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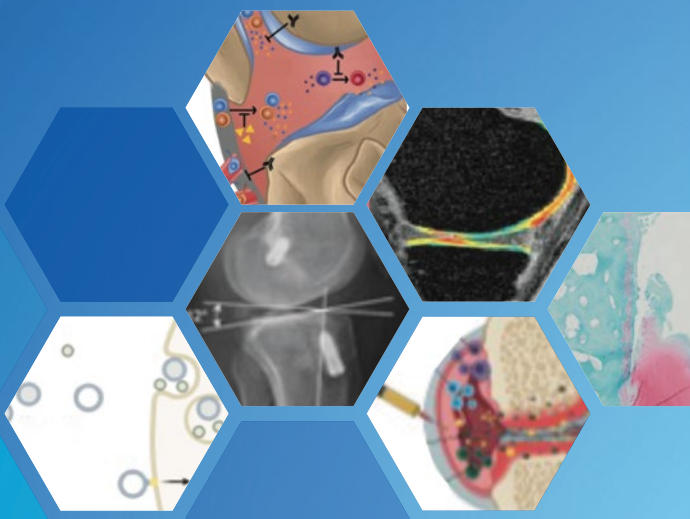

Early Osteoarthritis

State-of-the-Art Approaches to Diagnosis, Treatment and Controversies

ISAKOS
International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine

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International Cartilage Regeneration & Joint Preservation Society

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Preface

The definition and understanding of osteoarthritis have changed in the last decade, as we have learned to understand osteoarthritis as a continuous process that, once initiated, progresses. We have come to understand that this process is fuelled by mechanical as well as non-mechanical, inflammation or genetics-related factors and that many of our patients present to us are already on a spectrum of osteoarthritis, even though they may still be in the very early stages of this disease process. For this reason, we chose to create a dedicated book on "Early Osteoarthritis". This is a compendium for all physicians treating young patients with old knees.

As we understand the early osteoarthritic process differently now than when we first were trained two decades ago, we also see new treatment approaches rise to the occasion. This book on Early Osteoarthritis will take a deep dive into the origin and detection of early osteoarthritis by approaching this topic in three parts:

1. Epidemiology and Risk
2. Basic Science
3. Clinical Treatment of Early OA

Part I "Early Osteoarthritis Definition, Epidemiology and Risk" is dedicated to methods for early detection as well as epidemiological and economic aspects of the disease process in young individuals. Specifically the role common sports injuries, such as an ACL injury, play as a starting point for early osteoarthritis is being examined and introduced.

In Part II "Basic Science of Early Osteoarthritis", we focus on lesser known but important aspects of early OA that inform us about potential future treatment approaches. The role of inflammation and the immune system in early OA, the role of micro- and macro-biomechanics, injury contribution to the process, the role of macrophages and vesicles, such as exosomes, in the disease process and their potential application will be covered.

Part III "Clinical Treatment of Early OA" focuses on the clinical aspects of early osteoarthritis and potential emerging treatment options. The role of orthobiologics investigated from early anti-inflammatory approaches to the use of bone marrow aspirate concentrate, fat-derived or iPS stem cell therapy will be covered. Early osteoarthritis is often a domain that requires surgical intervention due to functionally disabling joint changes, such as chondral defects, or the complete loss of a weight-bearing surface. Many of these

patients are too young and active for joint replacement technologies to be a realistic option. We therefore focused on the latest in our understanding of joint preservation in patients with early osteoarthritis. We cover the use of alignment correction to meniscus preserving to regenerating strategies. The latest in chondral repair strategies such as osteochondral allografts, cell transplantation technologies or coral-based implants in conjunction with orthobiologics approaches will be covered in this final part.

Early osteoarthritis is an early manifestation of a long process that challenges both physicians and patients to think proactively, be engaged and often think out of the box. The input, counsel and deep dedication of basic science researchers, clinicians and patients are needed in order to move this field forward. We hope to provide a guide with this book, a starting point to improve and set our sights on the next generation of treatments and approaches in order to be able to provide the best and most comprehensive care for our young future patients.

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Part I

Early OA Definition, Epidemiology and Risk

Definition of Early Osteoarthritis

1

Henning Madry

1.1 Introduction

Osteoarthritis (OA) is a principal cause of chronic disability in adults [1]. Debilitating pain and progressive loss of joint function are the two major clinical signs, both of which lead to a considerably impaired quality of life. Irreparable degeneration of the articular cartilage, the gliding tissue covering the bony ends of all joints, represents the hallmark of OA. However, OA is not a simple degenerative disease where the cartilage wears away with time. Rather, it is a progressive and complex imbalance of the entire osteochondral unit and other tissues constituting a joint, among which are the subchondral bone, synovium, ligaments, menisci (in the case of knee OA), acetabular labrum (in the case of hip OA), and muscles.

An estimated more than 500 million people suffer from the debilitating clinical symptoms of OA across the world [2]. About 80% of the population shows radiographic signs of OA. Because of the aging population and the epidemic of obesity, especially in more economically developed countries, its incidence is further rising [3]. OA represents a high-burden noncommunicable disease, since its numbers significantly increased in terms of total burden and age-standardized disability-adjusted life-years (DALY) rates. A recent study recognized that the total DALY rates considerably rose by 35% and age-standardized DALY rates by 4% between 1990 and 2015 [4]. The total costs associated with the disease exceed $3 billion annually [5]. Although OA affects all joints, knee OA accounts for ~83% of the total OA burden [6]. These data attest to the ever-growing epidemiological and socioeconomic priority of OA.

However, a "no man's land" of OA exists, a "shadow zone" of early OA where the degenerative processes just begin, as beautifully articulated in an Editorial by Elizaveta Kon, Giuseppe Filardo, and Maurilio Marcacci [7]. Here, the cartilage might retain some of its regenerative ability, which is permanently lost in the more advanced stages of the disease [7]. Despite its clinical importance, this early phase of OA is sometimes overlooked. It represents, however, both from a diagnostic and therapeutical standpoint, perhaps the most fascinating period during the long process of the disease. Although patients experience often no or only reduced symptoms such as pain and disability, this is the phase where conservative preventive or new regenerative treatments such as efficient disease-modifying drugs (DMOADs) aiming to slow or even to reverse the progression of the disease may have a higher probability to meet success. Because changes of the articular cartilage and subchondral bone microstructures just begin, regenerative

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treatments would meet a joint that is much less damaged compared to the advanced stages of OA, when most cartilage volume is lost, the subchondral bone severely pathologically transformed, and the disease unravels its full clinical and radiological picture. A proactive modification of the course of the disease, before the destructive processes irreversibly compromise the entire joint, could possibly prevent the development of chronic pain before it elicits sensitization locally and centrally, and before functional impairment leads to deconditioning [8]. Such contemplations are supported by findings of a recent study applying a potential DMOAD to patients with Kellgren–Lawrence (K–L) grades 2 or 3 knee OA. Here, a dose-dependent and statistically significant reduction of cartilage loss was reported for the lesser affected lateral tibiofemoral compartment, while no significant effects were detectable in the clinically more frequently affected and more severely destroyed medial tibiofemoral compartment [9, 10]. In this context, Lohmander et al. suggested that the cartilage status differs between the medial and lateral tibiofemoral compartment [9]. A detailed assessment of such spatial osteochondral heterogeneity in advanced varus knee OA identified an unresponsive overloaded medial tibial plateau with severe cartilage and subchondral bone changes, while the lesser loaded lateral tibial plateau remained biologically responsive [11]. Therefore, DMOADs are probably much more effective in a compartment affected to a lesser degree by OA as opposed to the terminally impaired osteochondral unit in advanced OA, where most of the articular cartilage is eroded down to the calcified cartilage and the subchondral bone is sclerotic and even partially exposed. This theory is supported by observations of cartilage thickening and joint space width increases in knees with end-stage OA that are shielded from load by an external fixator [12].

When using the term "early OA," it appears important to declare the meaning of this term. First, and in the context of this book, "early OA" is the earliest chronological phase of OA, referring to the time when the cartilage degeneration, subchondral bone alterations, and other changes just begin. Such early OA therefore may occur at all patient ages, as it represents the onset and early face of the disease. Likewise, in the context of this book "early OA" does not refer to "early onset OA," a term sometimes used to describe the onset of OA at an early age of the patient, for example, such as occurring in adolescent patients with hip dysplasia [13–15]. The focus of this chapter is placed on the knee joint although early OA affects all joints including the hip [16].

Being able, both from a clinical and structural point of view, to define early OA will permit the early identification of patients at risk of OA development and progression while also enabling early treatment. This need has been recently recognized, and significant progress has been made to refine the evolving definition of early OA.

1.2 Clinical Presentation of Early OA

Early OA is an insidious phase of the disease since patients are either asymptomatic or present with only reduced clinical symptoms. Pain and a reduced range of motion, the two key clinical indicators for OA, are often present at a reduced intensity in early OA. OA pain is a major symptom, and pain on most days with radiographic features consistent with knee OA occurs in ~12% of those aged over 55 [17, 18]. The pain affecting the joint in OA is typically described as related to an activity of a deep and not well-localized nature [18]. A recent study identified weight-bearing activities involving bending of the knee, such as using stairs, to be the first patient-reported activity that is associated with knee pain [19]. However, it is unclear whether early OA may have been already present in an asymptomatic patient who is free of pain. In advanced OA, the pain is often constant, present at rest and in the night, often exacerbated by activity and relieved by rest [18].

The loss of the range of motion of an affected joint is often slow, asymptomatic, and may not always be recognized by the patient at early stages. Painless crepitus originating from the affected cartilage with movement of the joint and

joint line tenderness can be present. Although osteophyte formation is an early event in OA, considerable deformity based on bony enlargement such as Heberden's nodes, muscle atrophy, and joint effusion aremore characteristic of advanced OA stages. Other signs of OA, such as joint instability, may be present in early OA as in the case following ACL injury, which represents a considerable risk factor for early OA development. Perhaps because of these reduced symptoms, patients often do not seek medical diagnosis and treatment in this early phase [20].

The clinical fact that there is no direct correlation between the severity of structural OA damage to the osteochondral unit and the intensity of the clinical symptoms further complicates this dilemma. A recent clinical trial identified a statistically significant less reduction of articular cartilage thickness over time as assessed using an MRI, although these structural improvements did not translate into clinical importance because clinical measures of OA pain and function obtained in parallel were not significantly different from placebo [21]. This suggests in the context of the clinically well-known observation of pain-free phases during structurally confirmed knee OA that "pain" does not always correlate with (onset and progression of) joint damage, which is a key problem when aiming to identify clinically asymptomatic patients that already have structural changes comparable to early knee OA. Moreover, knee OA might progress slowly over a period of 10 or more years, rapidly, or not at all [22].

1.3 Structural Definition of Early Osteoarthritis

Structural changes of early OA chiefly occur in the articular cartilage and the subchondral bone, although early OA affects all other structures of the knee joint, such as the synovial membrane, the infrapatellar fat pad, the menisci, the joint capsule, ligaments, and muscles. A structural definition of early OA based on the histopathological grading and staging system from the Osteoarthritis Research Society International (OARSI) was recently proposed [23]. The OARSI system includes both the grade and the stage of OA, where grade reflects the depth progression of OA into the articular cartilage and is hence relevant for early OA. This is based on the assumption that the progressive involvement of the cartilage layers starts from the articular surface to the calcified cartilage, which indicates the advancement of the disease. In early OA, the articular cartilage surface shows signs of discontinuities, with fibrillations, and vertical fissures that maximally extend into the mid-zone. Early changes in the subchondral bone mainly comprise a progressive increase in subchondral plate and structural changes of the subarticular spongiosa. The OA grade is assessed in the region of the osteochondral unit that shows the most advanced manifestation of (early) OA. Grade is therefore unrelated to the horizontal extent of the disease. The horizontal extent of the involvement of the articular cartilage (within one part of the joint compartment) is termed stage.

Early OA was defined from a structural standpoint to be represented by OARSI grades (reflecting of progression into cartilage) of 1.0–3.0 [23]. In OARSI grade 1.0, the articular cartilage surface is intact. A surface discontinuity and fibrillation through the superficial zone represents the first structural change in early OA (OARSI grade 2.0). This is then followed by surface abrasion and cationic matrix stain depletion within the superficial zone (OARSI grade 2.5) and the occurrence of simple or complex vertical fissures that extending into the mid-zone of the cartilage in OARSI grade 3.0, but not deeper. The later stages of erosion (OARSI grade 4.0 and higher) are not considered early OA anymore as they reflect articular cartilage delamination, excavation, and complete erosion until the level of the calcified cartilage.

The changes of the subchondral bone in early OA start with an undulation of the subchondral bone–cartilage interface, a progressive increase in the thickness of the subchondral bone plate, and a remodeling of the subarticular spongiosa. Osteophytes form at the regions of the joint that are subjected to higher loads and are also a feature of early OA [24]. Angiogenesis within the

subchondral bone plate occurs possibly in conjunction with the remodeling process of the subchondral bone plate that involves both the action of osteoclasts and osteoblasts. Interestingly, the histopathological grading and staging system from the OARSI and the Mankin score do not account for histopathological changes of the subchondral bone in early OA, which therefore cannot be graded using these classification systems. In the OARSI system, grades 1–4 involve articular cartilage changes only. Aho and coworkers recently proposed a subchondral bone grading system that is also applicable to early OA [25]. In this system, grade 0 was defined to represent normal subchondral bone or very early OA changes with no evident subchondral sclerosis and subchondral plate thickening. Grade 1 represents mild subchondral bone sclerosis, an increase in bone volume, and thickened trabeculae in the subarticular spongiosa. Grade 2 was characterized by a distinct increase in subchondral bone sclerosis and volume. Grade 3 represents late-stage OA with severe sclerosis and a massive increase in subchondral bone volume. Another histological scoring system for subchondral bone changes in aging and OA was recently published [26]. How these grades of subchondral bone involvement correspond to the cartilaginous early OA changes remains to be determined.

1.4 A Short History on the Progress in the Clinical Definition of Early Osteoarthritis

Although the term "early osteoarthritis" has been in use since several decades [13–15, 27, 28], no definition was initially provided. The American College of Rheumatology (ACR) criteria for the classification and reporting of OA published in 1986 do not provide specific criteria for the diagnosis of early OA. They include pain and either morning stiffness (<30 min) or crepitus and objective findings such age over 50 years, osteophytes, and radiologically detectable joint space narrowing corresponding to grade 2 of the K–L classification) [29]. The ACR criteria served as a starting point for the definition of early OA.

The European League against Rheumatism (EULAR) provided in 2010 six key symptoms and signs for the diagnosis of established knee OA [30]. The three symptoms were persistent knee pain, limited morning stiffness, and reduced function, while the three signs included crepitus, restricted movement, and bony enlargement. EULAR acknowledged the need to develop diagnostic criteria for early symptomatic knee OA in individuals who, several years later, will fulfill the criteria of established knee OA [30].

The very first consensus meeting focusing on early OA with the title *Early Degenerative Arthritis of the Knee: Biological Solutions* was organized by Elisaveta Kon and Maurilio Marcacci of the Cartilage Committee of the European Society of Sports Traumatology, Knee Surgery & Arthroscopy (ESSKA) in 2011, bringing together international experts at the Rizzoli Orthopaedic Institute in Bologna, Italy (Fig. 1.1). The goal of the meeting was to improve the characterization of the patient populations suffering from the early phases of OA. Especially from a view of performing clinical studies, it was seen worthwhile to generate homogenous patient populations based on a standardized definition of early OA. The individual speakers discussed biological aspects of early OA, treatment options, and the role of the meniscus and osteotomy in preventing early OA. For the first time, and in addition to the classification criteria of established OA of the knee, criteria to classify patients with signs of emerging early knee OA were established and published [31].

In 2014, the *First International Early Knee Osteoarthritis Workshop* in Tokyo, Japan, hosted by the Japanese Society for Early Osteoarthritis and organized by Stefan Lohmander, Frank P. Luyten, Ken Nakata, and Ichiro Sekiya took place [8]. It gathered an international group of basic scientists, physician-scientists, rheumatologists, orthopedic surgeons, and physiotherapists and participants to develop a first draft of potential classification criteria for early knee OA (Fig. 1.2). A consensus was reached for three classes of criteria including (1) patient-reported outcomes such as pain and function, (2) clinical signs, and (3) K–L grades 0–1 on radiographs [8].

Fig. 1.1 Cover of the program for the consensus meeting "Early Degenerative Arthritis of the Knee: Biological Solutions" taking place at the Rizzoli Orthopaedic Institute in Bologna, Italy in 2011. (With permission from ESSKA and SIAGASCOT)

Fig. 1.2 Cover of the program for the "First International Early Knee Osteoarthritis Workshop" in Tokyo, Japan in 2014

To further seek agreement about the clinical features of early OA, an international panel of experts from various fields of OA research produced a core set of outcome criteria for early OA [32]. This opinion paper published in 2019 concluded that multiple factors must be considered to facilitate the development of predictive models for early knee OA, and further research validating outcomes in individuals at risk of early OA is required [32].

1.5 ESSKA 2012 Consensus Criteria for a Definition of Early Osteoarthritis

The ESSKA 2012 consensus criteria published in 2012 represent the first definition of early knee OA [31]. These are based on expert opinions while referring to the ACR criteria for knee OA. Defining such classification criteria was challenging, because no or only little structural changes can be seen on radiographs, also when applying the K–L criteria. This classification includes clinical (knee pain), radiological (K–L grading) and macroscopic cartilage surface analyses (arthroscopic findings), and nondestructive imaging parameters (MRI findings). The definition of early OA was based on the three items: (1) knee pain, (2) K–L grade 0 or I or II (osteophytes only), and (3) structural criteria, including either arthroscopic or MRI findings of cartilage lesions (Table 1.1). For practical ease, these three classification criteria were described in detail (Table 1.1). They are not related to microstructural histopathological parameters.

1.6 First International Early Knee Osteoarthritis Workshop 2014 Toward Classification Criteria for Early Osteoarthritis of the Knee

The First International Early Knee Osteoarthritis (IEKO) workshop aimed to improve the definition of early OA [8]. A consensus was reached for three classes of criteria including (1) patient-reported outcomes such as pain and function, (2) clinical signs, and (3) K–L grades 0–1 on radiographs (Table 1.2). For the (1) patient-based questionnaire, the Knee Injury and Osteoarthritis Score (KOOS) was chosen because of its domain relevance to early OA, extending the item "knee pain" of the ESSKA criteria. The second (2) set of criteria refer to the two clinical signs "joint line tenderness" and "crepitus" (to reflect also patellofemoral early OA). Finally, the (3) radiological criteria to be obtained during standardized weight-bearing X-ray examination included K–L 0 or 1. Two views were recommended, weight-bearing posterioranterior (PA) fixed flexion of both knees, and a bilateral skyline (supine) view that should be aligned with the OARSI Clinical Trials Recommendations for knee imaging in OA [33].

1.7 Establishing Outcome Measures in Early Knee Osteoarthritis

A recent perspective article published in the July 2019 issue of *Nature Reviews Rheumatology* highlighted considerations for best practice in the selection of outcome measures for use in clinical practice and the research setting to evaluate patients at initial presentation of early knee OA [32]. The authors proposed measures have been evaluated and published primarily for established OA, emerging evidence, and clinical expertise that, in the future, will have to be validated and possibly modified. These criteria include (1) patient-reported outcomes, (2) clinical features, (3) physical function outcomes, and (4) modifiable lifestyle-related outcomes (Table 1.3). Provisional criteria for early knee OA based on patient-reported outcomes of pain and function, together with clinical signs and a radiographic K–L grade of 0 or 1 were matched. As patient-reported outcomes may be affected in the early phase of knee OA, for example, by compensatory adaptations of performed activities [32, 34], such possible adaptive behavior has to be also considered [32].

Table 1.1 ESSKA criteria for the assessment of early OA (2012)

Criterion	Explanation
1. Knee pain	At least two episodes of pain for more than 10 days in the last year
2. Kellgren–Lawrence grade 0 or I or II	Kellgren–Lawrence scoring of standard radiographs may reach up to II (osteophytes only) in standing weight-bearing position with knees in ~20° of flexion and the feet in 5° of external rotation. The radiographs should be done bilaterally from a posteroanterior view in the frontal plane Kellgren–Lawrence grade 0 refers no abnormalities Kellgren–Lawrence I is defined as doubtful narrowing of the joint space and possible osteophytic lipping Kellgren–Lawrence II is defined as definite osteophytes with joint space narrowing. Kellgren II/osteophytes (osteophytes only, no joint narrowing) has been introduced into the category early OA
3. Structural criterion: Arthroscopic findings	Arthroscopic findings are based on the ICRS classification. The arthroscopic findings include either ICRS grades I–IV in at least two compartments or ICRS grade IIIV in one compartment with at least surrounding softening and swelling of the cartilage
4. Structural criterion: MRI findings	MRI findings include evidence of degenerative changes of the cartilage, meniscus, and/or BMLs. The definitions are based on the BLOKS and WORMS scores and their comparisons. A minimum of two of the four following scores should be fulfilled: (a) Cartilage morphology scores grade 3 or higher (WORMS grades 3–6): Minimally multiple areas of partial-thickness defects with intermittent areas of normal thickness to diffuse full-thickness loss in region (more than 75%; grade 6) (b) Cartilage score 1: Minimally grade 2 (BLOKS grades 2 and 3): 10–75% of cartilage loss in a region (medial, lateral, patellofemoral) to more than 75% cartilage loss in a region (c) Meniscal tears: Grade 3 or higher (BLOKS grades 3–4): From displaced tears or partial resection (grade 3) to complete maceration, destruction, resection (grade 4) (d) BMLs, typically scored as BMLs size: Minimally WORMS grade 2, i.e., 25% or higher BMLs in any one compartment

Adapted from Ref. [31]
Abbreviations: *BLOKS* Boston Leeds osteoarthritis knee score, *BML* bone marrow lesion, *ICRS* International Cartilage Regeneration & Joint Preservation Society, *MRI* magnetic resonance imaging, *OA* osteoarthritis, *WORMS* whole-organ magnetic resonance imaging score

Table 1.2 Proposal for classification criteria for early knee OA (2017)

Criterion	Explanation
A. Patient-based questionnaires	Knee Injury and Osteoarthritis Outcome score: 2 out of the 4 KOOS subscales need to score "positive" (≤85%) 1. Pain (9 items, including information on pain intensity, frequency, and duration) 2. Symptoms, stiffness (7 items) 3. Function, daily living (short version: 7 items) 4. Knee-related quality of life (QOL: 4 items)
B. Clinical examination	At least 1 criterion needs to be present: • Joint line tenderness • Crepitus
C. X-rays	Kellgren–Lawrence grades 0–I standing, weight bearing (at least 2 projections: Posteroanterior fixed flexion and skyline for patellofemoral OA)

Adapted from Ref. [8]
Abbreviations: *KOOS* Knee Injury and Osteoarthritis Outcome Score, *OA* osteoarthritis, *QOL* Quality of life

Table 1.3 Proposed outcomes for the assessment of early pre-radiographic OA in clinical practice and research settings (2019)

Outcome	Explanation
Patient-reported outcomes	The Knee Injury and Osteoarthritis Outcome Score (KOOS) can be used to measure pain during activity, other symptoms (for example, stiffness, grinding, catching, swelling, knee flexion and extension), function in daily life and during sport and recreational activities, and quality of life across different age and treatment groups. The Intermittent and Constant Assessment of Pain (ICOAP) questionnaire can be used to evaluate constant and intermittent pain
Clinical features	A clinical assessment including joint line tenderness should be performed in individuals with new-onset symptoms of knee pain, stiffness, crepitus, or a feeling of "giving way"
Physical function outcomes	Three measures seem promising for use in the clinical setting on the basis of their reproducibility, patient acceptability, and the equipment and expertise required: The single-leg hop test, the 30-s chair sit-to-stand test, the star excursion balance test, and measures of quadriceps strength. Multiple additional functional measures have been validated for use in the research setting
Modifiable lifestyle-related outcomes	Adiposity can be assessed by measuring body fat percentage or fat mass index (fat mass in kg/height in m^2) using dual-energy X-ray absorptiometry or bioelectrical impedance analysis if available. BMI is more feasible in the clinical setting, although it has limitations for use in athletes. Levels of physical activity can be assessed using a validated physical activity monitor or a validated questionnaire if objective methods are not available. Nutrition outcomes are not currently suggested for use in routine clinical care; however, the 3-day dietary record provides reliable estimates of nutrient intake

Adapted from Ref. [32]
Abbreviations: *BMI* body mass index, *ICOAP* Intermittent and Constant Assessment of Pain, *KOOS* Knee Injury and Osteoarthritis Outcome Score, *OA* osteoarthritis, *QOL* Quality of life

Clinical features of early OA, such as joint-line tenderness and crepitus, are relatively easy to examine and may be present in the absence of radiological findings. A standardized clinical examination of the knee (including those features) provides a good interobserver reliability in patients with established knee OA [32, 35]. Although the physical function outcome measures will complement the clinical evaluation of patients with early OA, there is currently no consensus answering the question, which outcome measures are most relevant for early OA [32, 36]. Both performance-based and physical impairment measures may be needed to assess the multidimensional nature of physical function. Performance-based measures include the single-leg hop for distance test [37], the 30-s chair sit-to-stand test [38], and the 6-min walk test [39], while quadriceps muscle strength is the most commonly reported outcome of physical impairment [40]. However, insufficient information is available to advocate a specific mode (e.g., isotonic, isokinetic, or isometric) or type of contraction (concentric or eccentric) [32].

Modifiable lifestyle-related outcomes such as obesity, dietary inadequacies, and physical inactivity might accelerate onset and progression of early OA through a combination of mechanical and systemic mechanisms [41]. Measures of adipositas such as body mass index (BMI) and waist-to-height ratio are important [42]. As physical activity is essential for normal joint health, low- or moderate-intensity physical activity might reduce the risk of early OA and may reduce the risk of disability [43]. Wearable monitors help to quantify physical activity and energy expenditure. Nutrition interventions achieve weight loss, although the scientific base for possible effects of nutritional factors on OA development is not clear [32].

For research, measures of (1) biomechanical outcomes, (2) imaging features, and (3) biomarkers are useful, but require further research and may not be appropriate (yet) for a routine clinical care setting (Table 1.4). Biomechanical outcomes include joint movement, loading, or muscle activation patterns [32]. As axial malalignment and alteration in the external knee adduction moment

Table 1.4 Proposed outcomes for the assessment of early pre-radiographic OA in research settings only (2019)

Outcome	Explanation
Biomechanical outcomes	Measures of biomechanical outcomes require further research and are not currently suggested for use in routine clinical care. However, such outcomes are ideal for informing the underlying mechanisms of OA progression and treatment interventions in the research setting
Imaging features	The utility of plain radiography in early OA is limited. Although MRI has superior sensitivity to change, has validity in the context of early OA, and is hence ideal in the research setting, MRI is not thought to be appropriate for the routine clinical care setting because of its high cost and potential risk of overdiagnosis
Biomarkers	No biomarkers are currently of use in routine clinical care; however, further validation of proteomic, lipidomic, and metabolomic tools in the research setting could lead to informative cartilage and synovial fluid profiles and provide important insights into OA progression

Adapted from Ref. [32]
Abbreviations: *MRI* magnetic resonance imaging, *OA* osteoarthritis

are associated both with an increased incidence and progression of knee OA, they are of value for early OA. Additional risk factors such as loss of joint stability and muscle weakness [32, 44] may also alter compartmental loading [32]. Imaging features chiefly include plain radiography and MRI, although arthro-CT has also a potential diagnostic value for early OA. Though radiography will remain the primary imaging modality in daily surgical and medical practice and clinical trials, limitations exist especially when aiming to visualize early OA features. For example, a recent study evaluating early OA in patients with otherwise stable knees undergoing knee arthroscopy for traumatic medial meniscal lesion visualized macroscopic early OA in the medial tibial plateaus whereas their K–L score was not significantly different from normal subjects [24]. MRI techniques become increasingly important as they allow to quantify morphological changes of early OA in topographic subregions [45], while also identifying meniscal damage and meniscal extrusion, synovitis, bone marrow lesions (BMLs), and structural damage to the joint. Cartilage composition and quality can be also estimated [32].

Biochemical markers in the blood, urine, or synovial fluid might be associated with incident radiographic OA. For early OA, they need to clearly differentiate between physiological normal and pathological early OA tissue turnover. Ideally, biomarkers would also distinguish different stages of the disease [32]. However, soluble biochemical markers require further study, validation, and qualification as markers of susceptibility to, or risk of, early OA before being adopted for widespread clinical use [32].

1.8 Application of the Definition of Early OA

Sasaki et al. [46] applied these new criteria to investigate the prevalence of early knee OA and possible risk factors in a cross-sectional study of the Japanese general population. The data showed that the prevalence of early knee OA was 9.5% in males and 15.0% in females, that the prevalence increased with age, and the highest prevalence was noted in females aged 50–59 years. Risk factors for early knee OA were also identified, among which female sex, ageing, obesity, and a history of knee injury, all of which were similar to those of definitive knee OA.

1.9 Outlook

Future research will focus on further refining the classification criteria in patients and defining the role of MRI and other imaging techniques in early OA detection, including anatomical and functional correlations. MRI may probably remain primarily a research tool for clinical

investigations, not for the daily standard clinical practice given its potential risk of overdiagnosis, relatively high costs, and more difficult availability compared with radiography [8, 32]. MRI is a valuable tool to shed more light into the relationship between synovial inflammation and BMLs that may be associated with focal cartilage (and meniscal) defects and the development of structural features of early OA. Also, how the clinical features of early OA correspond with the grades of microstructural changes of the cartilage but also the subchondral bone remains to be determined.

A recent study asked the thought-provoking question if early OA can be diagnosed when an asymptomatic patient shows radiological signs of early OA; thus what represents the difference between a naturally aging knee and early knee OA [47]? This study on 340 subjects aged 45–55 years that were free of radiographic knee OA at baseline identified common structural changes of the knee irrespective of the presence of pain or other (early) OA risk factors. The data showed that the development of features consistent with structural OA was similar irrespective of the presence of OA risk factors, while the overlap with clinical symptoms was modest. These findings suggest that structural changes seen on X-rays or MRI without clinical symptoms may not be regarded as early OA, but rather as risk factors for the development of early OA, as they are highly prevalent and associated with normal ageing [47]. Early knee OA needs however be ruled out in patients that present with long-term knee pain without radiographic evidence, such as K–L grade 0.

Future work will further integrate the structural changes with the clinical signs of early OA to refine the definition, perhaps in a joint-specific manner. Progress in the definition of early OA will lead to a better understanding of the disease process, allow identifying patients who are at a high risk of OA progression in the affected joint, and help evaluating the effectiveness of novel preventive and regenerative therapies aiming to prevent or to delay the onset of early OA.

Competing Interest Statement HM is the Editor-in-Chief of *Osteoarthritis and Cartilage Open*. The author has no financial conflicts to declare in connection with this chapter.

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MRI of Early OA

2

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2.1 Morphological MRI

Osteoarthritis (OA) is a slowly progressing, chronic degenerative disease that often affects the knee joint. Early diagnosis is essential for timely therapeutic and medical intervention in order to delay the progression of the disease, relieve pain, and prevent disability. While OA affects various tissues in the knee joint, articular cartilage, which is composed of hyaline cartilage, plays an important role in the pathogenesis of the disease.

In general, articular cartilage is essential for various biomechanical properties, i.e., to support and distribute loads as well as to provide lubrication [1]. However, as the disease progresses, the cartilage deteriorates, resulting in the loss of these biomechanical properties and leading to restricted mobility and pain for the patient, which can significantly affect the overall quality of life. Since cartilage is avascular, it has no significant regenerative capacity [2, 3], therefore making early diagnosis, and thus, early intervention, especially through conservative interventions such as physiotherapy and lifestyle changes, all the more necessary.

The method of choice for the detection of articular cartilage damage is magnetic resonance imaging (MRI), which provides the best soft-tissue contrast of all imaging modalities. Conventional qualitative MRI can reveal morphological changes in the cartilage, such as a reduction in cartilage volume, contour irregularities, fissures, and thinning, with high specificity and sensitivity [4, 5].

MRI has taken the central role in the vast field of osteoarthritis (OA) research and is a crucial modality in the routine diagnostic workup of OA and other musculoskeletal pathologies [6, 7]. This can be attributed to its ability to visualize articular cartilage, menisci, structures of the joint capsule, fluid collections, synovium, bone marrow, and all surrounding ligaments and soft tissues [8].

As there is still some controversy regarding the optimal MR protocol and the best possible evaluation, there have been attempts to standardize the MRI examination for the evaluation of knee OA, as published in 2006 by the

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Osteoarthritis Initiative (OAI) of the International Cartilage Repair Society (ICRS) [9].

To conduct a thorough assessment, the MRI protocols should be planned according to the available field strength and extremity coil. Cartilage defects and bone marrow lesions (BMLs) should be evaluated on fat-suppressed, fluid-sensitive (fs) proton-density-weighted (PDw) or T_2-weighted (T_2w) sequences [10]. Alternatively, a short tau inversion recovery (STIR) sequence can be used [11]. For over a decade, fs PDw sequences have been the established sequence because of the high sensitivity for articular cartilage damages and should be used with an echo time (TE) of ~40–60 ms and a spatial resolution of 0.3 × 0.3 mm in-plane resolution or better [12]. While three-dimensional gradient-echo (3D-GRE) sequences, such as the "Fast Low Angle Shot" (FLASH), "Dual-Echo Steady-State" (DESS), or "Spoiled Gradient Echo" (SPGR), can produce high-resolution isotropic images, they also carry the risk of underappreciating bone marrow edema or cartilage defects [13]. Also, the susceptibility artifacts in GRE sequences may be misinterpreted as cartilage thinning or meniscal defects [14]. It is noteworthy that the three-dimensional turbo spin-echo (3D-TSE) sequences are still under development and show great potential. However, they suffer from long acquisition times and are not yet validated sufficiently for the assessment of knee OA [15].

If present, both full- or partial-thickness defects found in the articular cartilage contribute equally to the development of further cartilage degradation in OA of the knee [16]. An example of a 29-year-old male patient with multiple grade IV defects is given in Fig. 2.1.

Apart from the articular cartilage, the meniscus is an important structure in the knee joint that can be used as a target structure in the assessment of patients at risk for the development of OA. In a prospective study of 407 middle-aged females, meniscal extrusion at baseline was associated with a higher likelihood of demonstrating knee OA radiographically 30 months later [17]. An example of a 33-year-old male patient with corresponding findings is illustrated in Fig. 2.2.

Also, if a meniscal root tear is found on an MRI, it is of particular importance as it is associated with a higher Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Index [18, 19]. Another risk factor is partial meniscectomy, as shown in a study by Roemer et al., which

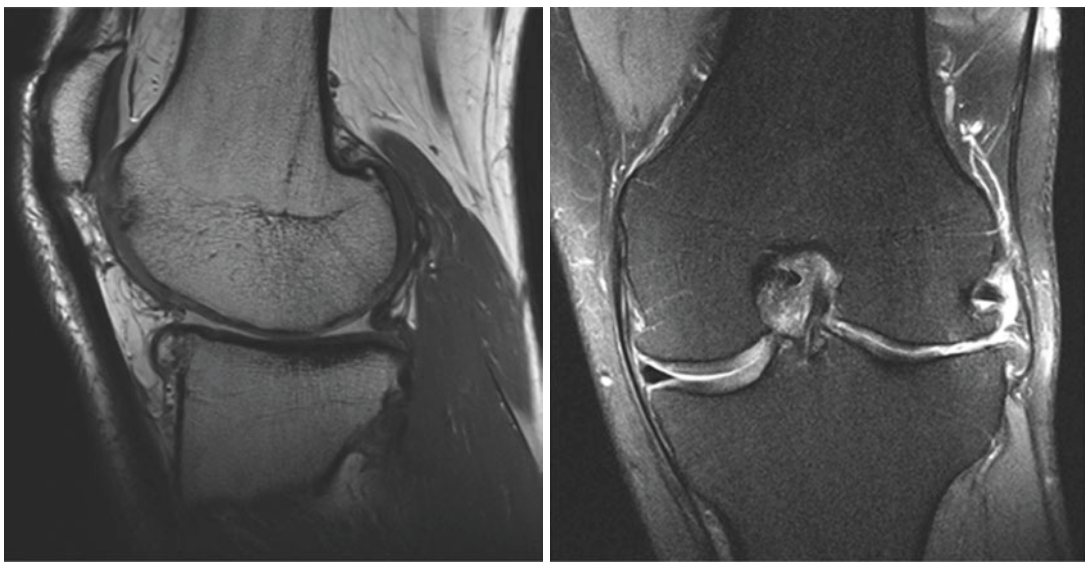

Fig. 2.1 A 29-year-old male patient with multiple grade IV cartilage defects in the lateral compartment and clear signs of early osteoarthritis of the lateral compartment

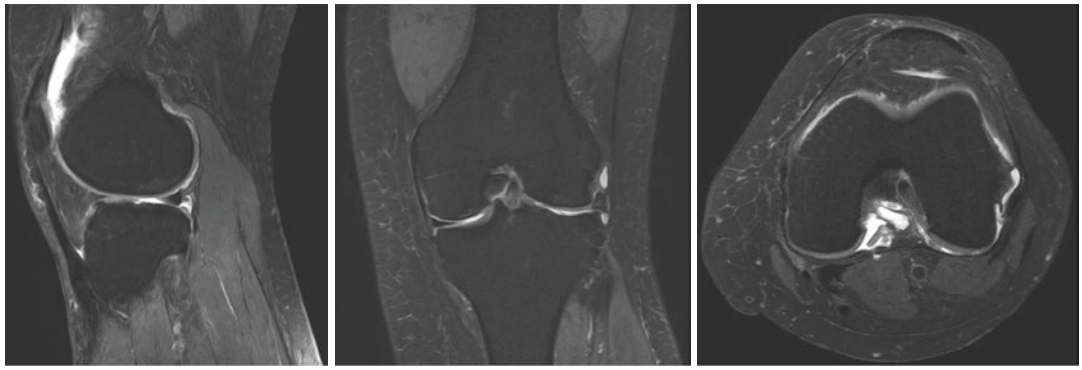

Fig. 2.2 Proton-density-weighted TSE images with fat saturation in three planes of a 33-year-old female patient with a grade IV defect on the medial femoral condyle with fluid-like signal in the defect area. Additionally, there is a smaller defect grade III–IV on the lateral femoral condyle with a corresponding grade III–IV defect of the lateral tibial cartilage. The anterior lateral meniscus is narrowed and of hyperintense signal with a central meniscal injury on the lateral side, which shows clear signs of meniscal extrusion. Osteophytes are visible on the lateral femoral condyle on the transversal image

reported a strong association of prior partial meniscectomy and the prevalence of knee OA after 12 months [20].

Synovitis is another important finding that can be seen on MRI with or without an intravenous contrast agent. Signs of a Hoffa synovitis with intra-articular effusion and MRI signal changes within the Hoffa's fat pad are associated with knee OA, as well as higher pain scores [21, 22].

In addition, bone- and bone-marrow-related changes are important in the process of knee OA development. Small osteophytes have already been associated with higher pain scores longitudinally and in cross-sectional studies [23]. BMLs are predictive of advancing knee OA and can even be associated with future cartilage damage. Also, they are accompanied by elevated inflammatory markers that may further facilitate the progression of knee OA [24].

There are multiple semiquantitative scoring systems that divide the joint into multiple subregions to grade different features of OA, mostly incorporating the aforementioned structures alongside other specific findings. For example, there are the MRI OA Knee Score (MOAKS) [25], the Whole-Organ MRI Score (WORMS) [26], the Anterior Cruciate Ligament Osteoarthritis Score (ACLOAS) [27], or the Knee Inflammation MRI Scoring System (KIMRISS) [28], among other novel scoring systems.

2.2 T_2 Mapping

Early cartilage degeneration, which is usually characterized by a loss of glycosaminoglycans and a loosening of the collagen fiber matrix, is more difficult to detect with conventional morphological MRI, as the cartilage may still look morphologically normal or relatively normal, although it may already suffer from early pathological changes [5].

Over the last few years, quantitative MRI methods (qMRI) have gained increasing interest, as they may be able to detect structural changes in tissue earlier in the course of the disease than morphological MRI. In this context, T_2 mapping is probably the most frequently used and investigated qMRI method.

Transverse (spin–spin) relaxation time mapping of the time constant T_2, in short T_2 mapping is a technique that does not require any contrast agents. It is typically measured using sequences of the "spin echo" class [29], which use a 180° radio frequency (RF) pulse for refocusing of the static dephasing effects, which is the main distin-

guishing feature of the time constant, T_2*, which is measured with MR sequences where this factor is not refocused [30].

It has been demonstrated that T_2 values in cartilage reflect the status of the cartilage ultrastructure (water content, collagen integrity, and collagen fiber orientation) [5, 31]. Furthermore, it was shown that T_2 values also correlate with histological grade [32], that can predict both the onset [33] and progression of knee OA [34], and that an increase in T_2 values is associated with an increase in OA severity [35].

Many of these insights into the relationship between early OA and T_2 values were gained from the Osteoarthritis Initiative (OAI) cohort datasets. The OAI includes 4800 participants with symptomatic knee OA or who are at risk of developing OA [36]. Using OAI data, for example, it was shown that, in regions with newly appearing cartilage lesions, the T_2 values locally (at the site where the lesion will occur) were already significantly higher 4 years prior to lesion onset compared to the surrounding cartilage regions [37]. In another study that used data from the OAI, it was shown that T_2 values correlated strongly with BMI and weakly with gender and age, with T_2 values increasing with increasing BMI and age and slightly higher in women [38].

The majority of studies on T_2 mapping employed manual ROI analysis or segmentation of the entire cartilage volume and used mean values for the entire tissue compartment. However, the cartilage has a very complex structure and consists of different layers, which can be roughly divided into the superficial, the transitional, and the radial zone. These layers differ both in their composition (collagen content and hydration) and collagen fiber orientation. The complex structure and the diversity of the layers in the cartilage are related to the biomechanical tasks it has to perform [39]. In addition, cartilage does not degenerate evenly across all layers in the course of early OA, but in a very complex manner, often starting in the superficial layer as surface discontinuity, glycosaminoglycan loss, and collagen matrix loosening [40].

Articular cartilage is an ordered collagen tissue and, as such, its T_2 values are prone to orientation dependence of the collagen fiber—with respect to the magnetic field direction as a result of the residual dipolar interaction of protons, which means that the T_2 values of cartilage tissue vary for every angle and are modulated by the expression: $D \sim 3\theta - 1$, of the secular dipolar coupling Hamiltonian [41].

At an angle of =54.7°, the so-called magic angle, the residual dipolar coupling is zero and the T_2 values reach their maximum; vice versa, at $\theta = 0^{\circ}$, the dipolar coupling is at a maximum and the T_2 values are the shortest [42].

These factors contribute to the fact that the different layers also have different T_2 values. As has been shown in in vitro studies, the shortest values are usually found in the deep zone, which consists of calcified cartilage and usually has lower hydration, while the overlying layers (intermediate and surface layer) tend to have longer T_2 values. That the values in the different layers are also dependent on the fiber-to-field angle, as in any collagen fiber tissue, was shown by Xia [43] and Hänninen et al. [44].

Considering the complexity of the cartilage structure, on the one hand, and the complexity of the degenerative changes in the cartilage, on the other hand, as known from histological studies [40, 45], it makes sense to also examine the spatial distribution of T_2 values.

