TKA performed for OA [25–29]. Patients with total ankle arthroplasties (TAA) performed for PTA similarly had higher complication rates compared to TAA patients with OA, and PTA ankle patients also had more operative procedures [30]. For patients undergoing total shoulder arthroplasties (TSAs), patients who had the diagnosis of primary OA had longer 10-year survival compared to those diagnosed with previous fracture (94.2 % vs. 76.8 %), lower complications (primary OA 8.9 % vs. PTA 24.7 %), and higher outcome scores as determined by the Constant-Murley score (primary OA 93.7 % vs. PTA 62.7 %) [31, 32]. Patients with rheumatoid arthritis (RA) undergoing total elbow arthroplasties (TEAs) had lower outcomes scores, as measured by the Mayo Elbow Performance Score (MEPS), than for TEAs performed in patients with PTA [33, 34]. Thus, patients with the diagnosis of PTA who are undergoing arthroplasty for hips, knees, ankles, shoulders, and elbows may have poorer outcomes than patients who have primary OA or RA.

Conclusion

Performing a delayed THA for PTA may be a difficult undertaking. Performing preoperative planning and addressing deficiencies intraoperatively when performing delayed THAs can provide good outcomes and improved function in patients with PTA. However, performing arthroplasty in patients with PTA must be done with caution, as results may not be as good as performing primary arthroplasty in patients with OA.

Conflict of Interest None of the authors listed above have a financial interest in this chapter.

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Section V

Developing and Future Assessment and Therapies

Measurement of Severity of Injury After Articular Fracture and Correlation with Post-Traumatic Arthritis Development

24

Donald D. Anderson and J. Lawrence Marsh

Introduction and Background

Articular fractures are a fascinating subset in the spectrum of all joint injuries that lead to post-traumatic osteoarthritis (PTOA) because of their severity and propensity to rapidly lead to joint degeneration. The treatment of articular fractures has been refined over the last two or three decades by focusing on techniques to reduce and internally fix the displaced articular surface, thereby restoring congruity and minimizing alterations in post treatment joint contact stress. Unfortunately, there are limits to the benefit of further advancements in surgical reconstruction; continued refinement of techniques has failed to improve patient outcomes [1], and PTOA after articular fracture remains common and disabling [2].

There has been recent interest in assessing and mitigating the deleterious effect of the acute injury to the joint. Unlike the associated bony fracture, the injured articular surface is less able to successfully repair. Indeed, despite accurate reduction of articular fractures, the initial injury to the articular surface often leads to a spreading zone of cell death [3]. Clinical observations suggest that the more severely the joint is injured the greater the likelihood and severity of PTOA and joint degeneration. The logical conclusion is that injury severity may be a key determinant of joint outcome after articular fracture.

Currently we are on the verge of developing new biological treatments that hold promise to improve the patient's mid and long term outcome after articular fracture [4]. When considering future clinical trials, the ability to identify which patients are at risk for development of PTOA is critical. High energy articular fractures, with their propensity to rapidly develop PTOA, are ideal clinical models to assess the effect of new pharmaceutical interventions. However, the deleterious mechanical forces and subsequent physiologic responses leading to PTOA are poorly understood and not amenable to clinical assessment, which hampers meaningful study.

The intensity of joint trauma at the time of fracture (injury severity) is one of the most important factors contributing to PTOA. For axial loads to extremities that lead to fracture, the severity of the fracture directly reflects the mechanical force delivered to the articular surface. The relationship between the amount of comminution and energy is often referenced in the orthopedic trauma literature [5]. Experienced clinicians generally describe fractures with large

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numbers of fragments as "high energy" fractures. Basic fracture mechanics principles indicate that in brittle materials there is a direct correlation between the fracturing energy and the quantity of liberated (fracture) surface area exposed by the bony disruption.

Over the past decade, enabling technologies have been developed based on validated digital image analysis from clinical CT scans to objectively stratify several biomechanical indices of fracture severity [6–8]. The severity of the initial joint injury is stratified primarily on the basis of the energy released at the time of articular fracture. This chapter will describe the development and validation of these techniques, speculating on their role in future research on PTOA and on the clinical care of patients with severe articular fractures.

Articular Fractures and PTOA: The Role of Injury Severity

Many patients that sustain fractures of the articular surface of weight-bearing joints develop PTOA, chronic pain, and subsequent poor joint function [9, 10]. PTOA occurs following a variety of joint injuries, but it is most common and most severe after comminuted articular fractures because of the severity of the initial joint injury. Unlike other forms of PTOA, following the most severe fractures, PTOA presents within a relatively short time frame. Modern treatment principles emphasize precise articular reduction, but despite optimal treatment, PTOA after fracture of the acetabulum occurs in more than 25 % of patients [11, 12], after tibial plateau fracture in between 23 and 44 % of patients [13, 14], and after fractures of the tibial plafond in more than 50 % of patients [15–18]. In tibial plafond fractures PTOA is easily detected radiographically in over 30 % of ankles within 2–4 years of fracture [19] and by 5–11 years after injury, the incidence increases to 74 % [20]. Most patients with PTOA of the ankle have decreased general health status and ankle pain and poor function [21].

Although the fundamental mechanisms that lead to PTOA are not well understood, the severity of the articular fracture plays a critical role and interacts with the degree of post-fracture joint incongruity. Both of these mechanical factors correlate with the development of PTOA [7, 10, 22–26]. It is a broadly accepted viewpoint within the orthopedic trauma community that "the extent of bone, cartilage, and soft tissue damage is directly related to the energy imparted to these structures" (Fig. 24.1) [5]. Greater energy leads to greater damage to the articular cartilage, thereby increasing the risk for PTOA.

Limitations of Traditional Assessment of Articular Fracture Severity

The energy involved in producing a fracture has not been a quantifiable variable, making assessment of the severity of the injury inexact, subjective, and largely empirical. An inability to control for the influence of injury severity has been a major confounding factor in clinical studies of intra-articular fracture treatments. In current clinical practice and for clinical research, fracture severity has been subjectively assessed by surgeons or investigators on radiographs, via categorical classifications. Fractures are placed in groups often defined at least in part by assumed severity. These classifications at best allow only crude assessments of injury severity, do not attempt to assess the energy of injury and have very poor interobserver reliability [27–29]. This seriously limits their use for clinical research and even for assessing prognosis or for decision-making about optimal treatments. For these reasons, the relationship between fracture severity and eventual outcomes remains very poorly understood. To scientifically assess the effect of treatment of any condition, an investigator must be able to measure pertinent variables. Techniques that are fundamentally objective and quantitative are needed to assess the mechanical risk factors for developing PTOA.

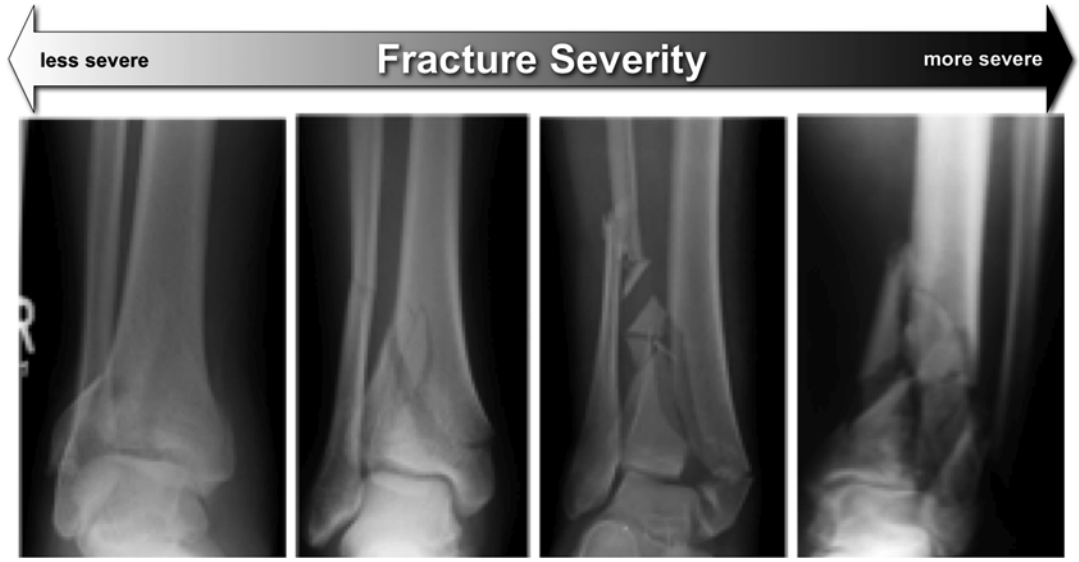

Fig. 24.1 These radiographs illustrate the tibial plafond fracture severity spectrum. Simple intra-articular fractures result from low energy impacts (*left*). As energy increases (moving from *left* to *right* in the figure), the fractures become more complex, with greater comminution [Taken with permission from Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. *Iowa Orthop J.* 31:1–20, 2011]

Theory of Measuring Fracture-Liberated Surface Area to Assess Injury Severity

Although poor observer agreement is common for categorical classifications, studies by our group and others have shown that if experienced clinicians rank order fracture radiographs by severity, the agreement between observers is very high. This technique of rank ordering fractures for severity has been used in several studies which have demonstrated that observer agreement is high when clinicians stratify injury severity using simple comparative rank ordering [30, 31]. Clinicians see information on radiographs that they correlate with severity. In this technique clinicians review a series of radiographs from patients with a specific injury under investigation. The clinicians then "rank" the injuries according to their relative severity within that group of injuries. In assessing relative severity of a set number of fracture cases, clinicians have a high level of concordance with each other [6]. The problem with categorical classifications, such as conventional fracture classifications, is the inherent overlap of categories and their definitions, not the ability of clinicians to agree on radiographic severity. Clinicians assess fractures as more severe based on increased comminution and displacement, and for articular fractures when the fractures have greater involvement of the articular surface. However, rank order techniques do not allow the clinician to apply an injury severity metric to a fracture that is not part of the rank order group. Unfortunately in the absence of a rank ordered series of cases there has been no way to measure any of these variables. Current image analysis technology allows these features of a fracture observed by clinicians to be objectively measured.

In 1998 our group first published the concept that the amount of comminution highly correlated with the amount of energy imparted to the bone to produce the fracture [32]. The idea fits with basic principles of fracture mechanics. During fracture, the mechanical energy absorbed by the bone is converted to new or liberated surface area of the

fracture fragments. More comminution means more new surface area. Direct measurement of new fractured surface is possible using routine CT scans acquired for many articular fractures during clinical care. A CT scan is made of the normal contra-lateral limb and the injured extremity. The external cortical and endosteal surfaces of the normal limb are measured as free bone surface area. The injured limb has this same area plus the addition of liberated surface area of fracture fragments seen on each image of the CT. This new, or fracture-liberated, surface area provides the basis for quantifying the fracture energy. Fracture energy serves as a metric of the energy pulse across that cartilage required to create the fracture. This fracture energy measurement provides a novel means to quantify injury severity in intra-articular fractures.

Development of the Analysis Techniques

Image analysis capabilities based on CT images were developed to measure the fracture-liberated surface area [33]. Accurately segmenting bone from other neighboring tissues was a significant technical challenge, due to similar attenuation characteristics. Metaphyseal articular fracture fragments without clear cortical margins and poorly defined fracture lines were particularly challenging.

The initial technique to segment fracture fragments used a seeded region-growing algorithm [34], which was geometrically accurate, but was too slow to be applied clinically. This analysis routine operates with conventional CT image data encoded in standard DICOM file format and can be run on a desktop personal computer.

The technique is used to analyze clinical CT datasets by identifying bone margins slice-by-slice (Fig. 24.2). The bone perimeters (endosteal, periosteal, and subchondral) are multiplied in a given CT slice by that slice's thickness, which yields the bone surface area through that slice volume. The bone surface areas are summed across all slices to determine the total amount of free surface area. The final step subtracts the pre-existing intact bone surface area from the fractured area to determine the liberated interfragmentary surface area. The accuracy of these measurements was originally established on precisely machined cubes of the polyurethane foam surrogate with a known surface area [34].

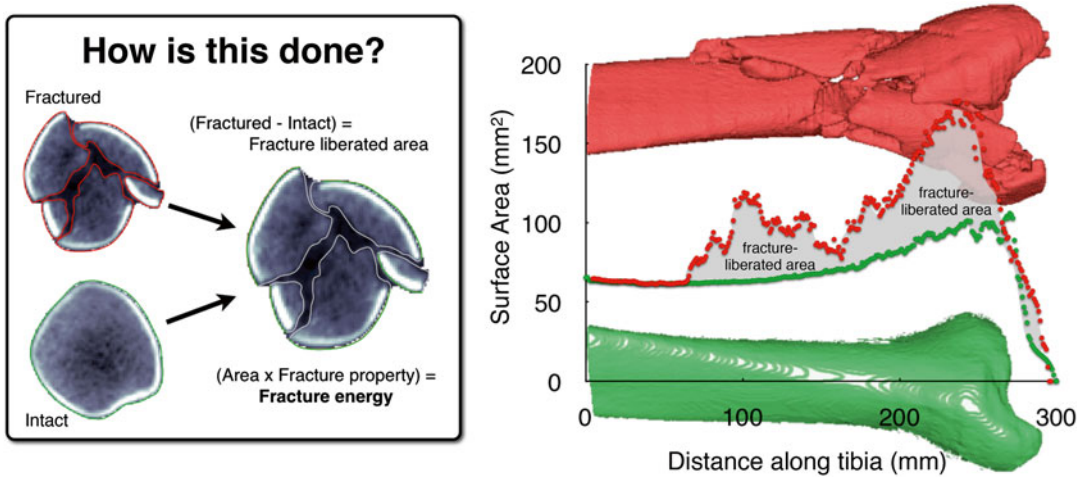

Fig. 24.2 Bone perimeters (matched intact and fractured), plotted along the length of the distal tibia, show how the fracture energy measure is calculated. *Inset*: CT slice from fracture case, with identified tibia bone fragment edges [Taken with permission from Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. *Iowa Orthop J.* 31:1–20, 2011]

Controlling for Other Factors

Human bone tissue is a very heterogeneous material. To account for the density and age-dependent energy-absorbing capacity of bone [35–37], bone density-based weighting was integrated into the algorithm. Fracture energy was calculated by multiplying the interfragmentary surface area by the energy release rate (G, units of J/m^2). This material property quantifies the amount of energy required to liberate a given surface area and is directly proportional to the first power of apparent density [37].

Bone density would ideally be based on CT Hounsfield intensities along fragment edges, regressed pixel-by-pixel. However, practically this approach is not reliable due to partial volume effects and high intensity gradients at the fracture edge. To deal with this challenge, G was partitioned according to densities found for the three dominant classes of bone: dense diaphyseal cortical, less dense metaphyseal cortical, and cancellous. The densities of these three bone classes are determined on a patient-specific basis [38], regressing from mixed Gaussian distributions. The final energy released by the fracture is determined by scaling (previously measured) impact energy/density data to the bone density values specific for each patient.

Fragment displacement/dispersion also influences the outcome of intra-articular fractures. Clinicians believe that widely displaced fractures are more severely injured than minimally displaced fractures, for good reason. Fragment displacement injures soft tissues and increases the complexity of surgical repairs. Similar to fracture energy, fragment displacement can be objectively quantified from CT studies.