In this context, using data from OAI and focusing on the femorotibial part of the articular cartilage, it could be shown, for example, that healthy reference cohort knees have significantly lower T_2 values in the superficial cartilage layer compared to subjects with early radiographic OA (ROA) or subjects with risk factors for ROA. In addition, healthy knees showed significantly lower deep cartilage layer T_2 values compared to subjects with risk factors for ROA. After a 1-year follow-up, both superficial and deep cartilage layers of the healthy reference cohort without risk factors showed an increase in T_2 values, but not in knees with early ROA or in knees at risk of developing OA. The authors of this study interpret their results to suggest that the previously measured differences in cartilage T_2 values between ROA and non-ROA cartilage may be due to differences in risk factor profiles between

cohorts rather than actual differences in the status of ROA [46].

The distribution of the T_2 values can also be evaluated using texture analysis, as introduced by Haralick et al. using a gray-level co-occurrence matrix (GLCM) [47]. This technique gives different GLCM factors of distribution, such as entropy, variance, and contrast.

Using GLCM, Schooler et al. studied the longitudinal changes in the laminar and spatial distribution of the joint cartilage T_2 values and found that, in subjects with cartilage lesions, the T_2 levels were higher than in those without lesions. In addition, GLCM contrast and variance in the group with lesions were significantly higher, suggesting that GLCM calculations may provide increased sensitivity that would not be detectable by analyzing the mean values of the entire tissue compartments alone [48].

Similarly, Joseph et al. [49] showed that, in subjects with risk factors for OA compared to healthy controls, the T_2 values, as well as the GLCM parameters (variance and contrast), were significantly elevated, which means that the elevated T_2 values in subjects at risk are also more heterogeneous. Taken from the same study, Fig. 2.3 shows two representative T_2 maps. One from a subject of the control cohort and one from a subject from the incidence cohort. Although neither of these two subjects has a cartilage abnormality (both have WORMS = 0), the T_2 values are significantly elevated in the subject of the incidence cohort (right), compared to the subject of the control cohort (left).

A main advantage of GLCM is that it is performed during image post-processing, and consequently, there is no additional time required to measure the subject.

T_2 mapping has shown great potential for the detection of early OA, but it will remain a major challenge in multicentre and in particular in multi-vendor OA trials in the future due to difficulties in the standardization of T_2 values, the orientation dependence of collagen, and the complexity of the course of OA in cartilage, which can vary greatly from subject to subject. As a result, it is still uncertain how or when T_2 mapping will finally make the definitive leap into the clinical routine as a biomarker for an early OA.

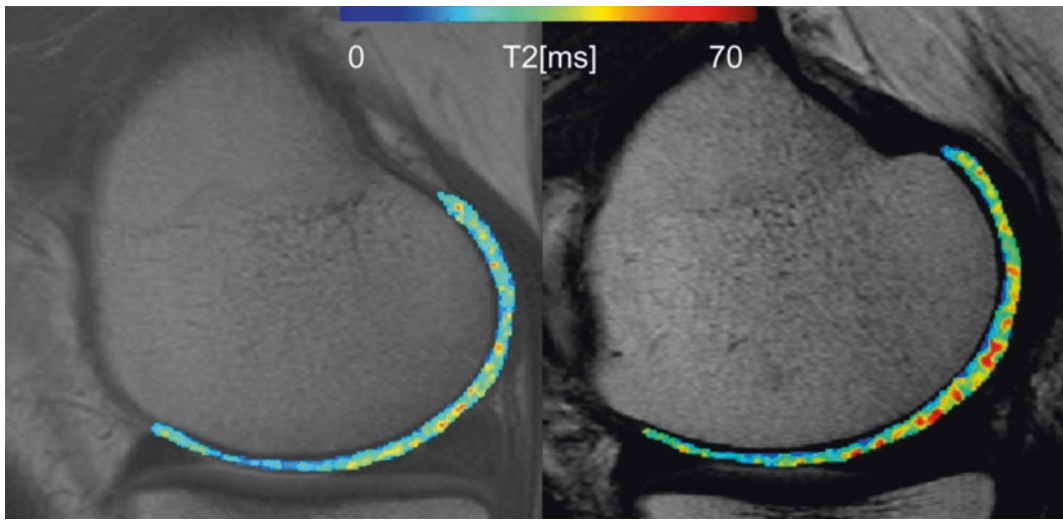

Fig. 2.3 (Left) Representative T_2 map of a subject from the control cohort. (Right) Representative T_2 map of a subject from the incidence cohort. Although neither subject shows cartilage abnormalities (both have WORMS score = 0), the mean T_2 values of the subject from the incidence cohort are elevated compared to the control subject (39.12 and 33.39 ms, respectively). In addition, the GLCM factors variance, contrast, and entropy are also elevated. (Reproduced with permission from Ref. [49])

2.3 Sodium (^{23}Na) MRI

Quantitative proton (^{1}H) MRI methods provide different information about the morphology of the knee joint, but biochemical changes in the joint often occur before morphological changes appear. Therefore, there is an urgent need for biochemical and quantitative MRI techniques that are feasible for use in the evaluation of the complex biochemical composition of an articular cartilage.

One of those techniques is sodium (^{23}Na) MRI, which can assess changes in the sodium ion content linked to glycosaminoglycan (GAGs) molecules. The negatively charged GAGs are the most important structural molecules of the cartilage, providing strong electrostatic and osmotic forces within the tissue. Furthermore, the GAG content has been highly correlated with the biomechanical properties of cartilage, in particular, compressive stiffness [50]. In articular cartilage, the negatively charged GAGs are surrounded by positively charged sodium ions. Hence, changes in sodium concentration can be used as an indirect indicator of the GAG concentration, which, in turn, can be noninvasively and quantitatively assessed using sodium imaging. ^{23}Na-MRI has been shown to be very successful in this task, mainly due to the more frequent utilization of ultra-high-field MR scanner. A substantial increase in the signal-to-noise ratio (SNR) compared to the lower field strengths can be achieved; therefore, the possibility for molecular investigation of the tissue, with higher sensitivity and specificity, was apparent.

One of the first studies that used sodium MRI for molecular investigations of OA patients at ultra-high field (7T) was reported by Wang et al. [51]. The authors performed a study that included volunteers with no symptoms of disease and patients with diagnosed and confirmed OA. These authors analyzed three different cartilage regions: patellar, medial femorotibial, and lateral femorotibial cartilage. The results demonstrated that the tissue sodium concentration (TSC) in OA patients was significantly lower (from 30 to 60%) compared to healthy subjects.

One of the main issues of cartilage sodium quantification is that signal from cartilage is usually contaminated by signal from the surrounding synovial fluid. Another study performed at 7T was published by Madelin et al., who evaluated cartilage sodium concentration in healthy volunteers and OA patients. Examinations were performed with and without fluid suppression techniques [52]. These authors showed that the TSC over all cartilage regions, measured with a radial sequence without fluid suppression, was similar between healthy subjects and OA patients; however, after fluid suppression was applied, the difference in TSC values between healthy subjects and OA patients was higher.

Zbyn et al. recently demonstrated that in vivo ^{23}Na-MRI is a feasible method that allows differentiation between low-grade cartilage lesions and normal-appearing (weight bearing, WB and non-weight bearing, NWB) regions of the articular cartilage [53]. In this longitudinal study, participants were assessed at four different time points (baseline, 1 week, 3 months, and 6 months) using morphological MRI at 3T and 7T, and compositional ^{23}Na-MRI. All patients underwent the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire immediately after the MRI exams. Significantly lower sodium signal intensities (SI) were observed in lesions compared to those in the WB and NWB zones, at all time points (Fig. 2.4). Results demonstrated that a significant decrease from baseline SI values in lesions was found at the 3-month visit; however, no substantial change was observed at 6 months. KOOS scores improved in all subscales at the 3- and 6-month visits, with a significant increase observed only in the quality-of-life subscale.

The hypothesis that early OA is associated with a loss in GAGs and a disorganization of the collagen network even before morphological changes become apparent is supported by sodium MRI results obtained from the first clinical investigations of osteoarthritis. Biochemical information about the condition of the joint may greatly help clinicians to achieve a comprehensive management of early OA, including improved detection, monitoring of progression, and different treatment procedure evaluations.

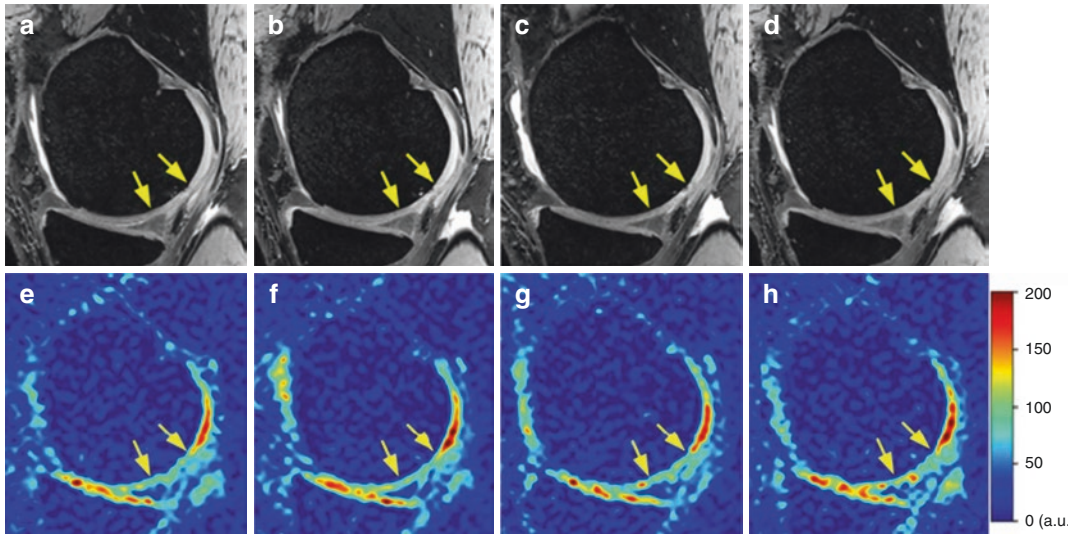

Fig. 2.4 Follow-up sagittal MRI scans of a 26-year-old patient with a low-grade chondral lesion (between yellow arrows) in the weight-bearing region of the medial femoral condyle. Morphological DESS images (**a**–**d**) and corresponding color-coded ^{23}Na images corrected for coil sensitivity (**e**–**h**) acquired at baseline (**a**, **e**), 1-week (**b**, **f**), 3-month (**c**, **g**), and 6-month (**d**, **h**) follow-up visits at 7T. The color bar represents ^{23}Na values corrected for coil sensitivity in arbitrary units. Please note that the ICRS grade assigned to the lesion was based only on the clinical 3T turbo spin-echo images. The 7-T DESS images were acquired only to provide an accurate measurement of cartilage thickness and a morphologic reference for ROI positioning. (Reproduced with permission from Ref. [53])

2.4 dGEMRIC in OA

The dGEMRIC and sodium (^{23}Na) MRI techniques are based on similar principles, with positive sodium ions attracted by the negatively fixed charged density (FCD) of the GAG side chains. These electrostatic forces are responsible for a direct relationship between the local sodium concentration and the FCD, with a strong correlation between the FCD and the GAG content.

Intravenously administered gadolinium diethylenetriamine pentaacetate anion (Gd-DTPA2−) penetrates the cartilage through both the articular surface and the subchondral bone. The contrast equilibrates in inverse relation to the FCD, which is, in turn, directly related to the GAG concentration. Therefore, T_1, which is determined by the Gd-DTPA2− concentration, becomes a specific measure of tissue GAG concentration, suggesting that Gd-DTPA2−enhanced MRI has the potential to monitor the GAG content of cartilage in vivo. Thus, T_1 mapping, enhanced by delayed administration of Gd-DTPA2− (dGEMRIC), can be considered the most widely used methodology for the detection of proteoglycan depletion in articular cartilage and has shown promising results.

dGEMRIC is sensitive to cartilage proteoglycan content and may predict the development of OA [54] (Fig. 2.5). It was recently demonstrated that T1Gd values in the medial tibiofemoral compartments decrease as the radiographic Kellgren–Lawrence grade increases [55]. Prescribed immobilization after injury of only 6 weeks has been shown to result in biochemical changes in the cartilage, measurable by dGEMRIC, with a mean decrease seen in T1 relaxation time (T1Gd) at 4 months, which persisted for up to a year [56]. In a longitudinal study, Owman et al. found that the low baseline T1Gd, using dGEMRIC in the medial and lateral femoral cartilage, was associated with a higher grade of joint-space narrowing after 11 years, and also with the development of osteophytes [57]. A study by Crema et al. found high-grade medial meniscal damage to be asso-

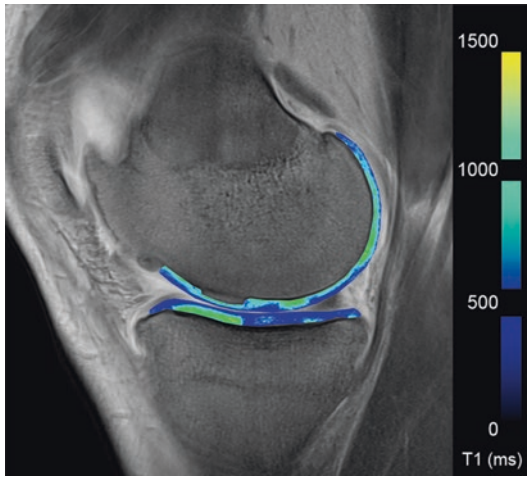

Fig. 2.5 On this sagittal postcontrast T_1 map (dGEMRIC) in a patient with moderate OA across the medial femorotibial compartment, several areas of lower T_1 values (blue color-coding) in femoral as well as in tibial cartilage layer can be seen with these lower T_1 values corresponding to lower glycosaminoglycan content due to early stages of cartilage degeneration in OA in different joint compartments and in different regions of cartilage layers, although cartilage thickness is still preserved and the surface looks mostly intact

ciated with low T1Gd times in the medial tibiofemoral compartment [58]. Lattanzi et al. recently demonstrated that dGEMRIC was accurate in detecting cartilage damage at the hip due to femoroacetabular impingement [59]. In an earlier study, Kim et al. also showed that the dGEMRIC index was significantly different in subgroups with mild, moderate, and severe grades of hip dysplasia [60], which suggests the ability of dGEMRIC to detect varying cartilage degeneration among these groups. In a study of 111 obese adults, dGEMRIC showed that weight loss over the course of a year resulted in an increased cartilage proteoglycan content [61]. A recently published study evaluated the articular cartilage in patients with an early knee OA using dGEMRIC after viscosupplementation with hyaluronic acid [62]. The study found no change in the structural composition of cartilage after 14 weeks, even though the symptomatic improvement was reported.

2.5 Conclusion/Future Perspective

Due to the aneural nature of the articular cartilage, patients frequently exhibit already significant loss of articular cartilage at their first orthopedic consultation. Thus, in the clinical orthopedic setting, the initial diagnosis continues to be made by plain X-rays due to cost-effectiveness and widespread availability and is classified according to Kellgren and Lawrence. In the majority of cases of end-stage three-compartment osteoarthritis, the indication to perform joint replacement is made without further performance of an MRI. However, there are different descriptions of the Kellgren and Lawrence classification, which differ in terms of their inter- and intrarater reliability for the classification of OA on plain X-rays. Morphological MRI allows for a more comprehensive evaluation of cartilage integrity and provides additional information on overall joint integrity. Hence, morphological MRI is increasingly used for the preoperative assessment as well, for instance, to assess the integrity of the cruciate ligaments in patients that may be eligible for unicompartmental knee arthroplasty. Although currently mostly limited to special centers and clinical studies, quantitative MRI that employs sequences sensitive to GAG content, collagen structure, and content may play a significant role in the subset of patients that present with an early osteoarthritis. In this patient cohort, it might enable the treating physician to monitor and quantify the loss of cartilage integrity that precedes morphological changes and assess the efficacy of emerging treatment options.

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3 MRI Relaxometry as Early Measures of OA

Xiaojuan Li, Carl S. Winalski, and Thomas M. Link

3.1 dGEMRIC: Delayed Gadolinium-Enhanced MRI of Cartilage

Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) quantifies the fixed charge density of cartilage, thus indirectly measuring glycosaminoglycan (GAG) content. Developed 25 years ago, it relies on MR image derived spin-lattice (T_1) relaxation measurements to show the equilibrium distribution of an ionic gadolinium chelate contrast agent, most commonly gadopentetate [$Gd(DTPA)^{2-}$], to reflect local cartilage GAG content [1–4]. At equilibrium, electrorepulsive forces of the contrast agent and the negatively charged carboxyl and sulfate groups of GAG result in more contrast agent in regions of low GAG content than in normal regions. Since MR contrast agents shorten T_1, damaged cartilage regions, with higher contrast agent, have a shorter T_1 than relatively healthy cartilage with lower local contrast concentration and higher T_1. Cartilage GAG concentration can be calculated from the change in T_1 after equilibration of the cartilage in $Gd(DTPA)^{2-}$ based on Donnan equilibrium theory [1, 5].

dGEMRIC has been applied to ex vivo cartilage samples and in vivo animal and clinical studies. Ex vivo, MR T_1 measurements are usually performed before and after soaking the samples in a $Gd(DTPA)^{2-}$ solution to equilibrium. The equilibration time depends on cartilage thickness, and full equilibration may take many hours [6]. Cartilage GAG concentrations calculated by dGEMRIC have been validated by direct biochemical and histologic measurements [1, 3, 7]. Correlations between dGEMRIC measurements and cartilage biomechanical properties have been shown [8–10]. Additionally, in an ovine model of femoroacetabular impingement surgery, dGEMRIC measurements correlated with Mankin histologic scores while there was no correlation between the cartilage T_2 and histological scores [11]. However, there are pitfalls to be considered with dGEMRIC, even with ex vivo studies. The contrast agent relaxivity, i.e., the strength with which the agent shortens T_1, may vary with tissue degeneration [12], and slow or variable diffusion rates into cartilage may cause uneven contrast equilibrium [6].

When performing in vivo clinical or animal dGEMRIC studies, the contrast may be injected either intravenously (IV) or intra-articularly (IA)

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with transport of the agent into the cartilage by diffusion. Following IA injection, enhanced joint fluid diffuses directly into the cartilage, while after IV administration, contrast distributes via synovial vasculature into the joint fluid of all joints. With IV contrast, diffusion into cartilage is mostly from the articular surface but also from subchondral bone. Equilibration after IA injection is slower since diffusion is only from articular surface. Although the significance of cartilage enhancement through subchondral bone is debated, uniform cartilage enhancement occurs more quickly following IV injection [2, 6]. With IV dGEMRIC, double or triple clinical dose is recommended for optimal differentiation of low GAG and normal cartilage [13]. Joint fluid, and thus cartilage, enhancement is greater if the imaged joint is moved through range of motion for 10 min immediately after injection to promote uniform distribution and equilibration while the serum contrast agent concentration is highest [14, 15]. The optimal delay between injection and MR imaging, i.e., the contrast "equilibration time," is cartilage thickness dependent. For thin hip cartilage, only 15–30 min delay following IV or IA injection has been recommended, while for knees, 2 h is suggested for the patella, but 45 min may be adequate for femoral cartilage [14].

In vivo quantification of cartilage GAG by dGEMRIC techniques, compared with ex vivo, have even more unknown and unmeasurable experimental variables potentially confounding measurements. Variations in postinjection contrast agent blood concentrations and delivery rates to the joints between patients as well as differential transport rates of contrast agent into cartilage may affect the cartilage concentration "equilibrium" [4, 14]. Unlike ex vivo experiments where a constant concentration solution bath is used, in vivo, the IV, joint fluid, and cartilage contrast concentrations change continuously with time and no true equilibrium state is reached. Only a "dynamic" cartilage contrast equilibrium can be achieved. As a practical matter, complete in vivo dGEMRIC imaging requires a long imaging session (pre- and postcontrast imaging and equilibration time) that most investigators find costly and inconsistent with patients' tolerance. Since nonsurgically treated cartilage has a long T_1 relaxation time with only small regional variations, most studies have excluded the precontrast T_1 imaging and reported only the "dGEMRIC index," i.e., the direct measurement of postcontrast cartilage T_1 [4]. The error caused by ignoring the local nonenhanced cartilage T_1 variations has been considered "probably not significant" [14].

Image acquisition options for T_1 quantification in dGEMRIC studies include: 2D inversion recovery fast spin-echo (IR-FSE), 3D IR-spoiled gradient echo (IR-SPGR) [16], 3D Look-Locker [17], and 3D variable flip angles with spoiled GRE imaging [18]. The reproducibility of the methods vary with superior results reported for 2D IR-FSE and 3D Look-Locker methods (RMS-CV: 5.8–8.4%) compared with the 3D variable flip angle method (RMS-CV: 9.3–15%) [18]. Since nearly all MR systems can perform at least one of these acquisitions, dGEMRIC is widely available, however, image postprocessing for the T_1 maps may require special software packages.

Clinical reports have shown the dGEMRIC index sensitive to early knee cartilage degeneration following acute anterior cruciate ligament (ACL) injuries (Fig. 3.1) [19, 20], and after meniscectomy [21]. Hip studies showed lower dGEMRIC indices with femoroacetabular impingement [22–25], suggesting early cartilage degeneration and an implied higher risk for osteoarthritis (OA) development. Several longitudinal studies showed that baseline dGEMRIC indices predicted radiographic OA changes in the knee [26, 27] and hip [22, 28]. A lower dGMERIC index of femoral cartilage at 2 years following anterior cruciate ligament rupture was found prognostic of both radiographic and symptomatic knee OA at 14 years [29]. The dGEMRIC index of patients with developmental hip dysplasia correlated with the severity of hip pain and deformity [30], and was better than other factors including age, radiographic arthritis severity, and dysplasia severity for prediction of surgical failure [31]. The dGEMRIC index of surgical cartilage repair tissue has correlated with clinical outcomes (Fig. 3.2) [32–34].

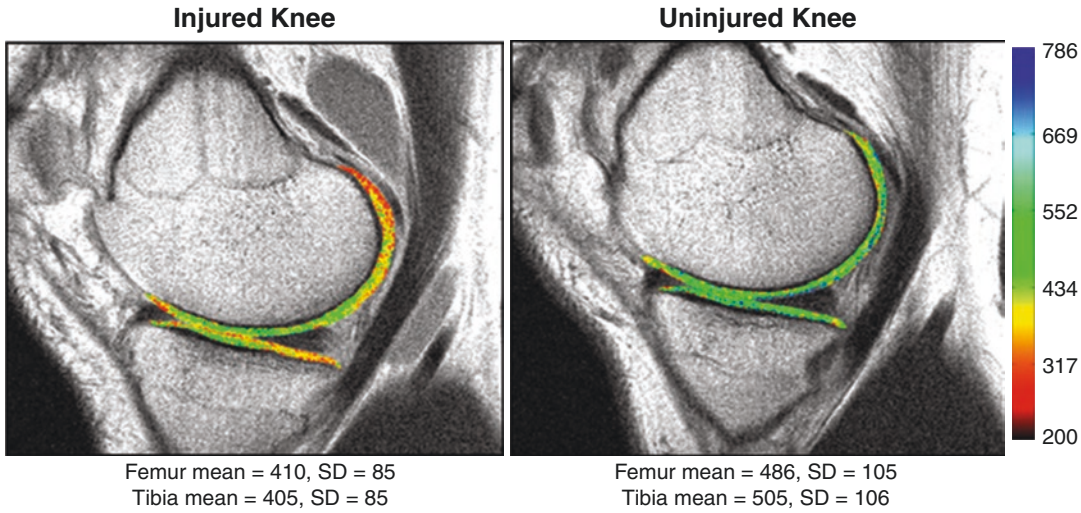

Fig. 3.1 The dGEMRIC index maps of medial compartments of an ACL-injured (left) and the contralateral uninjured knee (right). The blue and red regions denote high and low GAG concentrations, respectively. (Figure from Reference [19] with permission)

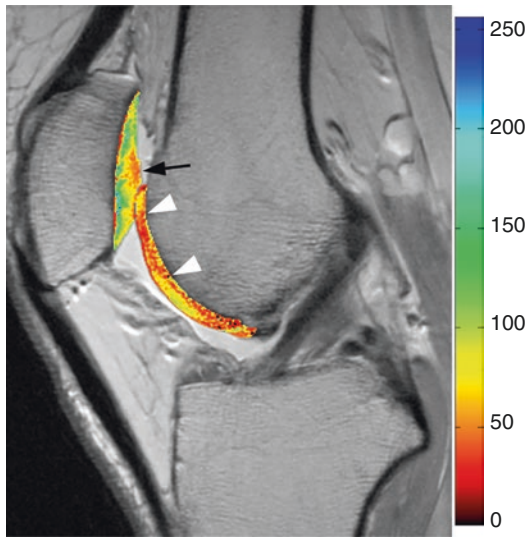

Fig. 3.2 dGEMRIC analysis of the patellofemoral compartment of a 40 woman 6 years following microfracture of the trochlea (repair marked with arrowheads) shows lower GAG content (lower dGEMRIC index, shorter T_1) of the repair tissue. Black arrow denotes a focal region of patellar cartilage degeneration

3.2 T_2 and T_2* Relaxation Time Measurements

The spin–spin relaxation time, T_2, is the process by which the transverse magnetization decays; this is caused by loss of phase coherence related to a preceding radiofrequency pulse. The T_2 reflects the ability of free water proton molecules to move and to exchange energy inside the cartilage matrix. Cartilage T_2 is primarily dependent on water and collagen content of the extracellular matrix as well as the orientation of the collagen fibers [35]. The lower the water content and the denser packed the collagen fibrils are, the faster is the T_2 decay and the shorter is the T_2 relaxation time. To measure T_2, images are acquired with different echo times (TE) and the signal is quantified in the cartilage matrix. A mono- or multi-exponential decay curve is fitted to the cartilage signal which provides the T_2 value.

There are multiple techniques for T_2 measurement with either gradient or spin echo sequences.

However, it is important to note that T_2 measurements obtained with different techniques and different scanners cannot be directly compared [36]. T_2 measurements were validated in a number of specimen studies. David-Vaudey et al. were among the first to compare T_2 measurements in cartilage-bone plugs from fresh cadaveric knees and specimens obtained after knee replacement with cartilage histology [37]. They found significant differences in T_2 values between normal and degenerated cartilage with a significant correlation between T_2 values and histological scoring based on toluidine blue and Masson Goldner stains [37].

While many studies have shown cartilage T_2 typically increases with the tissue degeneration [38], once cartilage degeneration is more advanced and associated with more substantial tissue loss, T_2 values may no longer correlate with the degree of degeneration [39], indicating that T_2 measurements may be less well suited for advanced disease stages.

Clinical studies in the knee have shown that cartilage T_2 measurements can predict the development of radiographic OA [40] and cartilage loss [41]. Liebl et al. [40] analyzed 50 knees with baseline Kellgren–Lawrence (KL) grade 0 that developed a KL grade ≥2 over a 4-year period and 80 control knees with KL grade 0 unchanged over the 4 years. The baseline cartilage T_2 in all compartments except the medial tibia were significantly higher in knees that developed OA compared with controls, in particular in the superficial cartilage layers ($P < 0.05$). Adjusted Odds ratios per 1 standard deviation increase in T_2 were 3.37 (95% CI 1.72–6.62) for the patella, 1.90 (1.07–3.39) medial femur, 2.17 (1.11–4.25) lateral femur, and 2.23 (1.16–4.31) for the lateral tibia. Joseph et al. developed a tool for OA prediction [42] based on 8-year data from the Osteoarthritis Initiative (OAI) (Fig. 3.3). Using clinical, radiographic, and MRI (including T_2 measurements) data, they could predict moderate to severe OA development over 8 years with an Area Under Curve value of 0.72, which is on par with the WHO fracture risk assessment tool (FRAX) widely used for osteoporotic fracture prediction [43]. They also developed a reference database for knee cartilage T_2 based on OAI subjects without MRI visible structural cartilage degeneration [44].

Investigators have used T_2 measurements for multiple clinical applications. Kijowski et al. [45] found that adding T_2 measurements to routine knee MRI improved the sensitivity cartilage lesion detection. Su et al. [46] found that higher baseline cartilage T_2 values in the femoral trochlea after ACL injury prior to surgical reconstruction were associated with worse Knee-injury and Osteoarthritis Outcome Scores (KOOS) after 1 year. Studies have found associations between knee pain and cartilage T_2 measurements [47, 48]. T_2 measurements were also used to investigate the impact of weight loss in obese and overweight individuals [49] and of diabetes on the cartilage matrix [50]. A recent systematic review and meta-analysis of cartilage T_2 measurements concluded that T_2 relaxation can distinguish those at risk for knee OA from healthy controls [51].

T_2* mapping has also been applied to analyze cartilage matrix changes [35]. T_2* is sensitive to change in T_2 as well as global and local inhomogeneity of the magnetic field. Alterations in T_2* due to microscopic inhomogeneity can reflect tissue structural properties and provide valuable information not present in T_2 measures. However, higher sensitivity to susceptibility artifacts and imperfect magnet shimming present technical challenges for T_2* imaging and complicate data interpretation. Unlike T_2 imaging, T_2* imaging uses gradient-echoes with small flip angles rather than spin-echoes which permits fast acquisition. This allows for 3D acquisition and higher spatial resolution with a shorter imaging time for T2* mapping.

T_2* values were reported to inversely correlate with the degree of hip cartilage degeneration as confirmed by histology [52]. In patients with femoroacetabular impingement, cartilage in regions with arthroscopically confirmed degeneration showed significantly lower T_2* as compared to normal appearing cartilage [53, 54]. However, elevated cartilage T_2* values have also been observed in OA in patients [55] and after ACL tears [56]. Such discrepancies, caused by either different imaging protocols used or the real differences in matrix changes associated with the

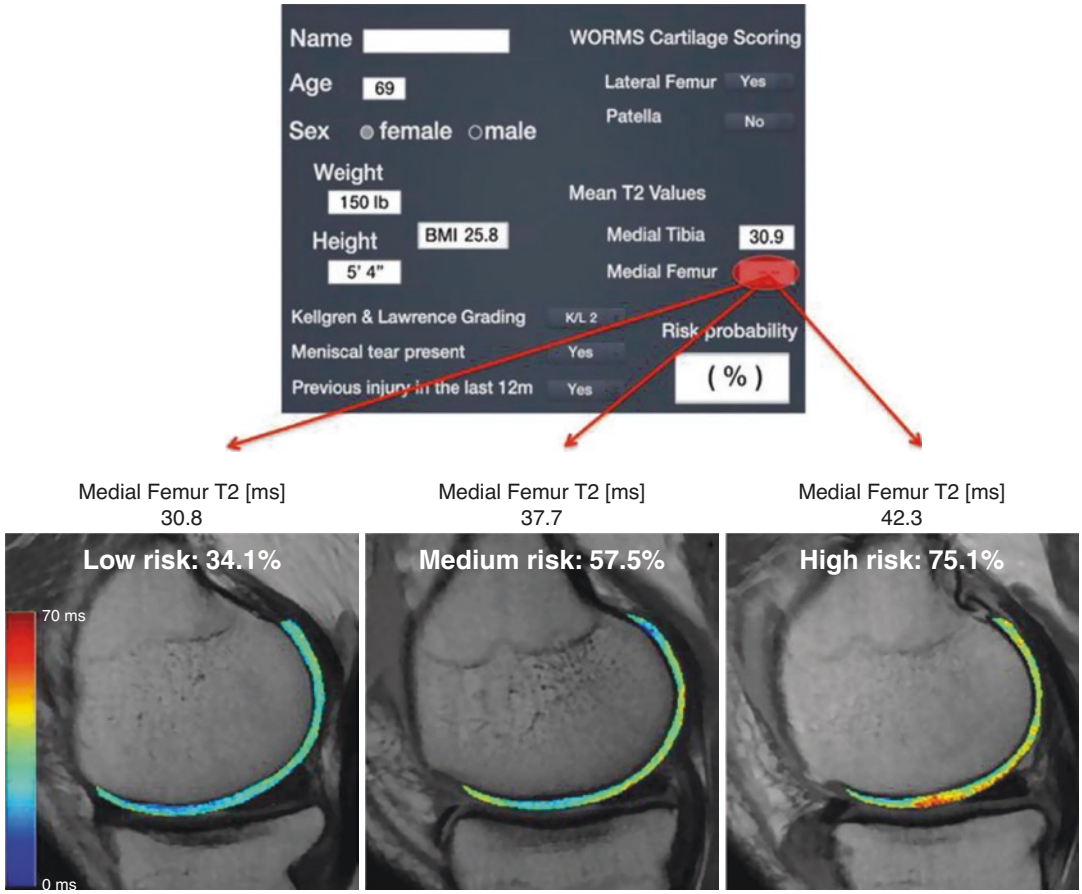

Fig. 3.3 A graphic of the Risk Score calculator developed by Joseph et al. is shown, which also demonstrates the impact of cartilage T_2 measurements on the OA risk prediction, while keeping the subject characteristics including Kellgren–Lawrence grade and Whole Organ Magnetic Resonance Imaging Score (WORMS) constant. As cartilage T_2 increases in the medial femur from 30.8 to 42.3 ms, the risk for moderate to severe OA increases from 34.1 to 75.1%. (Figure from Reference [42] with permission)

specific specimens/cohorts studied, present challenges when interpreting cartilage T_2*.

Combined with ultra-short TE (UTE) techniques, UTE-T2* imaging enables compositional evaluation of tissues with very short T_2 and T_2*, such as the deep cartilage layer, menisci, ligaments and tendons. Williams et al. reported the RMS-CV of in vivo UTE-T_2* measures in cartilage to be 8%, 6%, and 16% for full-thickness, superficial, and deep layers of medial femoral condyle cartilage, corresponding to absolute errors (SD) of 1.2, 1.5, and 1.5 ms, respectively [57]. Du et al. and Qian et al. identified multiple relaxation components using UTE T_2* in cartilage specimens, with short T_2* ranging 0.48 ~ 6 ms and long T_2* 22 ~ 35 ms [58, 59]. Elevated UTE-T_2* for cartilage and meniscus were reported after ACL injury and at 2 years after ACL reconstruction (ACLR) compared to uninjured controls [60, 61]. Over a 2-year period following ACLR, deep layer cartilage and meniscal UTE-T_2* decreased in subjects without meniscal tears, suggesting healing [60].

3.3 $T_{1\rho}$ Imaging

$T_{1\rho}$ relaxation time is the time constant of spin-lattice relaxation in the rotating frame, and can be measured by the spin-lock (SL) technique,

first described by Redfield [62]. $T_{1\rho}$ relaxation is sensitive to physicochemical processes with inverse correlation times on the order of the nutation frequency of the SL pulse (ω_1), which normally ranges from a few hundred to a few thousand Hertz. Thus, $T_{1\rho}$ is sensitive to macromolecular activities in biologic tissues occurring in this low-frequency range, including the collagen–proteoglycan matrix changes of cartilage degeneration. $T_{1\rho}$ increases with increased SL frequency (ω_1), a phenomenon termed as $T_{1\rho}$ dispersion, similar as T_1 dispersion. When no SL pulse is applied, $T_{1\rho}$ is equal to T_2; when the SL ω_1 increases towards the frequency of the main magnetic field, $T_{1\rho}$ approaches T_1. Thus, $T_{1\rho}$ is always greater than T_2, but lower than T_1.

In vitro studies have shown that $T_{1\rho}$ increases with proteoglycan depletion and correlates with histologic evaluation, and is more sensitive to GAG changes compared to T_2 [63–67]. $T_{1\rho}$ has also been correlated with arthroscopic evaluation [68, 69] and cartilage biomechanical properties [70, 71], suggesting $T_{1\rho}$ is a promising surrogate for functional cartilage evaluation.

Compared to T_2, cartilage $T_{1\rho}$ measurements are less sensitive to collagen fiber orientation with reduced magic angle artifacts because the SL pulse decreases dipolar interactions. The orientation dependency of $T_{1\rho}$ primarily diminishes when SL frequency >1 or 2 kHz [63, 72]. However, for clinical $T_{1\rho}$ imaging, the SL frequency is normally around 500 Hz and magic angle artifacts are present, but to a lesser extent than for T_2 measurement [63, 66, 73]. Matched regional analyses can be used to account for such effects. Adiabatic $T_{1\rho}$ imaging has shown less collagen orientation dependency than continuous wave $T_{1\rho}$ [74].

In a systematic review and meta-analysis, MacKay et al. reported excellent intra-observer, inter-observer and test–retest reliability of in vivo cartilage $T_{1\rho}$ and T_2 measurements, with most intraclass correlation (ICC) values >0.8 and coefficients of variation (CVs) <10% [75]. Gupta et al. reported the inter-observer ICC and the root mean square CV (RMS-CV) of subcompartment $T_{1\rho}$ quantification of 0.961 and 3.9%, respectively [69]. Using single vendor MR systems with identical sequences and parameters, the inter-site variation of $T_{1\rho}$ and T_2 were comparable or slightly higher than single site scan/rescan reproducibility (CVs in phantoms 2.8–6.9%) [76, 77]. Using 2D multiecho spin-echo T_2 techniques, Balamoody et al. reported inter-vendor mean T_2 differences from 5.4 to 10 ms (10~25%) in human knee cartilage [78]. A 3D $T_{1\rho}$ and T_2 mapping approach, MAPSS (magnetization-prepared angle-modulated partitioned k-space spoiled gradient echo snapshots) has been implemented on three major MR systems (GE, Philips, and Siemens), with mean inter-vendor inter-site CVs of 8.14% and 10.06% for $T_{1\rho}$ and T_2 values in human knee cartilage, respectively [79].

Numerous studies have shown elevated cartilage $T_{1\rho}$ values in subjects with OA [75], and in subjects at risk for OA [51]. $T_{1\rho}$ and T_2 relaxometry can discriminate between subjects with OA and controls ($P < 0.001$) with $T_{1\rho}$ being a better discriminator than T_2 for mild OA (standardized mean difference [SMD] [95% CI] 0.73 [0.40–1.06], $P < 0.001$) [75]. Atkinson et al. found subjects at risk for knee OA, i.e., those with knee injuries, low grade cartilage lesions, obesity, or in the OAI Incidence cohort, had a higher cartilage $T_{1\rho}$ in the femoral compartments, and a higher T_2 in all compartments [51].

Significantly elevated knee cartilage $T_{1\rho}$ and T_2 values have been observed after acute ACL injury and ACLR (Fig. 3.4) [80–82]. In a cohort of 40 patients with acute ACL tears, medial femoral cartilage showed the largest increase of $T_{1\rho}$ from 38.8 ± 2.3 ms preoperatively to 41.6 ± 2.9 ms 6 months after ACLR, corresponding to a standardized response mean of 1.1 for 6 months [83]. Greater cartilage $T_{1\rho}$ values have been cross-sectionally correlated with worse patient outcomes as evaluated with KOOS at 6 and 12 months after ACLR [84, 85]. Baseline $T_{1\rho}$ and T_2 values predicted patient outcomes at 6 and 12 months after ACLR [46]. Using $T_{1\rho}$ and T_2 as sensitive markers of cartilage health, researchers have identified factors that may contribute to posttraumatic OA (PTOA) development after ACLR, including meniscal injury [86], altered

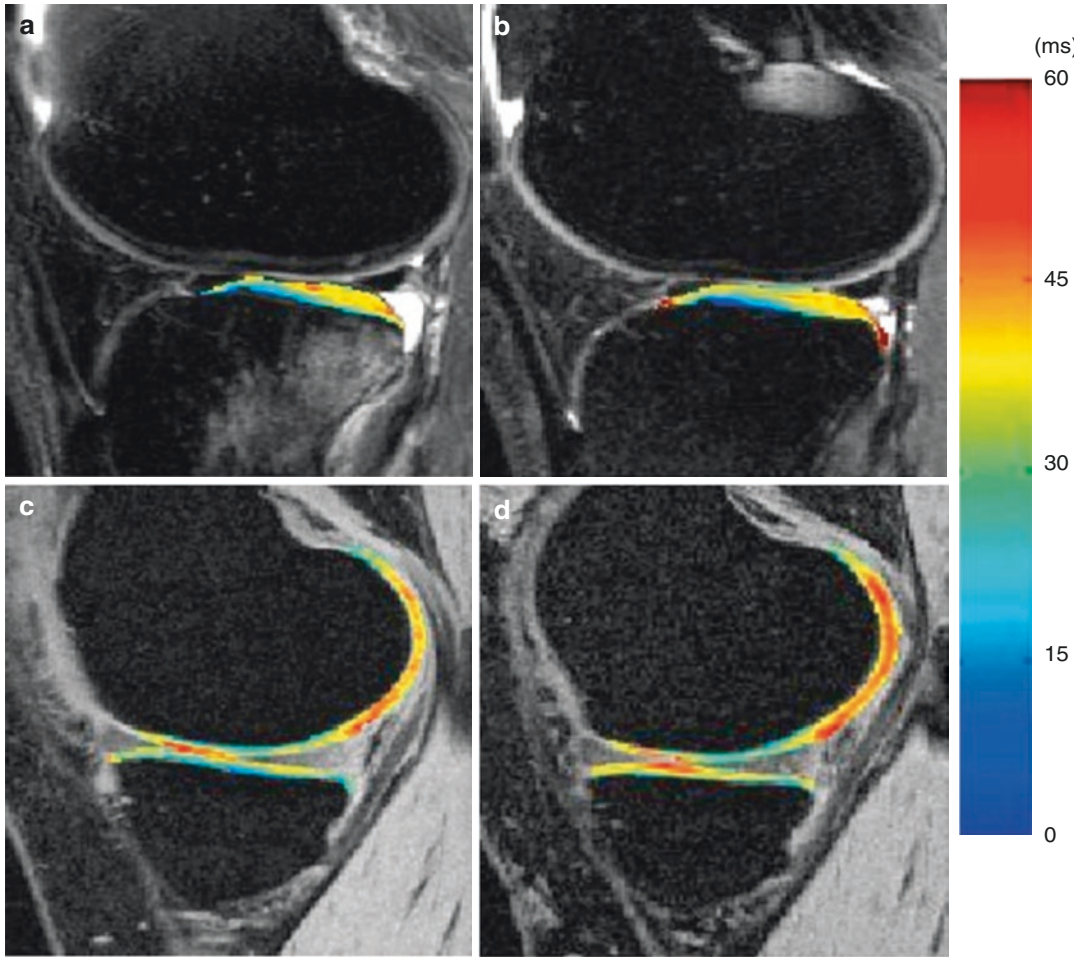

Fig. 3.4 $T_{1\rho}$ maps of (**a**, **b**) lateral and (**c**, **d**) medial sides of an ACL-injured knee at (**a**, **c**) baseline and (**b**, **d**) 1-year after ACLR. $T_{1\rho}$ values in posterior lateral tibia were elevated significantly in ACL-injured knees at baseline and remained high at 1-year follow-up despite resolution of bone bruise in the lateral tibia. $T_{1\rho}$ values in medial femoral condyle and medial tibia were significantly elevated in the ACL-injured knee at 1-year follow-up. (Figure from Reference [80] with permission)

biomechanics [87], synovial fluid inflammatory and cartilage breakdown biomarkers [88], muscle weakness [89], bone shape [90], and surgical factors [84, 91]. Thus, $T_{1\rho}$ and T_2 can serve as useful outcome measures for evaluation of novel interventions for slowing or avoiding the development of PTOA.

In addition to cartilage, elevated meniscal T_2 and $T_{1\rho}$ values have been reported in subjects with OA [92], meniscal tears [93], and ACL tears [94]. Recent studies showed feasibility of quantifying multicomponent $T_{1\rho}$ relaxation [95, 96] and $T_{1\rho}$ dispersion [97] in human subjects, which may provide more specific information regarding macromolecular changes in the cartilage matrix. Fast $T_{1\rho}$ imaging with novel reconstruction techniques will help reduce acquisition times without loss of accuracy [98, 99].

3.4 Summary

MRI relaxometry including dGEMRIC, T_2, T_2*, and $T_{1\rho}$ imaging provide valuable information on collagen-proteoglycan cartilage matrix changes that occur at early stages of OA. Such quantita-

tive measures may serve as promising imaging biomarkers for early detection, monitoring, and prediction of disease progression, and sensitive outcome measures in clinical trials of evaluating novel therapeutic strategies for OA. To date, however, these techniques are primarily used in research studies. Several factors are critical for clinical translation of MRI relaxometry for improved early diagnosis and prognosis of OA: (1) standardized acquisition protocols and multi-vendor multisite cross validation. The RSNA Quantitative Imaging Biomarker Alliance (QIBA) MSK subcommittee has worked on standardizing T_2 and $T_{1\rho}$ measurements for clinical and research use [100]; (2) fast imaging acquisition for incorporation into routine imaging protocols; and (3) standardized and automated postprocessing built into the clinical workflow. Synergized efforts between academic institutes, clinical sites and industrial partners are critical to move the field forward and to improve OA management with quantitative radiology.

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4 Epidemiology of Post-traumatic Osteoarthritis of the Lower Extremity: Premature Aging of Youthful Joints

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4.1 Epidemiology of Osteoarthritis

Osteoarthritis (OA) is a prevalent, costly disease. 45 million US adults over the age of 45 have radiographic evidence of OA [1–3]. More than 14 million individuals suffer from symptomatic, radiographic knee OA in the United States, and this number continues to grow as a result of the aging population and rising levels of obesity [2, 4]. The hip, with a 10% prevalence of symptomatic, radiographic OA in individuals over 45, and the hand (~7% prevalence) are also commonly affected [2, 5]. Osteoarthritis affects individuals across the globe with a worldwide prevalence of symptomatic OA estimated at 240 million people [6].

Several health conditions are associated with higher lifetime risk of OA [2, 6, 7]. Diabetes mellitus serves as an independent risk factor for the development of OA, with one study reporting that individuals with Type 2 Diabetes Mellitus (T2DM) have a 52% risk of developing OA, as compared to 27% among non-T2DM controls [8–11]. The prevalence of hypertension is twice as high among persons with OA as compared to matched controls [6]. In persons with body mass index greater than 30 kg/m^2, the lifetime risk of developing symptomatic knee OA increases to more than 60% [2, 12]. Gender also plays a role in OA development, with females having 25% greater risk than males for hand OA and 58% greater risk for knee OA [2, 5]. Further, symptoms and joint space narrowing are often more severe in women than in men [13].

Osteoarthritis typically interferes with daily activities and reduces quality of life. Indeed, 43% of persons with OA in the US experience limitations in activities of daily living and 9% are unable to work as compared to 5% of the general population [14, 15]. Consequently, OA gives rise to substantial direct and indirect costs. Updated to 2019 dollars, an estimated $112 billion dollars are spent each year treating OA in the USA, with indirect costs estimated at approximately $65 billion dollars [6, 16].

4.2 Overview of Mechanisms Linking Trauma to Osteoarthritis

It is estimated that 12% of cases of lower extremity symptomatic OA result from traumatic injury [17]. Traumatic injury can increase an individual's risk of OA by more than ten-fold. On average, patients with post-traumatic OA (PTOA)

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develop knee OA 10.4 years earlier, hip OA 9.0 years earlier, and ankle OA 14.0 years earlier than their primary OA counterparts [17]. The significantly higher risk and earlier age of onset of post-traumatic OA, as compared to OA from other etiologies, emphasizes the importance of injury prevention and the need for a clear understanding of the relationship between trauma and OA to inform prevention strategies (See Fig. 4.1 and Table 4.1).

Several mechanisms have been proposed to link acute injury to early onset of OA [18–21]. Articular cartilage is designed to distribute loading forces and to tolerate loads typical of daily activities. However, greater mechanical impacts may result in loading forces that exceed what the cartilage matrix can handle [21]. Previous research suggests that, in general, forces greater than 25 MPa can lead to joint damage, particularly when high loads are applied rapidly, which prevents the cartilage from redistributing the impact over a greater area [20].

Buckwalter et al. identify three types of articular cartilage injuries that may develop as a result of either acute or repeated mechanical trauma: (1) damage to the chondral matrix without disruption of the articular surface, (2) damage to the cartilage, and (3) damage to both the cartilage and subchondral bone [20, 21]. Even when the subchondral bone is unaffected, these injuries promote cartilage death, which in turn accelerates chondrocyte aging and matrix degradation, ultimately contributing to osteoarthritis [18, 22, 23]. Furthermore, injury may lead to altered mechanics, increasing the stress on articular cartilage [18, 22].

Chondrocytes can repair cartilage damage incurred from injury to some extent. But beyond that, the damage becomes irreversible [20]. Chondrocyte senescence, which results from increased metabolic stress on chondrocytes post-injury, decreases the ability of cells to maintain and repair damaged tissue, contributing to the risk of post-traumatic OA [18]. Healing and repair following injuries that extend into the subchondral bone are often incomplete. Chondral repair tissue is generally less stiff and more permeable than native articular cartilage, and more easily deteriorates over time [18, 21]. This results in a permanent defect, exposing the joint to greater mechanical loads that promote further degeneration [18, 21]. Additionally, intra-articular bleeding often associated with subchondral bone injuries has been shown to induce inflammation, which may contribute to the development of post-traumatic osteoarthritis [20, 23, 24]. Recent studies suggest that when activated, chondrocytes and cells in the synovium produce pro-inflammatory cytokines such as IL-6, IL-8, and tumor necrosis factor-alpha, which may result in apoptosis and abnormal expression of inflammation-related genes [25, 26]. This inflammatory response increases the risk of greater cartilage degradation and the onset of chronic disease; it may also exacerbate pain, swelling, and stiffness of the joint [24–26].

In addition to the joint damage arising directly from the impact of injury, the instability that may follow injury also contributes to post-traumatic OA. Two of the most common scenarios in which instability may result from injury include ACL rupture and meniscal tear, as discussed in the following section.

4.3 Post-traumatic Knee Osteoarthritis

Of the 14 million cases of symptomatic radiographic knee OA in US adults, 10% can be attributed to previous injuries, notably ACL or meniscal tear, each of which accounts for about 25% of serious knee injuries [27]. Prior knee injury renders an individual 4.2 times more likely to develop osteoarthritis [27].

4.3.1 ACL Injury

ACL rupture primarily affects young, physically active individuals, with particularly high incidence rates among individuals engaged in sports that require pivoting [22]. ACL rupture is often accompanied by meniscal tear, both of which can lead to decreased activity, worse knee function, and a substantially higher risk of knee OA [28, 29]. ACL tears are estimated to occur in 68.6 per

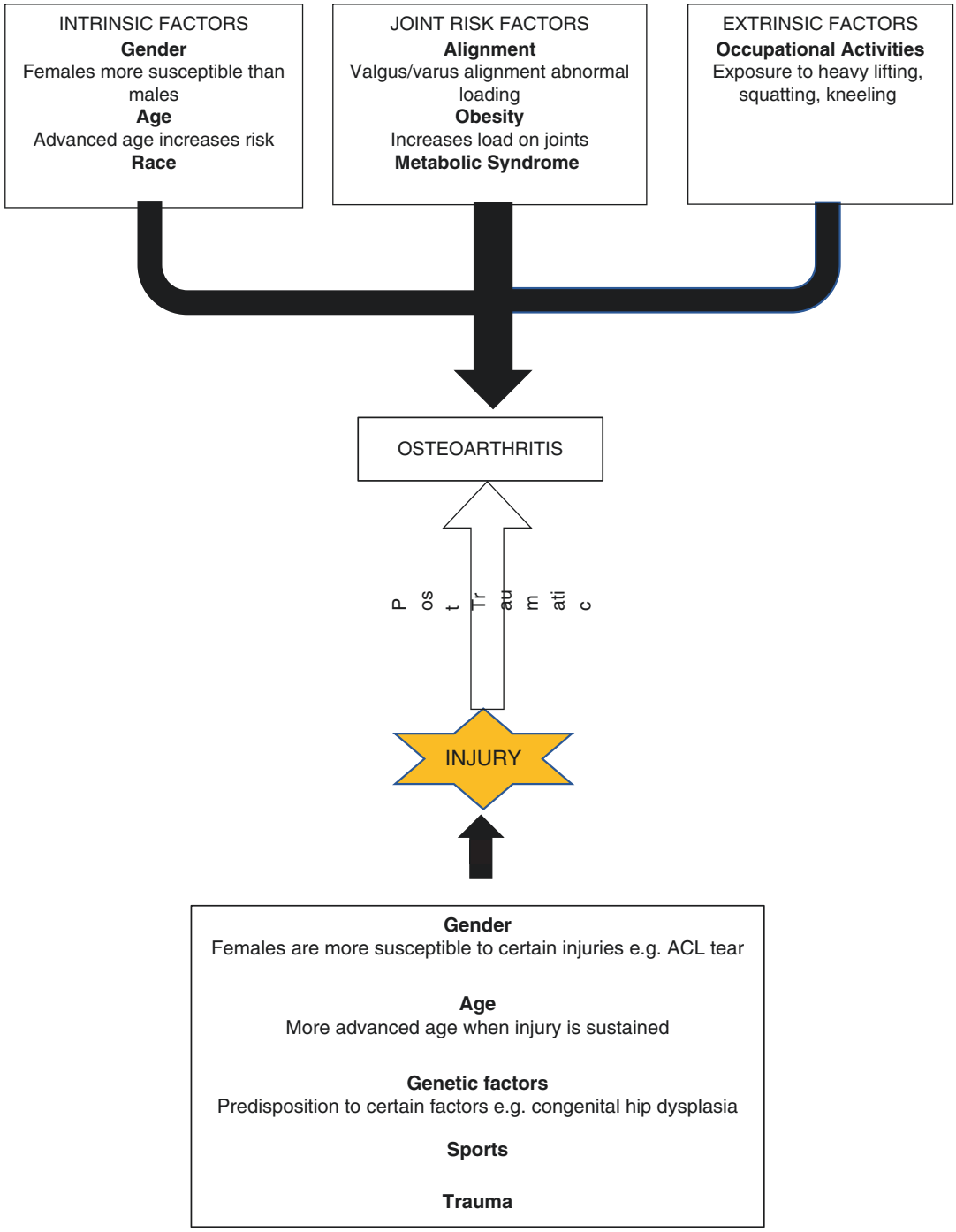

Fig. 4.1 Post-traumatic OA risk factors include risk factors for joint injury as well as risk factors for osteoarthritis (intrinsic, joint-level, and extrinsic). (Copyright by The Radiological Society of North America (RSNA®))

Table 4.1 Risk factors of post-traumatic OA

Risk Factor	Comments	References
All sites		
Gender	Females have a greater risk of OA than males – 25% greater risk for hand OA – 58% greater risk for knee OA	[2, 5, 14]
Diabetes	OA 2.0× more likely for Type-2 diabetes	[9–12]
Hypertension	OA 2.0× more likely	[7]
Body mass index ε 30 kg/m^2	Lifetime risk OA ~60%	[2, 13]
Knee		
Knee injury (all types)	Knee OA 4.2× more likely in those with knee injury history	[28]
ACL tear	40–50% of those with ACL tear developed radiographic OA within 14 years of tear – Females 3–5× greater risk of ACL tears than males	[23, 32, 33]
Meniscal tear	Knee OA more likely in those with meniscal tear (OR: 5.7)	[20]
Meniscectomy	Knee OA more likely in those with meniscectomy – RR: 4.8–9.8 (index knee) – RR: 2.6 (contralateral knee)	[30, 42]
Hip		
Acetabular fracture	48% of individuals developed OA within 2 years f/u	[57]
Femoroacetabular impingement (FAI)	Greater risk among athletes	[58–61]
Genetic factors	Including slipped capital femoral epiphysis and congenital hip dysplasia	[63, 64]
Ankle		
Type of ankle fracture	Risk of OA 70–80% in those with ankle fracture or severe acute ankle sprain/chronic instability	[65–68]
Extent of cartilage damage	Onset of OA after intra-articular fracture is much faster than extra-articular fracture (19.3 vs 32–50 years between fracture and OA onset)	[67]

100,000 person-years, with a cumulative incidence over 5% in persons between 10 and 64 years old [22]. Nearly 50% of individuals with ACL rupture and/or meniscal tear exhibit symptomatic OA within 10–20 years of injury [22, 30]. Given that the majority of individuals who tear their ACL do so before the age of 30, most of these individuals will be diagnosed with post-traumatic OA before the age of 50, which is, on average, 5 years younger than the median age of onset of OA in the general population (estimated to be 55 years old) [7]. Some studies have reported the age of post-traumatic OA diagnosis to be even earlier, with previously injured athletes showing signs of OA in their 30s [31, 32]. Lohmander et al. identified radiographic tibiofemoral or patellofemoral OA in 51% of female soccer players 12 years following ACL injury, as compared to 8% in the contralateral knee. These authors noted mild radiographic changes, defined as joint space narrowing grade or osteophyte grade ε1 in the patellofemoral and/or tibiofemoral compartments, in 82% of index knees versus 37% in the contralateral knee [31]. A similar study found that 41% of male soccer players who sustained tear exhibited radiographic OA 14 years after injury [32]. The majority of these study participants also experienced symptoms consistent with knee OA.

Overall, females are at high risk for ACL ruptures than males. A recent study noted that female athletes experienced a three- to five-fold greater likelihood of ACL tear than male athletes, likely due to gender-specific anatomical differences that influence landing mechanics, torsional stiffness, and joint laxity [22]. Several additional factors contribute to the predisposition to ACL tears among women including neuromuscular control deficits, which affect their lower extremity frontal plane when landing, preferential use—and

significantly greater strength—of quadriceps over hamstrings, as well as a weakened central core [33].

4.3.2 ACL Reconstruction Surgery

The indications for ACL reconstruction surgery are debated. Some studies, notably the KANON trial, suggest that patient-reported pain and functional status are similar among ACL-injured patients who undergo ACL reconstruction surgery plus structured rehabilitation as compared with those who undergo rehabilitation without surgery [28]. Among patients with ongoing objectively demonstrable instability following ACL tear, surgery is indicated. In the absence of subjective or objectively demonstrated instability, however, the treatment strategy is not clear-cut. When comparing individuals who underwent early ACL reconstruction surgery to those who received rehabilitation with the option of delayed surgery in a 5-year follow-up of the KANON trial, Frobell et al. found no statistically significant difference in the number of individuals who displayed tibiofemoral narrowing (KL ε 2) between the two groups. However, the proportion of subjects undergoing meniscectomy over the 5-year period was significantly lower in the early ACL surgery group as compared to the delayed surgical group, suggesting that reconstruction may reduce the risk of subsequent meniscal tear and ultimately improve knee stability [34]. Long-term follow-up will be required to determine whether reconstruction exacerbates or attenuates the development of osteoarthritis in persons with an ACL injury.

The lack of clarity about the effect of ACL reconstruction on OA incidence reflects the two primary proposed mechanisms, outlined above, by which injury leads to OA onset and progression. On the one hand, reconstructive surgery may further exacerbate the initial damage of the injury, setting the stage for OA. On the other hand, ACL reconstruction may reduce secondary instability due to ACL insufficiency, thereby minimizing the risk of future OA. These observations suggest that the management of persons with ACL tear should be directed both toward maintaining joint stability and minimizing the amount of damage to the joint.

As previously addressed, acute injury promotes the apoptosis of articular chondrocytes and the release of inflammatory mediators [23]. Surgery and associated intra-articular bleeding may prolong the inflammatory response [22]. However, surgery can reduce instability, restore the original mechanics of the knee, and diminish the force translated onto the cartilage, thus minimizing the pressures that lead to cartilage degeneration [35]. Furthermore, reconstruction may protect against subsequent meniscal tears, subchondral injury, and reoperation [35, 36]. In a study comparing the risk of reinjury between conservative and surgical treatment, Dunn et al. found that ACL reconstruction was associated with a 60% lower risk of subsequent reoperation, defined as any surgical procedure to treat meniscal or cartilage injury occurring more than 6 weeks after the index case (32.6% operation rate among previously conservatively treated subjects versus 12.7% reoperation rate for surgically treated subjects) [36]. More specifically, as compared to those treated non-operatively, persons treated with ACL reconstruction had a 56% reduction in lateral meniscal reoperation, 42% reduction in medial meniscal reoperation, and 35% reduction of surgery to repair cartilage injury [36]. These data beg the question of whether the mechanical stability gained from surgery outweighs the further injury that may result from surgery. With over 200,000 ACL surgeries performed each year in the USA, amounting to more than $3 billion annually, it is critical to continue to investigate the role of ACL reconstruction in the initiation and development of osteoarthritis and to identify individuals for whom surgery is most appropriate [28, 37].

4.3.3 Meniscal Tears

Meniscal tears may also contribute to the onset of radiographic and symptomatic osteoarthritis. Unlike ACL ruptures, which are almost always the result of traumatic injury, meniscal tears can

result either from trauma (typically in younger patients) or from degenerative changes (typically arising insidiously in older patients). Meniscal tears resulting from trauma to a healthy knee may lead to the development of osteoarthritis through joint damage and altered loading pattern mechanisms [29]. This mechanism mirrors the process involving ACL injury; in fact, often times, meniscal tears appear in concert with ACL tears [38–40].

Alternatively, a degenerative meniscal tear, which is associated with higher rates of symptomatic OA, typically is accompanied by other degenerative changes [29]. In these instances, failure of damaged cartilage to bear load adequately places greater stress on the meniscus, causing it to fail structurally. Because the meniscus contributes to joint stability, redistribution of loads, shock absorption, and cartilage lubrication, meniscal damage can exacerbate cartilage damage [29]. Thus, degeneration of cartilage can damage menisci while degeneration of menisci can also damage cartilage and lead to further progression of OA [29].

Meniscal tears are relatively common, with a cumulative prevalence of symptomatic meniscal tear between the ages of 10 and 64 of at least 15%, 2.5-fold greater than the cumulative incidence of ACL tear [22]. Meniscal tear contributes substantially to the overall incidence of post-traumatic knee OA, even when treated nonsurgically [19, 22]. In one study of knees with normal (KL 0) radiographs at baseline, 55% of those with meniscal tear at baseline transitioned to KL grade ε 2 over 30 months as compared with 19% of those with normal menisci at baseline [19]. Based on these findings, the adjusted odds of developing radiographic tibiofemoral OA with meniscal tears, as compared with knees without tears, is 5.7 (95% confidence interval 3.4–9.4) [19].

Undergoing either a partial or total meniscectomy also increases the risk of developing OA, not only in the index knee (RR: 4.8), but also in the contralateral knee (RR: 2.6), likely due to altered mechanics [29]. For example, Englund et al. showed that between 43 and 48% of knees that underwent surgical intervention (including partial and subtotal meniscectomy) and 27–29% of contralateral, unoperated knees demonstrated tibiofemoral radiographic OA 15–22 years after meniscectomy. Over the same period, just 9% of knees in persons who had no surgery developed radiographic OA [29, 41]. Others have reported the relative risk of developing mild radiographic changes (KL grade ε1) to be 14-fold (95% CI: 3.5–121.2) higher in persons who underwent meniscectomy than in age- and sex-matched controls without tears. Similarly, the risk of developing OA with KL grade ≥2 was 9.8-fold higher (95% CI: 3.5–37.6) in persons with the previous meniscectomy in comparison to age- and sex-matched controls [42]. In ACL-injured individuals, those who underwent meniscus surgery displayed even higher rates of radiographic rates of knee OA (69% versus 39% who did not have meniscus surgery) [31].

Currently, there are no approved disease-modifying osteoarthritis drugs. In addition to lifestyle modifications, several surgical strategies have been proposed in the last several decades to mitigate or delay the onset of osteoarthritis. The low-cost, single-stage microfracture (MF) procedure is performed most commonly in this setting. Several trials have investigated autologous chondrocyte implantation (ACI), a more costly two-stage operation, to manage post-traumatic or degenerative cartilage lesions [43–46]. There is conflicting evidence on the efficacy of ACI as a potential avenue to regenerate damaged cartilage [47–52]. In a randomized trial comparing ACI with MF, Knutsen et al. found no statistically significant differences in Lysholm and VAS pain scores between the two treatment groups at 2–, 5–, and 14–15 years follow-up [48–50]. Furthermore, both the ACI and MF groups exhibited high failure rates, defined as individuals who required reoperation due to persistent symptoms after the original cartilage defect repair (32.5% for MF, 42.5% for ACI) [49]. However, data from the 2-year follow-up of the SUMMIT randomized control trial suggest statistically significant improvement in KOOS pain

and function scores in individuals who underwent matrix-induced autologous chondrocyte implantation as compared to MF [51]. Further investigation is necessary to determine whether these treatment options prove to be clinically and radiographically effective both in the short- and long-term.

The use of joint stabilizing braces or implants may also help to reduce the risk of OA and the symptoms associated with OA [53]. In the knee, unloading braces and unloading implants serve to transfer load away from the affected compartment, alleviating stress on the cartilage and improving the biomechanics of the knee. Preliminary trials of unloading implants have been promising and may provide a minimally invasive alternative to osteotomy [43, 54].

While the above treatments may help to attenuate osteoarthritis, the most straightforward solution to avoid post-traumatic OA is injury prevention. In recent years, neuromuscular training has proven effective in reducing primarily lower extremity injuries, most notably ACL rupture. A meta-analysis reported by Donnell-Fink and colleagues in 2015 suggests that neuromuscular and proprioceptive training do indeed protect against general knee injury (27% reduction in injury rate) and specifically against ACL injury (51% reduction) [55]. ACL injury often results from landing or pivoting on an extended hip or knee when unbalanced. Neuromuscular training offers a combination of power, strength, balance, and coordination exercises that can improve joint stability and teach athletes to land and maneuver with control [33, 56]. In the event that injury has already occurred, this training technique may also help an individual to regain motor control and proprioception after ACL reconstruction [56]. In view of the high risk of reinjury after initial ACL rupture (25% of individuals experience a second rupture after ACL reconstruction), neuromuscular training has an important role both in rehabilitation (secondary prevention) and in primary injury prevention [33, 56]. These training programs should be implemented at an early age and maintained into adulthood [33].

4.4 Post-traumatic Hip Osteoarthritis

Post-traumatic hip OA accounts for 2% of all cases of hip OA [27]. Tveit et al. found elite male athletes to be twice as likely to develop hip OA and 2.5 times as likely to undergo hip arthroplasty, as compared to controls [57]. These greater risk ratios were primarily driven by impact sports [57]. Several specific entities appear to link trauma to hip OA, as discussed below.

Acetabular fractures are one of the leading causes of post-traumatic hip OA and are becoming increasingly common due to a growing incidence of car crashes [58]. In a study conducted by Cahueque et al., 48% of individuals with one of these complex, high-energy fractures developed post-traumatic OA within 2 years of follow-up [58]. Furthermore, complicated fracture patterns, along with the type of fracture (isolated posterior wall fractures or those coupled with transverse fractures) are more commonly associated with PTOA. Poor fracture reduction (non-anatomic as opposed to anatomic reduction) is also associated with a higher risk for PTOA development [58].

Femoroacetabular impingement (FAI), which arises from abnormal contact between the femur and acetabulum, may also increase the risk for the development of hip osteoarthritis [59]. In general, cam-type deformities, as opposed to pincer or mixed deformities, result in the premature development of hip OA [60]. There is currently insufficient evidence to determine whether FAI arises from genetic differences, participation in high-impact sports, or a combination of both, although arguments have been made for both etiologies.

The stress of high-impact sports on the hip socket likely also contributes to the development of FAI. Athletes, especially collegiate football players and professional hockey players, exhibit especially high rates of impingement, with one study reporting a three-fold increase in femoral tilt deformities, the most common femoral head deformity in hip OA, in athletes as compared to controls [59, 61, 62]. Likely, the “repetitive

microtrauma" that occurs during high-impact sports, especially as the growth plate closes during adolescence, causes the formation of reactive bone and cam lesions. This impingement may consequently lead to structural damage, including labral tears, thereby increasing the risk of developing hip OA [59, 60].

Other risk factors associated with the development of OA in younger individuals include slipped capital femoral epiphysis and congenital hip dysplasia, both of which can result from genetic factors and contribute to cartilage degradation [63, 64].

4.5 Post-traumatic Ankle Osteoarthritis

Although osteoarthritis of the ankle occurs in less than 1% of the population, OA develops in 70–80% of subjects sustaining ankle fracture or severe acute ankle sprain or chronic instability [65–68]. By contrast, the proportion of OA due to trauma is estimated at just 10% for the knee and 2% for the hip [2, 5, 27]. These higher rates in the ankle likely occur because of the ankle's structure: the cartilage within the ankle is much thinner and stiffer than that of the knee or hip and is, therefore, unable to adapt to contact stresses and changes to the articular surface [65]. With more individuals engaging in high-risk sports and active recreational activities, in addition to an aging population, the incidence of ankle fractures is rising [67]. This growing prevalence of ankle-related post-traumatic osteoarthritis is associated with a lower quality of life for more individuals because ankle injuries often result in long-term negative effects on pain and function [66].

Several important factors contribute to the risk and severity of post-traumatic ankle OA, the most pertinent being the type of ankle fracture and the extent of articular cartilage damage [69]. The two most frequent ankle fractures leading to OA are pilon fractures (29% of cases), which are caused by high-energy trauma from axial compression, and malleolar fractures (53% of cases) [67, 70, 71]. Alternatively, OA develops less frequently (about 10% of the time) following tibial, fibular, and talus fractures. The average latency time between injury and end-stage ankle osteoarthritis is approximately 41 years for tibial shaft fractures and 9 years for talus and combined fractures [67].

The difference between extra- and intra-articular fractures also influences the time to onset of osteoarthritis. While osteoarthritis often develops between 32 and 50 years following an extra-articular fracture, the interval between fracture and OA onset is nearly half that for intra-articular fractures (19.3 years) [67]. All intra-articular fractures can be defined as pilon fractures, and as such, are the result of high-intensity forces. By contrast, extra-articular fractures are often less severe, generally resulting from low-energy rotational or axial loads [72]. The occurrence of complications, such as non-union or osteomyelitis, during the healing process as well as fractures sustained at older ages have also been shown to be associated with worse outcomes and shorter time to the progression of OA [67].

4.6 Summary

Osteoarthritis is a serious, costly disease and is increasingly prevalent as a result of an aging population and higher rates of obesity. Approximately 12% of lower extremity symptomatic, radiographic OA can be attributed to traumatic injury, accounting for 10% of all cases of symptomatic, radiographic knee OA, 2% of cases of hip OA, and 70–80% of cases of ankle OA. Not only does traumatic injury increase the risk of developing OA, but also often leads to a more rapid progression, with persons presenting with post-traumatic OA several years earlier than those who develop OA from other etiologies. ACL and meniscal injury are most salient injuries leading to knee OA. Further research is necessary to better understand the role of ACL reconstruction surgery in the development of post-traumatic osteoarthritis and the balance between the risk of further structural damage and the need for greater stability. Given that there are currently no treatments available to

reverse joint degeneration and the onset of OA, the most effective prevention strategy for post-traumatic OA is to prevent injury (often the result of high-impact trauma) to the knee, hip, and ankle joints, as well as the soft tissue structures that surround them.

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5 Economic Aspects of Early Osteoarthritis

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5.1 Introduction

In this chapter, we will discuss osteoarthritis and its current economic implications in the United States. With the emergence of a younger population being diagnosed with osteoarthritis, we will also explore economic impacts specific to early osteoarthritis. Next, we will discuss the effect of socioeconomic factors on the development of early osteoarthritis. Finally, we review the current literature on the cost-effectiveness of novel surgical interventions for early osteoarthritis.

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5.2 Current Economic Burden of Osteoarthritis

Currently, articular cartilage injury is one of the most common and debilitating musculoskeletal conditions in the United States [1, 2]. Likely in part due to an aging population and an increasing rate of obesity, the annual prevalence osteoarthritis (OA) is increasing at a rapid pace [3]. Recent studies have estimated that between 27 and 31 million people are currently afflicted with osteoarthritis in the United States [4], with hip and knee osteoarthritis accounting for 17 million disability years [4]. Commonly referred to as "wear and tear" arthritis, osteoarthritis is thought to normally affect older adults who have accumulated years of repeated stresses that, eventually, contribute to joint aging and degeneration. Indeed, age seems to be the most significant independent risk factor for developing osteoarthritis [5, 6]. However, several other risk factors such as obesity, prior joint trauma, and developmental abnormalities may predispose certain individuals to early onset development osteoarthritis.

Arthritis places significant impact of the medical system, and the burden on society will only increase in the immediate future. In 1995, 6.5 million of visits were directly related to arthritis, as well as 265,000 Emergency Department visits, and 619,731 outpatient hospital visits [7]. In addition, those who were hospitalized spent in excess of 2.3 million days in inpatient care [7]. The National Health Interview

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Survey estimated in this year, 1995, than more than 20 million people in the United States had arthritis [8] with a prevalence rate of approximately 125 per 1000 persons [7]. As of 1999, patients with arthritis will visit the doctor 39 million times and be hospitalized more than half a million times per year [9]. Total costs of care for patients with arthritis in the year 1999 accounted for approximately 1.1% of the Gross National Product in the United States [9]. Direct medical costs associated with arthritis were about $15 billion, not accounting for the additional $65 billion in secondary disability and debilitating costs caused by loss of working aged individuals due to arthritis [9]. In such a short time period, there was an increase in the economic burden of arthritis on society, which will only continue to worsen. Census data predict that by the year 2030, the total US population will increase by 28%, the population aged 55 or older will increase by about 10%, and the population of those aged 65 years or older will nearly double [10]. Especially worrying is the amount patients with symptomatic arthritis pay for managed care plans, versus those without arthritis. A 20,000 subject study found that patients with symptomatic arthritis pay direct medical costs at two times the cost of those without arthritis after being matched for age, gender, and insurance claims [9]. On top of those direct costs, those with arthritis typically need special care which is not covered by insurance, including medication, home modifications, personal care, and transportation [11]. The costs of care for patients with arthritis, both direct and indirect, is a serious issue which needs to be addressed in order to lessen the economic burden on society in the near future.

5.3 Special Issues Associated with Early Osteoarthritis

In young patients, the development of osteoarthritis can be classified as either idiopathic or post-traumatic [12]. Idiopathic osteoarthritis in a young patient is rare, as idiopathic osteoarthritis develops through repeated wear and subsequent degeneration of articular cartilage. Normally this process occurs over many years; however, specific occupations and individual risk factors may accelerate this process. Post-traumatic osteoarthritis (PTOA) develops after an acute joint injury, such as a fracture, ligament tear, or articular insult. Most notably, several studies have linked anterior cruciate ligament (ACL) injury to the development of OA, with some studies reporting the incidence of PTOA following ACL injury to be as high as 87% [12, 13]. The exact mechanism of PTOA is currently not well understood and is thought to multifactorial in nature.

The increase in incidence of osteoarthritis in younger patients introduces an additional economic concern. Unlike older patients who are likely retired by the time they develop osteoarthritis, the development of osteoarthritis in younger adults leads to a likely loss of economic productivity, due to the pain and mobility limitations in younger populations. Early withdrawal from the workforce due to arthritis, along with a subsequent increased utilization of medical care, represent a potentially significant and growing societal burden in the United States. Previous studies have established that the development of arthritis increases one's risk of being out of the labor force by 64% [14]. The United States Bone and Joint Initiative (USBJI), in their 2016 Burden of Musculoskeletal Diseases report, found that from 2013 to 2014, adults of osteoarthritis reported 180.9 million total lost work days, which represented 34% of total lost work days for any medical condition [15]. There is also evidence that direct medical costs associated with osteoarthritis are higher for younger patients than older ones. MacLean et al. reported that direct medical costs attributable to osteoarthritis and associated comorbidity was $2827 a year for patients under the age of 65, compared to $1963 per year for patients older than 65 in 1991–1993 [1, 16]. Patients who develop osteoarthritis at a younger age must live with the debilitating effects of the disease for far longer than older patients with osteoarthritis, incurring hefty cumulative costs over a lifetime.

In 1980, 101,235 total knee arthroplasties (TKAs) were performed in patients under the age of 50 [17]. In 1990, that number increased to 137,673 [17]. In 2000, 158,108 TKAs were performed in patients under the age of 50 [17]. In

2010, an estimated 174,170 TKAs were performed on people younger than age 50 [17]. The definitive growth of younger populations who suffer from osteoarthritis of the knee creates novel concerns from both a medical and an economic viewpoint. Considering that in 2004, the median hospital charge for a primary TKA was $29,509 [18], the medical burden of osteoarthritis can be expected to significantly increase in the future.

Similar to the incidence of knee osteoarthritis, the incidence of hip osteoarthritis in younger populations in the United States is growing, perhaps at an even more rapid rate. In 1980, 71,175 total hip arthroplasty (THA) was performed in patients under the age of 50 [17]. In 1990, that number increased to 145,298 [17]. In 2000, 150,203 patients under the age of 50 underwent THA [17]. Finally, in 2010, an estimated 222,276 THA were performed on people younger than age 50, a greater than 200% growth over 30 years [17]. This exponential increase in THA is likely to continue in the future, with experts predicting a significantly increasing demand for total hip arthroplasty for younger patients by 2030 [19]. A 2009 analysis predicted that, by 2030, over 52% of primary THAs and 62% of primary TKAs will be performed on patients less than 65 years old [20]. This increased demand for THAs in younger patients will likely result in a significantly growing medical burden, considering the median hospital charge for a primary THA of $32,571 [18].

Currently, there is a lack of research on the economic impact of hip osteoarthritis in young adults in the United States. However, a study performed by Gupta et al. examined the economic burden of both hip and knee osteoarthritis in patients living in Ontario, Canada. The study found that younger age was an important predictor of employment-related indirect costs, making up 32.9% of total economic burden for patients less than 65 years old [21]. Understandably, younger patients who cannot perform their job due to severe pain and restriction from hip osteoarthritis are more likely to experience loss of wages and more significant financial losses compared to older, retired patients.

5.4 Socioeconomic Characteristics of Patients with Early Osteoarthritis

Risk factors for early arthritis include gender and socioeconomic status (SES). SES is a general measure for an individual's social and economic position relatively to others, and usually encompasses factors such as occupation, education, and income. Women tend to have early osteoarthritis had nearly twice the rate men do in the United States [11]. There is also a strong correlation between SES and the prevalence of osteoarthritis. Lower SES is greatly associated with higher rates of osteoarthritis with an inverse relationship as SES increases. In addition, less than 12 years of education and lack of a support system are also associated with higher rates of osteoarthritis [11].

As a surrogate reflection for the impact of early osteoarthritis, total knee arthroplasty due to osteoarthritis is a prevalent procedure performed in the United States that will only continue to grow in popularity [22]. A multitude of studies have been conducted, both retrospectively and prospectively, to investigate the effects of socioeconomic factors on likelihood of total knee replacements later in life [3, 4, 6, 23–33]. Socioeconomic factors include level of income, community, occupation, education, lifestyle, among others. Trends among demographic such as race, ethnicity, gender, etc., are also important when identifying potential risks for knee osteoarthritis. These socioeconomic traits are often related to body mass index, occupation risks and benefits, community poverty, and disability. All of these factors were investigated to find relationships between SES and the rates of total knee replacements.

5.4.1 Socioeconomic Distribution

The most important factor socioeconomic factor is the distribution of income and wealth. Evidence in recent years has shown that lower levels of SES are associated with higher cases of radiographic and symptomatic knee osteoarthritis in the developed world. In Australia, there was an

overall decrease of total knee replacements as SES increased according to the Index of Relative Socioeconomic Advantage and Disadvantage (IRSAD) [27]. In Spain, there is an incidence rate of knee osteoarthritis of 206.7/10,000 persons-year in the most disadvantaged communities. In contrast, the rate was 133.3 persons-year for individuals in the least disadvantaged communities [28]. As hypothesized in recent studies, this disparity could be because of difference in occupation, as well as access to other forms of care and medical literacy which typically come with higher SES. Callahan et al. explored how community poverty affected rates of knee osteoarthritis as part of the Johnson County, NC project [29]. The authors discovered that communities with a poverty level of greater than 25% correlated significantly with knee osteoarthritis [29]. In addition, African American communities were more likely to be suffering from these communal factors. There is evidence to show that obesity, injury, malalignment, childhood SES, and childhood abuse are linked to knee osteoarthritis later in life [30]. Other factors affecting development of knee osteoarthritis are diet and psychological factors. Direct correlations between disability and anxiety, fatigue, helplessness, and depression have been found in lower income communities [29, 31]. These psychological factors, in addition to low income and wealth, often lead to poor diet and/or obesity [31, 34]. In contrast, years of formal education, self-efficacy, and perceived quality of life were inversely correlated to disability and symptomatic knee osteoarthritis [31]. SES and wealth are often highly associated with education and types of jobs, as well, which can have a significant impact on the prevalence of knee osteoarthritis.

5.4.2 Education

Another important socioeconomic factor related to incidence of knee osteoarthritis in the general population is educational attainment. Several studies have reported a significantly higher incidence of knee osteoarthritis in populations with low levels of education [29, 32, 35]. Individuals with educational attainment of less than 12 years also scored higher on the Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire than those with more than 12 years of formal education at a significant level [33]. The WOMAC scale uses subsets of function, pain, stiffness, and total to identify both broad and specific effects of osteoarthritis. Data from the Johnson County, NC Osteoarthritis Project indicates that among those with less than 12 years of education, women are even more likely to have symptomatic and radiographic knee osteoarthritis. Levels of education can also impact the readiness for an individual to seek treatment and medical literacy. In Sweden, statistics show that individuals with low levels of education have higher rates of knee replacements; however, those with higher levels of education had knee osteoarthritis diagnosed at an earlier age on average [23]. This implies that those with higher levels of education are quicker to seek out care for their pain, while individuals with lower levels of education may choose to live with their pain. Although access to health care is available to all in Sweden, education and SES still play a deciding role in the care and risks of total knee replacements. Another study performed in Sweden investigated the differences between a guided treatment named “Better Management of Patients with Osteoarthritis” (BOA) and the reference group. It found that those in the BOA group had a higher level of SES, including educational attainment. Due to the extensive care involved in the BOA (training from a physical therapist, a self-management program, and an evaluation if they were recommended), it was harder to reach the lower SES individuals [24]. This may be because of the time involved in this type of treatment, self-efficacy, or the medical literacy that is associated with higher SES. Those with a bachelor’s degree or higher are also more likely to seek out physical therapy for knee osteoarthritis [25]. Education ultimately leads to the kind of job or profession one enters, which has a significant impact in possibly developing knee osteoarthritis leading to total knee replacements.

5.4.3 Work Status

Many studies into the correlations of SES and knee osteoarthritis investigated the associations of certain kinds of occupations. Nonmanagerial positions showed associations with higher scores of WOMAC function, pain, and total [33]. On a broad scale, those with manual labor/industrial jobs which involve continuous, repetitive motions have higher rates of knee osteoarthritis, both radiographic and symptomatic [36]. Occupations which are very physically demanding are associated with knee osteoarthritis after 10 or more years of exposure to those tasks [37]. For radiographic knee osteoarthritis, there was a significant correlation with sitting, climbing steps, daily lifting/carrying for women, and kneeling/squatting [26, 36]. In addition, there were multiple other activities which were linked to symptomatic osteoarthritis: walking, lifting greater than 10 lb, standing, crawling, and doing heavy work while standing [36]. Gender can also have an effect on which occupations have higher risks for knee osteoarthritis. In men, construction workers/masons, electricians, plumbers, service workers, and agricultural workers were at higher risk for knee osteoarthritis [37–39]. In women, cleaning services, caretaking, assistant nurses, and kitchen workers were at higher risk for knee osteoarthritis [37–39]. Occupational hazards and tasks linked to 12 years or less of education were significantly associated to knee osteoarthritis in both genders. In women, there is a significantly higher risk of symptomatic and radiographic knee osteoarthritis, while in men there is an 85% higher chance of symptomatic knee osteoarthritis [29]. This data should be used to make significant safety measures for the longitudinal health of individuals of all socioeconomic statuses to help prevent the development of knee osteoarthritis later in life.

5.5 Cost-Effectiveness of Treatments for Early Osteoarthritis

5.5.1 Knee

Degenerative knee osteoarthritis in younger adults has historically been rare, but its incidence in the United States is increasing. A study performed with epidemiological data from 2007 estimated the median age of a knee osteoarthritis diagnosis to be 55 years [40], compared to an average age of diagnosis of 69.4 in 1991 [41].

The economic burden of OA can be divided into arthroplasty and nonarthroplasty treatments. In terms of nonarthroplasty treatment options, three common types of procedures have been studied extensively: microfracture (MF), autologous chondrocyte implantation (ACI), and osteochondral autograft transplantation (OCA). These techniques are most effective by repairing hyaline cartilage in the knee, the principal preventor of knee osteoarthritis, thus making them the primary interest of research [42]. Additional procedures have been studied to examine their cost-effectiveness and functionality when considering the incidence of knee osteoarthritis later in life. These techniques were examined for cost-effectiveness, both in isolation and in comparison, to treatment options. For example, the effect of timing of anterior cruciate ligament (ACL) reconstruction (whether delayed or early) on resultant cost-effectiveness has been studied in long-term models [43, 44]. In order to test the cost-effectiveness, many studies have report cost-effectiveness as Incremental Cost-Effectiveness Ratios (ICER) and Quality Adjusted Life Years (QALY). ICER takes into account the cost difference of the strategies as well as their effectiveness to find the most efficient procedure while also keeping costs as low as possible for the patient [22]. QALY indicates the value of medical intervention dependent on the seriousness of the affliction [45]. These cost-effectiveness analyses and long-term economic models allow researchers to better understand the longstanding impact of the procedures.

Microfracture is an arthroscopic procedure in which chondral defects are repaired through the creation of small networks of fractures, allowing for increased vascular access and cartilage growth. A systematic review of data including 730 knee cases found that microfracture (MF) was the cheapest and most cost-effective, while first-generation autologous chondrocyte implantation [46]. Although these findings indicate that there is superiority in the cost-effectiveness of

MF versus the others, all of these saw an increase in functional outcome scores [46, 47]. Osteochondral allograft was a more expensive option in a study, but it is very cost-effective; the cost per QALY is €4692 ($5102.90) [48].