Fragment displacement was quantified from the bone surfaces defined in the fracture energy analysis [8]. With fracture fragment displacement in given cross sections, bone fragments are translated away from their intact positions, disrupting the native shape and alignment of the bone. The intact proximal portion of the fractured tibia was aligned with a mirrored image of the uninjured contralateral side (Fig. 24.3). Fragment displacement relative to their pre-fracture position was calculated by determining the volume of tissues through which fracture fragments were dispersed. For each CT slice, a convex hull (the smallest convex polygon circumscribing a given object) was determined for a composite of the aligned intact and fractured tibias (inset, Fig. 24.3). The increase in volume provided a metric of the amount of fragment displacement and dispersion.

Since PTOA is the outcome of greatest interest, the degree of comminution of the articular surface, as opposed to the metaphysis or diaphysis, is a key radiographic feature associated with injury severity. To quantify this variable the amount of fracture-liberated surface area located within 1.5 mm of the articular surface of the injured tibia was separately assessed and expressed as a percentage of the intact/contralateral surface area over a similar region of the distal tibia. This provides a separate severity measure exclusively focused on articular surface injury. This can also be assessed visually on plots of the local liberated surface area (energy) along the length of the distal tibia.

Results and Validation

New techniques need to be validated against accepted techniques, and the measured results need to be correlated with outcomes of interest. As an initial step to validate the CT-based injury severity metric, the technique was compared to clinician opinion of fracture severity using rank order assessment of radiographs of the same cases [6]. The radiographs of 20 tibial plafond fractures were chosen to span the spectrum of injury, from mild partial articular fractures to severely comminuted total articular fractures. Three experienced fracture surgeons ranked the cases for injury severity based on the radiographic appearances. The raters were instructed to order the cases from least to most severely fractured.

Inter-rater agreement and agreement between the rater's assessments of fracture severity and the CT-based fracture severity metrics were assessed using concordance rates. This statistical measure estimates the probability that any two

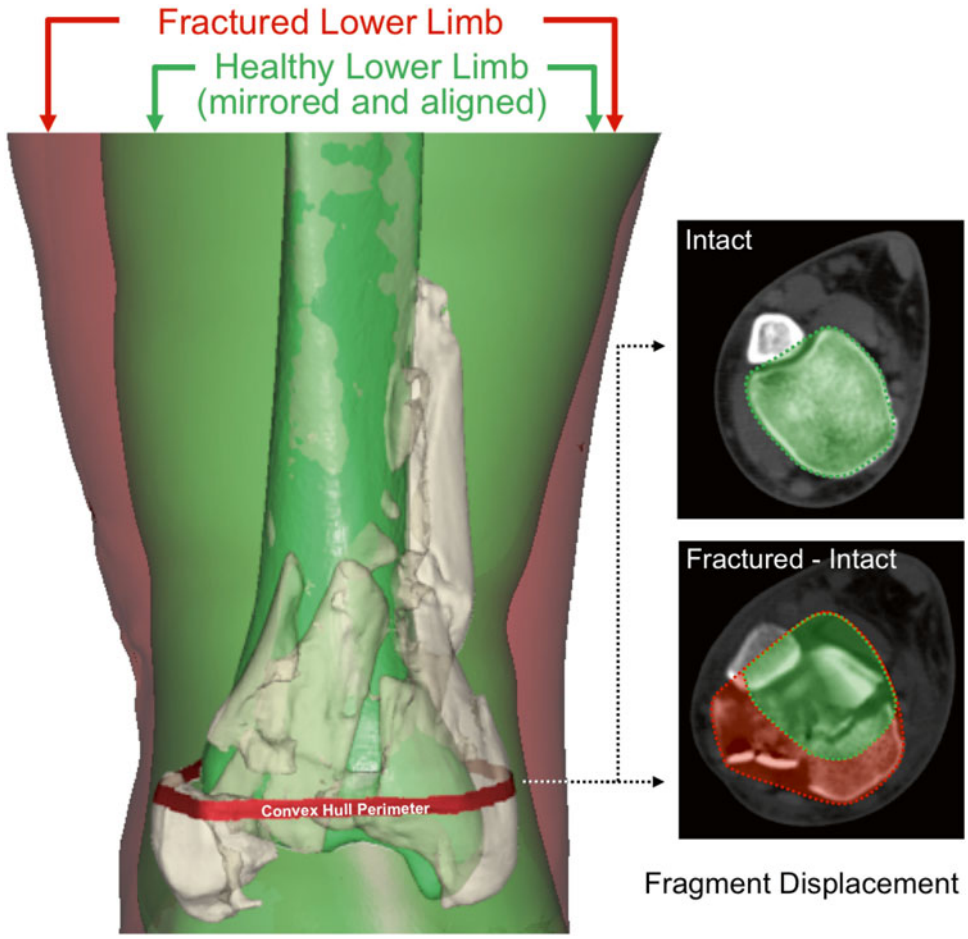

Fig. 24.3 Depiction of the fragment displacement/dispersion metric calculation [Taken with permission from Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. *Iowa Orthop J.* 31:1–20, 2011]

fracture cases would be ranked in the same order. Two ranked cases were concordant with each other when a case ranked higher by one rater also had a higher ranking for a second rater.

The range of different fractures encountered in the study is illustrated via plain radiographs in Fig. 24.1. This figure also illustrates the visual differences present in routine fracture radiographs that allow clinicians to reliably distinguish severity. Eight to ten hours was required to image process one CT dataset (Fig. 24.4), and provide the fracture energy data. Fracture energy ranged from 11 to 53 J, and fragment displacement volumes ranged from 3.4 to 47.4 cm^3, reflecting a wide range of fracture severity of these cases.

As expected, the three raters had high concordance with each other (Fig. 24.5) ranging from 87 to 91 %. The fracture energy metric had good concordance with the raters' ranks, ranging from 73 to 76 %, and concordance with the aggregate fragment displacement metric ranged from 82 to 89 %. The metric and clinician opinion are in high concordance with each other. This result provided initial validation of the image analysis approach to objectively measure fracture severity, but unlike the clinician ranks it provided a quantifiable metric of severity for each case.

To demonstrate that the CT fracture severity index correlates with meaningful outcomes and with PTOA development, a series of 36 tibial

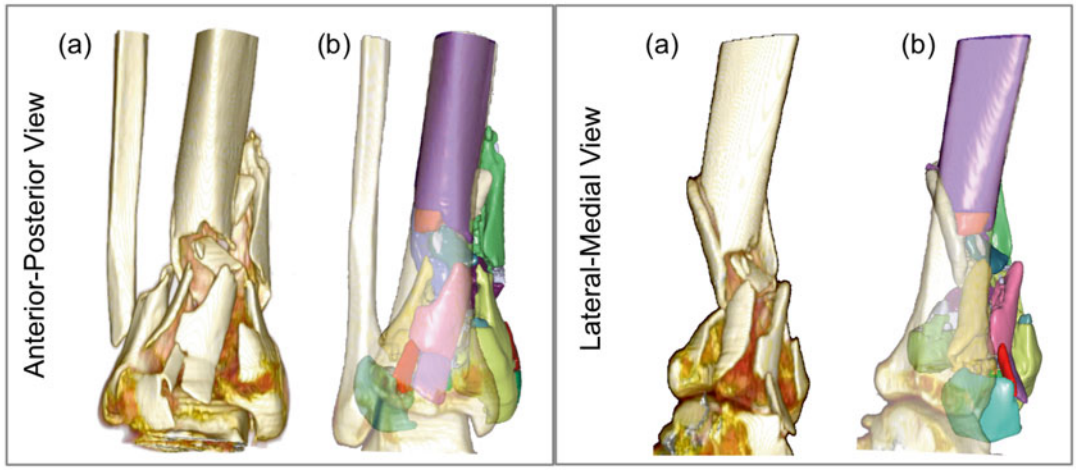

Fig. 24.4 (**a**) Standard, unsegmented rendering from radiology workstation: visually informative, but with no active functionality. Following segmentation, (**b**) individual fragments (49 of them in this case) may be readily, and independently studied (transparent surface is intact contralateral, mirrored and aligned proximally) [Taken with permission from Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. *Iowa Orthop J.* 31:1–20, 2011]

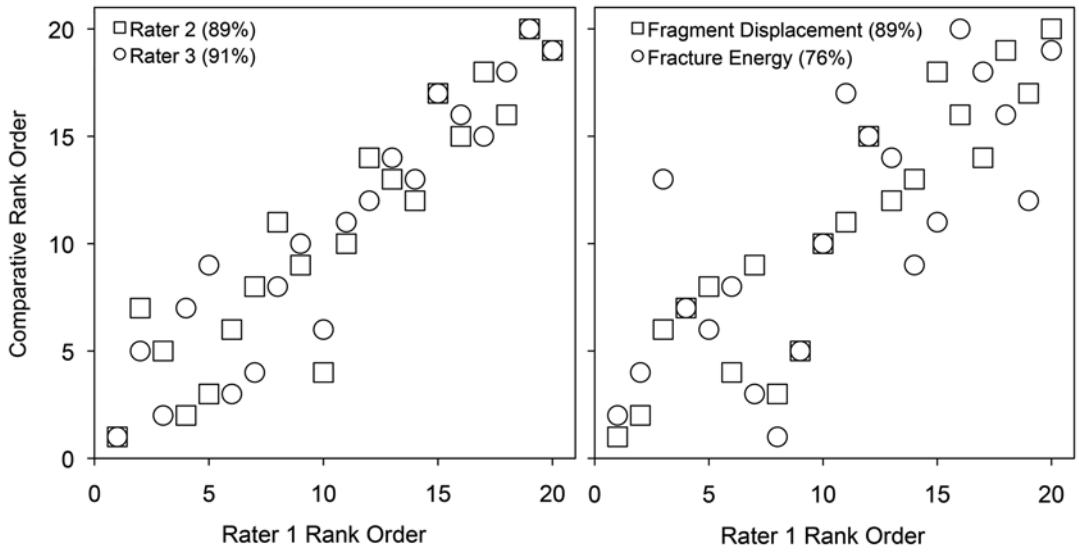

Fig. 24.5 Agreement between injury severity rankings and CT-based metrics. The graphs compare the rank ordering of rater 1 versus that of raters 2 and 3, and of the individual CT-based metrics. Concordance values are enclosed in *parentheses* following the rater/metric [Taken with permission from Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. *Iowa Orthop J.* 31:1–20, 2011]

plafond fracture patients who were uniformly treated were prospectively followed [8]. The goal was to assess if functional deficits, symptoms, and the degree of cartilage degeneration on radiographs in articular fracture patients correlate with the CT metrics of the acute mechanical insult. In addition, this study attempted to identify a threshold of acute injury severity that predicts the onset of PTOA.

At a minimum 2 year follow-up, Kellgren–Lawrence (KL) grades were assigned to the ankle radiographs. The Ankle Osteoarthritis Scale

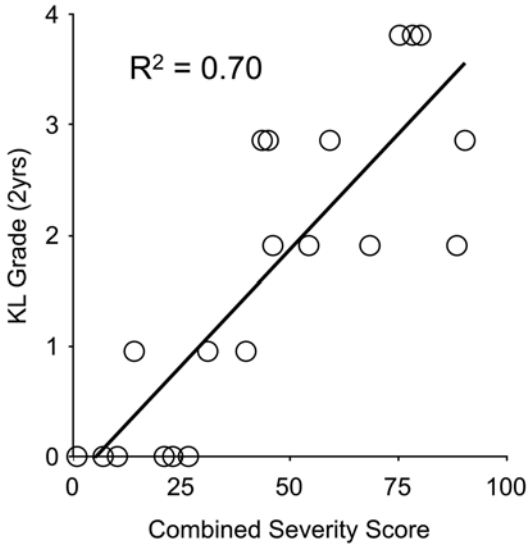

Fig. 24.6 A combined severity score including fracture energy and articular comminution predicted 70 % of the variation in KL arthrosis grade at 2-year follow-up [Taken with permission from Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. *Iowa Orthop J.* 31:1–20, 2011]

(AOS), was used to measure patient symptoms and disabilities related to ankle arthritis and to assess functional outcomes [39]. Relationships between the CT based fracture severity metric, PTOA severity, and AOS scores were determined by linear regression [8].

At follow up, 13 % of the patients had developed mild PTOA (KL=2), and 31 % had developed moderate to severe PTOA (KL≥3). Together, fracture energy and articular comminution explained 70 % of the variation in PTOA severity (Fig. 24.6). Fragment displacement/dispersal had less strong correlations with PTOA ($R^2=0.42$). A combined energy and comminution metric was developed that was a better predictor of PTOA than clinician assessment of the radiographs (0.70 vs. 0.47, respectively).

The clinical scores also correlated with both the KL scores of PTOA and with the combined fracture severity score. Patients with KL scores of ≤2 (no PTOA) had an average AOS score of 21.4 ± 20 and an average fracture severity score of 43.5 ± 11. Patients with a KL grade >2 (significant PTOA) averaged 40.8 ± 18 (greater score means greater disability and pain) for AOS and 69.8 ± 20 for fracture severity. These data suggest strong correlations between KL grade, fracture severity and AOS scores ($R^2=0.68$).

A threshold of injury severity that predicts whether a joint will develop PTOA has broad implications for the future treatment of intra-articular fractures. The data on the CT-based metrics suggests that such a threshold exists and is shown in Fig. 24.7. This illustration orders the cases by the combined acute fracture severity measure and demonstrates the cases with PTOA. These data support the existence of a severity threshold for the combined fracture severity metric, above which joint degeneration is likely. This is potentially very important information that allows fractures to be optimally targeted for early interventions based on the predicted likelihood of PTOA. These interventions could include traditional (accurate reductions in the borderline cases), new mechanical interventions (joint distraction in joints highly likely to develop PTOA) and/or biologic (new pharmaceuticals designed to enhance joint preservation). Prospective studies aimed at assessing new interventions to minimize PTOA would need to stratify patients for injury severity according to their otherwise-expected risk for joint degeneration.

It is interesting but not entirely surprising that fragment displacement/dispersal was not a significant predictor of PTOA. The high concordance between displacement/dispersal and clinician rank ordering suggests that the surgeons were influenced by the degree of fragment displacement in their judgments of overall injury severity. Surgeons may associate displaced fractures with increased soft tissue damage, and with difficulty accurately reducing fractures. These important factors for patient management were not highly predictive of PTOA in this patient series.

Expedited Techniques

Obtaining the results described above involved an analysis process that was deemed to take too long to be applied in the clinical setting. Eight to ten hours was required to image process one CT dataset, and provide the fracture energy data.