Another review found that Matrix-induced autologous chondrocyte implantation (MACI), a 2-stage procedure in which a patient's previously harvested chondrocytes are seeded on a collagen scaffold and re-implanted, was vastly outperforming MF in terms of clinical outcomes (e.g., delayed subsequent surgery, delayed knee replacements, etc.) [49]. Total MACI costs were investigated versus MF in another study, which found that MACI had long-term cost-effectiveness compared to MF [50]. In the first year, MACI was significantly higher than MF, but after the 5-year period the costs merged. MACI has had more effectiveness, though, thus lowering the ICER per QALY to €5000 ($5437.88) when compared to MF [50]. MACI also has lower costs for revision surgery than MF [50]. The ICER for ACI relative to mosaicplasty was €16,349 ($17,780.76) [51]. In addition, the cost per QALY gained for ACI over MF was €16,229 ($17,650.25) [52], and the cost per QALY gained for ACI with collagen cap compared to ACI with periosteal flap was $13,443 [53]. Economic models thus conclude that ACI is most cost-effective in long-term outcomes [42]. In a prospective study, Vilchez et al. discovered that after 1 year using an implant of a semisolid collagen, WOMAC, Knee Injury and Osteoarthritis Outcome Score (KOOS), and Oxford Knee Scores were improved versus the liquid form of the implantation [54]. This innovation shows that there is always room for more research to make these procedures as efficient as possible in the prevention of future injury, destabilization, and/or osteoarthritis.

In addition to these specific nonarthroplasty procedures to treat and prevent the onset of knee osteoarthritis, other common procedures in young athletes have been investigated for its cost-effectiveness, respectively. Specifically, ACL reconstructions are widely investigated for the cost-effectiveness, lower costs, and clinical results. ACL reconstructions generally demonstrate lower costs with greater benefits when compared to nonsurgical methods, such as rehabilitation and pain management [43, 44]. Additionally, early ACL reconstruction was substantially advantageous for both QALY and reduced cost/cost-effectiveness [32]. In a study by Mather et al., the early reconstruction group had an incremental gain of 0.28 QALYs and a lower overall cost to society of $1572 when comparing 928 patients [44]. Based on the model's prediction and Medicare rates, those who choose early ACL reconstruction could save anywhere from $956 to 2417 depending on when and where they get surgery (e.g., surgery center, hospital, outpatients) [44]. Additionally, femoral press-fit fixation in ACL reconstruction using bone-patellar tendon-bone autograft received good reports in 75% of patients at 15 years post-op [55]. These studies indicate that there is an immediate, growing need for more research into these techniques as well as innovative new techniques. This continued research will continue to help discover the societal and medical costs of procedures in the knee and what is most cost-effective, especially when pertaining to the increasing risk of knee osteoarthritis in the United States.

There is no consensus on the optimal management of younger patients with knee osteoarthritis. Historically, TKAs have not been indicated for patients younger than 60 years of age due to increased likelihood of future revision, as well as their increased activity demand [56]. In recent years, there has been a paradigm shift, with increased physician willingness to perform TKAs in younger patients with severe, recalcitrant osteoarthritis [57]. To assess the long-term cost-effectiveness of different treatment options, Bedar et al. performed a Markov model analysis in 2014 that analyzed the total economic cost to society of nonoperative treatment for a theoretical 50-year-old patient with end-stage osteoarthritis versus an early TKA [14]. This model accounted for factors such as lost wages, direct medical costs related to nonoperative treatment, direct medical costs related to TKA, and cost of potential revisions and operative complications. Ultimately, the financial analysis found that the implementation of an early TKA for younger patients with osteoarthritis resulted in markedly

lower total economic cost to society [14]. While the initial cost of a TKA is higher, over 30 years the benefit of TKA was found to be $69,800 (2012 U.S. dollars) on an individual level [14].

5.5.2 Hip

The economic burden of hip labral tears and early osteoarthritis can be divided into arthroplasty and nonarthroplasty treatments. THA is the most well-established, effective surgical approach to treating severe, end-stage osteoarthritis. However, this procedure is generally not recommended in younger patients due to high rates of future revision [58]. Currently, there are several alternative surgical approaches are currently being considered as preferable alternatives to THA in younger patients.

Resurfacing arthroplasty is a surgical technique in which the femoral neck is retained, and the femoral head is resurfaced rather than resected, conserving patient bone stock. In 2010, Bozic et al. compared both the clinical effectiveness and cost-effectiveness of metal-on-metal HRA compared to THA [59]. Using a Markov decision model analysis, the authors found that patients who underwent MoM HRA, over a 30-year follow-up period, experienced modestly higher lifetime gains in QALYs along with higher overall health care costs. Overall, MoM HRA was found to be more cost-effective than THA for men than for women, and for younger patients than for older patients. In 2012, Edlin et al. reported that, in an analysis of 126 patients who had undergone resurfacing arthroplasty for arthritis of the hip, surfacing arthroplasty offered short-term cost efficiency benefits compared to THA [58]. Patients who had undergone resurfacing had higher quality of life at 12 months. Although resurfacing was incrementally more expensive than THA, resurfacing arthroplasty offered higher quality of life at only £17,451 per QALY in the first year (£564/0.032 QALY). As this is lower than the standard UK willingness to pay of £20,000 per QALY, resurfacing arthroplasty may be considered as a cost-effective tool. In 2013, Heintzbergen et al. constructed a Markov decision analytic model to compare estimated cost per quality-adjusted life-year (QALY) of metal-on-metal hip resurfacing arthroplasty (HRA) compared with conventional THA [60]. The model determined that, for younger patients, metal-on-metal HRA was superior to THA. On average, HRA was estimated to be CAN $583 cheaper than THA with 0.079 higher mean QALY. In 2016, Pulikottil-Jacob et al. analyzed the cost-effectiveness of metal-on-metal hip resurfacing by performing a cost-utility analysis using individual patient data from the National Joint Registry for England and Wales from April 2003 to December 2012 [61]. The study found that resurfacing arthroplasty was unlikely to be a cost-effective treatment for hip arthritis as compared to THA at standard UK willingness to pay of £20,000 per QALY [61]. This was in large part due to the high revision rate of hip resurfacing, with an estimated revision rate of 13% at 10 years compared to <4% for most THA implants [61].

Another surgical option in the treatment of hip osteoarthritis is periacetabular osteotomy (PAO). This procedure is principally used to treat developmental hip dysplasia, a musculoskeletal deformity that may lead to the development of early onset osteoarthritis in younger patients. In these patients, the utilization of PAO vs THA remains controversial. In 2008, Sharifi et al. analyzed the long-term cost-effectiveness of PAO as compared to THA using a cost-utility analysis [62]. The study found periacetabular osteotomy was associated with lower total costs and higher cost-effectiveness for Tönnis grade-1 and grade-2 hips, with cost-effectiveness ratios of $7856/QALY and $10,807/QALY, respectively. However, THA was more cost-effective for Tönnis grade-3 hips.

Hip arthroscopy is also an appropriate surgical option for symptomatic acetabular labral tears, femoroacetabular impingement (FAI), acetabular dysplasia, hypermobility, and trauma [63]. As several studies have connected FAI as potential causative factor for the development of hip osteoarthritis, arthroscopy presents as an effective technique in the prevention of future incapacitating osteoarthritis. Few studies have scrutinized the economic impact of this surgery.

Shearer et al. found that among patients with FAI but no radiographic evidence of arthritis, the estimated incremental cost-effectiveness ratio (ICER) was $21,700/QALY compared to observation [64]. For patients with preoperative arthritis with a higher rate of progression to poor hip function, the estimated ICER for arthroscopy was higher at $79,500/QALY, indicating decreased cost-effectiveness. The high cost-effectiveness of arthroscopy in the treatment of FAI was also supported by a study performed in 2018 by Mather et al. [65] In this analysis of direct and indirect medical costs of 102 patients who underwent arthroscopy as well as reimbursement records of 32,143 individuals, hip arthroscopy was found to confer an average gain of 2.03 QALY per patient over a 10-year period [65]. In addition, arthroscopy was associated with a societal savings of $67,418 per patient versus nonoperative treatment [65]. Savings in cost were attributable to a decrease in indirect costs associated with lost wages and decreased workplace productivity, which were underscored by the young age of patients with FAI syndrome and potential for large functional restoration in these patients.

5.6 Conclusion

Early onset osteoarthritis and related surgical treatment represent a large economic burden in the United States, a burden that is expected to grow in the coming years. There are several socioeconomic factors that may predispose certain populations to a diagnosis of osteoarthritis at younger ages. Additionally, trauma and congenital deformity contribute significantly to the development of osteoarthritis at unusually early ages. While total knee and hip arthroplasty have been established as effective interventions, there has been much research into alternative procedures that may prove to be more cost-efficient and equally clinically effective. Further research into the cost utility of these procedures may further elucidate and inform policy makers, health insurers, as well as clinical decision making.

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6 Early Osteoarthritis: Frequency, Epidemiology, and Cost of ACL Injuries

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6.1 Introduction

Anterior cruciate ligament (ACL) injuries are one of the most common knee injuries in the United States, especially in younger and active individuals [1]. According to the Centers for Disease Control (CDC), there are upward of 250,000 ACL injuries per year, although some affected patients may not undergo clinical evaluation [2]. Recently, the epidemiology of ACL injuries has been more thoroughly analyzed in different patient populations and by sport.

This research can be used to better predict health care utilization, accurately assess cost-effectiveness, and assist in developing injury prevention programs.

There is a significant cost burden for treating ACL injuries through both surgical and conservative approaches. ACL reconstruction is considered the gold standard treatment for ACL injuries [3, 4]. However, conservative management through bracing and physical therapy can be utilized in some patients, especially those with lower activity demands [5]. While treatment should always be patient-specific, cost-effectiveness is an important consideration. Within ACL reconstruction, a wide range of factors can affect costs. For example, surgical setting, technique, such as a single- versus a double-bundle approach, and graft choice, can significantly alter cost-effectiveness [6–8]. This chapter will explore the frequency and incidence of ACL injuries. Subsequently, costs associated with ACL injuries and approaches to maximize cost-effectiveness will be discussed.

6.2 Frequency and Incidence

A thorough understanding of the frequency and incidence of ACL injuries is needed prior to developing patient-population specific strategies to minimize injuries and maximize treatment cost-effectiveness. In a long-term population study, Sanders et al. identified 1841 patients with a complete ACL tear from the Rochester Epidemiology Project, which uniquely allows for longitudinal medical record tracking [2]. The authors reported an annual incidence of 68.6 per 100,000 person-years. Interestingly, they reported a significantly higher annual incidence of ACL injuries in males compared to females (86.6 vs 55.3 per 100,000, $P < 0.01$), although ACL injuries in males decreased from 1990–1994 to 2005–2010 ($P < 0.01$). In terms of treatment, ACL reconstruction has become the mainstay treatment in recent years. The authors reported a significant increase in ACL reconstructions within 1 year of injury, with 43% of patients undergoing reconstruction in 1990–1994 versus 75.9% in 2005–2010, demonstrating a general shift towards surgical management.

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6.2.1 Skeletally Immature Patients

Limited literature has been devoted to defining the incidence of ACL injury in young patients and those with open physes with many studies instead focusing on the epidemiology of high school and collegiate athletes. One study by Funahashi et al. identified a total of 71 skeletally immature patients who experienced complete ACL tears [9]. At age 11, male incidence was 0.30 ± 0.20 and female incidence was 0.10 ± 0.20 per year per 10,000 lives. In comparison, by age 12 the incidence increased to 0.77 ± 0.63 in males, and 0.3 ± 0.38 per year per 10,000 in females. Due to the small sample size in this study, future investigations are needed to further define the epidemiology of ACL injuries in young, skeletally immature patients.

6.2.2 Competitive Athletes

Since ACL injuries are more common in athletic individuals, numerous investigations have been performed to quantify the frequency and incidence across various sports. Granan et al. performed a large cohort analysis of 10,958 ACL reconstructions to determine frequency of sport-specific injury [10]. The most commonly affected sport was soccer (33.8%), followed by skiing (16.6%). In terms of all ligamentous injuries, when controlling for time from injury to surgery, age, and sex, skiing injuries were more likely to result in an isolated ACL tear (1.13 times) and concurrent posterior cruciate ligament (PCL) tear (2.05 times) compared to soccer. In contrast, football players had a higher likelihood of medial collateral ligament (MCL) involvement (2.72 times). Soccer and skiing are more likely to have ACL tears compared to other sports, with skiers at an increased risk for concurrent PCL tears.

Multiple studies have reported that competitive level and match play are risk factors for ACL injury. For example, in the professional Italian soccer league, the risk of injury has been defined as 0.0618 injuries per 1000 h of total play with higher ranking teams (1st through 4th) at higher risk [11]. Similarly, Walden et al. reported a 20-fold higher match ACL injury rate compared to training rate (0.340 vs 0.017 per 1000 h of play) in European soccer teams [12]. In skiing, from 1980 to 2005, the overall incidence of injury was 8.5 per 100 skier-seasons with primary injuries occurring at a rate of 5.7 per 100 skier-seasons. Those who were ranked Top 30 in the world had a higher risk of injury ($P < 0.01$) [13]. These studies demonstrate a high incidence of ACL injury with high level of competitive play being a significant risk factor.

In many sports, including basketball and soccer, female players are at a higher risk for ACL injury than their male counterparts. When analyzing the role of sex in each sport, a meta-analysis demonstrated that sports with the highest female to male ratios of ACL injury were basketball (3.5), soccer (2.67), lacrosse (1.18), and alpine skiing (1.0), demonstrating that depending on the sport, females can over a three times greater risk of ACL injury [14]. Similarly, a meta-analysis of collegiate and high school athletes demonstrated higher injury rates for females than males (injury rate ratio [IRR] = 2.30–2.49) across five sports (basketball, ice hockey, lacrosse, soccer, and baseball/softball) [15]. This study also reported that female basketball players were at the highest risk (IRR = 3.25). However, the rate of female injuries in other sports, specifically soccer, may be increasing [16]. When comparing female basketball injury mechanisms, Agel et al. reported that female basketball players sustained 100 contact injuries and 305 noncontact injuries compared to female soccer players who sustained 115 contact injuries and 161 noncontact injuries, illustrating the differences in injury mechanism between sports [17, 18]. ACL injuries due to noncontact injuries may be related to anatomical risk factors such as an increased posterior-inferior lateral tibial plateau slope in males and decreased volume and height of the medial tibial spine [19]. These studies support that women are at a significantly higher risk than males in many sports, particularly in basketball.

6.2.3 Recurrent ACL Injury

Recurrent ACL injury is common and there are a wide range of risk factors for recurrent ACL injury. Similar to primary ACL injury, sport type and competitive level appear to play a large role. A study by the Multicenter Orthopedic Outcomes Network (MOON) group reported that the contralateral knee (3% risk) is at a similar risk of injury to the ipsilateral knee (3% risk) based on a 2-year cohort study in patients who had undergone ACL reconstruction [20]. Slater et al. investigated risk factors for recurrent ACL injury in National Collegiate Athletic Association (NCAA) athletes and high school athletes [21]. Between these two populations, a total of 644 first-time injuries and 61 recurrent injuries were identified (recurrence rate of 8.7%). NCAA athletes had a 4.6 times likelihood of suffering a recurrent injury compared to high schoolers. When investigating noncontact injuries, recurrent tears were more likely to occur in the preseason (odds ratio [OR] = 2.8) or postseason (OR = 4.5). An additional study investigated the recurrence rates within the NCAA over a 10-year period (2004–2014) [22]. During this time, 1105 ACL tears were identified, of which 126 were recurrent (recurrence rate of 11%). Sub-analysis demonstrated that the sports with the highest rates of recurrent ruptures were men's football (15 per 100,000 athletic exposure [AE]), women's gymnastics (8.2 per 100,000 AE), and women's soccer (5.2 per 100,000 AE). In addition, while men had significantly higher rate of recurrent rupture ($P = 0.04$), both genders demonstrated a significant decrease in the ratio of recurrent to primary ACL injuries during the study period. These findings suggest that risk for recurrent ACL injury is sport-dependent and may increase with higher competitive levels.

Additionally, younger patients may be at increased risk of recurrent ACL injury. A meta-analysis by Wiggins et al. of 14 pooled studies demonstrated an overall reinjury rate of 7% but they demonstrated that patients under 25-years old were at a significantly higher risk for ipsilateral reinjury (10%). Those who returned to sport were at even higher risk (20% reinjury rate) [23]. Similar findings were also reported by Webster et al. [24]. In 561 patients who had undergone ACL reconstruction, subsequent ipsilateral injury occurred in 4.5% of patients ($n = 25$), while contralateral injury occurred in 7.5% ($n = 42$). Patients younger than 20 (ipsilateral: OR = 6.3, $P < 0.01$, contralateral: OR = 3.1, $P < 0.01$) and those who returned to pivoting sports (ipsilateral: OR = 3.9, $P = 0.01$, contralateral: OR = 4.9, $P < 0.01$) had an increased risk of both ipsilateral and contralateral injury. An additional study of 2915 ACL reconstructions reported the incidence graft ruptures to 3% after primary reconstruction at a mean follow up of 5.0 ± 1.1 years [25]. Similarly, male sex ($P < 0.01$) and age under 25 was associated with a higher risk (OR = 6.0) of rupture. Younger patients are likely at increased risk of reinjury for both their ipsilateral and contralateral knee, which is likely related to their return to high-risk sporting activities.

6.3 Cost-Effectiveness

Costs of ACL injuries have been analyzed from numerous angles, from the expenses of nonoperative management compared to surgery, to the costs of different ACL reconstruction techniques. Studies have suggested that the most cost-effective approach is to use a single-bundle, outpatient, autograft for ACL reconstructions [26].

6.3.1 Costs of ACL Reconstruction and Conservative Management

ACL reconstruction is the most common treatment for ACL injuries and its associated costs range from under $5000 to over $20,000. This large difference likely stems from different factors including surgery setting (inpatient vs outpatient), graft source, state of operation, and concomitant procedures. Furthermore, costs reported in 2010 are likely to differ from costs reported in 2019. In an outpatient assessment of ACL costs in patients under 65 years old from 2005 to 2013, the median immediate costs of

229,446 outpatient ACL reconstructions was $9,399.49 [27]. Median total health care utilization cost for ACL reconstructions was $13,403.38. Both immediate costs and total health care utilization costs increased over the duration of the study period. In contrast, in an analysis of 14,713 ACL reconstructions, Bokshan et al. reported a mean cost of $24,707 [28]. In a state sub-analysis, costs were highest in Florida (mean = $31,281) and lowest in Maryland (mean = $11,429). Multivariate analysis demonstrated that general anesthesia (coefficient: $2,049), Hispanic ethnicity (coefficient: $1,828), the presence of comorbidities (coefficient: $1,749), male gender (coefficient: $1,126), increased operating room (OR) time (coefficient: $108/min), and age (coefficient: $54/year) significantly increased total reconstruction costs ($P < 0.01$ for all). In addition, a smaller analysis of 434 outpatient ACL reconstructions also found graft type to be predictive of costs, with allografts ($n = 100$) costing 44.5% and hybrid reconstructions ($n = 31$) costing 33.1% more than autografts ($n = 293$) ($P < 0.01$ for both) [29]. Not surprisingly, OR time increased costs by 0.3% per minute. Concomitant procedures such as meniscal repair (24.4%) and other procedures (e.g., chondroplasty, osteochondral autograft) significantly increased costs by 15.9% on average ($P < 0.01$ for all). These studies show that a wide range of factors such as location, graft type, and demographic factors influence the cost of ACL reconstruction.

There is a large variance in costs between ACL reconstructions. Seiffer et al. analyzed the cost of an ACL reconstruction based on device and implant costs based on the practices of 11 orthopedic surgeons over a 6-month period [30]. In 2013, the mean procedural cost was $2,039.09 (range: $392.80–$4,670.31), which is lower than other studies and may be related to the location of the study (Idaho). A breakdown of costs demonstrated the highest mean cost was for the allograft, with a mean cost of $1,976.43 (range: $1,275.00–$2,545.75). Tools and disposable costs were the second largest component at a mean of $452.33 (range: $40.10–$2,136.00). Tibial fixation cost a mean of $293.52 (range: $95.00–$760.00), femoral fixation cost a mean of $367.14 (range: $95.00–$865.00), and each suture was $18.26 (range: $1.19–$46.00). This study demonstrates the wide range of costs associated with each ACL reconstruction.

Despite the widespread use of ACL reconstruction, conservative management may still be preferred in some patients, especially those with low activity levels; however, this approach is not the most cost-effective. For instance, a comparison of costs for rehabilitation versus ACL reconstruction was performed by Mather et al. using the MOON cohort and data from the knee anterior cruciate ligament, nonsurgical versus surgical treatment (KANON) study [31]. Early outcomes were based on Short Form (SF) 36 scores, while long-term outcomes were based on osteoarthritis development. In the short-term analysis (6-years follow up), ACL reconstruction was reported to be the dominant strategy, providing a gain of 0.18 quality-adjusted life year (QALY) while costing $4,503 less than rehabilitation. The effectiveness of ACL reconstruction was exaggerated in the long-term model, which demonstrated that ACL reconstruction provided a QALY gain of 0.72 with a cost savings of $50,417. In 2009 study, Lubowitz et al. investigated cost-effectiveness where QALY was based on life expectancy and quality of well-being (difference between preoperative and postoperative score) [32]. Based on this, ACL reconstruction was also deemed more cost-effective at $5,783 per QALY. While not directly applicable to the United States healthcare system, a study out of Sweden directly compared cost-effectiveness of conservative management to ACL reconstruction, where QALY was based on activity level. The authors reported that conservative management cost $15,466 (USD) and had a QALY of 0.66 versus reconstruction, which cost $16,038 with 0.78 QALYs. Therefore, reconstruction had an incremental cost-effectiveness of $4,890/QALY. In addition, Stewart et al. investigated the cost-effectives of physical therapy versus ACL reconstruction in a competitive athlete cohort by using published data on return to preinjury level of play in both treatment options [33]. The model proposed by the authors demonstrated that ACL

reconstruction as a preferable option at a cost of $22,702 per QALY gained. Quality adjusted life years (QALY) was based on EuroQol 5-dimension (EQ-5D) survey and SF-36 responses. In addition, a two-way sensitivity analysis demonstrated a higher net monetary benefit for ACL reconstruction for both quality of life of return to play and no return to play. These studies demonstrate that ACL reconstruction is a more cost-effective treatment strategy than conservative management.

Recently, ACL repair has gained popularity, especially in pediatric patients and those with avulsion fractures. Despite demonstrating promising clinical outcomes and survivorship in these populations in clinical trials, there is no literature investigating the costs and cost-effectiveness of ACL repair [34]. Future research is needed to investigate the cost-effectiveness of ACL repair compared to ACL reconstruction in these patient populations.

6.3.2 The Role of Time to Intervention

When analyzing the role of time to intervention, it is important to consider a wide range of factors that can affect both patient outcomes and costs. Early surgery, for example, may increase the risk of arthrofibrosis, while delaying surgery can increase the risk of cartilage and meniscal pathology and the rate of OA [35–37]. In a study out of Sweden, an analysis of 5-year cost-effectiveness of early (within 10 weeks) (n = 62) versus late (n = 59) reconstruction was performed by Kiadaliri et al. using data from the KANON trial [38]. The authors reported that early reconstruction provided 0.13 more QALYs (P = 0.11), based on SF-36 scores, but at increased cost of $4,695 ($P$ = 0.19). These results suggested that early reconstruction did not result in increased cost-effectiveness. However, this cohort was limited to only 121 participants and therefore may have been underpowered. Mather et al. compared early reconstruction to rehabilitation and delayed reconstruction and demonstrated that the early reconstruction group gained 0.28 QALYs over the delayed group and decreased total costs (including direct and costs to society) by $1572 [39]. Outcome data was utilized from the MOON group and KANON trial, with QALYs based on SF-6D and SF-36 scores. The delayed reconstruction group not only had a higher mean cost ($21,454 vs $19,883) but also lower QALYs gained and thus had a higher cost-effectiveness ratio ($4,434 vs $3,881). From a cost-effectiveness standpoint it is unclear which time to intervention is superior, especially because neither of these studies included the development of osteoarthritis in their models.

6.3.3 Costs of Concomitant Meniscus Pathology

In 31–82% of cases, ACL injuries occur with concomitant meniscal pathology [40–43]. To define how the addition of surgically treating meniscal pathology affects ACL reconstruction cost-effectiveness, Lester et al. compared the cost-effectiveness of meniscectomy to meniscal repair. The authors reported that the direct cost of a concomitant meniscectomy was $24,768 with 17.16 QALYs compared to a cost of $17,898 with 18.00 QALYs, where QALYs were based on health benefits and costs with particular attention devoted to the development of OA and the need for a TKA [44]. This study demonstrated that meniscal repair not only increased QALYs by 0.77 but also decreased costs by $8178.57. Despite these findings, future cost-effectiveness research is needed to specifically define which types of meniscal tears will have the greatest benefit from repair, and which should be treated with debridement.

6.3.4 The Role of the Surgical Setting

The setting of ACL reconstruction has significantly changed over time with 43% of procedures performed in the outpatient setting in 1994 versus 95% in 2006 [45]. From 2003 to 2011, in the National Inpatient Sample data set, the number of

inpatient ACL reconstructions decreased from 1,963 to 929 [46]. However, the mean length of stay and total cost increased from 2.6 to 3.3 days and $27,266 to $72,559, respectively. Similarly, within the National Inpatient Sample database a total of 104,740 patients were who underwent inpatient ACL reconstructions were identified and a significant decrease from 1998 to 2010 in inpatient admissions was reported ($P < 0.0001$) [6]. Furthermore, there was significant increase in mean length of stay from 1.65 days in 1998 to 2.36 days in 2010 ($P < 0.01$). Accordingly, the cost of inpatient admission rose from $11,103 in 1998 to $46,528 in 2010 ($P < 0.01$), a large increase even when considering inflation [6]. In addition, outpatient surgical centers may further decrease costs. In an analysis of 49 ACL reconstructions, those performed at an outpatient surgical center had significantly shorter OR times ($P < 0.01$) and required less staff ($P < 0.01$) [47]. These studies suggest that ACL reconstructions have shifted toward predominately being performed in an outpatient setting, which is a more cost-effective approach. Future research is needed to investigate if outpatient ambulatory surgical centers further reduce costs.

6.3.5 The Role of Anesthesia

As there has been a massive shift from inpatient procedures in the early 1990s to outpatient procedures in the 2000s, studies have investigated whether changes in anesthesia can reduce procedural costs. An early study by Williams et al. investigated the costs of using nerve block after ACL reconstruction [48]. In this nonrandomized control trial, the authors found that patients who received a nerve block had a lower hospital admission rate (4% vs 17%). Furthermore, hospital admissions were associated with an increase cost of 11% ($P < 0.01$). Thus, the authors concluded that this approach could decrease costs. In addition, Hall-Burton et al. investigated the use of nerve blocks in a pediatric cohort of ACL reconstructions [49]. In their study, 115 patients who received a nerve block (either a single-injection femoral and sciatic block [$n = 59$] or femoral perineural catheters and a single-injection sciatic nerve block [$n = 57$]) were compared to 39 who did not. The authors reported a significantly lower rate of unplanned hospital admissions ($P = 0.045$), opioid consumption ($P < 0.001$), and time in in the postanesthesia care unit ($P = 0.013$). Furthermore, they reported that the most cost-effective scenario (saving $250 per ACL patient) was performing a single injection in a dedicated injection room. While nerve blocks may be cost-effective, they may also cause postoperative quadriceps weakness and atrophy [50]. Future studies are needed to evaluate the cost-effectiveness of nerve blocks when including postoperative quadricep weakness in their model.

6.3.6 Costs Based on Graft Type

Graft source (allograft versus autograft) plays a significant role in cost. Using an allograft requires a cost for purchasing the graft, while an autograft increases indirect costs such as OR time. A comparison of costs for 50 allograft ACL reconstructions was compared to 105 autografts [51]. While OR time (110 vs 97 min, $P < 0.01$) and total OR costs ($3,512 vs $3,121, $P < 0.01$) were significantly greater for the autograft group, the autograft was still the less expensive option ($4,872 vs. $5,465, $P < 0.01$). Furthermore, the complication rate was similar in the allograft (4%) vs autograft (5.7%) groups. Similarly, a comparison of BTB autografts, hamstring autografts, and hamstring allograft also demonstrated that autografts were more cost-effective [7]. A hamstring autograft was deemed most cost-effective (cost = $5,375, QALY = 0.912, CE = $5,892), followed by a BTB autograft (cost = $5,580, QALY = 0.906, CE = $6,157) and lastly an allograft (cost = $6,958, QALY = 0.904, CE = $7,694).

An additional analysis by Greis et al. comparing hamstring autograft to tibialis anterior or posterior allografts reached a similar conclusion [52]. The total cost of the allograft surgery was $4,587 compared to $3,849 for autografts, despite autografts having longer OR times (125 vs 92 min). Furthermore, while reimbursement was

lower ($P = 0.02$), the percent margin on autograft surgeries was higher (45% vs 41.5%). Similar results were reported by Barrera et al. when analyzing 106 BTB autografts versus BTB allografts used for ACL reconstructions performed at an ambulatory surgery center [53]. The authors reported total costs at \$4,147 ± \$943 and \$3,154 ± \$704 for the allograft and autograft cohorts, respectively ($P < 0.01$). Cooper et al. also reported higher costs associated with a tibialis anterior allograft ($n = 49$) versus a BTB autograft ($n = 49$) [54]. In their study, allografts increased costs by a mean of \$1,123.16 ($P < 0.01$). These studies demonstrate that while autografts increased OR time and staffing, it did not equate to the cost of purchasing an allograft. Thus, autografts have been shown to be a more cost-effective approach.

6.3.7 Costs Based on Technique

While some orthopedic surgeons may prefer a double-bundle to single-bundle reconstruction for certain patients, it is a much more time intensive and technical approach, and therefore is less cost-effective. Brophy et al. investigated the economic impact of widespread adoption of a double-bundle technique and how to offset this potential cost [8]. Using previously reported cost assumptions, the authors reported that switching to a double-bundle technique would cost the United States between \$36 and \$792 million annually. To offset this cost, based on modeling the double-bundle approach would have to reduce the revision rate from 4 to 1.5% at a minimum. A study in Sweden also compared the single ($n = 50$) versus double-bundle ($n = 53$) technique [55]. While the absolute costs are not directly comparable to costs in the United States, the authors reported that the double-bundle technique was associated with significantly higher costs ($P < 0.01$) without any significant improvement in QALYs (based on the EQ-5D patient reported outcome). Moreover, this was likely due to the significant longer OR time needed for the double-bundle technique ($P < 0.01$). In contrast, Paxton et al. reported that double-bundle technique may be more effective. In their study, the authors directly compared the costs of single and double-bundle ACL reconstructions based on effectiveness, which was defined using revision rate and IKDC score [56]. Based on published clinical studies and IKDC score achievement, the authors reported that according the incremental cost-effectiveness ratio of double to single bundle ACL reconstructions was \$6,416 per QALY. However, this model utilized outcome information from a few studies published in the early 2000s, thus the model may not accurately capture today's cost-effectiveness of these approaches. Through a variety of models and clinical outcome data, these studies suggest that a single-bundle technique may be more cost-effective.

6.3.8 Injury Prevention

In an attempt to preemptively decrease costs associated with ACL treatment, multiple investigations have been conducted investigating how injury prevention can decrease costs, especially in athletic populations. Swart et al. reported that in their model using previously published data, a universal neuromuscular training for athletes could save \$100 per player per season, in part by reducing ACL injuries from 3% to 1.1% [57]. In addition, the training program resulted in a net gain of 0.05 QALYs compared to no screening. An Australian study demonstrated that the high-risk sports program for 18–25 year-olds (HR 12–25) for ACL injury prevention could decrease costs by US \$693 per lifetime [58]. The program decreased ACL injuries by 40% and decreased the number of lifetime cases of osteoarthritis and total knee arthroplasty by 842 and 584 per 10,000, respectively. In addition to primary ACL injury prevention, costs can also be reduced by preventing subsequent ipsilateral or contralateral injury. DeFrancesco et al. evaluated the cost-effectiveness of an enhanced return to play protocol [59]. Enhanced return to play protocols included neuromuscular retraining, advanced testing (both quantitative and qualitative) and/or increased frequency of clinic visits. In their

model, the enhanced protocol provided slightly more QALYs (0.764 vs 0.756) and was less expensive ($7,388 vs $7,687) than standard protocols. The enhanced protocol also reported reducing the incidence of contralateral ACL injury by over 13.8%. Injury prevention is key to reducing healthcare costs associated with ACL injury. Future studies are needed to investigate which patient populations would receive the greatest benefits from each type of injury prevention program.

6.4 Conclusion

ACL injury is a common injury often affecting athletes, especially those who play soccer and basketball, with higher incidences reported at higher levels of play. Both nonoperative management and ACL reconstruction surgery result in significant costs. The most cost-effective approach is likely an ACL reconstruction with an autograft, single-bundle technique, that is performed at an outpatient surgery center. However, modeling in the current literature uses a variety of outcome measures making it difficult to directly compare studies. Given the high long-term costs associated with ACL injury, meniscus tears, and related OA, injury prevention may be key for lowering costs. Further research is needed to define the ideal injury prevention program for each individual.

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7 The Human Anterior Cruciate Ligament Injury Model of Early Osteoarthritis

Cale A. Jacobs and Emily R. Hunt

7.1 Prevalence of Osteoarthritis After Anterior Cruciate Ligament Injury

Osteoarthritis (OA) in people under the age of 50 is estimated to cost society $200 billion annually [1]. While the development of treatment approaches for established OA has been studied for many years, the results have ranged from being somewhat encouraging to disappointing. Numerous failed clinical trials would now suggest that late drug intervention, once tissue damage is fully established, will allow for symptom but not disease modification [2, 3]. As a consequence, the research focus has shifted to early detection and early treatment of OA. Idiopathic OA has a chronic etiology that can take decades to assess factors related to progressive changes and/or the potential efficacy of therapeutic interventions. On the contrary, acute injuries with a high prevalence of subsequent OA like anterior cruciate ligament (ACL) injury offer a clear triggering event that initiates the OA process, thereby making ACL injury a very attractive model to elucidate the underlying factors related to OA onset and progression as well as to assess the efficacy of novel treatment options.

The prevalence of posttraumatic osteoarthritis (PTOA), which occurs secondary to a traumatic joint injury, is a common occurrence in young patients usually at the beginning or in the middle of their productive work-life [1, 4–8]. Furthermore, the prevalence of PTOA continues to increase and now represents the most common cause of military disability [9, 10]. In the last decade, it has become apparent that patients who suffer ACL injuries have a very high risk of progression to radiographic and clinically symptomatic OA as early as 2–5 years after ACL injury [11, 12]. The rupture of the ACL is a dramatic event that usually does not go unnoticed and, in the United States, almost always leads to a surgical reconstruction of the ACL. The prevalence of ACL injury in the United States is high and increased by 36% from 1994 to 2006, with the current estimate for the number of ACL reconstructions performed in the United States of 45/100,000 capita [13].

ACL reconstruction successfully improves clinical outcomes out to 10 years following surgery [14]; however, surgery is not protective against the progressive PTOA changes. As such, PTOA has been described as a “silent killer” of the joint since cartilage degradation continues despite a lack of pain and/or functional limitation [15]. In fact, more than 80% of those with combined ACL and meniscus injuries have PTOA

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within 10–15 years [16, 17], and over 85% of young female athletes have radiographic changes during this time period as well [18].

7.2 ACL Injury as a Model of Early Osteoarthritis

Because of the high prevalence of subsequent PTOA, ACL injury has been adopted as a human model of early osteoarthritis. This approach has become widely utilized, and as of May 25, 2020, there were 112 studies listed in a search of clinicaltrials.gov with the terms ("ACL" AND "osteoarthritis").

There are multiple mechanisms by which ACL injury promotes PTOA progression. The injury affects the joint as an organ impacting not only the articular cartilage but also the synovium, underlying bone, and surrounding musculature with similar pathways implicated in the development and progression of idiopathic knee OA [19–21]. The response to acute ACL injury involves a complex dynamic interaction of multiple pathways including the NF-κB, cytokine-cytokine receptor interaction, and osteoclast differentiation pathways, among others [22–25]. As such, the injury results in a persistent inflammatory response and cartilage degradation that in time turns into a chronic sequela with poor long-term outcomes. Furthermore, data suggest that not only is the articular cartilage affected after ACL injury but so are the subchondral bone and surrounding musculature that also contribute to PTOA progression. In addition, both patient and injury characteristics may also contribute to PTOA progression after ACL injury.

7.2.1 Persistent Inflammatory Response and Cartilage Breakdown

ACL injury triggers a biochemical cascade leading to cartilage degradation. The current standard of care for patients with combined ACL and meniscal injuries consists of surgical treatment, which successfully restores joint stability; however, this approach does not address the persistent inflammatory process that promotes cartilage degradation and PTOA progression. After ACL injury, the ensuing inflammatory response undermines the lubricating mechanisms of knee [26]. Persistent, elevated levels of proinflammatory cytokines and degradative matrix metalloproteinases (MMPs) followed by progressive cartilage catabolism have been shown soon after ACL and meniscus injury [15, 23, 27–29].

While the inflammatory response is not an unexpected event immediately following injury or surgery, persistent elevations in proinflammatory cytokine concentrations in the synovial fluid remain elevated 5 years after surgery [16]. This is not a benign finding as proinflammatory cytokine concentrations prior to or on the day of ACL reconstruction have been predictive of both progressive cartilage changes and inferior patient-reported outcomes [30, 31]. PTOA progression is multifaceted and includes activation of the proinflammatory NFκB pathway, an increase in proinflammatory M1 macrophages, cellular senescence, and bone remodeling [32–36]. This process involves both an upregulated proinflammatory response with a dysregulated anti-inflammatory response, providing pivotal information about potential therapeutic targets [23, 37]. Reducing MMP and cytokine activity after ACL and meniscus injury may alter the progression of PTOA for this at-risk patient population [38].

7.2.2 Changes to Underlying Bone

The initial insult of ACL injury is commonly associated with subchondral bone marrow lesions [39, 40]. We have reported an upregulation of the osteoclast differentiation pathway in the synovial fluid proteome after ACL injury [23], which may either be a result of bony trauma suffered at the time of ACL injury or may potentially be contributing factor in the progression of PTOA. While midterm outcomes for younger patients do not appear to be impacted by the presence of bone marrow lesions, older patients have demonstrated

self-reported pain, functional limitations, and greater cartilage degradation [35, 40, 41]. The size of bone marrow lesion has been associated with progressive cartilage degeneration and poor patient-reported outcomes after ACL injury [35]. The combined presence of a bone marrow lesion with local articular cartilage changes has been associated with self-reported pain and functional limitations consistent with symptomatic OA, especially in the high-risk group of older ACL patients [40].

Bone mineral density is significantly decreased after ACL reconstruction not only in the distal femur and proximal tibia [42, 43], but in the hip and ankle as well [44, 45]. The loss of bone mineral density may then increase the risk of subsequent fracture, but when losses of bone mineral density are combined with increased mechanical loading of the bone as patients resume daily work and sporting activities, patients may also be predisposed to changes in the shape of the distal femur and proximal tibia. Indeed, shape changes have been documented after ACL reconstruction. The area of the medial femoral condyle increases within the first few months after ACL reconstruction, with the condyle becoming wider and flatter similar to what is seen in patients with end-stage idiopathic OA [46]. Furthermore, bone shape changes within 6 months of ACL reconstruction correlated with subsequent patient-reported outcomes and MRI markers of cartilage quality at 3 years [46, 47].

7.2.3 Muscular Changes

Despite significant time spent in postoperative rehabilitation, many patients have persistent quadriceps weakness and atrophy lasting years after ACL injury and/or reconstruction [48, 49]. Prolonged quadriceps weakness has widespread implications for the patient including elevated reinjury risk [50], decreased quality of life [48, 51, 52], early-onset osteoarthritis, and reduced life-long physical activity levels [53–55]. Quadriceps weakness has both neurological [56–58] and cellular mechanisms that underlie muscle atrophy [59–61], and the response to injury and role in OA progression may also differ between the sexes [62, 63].

Persistent quadriceps atrophy after ACL injury and/or reconstruction has been, in part, attributed to alternations in neurological function, as patients are often unable to fully contract their muscle after injury. Though the exact mechanisms driving this response are unknown, altered activity after ACL injury has been well documented at the spinal [56, 58] and cortical level [58, 64, 65], negatively influencing the ability to generate a muscle contraction. While the nervous system and skeletal muscle are known to have many inherent physical (neuromuscular junction) and biological links, the extent to which alterations in neural activity directly contribute to muscle atrophy is unclear, as neither its time course, nor true prevalence, in human or animal models of ACL injury has been determined.

While neurological adaptions contribute to persistent weakness, the force-generating capacity of individual muscle cells also impacts muscle function independent of alpha motor neuron activation [66]. Cross-sectional studies have demonstrated that cellular alterations after injury may also contribute to persistent quadriceps weakness after ACL injury. After ACL injury, patients demonstrate greater quadriceps muscle collagen content, and fibrosis, as well as increased abundance of inflammatory markers like tumor necrosis factor alpha (TNFα), both in the muscle and circulating serum [59–61, 67]. Alterations in quadriceps muscle volume and cross-sectional area are common after ACL injury [59, 68–71], corresponding with reduced whole muscle strength [66].

Furthermore, these mechanisms may differ between the sexes. Quadriceps force normalized to body mass has been reported to be significantly lower for female ACL reconstruction patients as well as reduced rates of torque development [62]. Both weakness and the rate of torque development have been implicated in the progression of OA [72, 73] and have been shown to impact the risk of subsequent OA diagnosis more for females than males [63]. Studies are underway to not only assess the underlying etiology of muscular weakness following ACL injury and whether these

mechanisms differ between the sexes, but also of rehabilitation-based and pharmacological interventions to mitigate muscular changes to alter the progression of PTOA.

7.3 Patient and Injury Factors That Increase the Likelihood of Osteoarthritis After ACL Injury

Human ACL injury provides a clinical model of early PTOA; however, much like idiopathic OA, the progression can be affected by patient and injury-specific factors. Patient age, sex, and body mass index (BMI) as well as meniscus status have been reported to increase the likelihood of radiographic and symptomatic OA after ACL injury [74, 75].

7.3.1 Concomitant Meniscus Injury

The presence of concomitant meniscus injury has repeatedly implicated as a risk factor for OA after ACL reconstruction [74, 76–78] with both ACL and meniscus injury significantly increasing the odds of undergoing total knee arthroplasty [79, 80]. As described in an earlier section, the role of the meniscus injury on PTOA progression may be related to the persistent intraarticular inflammatory response seen after ACL reconstruction [16, 81]. Proinflammatory stimulation of meniscus cells increases matrix metalloproteinase and cytokine activity [28, 81, 82]. The combination of proinflammatory cytokines and compressive loading, similar to the loading seen during sports activities, further promotes degradative enzyme activity and an increase of proinflammatory mediators [83]. Moreover, meniscus injury was associated with increased subchondral bone plate thickness of the lateral femoral condyle consistent with changes seen in OA were reported within 5 years of ACL reconstruction [84]. The meniscus thereby plays an active role after ACL reconstruction in promoting the cycle of articular cartilage degradation and PTOA progression.

Lateral meniscus injuries are more common with acute ACL injury than injuries to the medial meniscus [85, 86], and lateral meniscus injuries appear to have a number of different pathways to promote progressive cartilage degradation. In a finite-element study, lateral meniscectomy increased contact and shear stresses more than 200% greater than after medial meniscectomy [87]. Not only is there increased force borne by the articular cartilage that may accelerate OA changes, lateral meniscectomy results in greater effect on both anterior and rotational instability than medial meniscectomy [88]. Persistent instability has been identified as a risk factor for OA after ACL reconstruction [74], but also increases the risk of subsequent medial meniscus injury. Whether it be by directly increasing articular cartilage forces or by increasing the likelihood of persistent joint laxity that may result in subsequent articular cartilage or meniscus injury, lateral meniscus injuries appear to have an important role in the progression of OA after ACL injury.