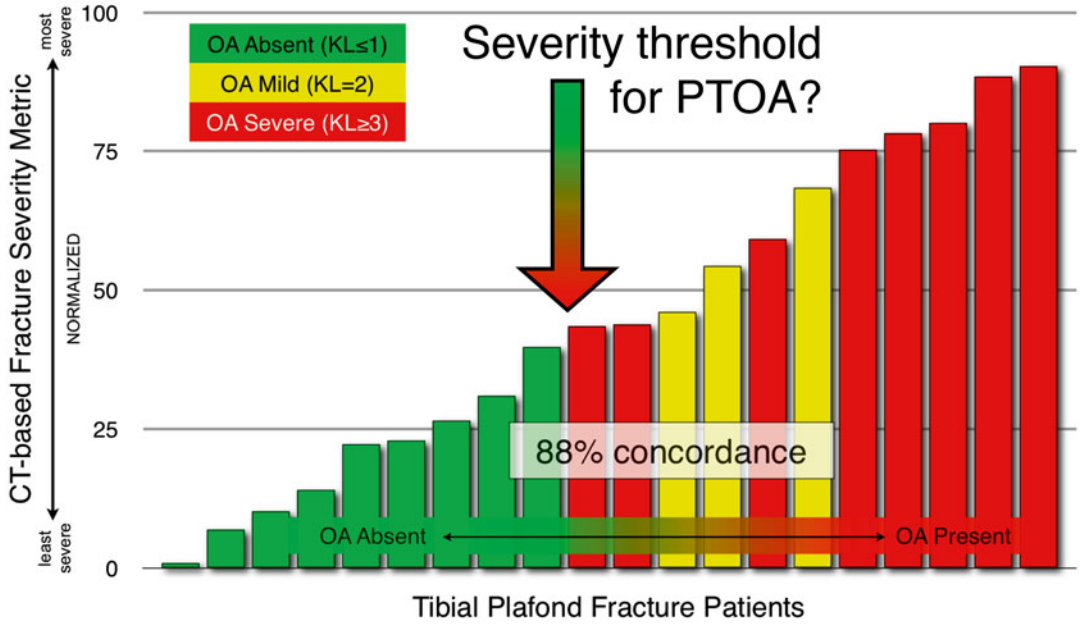

Fig. 24.7 The CT-based severity metric successfully discriminated between cases that developed PTOA and those that did not, in a threshold-like manner [Taken with permission from Anderson DD, Marsh JL, Brown TD. The pathomechanical etiology of post-traumatic osteoarthritis following intraarticular fractures. *Iowa Orthop J.* 31:1–20, 2011]

To deal with this problem, textural image analysis has been developed as a technique to provide an expedited assessment of fracture severity [40]. Textural image analysis quantifies "disorder" in a CT slice based on the gray level co-occurrence matrix (GLCM). The GLCM indexes the spatial homogeneity of pixel intensities (image texture).

Using this technique, fracture severity assessment was reduced from roughly 8–10 h to about 10 min, and excellent agreement (linear regression $R^2 = 0.80$) with the area-based energy metric was maintained. The expedited technique requires absolutely no human analyst intervention.

Another important step toward widespread use in orthopedic practice is to avoid the need to scan the intact contralateral limb. The intact limb is not routinely scanned, so this is an important obstacle to broad clinical use of these techniques. Even for clinical research relying upon the opposite limb CT anatomy is difficult if it is at variance with routine radiology protocols. To begin to solve this problem a study was designed to determine a normative anthropometric model of the intact distal tibia, from which to derive normative bone surface area data [41]. The goal was for an allometrically scaled tibia model to serve as a surrogate data set allowing accurate measurement of liberated interfragmentary surface area in a fractured limb. The free bone surface area of the intact distal tibia of 22 subjects was regressed from pre existing CT data of their uninjured limb. When the regression data were applied to actual distal tibia fracture cases, the concordance between fracture energy for the regressed versus true bone surface areas was 90 %. These data suggest that normative bone surface area can be substituted for measured intact-contralateral surface area, opening the door to eliminating an important obstacle toward wider applicability of these techniques.

Next Steps and Future Work

Currently this technique has only been validated in a clinical series involving small patient numbers from a single institution. A multi-institutional study focusing on distal tibia fractures is currently underway. This should lead to valuable additional information about the effect of quantified injury

severity on patient outcome and PTOA and hopefully result in more routine use of these metrics.

Further developmental work is necessary to achieve broader use of CT-based fracture energy measures. First the technique needs to be assessed in other articular fractures. The focus has been exclusively on distal tibia fractures, and although the CT-based technique can be easily adapted to other articular segments, the validating clinical work needs to be repeated. The necessity for a CT of the opposite limb will need to be circumvented for the technique to be widely applicable. The normative bone surface technique described above needs to be further validated. Finally the technique needs to be further automated and the mechanisms to make it easily available, not only for research teams but for routine care, need to be developed. We envision that a fracture energy calculation could be routinely provided as part of obtaining a CT scan of an articular fracture.

Objectively quantifying acute fracture severity holds promise to improve clinical research and set the stage for meaningful trials of new biologic agents to preserve articular surfaces. It also will improve patient care by guiding treatment and providing risk stratification and determining prognosis.

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Biomarkers of PTA

25

Yvonne M. Golightly, Samuel B. Adams Jr., and Virginia Byers Kraus

Abbreviations

ACL	Anterior cruciate ligament
DAMPs	Damage-associated molecular patterns
MRI	Magnetic resonance imaging
OA	Osteoarthritis
PTOA	Post-traumatic osteoarthritis

Introduction

Biomarkers of progression indicate how likely or how quickly a patient's disease will progress. A second type of prognostic biomarker predicts the likelihood of response to a treatment intervention; this is generally referred to as a predictive marker in guidance provided by the Food and Drug Administration (FDA) [1]. The availability of a prognostic biomarker for clinical research or clinical use would represent a major advance for patients with joint injury. Such use would require biomarker qualification, a process linking a biomarker with biology and clinical end points [2, 3].

Current data suggest that a large proportion (14–80 %) of severe joint injuries result in post-traumatic osteoarthritis (PTOA) [4–7]. In two meta-analyses from 2010 and 2011 [8, 9], the odds of knee PTOA were reported as 3.86 and 5.95 times greater for individuals with a history of knee injury compared to those without knee injury. The time to onset based on radiographic criteria can range from 10 to 20 years [4–6], but may be accelerated considerably in populations experiencing extreme injuries in combination with occupational stresses, such as the military. In combat-injured warriors, PTOA may develop in as little as 2–3 years after traumatic injury [10, 11]. This variability in time to PTOA and PTOA susceptibility represents a major obstacle to the future implementation of targeted therapies for PTOA. The identification of biomarkers to predict the likelihood of progression to disease would be of great importance. [12]. Qualification of a prognostic biomarker to predict the likelihood of radiographic PTOA in subsequent years or decades would require a large, long, and financially daunting prospective trial. Thus, a critical need of the field is to overcome this roadblock in biomarker qualification, which in turn would lead

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to the establishment of intermediate outcomes indicative of long-term PTOA status.

A conceptual framework for PTOA pathomechanics is instructive for providing insight into the origins of biomarkers during the course of PTOA (Fig. 25.1). Biomarkers may be generated at two key points: pre-injury and after injury.

Pre-injury biomarker generation. As illustrated in Fig. 25.1, biomarker generation may begin in the pre-injury phase among individuals who exhibit abnormal biomechanical movement patterns, leading to aberrant mechanical joint loading and biological changes. Cartilage degeneration may occur because of changes in loading patterns. A study from the U.S. Military Academy supports these pre-injury differences in serum biomarker levels [14]. Serum C-terminal propeptide of Type II collagen (CPII), Type I and II collagenase-generated cleavage epitope (C1,2C), and Type II collagenase-generated cleavage epitope (C2C) were compared pre- and post-injury in 45 anterior cruciate ligament (ACL) injured and 45 control participants who were matched on age, sex, height, and weight. Because these biomarkers in subjects of this age can be generated from open growth plate cartilage, it is particularly important, as done here, to match subjects in the injury and non-injury groups by age. Biomarker levels were expected to be comparable at baseline/pre-injury for ACL injured cases and controls, diverging at follow-up/post-injury. However, cases and controls differed not only at follow-up/post-injury but also at baseline/pre-injury, with biomarker levels of serum CPII and C1,2C tending to be higher among those participants who subsequently injured their ACLs compared to those without injury. These differences are consistent with the concept of a pre-injury biomarker risk profile that may be related to cartilage metabolism and ACL injury risk.

Post-injury biomarker generation. The link of injury and osteoarthritis (OA) is well-supported [13, 15–17]. The acute inflammatory response from the injury itself, along with the trauma associated with surgical repair, may contribute to biomarker generation, mainly biomarkers of inflammation, cartilage turnover, and joint metabolism [18]. After the initial injury or surgical repair, changes to the joint tissues, particularly in a weight-bearing joint, may result in a decline in the tissues' ability to manage dynamic forces, particularly with movements requiring rapid acceleration and deceleration, such as running and jumping. Alterations in movement and joint loading patterns may contribute to an uneven distribution of joint forces during daily activities or sport, with overloading of some cartilage regions and insufficient loading of others [13, 16, 17].

Herein, we summarize current efforts to validate and establish criteria for identifying the trajectory to PTOA early in its course; these would serve as endpoints for biomarker qualification. Secondly, we summarize preclinical and clinical studies that report biomarker data and in keeping with the theme of the book, discuss these from the perspective of the insights they can provide to disease pathogenesis, diagnosis and management. Finally, we discuss what biomarkers tell us about pathogenesis in PTOA compared to idiopathic OA.

Advances in Qualification Endpoints for PTOA Biomarkers

When using a biomarker as a substitute for a clinically meaningful endpoint, one must first be clear about the clinically meaningful endpoint for which the biomarker is a proposed surrogate [19]. The insensitivity of radiographic endpoints for OA has led to ongoing intensive efforts to evaluate other imaging and biochemical biomarkers for their ability to detect worsening of OA more quickly and meaningfully [20]. This same issue has plagued attempts to prevent PTOA. Namely, improvement of the long-term outcomes of joint injury have been hampered by the lack of qualification endpoints for early events that identify an adverse trajectory, i.e., one headed to PTOA 5–20 years later. Recently the Arthritis Foundation has launched an OA Flagship Initiative—the Anterior Cruciate Ligament (ACL) Intervention study (https://www.arthritis.org/research/funded-research/acl-feasibility-trial/) to test the feasibility of multi-site coordination of T1rho and other magnetic resonance imaging in the very acute and subacute setting of an ACL injury. This study could be pivotal to the establishment

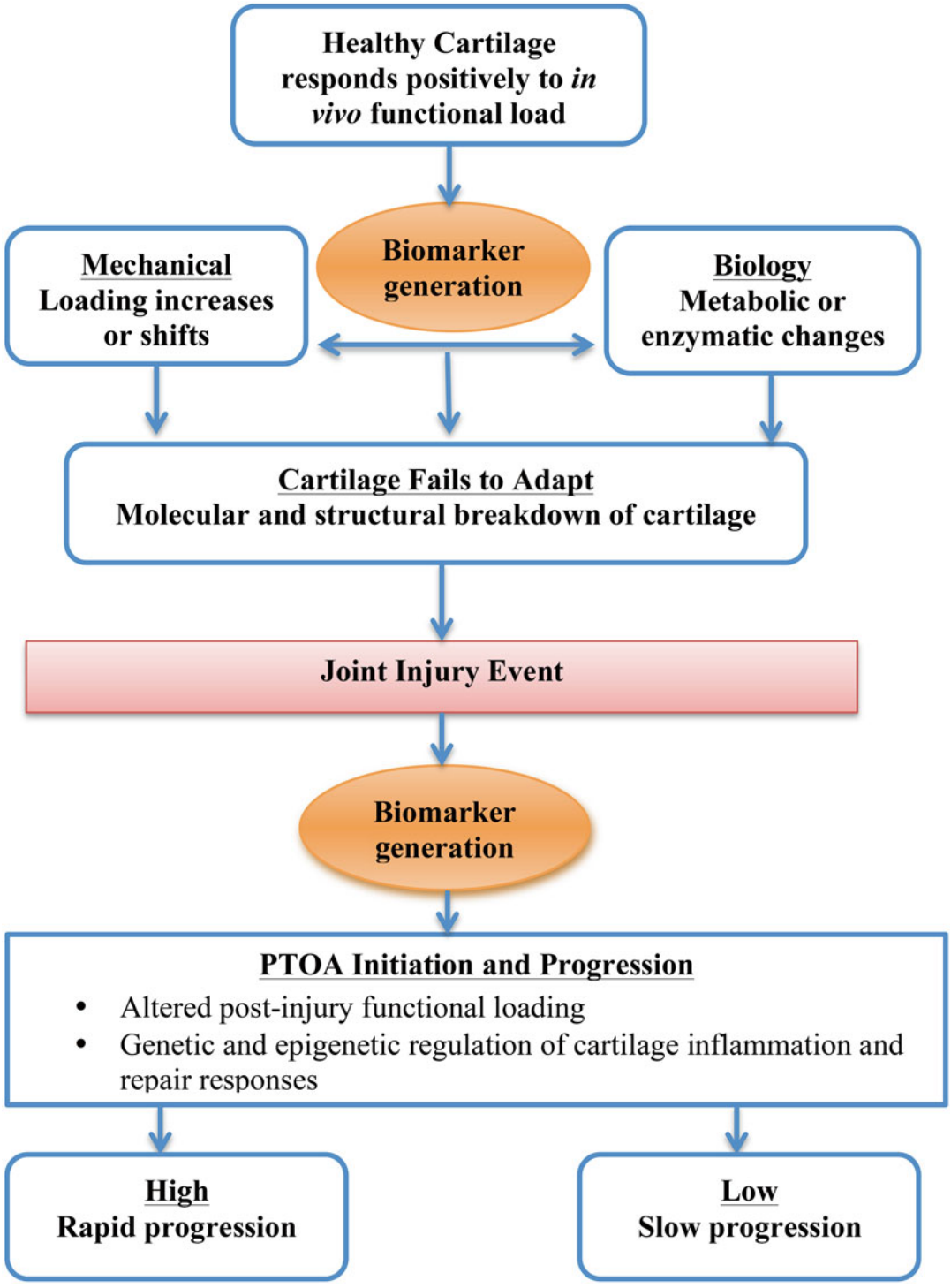

Fig. 25.1 Framework for PTOA Pathomechanics. Modified from: Andriacchi and Mundermann 2006 [13]

of a clinical trial paradigm for testing adjunctive early therapeutics, and monitoring with biomarkers from the onset of injury to improve and someday hopefully fully prevent PTOA.

The most useful biochemical biomarkers for diagnosing and monitoring susceptibility to PTOA are macromolecules originating from joint structures whose levels in serum, urine, and synovial fluid reflect processes taking place locally in the joint. The test of the ability of a biomarker to report on local events is best addressed by analyses of synovial fluid and matched serum; an optimal candidate biomarker is likely to have synovial fluid concentrations that are equal to or greater than serum concentrations and serum concentrations that correlate with synovial fluid concentrations. A few biomarkers to date seem to meet these criteria [21] but a great deal more work is needed to expand the armamentarium of systemic biomarkers that could be used to monitor joint health and metabolism pre- and post-injury to the joint.

PTOA Biomarkers in Preclinical Studies

Both in vitro and in vivo PTOA models are useful for defining biomarkers of early OA and for monitoring response to pharmacological and non-pharmacological (including surgery) therapy. In vitro loading of cartilage explants can provide useful insights into the biomarkers generated by injury and a system in which to test the efficacy of interventions designed to prevent the development of PTOA. In one study, the magnitudes of mechanical stress in a range of physiological to hyperphysiologic strains increased the release of cartilage oligomeric matrix protein (COMP) in proportion to the magnitude of dynamic mechanical stress, increased keratan sulfate, chondroitin sulfate and glycosaminoglycan (GAG) in a bimodal pattern, and decreased protein and proteoglycan synthesis at the highest level of stress [22]. In a recent study, a single impact led to detrimental effects on cell viability, and release of GAG and prostaglandin E2 to the media, which were primarily strain dependent [23].