7.3.2 Increased Age at the Time of Injury or Surgery

Because of the prevalence during team sports, ACL injury is often considered to impact younger patients more often than relative older patients. However, in a recent analysis of nearly 110,000 patents that underwent ACL reconstruction from a large insurance claims database, we were surprised to see that nearly half of the patients (48%) were age 25 years or older [89]. Interestingly, the response to ACL injury may differ based on patient age. Similar to meniscus injury, increased age at the time of surgery was identified as an independent risk factor for radiographic PTOA in cohort studies [74, 76, 77]. Additionally, the potential role of bone marrow lesions on PTOA may differ by age, as older patients have demonstrated self-reported pain, functional limitations, and greater cartilage degradation [35, 40, 41]. Patients ≥25 years of age may then display worse symptoms and more rapid PTOA progression,

thereby potentially providing a signal that may be more readily monitored when compared to younger patients.

7.3.3 Increased Body Mass Index (BMI)

Much like age, obesity and increased BMI may exacerbate the progression of OA after ACL reconstruction [75, 90]. Obesity has long been linked with idiopathic OA progression, and while this has historically been attributed to increased loading of the articular cartilage secondary to increased body mass, the "wear and tear" model of OA has been further developed to include metabolic mechanisms as well. Mechanical loading of the cartilage may very well be implicated in OA progression; however, despite involving non-weight-bearing joints, more rapid hand OA progression was seen obese individuals [91]. Obesity is associated with increased adipokine activity, increased adipose deposits in the infrapatellar fat pad, systemic increases in proinflammatory cytokines, and disturbed lipid metabolism, and these factors have been linked to OA progression [92]. After ACL reconstruction, increased BMI has been associated with greater type 2 collagen turnover [93]. While BMI at the time of ACL reconstruction is predictive of subsequent OA progression, BMI also continues to increase in the 10 years after ACL reconstruction [14]. Increased BMI is then confounded with a decrease in physical activity during this time period [14] and is indicative of a cycle of inactivity and injury leading to a chronic metabolic state which is likely a driving factor in the progression of OA.

7.3.4 Female Sex

There is mixed evidence as to whether sex influences the prevalence of OA after ACL injury or reconstruction. From a study of an insurance claims database, Bodkin et al. identified that female sex was associated with significantly increased likelihood of OA diagnosis within 5 years of ACL reconstruction [75]. Similarly, in a long-term follow-up of a randomized clinical trial comparing the differences between hamstring and bone-patellar tendon-bone autografts, Barenius et al. reported that the OA was more common amongst female patients [94]. Female sex was also found to be an independent predictor of increased Kellgren–Lawrence grade by Li et al. [78].

Mechanistically, there are several potential rationales to explain the increased risk. First, the chemokine response to injury appears to differ between the sexes with females demonstrated increased chemokine production after ACL injury when compared to males. Additionally, persistent muscle dysfunction has been implicated in the progression of OA after ACL reconstruction, and females have demonstrated inferior muscle strength and reduced rate of torque development [62, 63]. Females have also demonstrated asymmetrical knee biomechanics and joint loading after ACL reconstruction [95], which may further increase the risk of progressive cartilage loss.

However, while some reports have found that females are at increased risk, and there may be mechanistic rationales to potentially explain these differences, other groups have not found female sex to be associated with increased OA risk. In the Multicenter Orthopaedic Outcomes Network cohort and in a population-based study in Sweden, sex was not identified as a predictor of early joint space narrowing or symptoms consistent with OA [76, 77, 96]. Furthermore, male sex was reported to be a predictor of both early cartilage changes and the progression to total knee arthroplasty (TKA) [97, 98]. As such, additional work to determine the role of sex on PTOA progression after ACL reconstruction is needed.

7.4 Conclusion

Because of the high prevalence of subsequent PTOA, ACL injury has been adopted as a human model of early osteoarthritis. There are multiple mechanisms by which ACL injury promotes PTOA progression. The injury affects the joint as an organ impacting not only the articular carti-

lage but also the synovium, underlying bone, and surrounding musculature with similar pathways implicated in the development and progression of idiopathic knee OA. In addition, both patient and injury characteristics may also contribute to PTOA progression after ACL injury.

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Part II

Basic Science of Early OA

8 Biomechanics of Instability and Its Relationship to OA

Benjamin B. Rothrauff, Michael A. Fox, Ryan S. Murray, Philipp W. Winkler, and Volker Musahl

8.1 Introduction

Degenerative joint disease, or osteoarthritis (OA), entails deterioration of a synovial joint. Although cartilage loss has classically defined OA, the disease involves a complex interplay of all joint tissues, also including bone, ligament, tendon, synovium, and meniscus. Therefore, the synovial joint is better conceptualized as an organ system, with OA ultimately progressing to joint failure. As with all examples of organ failure, the pathogenesis of OA is incompletely understood but includes a multifactorial etiology. These mechanisms involve direct damage to joint structures and the resulting inflammatory response to conservative and/or operative treatment, mediated in the context of personal factors such as age, obesity, lifestyle, pain processing, and genetics [1]. In contrast to the inflammatory arthritides driven by pathologic autoimmune processes, the myriad biological mechanisms underlying OA are most strongly influenced by the mechanical microenvironment of the joint. In understanding the mechanics of the healthy and diseased joints resulting from acute structural damage due to traumatic injury, as well as attritional damage due to age-associated 'wear and tear', it may be possible to intervene surgically and/or pharmacologically to preserve or restore joint health, in turn preventing the ultimate sequela of OA as total joint failure.

Given the frequency of knee injuries, coupled with the prevalence of knee OA, joint mechanics of the knee have arguably been better characterized than any other joint. Further elucidating the biomechanics of the knee and its relationship to OA will also likely hold insights for all synovial joints. To that end, it has long been recognized that joint instability resulting from injury or surgery on knee structures, such as the anterior cruciate ligament (ACL) and meniscus, can accelerate the onset and progression of OA [2–4]. Surgical interventions intended to restore native knee kinematics often fall short in completely achieving this goal, which at least partly explains the elevated risk for OA even when surgical repair or reconstruction of damaged knee structures is performed [5, 6]. Although the orthopaedic surgeon is principally interested in restoring native knee stability through reconstruction and/or repair of the passive constraints of the knee, such as ligaments and menisci, there is growing appreciation that aberrant dynamic stability of the knee due to knee muscle weakness, neuromuscular coordination, and proprioception, also contributes to the increased risk for OA following

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injury and/or surgery [7, 8]. Herein, the relationship of knee instability, secondary to isolated or combined injury of knee structures, with OA is reviewed. The development of animal models, advancing imaging modalities, and biomarkers of OA progression, are also briefly highlighted, as these emerging technologies further our understanding of the causal relationships between knee instability and OA, which may, in turn, facilitate the creation of targeted interventions to prevent or reverse OA.

8.2 Injury Patterns and the Prevalence of OA

Traumatic injury to the knee and associated instability has been linked as a major contributor to OA. Population studies estimate that approximately 12% of knee OA is attributable to traumatic injury, which includes injuries to the menisci, cruciate ligaments, collateral ligaments, or a combination of multiple knee stabilizers [9]. As highlighted below, injury to these structures increases the risk of OA onset and progression, with surgical reconstruction and repair often mitigating, but not eliminating, the elevated risk.

8.2.1 Meniscus

The menisci of the knee are crescent-shaped fibrocartilaginous tissues interposed between the articular surfaces of the femur and tibia, which principally serve to distribute tibiofemoral contact stresses. As early as 1948, Fairbank associated meniscal deficiency (following total meniscectomy) with radiographic joint space narrowing [10]. Subsequent studies confirmed that tibiofemoral contact stress increased after meniscectomy, thereby providing a mechanism by which the meniscus prevents joint degeneration [11]. In particular, meniscal tears can increase cartilage strain, with supraphysiological deformation of the cartilage causing matrix catabolism [12]. Given the established function of the meniscus in preserving joint integrity, complete meniscus excision (i.e. total meniscectomy) is now performed infrequently, with every effort made to preserve as much meniscal volume as possible [13]. That said, the meniscus is still the most commonly injured knee structure, and partial meniscectomy is the most commonly performed orthopaedic procedure, with up to one million operations performed annually in the USA [14].

While partial meniscectomy seeks to preserve healthy meniscal tissue, biomechanical studies consistently demonstrate decreased contact area and increased stresses with the removal of even small volumes of tissue [15, 16]. Partial meniscectomy of an isolated meniscus tear results in a 14-fold increase in long-term risk of developing severe OA [17], while partial meniscectomy concomitant with ACL reconstruction, an independent risk factor for OA progression, further increases this risk 7- to 11-fold [18]. Given the importance of meniscal preservation, there has been an increased performance of meniscus repairs in the past decade [14]. Under particular indications, suture repair appears to be chondroprotective. Following an isolated traumatic medial meniscal tear, 81 patients underwent either suture repair (n = 42) or partial meniscectomy (n = 39) [19]. At long-term follow-up (mean of 8.8 years post-operatively), osteoarthritic progression was detectable in 19% of patients after repair compared with 60% after meniscectomy [19]. As compared to subtotal/total lateral meniscectomy, meniscal allograft transplantation was also found to be chondroprotective [20]. Nevertheless, the criteria for meniscal transplantation are stringent, and the indications for suture repair also remain relatively narrow. Notably, repairs of tears located in the periphery of the meniscus exhibit an excellent healing response, given their proximity to the vasculature [21], while failure rates approaching 75% have been reported for repairs in the avascular inner region [22, 23]. Novel surgical techniques and suturing devices have been developed in an effort to improve the mechanical properties of the surgical repair, but in vivo and long-term data are lacking to support improved outcomes and OA prevention.

Finally, there is an increasing understanding of the role of the menisci as secondary restraints to tibiofemoral translational and rotation, especially in the context of concomitant ACL injury [24]. The lateral meniscus, which is circular in morphology and more mobile than the medial meniscus, works synergistically with the ACL and the structures of the anterolateral complex (ALC, discussed below) of the knee to control rotatory knee stability, as commonly examined through the pivot shift test. A quantitative pivot shift test revealed that a concurrent lateral meniscus tear increases rotational laxity in ACL-deficient knees [25, 26]. Even absent ACL injury, partial lateral meniscectomy was found to affect knee translational and rotatory laxity, as measured with a robotic testing system [27]. Further work has confirmed that the lateral meniscus, anterolateral capsule, and iliotibial band (ITB), in concert with the morphology of the lateral tibial plateau (i.e. increased posterior tibial slope and small size) contribute to a high-grade pivot shift [28]. Given the interactive relationship of bony morphology and mechanical alignment with meniscal function, slope-altering osteotomies are often required when performing concomitant meniscal allograft transplantation so as to optimize clinical success (Fig. 8.1).

The medial meniscus, possessing a C-shaped morphology and being relatively immobile as it possesses robust capsulomeniscal attachments and deepens the concave medial tibial plateau, is especially important as a secondary restraint for the ACL in resisting anterior tibial translation. Given this role, the medial meniscus is commonly torn following ACL injuries treated non-operatively, as the medial meniscus becomes the primary translational restraint absent a functioning ACL [29]. Even absent ACL injury, the medial meniscus plays an important role in knee stability. The creation of posterior medial meniscus tears in cadaveric specimens has been shown to increase anteroposterior instability, with associated increases in peak tibiofemoral contact stresses [30]. Similar adverse effects on in vivo knee kinematics were seen in patients with isolated medial meniscus root tears [31]. As could be expected, partial medial meniscectomy also disrupts knee kinematics, with increasing instability correlated with the volume of resected meniscal tissue [32, 33].

Persistent changes in kinematics appear to exist even following ACL reconstruction (ACLR) in the context of concomitant ACL and meniscal injury. Using dynamic stereo radiography to evaluate in vivo knee kinematics during downhill running, a history of medial and/or lateral meniscal tears (treated with partial meniscectomy or repair) was associated with increased anterior tibial translation 24 months following ACLR [34]. As illustrated by these recent studies, the menisci are increasingly recognized for their central function in knee stability, in combination with their established role in distributing tibiofemoral contact stresses. Given the central role of the menisci in normal knee function, preservation of the menisci whenever possible should be performed in an effort to restore knee stability and (hopefully) prevent early arthritic changes.

8.2.2 Anterior Cruciate Ligament (ACL)

The ACL is the primary restraint to anterior tibial translation and internal tibial rotation, especially at low knee flexion angles (i.e. closer to extension). As such, it is often injured during pivoting sports (e.g. football, basketball, rugby). Given the popularity of these sports, the ACL is one of the most commonly injured knee structures, with ACL tears accounting for over 20% of knee injuries in athletic populations [35]. As affected patients are often young and sometimes prominent public figures (i.e. elite athletes), ACL injury and treatment has been one of the most intensively researched topics in orthopaedic surgery. It has been consistently reported that ACL rupture alters knee kinematics, which frequently persists over time especially if operative treatment is not performed, resulting in accelerated onset of OA. However, the range of estimations for the development of post-traumatic OA (PTOA) following ACL injury has been highly variable, with the prevalence of OA reported anywhere between 10 and 90% [2, 36]. This value decreases when

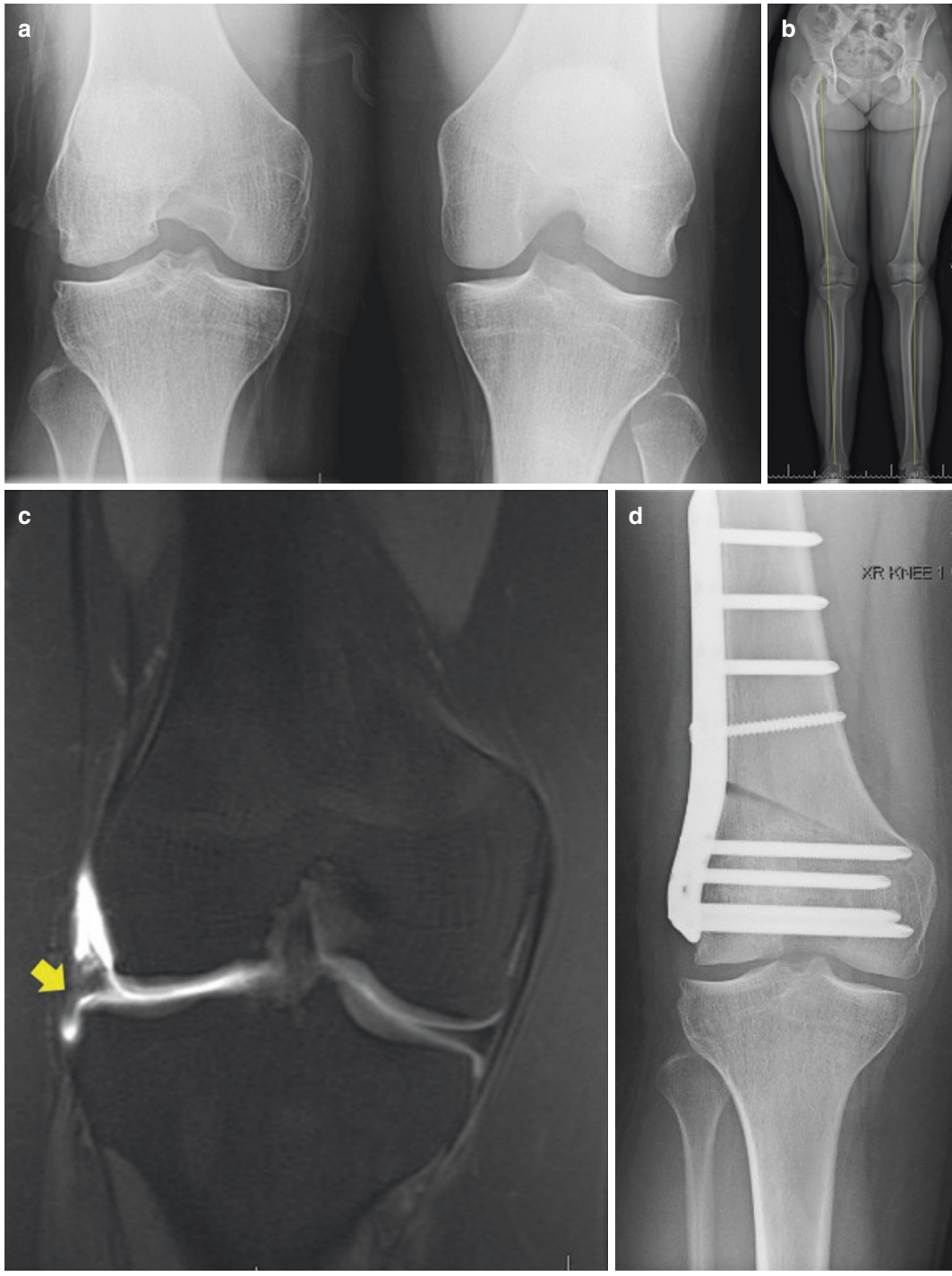

Fig. 8.1 Combined distal femoral osteotomy and lateral meniscus allograft transplantation (MAT). (**a**) Bilateral, weightbearing X-rays demonstrate early arthritic changes (osteophytes, tibial spine spiking) but relatively preserved joint space of affected (right) lateral compartment. (**b**) Standing limb-length X-ray showing bilateral valgus alignment (right 6° vs left 4°). (**c**) Pre-operative T1-weighted fat-suppressed MRI demonstrating lateral meniscus extrusion, insufficiency (yellow arrow). (**d**) Immediate post-operative X-ray of lateral femoral opening wedge osteotomy and lateral MAT. (**e**) T2-weighted fat-suppressed MRI at 6 months post-operation demonstrating intact, remodelling lateral meniscus allograft. (**f**) X-ray at 6 months post-operation demonstrating intact hardware and union of osteotomy site

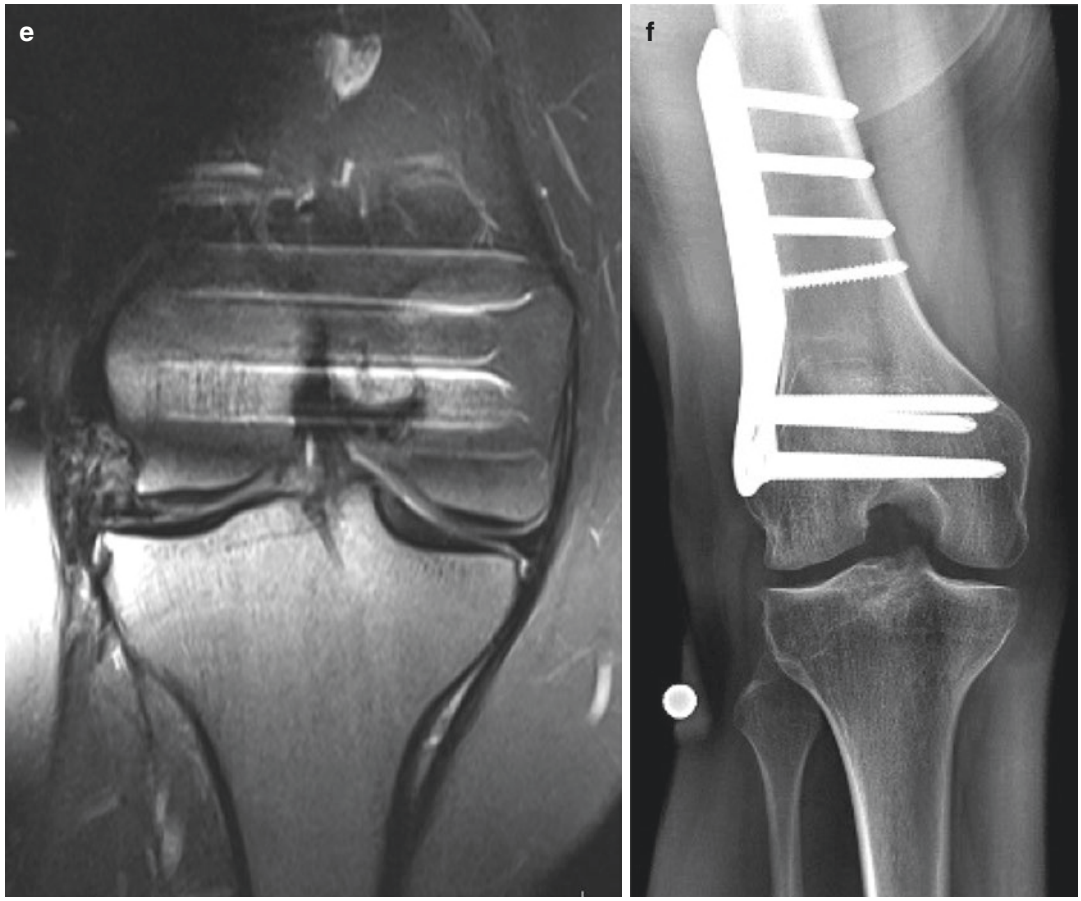

Fig. 8.1 (continued)

focusing on isolated ACL injures, with values ranging from 0 to 13% [36, 37]. In one of the few prospective studies looking at long-term outcomes after non-operative management for ACL injuries, the prevalence of OA was 16% at 15-year follow-up [38]. Other studies attempted to achieve a more homogeneous patient population by looking at injuries in select groups of athletes. A retrospective review of 219 male soccer players with an average of 14 years follow-up after an ACL injury found that 41% of patients demonstrated radiographically apparent OA [39].

Variability in OA incidence following ACL injury may in part be attributable to differences in activity levels, whereby less active individuals, or those who voluntarily reduce activity following injury, mitigate the risk for further injury to secondary restraints, and in turn, OA risk. However, for those seeking a return to pre-injury activity levels (often including sport), ACL reconstruction has been the standard-of-care. While ACLR has been shown to improve knee laxity and facilitate return to sport, systematic reviews of past literature suggest an inconsistent reduction in OA risk [2, 6]. However, the majority of studies in past decades that examined OA prevalence following ACLR utilized a transtibial technique, which frequently fails to place the femoral and tibia tunnels within the anatomic ACL footprints [40, 41]. As compared to drilling the femoral and tibial tunnels independently with the explicit intention of achieving anatomic ACLR, transtibial drilling is inferior in restoring normal joint kinematics [42–44]. Therefore, it has been hypothesized that anatomic ACLR, through independent tunnel drilling, may better protect against

PTOA. To that end, a small cohort study with less than 2-year follow-up found that non-anatomic graft placement through transtibial drilling led to a significant decrease in cartilage thickness as measured by magnetic resonance imaging (MRI), a phenomenon not seen with anatomic graft placement through independent tunnel drilling [45, 46]. As independent tunnel drilling and anatomic ACLR have only gained popularity in the past 15 years, there are no long-term comparative studies evaluating OA prevalence following anatomic vs non-anatomic ACLR. However, a recent systematic review of all studies with a minimum 10-year follow-up found a pooled OA prevalence, as defined radiographically, of 23% following anatomic ACLR compared to 44% following non-anatomic ACLR, suggesting the importance of restoring knee stability to reduce OA risk (Fig. 8.2) [47].

The role of the ALC of the knee in rotatory knee stability, especially in the context of ACL injury, has also been the subject of recent debate [48]. Cadaveric biomechanical studies have shown combined ACLR and lateral extra-articular tenodesis (LET) may better restore joint kinematics compared to isolated ACLR in the presence of frank ALC injury [49]. On the other hand, LET has been reported to have no added benefit absent capsular injury [50] and may actually overconstrain knee motion [51], resulting in elevated contact pressures in the lateral compartment and increased lateral patellar tilt if the tenodesis is

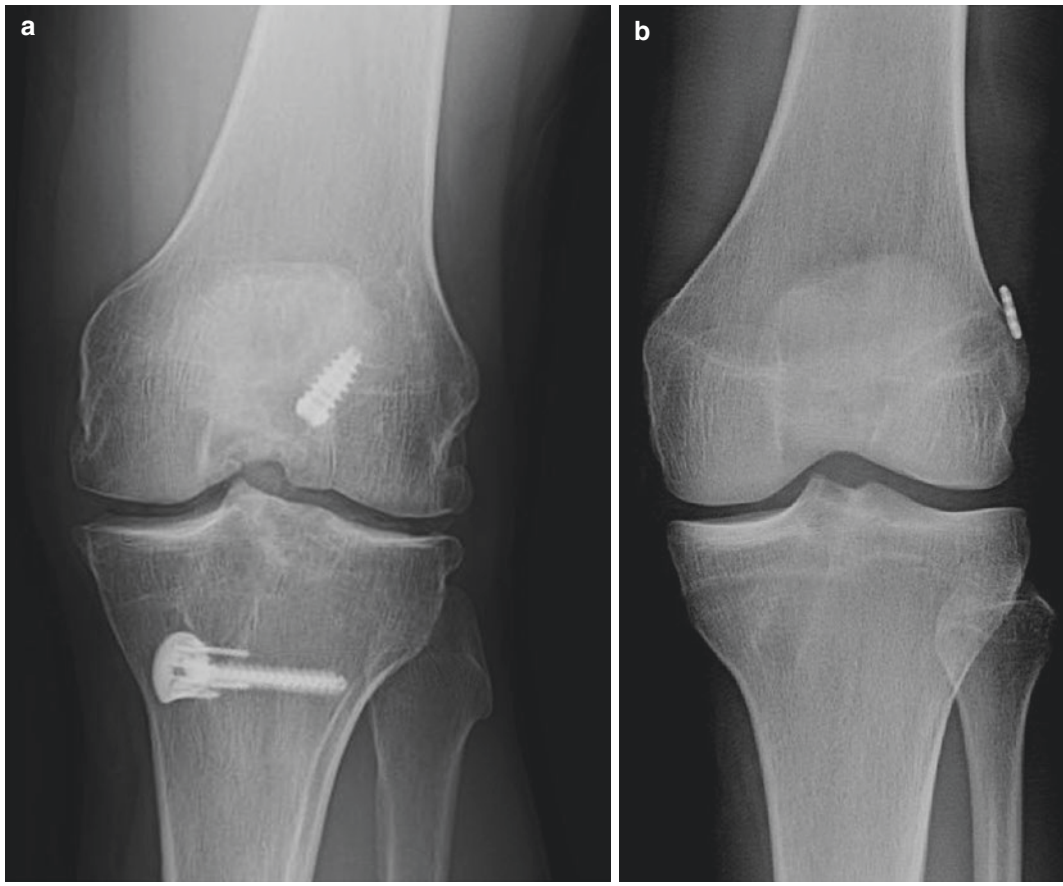

Fig. 8.2 Comparison of non-anatomic and anatomic ACLR on long-term OA prevalence. (**a**) Ten-year follow-up X-ray of non-anatomic ACLR performed by transtibial drilling demonstrating osteoarthritic changes, including joint space narrowing, osteophyte formation, and subchondral bone sclerosis. (**b**) Ten-year follow-up X-ray of anatomic ACLR performed by independent tunnel drilling demonstrating minimal degeneration changes

over-tensioned [52]. The in vivo effects of LET procedures remain less clear. A prospective randomized study involving high-risk young athletes who underwent anatomic ACLR with or without LET found that the combined procedure reduced graft failure (4%) compared to isolated ACL reconstruction (11%) [53]. As ACLR was performed exclusively with hamstring autografts, it is not clear if alternative grafts (e.g. bone-patellar tendon-bone and quadriceps tendon autografts) would yield such findings. Similar, this study only included 2-year follow-up. It is therefore unknown if the reported benefits of LET persist over time or if there is an increased risk of long-term OA. Given these uncertainties, the indications for LET are yet to be clearly defined [48, 54].

The bony morphology of the tibia and femur also contribute to knee stability and are important considerations especially in the setting of revision ACLR. A steep posterior tibial slope, deep posterior lateral femoral condyle, and varus malalignment are common findings in multiple ACL failures [55–58]. As shown through cadaveric biomechanical testing, increasing posterior tibial slope strongly correlates with increasing ACL graft forces [59]. Combined high tibial osteotomy (HTO) and ACLR can be employed to correct excessive angulation (i.e. posterior tibial slope, coronal alignment) and replace the deficient ACL (Fig. 8.3) [58, 60]. A recent systematic review that included seven studies found that combined HTO and revision ACLR, most commonly performed for a posterior tibial slope ≥12° with or without concomitant varus malalignment defined as a hip-knee-ankle angle >180°, produced good post-operative functional outcomes, low complication rates, and no reported re-ruptures [61]. However, the studies were limited by the small sample size and short-term follow-up.

8.2.3 Posterior Cruciate Ligament (PCL)

Mirroring the ACL, the PCL is the primary constraint to posterior translation of the tibia. But in contrast to the ACL, significant, isolated, injury to the PCL is relatively rare and more often occurs in the context of multi-ligament knee injuries [62]. Often injured during high-energy trauma (e.g. motor vehicle accidents), the incidence of PCL injury has been reported at rates as high as 44% in a cohort of trauma patients with acute knee hemarthrosis [63]. However, isolated injuries to the PCL are quite rare, representing less than 1% of knee injuries [35]. In contrast to ACL injuries, isolated PCL injuries have historically been better tolerated, with good subjective outcomes and high rates of return to sport achieved with conservative treatment [64]. Nevertheless, knee kinematics remained altered with conservative management.

Numerous cadaveric and in vitro studies have demonstrated that PCL deficiency results in increased posterior translational of the tibia, with increased contact pressures in the medial and patellofemoral compartments [64, 65]. Examination of tibiofemoral motion by MRI during a pseudo-static squat in PCL-deficient patients further revealed posterior subluxation of the medial tibial plateau through an arc of flexion from 0° to 90°, without a change in kinematics of the lateral compartment [66]. In a related study, it was found that PCL deficiency led to a more anterior and medial location of peak cartilage deformation on the medial tibial plateau between 75° and 120° of knee flexion, with no alteration in the location of peak cartilage deformation seen on the lateral tibial plateau [67]. PCL deficiency was also found to increase patellar tilt and lateral patella shift with increasing knee flexion [68]. These kinematic changes, principally involving the medial and patellofemoral compartments, correlate with the increased incidence of arthritic changes in these compartments following PCL injury [64]. Even young athletes with asymptomatic PCL deficiency who successfully return to sport demonstrated unexpected increases in T1ρ values of knee cartilage on MRI, consistent with subclinical cartilage degeneration despite a well-functioning knee [69]. T1ρ is a quantitative composition imaging technique for which increasing values are inversely related to cartilage proteoglycan concentration (i.e. increased T1ρ = increased proteoglycan loss).

Due to the low overall incidence of isolated PCL injuries, current studies on long-term outcomes are primarily limited to small case series, with few studies assessing long-term OA prevalence (≥10 years from injury). In a population-based retrospective study of 48 patients with an isolated PCL tear treated conservatively, patients were 6.2 times more likely to have symptomatic OA as compared to individuals without a PCL tear [62]. In a similar retrospective case series, 68 patients with an isolated PCL tear treated non-operatively were followed for a mean of 17.6 years [70]. Of the 44 patients who underwent both objective and subjective evaluation (at a mean follow-up of 14.3 years), only five patients (11%) had radiographic evidence of

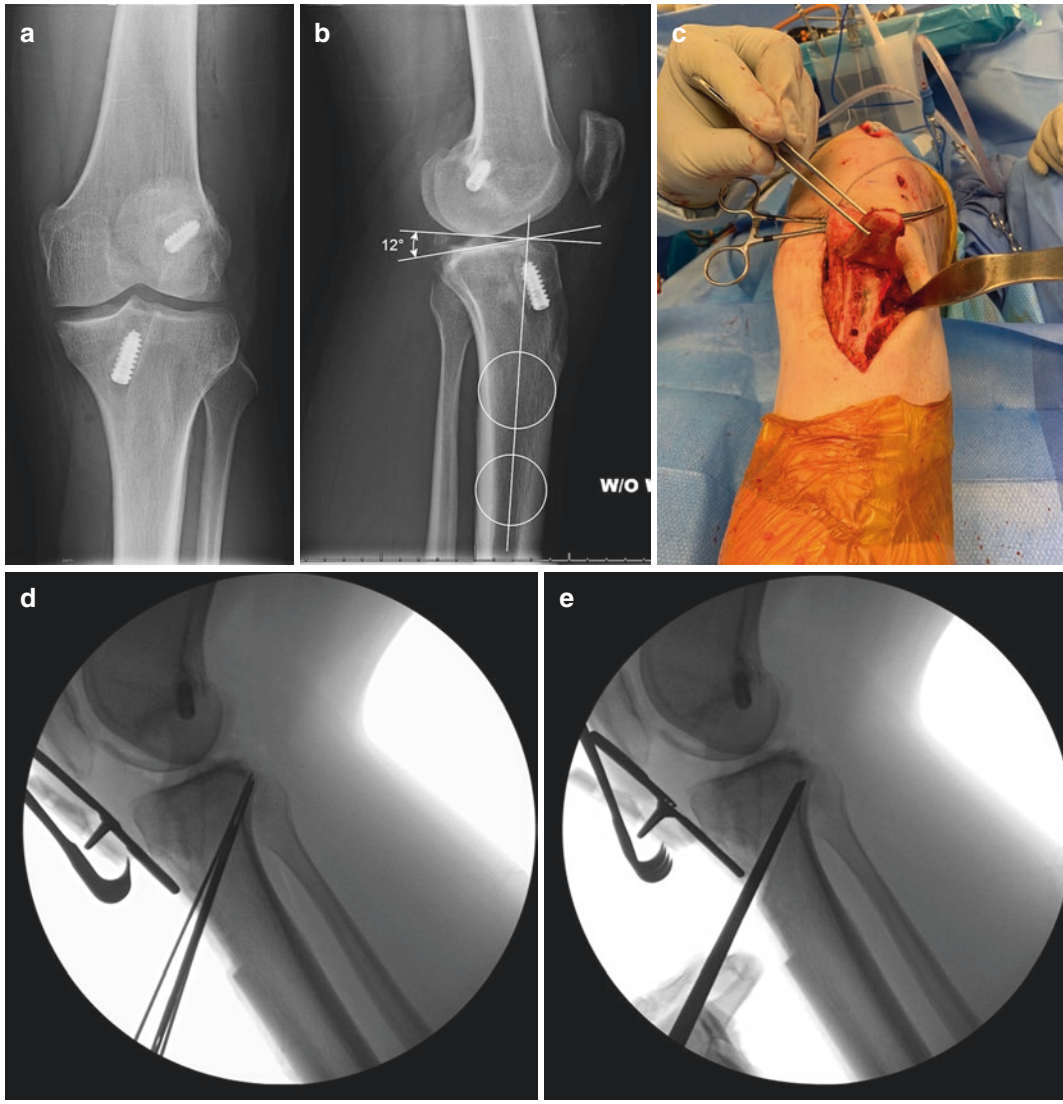

Fig. 8.3 Combined HTO and ACLR for multiple ACL failures. (**a**, **b**) AP and sagittal X-rays of multiply revised ACL-reconstructed knee, again ACL deficient, with posterior tibial slope of 12°. (**c**) A tibial tubercle osteotomy is performed to access the HTO site of the anterior tibial cortex. (**d**, **e**) Intra-operative radiographs of slope-correcting HTO. (**f**) Photograph of secured anterior tibial plate (TomoFix®, DePuy Synthes) and insertion of ipsilateral quadriceps tendon autograft for arthroscopic revision ACLR. (**g**) Post-operative sagittal X-ray demonstrating corrected posterior tibial slope of 6°

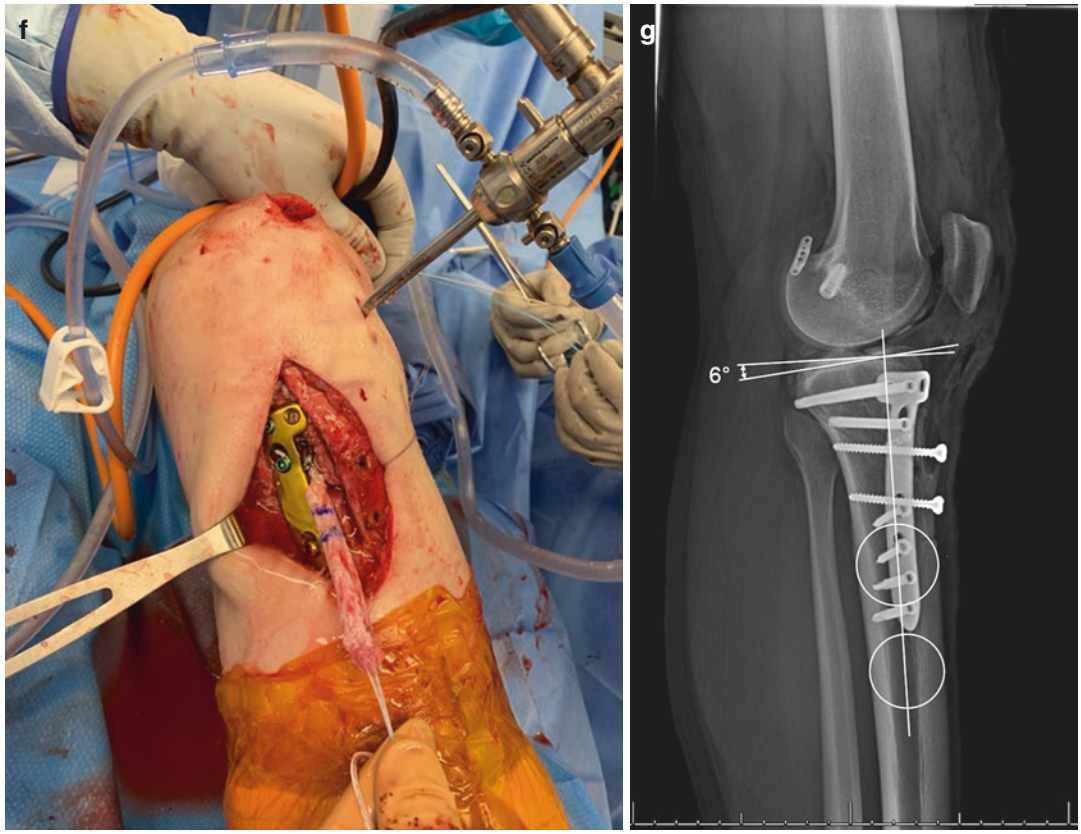

Fig. 8.3 (continued)

moderate to severe OA. Furthermore, patients were found to largely have remained active, regained quadriceps strength and full ROM nearly equal to the contralateral (healthy) limb, and reported good subjective scores (as measured by International Knee Documentation Committee (IKDC) and Cincinnati Knee Rating System, CKRS) [70]. Across the literature, the rate of OA prevalence in the medial and patellofemoral compartments following PCL injury ranges from 11 to 53% [64].

Given the persistent changes in knee kinematics, with an increased risk of subsequent OA following PCL injury, PCL reconstruction (PCLR) has been performed in an effort to restore knee stability and prevent future joint degeneration. As with ACL reconstruction, PCLR was historically performed with a single-bundle (SB) technique. As described primarily in case series, PCLR provides significant improvement in subjective outcomes, often equal in magnitude regardless of whether the PCL injury is isolated or concomitant with damage to other knee structures [71–73]. Nevertheless, no study has directly compared outcomes following PCLR vs conservative management. Furthermore, while PLCR can improve the posterior drawer by an average of 1 grade, it has not reliably restored normal knee stability, nor has it been shown to prevent OA [74, 75]. Similar to the ACL, there has been a recent interest in anatomic double-bundle (DB) PCLR, which has produced significant improvement in functional and objective outcome measures [76]. Whether DB PCLR yields better outcomes, including a reduction in OA prevalence, as compared to SB PCLR remains an open question, with at least one case series finding no difference between techniques at minimum 10 years follow-up [77].

8.2.4 Multi-ligament/Knee Dislocation

Multi-ligament knee injury is defined as an injury to two or more of the soft tissue stabilizers of the knee, including the cruciate ligaments, collateral ligaments, and posterior medial and lateral complexes (i.e. PLC and PMC, respectively). Multi-ligament knee injuries are also frequently referred to as knee dislocations, as the ligamentous disruption causes multidirectional instability that can result in dislocation and subluxation of the knee joint. These are extremely rare, often high-energy injuries, representing just 0.02% of orthopaedic injuries [78]. As a result, the available literature regarding surgical reconstruction and long-term outcomes is limited to small case series. One case series followed 26 patients who underwent surgical reconstruction after sustaining a multi-ligament knee injury, finding 69% of patients demonstrated radiographically significant OA in the reconstructed knee when graded with the Kellgren–Lawrence (KL) classification at an average of 8-year follow-up [79]. A similar series of 44 patients found a lower prevalence of OA using the KL classification, with 23% of knees demonstrating radiographic OA after 5-year follow-up [80].

8.2.5 Collateral Ligaments

The medial collateral ligament (MCL) and lateral collateral ligament (LCL) are the primary restraints to valgus and varus angulation of the knee, respectively. When included with additional structures comprising the posteromedial corner (PMC) or posterolateral corner (PLC), the structures provide additional rotatory support. Given their extra-capsular location, the collateral ligaments possess a superior innate healing capacity as compared to the cruciate ligaments. Furthermore, the collateral ligaments are often injured concurrent with a cruciate ligament and/or meniscus, obscuring their isolated role in knee instability and OA pathogenesis. The indications for operative treatment of isolated and cruciate-combined collateral ligament injuries are debated [81, 82], with both a multi-centre cohort study [83] and prospective randomized study [84] demonstrating equivalent short-term subjective and objective outcomes following ACLR with operative vs non-operative treatment of a concurrent MCL tear. That said, operative treatment of a combined ACL and PMC/PLC injury has been associated with poorer outcomes as compared to reconstruction of an isolated ACL tear [85–87]. As multi-ligament injury is often exclusion criterion for studies examining the relationship between cruciate reconstruction and OA, the role of concomitant MCL/LCL/PMC/PLC injury as a risk factor to PTOA is not clearly defined [36]. Recognizing the inherent limitations in the study design, patients with a partial MCL tear treated conservatively demonstrated no signs of radiographic OA at 10-year follow-up, compared to 50% of patients with a combined ACL and complete MCL rupture, both of which were treated operatively [88].

8.3 Limitations of Clinical Studies

Long-term outcomes after traumatic knee injuries have been a heavily researched topic in the orthopaedic literature. However, studies continue to produce a broad range of clinical outcome data for similar injuries, making it a challenge to draw generalizable conclusions from the available information. This is likely attributable to many limitations in long-term studies, including diversity in study populations, injury patterns, outcome criteria, follow-up duration, and surgical management strategies.

8.3.1 Concomitant Injuries

While knee injuries are a common orthopaedic presentation, injury to a single stabilizing structure is relatively rare with most cases presenting with combined injuries to multiple structures. Population studies estimate that only 20% of all ACL tears represent isolated injuries, with the menisci and MCL tears representing the most commonly associated injuries [89]. This compli-

cates outcome studies as concomitant injuries have been shown to have worse long-term morbidity when compared to isolated injuries. In a systematic review of studies investigating OA following ACL injury, the prevelance of OA increased from 0 to 13% after isolated ACL injuries compared to 21–48% in patients with concurrent ACL and meniscus injuries [90]. A prospective study by the same group found a similarly increased trend towards a higher prevelance of OA with combined injuries compared to isolated ACL injuries (80% vs 62%) [37]. It is also thought that an isolated injury to a single knee structure can cause changes in the loading of the knee joint, which can lead to subsequent injury and degeneration of other knee structures, compounding the risk for subsequent OA (such as a degenerative meniscal injury after sustaining an isolated injury to the ACL).