Nearly all animal models of OA are PTOA models. Therefore, close attention to the results of biomarker analyses and interventions in these models could provide major insights for monitoring and prevention of PTOA in humans. A recent study in minipigs demonstrated that upregulation of genes coding for proteins capable of degrading cartilage extracellular matrix occurred within the first few days after anterior cruciate ligament injury; this response was in chondrocytes, cells in the synovium, ligament and scar tissue located between the torn ends of the ligament [24]. Matrix metalloproteinase (MMP)-1 gene expression was upregulated in the articular cartilage, synovium and ligament, MMP-13 expression was suppressed in the articular cartilage, but upregulated 100-fold in the synovium and ligament, and ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs 4) was upregulated in the synovium and ligament but not in the articular cartilage. They noted that the concentration of collagen degradation fragments (C2C) in the synovial joint fluid nearly doubled in the first 5 days after injury. In the superhealer MRL/MpJ mice compared with non-superhealer mice, protection from PTOA was associated with lower protein levels of IL-1alpha and IL-1beta in the synovial fluid, serum, and joint tissues; higher systemic levels of the anti-inflammatory cytokines IL-4 and IL-10 [25]; lower gene expression of tumor necrosis factor alpha, IL-1beta, macrophage inflammatory proteins and macrophage-derived chemokine (CCL22) in the synovial tissue; and reduced acute and late-stage infiltration of synovial macrophages [26]. The synovial fluid biomarker analyses in mice were made possible by the use of a novel synovial fluid recovery method suitable for very small joints [27]. These data show strong associations of joint tissue inflammation with the development and progression of PTOA in mice. Taken together, these results demonstrate a holistic response of the whole joint unit to injury. They also support the hypothesis that acute events, arising immediately at the time of injury, play a key role in susceptibility to PTOA in the long-term and may need to be appropriately neutralized to prevent PTOA.

A mouse fracture model supports the role of inflammation and cytokines in acute joint injury, showing that acute joint pathology and synovial inflammation are associated with increased intra-articular fracture severity in the mouse knee [28]. This model has provided further support for the hypothesis that immediate and early events after injury play a role in PTOA development [29, 30]; in this regard, a one-time intra-articular delivery of a small amount (0.9 mg) of IL-1Ra immediately after fracture, but not 4 weeks of continuous systemic delivery (1 mg/day), dramatically reduced cartilage degeneration and synovial inflammation [29]. Moreover, intra-articular delivery of purified mesenchymal stem cells in this model system prevented PTOA [31]. Interestingly, stem cell therapy improved OA scores without reducing the degree of synovial hyperplasia after fracture. Because the mesenchymal stem cells were capable of inhibiting the proliferation of in vitro stimulated splenocytes, the authors proposed their mode of action was immunomodulatory.

In an animal model, intra-articular IL-1 superimposed on previous joint injury caused a more rapid and more severe arthritis [32]. IL-1 is released as part of the acute inflammatory response following tissue damage, causing local increases of the proinflammatory cytokines IL-6, tumor necrosis factor alpha (TNF-α), and transforming growth factor beta (TGF-ß1) [33]. TGF-ß1 is a profibrotic cytokine that plays a key role in normal wound healing and in the development of progressive tissue fibrosis [34–36]. These cytokines contribute to inflammation and fibrogenesis by stimulating myofibroblasts, fibroblasts, and extracellular accumulations of collagen and fibronectin [37, 38]. Taken together, these data show that inflammation and biological factors play a key role in PTOA development following injury. A comprehensive understanding of these immediate and early molecular events and their timecourse will go far to establishing a molecular biomarker profiling that could be used to prognosticate, at particular times, the anticipated susceptibility to a PTOA trajectory. Moreover, the growing pharmacopeia of biological agents to inhibit these factors, including for instance the key OA-related cytokine IL-1, provides new avenues for prevention of PTOA.

PTOA Biomarkers in Clinical Studies

A cascade of biomarker changes occurs after injury. This timecourse of biomarker changes has been noted to recapitulate the degradation of matrix components observed in cartilage explants in vitro upon addition of proinflammatory cytokines [18]; namely, changes are preceded by proteoglycan loss followed by collagen degradation, considered an irreversible insult to joint integrity. These observations reveal that the onset of pathological cartilage catabolism is immediate, like a "heart attack of the joint". This timecourse of joint metabolic disturbances suggests, as mentioned above, that immediate action may be required to alter this course of deleterious events.

After knee injury, cartilage degradation is favored over repair, with increased collagen cleavage [39]. Within the first month after joint injury in humans, Lohmander has documented synovial fluid elevations of cartilage proteoglycan fragments and metalloproteinases [40], collagen fragments [41] and persistent elevations of these molecules *over decades* [42–46]. Lohmander 1993 [42] showed sustained, 20-year elevations following joint injury, of synovial fluid proteoglycan fragments, MMP-3 (Stromelysin-1), and the ratio of MMP-3/TIMP (tissue inhibitor of metalloproteinases-1). The sustained increased release of cartilage macromolecular fragments is thought to be responsible for the frequent development of PTOA in patients with injuries. Joint instability in addition to the constitutive and excess loss of cartilage macromolecules into synovial fluid after severe joint injury likely contribute to episodic clinical flares and inflammation that further contribute to OA progression [47]. Of note however, the timecourses of the intra-articular cytokine levels appear to vary widely in any given individual [48]; further study of inter-individual variations in response to injury will likely yield insights into risk profiles and mechanisms for effectively neutralizing the trajectory to PTOA.

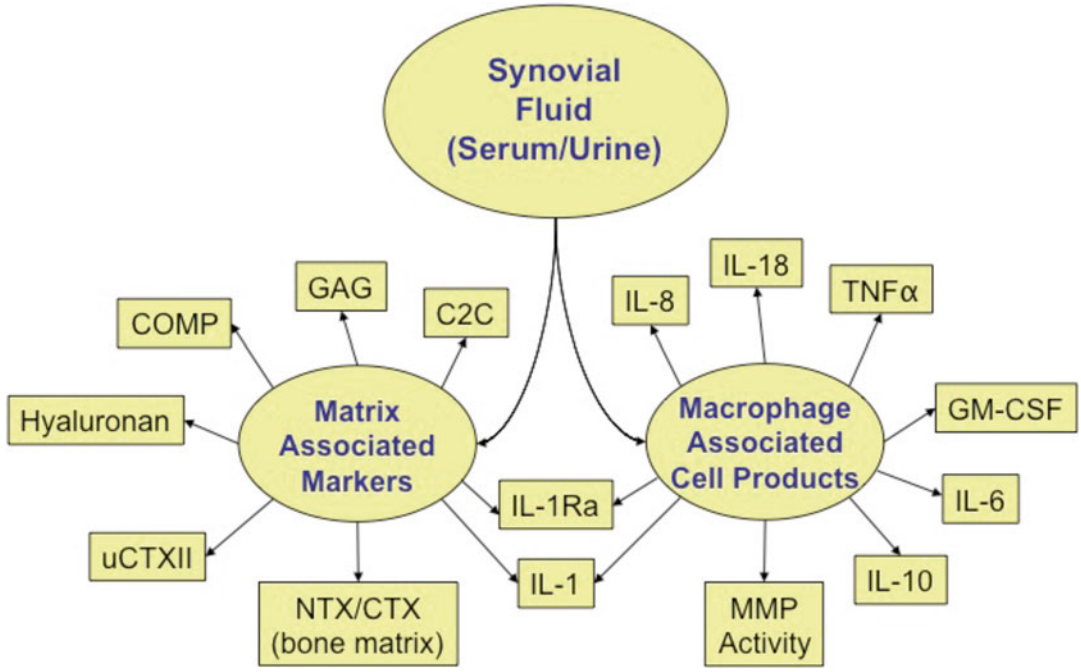

Fig. 25.2 Candidate biomarkers for injury studies. *C2C* type II collagenase-generated cleavage epitope, *COMP* cartilage oligomeric matrix protein, *GAG* glycosaminoglycan, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *IL* interleukin, *MMP* matrix metalloproteinase, *NTX/CTX* N-terminal telopeptide /C-terminal telopeptide, *TNF* tumor necrosis factor alpha, *uCTXII* urinary C-terminal telopeptide of type II collagen

Figure 25.2 provides a graphic of some potentially useful biomarkers for monitoring susceptibility to PTOA and the impact of interventions. These biomarkers include macrophage-associated cell products (IL-1α, IL-1β, IL-1Ra [receptor antagonist], IL-6, IL-8, IL-10, IL-18, granulocyte-macrophage colony-stimulating factor [GM-CSF], TNFα, and MMP activity); other relevant biomarkers include matrix-associated joint tissue markers: total GAG levels, type II collagen degradation (C2C, urinary C-terminal telopeptide of type II collagen [uCTXII]) and synthesis (CPII) epitopes, COMP, and hyaluronan, and bone related N-terminal telopeptide (NTX-I), to indicate the degree of cartilage degradation and bone turnover in the setting of acute injury [41, 49–56]. In addition, high sensitivity C-reactive protein can be monitored as a marker of inflammation. Total GAG levels correlate with severity of chondral damage in the setting of acute injury [57, 58]. GAG is also released from cartilage in the presence of inflammation induced by IL-1 injected into rabbit joints [59]. Therefore, total GAG is a useful measure to indicate degree of aggrecan breakdown. Another GAG epitope, the serum WF6 epitope, representing a specific pattern of sulfation in chondroitin 6-sulfate was higher in ACL injured compared to healthy controls [60]. In a placebo controlled dog OA model, C2C levels were elevated in the first 4 weeks after induced injury and remained elevated for 16 weeks, indicating increased cartilage turnover leading to onset of OA [54]. C2C can be detected in urine, serum, and synovial fluid and therefore serves as valuable potential marker determining the extent of cartilage loss [61]. Urinary CTXII has the most data supporting its use as a cartilage degradation marker [62]. Recent data suggest urinary CTXII may be most indicative of the turnover in the mineralized cartilage layer [63] and be prognostic for PTOA after ACL injury as decreasing concentrations were associated with decreasing knee pain and improving function [64]. Hyaluronan levels have been shown be predictive

of OA progression (summarized in [65] In our pilot trial of ACL injury in humans, synovial fluid IL-1α and serum HA decreased significantly in response to intra-articular IL-1Ra [66]. Other biomarkers are associated with acute joint injury, such as lubricin, stromal cell-derived factor (SDF-1), cartilage intermediate layer protein (CILP), and fibril-associated collagens with interrupted triple helices (FACIT) and fibrillar collagens), but it remains to be seen whether they are predictive of long-term structural damage [12].

Metabolic Profiling and Metabolites as Biomarkers

Metabolic profiling (metabolomics, metabonomics) is the qualitative and quantitative analysis of small molecules in a system under a given set of conditions (such as healthy or diseased joints). Metabolites are the end-products of cellular regulatory processes, and their levels can be regarded as a global assessment of a cellular state and the ultimate response of biological systems to environmental changes, such as those that might occur in PTOA (intra-articular environment after trauma), taking into account genetic regulation, altered kinetic activity of enzymes, and changes in metabolic reactions [67]. The science of metabolomics was developed decades ago to study inborn errors of metabolism, toxicology, and functional nutrigenomics [67]. More recently, metabolic profiling has been validated as a diagnostic tool and was used to discover citrate and choline as biomarkers for prostate and breast cancer, respectively. In fact, both tests are now covered by health insurance providers [68–70].

The last decade has seen an increase in the use of metabolic profiling as a predictive tool for OA. Lamers et al. [71] used nuclear magnetic resonance (NMR) to study urine from Hartley outbred guinea pigs that spontaneously develop OA. Lactic acid, malic acid, hypoxanthine and alanine were found to contribute heavily to the metabolic profile of OA. In a follow-up patient-based study [72], they demonstrated that the NMR spectra could discriminate between healthy and OA groups. Recently, Zhai et al. [73] employed metabolic profiling on human serum and demonstrated that ratios of valine and leucine to histidine were predictive of OA. While these studies measure metabolites in serum or urine, which are more reflective of systemic arthritis burden, PTOA is joint specific and may more accurately be characterized by a joint-specific profile, such as that found in the synovial fluid. Indeed, metabolic profiling has been performed on synovial fluid from experimentally induced OA in canine knee joints using NMR [74] and demonstrated a hypoxic and acidotic environment with OA that uses fat metabolism as an energy source.

Likewise, Adams et al. [75] performed metabolic profiling on the synovial fluid from patients with end-stage ankle PTOA. Synovial fluid from patients without ankle pathology was used as a healthy control. Metabolic profiling identified 182 metabolites across all synovial fluid samples. Of these, 106 (58 %) were found to be significantly elevated in the PTOA group and one was significantly higher in the control group (threonine). A random forest analysis was performed on the data to determine whether healthy and PTOA samples could be differentiated from one another based on their metabolic profile, and to determine which metabolites were most influential to differentiate between groups. Random forest analysis yielded a predictive accuracy of 90 % when using the metabolic profiles to distinguish between groups. Glutamate, which was >7-fold higher in the PTOA group, ranked number one overall in the random forest analysis and has previously been associated with arthritis [71, 72].

Additionally, significantly elevated levels of proline, *trans*-4-hydroxyproline, and the dipeptide prolyl-hydroxyproline were found in the PTOA group; these data support increased extracellular matrix turnover and collagen breakdown. Evidence of an increased oxidative environment in PTOA was provided by the significantly increased levels observed for oxidized glutathione (GSSH), cysteine disulfide, cystine, cysteine-glutathione disulfide, threonate, and alpha-tocopherol. An increased inflammatory environment in PTOA was evidenced by elevated levels of tryptophan, kynurenine

(the inflammatory cytokine-responsive metabolite of tryptophan) and the fibrinogen cleavage peptide DSGEGDFXAEGGGVR, which was significantly higher (>10-fold) in the PTOA group and was the second most important metabolite for differentiating between arthritis and control groups in the random forest analysis. Fibrinogen fragments have been shown to be elevated in the SF of inflamed joints [76], and the citrullinated DSGEGDFXAEGGGVcR peptide has recently been shown to be elevated in patients with rheumatoid arthritis [77]. The kynurenine pathway, which is responsive to immune and inflammatory stimulation, is altered in a variety of human disorders and diseases, including cancer, depression, dementia, and several other neurodegenerative and central nervous system disorders [78–80]. Elevated tryptophan metabolism and kynurenine levels have also been shown in primary synovial cell cultures in response to elevated IFN-γ, suggesting altered or increased tryptophan metabolism in response to inflammatory cytokines associated with arthritis [81]. Additional metabolites including lactate, malate, hypoxanthine, glycerol, isoleucine, hydroxybutyrate, and hydroxyisobutyrate were significantly elevated confirming results from previously reported metabolic studies of synovial fluid [71, 72]. Similar to OA, these results suggest an inflammatory, oxidative, hypoxic and acidotic intra-articular environment for PTOA. These data support the use of metabolites as biomarkers in PTOA.

PTOA Pathogenesis and Timecourse from the Standpoint of Biomarkers

Is PTOA the same as idiopathic OA only more dramatic and with a truncated timecourse? One study of a large cohort of retired National Football League (NFL) players [82] suggests that the two conditions are similar. The overall prevalence of arthritis (mostly OA [87 %]) was higher in retired NFL players ($N = 2{,}538$) than in the general United States male population. The retired NFL players likely had PTOA as a consequence of the high incidence of joint injury that occurs with football. Looking at the prevalence of arthritis by age group shows that differences between the retired NFL players and general male population were most apparent at younger ages, but the disparities attenuated with advancing age, and were not significant by age 65+ years. The remarkable similarities in arthritis prevalence in the older age groups of the two populations suggest that PTOA (retired NFL players) and idiopathic, age-related OA (general male population) are comparable conditions, with injury accelerating arthritis onset, but not increasing the occurrence of arthritis across the life course (Fig. 25.3) [82].

Conversely, in their 2013 review paper, Little and Hunter [83] stated that PTOA and idiopathic OA may differ by molecular pathophysiology. The authors pointed to the poor translation of preclinical animal studies to Phase II and III human clinical trials, suggesting that the lack of compatibility of the OA animal model (injury-induced in young animals) with human OA (older adults with spontaneous disease) may be a factor in the failure of an effective preclinical intervention in human clinical trials.

Maintenance of cartilage extracellular matrix and suppression of catabolism involves mechanosensing by the transient receptor potential vanilloid 4 (TRPV4), a Ca^{2+}-permeable osmomechano-TRP channel that is highly expressed in articular chondrocytes [84]. Cartilage wounding, as in injury, triggers a wound healing response [85, 86] that is also sensed mechanically although the precise mechanistic details have yet to be fully elucidated. This is made evident by the fact that no OA ensues if the wounded limb is immobilized [87]. The classic wound healing response includes an inflammatory phase, a proliferative phase and a wound remodelling phase with scar tissue formation [88]. Mechanosensing seems key to initiating the inflammatory phase of wound healing.

What do biomarkers tell us about disease pathogenesis in the context of joint injury? For one, PTOA, like OA, appears to involve the innate immune inflammatory response with damage-associated molecular patterns (DAMPs) involved in both [89, 90]. DAMPs are breakdown products of endogenous molecules, such as

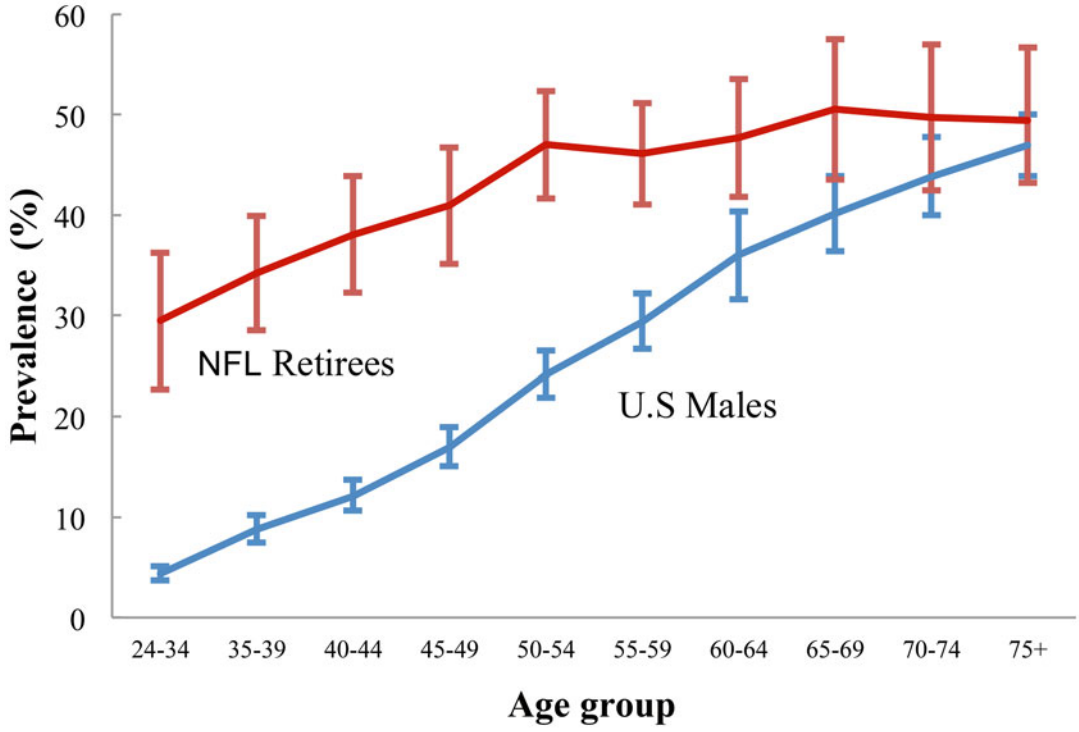

Fig. 25.3 Prevalence of arthritis in retired professional football players and general population of US males. Adapted, by permission, from Y.M. Golightly, S.W. Marshall, L.F. Callahan, and K. Guskiewicz, 2009, "Early-onset arthritis in retired National Football League players," *Journal of Physical Activity and Health* 6(5): 648–643 [82]

fibronectin [89] and hyaluronan [90], and molecules released by activated, stressed or dying cells, such as high-mobility group protein 1 (HMGB-1) [90]. DAMPs activate a primitive and powerful innate immune response [91] that accentuates the catabolic response to injury. The surface area of exposed cartilage matrix after fracture is thought to enhance the release of cell debris and inflammatory molecules from the cartilage into the surrounding area, thereby serving as a stimulus for acute and chronic inflammatory processes contributing to the likelihood of PTOA [92]. Therefore, another potentially fruitful area for future development of novel treatments for the prevention of PTOA would be the sequestration of DAMPs [92].

Biomarkers are generally considered indirect measures that may fail to provide reliable evidence about the benefit-to-risk profile of interventions [19]. This would generally be true for biomarkers that are not in the causal pathway of the disease process (indirect biomarkers). However, the mediators of the trajectory to PTOA after joint injury include DAMPs as described above. Many DAMPs can be measured as biomarkers and are directly in the causal pathway of disease (direct biomarkers) (Fig. 25.4). Thus, joint disease is one field in which the development of direct biomarkers appears eminently feasible.

Prospects for the Future

Unanswered questions remain. Can biomarkers identify the subset of individuals on a trajectory to PTOA? What role does intra-articular bleeding play in the eventual evolution to PTOA? Can biomarkers be used to monitor the efficacy of interventions for PTOA prevention? Current efforts are examining these questions, including an innovative study of intra-articular steroid treatment for acute ACL injuries.

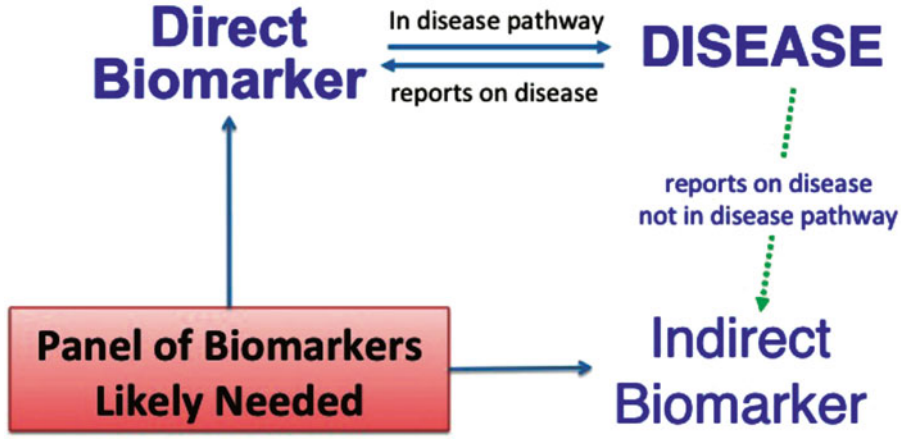

Fig. 25.4 Conceptual diagram of direct and indirect biomarkers of a disease process. Danger signals, in the form of disease associated molecular patterns (DAMPs) are generated in PTOA. These have the potential to report on the disease process as well as activate it through stimulation of pathways of the innate immune system. Although to date, no studies have reported genetic or epigenetic factors associated with susceptibility to PTOA [12], based on results from genetically engineered mice [83] it is likely that a number of genetic modifications provide protection against or worsen PTOA. For instance, the intensity of response of the innate immune system in an individual may in part be controlled by genetic determinants [93]. Indirect biomarkers may report on the disease process but may not be directly involved in disease pathogenesis. Given the complexity and multitude of joint tissues involved in development of PTOA, it is likely that a panel of several, rather than a single biomarker, will be required for adequately predicting susceptibility to PTOA in the pre-injury and post-injury periods

Summary

For a biomarker to have full utility, as summarized by Fleming et al. [19], there needs to be a strong correlation between the biomarker and the clinical efficacy measure, and the biomarker must fully capture the net effect of the intervention on the clinical efficacy measure. These requirements could be met by determining the following per Fleming et al. [19]: (1) the principal pathways through which the disease process affects how a patient feels, functions or survives; (2) the extent to which effects on the biomarker capture the meaningful 'on-target' effects of the intervention on those causal pathways of the disease process; and (3) any 'off-target' effects of the intervention that would meaningfully affect the clinical efficacy measures and yet would not be captured by the biomarker. These steps comprise the research agenda of the future for development of biomarkers to identify pre-injury and post-injury susceptibility to PTOA.

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26 Potential Targets for Pharmacologic Therapies for Prevention of PTA

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Joint Injuries and the Risk of Post-Traumatic Osteoarthritis (PTOA)

Joint injuries are progressive and debilitating, often life-changing events, that can result in osteoarthritis (OA). Epidemiologic studies reported that 13–18 % of patients that underwent total joint replacement had an identifiable acute trauma to the joint [1]. It has also been shown that early onset of OA can occur within 10 years after injury [2] indicating that the patients with PTOA are much younger (18–44 years) than those with idiopathic OA. Just in the USA, there are about 5.6 million people suffering from PTOA that translates in to $3.06 billion annual burden on the health system.

The key difference between primary or idiopathic OA and PTOA is the presence of precipitating insult to the joint in patients that suffer from PTOA, where the extent of cartilage damage depends on the intensity and force of the impact [3–5]. Regardless what causes joint injury PTOA develops as a result of poor intrinsic regenerative ability of hyaline articular cartilage [3, 6]. Biomechanically, these patients have a decreased tensile strength and compressive stiffness of their cartilage [5]. Furthermore, even if cartilage is spontaneously repaired it may be challenged with: (1) its inability to adapt to the stiff environment of the host adult cartilage; (2) changes of the intra-articular joint environment; (3) limited regional specialization; (4) lack of (or limited) proper structural organization impeding production of proper matrix proteins and their assembly; (5) altered metabolism of repaired cartilage; and (6) its inability to withstand the load and compression resulting in a higher susceptibility to reinjury.

Current biological surgical approaches treat the developed disease but fail to regenerate normal articular hyaline cartilage. The idea of biological interventions or pharmacological treatment is based on the premise of arresting and/or preventing the onset and progression of the disease. Ideally, biologic interventions should be applied immediately or soon after the trauma incident. Research on Early ARthritis THerapies (EARTH) has emphasized the need of studying PTOA to advance our understanding of and treatment options for all forms of osteoarthritis [7].

Understanding cellular responses to joint trauma and profiling of the released biomarkers will help develop biologic-based intervention strategies to halt the disease immediately or soon after injury [8].

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Phases of Immediate Cellular Responses as Potential Targets for Biologic Therapy

The literature on PTOA that includes in vivo, in vitro, and limited clinical studies consistently points to three overlapping phases of cellular and molecular responses that occur after acute cartilage or joint injury: an *Early Phase* characterized by cell death/apoptosis and inflammation; an *Intermediate Phase* with a temporary balance between subsiding catabolic and initial anabolic responses; and a *Late Stage*, characterized by prevailing anabolic/remodeling processes (in many cases with aberrant repair) that may also include episodes of catabolism (all reviewed and summarized in Anderson et al. [3]). All these events may help identifying *intervention strategies* that are based on specific molecular and metabolic pathways. The ideal therapy must probably be multi-varied and include anabolic and anti-catabolic approaches with the attraction of appropriate cells (whether stem cells or chondrocytes). This therapy should also be able to stimulate chondrocyte metabolism and intrinsic repair while protecting integrity of cell membrane and inhibiting catabolic pathways that lead to chondrocyte death and matrix loss. It may not be able to stand on its own, but should be able to improve the outcomes of surgical interventions. Based on today's knowledge, the following are the key mechanisms that need to be considered in the development of biologic intervention therapies: (1) Chondroprotection; (2) Matrix protection; (3) Anti-inflammatory and anti-catabolic; and (4) Pro-anabolic inducers of repair. Specific focus of this review is on biologics that are already approved for clinical use or in preclinical or clinical testing.

Chondroprotection

Cell death is the first response to injuries. There are two main mechanisms of cell death: necrosis, in which increased fluid uptake causes cell swelling and rupture resulting in the release of the intracellular components and activation of an inflammatory cascade; and apoptosis, in which chromatin condensation, DNA fragmentation, cell shrinkage, and membrane blebbing lead to self-destruction of the cell. Oxygen and reactive oxygen species (ROS), though important for cartilage homeostasis [9], in excess amounts induce chondrocyte death and matrix degradation. Mechanical injury has also been associated with an increase in production of ROS and decreased antioxidant capacity [10]. Together this suggests that chondroprotection can be achieved via targeting different mechanisms and pathways: preservation of cell membrane integrity, protection of mitochondria, antioxidant therapy, and inhibitory therapy against caspase signaling, inducible nitric oxide synthase, calcium quenching, and others.

Effect of Antioxidants on Chondrocytes Survival

Vitamin E, *N*-acetyl-L-cysteine (NAC), rotenone, and superoxide dismutase are among exogenous antioxidants that were used in experimental settings as chondroprotective agents. NAC can prevent apoptosis and promote cell survival by activating extracellular signal-regulated kinase pathway [10]. When combined with vitamin E [11, 12] the effect was dependent upon the experimental model, the type and degree of damage, the species, and the interval between the injury and drug administration. As a pretreatment, NAC was superior to vitamin E and increased chondrocyte survival by about 50–80 % [11]. However, pretreatment option is very unlikely in a real life scenario. Post-injury treatment with antioxidants seems more appropriate, especially if antioxidants are administrated intra-articular immediately or soon after the injury. Immediate treatment with NAC improved chondrocytes viability by up to 74 %, while a delayed treatment had a lesser, though still relatively high, effect (59 %). Vitamin E on its own was ineffective [10]. Superoxide dismutase was also shown to affect apoptosis in a dose-dependent manner [10]. These studies indicate that a window of opportunity for treatment does exist and mechanism-based timely delivery

Table 26.1 Chondroprotection with Antioxidants

Drugs	Mechanism of action	In vitro effect on chondrocytes	Pathology used for	Effects reported in clinical studies	Country performed at
Oral vitamin E	Antioxidant	Improved chondrocyte viability	Prevention of knee OA	No benefits [48, 49].	Australia
NAC	Antioxidant	Improved chondrocyte viability	No clinical studies reported		
Oral iNOS Inhibitor (Cindunistat, SD-6010, Pfizer)	Antioxidant	Improved chondrocyte viability	Knee OA	No clinic benefit on progression of OA [50].	Multicenter-multinational

OA osteoarthritis, *NAC* *N*-acetyl-L-cysteine

of biologics can provide necessary protection in post-traumatic degenerative events.