8.3.2 Host-Related Variables

Several patient-related factors, including age, gender, body mass index (BMI), and physical activity level have been proposed as potential risk factors for OA after traumatic knee injury. Obesity has been shown to be a risk factor for the development of OA in the general population, with a 5-unit increase in body mass index (BMI) corresponding to a 35% increased risk for the development of OA [91]. The longitudinal prospective cohort study performed by the Multicenter Orthopaedic Outcomes Network (MOON) Research Group looked at these patient-related factors in 421 primary and revision ACL reconstructions with at least 2-year follow-up, finding a slight association between radiographically apparent OA and age (OR = 1.06) and increasing BMI (OR = 1.05) [92]. A case-control series of 249 patients who underwent ACLR found greater elevations in risk for radiographic OA in overweight (BMI > 25, OR = 2.04) and obese patients (BMI > 30, OR = 3.24). [93] However, larger reviews have failed to find any consistent association between these variables in the broader literature [94]. While higher activity and return to sport is thought to be a risk factor due to the increased stress on the knee joint during competitive athletics, this association has not been shown in the literature [94]. Similarly, a prospective cohort study of 56 patients who underwent ACLR found no association between radiographic OA (according to the KL classification) and return to sport [95].

8.3.3 Operative vs. Non-operative Treatment

The association between surgical and conservative management of ACL injuries and long-term outcomes has been extensively discussed in the orthopaedic literature. The management strategy itself is a challenging variable to study objectively, as the decision for aggressive or conservative management is heavily dependent on numerous patient- and surgeon-related factors. While individual studies have shown trends towards increased risk of OA with either operative or non-operative management [96, 97], systematic reviews have failed to demonstrate any statistically significant association in high-quality studies [37, 98, 99]. No association has also been shown between the timing of eventual surgical intervention and the development of OA [94].

8.3.4 Variation in Outcome Metrics

Numerous means of assessing the severity of OA in terms of both radiographic and clinical symptoms have been proposed in the literature, including clinical questionnaires, radiographic metrics, and biomechanical markers. The oldest and most widely used radiographic assessment scale was first proposed by Kellgren and Lawrence in 1957, which classifies radiographs on a grading scale of 0–4 with a score of 2 or greater corresponding to significant arthritis [100]. Several other classification systems have been validated for use in the setting of traumatic knee injuries, including the International Knee Documentation Committee (IKDC) system, the Ahlbäck system, and the Osteoarthritis Radiographic Severity Index (OARSI) [101–103]. A review of long-term out-

comes after ACL injury found over ten different classifications utilized across the reviewed studies [2]. Each system attempts to use these measures to categorize patients based on the severity of disease. However, differences in the content and relative weighting of different measures in the system can lead to alternative conclusions for the same patient population. These discrepancies can be further exaggerated by interobserver variability, which also varies depending on the employed scoring system. A study by the Multicenter ACL Revision Study (MARS) Group attempted to assess the reliability of the most common scoring systems by comparing the preoperative radiographic assessment with intraoperative assessment of the articular cartilage at the time of arthroscopy, finding that the IKDC classification system with 45° weight-bearing radiographs had the highest correlation between the radiographic scoring classification and arthroscopic findings ($r = 0.66$) [104]. No scoring system had higher than moderate inter-observer reliability.

8.4 Future Perspectives

8.4.1 Animal Models

Even with narrow inclusion criteria, clinical studies with human patients inherently entail a modest level of heterogeneity in experimental parameters (e.g. patient demographics, concurrent injury, time to surgery, etc.) and a limited (typically non-invasive) number of outcome measures, often preventing inference of causal relationships between instability and OA. Animal models overcome many of these limitations, at the obvious loss of some clinical validity. Nevertheless, animal models have been instrumental in elucidating the molecular mechanisms mediating OA in the context of knee instability [105]. Both invasive and non-invasive models have been developed, with ACL transection and medial meniscectomy being the most widely used and characterized model [106]. Model animals have spanned from mouse to non-human primates, with small animals enabling targeted modulation of putative molecular mediators (e.g. genes, growth factors, cytokines) while the larger animals share greater homology to humans with regards to anatomy and surgical approaches [105, 106].

In addition to their utility for elucidating the mechanistic underpinnings of instability-induced OA, animal models have been commonly used to investigate novel surgical and biological therapies. For example, a non-surgical mouse model of ACL rupture was used to explore the efficacy of doxycycline, an inhibitor of matrix metalloproteinase 13 (MMP-13), in preventing the progression of OA, a process which is driven in part by MMP-13-induced chondrogenic hypertrophy [107]. In a dose-dependent manner, doxycycline treatment reduced cartilage damage and synovitis [107]. As a second example, increased posterior tibial slope is a known risk factor for ACL injury and poorer post-operative outcomes in human patients [108, 109]. In many quadrupeds (e.g. dogs, cats), there is a large (>12°) posterior tibial slope of the native knee, which is thought to contribute to the rapid onset and progression of OA following ACL tears in these animals [110, 111]. To that end, joint-levelling osteotomy in these animals has shown promising results in slowing OA progression and improving joint function, even without concurrent ACLR [112, 113]. Reducing posterior tibial slope has been shown to reduce the magnitude of the pivot shift in ACL-deficient cadaveric knees [114], but the indications for levelling osteotomy in human patients, and its effect on instability and ensuing OA progression, remain the subject of future research. As a final example, there has been renewed interest in primary ACL repair (as compared to standard-of-care ACL reconstruction), with recent clinical studies showing heterogeneous results, including reports of high failure rates [115–118]. Largely absent from the literature are long-term studies of ACL repair, which are needed to determine the rates of OA following ACL repair. On the other hand, recent work using a minipig model has found that cartilage damage is inversely proportion to the degree to which the anatomic and structural

properties of the native ACL are achieved following ACL repair [119, 120]. Therefore, animal models may be useful to further optimize ACL repair strategies and monitor OA progression (on an accelerated timescale) before these novel surgical approaches are broadly adopted in clinical practice.

8.4.2 In Vivo Kinematics

At present, the relationship between knee instability and OA is largely correlative, with cadaveric biomechanical studies demonstrating perturbations in joint kinematics following iatrogenic damage to knee structures offered as an indirect explanation for the increased rates of OA seen in patients sharing these injury patterns. Historically, the ability to determine in vivo joint kinematics during dynamic movement has been limited by technology, with the most common methodology for this task (video-based motion capture) lacking the necessary precision and repeatability to detect small but clinically significant changes that distinguish the healthy from the diseased (or to-be-diseased) knee. Although relatively limited by expense, processing speed, and the necessity of expert personnel, stereo radiography through dynamic biplanar fluoroscopy has been a powerful tool to accurately measure in vivo kinematics [34, 121, 122]. Advances in computing speed and power will only further increase the utility of these technologies. Similarly, video-based technologies continue to evolve, now capable of more accurately measuring joint kinematics without the use of skin markers, which are prone to motion artifact [123]. For example, the markerless Microsoft Kinect V2 system demonstrated excellent correlation with the conventional marker-based Vicon system when measuring knee kinematics during a drop vertical jump [124]. With continued improvement in technologies to measure dynamic joint kinematics, our understanding of the relationship between joint instability and OA will evolve from one of correlation to causation.

8.4.3 Advanced Imaging and Biomarkers

Like the emerging tools for studying in vivo kinematics, both advanced imaging modalities and biomarkers will play an expanding role in understanding and predicting OA risk following injury and/or with chronic instability [125]. Already, conventional clinical MRI sequences (T1, T2) are being used to evaluate cartilage morphology within a few years of acute knee injury, as opposed to at least a decade for radiographic evidence of OA on X-ray to appear [126, 127]. However, quantitative MRI sequences (e.g. T1ρ, UTE-T2*, dGEMRIC) will permit evaluation of the biochemical composition and integrity of joint tissues within weeks to months of injury [128–130]. Similarly, advances in gene/transcript sequencing and proteomic analyses will likely permit the identification of biochemical biomarkers that are both diagnostic and prognostic of the severity of the joint injury and the sequela likely to ensue, with or without intervention [131–133].

8.5 Conclusion

Joint instability is a known risk factor for OA, as richly demonstrated through clinical studies of outcomes following injury to knee structures, including menisci, cruciate ligaments, and collateral ligaments. The literature supports a causative role of instability in promoting and accelerating OA, but the molecular mechanisms underlying instability-mediated joint degeneration must still be elucidated. There have also been equivocal results regarding the benefit of surgical reconstruction or repair of injured knee structures in mitigating or preventing OA. However, more recent results suggest that anatomic reconstruction, as exemplified in review of anatomic vs non-anatomic ACL reconstruction, may mitigate OA risk. Therefore, restoration of native joint kinematics, achieved either through operative or conservative treatment, should be pursued following injury. Emerging technologies will fur-

ther our ability to investigate in vivo kinematics and the biological response to injury and repair, from which our improved understanding of the biomechanics of instability will facilitate the development of interventions to more successfully prevent or reverse OA.

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9 Early OA Following Synovial Joint Fracture

Don Anderson, James Martin, J. Lawrence Marsh, Jessica Goetz, Mitchell Coleman, Todd McKinley, and Joseph Buckwalter

9.1 Joint Fractures and Post-traumatic Osteoarthritis

Post-traumatic osteoarthritis (PTOA), the osteoarthritis (OA) that develops following joint injury, causes pain and disability for millions of people [1–4]. It often afflicts young adults who may not be optimal candidates for joint replacements [1, 2, 4, 5]. A substantial fraction (approximately 12%) of the advanced OA in hips, knees, and ankles arises secondary to joint trauma [1, 6].

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Acute joint injury and post-traumatic residual joint abnormalities, primarily instability and articular surface incongruity [7–10], lead to progressive loss of articular cartilage, bone remodeling, and changes in the joint soft tissues, resulting in pain and loss of joint function, the clinical syndrome of OA [4, 11, 12]. A wide range of joint injuries including contusions, dislocations, ligament, meniscal, and joint capsule tears can lead to PTOA [1, 3, 4, 13–15]. However, intra-articular fractures (IAFs), fractures that extend from the articular surface into the subchondral bone [16–27], are the injuries that most predictably lead to PTOA [3, 14, 18, 19, 22, 24, 27–31], presumably because unlike other joint injuries, they subject the articular surface to loads sufficient to disrupt articular cartilage and fracture underlying bone [19]. Although IAFs occur less frequently than less severe joint injuries, they are the most common cause of combat-related disability in US military service personnel [32], and ankle fractures are the most common cause of disabling ankle OA [2, 5]. Unfortunately, current treatments of all types of joint injuries all too often fail to prevent OA.

Once OA is established by observable structural changes in the joint, PTOA and OA attributed to most other causes, or of unknown etiology, cannot be distinguished except by the history of joint injury in PTOA [11, 33–36]. The pain, loss of function, and physical changes in the joint are identical [4, 11, 12, 33, 35–39]. Because the OA that develops following IAF has a known initiat-

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ing event, it is possible to study the onset and progression of the disease from a known point in time and to develop and test interventions that have the potential to prevent or decrease the severity of the disease. Unlike many other joint injuries that may not lead to symptomatic PTOA until decades after the injury, IAFs frequently lead to symptomatic disease within a few years, which makes it possible to test new treatments in a relatively short period of time [3, 37]. For these reasons, better understanding of how IAFs lead to PTOA and the testing of new treatments in patients with IAFs will provide valuable insights into synovial joint biology and the pathogenesis of all forms of OA.

Prior to the development of clinically useful radiography in 1895 by William Roentgen, physicians and bonesetters had little understanding of IAFs [40–42]. Like most patients with IAFs, patients with joint dislocations and peri-articular fractures also present with a history of injury and localized pain, swelling, and deformity, making distinguishing IAFs from these other injuries difficult if not impossible. In the past, all these injuries were generally treated with manipulation or traction to correct deformity followed by immobilization. The use of radiographs led to the understanding of IAFs as a distinct entity and the ability to distinguish them from other injuries. Relying on radiographic images, orthopedic surgeons developed systems of classifying these injuries based on the patterns of the fracture lines in the bones; systems that have been used to guide treatment [21, 43–45]. Modern anesthesia and sterile techniques made the surgical reduction of IAF feasible. Devices for fixation of IAFs made it possible to restore normal or near-normal joint alignment and congruity for many IAFs, and to minimize the length of joint immobilization. The more recent availability of pre-operative CT scans has allowed surgeons to evaluate complex IAFs and improve planning for reduction and stabilization. Taken together, these advances have dramatically improved the treatment of IAF.

However, even with current optimal treatment, as many as one in four patients develop OA after fractures of the acetabulum [46, 47], between 23% and 44% of patients develop knee OA after intra-articular fractures of the knee [27, 48, 49], and more than 50% of patients with fractures of the distal tibial articular surface develop OA [50–52]. Because it is not always possible to restore perfect joint congruity following IAF, a substantial number of patients are left with residual joint incongruity and instability secondary to incongruity that also increases the risk for OA. The time from injury to the onset of OA following IAF varies. Following severe IAFs, that is higher-energy comminuted IAFs with disruption of the articular surface that cannot be restored to anatomic alignment, OA may develop in less than a year. Following less severe injuries, including lower-energy IAFs and disruptions of the articular surface that can be restored to anatomic alignment, OA may not develop for many years, or may never develop [19, 22, 37, 47, 49, 52]. Despite the advances in joint imaging and surgical treatment of IAFs, the risk of OA following IAFs has not decreased substantially in 50 years. It seems unlikely that further progress in joint imaging and surgical techniques will dramatically reduce the risk of OA following IAF [1, 3, 24].

For these reasons, there is a clear need to advance understanding of the biologic pathways that lead to PTOA and to develop better methods of assessing the risk of OA following IAF so that treatments can be individualized [3, 37]. That is, patients at low risk of PTOA can be appropriately treated with current methods, but new approaches will be needed for those patients who will otherwise rapidly develop disabling PTOA following IAF.

The following sections discuss innovations in the evaluation of OA risk following IAF, biologic pathways responsible for OA following IAF, biologic strategies to minimize OA risk or severity following IAF, the importance of improved rehabilitation following IAF, and future advances that are needed to dramatically reduce PTOA risk.

9.2 Evaluation of OA Risk Following Joint Fracture

Reliable methods of predicting who is at risk of a disease frequently lead to success in preventing or in decreasing the risk or severity of that disease. Identifying people at high risk makes it possible to test new treatments in those who are most likely to develop the disease. Specifically, the ability to predict the risk of PTOA following IAF will make it possible to conduct rigorous clinical trials of new treatments within a relatively short period of time and to devise individualized patient treatments. Prospective research on interventions to decrease the incidence and severity of PTOA requires that potentially confounding mechanical factors be assessed and quantified so the effect of the intervention can be identified. Two key confounding mechanical PTOA risk factors are the severity of the acute injury and ongoing chronic aberrant joint loading that arises from imperfect restoration of the articular surface. Recent work has shown that it is possible to predict PTOA risk following IAF [4, 7–10, 24, 53–56] by measuring the acute fracture energy and the elevated joint contact stresses caused by residual joint incongruity [3, 23, 28, 37, 55, 57–62].

9.2.1 Acute IAF Severity (Fracture Energy)

The mechanisms of joint injuries range from simple falls and sports injuries to high-speed motor vehicle accidents, falls from heights, and battlefield injuries [13, 15, 31, 32]. These various mechanisms produce a spectrum of injury that includes sprains, partially torn ligaments, and minimally displaced IAFs on the low end of the spectrum to devastating IAFs which explode the articular surface into multiple osseous-chondral fragments. It is no surprise that the risk, timing of onset, and severity of PTOA closely follow this spectrum of injury and that injury severity is a critical factor in determining the risk for PTOA after IAF.

Clinicians typically assess IAF injury severity using radiographs, CT, and MRI. These assessments have been aided by fracture classifications that segregate IAFs in groups by apparent severity of injury [21, 43–45]. However, the scientific utility of these classifications has been hampered by the inherent subjective nature of the assessments leading to poor inter-observer reliability of fracture classification. Recently, accurate and objective quantitative methods to measure the fracture energy, a quantitative measure of IAF severity, have been developed utilizing image analysis methods, and this computationally derived fracture energy predicts the risk and severity of PTOA following IAFs.

These computational image analysis methods [23, 28, 55, 57–63] have enabled objective metrics of IAF energy to be measured from standard-of-care CT scans (Fig. 9.1). These methods rely upon principles of fracture mechanics relating the energy involved in causing a fracture to the amount of liberated inter-fragmentary bone surface area. Bone density, which can be deduced from CT scan data, also influences the fracture energy. Additionally, liberated bone surface area near the articular surface can be weighted more heavily to incorporate the understanding that injury localized to the articular surface can be especially harmful. Automated approaches now hold promise to allow routine measurement of IAF energy in clinical practice as an indicator of severity and therefore prognosis.

This objective IAF severity metric, obtained from analysis of pre-operative CT scans, was originally shown to reliably predict the risk and severity of PTOA in tibial pilon fractures [23, 28, 55, 61, 62]. More recent work has involved computing this fracture severity in over 400 IAFs of the calcaneus (subtalar joint), the tibial plateau (knee), the acetabulum (hip), and distal radius (wrist). These joints differ greatly in the amount of articular surface over which load transfer occurs, so perhaps not surprisingly, the raw fracture energy does not directly predict PTOA risk across them all. Taking a tissue-level, rather than the whole organ-level, approach helps to explain this lack of predictive ability; when corrected for

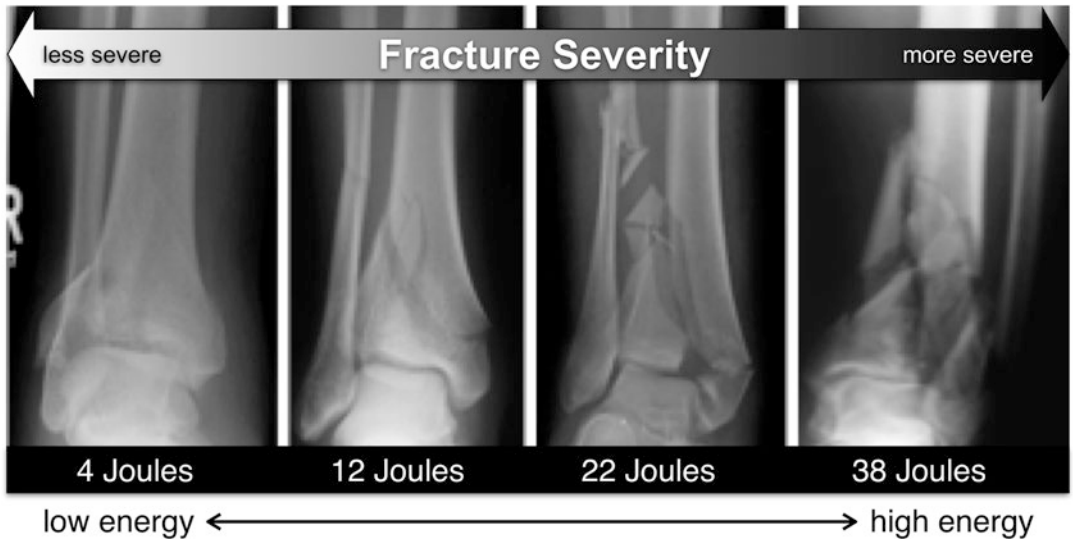

Fig. 9.1 Plain radiographs of four tibial pilon intra-articular fractures. Acute intra-articular fracture severities span a wide spectrum. The principles of fracture mechanics offer an objective basis for quantifying injury severity based on the amount of energy liberated, expressed in Joules, calculated from inter-fragmentary bone surface area measured on standard CT scans

the varied contact area across joints of different sizes, fracture energy predicts the PTOA risk in the subtalar, ankle, hip, and wrist joints (Fig. 9.2).

9.2.2 Chronic Overloading (Residual Joint Incongruity and Instability)

Imperfectly reduced IAFs alter the shape and congruity of the articular surface. Over time the resulting abnormal joint mechanics, including elevated peak contact stresses in some regions and instability or abnormal motion, increase the risk and accelerate time course to PTOA. Cadaveric testing and in vivo preparations have quantified joint-level changes in stresses due to incongruity [8, 9, 64], stress rates resulting from instability associated with incongruity [9], and numerical correspondence between instability and PTOA in vivo [10]. In current practice, the goal of surgical treatment of most IAFs is to, as much as possible, restore the articular surface to its pre-injury configuration [22]. Progress in the treatment of IAFs over recent decades has therefore focused on optimizing techniques of surgically reducing and fixing fractures. However, for severely displaced comminuted IAFs, perfect joint restoration remains elusive and further advances in reduction techniques seem unlikely. Furthermore, measuring the accuracy of IAF reduction on radiographs is limited. CT scans provide more detail about residual fracture fragment displacement, but residual displacement is a poor surrogate for cartilage overloading from increased contact stress. For this reason, progress in computational modeling was needed to more accurately predict deleterious joint loading following IAF.

Fortunately, modern computational stress analysis methods provide the capability to calculate the articular surface contact stress in a specific joint during functional loading of the extremity. This means that a patient-specific modeling approach, working from post-operative CT imaging that allows determination of the incongruous joint surface, can capture the mechanical effect of a residual articular incongruity. The insight provided by this modeling approach demonstrates how chronic elevation of contact stress compounds PTOA risk and severity [23, 53, 55, 57–59, 61, 65] (Fig. 9.3). Furthermore,

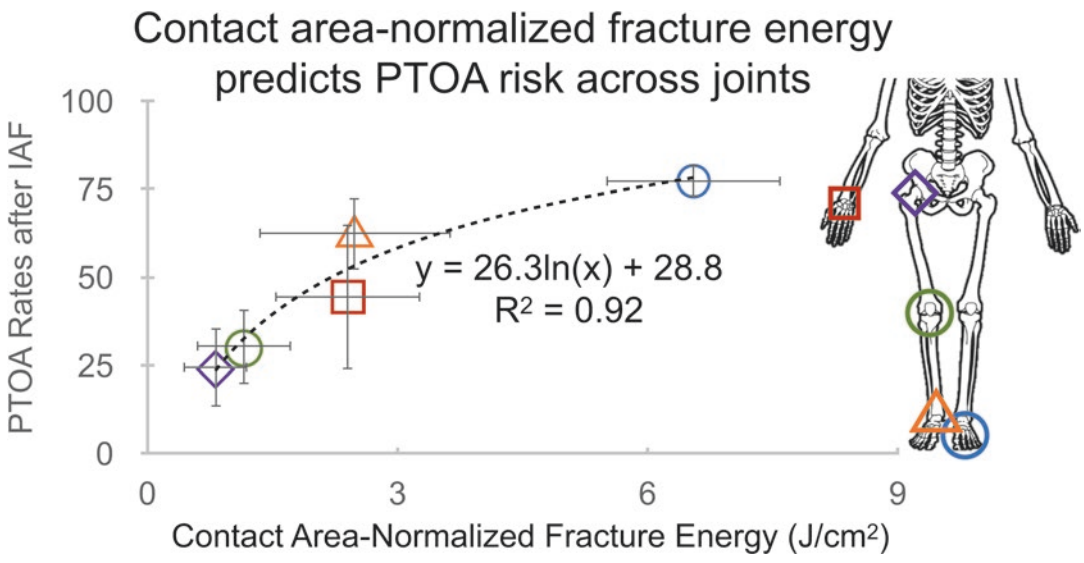

Fig. 9.2 Contact area-normalized intra-articular fracture energy. A contact area-normalized intra-articular fracture energy metric (J/cm^2) was shown to be highly predictive of PTOA rates across subtalar, tibial pilon, knee, hip, and wrist joint fractures

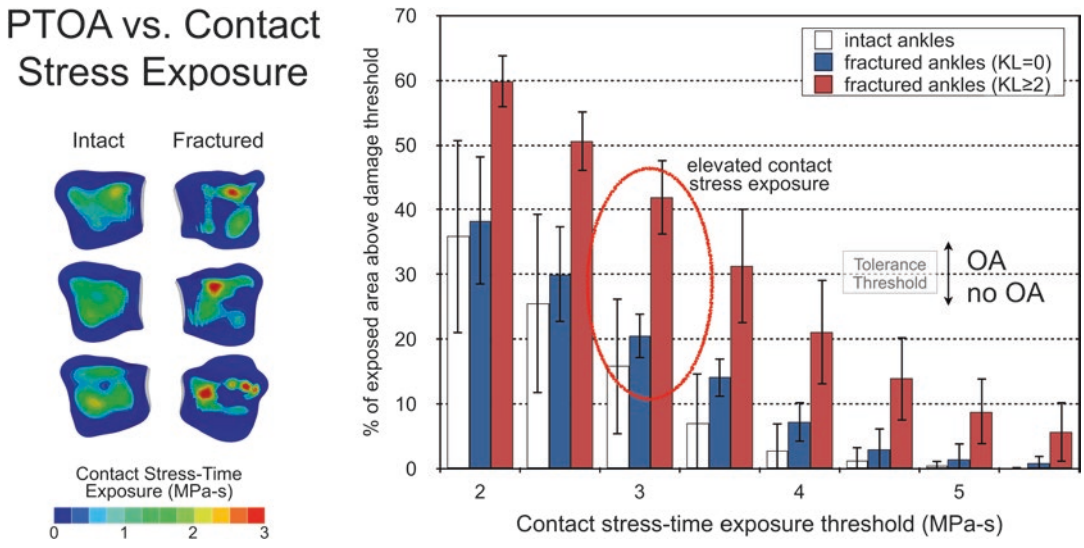

Fig. 9.3 Tibial pilon intra-articular fracture chronic contact stress exposure. Patient-specific computational stress analysis provides a metric of contact stress-time exposure that can discriminate between fractured ankles that did (KL ≥ 2) versus those that did not (KL = 0) later go on to develop PTOA. There appears to be a tolerance threshold (percent of exposed joint surface area above the damage threshold) above which elevated chronic contact stress times correlate with PTOA risk

this approach can also guide the development of new interventions such as modifications of rehabilitation methods following surgical treatment of IAFs that might alleviate deleterious contact stresses during or after joint healing.

9.3 Biologic Mechanisms Responsible for PTOA Following Joint Fracture

A series of investigations focused on a variety of joint overloading and injury scenarios have revealed that extreme acute overloading of articular surfaces, like that seen in IAFs, triggers chondrocyte mitochondria to produce excessive reactive oxygen species (ROS). The resulting oxidative injury mediates chondrocyte dysfunction, death, and chondrocyte senescence associated with PTOA.

9.3.1 Mitochondrial-ROS Pathway

Extreme impacts cause abrupt and intense bursts in chondrocyte ROS production followed by chondrocyte death [66–70]. To confirm the role of the ROS in mechanically induced chondrocyte death, the antioxidant *N*-acetylcysteine (NAC), which has been shown to protect numerous other tissues from oxidative damage [71], was applied to cartilage explants after injury. Even when applied 4 h after impact, NAC treatment more than doubled cell survival and prevented loss of matrix proteoglycans [69]. Cyclic compressive overloads also caused articular cartilage chondrocyte death and dysfunction that could be prevented with NAC [70, 72]. Protection of chondrocytes by NAC from both acute impact and repetitive overloading of the articular surface suggests that oxidative damage is a common pathological result of excess acute and repetitive mechanical stress applied to articular surfaces.

To study the distribution of cell injury and death following IAF in humans, fresh normal human ankle joints were subjected to impacts that caused tibial pilon IAFs in patients [73]. Superficial zone chondrocyte viability was measured over 48 h following IAF. Despite the severity of the injury, there was minimal cell death immediately after IAF. In the region within 500 μm of joint fracture lines, an average of only 20% of the superficial zone cells died immediately. At more than 500 μm from the fracture lines, only a few percent of the cells died, and there was no evidence of cell death deep to the superficial zone. However, over the next 48 h, the proportion of dead cells in the entire articular cartilage superficial zone nearly tripled. This finding, taken together with NAC's in vitro efficacy up to 4 h after impact, suggests that preservation of chondrocytes by joint treatments administered after IAF is possible.

Observations that preventing mitochondrial ROS production could prevent tissue damage following traumatic brain damage and myocardial infarctions [74–77] led to the hypothesis that chondrocyte mitochondria were the source of the ROS observed following mechanical injury. Inhibition of mitochondrial electron transport with rotenone, an inhibitor of mitochondrial electron transport, dramatically improved chondrocyte survival following impact injury in explants [68]. This observation supports the premise that chondrocyte mitochondria are the source of damaging levels of ROS and provided more evidence that interrupting the mitochondrial-ROS pathway can minimize progressive articular chondrocyte death following injurious impacts.

To delineate the connections responsible for mechanically induced production of ROS and subsequent cell death, studies were initiated to determine if mechanical signals are transmitted from the articular surface through the matrix and then across the cell membrane to the mitochondria. In one set of experiments, the adhesion between the chondrocytes and the extra-cellular matrix was disrupted [78]. Osteochondral explants were treated with focal adhesion kinase (FAK) and sarcoma (Src) kinase inhibitors (FAKi, Srci, respectively), molecules that disrupt cell binding to the extracellular matrix, and then subjected to an impact known to cause chondrocyte death. Chondrocyte viability was assessed

by confocal microscopy immediately as well as 24 h post-impact. With no treatment, immediate post-impact cell viability was 59%. Treatment with 10 μM Srci, 10 or 100 μM FAKi improved viability to 80%, 77%, and 82%, respectively ($p < 0.05$). After 24 h viability declined to 34% in controls, 48% with 10 μM Srci, 45% with 10 μM FAKi, and 56% with 100 μM FAKi treatment ($p < 0.01$). These results confirmed that acute chondrocyte mortality was FAK- and Src-dependent, and implicated integrin-cytoskeleton interactions in activating the mitochondrial-ROS pathway.

To further support the concept that the connections between the cytoskeleton and the extracellular matrix are a critical link in the mechanical stress-ROS pathway, the connections between mitochondria and the cytoskeleton were cut by depolymerizing the chondrocyte cytoskeletal f-actin. Depolymerizing the cytoskeleton prevents the deformation of cellular contents after mechanical loading of the explant articular surfaces [79]. The articular surfaces were then subjected to injurious impacts and intracellular oxidant levels and cell viability assessed. Disrupting the cytoskeleton decreased the percent of superficial zone cells producing significant amounts of ROS from more than 70% to less than 20%, the same percent as the control samples. It also increased the number of cells that remained viable from less than 40% to more than 80%, the same percent as the control samples.

Together with previous findings, these data show that intense strains caused by impact loading of the articular surface induce chondrocyte death through a pathway whereby strain passing from the articular surface through the extracellular matrix, through cell adhesion receptors to the cytoskeleton, triggers mitochondrial ROS release. Disruption of this mitochondrial-ROS pathway, by administration of NAC and by cutting the connections between chondrocytes and the extracellular matrix and between the cytoskeleton and the mitochondria, markedly decreased impact-provoked ROS levels and cell death indicating that inhibition/disruption of this pathway might prevent injury-induced chondrocyte death in vivo.

9.3.2 Chondrocyte Senescence

The two universal risk factors for the development of OA are increasing age and mechanical joint injury such as IAF [38, 80–84]. There is reason to believe that chondrocyte senescence has an important role in both age-related OA and PTOA [80, 83, 85–88]. Senescent cells accumulate with age throughout the body and exert deleterious effects on tissues through secretion of pro-inflammatory chemokines and cytokines, and matrix proteases [89, 90]. A study of human articular cartilage showed that chondrocyte telomere length decreases and senescent chondrocytes accumulate with increasing age [91]. Increasing chondrocyte senescence and telomere erosion were associated with a marked decline in chondrocyte anabolic function [80, 86, 91]. Other studies have shown that the shortening of telomeres, the short sequences of DNA at the ends of chromosomes that protect the chromosomes from damage, is closely associated with cell aging, dysfunction, and senescence [92], and with osteoarthritis [88, 93, 94]. Chondrocytes in fractured joints are subject to brief but intense ROS exposure which causes irreversible mitochondrial damage and a senescence phenotype [53, 68, 69, 72, 79, 95, 96]; and, oxidative damage accelerates chondrocyte senescence and telomere erosion [83, 85, 87, 97–99]. Work with animal models provides additional support for an important role of chondrocyte senescence in PTOA [100, 101].

9.4 Minimizing the Risk of OA Following Joint Fracture

Based on the in vivo and in vitro studies showing that the mitochondrial-ROS pathway and accumulation of senescent cells have roles in the pathogenesis of PTOA, it is reasonable to consider methods of interrupting the mitochondrial-ROS pathway and removing senescent cells to prevent or minimize OA following IAFs.

9.4.1 Interruption of the Mitochondrial-ROS Pathway

Because the in vitro studies showed that interventions that interrupted the mitochondrial-ROS pathway preserved chondrocytes following injurious articular cartilage impact [3, 53, 67–70, 79], it is reasonable to ask if these or similar interventions could preserve articular cartilage following mechanical insult in vivo. To answer this question, the effects of NAC and amobarbital (a reversible mitochondrial electron transport inhibitor) treatments of two different types of in vivo injury were studied in animal models of PTOA. NAC and amobarbital were selected because they interrupt the mitochondrial-ROS pathway at different points, and they are approved for human use for other indications. The first model was a sub-fracture impact to the articular surface of the rabbit medial femoral condyle [66]; the second was a closed IAF of the distal tibia in Yucatan mini-pigs [37, 54]. If untreated, both types of in vivo joint injury consistently lead to progressive articular cartilage degeneration.

In the rabbit studies [37, 66, 102], the medial femoral condyle was exposed through a posterior arthrotomy and the load-bearing femoral articular cartilage surface was subjected to a sub-fracture impact that causes progressive cartilage deterioration. The animals were then treated with intra-articular injections of NAC or amobarbital in a hydrogel carrier. Seven days after injury, absolute chondrocyte viability, chondrocyte ATP content, and serum TNF-α were measured. Compared with controls, NAC-treated joints had more viable chondrocytes, 7500 cells/mm^3 versus 2000 cells/mm^3, and the chondrocytes in treated joints had more ATP, 4.5 nmol/mg versus 2.5 nmol/mg. Additionally, animals with NAC treated joints had lower serum TNF-α 5000 ng/mg BUN, compared to 8000 ng/mg BUN in untreated animals. Amobarbital also significantly improved chondrocyte survival and ATP levels. The serum TNF-α changes indicate that the joint injury caused a systemic response and that treating the joint injury with NAC decreased this response [37].

Because the rabbit knee differs considerably in size and loading position from human joints, it was appropriate to determine whether NAC and amobarbital could prevent OA after IAF in a joint that more closely resembles the size and load transfer through human joints. The Yucatan mini-pig animal model of distal tibial hock (ankle) IAF has notable value for the study of IAF because it allows surgical treatment that parallels the surgical treatments received by humans with these injuries [54]. Furthermore, the joints are large enough to test doses of pharmacologic treatments and methods of drug delivery like those that could be used in humans; and, these joints provide ample tissue for detailed analysis using multiple different methodologies (imaging, biochemistry, cell function, morphology, and histology) concurrently on a single specimen.

In the Yucatan mini-pig IAF model, IAF of the distal tibial is created intra-operatively using a closed-joint impact by a pendulum, and the fracture is treated with open reduction and internal fixation using plates and screws [54]. At a relatively early time point, 12 weeks after injury and treatment, histological cartilage degeneration begins to develop on the talus, even in joints fixed with anatomic reduction (p = 0.27-lateral side and p = 0.55-medial side differences in Mankin scores between anatomically reduced IAF and uninjured joints). As would be expected, more severe cartilage erosion develops on the talus in joints fixed with an intentional 2-mm step-off (p = 0.0001-lateral side and p = 0.036-medial side for differences in Mankin scores between step-off reduction and uninjured joints). All animals demonstrating cartilage degeneration had achieved bony union.

To test NAC and amobarbital IAF treatments in this large animal model, distal tibial IAFs in Yucatan mini-pigs were anatomically reduced and stabilized with a plate and screws [103]. The IAFs in two experimental groups of animals were additionally treated with either NAC or amobarbital suspended in a hydrogel at the time of surgical fracture fixation and then again 1 week later. Six months after injury the joints of animals treated only with surgical IAF reduction and fixation had extensive full-thickness cartilage ero-

sions and synovitis. In contrast, joints treated with surgical IAF reduction and fixation plus NAC or amobarbital rarely had cartilage erosions and did not have synovial inflammation 6 months after injury [103]. NAC and amobarbital did not suppress acute joint inflammation caused by IAFs, thus it is unlikely that their preservation of injured joints was due to anti-inflammatory actions. The presence of synovitis in the joints that did not receive NAC or amobarbital 6 months after injury suggests that the degeneration of these joints may have stimulated an inflammatory response. Overall, these results confirmed an in vivo role of the mitochondrial-ROS pathway in cartilage degeneration following joint injuries that closely resemble human IAFs.

Intriguingly, sham surgery, that is arthrotomy and fixation of plates and screws to the distal tibia in the absence of joint fracture, caused degenerative changes 1 year after surgery. This observation suggests that joint surgery alone causes deleterious biologic responses and that there is a need to find methods of minimizing or preventing the adverse effects of joint surgery.

9.4.2 Senolytics

Since senescent cells damage tissues through secretion of pro-inflammatory chemokines and cytokines, and matrix proteases [89, 90], use of senolytic agents, small molecules that selectively induce death, or lysis, of senescent cells [104, 105], offers a novel approach to preventing or minimizing PTOA. Senolytics may also have anti-inflammatory effects [101]. In one such experiment, a drug with senolytic and anti-inflammatory effects, decreased cartilage destruction, reduced subchondral bone plate thickness, and prevented synovitis in a mouse medial meniscus destabilization model [101]. Similar results were found in a study using a mouse anterior cruciate ligament transection model [100]. After anterior cruciate ligament transection, senescent cells accumulated in the articular cartilage and synovium. Elimination of these cells decreased the severity of PTOA and reduced pain. In addition, removal of senescent cells from in vitro cultures of chondrocytes harvested from the knees of human patients undergoing joint replacement surgery decreased expression of senescence and inflammatory markers and increased expression of cartilage extracellular matrix proteins. Encouraging results from studies of mice and cell cultures do not always translate into effective treatments in humans, but they do provide a rationale for more extensive in vivo studies in animals with joints that closely resemble human joints.

9.5 The Potential of Chondrocyte Progenitor Cells to Restore Articular Cartilage Following Joint Fracture

Although inhibiting the mitochondrial-ROS pathway and lysing senescent cells may lead to better outcomes of many IAFs, it is unlikely that these approaches will prevent or even significantly decrease the risk of PTOA following severe IAFs. Despite the best surgical reconstruction of high-energy comminuted IAFs, there are gaps in the articular surface and extensive regions of chondrocyte death. However, new methods of promoting joint healing offer the possibility of restoring a functional articular surface in these severe IAFs. One promising approach is the use of chondrocyte progenitor (chondroprogenitor) cells [106–113].

A study of osteochondral explants subjected to high-intensity articular surface impacts caused chondrocyte death in the impacted regions, but cells that migrated from the surrounding cartilage across the articular surface repopulated the non-viable areas within 7–14 days by [109]. In further studies to characterize this response of articular cartilage to injury, osteochondral explants were injured by blunt impact or scratching, resulting in localized chondrocyte death. Injured sites were then serially imaged by confocal microscopy, and proliferating migrating cells were evaluated for chondrogenic progenitor characteristics. Chemotaxis assays were used to measure the responses of these migrating cells to chemokines, injury-conditioned medium, dead cell debris, and

the alarmin high mobility group box chromosomal protein 1 (HMGB-1) (an endogenous molecule released from damaged tissue). The migrating cells were highly clonogenic and multipotent and expressed markers associated with chondrogenic progenitor cells. Compared with chondrocytes, these cells overexpressed genes involved in proliferation and migration and under-expressed cartilage matrix genes. They were more active than chondrocytes in chemotaxis assays and responded to cell lysates, conditioned medium, and HMGB-1. Glycyrrhizin, a chelator of HMGB-1. A blocking antibody to the receptor for advanced glycation end products (RAGE), inhibited responses to cell debris and conditioned medium and reduced the numbers of migrating cells on injured explants. In summary, these experiments showed that injured or dying chondrocytes release molecules that trigger the emergence, proliferation, and homing of chondrogenic progenitor cells that invade and repopulate regions of injury-induced chondrocyte death [109, 111].

In an in vitro study of full-thickness, articular cartilage defects filled with a fibrin hyaluronic acid hydrogel containing stromal-derived growth factor (SDF-1), chondrocyte progenitor cells migrated into the defect, assumed the morphology of chondrocytes and filled the defect with chondrocytic cells and an extracellular matrix containing type II college and proteoglycans [109, 111, 114]. This new matrix bonded to the surrounding normal articular cartilage. Other in vitro work has confirmed that chondrogenic progenitor cells can produce extracellular matrix molecules [106]. Additionally, further study of chondrocyte progenitor cells showed that they phagocytized cell and matrix debris much more efficiently than chondrocytes, supporting the hypothesis that they play a macrophage-like role in injured cartilage [115].

These basic studies indicate that focal articular cartilage restoration using recruitment, migration, and matrix production by chondrocyte progenitor cells is feasible [110]. To determine if chondrogenic progenitor cells can restore articular cartilage in vivo, focal full-thickness chondral defects were made in weight-bearing and non-weight-bearing areas of goat knee joints. Defects were filled with a fibrin-hyaluronic acid hydrogel either with or without chemotactic and chondrogenic factors. As Fig. 9.4 shows, chondrocyte progenitor cells restored articular cartilage in defects treated with the hydrogel containing the chemotactic and growth factors, and the new cartilage remained intact for at least 6 months. Defects treated with the hydrogel alone did not develop new cartilaginous tissue.

The use of chondrogenic progenitor cells to repopulate areas of chondrocyte death and to fill focal defects with cartilaginous tissue might be especially useful in the treatment of IAF. This approach could be combined with optimal surgical reconstruction and biologic interventions to preserve as many chondrocytes as possible by inhibiting the mitochondrial-ROS pathway. The use of mesenchymal stem cells is another potential approach to repairing articular cartilage damage in IAFs. But, since chondrocyte progenitor cells are the closest cell source to chondrocytes, they may have better potential to restore articular cartilage than other stem cells [107].