The beneficial effects of ROS scavenger NAC and superoxide dismutase on chondrocyte survival implicate chondrocyte death by apoptosis being secondary to the production of ROS, although the source of ROS excess remains unclear. An ability of superoxide dismutase to promote chondrocyte viability points to the role of mitochondria in cell survival, which was confirmed in studies with rotenone, an agent that suppresses the release of superoxide from the mitochondria and thus prevents cell death [13]. Though it is unlikely that rotenone itself might be a good candidate for clinical use due to its high cellular toxicity, this study identified an important mechanism that should be further explored for the development of targeted therapy.

Nitric oxide (NO), as reactive nitrogen species, and superoxide anion, as reactive oxygen species, are among main catabolic factors produced by the chondrocyte [14]. Both agents have been upregulated after trauma and contributed to cartilage degradation. This suggests a potential role for inhibitors of NO synthase (iNOS) in matrix protection, which was documented by in vitro and in vivo studies [11]. Pretreatment of human cartilage explants with nitric oxide synthase inhibitor *N*-Nitro-L-arginine methyl ester (L-NAME) resulted in significant increase in chondrocyte survival and reduction in apoptosis via interference with the IL-1β signaling pathway [6]. In an in vivo canine OA model, intra-articular injection of another iNOS inhibitor, *N-iminoethyl-l*-Lysine (L-NIL), decreased chondrocyte apoptosis and degenerative OA changes in comparison to the untreated control [6, 15]. In addition, a reduced level of caspase 3 and matrix metalloproteinase (MMP) activity was found in the L-NIL treated dogs suggesting that iNOS inhibitors reduce the progression of PTOA through the caspase 3 mediated inhibition of apoptosis that also results in the diminished MMP activity [6, 15]. All current clinical trials that use antioxidants are summarized in Table 26.1.

Inhibition of Caspases/Apoptosis to Promote Chondrocytes Survival in PTOA

Apoptosis is one of the main causes of chondrocyte death after mechanical injury [16–18]. It is mediated by cysteinyl aspartate-specific proteases called caspases and their inhibitors have been shown to reduce the level of apoptosis and the severity of cartilage lesion in vivo and in vitro. Intra-articular injections of the pan caspase inhibitor ZVAD-FMK (benzyloxycarbonyl-Val-Ala-Asp(OMe) fluoromethylketone reduced cartilage degradation via the inhibition of caspase 3 activity and p85 fragment and prevented the development of cartilage lesions in the Anterior Cruciate Ligament (ACL) PTOA model [18, 19]. In vitro, a protective effect of Z-VAD-FMK was demonstrated on cartilage from various species (bovine, rabbit, equine, and human) subjected to a single impact, static compression or blunt trauma [17]. However, in our studies on human cartilage acute injury model the effect of caspase inhibitors (inhibitors of caspase 3 and 9 or pan-caspase inhibitors [Z-VAD-FMK or Q-VD-OPh])

Table 26.2 Chondroprotection with anti-apoptotic drugs

Drugs	Mechanism of action	In vitro effect on chondrocytes	Pathology used for	Effects reported in clinical studies	Country performed at
Z-VAD-fmk	Anti-apoptotic	Increased viability	No clinical studies reported		
Caspase inhibitors	Anti-apoptotic	Increased viability	No clinical studies reported		

[20], was not as pronounced as in studies by D'Lima et al., [18] which used a lower peak stress during impaction (25 MPa vs. 14 MPa). Yet in both studies the cells that survived impaction showed elevated proteoglycan (PG) synthesis after the treatment with caspase inhibitors resulting in better matrix preservation (low Mankin score) especially in the areas adjacent to the impact. Both pan-caspase inhibitors tested in our laboratory demonstrated similar efficacy. Despite a wide range of effects, evidence suggests that caspase inhibitors could be and should be considered for targeted therapeutic intervention in PTOA, especially if they are used immediately or soon after joint injury before the fully blown apoptotic cascade takes place. Yet no clinical investigation has been made for caspase inhibitors for the treatment of PTOA (Table 26.2).

Cell Membrane Integrity and Its Role in Chondrocyte Survival

The integrity of cellular membrane is critical in preventing the development of PTOA. Its disruption by injury alters the capacity of the cells to maintain normal homeostasis leading to cell necrosis followed by the leakage of the intracellular components with subsequent catabolic activation [21]. For instance, altered intracellular calcium homeostasis has been implicated as an upstream event in progressive chondrocyte death after mechanical injury [22]. A reduction in extracellular calcium (by chelating calcium from the culture media using EGTA) has shown to decrease chondrocyte death following single impacted load, possibly through the prevention of an increase in cytoplasmic calcium [22].

Surfactants have hydrophilic and hydrophobic centers similar to the lipid bilayer composition of the membrane. Therefore, they can fill the holes formed as a result of the membrane disruption and thus promote membrane healing and prevent cell death. A number of laboratories (including ours) focused on the use of poloxamer 188 (P188) to prevent chondrocyte death in various in vitro and in vivo PTOA models. [10, 20, 23–25]. Initially, it was shown that P188 can significantly reduce the level of apoptosis in bovine chondrocytes in the ex vivo blunt impact model [25]. Then, the same effect was documented with early P188 administration in the in vivo rabbit model [24], where P188 was effective in a short- and long-term follow-up in preventing DNA fragmentation of injured chondrocytes. This study implied that P188 acutely repaired damaged plasma membrane, which precluded further degradation of traumatized chondrocytes. Contrary, in a similar study by Martin et al. [10] P188 was shown to be ineffective. One of the major limitations of early reports on P188 is that they focused only on chondrocyte survival without looking at its overall effect on cartilage metabolism and matrix integrity.

Our laboratory chose a different approach and investigated the mechanism of action of P188 in addition to its documented effect on cell survival and metabolism. We demonstrated that P188 was superior to caspase inhibitors 3 and 9 in promoting cell survival after acute injury [20]. We also found that a single treatment with P188 added immediately after injury was able to inhibit cell death by necrosis and apoptosis and, more importantly, was able to prevent horizontal and longitudinal spread of cell death to the areas that were not directly affected by the impaction. Though P188 was present in the explant culture only for the first 48 h, the effect was sustainable for 7 out of 14 days of the experiment. Furthermore, we identified the mechanisms through which P188 exhibited its effects [23]. P188 surfactant directly or indirectly inhibited phosphorylation of the key

Table 26.3 Chondroprotection with surfactants

Drugs	Mechanism of action	In vitro effects on chondrocytes	Pathology used for	Effects reported in clinical studies	Country performed at
P-188	Prevents membrane disruption	Prevents necrosis	No clinical studies reported		

mediators of the IL-6 signaling pathway: Stat1, Stat3, and p38. In addition, it also inhibited phosphorylation of another kinase involved in apoptosis, glycogen synthase kinase 3. Our biochemical and histological data suggested that p38 kinase may act upstream of Stats signaling and that activation of p38 kinase as result of injury may be partially responsible for initiation of IL-6/Stats mediated catabolism. The role of p38 was confirmed using specific p38 inhibitor, which not only inhibited IL-6 signaling but also reduced apoptosis. Interestingly, pretreatment with P188 or its multiple applications post injury were not superior to a single initial treatment suggesting that the protection of damaged cell membrane remains its primary function through which P188 prevents trauma-induced cell death. Together, data presented in this part of the review suggest that chondroprotective therapy should be considered as the first and the earliest step in biologic approaches to PTOA regardless which mechanism of cell death is targeted. When chondrocyte death is arrested or prevented there are more chances to trigger anti-catabolic and pro-anabolic responses in the remaining viable cells. Yet no clinical investigation has been made for P-188 for the treatment of PTOA (Table 26.3).

Inhibition of Proinflammatory Mediators or Anti-catabolic Therapy

Synovial inflammation has been observed at early stages of PTOA, especially after joint injury. Innate immunity has been implicated as an active player in the development of synovitis and activation of downstream inflammatory and catabolic events in articular cartilage and other tissues of the joint that may lead to PTOA onset and progression. It is unclear whether morphological changes are primarily due to whole joint trauma, or in less severe cases to a systemic immune response or occur secondarily to menisci tear, rupture of ACL followed by subsequent cartilage degeneration and subchondral bone lesions. Soluble inflammatory mediators are detected in synovial fluid of patients with OA and PTOA, including a variety of cytokines and chemokines such as IL-1β, TNF-α, IL-6, IL-8, and IL 10. The innate immune system plays an essential role in modulating multiple forms of tissue injury and repair. The role of innate immune players, including pattern recognition receptors (PPRs) and damage-associated molecular patterns (DAMPs) is still to be understood in the progression and development of PTOA.

Anti-catabolic therapy has been primarily tested for degenerative OA. Among anti-catabolic agents currently approved for clinical use (summarized in Table 26.4) are antioxidant NAC (described in detail above), interleukin-1 (IL) receptor antagonist (IL1-Ra), and TNF-α antagonist. IL-1 and TNF-α are the most studied cytokines in OA [26]. Both are potent activators of cartilage degradation and their activity and concentrations have been significantly increased after acute injury in correlation with the disease severity [27]. In addition, many other cytokines, including IL-6, IL-8, and IL-10, are elevated early after injury and play a role in cartilage loss and progression of PTOA [3, 20] justifying anti-catabolic therapy as a potential way to counteract PTOA.

IL1-Ra has been studied as the protein or gene in both in vitro and in vivo models. In the OA equine in vivo model IL1-Ra was injected as adenoviral gene construct intra-articularly [28] and showed marked clinical improvement in treated horses characterized by significant reduction in subchondral edema, joint fibrillation, and chondrocyte necrosis. Autologous conditioned serum enriched in endogenous IL1-Ra has been developed under the name "Orthokine" [29] and initial data suggested that its intra-articular injections reduce pain and increase joint function [29].

Table 26.4 Chondroprotection with proinflammatory inhibitors

Drugs	Mechanism of action	In vitro effects on chondrocytes	Pathology used for	Effects reported in clinical studies	Country
Intrarticular Anakinra (Kineret®)	Antagonist of IL-1 receptor	Prevents OA degradation	Acute ACL tears	Patients reported improved KOOS. Synovial inflammation biomarkers were lowered compared to placebo [51].	USA
Intrarticular Orthokine (Orthogen, Dusselford, Germany)	Autologous antagonist of IL-1 receptor	Prevents OA degradation	Knee OA	Patients randomized to Orthokine improved in KOOS symptoms compared to placebo [52].	Netherlands
Intrarticular Anakinra (Kineret, CA)	Antagonist of IL-1 receptor	Prevents OA degradation	Persistent knee effusion after arthroscopy knee surgery	Patients reported reduced effusion, improved ROM (reduced Knee Effusion) [53].	USA
Intrarticular Anakinra (Kineret, CA)	Antagonist of IL-1 receptor	Prevents OA degradation	Arthrofibrosis	All patients reported increased ROM [54]	USA
Subcutaneous Adalimumab (Abbott Lab, Illinois)	TNF receptor blocker	Prevents OA	IP OA of the hand	Less progression of radiological joint erosion compared to placebo [55].	Belgium
DLX 105 (ESBATech AG)	TNF receptor blocker	Prevents OA	Knee OA	RCT on knee OA. Study completed. Still waiting for results to be published.	NCT00819572 @clinicaltrials.gov Multinational (Germany/Switzerland)
SAR 113945 (Sanofi)	Inhibitor of NFKB receptor	Reduces MMP13	RCT on knee OA	RCT on knee OA. Study completed. Still waiting for results to be published.	NCT01113333 (@clinicaltrials.gov) Germany

Anakinra IL-1 receptor antagonist, *RCT* randomized controlled study, *ROM* range of motion, *IP* interphalangeal finger joints

In our laboratory using ex vivo acute trauma model on human cartilage explants IL1-Ra has been tested in two doses, low (20 ng/ml) and high (100 ng/ml). While low dose was ineffective, high dose promoted cell survival. Surprisingly, although low dose of IL1-Ra was not able to reduce chondrocyte death, it was able to increase PG synthesis by remaining viable cells. An overall effect of IL1-Ra was not sustainable and was lost soon after the agent was removed from culture.

TNFα is a second cytokine strongly associated with cartilage loss in OA and PTOA. We found TNF-α being elevated immediately after injury in the acute trauma model. Antagonist of TNF-α, PEGylated soluble TNF-α receptor I, alone and/or in combination, downregulated MMP-1, MMP-3, and MMP-13 expression and promoted cartilage preservation by reducing the release of PGs and increasing production of lubricin in the rat model of PTOA [30]. Collectively, the literature available on pro-inflammatory cytokines suggests that the inhibition of IL-1 and/or TNF-α, and perhaps IL-6 family of chemokines, may offer a useful therapeutic approach for the management of PTOA. We do think though that anti-inflammatory therapy might be secondary to chondrocyte protection in preventing PTOA, but is critical in reducing the disease progression. It is also important to recognize that acute inflammation may be necessary to trigger cellular and matrix remodeling responses, while chronic inflammation may be associated with the progression and manifestation of PTOA. Table 26.4 summarizes current clinical therapies with inhibitors of proinflammatory mediators.

Agents to Protect Cartilage Matrix

Degradation of cartilage matrix constituents occurs directly due to proteolytic enzymes of various families: Matrix Metalloproteinases (MMPs), A Disintegrin and Metalloproteinases (ADAMs), ADAMs with Trombospondin Motif (ADAM-TSs), cathepsins, and others. Therefore, to protect the matrix, two general approaches can be considered: inhibition of matrix-degrading proteinases with inhibitors of specific or general mode of action or by affecting factors responsible for their activation, such as ROS, NO, inflammatory cytokines, and matrix fragments. Inhibition of ROS and inflammatory cytokines has been already discussed above.

NO has been long implicated in cartilage degradation and patients with OA show elevated levels of nitrites in their biological fluids [31]. The increased NO production has been reported to inhibit aggrecan and total PG synthesis [31] and increase MMP and iNOS activity. The use of the iNOS inhibitor L-NIL has slowed the progression of PTOA in canine experimental OA model suggesting that iNOS can be a good target for matrix protection in PTOA [32].

Specific inhibitors of MMPs have been on the wish list as the disease modifying OA drugs for a long time, yet selective inhibitors are not widely available as of to date. Therefore, the number of studies that address their utility in PTOA is very limited and the majority of them focus on the inhibition of either MMP-13 or aggrecanases. To compensate for the lack of effective synthetic inhibitors often transgenic modifications are used to prove the importance of the inhibition of specific proteinases in preventing disease development. For instance, Little et al. [33] using MMP-13 knockout mice demonstrated cartilage protection in surgically induced OA model in the absence of MMP-13 gene. This was similar to the results obtained with an oral administration of the synthetic MMP-13 inhibitor in a rabbit PTOA model [34]. Inhibition of aggrecanases or ADAMTSs also received attention in experimental OA studies, especially after ADAMTS5 knockout mice have shown not to develop OA. Therefore, inhibitors of aggrecanases and cartilage specific MMPs with high specificity and low toxicity are clearly among future therapeutic agents for the treatment of PTOA.

Growth Factors and Matrix Remodeling in PTOA

One of the most developed directions in biologic approaches to PTOA is the use of growth factors to stimulate production of cartilage matrix and induce pro-anabolic responses. Amongst the mainly studied in vitro and in vivo growth factors

are the members of the Transforming Growth Factor-β (TGF-β) superfamily, especially bone morphogenetic proteins (BMPs), Fibroblast Growth Factors (FGF)-2 and 18, and Insulin-Like Growth Factor-1 (IGF-1) [35]. BMP-2 and BMP-7 appear to be extremely potent in cartilage and bone repair. Furthermore, Tissue Gen. Inc has recently developed TG-C (cartilage), which consists of allogeneic chondrocytes cells that have been genetically modified to produce the therapeutic growth factor (TGB1). At the moment there is a Phase II study in the USA being conducted for the treatment of knee OA with the use of this product (@clinical trials.gov/NCT01221441). BMP-7, also known as osteogenic protein-1 (OP-1), has been studied most extensively in vitro in our laboratory using human cartilage (reviewed in Chubinskaya et al.) [36, 37] as well as in OA and PTOA animal models [37, 38]. The results suggest that for adult articular cartilage BMP-7 may be the best candidate so far as a disease-modifying OA and even PTOA drug due to its pro-anabolic and anti-catabolic properties. Unlike TGF-β and other BMPs, BMP-7 upregulates chondrocyte metabolism and protein synthesis without creating uncontrolled cell proliferation and formation of osteophytes. BMP-7 prevents chondrocyte catabolism induced by pro-inflammatory cytokines or fragments of cartilage matrix components. It can induce synergistic anabolic responses with other growth factors, IGF-1, in normal and OA, young and old chondrocytes. It also regulates production of other growth factors (stimulates IGF-1 expression and inhibits BMP-2 expression) and their signaling pathways [39, 40]. In terms of IGF-1, BMP-7 restores the responsiveness of human chondrocytes to IGF-1 lost with ageing through the regulation of IGF-1, its receptor IGF-R1, binding proteins and downstream signaling mediators [36]. BMP-7 has been also extensively studied in various PTOA animal models in dogs, sheep, goats, and rabbits. In all these PTOA models (ACL transaction, osteochondral defect, and impaction), BMP-7 regenerated articular cartilage, increased repair tissue formation and improved integrative repair between new cartilage and the surrounding articular surface. In the impaction model [41], a window of opportunity for BMP-7 treatment has been identified. BMP-7 was most effective in arresting progression of cartilage degeneration if administered twice at weekly intervals either immediately after trauma or delayed by 3 weeks. If delayed by 3 months, the treatment was ineffective, suggesting that the development and progression of PTOA could be arrested and maybe even prevented if the right treatment is administered at the right time. Phase I OA clinical study (Table 26.5) produced very encouraging results by showing tolerability to the treatment, absence of toxic response, and a greater symptomatic improvement in patients that received a single injection of BMP-7.[42]

Members of the FGF family, FGF-2 and 18, have been also tested as potential disease modifying drugs. There is no consensus on the role of FGF-2 in cartilage homeostasis and responses greatly depend on the cell type, species and experimental model. FGF-2 can stimulate cartilage reparative responses, but its potent mitogenic effects may lead to chondrocyte cluster formation resulting in poor extracellular matrix organization due to a relatively low level of type II collagen [43]. Another member of the same family, FGF-18, appears to be a more attractive choice as pro-anabolic agent in PTOA [40, 44]. It has been shown to induce anabolic effects in chondrocytes and chondroprogenitor cells and to stimulate cell proliferation and type II collagen production [45]. At this point only two growth factors, FGF-18 and BMP-7 (Table 26.5), have been tested for cartilage repair in phase I clinical studies in patients with established OA. A clinical trial with FGF-18 on patients with PTOA is completed; however, results are not available as of yet. In considering growth factor therapy, there are a number of issues that need to be taken into account: choice of the growth factor, its formulation and dose, carriers and scaffolds, delivery methods (local via injections vs systemic vs gene delivery), time of intervention, and of course, possible adverse effects. Another important issue is that growth factors are expressed endogenously and production of many of them is elevated in response to injury. Therefore, their autocrine levels have to be considered in determining the dose and timing of growth factors administration. Table 26.5 summarizes current clinical therapies with growth factors.

Table 26.5 Chondroprotection with growth factors

Drugs	Mechanism of action	In vitro effects on chondrocytes	Pathology used for	Effects reported in clinical studies	Country
Intrarticular BMP-7 (Stryker)	Anabolic	Upregulates chondrocyte metabolism	OA of the knee	Patients improved WOMAC score compared to placebo [56].	USA
Intrarticular FGF-18 (Merck)	Anabolic	Upregulates chondrocyte metabolism	OA of the Knee	RCT on knee OA. Study completed. Still waiting for results to be published.	NCT00911469 @clinicaltrials.gov Sweden
Intrarticular APS (Biomet)	Anabolic-anticatabolic	Reduces MMP	OA of the knee	RCT on knee OA. Study recruiting patients.	NCT01773226 @clinicaltrials.gov Netherlands
Intrarticular PRP	Anabolic	Increase chondrocyte proliferation, proteoglycan and type II collagen deposition	OA of the knee	Patients improved knee and pain and function [57]. Other study reported similar results for Hip OA [57, 58].	Czech Republic (Knee study) Spain (Hip Study)
Intrarticular EMD (SERONO)	Anabolic	Increase chondrocytes proliferation and differentiation	OA of the Knee	RCT on Knee OA. Study completed. Still waiting for results to be published.	NCT: NCT01033994 @clinicaltrials.gov Canada
Intrarticular rhFGF-18 (Merck)	Anabolic	Upregulates chondrocyte metabolism	OA of the knee	RCT on Knee OA. Study completed. Still waiting for results to be published.	NCT: NCT00911469 @clinicaltrials.gov Bulgaria
Intrarticular rhFGF-18 (Merck)	Anabolic	Upregulates chondrocyte metabolism	Isolated chondral lesions of the knee	RCT on Isolated chondral lesions of the knee. Recruiting patients.	NCT: NCT01066871 @clnicaltrials.gov Switzerland

APS autologous protein solution, *EMD* enamel matrix derivatives

Platelet-Rich Plasma as Another Source of Growth Factors

The therapeutic use of autologous platelet-rich plasma (PRP) constitutes a relatively new biotechnology that has been a breakthrough in the stimulation and acceleration of soft-tissue, bone, and cartilage healing [46]. The efficiency of this process lies in the local and continuous delivery of a wide range of growth factors and proteins, mimicking the needs of the physiological wound healing and reparative tissue processes. In this process a preparation rich in growth factors (PRGF) combines the advantage of an autologous fibrin clot that will aid in hemostasis as well as provide growth factors in high concentrations to the site of a tissue defect. The source of the new PRP preparation consists of a limited volume of plasma enriched in platelets obtained from the patient. Once the platelet concentrate is activated a myriad of growth factors and proteins are released, progressively, into the local environment. The application of PRP in cartilage repair is relatively new. Chondrocytes and Mesenchymal Stem Cells exposed to PRP both have increased cell proliferation and cartilage extracellular matrix synthesis (PGs and collagen type II) compared with controls [47]. Synoviocytes from patients with OA cultured in PRP demonstrated increased hyaluronic acid (HA) production and secretion, suggesting that PRP could potentially serve as an endogenous source of chondroprotection and joint lubrication after intra-articular application [48]. In a rabbit model, osteochondral defects treated with PRP [49] demonstrated a higher extent of cartilage regeneration as well as an increased production of the glycosaminoglycans in comparison to controls. However, clinical

results on the application of PRP on cartilage regeneration have been controversial. Recently, a randomized controlled study comparing HA versus PRP in the short-term treatment for symptomatic knee OA showed plasma rich in growth factors being superior in alleviating symptoms of mild to moderate OA of the knee [50]. Recently, Gobbi et al. evaluated the effect of PRP in 50 patients with OA. At 12 months follow-up, patients had improved clinical symptoms with no adverse effects reported [51]. A separate study by Dr. Mei-Dan reported that osteochondral lesions of the ankle treated with intra-articular injections of PRP and HA resulted in a decrease in pain scores and an increase in function for at least 6 months, with minimal adverse events. In this study PRP treatment also led to a significantly better outcome than HA [52].

The use of PRP for cartilage repair continues to expand, yet still critical clinical questions remain to be answered: the optimal PRP formulation, standardization of its preparations, dosing, timing, and the number of injections, efficacy, long-term effect, and many others. The existence of different PRP preparations makes it even more difficult to compare the results. Prospective randomized studies that utilize advanced quantitative cartilage imaging techniques are necessary to assess the efficacy of this new promising biological treatment.

Conclusion

One of the fundamental questions in PTOA therapy is when and which agents have an indication for patients with PTOA and whether principally new treatments have to be considered. In the last 5–10 years a tremendous progress has been made in our understanding of the mechanisms that drive PTOA and key cellular and molecular pathways contributing to the process. A number of ex vivo approaches and in vivo animal models have been developed and characterized to reproduce joint injury followed by degenerative progression specific for PTOA. Innovative surgical methods have been brought to the clinic and now they include cell and tissue based treatments. However, well-defined clinical studies on large cohorts of patients are necessary to validate these novel techniques and therapies. Tables 26.1–26.5 provide a summary of ongoing clinical studies and registered clinical trials with interventional biological treatments for OA or PTOA. Analyzing biologic approaches for PTOA we believe that the ideal therapy must be multi-varied and target multiple mechanisms. Based on the existing knowledge we propose that this therapy should include chondroprotective agents in combination with pro-anabolic factors that preferably also possess anti-catabolic properties. In summary, the following are the key mechanisms that should constitute the basis for the design of intervention therapies to induce cartilage remodeling and regeneration in PTOA: (1) Chondroprotective; (2) Anti-inflammatory; (3) Matrix protective; and (4) Pro-anabolic. The most beneficial agents are those that target multiple pathways and mechanisms. However, one of the biggest remaining challenges is the translation of accumulated basic knowledge into the clinic and the development of appropriate effective therapies which can be administered within the window of opportunity.

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Stem Cell Therapies for Post-Traumatic Arthritis

27

Farshid Guilak, Brian O. Diekman, and Steven A. Olson

Introduction

Post-traumatic arthritis (PTA) is a common condition that occurs in more than 40 % of people who experience significant joint trauma, such as ligament injury, meniscal tear, or intra-articular fracture [1]. PTA is estimated to be responsible for 12 % of all osteoarthritis (OA) cases in the USA, resulting in an incidence of 5.6 million people and a large economic burden due to the young age of the PTA population [2]. While surgical repair of soft tissues and fractures is the most common treatment for PTA, there is little evidence that these procedures have a long-term disease-modifying effect [3, 4].

In this regard, there is great interest in the development of new therapies that can alter the course of PTA [1, 5]. Such therapies have generally involved synthetic or biologic molecules targeting specific anabolic or catabolic pathways, including inflammation, reactive oxygen species, cell death, growth factors, bone remodeling, degradative enzymes, or altered mechanotransduction (see review by Chubinskaya et al., Chap. 26). Both systemic and intra-articular molecular therapies are being developed in this regard, with increasing consideration for the development of drug delivery technologies for controlled release of these molecules as potential disease-modifying drugs [6].

More recently, however, there has been growing interest in the potential of stem or progenitor cells to perform some of these therapeutic functions as a regenerative therapy for a wide range of disease states, and particularly for osteoarthritis [7]. While most cell-based therapies have assumed that long-term engraftment and differentiation of stem cells are required for regenerative effects, exogenously delivered mesenchymal stem cells (MSCs) have also been shown to enhance regeneration by "trophic" mechanisms, such as the direct secretion of bioactive factors or by altering the cytokine and growth factor production of endogenous cells [8–11]. In this regard, there has been growing interest in the potential for stem cell-based therapies to prevent joint degeneration following injury. In particular, most approaches have focused on direct intra-articular injection of stem cells post-injury. Here we review the in vivo animal studies that have investigated the potential of stem cell therapies

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for reducing the severity of PTA, as well as some of the potential mechanisms that may be involved in these responses.

Animal Studies of Stem Cell Therapy for PTA

While a number of tissue engineering methods and stem cell-based approaches have been studied for cartilage repair and regeneration, relatively few studies have examined stem cell therapies for the prevention of PTA after soft tissue injury or articular fracture. The first reported study examined the ability of MSCs to enhance the repair or regeneration of osteoarthritic cartilage in a caprine PTA model [12]. In this study, autologous bone marrow MSCs were isolated, expanded in culture, and transduced to express green fluorescent protein. Six weeks following total medial meniscus and resection of the anterior cruciate ligament, ten million MSCs suspended in hyaluronic acid were injected intra-articularly. While the original goal of the study was to regenerate the OA cartilage through engraftment into fissures, this study showed marked regeneration of the medial meniscus that contained the implanted cells. Importantly, this treatment showed a reduction in cartilage degeneration and other OA characteristics. These findings show the potential for intra-articular MSC therapy to regenerate damaged tissues and to retard the progressive degeneration that occurs following joint injury [12].

More recently, a similar approach was used to investigate the ability of synovium-derived MSCs to enhance meniscal regeneration in rat massive meniscal defect [13]. Five million luciferase/LacZ+ synovium-derived MSCs were injected into the knees of wild-type rats following meniscectomy. After 12 weeks, the injected joints exhibited regenerated menisci that were LacZ positive, produced type II collagen, and showed meniscal features by transmission electron microscopy. Importantly, luminescence analysis in vivo showed that the injected cells were not detectable outside the joint, indicating that synovium-derived MSCs injected into a meniscectomized knee remained within the lesion and regenerated meniscal tissue without mobilization to distant organs. While the influence of this treatment on PTA was not reported in this study, two follow-up studies in other species explored the effects of synovium-derived MSCs on both meniscus regeneration and subsequent development of OA. In rabbits, a single delivery of ten million cells enhanced meniscus regeneration and limited the extent of subsequent OA as compared to contralateral joints that received saline after injury [14]. In a porcine model, three injections of 50 million cells were sufficient to enhance the quality of regenerated meniscus and reduce the severity of OA changes as compared to contralateral controls [15].

In a sheep model of meniscectomy coupled with ACL excision, an injection of two million bone marrow MSCs was found to reduce the severity of PTA after 6 weeks [16]. Of interest in this study was that both chondrogenically induced cells and non-induced cells showed gross evidence of inhibiting cartilage destruction, although greater meniscus repair was observed in the knee joint treated with chondrogenically induced cells. These findings show that a single intra-articular injection of MSCs, either chondrogenically induced or not, could retard the progression of osteoarthritis [16].

The ability of intra-articular injection of scaffold-free adipose-derived stem cells (ASCs) to diminish PTA severity was examined in several studies using a rabbit model of anterior cruciate ligament transection (ACLT). At 12 weeks following surgery, the experimental group received a single intra-articular injection of 1,000,000 ASCs derived from the knee fat pad [17] or subcutaneous fat [18]. At 20 weeks after surgery, either source of ASC resulted in a significantly reduced osteoarthritic degeneration as measured by Mankin grading of the joint. In other studies, a similar approach was used with either two million or six million subcutaneous ASCs injected immediately following ACLT, and also showed reduction of PTA at 16 or 24 weeks after surgery [19]. In a more recent study, a similar rabbit model showed that intra-articular injection of MSCs also reduced OA severity in a rabbit ACLT model [20]. These findings show that

ASCs, as well as MSCs, can reduce the severity of PTA following ACL injury.