9.6 Rehabilitation Following IAF

Advances in biologic treatments of IAF have the potential to significantly improve IAF treatment, but to gain the maximum benefit of these treatments it will be important to provide individualized optimized rehabilitation. Ideally, a rehabilitation program will promote healing, decrease pain and swelling, and restore range of motion, strength, endurance, and proprioception [116]. Some IAFs, depending on the specific injury and treatment, may require immobilization. However, experimental studies of the past several decades confirm the deleterious effects of prolonged immobilization and the beneficial effects of activity on the musculoskeletal tissues [117–120]. One of the most important advances in the promotion of musculoskeletal healing has come from understanding that treatment of injuries with prolonged rest delays recovery and adversely affects normal tissues and that con-

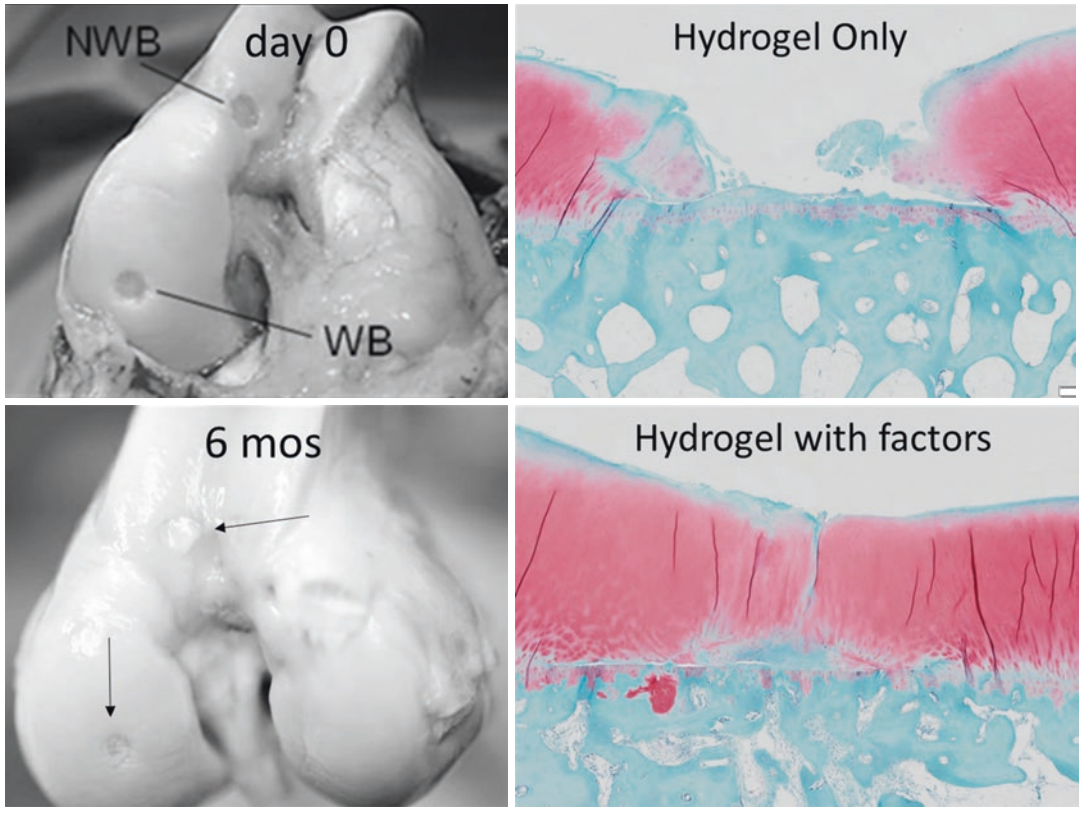

Fig. 9.4 Chondrocyte progenitor cell based cartilage regeneration in vivo. Focal full-thickness chondral defects were made in a weight-bearing (WB) area and non-weight bearing (NWB) areas of goat knee joints. Defects were filled with fibrin/hyaluronic acid hydrogel without chemotactic and chondrogenic factors (hydrogel only) or hydrogel with factors. The upper left panel shows the osteochondral defects at time zero and bottom left panel shows defects at 6 months following the creation of osteochondral defects treated with hydrogels containing chemotactic and chondrogenic factors. Safranin O histology of weight-bearing defects at 6 months following creation of the defects is shown in the two panels on the right. The defect in the upper right panel was treated with the hydrogel only. The defect in the lower right panel was treated with the hydrogel containing the chemotactic and chondrogenic factors

trolled early resumption of activity can promote restoration of function [117–119].

Prolonged immobilization of injured joints can lead to fibrosis within the joint and contraction of the joint capsule leading to restricted motion; and, prolonged immobilization adversely affects articular cartilage including loss of volume and decreased synthesis of matrix molecules, especially proteoglycans [33, 117–119, 121, 122]. Unfortunately, joint fibrosus can also occur following IAFs in joints that have not been subjected to prolonged immobilization. Post-injury joint fibrosus is associated with abnormal persistence of myofibroblasts that overproduce and contract collagen matrices, and in an animal model drugs that target myofibroblasts and collagen production reduced post-joint injury fibrosus [123].

The effects of loading and motion on IAF healing and joint fibrosus have not been defined but studies of a spectrum of extremity bone and joint injuries show that well-designed individualized rehabilitation programs can benefit patients by accelerating recovery and possibly lead to better ultimate outcomes. For example, an integrated orthotic and rehabilitation initiative improved physical performance, pain, and patient-reported outcomes in patients with severe, traumatic lower

extremity deficits, and these improvements were sustained for more than 2 years after injury [124]. New custom dynamic orthoses have the potential to improve function after severe ankle IAFs and possibly promote joint healing [125]. Regrettably, the optimal methods for facilitating the healing of IAFs and preventing joint fibrosus by use of controlled loading and motion have not been defined. How long should loading of IAF be limited? How soon should the motion be started after IAF? What guided joint loading and motion regimens promote healing and restoration of function? Is it possible to identify joints at risk for fibrosus and intervene before joint contracture is established? Clearly, there is a need to pursue answers to these questions.

9.7 Future Advances Needed to Decrease the Risk of OA Following Joint Fracture

Advances in assessing the risk of PTOA following IAFs, understanding of the biologic pathways responsible for PTOA, and developing therapies designed to treat IAFs have made it possible that PTOA risk and severity after IAF might be diminished in the near future. However, more research is needed to determine if safe, effective, and widely applicable treatments of IAF can be translated into routine clinical practice. Basic research is needed to clarify acute and chronic contributions of overloading to PTOA via the mitochondrial-ROS pathway as well as how this pathway interacts with other responses of joints to injury [3, 37, 53]. In addition, the safety of inhibition of the mitochondrial-ROS pathway must be tested in patients. Surgical treatment of IAF triggers a second deleterious biologic response that appears to contribute to the risk of PTOA [103]. Thus, finding ways to optimize joint health before surgery and minimize the damaging effects of surgery are important. Widely applicable methods of assessing PTOA risk will make possible rigorous clinical trials of new treatments of joint injuries. New measures of the early responses of injured joints to treatment will help guide treatment. Specifically, assessment of the responses of joints soon after treatment would help guide efforts to optimize interventions for specific patients and injuries. Basic and clinical research is needed to advance understanding of the effects of rehabilitation of IAFs with guided and controlled regimens of joint loading and motion. Effective treatment of the spectrum of IAF will require individualized patient treatments based on the reliable assessment of the risk of PTOA. In addition to the assessment of the injury, risk assessment should also include specific patient variables that influence the response to injury and potential for healing including age and metabolic status. Patients with a low risk of PTOA may be effectively treated with current methods of surgical IAF treatment coupled with inhibition of the mitochondrial-ROS pathway. Patients with a higher PTOA risk may benefit from augmentation of mitochondrial-ROS pathway inhibition with other biologic interventions including lysis of senescent cells and restoration of articular cartilage with chondrocyte progenitor cells and new approaches to post-injury joint rehabilitation.

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Inflammation After Anterior Cruciate Ligament Injury

10

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10.1 Biochemical Response to Injury

Partial or complete tearing of the anterior cruciate ligament (ACL) causes acute pain, instability, and swelling [1]. ACL rupture, regardless of accompanying damage to nearby bone and/or cartilage, initiates a prolonged deleterious cascade of inflammation and catabolic enzyme activity [2, 3]. Long-term consequences of ACL injury, include chronic pain, potential arthrofibrosis, recurrent instability, limited range of motion, and an increased risk of developing post-traumatic osteoarthritis (PTOA) [4–6]. More than 50% of all patients with ACL rupture will develop radiographic PTOA within 5–15 years after injury [7–9].

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There is an early onset of osteoarthritic biomarker profiles immediately after ACL injury as measured in synovial fluid. Cytokine concentrations in the synovial fluid early after ACL injury have been associated with degenerative cartilage changes over the ensuing 3 years [10]. These degenerative changes are precursors to the development of osteoarthritis (OA). OA is defined as a chronic, inflammatory condition with macrophages acting as mediators in the cycle of cartilage degradation [11, 12]. Synovial macrophages are activated by proinflammatory cytokines as a result of cartilage breakdown which then activate chondrocytes and production of matrix metalloproteinases (MMPs), creating a cyclical process of cartilage degradation [11].

Markers most closely associated with cartilage breakdown include cross-linked C-telopeptides of type II collagen (CTX-II), matrix metalloproteinase-3 (MMP3), and cartilage oligomeric matrix protein (COMP) and all indicate a progressive breakdown of cartilage matrix and collagen over the first 5 weeks after ACL rupture. There is also a dramatic 250% increase in CTXII in the first 5 weeks after injury, while inflammatory markers in the interleukin 1 family (IL1α, IL1ß, IL-1ra) show a decline over that time period. Several studies have reported synovial biomarker changes after ACL injury and reconstruction, [13] but unfortunately none of these studies have longitudinally reported the very early progression of biomarker profiles

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during the first 6 weeks after ACL rupture, before reconstruction. Studies including Wasilko et al. [13] reported on synovial fluid markers as early as 10 weeks after ACL reconstruction and Struglics et al. [14] reported their first measurements with a mean of 1.3 weeks after injury ranging from 1 to 42 days. Catterall et al., whose study included one of the earliest evaluated time points, reported on synovial fluid and serum markers an average of 15.2 days after ACL injury [3]. Recently some groups, such as Lattermann et al. [15], have been able to provide synovial fluid biomarker levels as early as 5 days after ACL injury, but this time point is far less common. Together, these studies highlight the evidence for early cartilage degenerative processes and its rapid progression, based on adverse joint tissue metabolism, after the initial ACL injury. The strict longitudinal design of these trials demonstrates the early progression of inflammation and subsequent cartilage breakdown after injury and thus augments the long-term data reported in these earlier studies [13, 14].

Besides just categorizing the inflammatory response following ACL injury, recent literature reveals that early biochemical changes after ACL rupture are indicative of poorer physical and self-reported outcomes 2 years after surgical reconstruction [16]. These data support the previously described cascade of increased IL-1α, which stimulates the production of MMPs, thereby reducing proteoglycan content and changing cartilage mechanical properties. Previous studies have established that ACL injury triggers a biochemical process that worsens over the first 4–6 weeks after injury [3, 17, 18]. Inflammation is initiated early on by the intra-articular hemarthrosis and subsequent pathogenic processes, including the down-regulation of proteoglycan synthesis and upregulation of MMPs [19–21]. This is important because IL-1 and IL-1ra are critical in the regulation of the pathological processes involved in joint tissue breakdown [22, 23]. Synovial fluid IL-1 levels are increased in patients with ACL injury and correlate with severity of chondral damage [24]. Synovial fluid levels of the IL-1ra cytokine, which is chondroprotective, decreases significantly after ACL injury, resulting in relatively unopposed increases in the activity of IL-1 [25]. Furthermore, levels of IL-1ra decrease as the severity of cartilage damage is increased [24]. Additionally, the superficial cartilage layers have been shown to be more susceptible to IL-1 induced damage compared with deeper chondral layers in vitro [26] and porcine cartilage explants are more sensitive to the chondrodegenerative effects of IL-1α than IL-1β [27].

Initial biochemical changes after injury may also serve as prognostic indicators of the long-term consequences resulting from ACL injury that is not mitigated by surgical stabilization alone. Inflammatory cytokine and degradative enzyme concentrations on the day of ACL reconstruction surgery have been shown to correlate with cartilage changes on magnetic resonance imaging 3 years after surgery, [10] demonstrating a connection between cytokine and degradative enzyme concentrations and the potential progression of post-traumatic OA. Further, cytokine and degradative enzyme concentrations have also been associated with worse patient-reported outcome scores on the Knee Injury and Osteoarthritis Outcome Score (KOOS). For example, both IL-1α and MMP-9 concentrations on the day of ACL surgery were significantly higher for patients that did not post-operatively achieve a satisfactory patient acceptable symptom state (PASS) score on KOOS quality of life subscale compared to those with better self-reported clinical outcomes [16]. In conjunction with increased IL-1α and MMP-9 concentrations, there were increased concentrations of biomarkers of bone and cartilage turnover (sf NTX-I and uCTX-II). This overall state of upregulated catabolic markers and inflammation may lead to recurrent effusions and/or persistent synovitis which appears to potentially have long-term consequences for knee joint health.

Increases in inflammatory synovial fluid biomarkers following ACL injury have been well established but inflammation following injury is a complicated process. An important response to joint injury includes activation of inflammatory pathways. However, it should be noted that prolonged activation of these pathways results in increased biomarkers associated with progressive

cartilage catabolism and matrix breakdown [28–30]. A recent study by King et al. [31] demonstrated upregulation of the cytokine–cytokine receptor interaction pathway following acute ACL injury. This is likely because cytokines play a crucial role in both innate and adaptive inflammatory host defenses as well as development and repair processes aimed at restoring homeostasis. Although this pathway has been previously linked to diseases, including juvenile idiopathic arthritis, this suggests a role in cartilage degradation and not solely a response to acute injury [32]. Along with upregulations in inflammatory pathways, King et al. also found significant increases in specific proteins related to the tumor necrosis factor (TNF) family and included TNF receptor superfamily members 3, (TNFRSF3), 4 (TNFRSF4), 9 (TNFRSF9), and 25 (TNFRSF25). Interleukin 17 receptor A (IL17RA) was also upregulated and has been shown to be involved in the pathogenesis of inflammatory and autoimmune diseases including rheumatoid arthritis (RA) [32]. Genes in the TNF family have been previously associated with both RA and OA, [33, 34] and this could be a potential therapeutic target. The changes observed [31] in markers associated with cartilage degradation and OA were consistent with prior studies, namely increased synovial fluid aggrecan fragments, MMP-1, MMP-3, and ADAM12 following knee injuries and in early-stage OA [28, 35]. Notably, King et al. [31] also found a number of RA-related markers that were upregulated following acute ACL injury. This suggests that the initial response to ACL injury may have specific biological pathways in common with RA. Proteins increased acutely after ACL injury included apoliopoprotein E and isoform E3, vascular cell adhesion protein 1, IL-34, and cell surface glycoprotein CD200 receptor 1. This is potentially suggestive of cartilage breakdown driven by the inflammatory process, which is similar to what is seen in the early stages of RA. Others have demonstrated the similarity in early stages of PTOA to the early stages of RA [36]. Both diseases are associated with synovial membrane inflammation including increases in inflammatory cytokines but exhibit no radiographic evidence of OA. These two diseases differ, however, as in RA the initial inflammatory response ultimately leads to T and B cell proliferation in the synovium that is not present in OA.

Acute ACL injury initiates a cascade of activity including activation of synovial macrophages and increases in concentrations of TNF-alpha, IL-1, and other proinflammatory cytokines, which results in increased markers of type II collagen turnover in the acute phase after injury [17, 18, 37–41], that could potentially be attenuated with treatment.

10.2 Post-operative Response

The current standard of care in the United States is to surgically reconstruct the ACL (ACLR) after injury in order to restore biomechanical stability [42]. A patient's ability to regain function after surgery can be difficult due in large part to the persistent quadriceps atrophy following ACLR. Patient-reported function is not different between patients treated non-operatively and patients treated with reconstruction suggesting that surgery is not necessary to be functional in all cases [43]. There is evidence to suggest that the severity of the injury may have a role in the development of quadriceps weakness but overall, those with a complete tear of the ACL had poor to fair muscular strength 8 years after injury [44]. Atrophy and weakness of the quadriceps muscle persists regardless of reconstruction, and patients who remain ACL deficient (ACLD) will likely continue to have quadriceps atrophy and joint dysfunction years following ACL injury. While non-operative treatment of ACL injury is less common, the question becomes whether patients that remain ACL deficient also undergo a similar inflammatory process after the initial injury and how that differs long term from patients who undergo ACLR surgery.

The ACL reconstructive surgery likely represents a "second hit" to the injured knee joint due to the disruption and loss of integrity of the joint capsule and other secondary insults to the joint structure [45]. There is a well-documented elevation in inflammatory and cartilage breakdown

markers in synovial fluid following ACL injury [3, 31, 37, 38, 46]. Despite pre-operative administration of intra-articular Triamcinolone acetonide (corticosteroids), pro-inflammatory cytokines concentrations were increased in the synovial fluid following ACLR. Moreover, cytokine concentrations 1 week after surgery were greater than what was observed approximately 1 week after the initial insult of injury [15]. These findings concur with previous findings of an increased inflammatory burden following ACLR [47].

Knee joint inflammation after ACLR increases after surgery, and while pro-inflammatory cytokine concentrations begin to decrease about 4 weeks after surgery, biomarker concentrations do not return to preoperative levels [48]. Larsson et al. found similar results, showing increases in synovial fluid pro-inflammatory cytokines up to 5 years post ACLR in the KANON trial. Higher levels of inflammatory cytokines years after ACLR was also true when comparing patients who had surgery early after injury to patients who had delayed reconstruction [49]. Interestingly, the increase in cartilage breakdown markers does not increase until 4 weeks or longer after the ACL reconstruction surgery [15, 16]. These data suggest a time-related response of chondral breakdown to the second "inflammatory hit."

A state of increased and/or prolonged inflammation following surgery likely contributes to the chondrodegenerative and bony changes observed during the first 2 years following ACLR, thereby potentially promoting the progression of PTOA. Chronic inflammatory synovitis [50, 51] and several pro-inflammatory cytokines, such as IL-1 and TNFα [52, 53], have been closely linked to the progression of idiopathic OA. Data from animal studies of OA further emphasizes the role of IL-1 and TNFα in the onset and progression of OA [54]. Similarly, pro-inflammatory stimulation of meniscus cells in vivo increases cytokine and MMP activity [55].

10.3 Inflammatype

As the underlying mechanisms of PTOA are becoming clearer, a trend is emerging whereby there are certain groups of patients that have a different and more robust inflammatory response to injury. A recent study by Jacobs et al. [56] demonstrates this and while the results of this study differ slightly from a previous paper by Amano et al. [10], it is becoming increasingly apparent that there is a subset of patients with dysregulated inflammatory responses after acute injury. A subset of patients following ACL injury not only had a more robust pro-inflammatory response but the corresponding innate anti-inflammatory response was muted [56]. This coincides with Dr. Scott Dye's theory that there may be phenotypical variations in the response to injury and potential genetic predisposition to dysregulation of molecular and/or cellular homeostasis [57]. In response to acute injury, whether ACL injury, polytrauma, or meniscus injury, there is a complex and dynamic interaction of multiple pathways including the NF-κB, cytokine–cytokine receptor interaction, and osteoclast differentiation pathways, among others [58–61]. Similar pathways are also implicated in the development and progression of knee osteoarthritis [62–64]. Because of the complex and variable nature of this individualized response, a better understanding of the dynamic interplay between multiple pathways may be necessary in order to improve both short- and long-outcomes in the future [65, 66].

When considering potential treatment paths in response to injury, it is pertinent to recognize that between 27 and 38% of patients who sustain a traumatic or ACL injury demonstrate dysregulated inflammatory responses [56]. These data suggest that the biologic response to injury does not fully explain the development of early onset PTOA after ACL injury because on average 50% of patients develop PTOA within 15 years of injury. This means that there is a sizable portion of patients that do not exhibit dysregulated inflammatory responses but still progress on to develop PTOA. The progression of PTOA is multifaceted in nature. It is likely that the combination of increased body mass and decreased activity seen in the years following ACLR [67] may contribute to the progression of PTOA even in those patients who do not exhibit inflammatory dysregulation immediately after injury. There is also the possibility of reinjury that could account for

the additional incidental 25% of PTOA. The progression to PTOA is variable and multifactorial, further justifying the need to assess personalized inflammatory signatures after injury in order to help mitigate the deleterious path of this complex condition.

10.4 Treatment Considerations

There are many factors to consider when determining the appropriate treatment options for mitigating the progression of PTOA. This includes the consideration of the inflammatory response, attenuation of chondral breakdown, and how increased joint loading and resumption of physical activities and sports influences these factors. Proteomic analysis also revealed large post-injury increases in RA-associated markers, and upregulation of the cytokine-cytokine receptor interaction pathway that is closely associated with inflammation [31]. Furthermore, it is particularly noteworthy that four protein analytes in the TNF family were upregulated following acute ACL injury. Genes in the TNF family have been previously associated with both RA and OA, [33, 34] highlighting its potential as a therapeutic target.

Intra-articular corticosteroid injections have already been used as treatment options in small randomized clinical trials [15, 48]. Corticosteroids have been demonstrated to reduce markers of type II collagen breakdown following acute ACL injury; [17] and it may be the underlying ability of corticosteroids to modulate macrophage function that is causing the reduction in collagen breakdown. Acute injury initiates a cascade of activity including activation of synovial macrophages and increased concentrations of TNF-alpha and other proinflammatory cytokines, which thereby results in increased markers of type II collagen turnover in the acute phase after injury [17, 18, 37–41]. Intra-articular corticosteroids have demonstrated the ability to disrupt this cycle by decreasing TNF and cytokine concentrations in chronic arthritis [68], although this reduction is transient.

Additionally, there is evidence that biologic agents targeting the inflammatory process can reduce inflammation and can potentially attenuate the early process of chondral breakdown. For example, IL-1ra can reduce chondral lesion size and improve outcomes in animal models and in an idiopathic OA population [69–71]. Furthermore, in a small randomized clinical trial IL-1ra has been shown to reduce effusions and pain after ACLR [72]. Other biologics such as platelet rich plasma (PRP) and even more so, bone marrow aspirate concentrate (BMAC) have been shown to contain an abundance of IL-ra and other anti-inflammatory cytokines and should be considered as potential treatment options following ACL injury [73, 74]. Other possible interventions that aim at reducing MMP and cytokine activity after acute knee injury may alter the progression of PTOA [75], suggesting that perioperative anti-inflammatory treatment may improve joint health following surgery.

An important consideration for biologic and injectable treatments is the timing of treatment. As was discussed earlier, ACL reconstruction surgery presents a second insult to the joint, reinitiating the inflammatory response. Previous studies looking at injecting corticosteroids [15, 48] before surgery mitigated the initial inflammatory response but that effect is likely washed out after surgery. Due to the "second hit" that surgery presents, biologic treatments are likely to be more effective when administered after surgery to combat the reignited and increased inflammatory and cartilage breakdown processes. Furthermore, it should be noted that single-dose injections have not been tremendously successful and therefore a longer release biologic or multiple injections may provide more consistent treatment over time and has the potential to be more effective. This could also be true because based on proteomic analysis of synovial fluid PTOA [31], PTOA presents more like RA than OA. Extended-release treatments are commonly used to treat RA symptoms [76] and show good efficacy and may be a viable option in combating PTOA.

Another aspect to consider for treatment of PTOA is how return to physical activity and joint loading effects the inflammatory processes and knee cartilage. After ACL injury, evidence reports both underloading (reduced

mechanical load) and overloading in walking [77, 78] running [79], squatting [80, 81], and jumping [82] across different phases of recovery. These altered loading strategies have also been associated with degeneration of articular cartilage and bone. Mechanical loading may influence cartilage degradation in several ways. For example, OA patients with greater cartilage turnover in response to light exercise demonstrated significantly greater cartilage thinning over 2 years [83]. Altered joint mechanics secondary to concomitant injuries treated with chondroplasty or partial meniscectomy can also accelerate the development of idiopathic OA [84]. Furthermore, high impact loading has the potential to increase activation of the pro-inflammatory NFκβ pathway, whereas low-intensity loading can decrease NFκβ activity [85]. This is important when determining return to physical activity guidelines and how individuals who are resuming functional activities may be increasing their inflammatory burden, ultimately affecting their long-term joint health. The progression of PTOA begins with an increased inflammatory burden in the knee after ACL injury. Marked increases in inflammatory cytokines following injury and surgery create a sustained inflammatory response that may contribute to chondral breakdown. There is the potential for biologic treatments to be efficacious but the timing of treatment and how increased loading in the period after surgery affects inflammation need to be considered.

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T Cells in Early Osteoarthritis

11

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11.1 Introduction

Historically, osteoarthritis (OA) was considered a wear and tear disease initiated and propagated by biomechanical processes resulting in degeneration of articular cartilage. However, there is also strong evidence demonstrating the involvement of inflammation and the immune system in the pathogenesis of OA throughout the disease process. Histological studies reveal that over 50% of patients with OA have a mononuclear cellular infiltrate in the synovial fluid and synovium that consists of lymphocytes, monocytes, and dendritic cells derived from peripheral blood [1, 2]. Acutely following joint injury, pro-inflammatory chemokines and cytokines are released from the cartilage and the synovium and attract a variety of immune cells to the joint, the majority of which are macrophages and T lymphocytes [3]. Through the release of cytokines and chemokines and cell-to-cell interactions, T cells modify the inflammatory joint environment and influence the progression of disease.

This chapter will focus on the role of T cells in *early* OA, because only in the early phases of OA can true disease intervention and disease prevention occur. Herein, we will lay the foundation for how cytokines and chemokines released from chondrocytes and synoviocytes home T cells to the inflamed joint acutely following injury, and how the specific T cell subtypes can influence disease progression. We will discuss T cell behavior within the synovium, including activation and proliferation in antigen-dependent or independent manners, and why these events in early OA are critical for sustained T cell responses within the joint. We will explore the biology of different T cell subsets within the joint that can act to mitigate or propagate disease progression dependent upon their phenotype, and how the cytokine environment of the joint can reciprocally polarize T cell phenotype, potentially exacerbating the T cell inflammatory response. Finally, we will discuss how further exploration of the interplay between T cells and joint dysfunction will inform the development and utilization of targeted immunotherapies early in disease to mitigate OA. Throughout this chapter, we will convey the need to further explore how T cell functions within the joint during early OA influence disease progression and can potentially be manipulated to mitigate OA to prevent joint destruction.

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11.2 Orchestration of the T Cell Response in OA

T cell infiltration into an inflamed joint is emerging as a hallmark of OA. This infiltration is considered abnormal because there are very few tissue-resident T cells within the synovium or synovial fluid of a healthy joint [4]. While a small population of T cells may reside in a healthy joint and play a role in maintaining joint homeostasis, an inflammatory event is needed to initiate infiltration of pathogenic T cells into the joint. T cell activation can occur in both an antigen-dependent and -independent manner. The presence of mono- and oligo-clonal populations of T cells within the synovium of OA patients points to an antigen-specific proliferation of T cells within the joint itself [2]. Additionally, T cells from the peripheral blood of some patients with OA activate and proliferate in response to chondrocyte and synoviocyte membrane antigens, suggesting that self-specific T cells exist in the circulation of OA patients as well [5]. Moreover, T cells from patients with OA have been found to recognize specific amino acid sequences from aggrecan, which is a major constituent of normal articular cartilage but can also function as an auto-antigen within the joint [6]. Taken together, these data suggest that OA is characterized by aberrant systemic and local joint T cells that are driven by joint-derived antigens.

T cells are part of the adaptive immune system. They are derived from hematopoietic stem cells in the bone marrow that differentiate into lymphoid progenitor cells that migrate to the thymus and commit to the T lymphocyte lineage. During development, diverse T cell receptors are generated through germline DNA rearrangement. These T cell receptors can recognize virtually any antigen. The process of negative selection largely deletes T cells that strongly recognize self-antigen, [7] but this process is not perfect, and some self-reactive T cells can develop [8]. While still in the thymus, T cells either mature into $CD4^+$ helper T (Th) cells, which are the predominant cell type in an OA joint, or $CD8^+$ cytotoxic T cells. These mature T cells that are still naïve to antigen then leave the thymus and travel to secondary lymphoid tissues where they can be activated by an antigen-presenting cell, typically a dendritic cell [9].

Three signals are required for the activation and proliferation of naïve T cells. First is the signal received when a T cell receptor recognizes its cognate antigen presented by an antigen-presenting cell in the context of major histocompatibility complex (MHC). Second is co-stimulatory signaling in which a co-stimulatory molecule on the T cell, like CD28, binds a member of the B7 receptor family on the antigen-presenting cell. Finally, the T cell must also encounter IL-2 for proliferation, and other cytokines that support activation and polarization. This three-step process creates a significant barrier for inappropriate T cell activation to occur, thus preventing the proliferation of T cells that might otherwise recognize auto-antigens. Moreover, if a T cell binds a specific antigen alone without receiving a co-stimulatory signal, it will become anergic and unable to respond to antigen in the future. Interestingly, T cells co-cultured with fibroblast-like synoviocytes that are able to present antigen-loaded MHC II adopt an anergic phenotype. This suggests that, although fibroblast-like synoviocytes are capable of presenting antigen to T cells, they are unable to activate naïve T cells because they lack co-stimulatory molecules [10].

Initial T cell priming in OA is likely to occur in a lymph node local to the joint. In this scenario, dendritic cells in the joint carry antigen to the lymph node, or alternatively, dendritic cells in tissues proximal to the damaged joint pick up antigens that have drained out of the damaged joint and then migrate to the lymph node [11]. However, during ongoing disease, there may be other modes of antigen presentation and persistent T cell activation. Lymphoid nodular aggregates and lymphoid follicles containing macrophages, T cells, and B cells can be found in the synovium of patients in all stages of OA [12]. There is evidence in RA that auto-antigens are presented to T cells by antigen-presenting cells within the synovium [13]. A rabbit medial meniscectomy model suggests that this may also be true for OA. At weeks 2 and 4 post-meniscectomy,

large numbers of mature dendritic cells were present in lymphoid aggregates within the synovium [14]. A recent study in a mouse model of load-induced arthritis found that the total number of T cells in the inguinal lymph node was significantly increased within 1–2 weeks of loading [15]. This suggests that while initial T cell activation by dendritic cells likely occurs in local lymph nodes, it may then be perpetuated in the synovium [11].

Cytokines in the micro-environment during priming determine the fate of $CD4^+$ T cells and polarize them to one of a number of functional subsets or fates [16]. These $CD4^+$ T cell fates include Th1, Th2, Th17, and Regulatory (Treg) (Fig. 11.1). Th1 cells develop in response to IFN-γ and IL-12, which cause downstream activation of the T-bet transcription factor and induce Th1 cells to secrete IFN-γ and TNF-α. They activate phagocytic cells and are involved in the elimination of intracellular pathogens. IL-4 activates the transcription factor GATA3 and directs naïve cells to a Th2 fate. Th2 cells coordinate the immune response toward extracellular pathogens including helminths and predominantly secrete IL-4, IL-5, and IL-13. Th17 cells are responsible for immunity against extracellular bacteria and fungi through the secretion of IL-17A, IL-21, and IL-22. IL-1β, IL-6, and IL-23 activate the RORγt transcription factor, driving the emergence of the Th17 phenotype. Treg cells develop in one of two ways. Natural Tregs develop in the thymus and

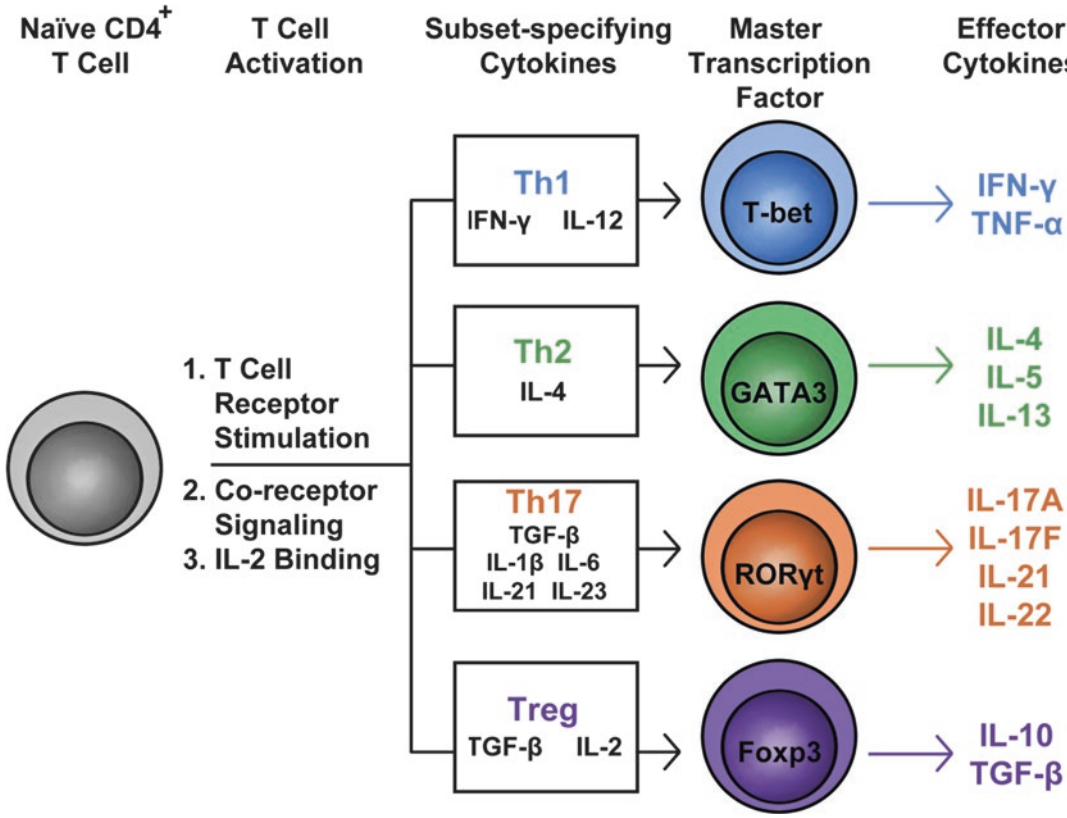

Fig. 11.1 $CD4^+$ T cells fates are determined by cytokine environment during differentiation. Naïve T cells must receive three signals in order to activate and proliferate. This includes binding of the T cell receptor to the appropriate MHC class and cognate antigen for activation, co-receptor signaling to increase survival signal to the T cell, and finally T cells must bind IL-2 in order to proliferate. Subset-specifying cytokines released by mature antigen-presenting cells activate subset-specific master transcription factors, determining T cell fate and effector functions

Induced Tregs develop in the periphery under the influence of TGF-β and IL-2. Treg development and function is directed by the transcription factor Foxp3 that supports downstream secretion of IL-10 and TGF-β. Tregs are critical for tolerance to self- and foreign-antigen and resolution of inflammation [17]. Activation by antigen recognition initiates T cell proliferation and differentiation prior to homing to sites of inflammation, such as an OA joint, where they carry out their effector functions.

As T cells are activated and adopt specific fates (see Fig. 11.1) [17], these subsets also express specific chemokine receptors that mediate the ability of T cells to respond to chemokines that direct immune cell migration to and within tissues. In the context of OA, chemokines produced from inflamed cartilage and synovium promote T cell homing to the joint. In addition, the vasculature of the inflamed synovium becomes highly positive for E-selectin, which promotes the extravasation of immune cells from the peripheral blood into the joint [2].

Recent studies have revealed that specific chemokines are key mediators responsible for immune cell homing in early OA, including CCL5, CCL17, CCL20, and CXCL12 [18, 19]. CCL5 is a potent T cell chemoattractant that binds to CCR1, CCR3, and CCR5, all of which can be expressed by T cells [20–22]. CCL5 knockout mice are partially protected from cartilage injury as a result of destabilization of the medical meniscus-induced OA compared to wild-type mice [23]. CCL17 induces chemotaxis in T cells through interactions with CCR4, which is expressed only on specific $CD4^+$ T cell subtypes, including Th17 and Tregs [24]. CCL17 blockade in mice with collagenase-induced arthritis resulted in reduced pain and OA [25]. Synoviocytes from OA patients secrete CCL20, which is strongly chemotactic for lymphocytes and binds to CCR6 [26]. CXCL12 is another potent chemokine for lymphocytes that are closely associated with the radiographic severity of OA [27]. Additionally, CXCL12 can enhance the effects of certain pro-inflammatory cytokines, including IL-17A, on fibroblast-like synoviocytes [28]. Because different T cell subsets exhibit specific receptors, the cytokines released by cartilage and synovium during early OA will affect which subtypes are homed to the joint, subsequently playing a role in disease pathogenesis.

The aforementioned chemokines and cytokines orchestrate priming and homing T cells to the joint where they elicit their effector functions through several mechanisms including secretion of cytokines and cell-to-cell interactions (Fig. 11.2). T cells can mediate the progression of OA by affecting both stromal and immune cells within the joint. These effector functions of T cells within the inflamed, early OA joint could be targeted therapeutically on a patient-to-patient basis to interrupt the course of disease before irreversible joint damage has occurred.

11.3 $CD8^+$ Cytotoxic T Cells (CTL)

Cytotoxic T lymphocytes (CTL) express the CD8 co-receptor and perform cell-mediated immunity. CTLs kill harmful cells, including cancer cells and cells carrying intracellular pathogens. CTLs recognizing self- and non-self-antigens presented by MHC class I, which is found on all nucleated cells. CTLs carry out their effector functions through two main actions. First is release of antiviral and anti-tumor cytokines, primarily IFN-γ and TNF-α. Second is by directly killing cells, either through release of cytotoxic granules or by Fas/FasL interactions [29]. In the synovium of OA joints, $CD8^+$ T cells are present, but at significantly lower numbers than $CD4^+$ T cells [30].

Interestingly, in OA, there is an increase in the $CD4^+$:$CD8^+$ ratio, and a decrease in the total number of CTLs in the patients' peripheral blood [30]. In a mouse model of anterior cruciate ligament transection, $CD8^+$ T cells infiltrated synovial fluid of afflicted joints within 30 days and persisted for 90 days. Additionally, $CD8^+$ T cells expressed TIMP1, a regulator of matrix metalloproteinases and disintegrin-metalloproteinases, which helps to maintain extracellular matrix composition, and the number of $CD8^+$ T cells expressing TIMP1 correlated positively with disease severity. Moreover, increased TIMP1, VEGF, and MMP13 in the synovium correlated

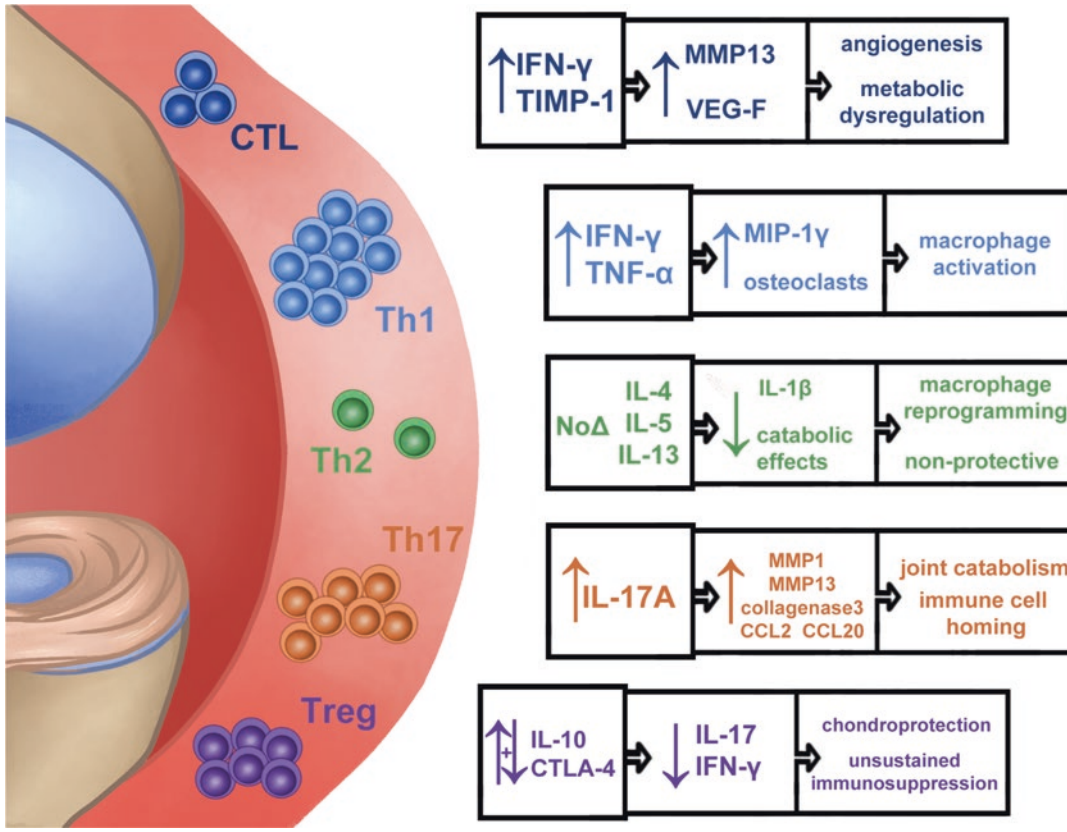

Fig. 11.2 T cells alter joint homeostasis in a subset-specific manner. T cells are homed to the joint by inflammatory cytokines released by joint tissues, where they then carry out their effector functions and contribute to loss of joint homeostasis. Cytotoxic T lymphocytes are not abundant in the joint but contribute to increased vascularization and matrix degradation. Th1 cells are the most abundant T cell subtype within the joint but appear to carry out their effector functions mainly thorough macrophage polarization and activation. Th2 cells are found sporadically and sparsely within the OA joint and do not appear to offer protection against cartilage breakdown. Th17 cells contribute significantly to matrix degradation as well as synovial inflammation while further contributing to immune cell homing to the joint. Tregs provide early immunosuppression but are unable to restore joint homeostasis and ultimately cannot sustain their effector functions in order to mitigate OA progression

with $CD8^+$ T cell activation [31]. $CD8^+$ T cells therefore may contribute to imbalance of joint metabolism through dysregulation of both TIMP1 and MMP13, and angiogenesis leading to synovial inflammation.

Aside from these studies, $CD8^+$ T cells in the OA joint have remained somewhat unexplored. In RA, $CD8^+$ T cells are detected in synovium prior to clinical symptoms [32]. Within RA synovial fluid, there is an accumulation of autoreactive $CD8^+$ T cells that are clonally related [33] and are associated with disease severity and breakdown of self-tolerance. Conversely, suppressor $CD8^+$ T cells in the joint may play a role in disease mitigation by inhibiting the functions of autoreactive $CD4^+$ T cells [34]. Additional studies into $CD8^+$ T cells will aid in understanding their contribution to OA initiation and progression, and potentially reveal new therapeutic options for OA patients.

11.4 T Helper 1 Cells (Th1)

Th1 cells, driven by IL-12 and IFN-γ and controlled by T-bet to produce IFN-γ, [17] are the most abundant T helper cell subset in the synovial fluid and synovium of patients with OA [4].

And although there are fewer Th1 cells in the synovium of patients with OA compared to RA, the Th1 cells present in both types of diseased tissue expressed similar transcript levels of IFN-γ when stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin [35]. Th1 cells in the synovial fluid of patients with OA also secrete higher concentrations of IFN-γ than circulating Th1 cells in the peripheral blood upon PMA and ionomycin stimulation [36]. Within 30 days of anterior cruciate ligament transection in a mouse model of OA, IFN-γ^+ cell numbers increased in the synovium, and subsequently decreased by 90 days post-induction. This was associated with an increase in MIP-1γ and number of osteoclasts, while CD4 knockout mice had lower concentrations of MIP-1γ and slower cartilage degeneration [37].

Importantly, while Th1 cells are the most abundant $CD4^+$ T cell subtype in the OA joint, they may not be the most inflammatory. In vitro, Th17 cells induce synthesis of IL-6, IL-8, MMP1, and MMP3 in synovial fibroblasts from patients with early RA, whereas Th1 and naïve $CD4^+$ T cells do not [38]. It has also been reported that in patients with RA, Th1 cells and related cytokines are only significantly increased in peripheral blood in late-stage disease, while Th17 cells and their related cytokines are significantly elevated throughout disease progression [39].

While the role of Th1 cells in the progression of OA remains unclear, their orchestration of the effector functions of macrophages and monocytes is likely partially responsible for continued inflammation within the joint. However, evidence from RA would suggest that the contribution of Th1 cells to rheumatic disease is more prominent during later disease stages and that other $CD4^+$ T cell subtypes, including Th17 cells, are more important drivers of pathogenesis early in the disease process.