In other studies, investigators have turned to mouse models to better examine specific mechanisms by which stem cell therapies could ameliorate PTA. Indeed, over 25 genetically engineered mouse strains have shown amelioration of at least one feature of PTA [5], which has led to the identification of several pathways for possible intervention. For example, several studies in the past decade have shown that the MRL/MpJ mouse strain possesses an intrinsic capacity for regenerating cartilage as well as other tissues [21–23]. Of particular interest was the finding that in a mouse model of articular fracture [24], these mice were protected from PTA [25], and that this regenerative behavior was associated with differences in the magnitude and duration of the inflammatory response following injury [26, 27]. To examine whether the stem cells from MRL/MpJ mice possess particular regenerative capacity, Diekman et al. examined the multipotential differentiation capabilities as well as the ability of a single intra-articular injection of MSCs to prevent PTA in mice [28]. Using a highly purified population of MSCs from bone marrow (CD45−/TER119−/PDGFRalpha+/Sca-1+), MSCs from C57BL/6 mice displayed greater adipogenic, osteogenic, and chondrogenic differentiation as compared to MSCs from MRL/MpJ mice. Nonetheless, the delivery of 10,000 MSCs from either strain of mice prevented the development of PTA following articular fracture. The levels of cytokines in the serum and synovial fluid were altered by treatment with stem cells, including elevated systemic interleukin-10 (IL-10), suggesting that intra-articular stem cell therapy can prevent the development of PTA after fracture potentially through modification of the inflammatory environment of the joint [28].

Potential Mechanisms of Action of Stem Cells

While the ability of stem cells to regenerate joint tissues such as cartilage and meniscus has been well documented, growing evidence suggests that the therapeutic effects of these cells following intra-articular delivery may be due to paracrine signaling and anti-inflammatory effects that act both systemically and locally in the joint (reviewed in [29]). For example, cytokines such as interleukin 1 (IL-1) and tumor necrosis factor alpha are upregulated with joint trauma [30, 31] and can contribute to joint degeneration and PTA by suppressing matrix synthesis and inducing catabolic matrix metalloproteinase (MMP) activity [32, 33]. In contrast, the presence of IL-1ra or other cytokine inhibitors can alter the inflammatory cascade by inhibiting IL-1 or other pro-catabolic cytokines [34]. Similarly, IL-4 and IL-10 have been identified as potentially important anti-inflammatory cytokines that can exhibit chondroprotective effects in several settings of joint disease (reviewed in [35]). Growing evidence suggests that MSCs can alter the balance of these pro-inflammatory and anti-inflammatory cytokines when given as therapeutic agents [28]. For example, MSC delivery after long-bone fracture has been shown to decrease systemic levels of IL-1β [36], and MSC therapy can alter macrophage response to cause an increase in IL-10 secretion in a sepsis model [37]. Other types of stem cells, such as ASCs, have been shown to have strong immunomodulatory characteristics that are likely to play an important therapeutic role [38, 39]. In addition to immunomodulatory roles, the growth factors secreted by stem cells can also alter the synthetic capabilities of endogenous cell types. For example, species-specific RT-PCR showed that the delivery of human synovium-derived MSCs caused an increase in type II collagen gene expression by host rat cells during meniscus regeneration [40].

Clinical Studies and Future Directions

In recent years, a number of clinical studies have been initiated for the application of stem cell therapies for osteoarthritis or other joint diseases (see reviews in [6, 41, 42]). However, very few of these studies have addressed PTA, rather than primary (nontraumatic) osteoarthritis, likely reflecting the

Table 27.1 Clinical trials of stem cell therapies for PTA (from www.clinicaltrials.gov)

Title: A phase I/II, randomized, controlled, double-blind, study of chondrogen—adult universal cell delivered by intra-articular injection following meniscectomy in patients 18–60 years
ClinicalTrials.gov Identifier: NCT00225095
Sponsor: Mesoblast International Sàrl
Purpose: The purpose of this study is to determine whether chondrogen is a safe and effective postoperative treatment of the knee following meniscectomy (the surgical removal of all or part of a torn meniscus)
Primary outcome measures: Meniscal volume
Secondary outcome measures: Quality of life; immunological endpoints; safety
Status: Completed
Title: A long-term follow-up study of chondrogen—adult human stem cells delivered by intra-articular injection following meniscectomy in subjects 18–60 years
ClinicalTrials.gov Identifier: NCT00702741
Sponsor: Mesoblast International Sàrl
Purpose: The objective of the present study is to establish the long-term safety of an intra-articular injection of human mesenchymal stem cells (hMSCs) (chondrogen)
Primary outcome measures: Comparison of treatment adverse event rates
Secondary outcome measures: Concomitant medications; visual analog scale
Status: Completed
Title: Phase 2 study to assess safety and tolerability of a single injection into the knee joint of two different doses of MSB-CAR001 combined with hyaluronan compared to hyaluronan alone in patients who have undergone an ACL reconstruction
ClinicalTrials.gov Identifier: NCT01088191
Sponsor: Mesoblast, Ltd.
Purpose: The purpose of this study is to evaluate safety and preliminary efficacy of MSB-CAR001 in subjects who have recently undergone an anterior cruciate ligament reconstruction
Primary outcome measures: To determine the overall safety of stem cell injections (MSB-CAR001) plus carrier using physical examinations, vital signs, treatment-emergent adverse events, and results of clinical lab tests (hematology, serum chemistry, inflammation, and immunology)
Secondary outcome measures: To evaluate the overall efficacy with MSB-CAR001 plus hyaluronan compared to hyaluronan alone using MRI scans and X-ray of the involved knee joint and access the change in outcomes (KOOS, SF-36) and pain (VAS)
Status: This study is ongoing, but not recruiting participants

fact that there are currently no therapies available beyond surgical management of joint injuries that are known to reduce the development of PTA. The safety profile of stem cells injected into joints has been excellent, with the only serious adverse events related to the procedure from over 800 cases involving bone marrow aspiration for cell isolation [43]. The results of these studies remain to be reported, although current trials are examining the therapeutic potential of MSCs for meniscal degeneration, partial meniscectomy, or ACL reconstruction (Table 27.1).

PTA serves as an attractive target for the development of novel therapeutic approaches such as the use of stem and progenitor cells. Interestingly, a majority of the preclinical work in the field of osteoarthritis has utilized animal models that are more representative of PTA as compared to primary OA [5]. In this regard, novel therapies may arise from a better understanding of molecular mechanisms involved in the development of PTA after joint injury, combined with a better understanding of the intra-articular action of stem cells. Increasing evidence suggests that the regenerative capabilities of stem cells may be through their influence on the inflammatory environment [28, 29]. Given the emerging body of evidence that stem cell therapies lessen the development of PTA after a wide range of joint injuries, it is likely that human clinical trials will be initiated to assess the ability of stem cell therapies to improve outcomes in carefully selected patient populations.

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Unanswered Questions and Future Directions in Post-Traumatic Arthritis

28

Steven A. Olson and Farshid Guilak

The clinical relevance of post-traumatic arthritis (PTA) has emerged over the past decade [1]. PTA has gone from a footnote in discussions of the burden of disease of arthritis to the level of being recognized in recent years as a cause of a significant proportion of the overall incidence of joint disease, as well as the most common cause of soldiers being unfit for active duty [2, 3]. Important advances in elucidating the pathophysiology of PTA have been made. The current focus on PTA research provides an important stimulus to find ways to translate these advances into clinical interventions to lessen the impact of PTA after joint injury. In many ways, arthritis after joint injury provides important advantages as a system of study [4]. For example, the time of joint injury is frequently known, and the mechanism and severity of injury can often be assessed. The time for PTA to develop after joint injury is much faster than traditional osteoarthritis, particularly PTA after intra-articular fracture, making interventional studies more feasible [3, 5]. Yet the numbers of published peer-reviewed investigations on PTA are significantly fewer in comparison to peer-reviewed investigations focusing on rheumatoid arthritis or osteoarthritis [5].

It is appropriate to consider why there are so many fewer investigations focused on PTA relative to other forms of arthritis. To provide perspective on the variation in numbers of peer-reviewed investigations in PTA as compared with other forms of arthritis a PubMed search in November 2014 gave the following numbers of published citations for the subject terms "Rheumatoid Arthritis"—119,704, "Osteoarthritis"—58,213, and "Post-Traumatic Arthritis"—950 [5]. A majority of our understanding of arthritic conditions comes from the work of rheumatologists and musculoskeletal basic science researchers; much of this work focuses on mechanisms and outcomes of medical therapy for various forms of arthritis. However, joint injury is unique among causes of arthritis in that the primary management is often surgical and is provided almost exclusively by orthopedic surgeons [6]. There are several aspects in the practice of orthopedic surgery that affect the ability to have an insight into the development of PTA [2]. Surgeons are trained to restore anatomy (reduction/fixation) or soft tissue function (reconstruction of the ACL or repair of the meniscus) following injury—this is the case with treatment of joint injuries. Orthopedic surgeons have used surgical management as the primary (and often only) form of treatment to prevent PTA after joint injury. In part this is because not only are the mechanisms that cause PTA incompletely understood,

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but there are no pharmacologic therapies currently available to limit PTA development after joint injury. With increasing specialization in practice, often-care for a patient's acute joint injury and care for the PTA that develops later are provided by different orthopedic surgeons. This lack of continuity of care has limited observation of the process of development of PTA.

The treatment of major joint injury is primarily evaluated and treated by orthopedic surgeons. For this reason, the primary onus for prevention and treatment of PTA should also belong to orthopedic surgeons [3, 6]. This important clinical area of PTA development is relatively unexplored. As with traumatic injury in general, PTA tends to occur in a younger population [7]. These patients are at risk for lifelong disability secondary to the effects of joint injury [7]. The impact on both the patients and on society is significant. Taking a leadership role in calling for an increased understanding of the effects of joint injury and encouraging development of therapies to prevent PTA is an important role for the Orthopedic Surgery community.

The traditional paradigm with which most orthopedic surgeons approach treatment of a joint injury can be paraphrased as "biomechanics trumps biology" [8]. This is reflected in the approach of surgical restoration of anatomy following joint injury. The observations highlighted in Chaps. 8 and 18 that increasing the magnitude of articular mal-alignment leads to higher contact stresses affecting a progressively smaller are a of the joint surface-points to one of the conundrums of PTA development. Why does a focal area of mal-alignment and its resultant localized cartilage damage lead to global arthritis throughout the entire involved joint? The work of Loeser, Goldring, and coworkers provides direction to begin to address this question [9]. They highlight the intra-articular response to joint injury as an organ system response within this local environment. Increasing numbers of investigators have begun to recognize the importance of the interactions between biological and biomechanical factors among the many cell and tissue types within the intra-articular environment in the pathophysiology of various forms of arthritis [10]. Orthopedic surgeons have classically focused on the effects of joint injury on articular cartilage and chondrocytes in isolation until recently. There is a paucity of data, both acute and chronic, regarding the intra-articular response to joint injury in humans. The focus of this text is to expand that understanding of the various aspects of joint injury and the intra-articular injury response.

The importance of understanding molecular mechanisms that are active in the intra-articular environment after joint injury that may lead to novel therapies has only recently been appreciated. The role of cytokines in PTA has recently been highlighted in the literature. Chapters 4–10 detail experimental models of cartilage and whole joint injury. The sophistication of articular injury models has increased significantly. Standardized models of joint injury in mice that progress to PTA have been developed in the past 10 years (see Chaps. 5–10). These models provide opportunity to identify molecular mechanisms and assess novel therapies in response to specific types of joint injury. The use of genetically modified or inbred strains of mice is beginning to lead to novel therapies that may prove clinically useful. It is likely that different mechanisms of joint injury may result in detectable differences in injury response within the joint.

Important progress has been made in understanding of the effects of joint injury and the subsequent intra-articular injury response. There are still important clinical questions that need to be addressed in order to effectively translate potential interventions into clinical practice. Future efforts to improve our ability limit or prevent PTA development after joint injury can be described in several key areas.

Who is at risk? An in depth understanding of how injury characteristics correlate with PTA development is needed to better identify patients at greatest risk. Development of registries to track treatment and long term outcome of articular fractures is a potential mechanism for addressing this issue. Both Norway and the UK have registries of hip fractures currently in operation [11, 12]. The Department of Defense has limited data on this subject. The MOON cohort as reported in Chap. 20 is a registry of patients with ACL tears and is focused on clinical outcome and development

of PTA [13]. In future efforts biobanking could be used to assess for underlying genetic factors that may predispose a patient to PTA development. Initial attempts by Anderson and colleagues to use energy of injury, and articular surface comminution have been proposed as the basis for a metric to predict the risk for PTA in distal tibia fractures (Chap. 24) [14]. Such efforts need to be expanded to include other joints and more user-friendly imaging techniques that can be readily adopted into practice.

How do we follow disease progression? Once a joint injury occurs today the patient is followed clinically with serial physical examination and radiographs. While this is enough to diagnose the establishment of PTA over time, it is insensitive in detection of subtle amounts disease progression. There is no established synovial or serum marker that can be used to follow or predict disease progression or improvement after a joint injury (Chap. 25). This is an area of research where human biosamples collected from patients who have sustained joint injury will be of great value. Data from such samples can be compared to basic science investigations of joint injury to detect measurable markers. This type of work will help establish the clinical value of these basic models of PTA, as well as providing a means to determine which compounds will be clinically useful as a marker of PTA onset or progression.

How can we modify the response to injury to prevent PTA? Recent work suggests that modification of the intra-articular post-injury response can reduce the development of PTA. In mice the administration of IL1-Ra immediately after a closed articular fracture prevented PTA changes (Chap. 8) [15]. This observation is important as it implies that there is an opportunity to modify the biologic response in a parallel way to that which surgeons use to modify the anatomic effects of joint injury. To be able to use this much greater knowledge concerning the innate and adaptive immune responses to joint injury is needed [16]. The field of solid organ transplantation has significantly advanced our knowledge of acquired immunity. Today, there are molecular probes to investigate the various aspects of acquired immunity and pharmacologic therapies to target specific aspect of the immune response [17]. A similar knowledge base for innate immunity in the intra-articular environment will be required to treat PTA. Equally as important is an understanding of when to intervene after joint injury. A better understanding of the time course of cellular and molecular mechanisms following joint injury is needed to understand how to optimize interventions to prevent PTA.

What is the effect of surgical repair on the post-injury response? Important questions about the how surgical repair of joint injury will affect the intra-articular injury response need to be addressed. Does the surgical repair of a joint injury alter the post-injury response? Does a surgical repair stimulate its own injury response? Does an open arthrotomy have a different effect than a percutaneous procedure? Is there a point after joint injury when intervention is no longer effective? An improved understanding of how surgical repair impacts the post-injury response is needed.

The goal of this text is to provide a comprehensive reference for those clinicians and researchers involved in clinical care of patients with joint injury and basic investigation of the effects of joint injury. As with all texts, at the time of publication new ideas and therapies are being developed and tested. We have made efforts to make the information as up to date as possible. The spectrum of information in this text has not been previously complied in one location. It is intended to be a valuable resource for those interested in this area of investigation.

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