11.5 T Helper 2 Cells (Th2)

Th2 cells that respond to IL-4 and produce IL-4, IL-5, IL-9, and IL-13 under the control of GATA3 are involved in mucosal immunity and the immune responses to extracellular pathogens and tissue repair, [40] but it is currently unclear whether or not Th2 cells are important contributors to the pathogenesis of OA. In the synovium of patients undergoing total knee replacement, neither IL-4 nor IL-5 mRNA transcripts were found in any of the 18 patients [35]. Moreover, synovial fluid cells from OA patients stimulated with PMA and ionomycin did not express levels of IL-4 that were detectable by RT-qPCR after 24 or 72 h of stimulation [41]. Additional studies have failed to find Th2-related transcripts within the joints of OA patients [42]. However, using flow cytometry, low numbers of $CD4^+IL\text{-}4^+$ cells have been found in the synovial fluid of OA patients [36] and appear in similar frequencies within the synovium when compared to RA patients [43]. $IL\text{-}4^+$ cells were also found in all three layers of the synovium using immunohistochemistry, albeit at very low numbers when compared to IFN-γ^+ cells or $CD4^+$ cells in total [4].

Therefore, Th2 infiltration into the joints of OA patients appears to be sparse and sporadic, but within these patient subsets, they may aid in disease mitigation through reprogramming of macrophages toward an anti-inflammatory and reparative phenotype, or by secretion of cytokines that can protect tissues of the joints from pro-inflammatory and catabolic cytokines. Overall, further investigation is required to elucidate the role of Th2 cells in early OA.

11.6 T Helper 17 Cells (Th17)

Th17 cells secrete IL-17 family cytokines, IL-22, and GM-CSF in response to IL-1β, IL-6, and IL-23 and under the control of RORγt, and are implicated in a variety of chronic inflammatory and autoimmune diseases, including rheumatoid arthritis (RA) and psoriatic arthritis (PsA) [44]. Th17 cells were discovered as a distinct T helper subset in 2005, and so have not been well scrutinized under the lens of OA, but in vitro and in vivo models of OA and RA indicate that they play a considerable role in OA initiation and progression [45].

In human patients with OA, IL-17A was significantly increased in synovial fluid compared to

undetectable in unmatched, healthy controls [30]. Furthermore, an increase in IL-17A within the joint has been positively correlated with pain and severity of disease in patients with knee OA [46]. In OA patients with inflamed synovium, gene and protein expression of IL-17A and IL-22 were increased in inflamed regions compared to non-inflamed OA synovium, and correlated with the release of IL-6 and IL-23 [47]. This indicates that not only are Th17 cells present and active within the joint but that joint inflammation is associated with their maintenance. Additionally, in early joint trauma (ACL tear), the soluble form of IL-17 receptor A, which transduces IL-17 signaling, was increased 128% in synovial fluid at a mean of 14 days post-injury compared to six days post-injury [48].

The presence of elevated IL-17A concentrations in the joint is thought to contribute directly to joint inflammation, tissue remodeling, and loss of function. In vitro, IL-17A treatment of human cartilage explants or synoviocytes activated NF-κB and led to increased synthesis of MMP1, MMP13, [49] and collagenase-3, all of which contribute to matrix loss [50]. Importantly, in a co-culture system of T cells and synovial fibroblasts from early RA patients, Th1 cells did not elicit the same catabolic responses that Th17 cells did, suggesting that Th17, not Th1, responses may be more responsible for joint destruction in OA [38]. In addition, IL-17A enhanced the expression of IL-6 and IL-8 from synovial fibroblasts, aiding in the maintenance of the Th17 phenotype and perpetuating immune cell homing to the inflamed joint [51]. Moreover, IL-17A further promoted T cell recruitment by upregulating the expression of CCL2 and CCL20 expression in synovial fibroblasts [52]. These data suggest that Th17 cells, particularly through their ability to produce large amounts of IL-17, are important players in the loss of joint homeostasis in OA.

Studies in murine models are consistent with these in vitro and ex vivo findings in humans. In murine models of collagen-induced arthritis, treatment with anti-IL-17A neutralizing antibody reduced, though did not eliminate, synovitis, and cartilage damage [53]. Furthermore, arthritis was considerably diminished in IL-17A-deficient mice compared to wildtype mice. Not only did fewer IL-17A-deficient mice develop arthritis, but those than did had lower arthritis scores [54]. Of note, other immune cell types, such as local synovial macrophages, participate in the orchestration of Th17 responses, where they promote differentiation and maintenance of Th17 cells within the synovium [55]. Mouse models have also shed light on the contribution of other Th17-derived factors, such as IL-22, to arthritic disease. For example, IL-22 mRNA and protein expression were increased during onset of antigen-induced arthritis in mice, while the use of an anti-IL-22 antibody and IL-22 deficiency in mice attenuated pain and reduced synovitis, suggesting that targeting of multiple cytokines released by Th17 can reduce arthritis symptoms [56].

Taken together, data from human OA and RA patients, as well as in vitro and in vivo models, suggest that Th17 cells and cytokines play a role in the establishment and progression of OA. IL-17A has been shown to have a direct inflammatory role on synoviocytes and chondrocytes by initiating and perpetuating catabolism and homing of additional immune cells to the inflamed joint. It will be critical to continue investigation of Th17 cells in early OA in order to find targets for which new therapeutics can be made, or for which existing therapeutics can be implemented to mitigate OA progression.

11.7 Regulatory T Cells (Tregs)

TGF-β and IL-2 induce activation of the Foxp3 transcription factor in Regulatory T cells, which can occur in the thymus or periphery to give rise, respectively, to natural or induced Tregs. Tregs produce immunosuppressive cytokines such as IL-10 and TGF-β and dampen immune activation through cell-to-cell interactions and by acting as an IL-2 "sink" to prevent IL-2-associated activation of auto-reactive naïve T cells. Tregs are key players in a multitude of autoimmune and inflammatory diseases, with disease emergence and progression often associated with a lack of Tregs

at critical sites or a failure of Tregs to control or arrest ongoing T cell activation [57].

In patients with mild to severe OA, there is an increase in the percentage of cells in peripheral blood that exhibit a Treg phenotype. However, when stimulated with PMA and ionomycin, these cells were significantly inhibited in their ability to secrete IL-10 [58]. The inability of T cells to carry out their effector functions can be indicative of overstimulation and subsequent exhaustion. Within the context of OA, this could be a potential consequence of chronic inflammation in the joint. Evidence from RA patients would also suggest that peripheral Tregs have a reduced ability to suppress aberrant activation of effector $CD4^+$ T cells through cell-to-cell interactions. This is due to defects in expression of the immune checkpoint molecule CTLA-4, which competitively binds to B7 family members on antigen-presenting cells, blocking effector T cell activation [59].

Not only are Tregs enriched within the peripheral blood of OA patients, they are enriched within the synovium and synovial fluid. There is evidence that Tregs infiltrate the joint during the acute phase of inflammation and are highly active in this phase. Following acute ACL tear, IL-10 increased in synovial fluid, but waned as early as 3 months post-injury [60]. Tregs within the synovium of patients with chronic OA displayed an activated effector memory phenotype compared to peripheral blood Tregs, which displayed a resting central memory phenotype [61]. Moreover, IL-10 transcripts were detected in the synovium of nearly all OA patients [35]. Taken together, these data indicate that Tregs are present during initiation of inflammation, persist in the joint, and may actively attempt to suppress inflammation but are unable to return to joint homeostasis.

Animal models support the role of Tregs and IL-10 in chondroprotection. IL-10 knockout mice with collagen-induced arthritis developed more severe arthritis scores than wildtype mice, which was associated with an increase in production of Th1 and Th17 cytokines, and polarization of macrophages toward an M1 phenotype [62, 63]. In a rabbit model of OA, intra-articular injection of synoviocytes overexpressing IL-10 through retroviral gene transfer five days post-excision of the medial collateral ligament plus medial meniscectomy improved histological scores compared to controls [64]. While absence of IL-10 leads to more severe arthritis, presence and overexpression of IL-10 do not appear to mitigate disease progression in the long term, suggesting that Treg cytokines alone are not sufficient to resolve inflammation.

The continued progression of OA suggests that Treg secreted factors and Treg cell-to-cell contact-mediated suppressor functions are not sufficient to mitigate disease progression. This is in spite of early Treg migration to and activation within the inflamed joint. Furthermore, evidence suggests that Treg activity is dampened as disease progresses, rendering these cells unable to mount suppressive functions that could help control inflammation in the joint to promote repair, thus contributing to OA pathogenesis and failure of disease mitigation.

11.8 Th17: Treg Phenotype Plasticity

$CD4^+$ T helper cell lineages were originally thought to be stable; however, plasticity between Th17 and Treg phenotypes has now been described in multiple contexts including uveitis and scleritis, as well as RA [65]. This instability, within the context of normal physiological conditions, aids in overcoming infections, preventing collateral tissue damage, and resolution of inflammation [66]. However, when plasticity becomes unregulated, it can lead to uncontrolled inflammatory T-cell responses. While transcription factors RORγt and Foxp3, respectively, drive Th17 and Treg phenotype and function, the cytokine microenvironment can activate the reciprocal transcription factor, leading to phenotype plasticity (Fig. 11.3) [67, 68]. The result is that T cell function is altered through simultaneous activation of both transcription factors, and cells are subsequently able to acquire the capabilities of both subsets; secreting Th17 cytokines while eliciting Treg suppressor functions [69].

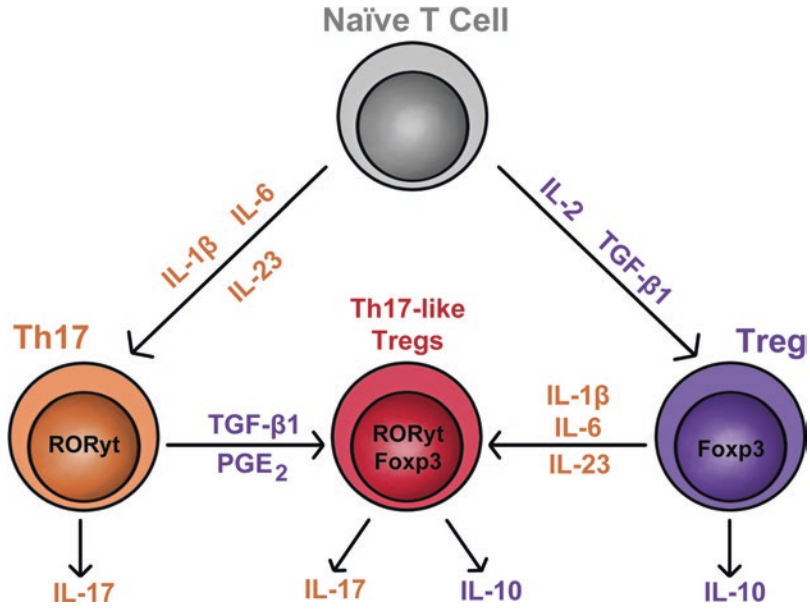

Fig. 11.3 Th17:Treg plasticity may contribute to disease pathogenesis. Although T helper cell phenotypes were thought to be terminal and stable following naïve T cell differentiation, there can be plasticity between Th17 and Tregs. Cytokines within the OA joint can activate the reciprocal transcription factor, leading to an intermediate cell type that secretes Th17 cytokines and, in some contexts, is also capable of carrying out Treg suppressor functions. Instability in Treg phenotype may play a role in loss of joint homeostasis and continued catabolism

Evidence from diseases specifically affecting the joint, including RA and PsA, suggests this plasticity could be involved in the failure of OA resolution as well.

In chronic diseases, persistence of inflammatory cytokines, including IL-1β, IL-6, and IL-23, can lead to destabilization of the Foxp3 transcription factor in Tregs. These cytokines promote the expression of RORγt, yet the resultant Th17-like Tregs also maintain Foxp3 expression, though these cell do not always fully maintain the effector functions of true Tregs [69]. Evidence in mice supports a role for synoviocytes during the induction of Treg phenotype plasticity. For example, in a mouse model of collagen-induced arthritis, $Foxp3^+$ T cells secrete IL-17 following incubation with rheumatoid fibroblast-like synoviocytes, indicating that fibroblast-like synoviocytes from the inflamed joint are sufficient to induce conversion of local $Foxp3^+CD4^+$ T cells to $Foxp3^+CD4^+IL\text{-}17A^+$ cells, exacerbating early inflammation [70].

Increased Th17-like Treg cells can be found in the blood of RA patients and is positively correlated with an increase in Th17 cells in the peripheral blood [71]. A parallel enrichment of Th17 cells in the peripheral blood of OA patients suggests that they also exhibit an increase in peripheral blood Th17-like Tregs [72]. Although these Th17-like Tregs begin to secrete IL-17A, they are still capable of suppressing effector T cell proliferation ex vivo. Conversely, Th17-like Tregs within the joint of RA patients do not maintain suppressor functions, and through secretion of IL-17A, likely contribute to disease progression. There is an upregulation of IL-1β and IL-6 in the synovial tissues following injury, and an upregulation of IL-23 in the peripheral blood of OA patients, further suggesting that Treg phenotypic switching is involved in the pathogenesis of OA [73, 74].

While pro-inflammatory cytokines can lead to Treg phenotype switching, during the resolution phase of inflammation, anti-inflammatory cytokines can induce Th17 cells to convert to a regulatory phenotype through upregulation of Foxp3. In a mouse model of colitis, during resolution of inflammation, high concentrations of TGF-β1

decreased RORγt activity in a dose-dependent manner and caused Th17 cells to transdifferentiate into IL-17A$^+$Foxp3$^+$ cells, which simultaneously secrete IL-17A and IL-10 [75]. Furthermore, TGF-β1 and PGE_2 secreted by mouse and human tumor cells induced Foxp3 expression and subsequent suppressor functions in Th17 cells [76]. Retinoic acid, a driver of Foxp3 activation in inducible Tregs, has also been implicated in suppression of Th17 phenotype [77]. However, it is not clear how retinoic acid concentrations vary within the joint, and whether increased retinoic acid contributes more strongly to cartilage destruction or immunomodulation in OA [78]. Regardless, increased concentrations of TGF-β1 and PGE_2 observed in joints of OA patients suggest that there is potential to drive infiltrating Th17 cells to express Foxp3 and limit the pro-inflammatory and pro-catabolic functions of Th17 cells [18, 79].

Observations made of IL-17A$^+$Foxp3$^+$ cells and Th17-like Tregs in other diseases suggest that phenotype plasticity between Th17 and Tregs may play a role in OA pathogenesis. Investigating plasticity in Th17 and Treg phenotype will potentially increase our understanding of how T cells respond to the local joint environment in a context-dependent manner, which will be an important step toward developing and applying immunotherapies for early OA.

11.9 T Cell-Targeted Immunotherapies for OA

During progression of OA, homeostasis is lost in favor of a catabolic state, where catabolism is defined as progressive and irreversible joint destruction and pain. It is now accepted that disease modification must occur early before this destruction becomes irreversible [80, 81]. Immunotherapy is the use of treatment that targets the immune response, which can include stimulation or suppression, in order to modify disease progression. In the case of autoimmune disease, including RA, immunotherapies that suppress and block aberrant immune function have been successfully implemented for several decades to protect the patient from chronic pain and joint destruction [82]. While some of the key pathways in OA could similarly be targeted using existing immunotherapies, thus far, immunotherapy has not been a mainstay of OA treatment due to inconsistent patient results. For example, use of the anti-TNF-α monoclonal antibody (mAb) therapy adalimumab failed to reduce pain and symptoms in patients with erosive hand OA but did improve joint stiffness and WOMAC pain in patients with knee OA [83]. Currently, there are therapies available and in use for other diseases that target several key areas of the T cell response, including T cell homing, T cell activation and maintenance, and T cell effector functions (Fig. 11.4). A number of these could be leveraged to treat various aspects of the inflammatory response in OA to limit or perhaps even reverse joint destruction. However, understanding more about T cells in the pathogenesis of OA will be important for the targeted use of immunotherapies in OA.

As stated previously, acutely following joint damage, chondrocytes and synoviocytes release a cascade of cytokines and chemokines that not only affect the local joint environment but also home immune cells, including T cells, to the damaged joint. One potential method to reduce T cell-induced inflammation within the joint is to stop T cell trafficking to the joint by either blocking chemokines or their receptors. In RA, treating patients with an antagonist against CCR1, which is expressed by T cells and binds CCL5, reduced the number of CD4$^+$ and CD8$^+$ T cells within the synovium after only 14 days of treatment and significantly reduced the number of tender and swollen joints [84]. Although all patients in the study had firmly established disease, application of such a therapy to early OA may restore homeostasis and promote complete repair of damaged tissue. In a murine model of collagenase-induced OA, treatment with mAb therapy targeting the T cell chemoattractant CCL17 ameliorated the pain and significantly reduced histological score and osteophyte size [25]. Thus, blockade of T cell trafficking chemokines and their receptors may present viable options for OA mitigation.

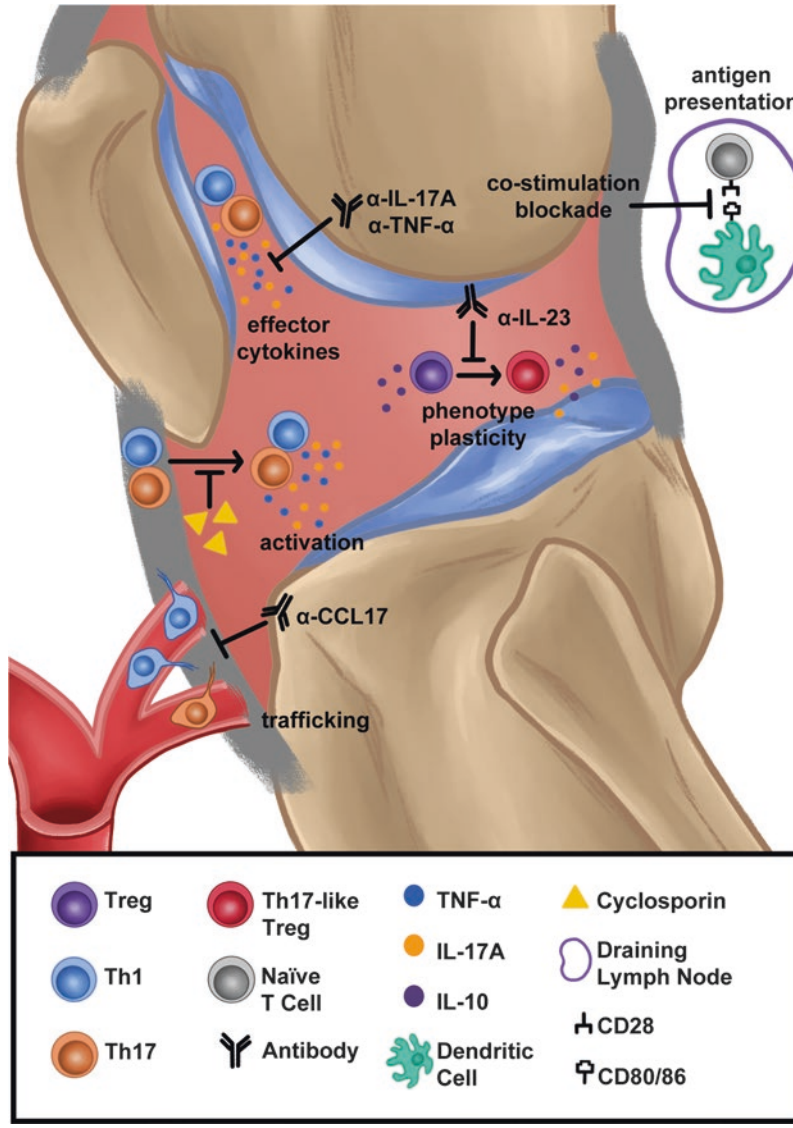

Fig. 11.4 Immunotherapies that target different aspects of T cell response offer new intervention options for OA mitigation. There may be missed opportunities to rapidly translate existing immunotherapies for use in OA. Therapies that target antigen presentation to T cells, T cell trafficking, activation, phenotype plasticity, and effector cytokines are already available and approved for use in other T cell-mediated diseases and could be used to limit or mitigate progression of OA if applied at the right time during disease imitation

Another approach to modifying disease progression in OA is to use therapies that target T cell activation. This can be achieved through several pathways, including blockage of extracellular signaling, or stopping downstream transcription factor activation. One approach to limit T cell activation is to block co-stimulation. Used for the treatment of RA, abatacept is a fusion protein composed of the extracellular domain of the immune checkpoint molecule CTLA-4 and the Fc portion of IgG1. Abatacept shuts down T cell activation by antigen-presenting cells, and within the context of RA, helps to prevent T cell recognition of self-antigen, a driving factor of the disease [85]. It is also possible to target T cell activation more directly. For example, cyclosporin is an immunosuppressant used in the treatment of chronic diseases, such as RA and Crohn's disease, that targets calcineurin, which is a signaling molecule critical for T cells to elicit effector functions [86]. Calcineurin activates nuclear factor of activated T cell cytoplasmic (NFATc), which upregulates downstream T cell responses. These therapies hold potential to mitigate and alleviate OA symptoms within the context of aberrant T cell activation. However,

considering previous failures and mixed results of immunotherapy in OA patients, it will be pertinent to target patients who actively present with T cell-driven OA or else intervention is likely to be ineffective.

A third approach is to target inflammatory cytokines that are secreted by activated T cells. For example, anti-IL-17A therapy has been met with success in the treatment of RA and PsA where, as with OA, IL-17A secreted by Th17 cells is increased in the synovial fluid compared to healthy patients. In PsA patients, treatment with anti-IL-17A therapy reduced radiographic disease progression, effectively inhibiting structural degeneration of the joint [87]. Furthermore, RA patients in some clinical trials who did not respond to anti-TNA-α antibody had reduced disease severity following treatment with anti-IL-17A mAbs [88]. Although mAb therapy targeting the Th1 cytokine TFN-α has previously produced mixed results in OA patients, it is possible that this is because treatment was applied too late in the progression of OA [83]. While treatment of anti-TNF-α therapy did not appear to mitigate disease nor reduce clinical symptoms of several cohorts of patients with end-stage hand OA, it did yield promising results in patients with knee OA with a Kellgren–Lawrence grade of 2–3. Treated patients had significant improvements in WOMAC pain score, stiffness, and function [89].

A final approach is the targeting of cytokines that maintain T cell phenotype and/or promote plasticity toward a pro-inflammatory phenotype. Of note, anti-IL-23 and combination anti-IL-12/23 antibodies that target the p40 region common to both cytokines have undergone phases II and III clinical trials in RA and PsA patients. IL-23 and IL-12 drive and maintain Th17 and Th1 phenotypes, respectively. In PsA patients, anti-IL-12/23 therapy inhibited radiographic progression of joint damage. However, in patients with RA, while there was numerically higher improvement in tender and swollen joints following anti-IL-12/23 treatment, neither the aforementioned treatment nor anti-IL-23 treatment significantly improved RA symptoms [90]. This may be partially explained by the findings that, in a murine model of collagen-induced arthritis, IL-12-driven Th1 activity was not responsible for collagen-induced arthritis, but IL-23 was responsible for T cell-mediated flare-ups, indicating that timing of anti-IL-23 is critical for mitigation of inflammation driven by T cells [91].

Taken together, findings from use of immunotherapies in OA and related diseases warrant further exploration of their application early in the OA disease process. This is before irreversible joint destruction has occurred when there is still the possibility of mitigating catabolism and returning the joint to homeostasis. However, the dynamic nature of OA also calls for a better understanding of T cell involvement during early stages and progression of disease, so that we are able to not only to identify targets for immunotherapies but also timing of when those therapies will be most effective at mitigating disease.

11.10 Conclusion

The immune response is one of a number of critical factors that contribute to disease pathogenesis of OA. There is mounting evidence that T cell populations are altered not only in the synovium and synovial fluid of those afflicted with OA but also within the peripheral blood. Although a variety of $CD8^+$ and $CD4^+$ T cells infiltrate the joint acutely following injury, work in human patients and in animal models indicates that Th17 and Treg effector functions and phenotype plasticity within the joint environment play critical roles in the balance between catabolism and anabolism subsequent to joint damage.

Understanding how T cells contribute to OA initiation and progression presents the opportunity to use immunotherapies that successfully modulate T cell activities as has been done in other inflammation-mediated diseases, including RA and PsA. To date, there have been mixed outcomes of clinical trials using immunotherapies in OA patients, perhaps because the application of these therapies targeted the wrong T cell population or because of T cell plasticity, or more simply, they were used too late in the disease process, when cartilage damage is complete and

irreversible. Therefore, it is important that the role of T cells in early OA continues to be investigated to yield new insights into OA as an immune-mediated disease. This will be critical for identifying novel immunotherapies that can truly modify the course of OA and mitigate disease progression.

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12 Monocytes, Macrophages and Joint Inflammation in Osteoarthritis

Renee T. Ormsby and Julia F. Charles

12.1 Introduction

Osteoarthritis was originally described based on changes to the composition, structure and function of the articular cartilage [1]. However, osteoarthritis (OA) is now considered a 'whole joint' disease, characterized by subchondral bone remodelling, stiffness, pain and synovitis, as well as cartilage degeneration [2]. In particular, studies have identified overexpression of mononuclear cell infiltration, blood vessel formation and pro-inflammatory mediators in patients with OA in the early stages of the disease [2]. Low-grade inflammation is a key marker of early OA [3], and is detectable in the joint by evidence of synovial hyperplasia and low-grade inflammatory infiltrates within the synovial lining. The early stages of osteoarthritis are often defined by an increase in inflammation, with increased presence of macrophages [2], evidence of subchondral bone loss and early stages of cartilage degradation.

12.1.1 Joint Inflammation in OA: A Role for Monocytes and Macrophages

Inflammation plays an important role in the body; the immune system protects the body from infection and defends against foreign pathogens or molecules. The innate immune pathway is activated by tissue and cellular damage, which activates damage-associated molecular patterns (DAMPs) that signal to the immune system to recruit immune cells to the damaged tissue. Pro-inflammatory cytokines, produced by tissue-resident cells, as signals to immune cells such as monocytes and lymphocytes, targeting the site of inflammation. Under normal physiological conditions, the immune cells promote normal tissue healing, reducing inflammation over time [4]; however, in OA, a low-level inflammatory state has been shown to persist [3]. This is likely due to unresolved cartilage damage, bone degradation and the on-going presence of synovitis in the synovial membrane.

The synovial membrane plays an important role in maintaining joint movement by the production of synovial fluid, which reduces friction between the articular cartilage of joints. Synovitis

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has been identified as an early marker of osteoarthritis, it involves inflammation of the synovial membrane which leads to joint swelling and effusion [1]. The synovial membrane also contains a large amount of pro-inflammatory and catabolic products, including metalloproteinases and aggrecanases, which contribute to the articular matrix degradation [5]. Histological analysis of the synovium in OA patients shows an increase in immune cells, including leukocytes, monocytes, macrophages and t-cell lymphocytes [6], which are thought to increase in response to the production of degraded cartilage fragments which are released into the synovial cavity, stimulating synovial inflammation [7].

Myeloid progenitor cells, monocytes and macrophages, play a key role in regulating inflammation. There are two types of macrophages present in the body, M1 and M2 macrophages, which play various roles during inflammation. M1 macrophages release pro-inflammatory cytokines and are activated in response to T helper cells, and M2 macrophages are important during repair and are anti-inflammatory. M1 and M2 macrophages have been identified in the patients with OA, with a larger number of inflammatory M1 macrophages present [8]. Furthermore, inflammatory markers have been detected in the surrounding tissue of OA joints, identifying tissue-resident macrophages which contribute to the early inflammatory signals. Both bone marrow-derived macrophages and tissue-resident macrophages play important roles in inflammation and contribute to the activation of bone resorption. In OA, the pro-inflammatory cytokines produced by macrophages have been identified as potential therapeutic targets, these include TNFα, IL-1 and IL-6.

12.1.2 Osteoclasts in Bone Marrow Lesions and Subchondral Bone Loss in OA

As mentioned above, monocytes and macrophages have been detected in large amounts in osteoarthritic joints; these cells also play a key role in bone resorption. Activated macrophages produce inflammatory cytokines that stimulate the differentiation of macrophages into multi-nucleated bone-resorbing cells, called osteoclasts [9]. Osteoclasts are formed when mononuclear cells, macrophages, fuse into a multi-nucleated giant cell. These cells are then capable of producing enzymes that degrade the bone and are activated during bone remodelling. Osteoclast activity and differentiation is regulated by key factors, including the release of macrophage colony-stimulating factor (M-CSF) from macrophages, and the production of receptor activator of nuclear factor-kappa-B ligand (RANKL) by osteoblasts and osteocytes, and by fibroblasts at lower levels [10]. Formation of osteoclasts is also regulated by the production of TNFα and IL-6, two pro-inflammatory cytokines expressed by monocytes and macrophages. Osteoclasts play a key role in regulating bone homeostasis and are coupled with the activity of osteoblasts, bone forming cells which balance resorption with bone formation. However, in early OA there appears to be an uncoupling between osteoclasts and osteoblasts, favouring bone resorption over formation, which leads to the destruction of the subchondral bone [11].

The activities of the myeloid cell lineage include the production of pro-inflammatory cytokines by monocytes and macrophages, and the activation of osteoclastic resorption. Together, myeloid cells play a very important role in regulating the immune response and bone remodelling that occurs in the early stages OA and are therefore key therapeutic targets that could alleviate the symptoms of OA, with the possibility of preventing further progression of the disease.

12.2 What Can Animal Models of Osteoarthritis Teach Us About the Role of Myeloid Cells in OA

The development of early OA in humans often occurs with little surgical management, making biopsy samples unattainable, thus animal models have been investigated to identify the changes

that occur on the cellular level during the initial stages of the disease. Various animal models have been used to investigate the development of osteoarthritis, including both naturally occurring osteoarthritis that occurs spontaneously, and invasive and non-invasive induced OA. Commonly, this has been investigated in mice, rats and guinea pigs, as well as larger animals including dogs and horses. These models have been used to investigate the changes in monocyte, macrophage and osteoclast activity, and the effects on inflammation and subchondral bone remodelling.

12.2.1 Naturally Occurring Osteoarthritis

In a process similar to humans, spontaneous occurring osteoarthritis develops in animals with aging. Spontaneous OA has been detected in small animals such as guinea pigs, rats and mice [12]. These animal models allow in-depth examination of the joint pathology that occurs with OA, including the degradation of the articular cartilage and subchondral bone remodelling, at a cellular level which is near impossible to achieve in patients with early OA. These models also closely mimic the progression of the disease in humans, as identified by the histological and biochemical changes that occur with progression of the disease.

One such animal model is the Dunkin Hartley (DH) strain of guinea pigs, which develop OA at 3 months of age [13]. This model closely simulates the slow progressive changes observed in human OA, prior to the animal reaching skeletal maturity at 6 months. This model has been used to investigate the changes in cartilage composition and degradation with development of the disease. In particular, guinea pigs express high levels of IL-6 at 12 months of age, a pro-inflammatory cytokine produced by monocytes and macrophages [14]. In fact, serum levels of IL-6 positively correlated with the histological score of OA in guinea pigs [15], and similarly, in humans, circulating IL-6 was shown to be a predictive value in the development of knee OA as detected by radiography [16], indicating that this model may be a good representative of the human condition. IL-6 also plays an important role in regulating osteoclast differentiation and bone resorption. The guinea pigs also expressed high levels of other cytokines including IL-8, IL-17, MIP-1α and TNF-α [14], which have important roles in both the immune and resorptive responses in OA. TNF-α has also been detected at high levels in patients with early OA [2].

Another animal model of spontaneous occurring OA is the STR/ort mouse model [17]. These mice display human-like cartilage lesions after 12–20 weeks of age, where 8 weeks of age is considered an adult mouse. The mice commonly develop OA in the knee, ankle, elbow and temporomandibular joint. Knee pathology includes degeneration of the medial tibial cartilage at the insertion of the cruciate ligament, articular cartilage (AC) fibrillation, osteophyte development and subchondral bone sclerosis. Analysis of the joint profiles of these mice identified increased levels of MMPs [18], inflammatory cytokines including IL-1b, IL-4, IL-10 and interferon γ [17].

STR/ort mice have been shown to have an increased population of myeloid cells compared to C57BL6J mice of the same age, with a twofold increase of CD11+Gr1+ cells in the peripheral blood and spleen, and were also detected in the synovial tissue [19]. These mice also have a twofold increase in the number of inflammatory macrophages ($CD11c^{+}F4/80^{+}CD11b^{+}$) within the synovial tissue of the diseased joint as well as increased TNF and MMP3 expression in synovial tissue [20]. Furthermore, these macrophages also express high levels of IL-1β, which have been shown to regulate the expression of the calcitonin receptor-like receptor (CLR). CLR is the receptor for calcitonin gene-related peptide (CGRP), which plays a role in modulating pain and neurogenic inflammation in OA, therefore activated macrophages in OA may play a role in regulating pain through the induction of cytokines [21].

The STR/ort murine model has also been used to identify changes in the extracellular expression of SOD, super oxide dismutase, which plays important roles in binding reactive oxygen

species (ROS). SOD has been shown to be decreased in the cartilage of humans with OA [22]. ROS and SOD also play important roles in osteoclastogenesis [23], ROS regulates both osteoclast differentiation and resorption. The binding of RANKL to the RANK receptor on osteoclasts produces ROS, which then acts as a secondary messenger, activating MAPK, NFκB, and Ca2+ mobilization pathways. These pathways stimulate the expression of NFATc1, a transcription factor that in turn promotes the transcription of key osteoclastogenic genes, including tartrate-resistant acid phosphatase (TRAP), CTR, CTSK and pro-fusion genes [24]. ROS also plays a role in osteoclast resorption. Osteoclasts produce the protease, Cathepsin K to degrade the bone mineral in the bone compartment, the degradation products are endocytosed by osteoclasts with cathepsin k into vesicles. The vesicles undergo transcytosis and are fused with TRAP-containing vesicles, co-localizing cathepsin k and TRAP. TRAP then undergoes proteolytic digestion by cathepsin k and produces ROS to complete the mineral matrix degradation [25, 26]. Mitochondrial ROS also plays a role in osteoclast differentiation through the mitochondria-targeted antioxidant (MitoQ), which has been shown to suppress osteoclastogenesis in vitro [27]. Therefore, ROS may play an important role in OA in regulating the subchondral bone loss evident in early OA.

Certain changes observed in the bone homeostasis of STR/ort mice are, however, different compared to bone remodelling in human OA. Female mice, in particular, have increased bone formation and impaired bone resorption, unlike humans [17]. However, the male mice do display a loss of femoral trabecular bone volume (BV/TV) at 10 weeks of age, which is then restored over time, consistent with the earlier initiation and progression of OA in humans. This suggests that only the male STR/ort mice are appropriate with regards to being informative for human OA. Furthermore, the STR/ort mice have been identified with an inherent endochondral ossification defect that likely influences the changes in bone formation and therefore the OA pathology observed [17].

Naturally occurring OA also occurs in larger animals including dogs and horses. The stifle joint in both dogs and horses is anatomically and histologically similar to the human knee [28]. The canine spontaneous OA model is caused by the significant activity levels of these animals, this leads to wear of the cartilage, similarly observed in active humans. This causes cracking joints, impaired mobility, stiffness and muscle wastage in the animals [29]. Dogs with spontaneous OA similarly have a loss of subchondral bone, followed by an increase in porosity and sclerotic bone formation at the subchondral plate [30]. One study observed increased levels of osteocalcin in the serum of canines with OA and was associated with age of the animal, increased detection of osteocalcin in the serum occurs with active bone resorption [31]. The canine model of spontaneous OA has predominantly been used to detect changes in the cartilage [32].

Horses also develop spontaneous OA due to the high levels of activity performed by these animals, OA is predominantly detected in the metacarpophalangeal (MCP) joint [33]. The disease in horses is characterized by synovial membrane inflammation and increased expression of metalloproteinases (MMPs), IL-1 and TNFα, similarly detected in the synovium of humans with OA [34]. Analysis of carpal bones from horses with post-traumatic OA showed a significant increase in the number of osteoclasts at the subchondral plate. Increased RANKL expression was observed in the cartilage of these animals and correlated with cartilage degeneration, osteoclast density and microcracks within the cartilage. This study suggests that RANKL in the cartilage plays a key role in stimulating both microcracks and subchondral bone resorption [35].

Studies comparing spontaneous occurring OA and the induction of OA through surgically induced damage have highlighted the changes that develop in the early stages with intentional joint injury. These changes include the development of synovitis and articular cartilage degradation occurring earlier with injury than with spontaneous occurring OA in these animals [36]. Active bone resorption is also an important marker of OA and is observed in patients with

early knee OA. In particular, patients who showed progression of the disease also showed upregulated markers of resorption [37]. This has been observed in animal models of induced OA with injury, in particular in the anterior cruciate ligament injury model which shows early resorption of the subchondral bone and is subsequently followed by increased bone formation [38].

12.2.2 Induced and Invasive Models of Osteoarthritis

A method of inducing non-invasive OA is the use of collagenase injections to stimulate degradation of the articular cartilage and subchondral bone. This model is often compared to the ACLT invasive model of OA where the surgical injury shows sustained damage over time [39]. The collagenase model is more often used for studying other forms of arthritis rather than OA.

As mentioned, OA often develops after joint injury in both humans and animals. In humans, joint injury is caused by increased activity and loading in athletes and/or through damage to the bone, ligaments and cartilage in the joint. Animal models of invasive model of OA is performed by surgically inducing damage to the joint tissue, specifically the medial meniscus or the anterior cruciate ligament, which have been shown to stimulate joint degeneration and OA in humans.

Non-invasive induction of post-traumatic osteoarthritis has also been developed in order to simulate the injury that occurs in humans, such as anterior cruciate ligament tears. Non-invasive techniques use externally applied mechanical loads that do not break the skin or disrupt the joint capsule [40].

The anterior cruciate ligament (ACL) injury model is based on ACL tears that occur predominantly in young active adults; this injury often predisposes the patient to developing post-traumatic OA. The surgically induced model of OA involves transection of the anterior cruciate ligament, which induces mechanical instability and leads to OA developing in the knee joint. This model causes the animal to favour the healthy limb over the injured leg, changing the mechanical loading and stresses on the bone, similar to patients who undergo the same injury. ACLT injury has been shown to cause cartilage degeneration, subchondral bone remodelling and osteophyte formation in animal models. This model has been investigated in mice, rats, dogs, goats and sheep [33].

Bone resorption is observed in this model, a study by Zhu et al. using the murine ACLT surgery at 2 months of age showed an increase in TRAP-positive osteoclasts after 1 and 2 weeks post-surgery [41]. Histological analysis of the ACLT damaged joints at 8 weeks post-surgery showed large bone marrow cavities visualized as fibrous lesions within the subchondral bone. The lesions were associated with an increase in tissue volume, as measured by μct. Pain is another key symptom associated with OA. This study also showed that the subchondral bone remodelling that occurred in the early stage of the disease was associated with the production of nocireceptors, which are sensory neurons. The mice with ACLT surgery showed increased number and density of nociceptive neurons (identified by immunostaining of CGRP, a neuropeptide) next to the subchondral bone, compared to sham-operated mice. This study then showed specific knockdown of RANKL, a key osteoclast activity differentiation marker in a Dmp1-Rankl$^{fl/fl}$ mouse model prevented the bone loss associated with ACLT surgery and reduced the number of sensory neuron fibres. Furthermore, results from in vitro culture of neurons showed that Netrin-1, produced by osteoclasts, regulated the number and outgrowth of neuronal fibres. Inhibition of Netrin-1 in a Trap-Ntn$^{fl/fl}$ mouse model reduced the number of neuronal fibres and reduced pain. Therefore, this model provides evidence that osteoclasts may play an important role in regulating pain in OA, particularly in the early stages of the disease when subchondral bone remodelling occurs [41].

The ACLT OA model has also been used to identify changes in TGFβ, which has important roles in regulating bone formation and maintaining articular cartilage. Mice with ACLT surgery showed elevated expression of TGFβ in the subchondral bone [42], TGFβ has also been observed at increased levels in the synovial fluid of patients

with OA [43]. TGFβ has been identified as a crucial factor that regulates the development of osteophytes, inhibition of endogenous TGF-β in a murine knee model prevented osteophyte formation [44]. Furthermore, synovial macrophages have been shown to play a key role in the development of osteophytes, removal of lining macrophages prevented the formation of osteophytes induced by TGFβ [45].

Induced ACL injury in mice has been shown imitate the human condition. C57BL/6J mice have been shown to be susceptible to the development of OA after ACL rupture. These mice display subchondral bone loss, cartilage erosion, osteophyte formation and a significant increase in OARSI score 12 weeks post-injury compared to the contralateral control [46].

ACLT in mice has been shown to cause synovitis, a study by Chen et al. showed a significant increase in synovitis score, as measure quantitatively [47], as well as increased cell infiltration and intra-articular synovial hyperplasia and abundant cell infiltration 8 weeks post-surgery [48]. Furthermore, these mice show a significant increase in pro-inflammatory cytokines, TNFα and IL-6, in the serum of the ACLT mice compared to sham control.

Similar results were shown in a study by Wang et al., where ACLT injury increased both synovitis score and OARSI score after 8 weeks post-surgery. Treatment with celecoxib, a non-steroidal anti-inflammatory drug, 10 mg/kg per day or an anti-inflammatory agent termed Tanshinone I (10 and 30 mg/kg per day), significantly reduced the synovitis and OARSI scores in mice with ACLT surgery compared to sham control [49].

In a similar model of ACLT injury, after the mice underwent ACL or Sham surgery, they were made to run on a treadmill at a speed of 16 m/min every day for 4 weeks. Analysis of the articular cartilage showed increased mRNA expression of inflammatory cytokines, including TNFα, IL-1β and IL-6, in these mice 4 weeks post-surgery. Increased protein expression of TNFα and IL-1β was also detected in the articular cartilage of these mice compared to sham control. Histological analysis of the joints showed extensive cartilage damage, disorganized chondrocyte formation and eroded cartilage surfaces, as determined by the Mankin score for OA cartilage evaluation, as well as significant inflammation and synovial hyperplasia [50]. This study replicates the damage that occurs with unrepaired ACL tears with joint loading, simulating the inflammation that would occur in humans pre-operatively.

Similarly, evidence of increased immunohistochemical staining for inflammatory cytokines, IL-1α and IL-1β, has been shown in the articular cartilage of mice 4 weeks after ACLT surgery, as well as increased mRNA expression of both IL-1α and IL-1β, and IL-6. Histology images of the joint show significant cartilage destruction visible by Safranin O staining. This study also showed increased oxidative stress, DNA damage and cellular senescence in articular cartilage of mice who underwent ACLT surgery compared to sham control [51]. This suggests that inflammatory markers are produced within the articular cartilage, potentially activating and recruiting macrophages to the site of injury.

In fact, in another model of ACLT surgery, the macrophage inflammatory protein 1γ (MIP-1γ) was increased in the synovium with an increased immune response in this model of OA. MIP-1γ plays an important role in recruiting inflammatory cells to the injured tissue and plays a role in wound healing. Analysis of CD4−/− mice with OA showed a significant decrease in expression of MIP-1γ and slower cartilage degeneration as well as decreased TRAP staining [52], therefore CD4+ cells are involved in the progression of OA by regulation of MIP-1γ. MIP-1γ regulates monocyte chemotaxis and is a chemoattractant, but importantly MIP-1γ has been shown to regulate osteoclastogenesis. In a study by Chen et al. induced MIP-1γ gene silencing via lentiviral gene transfer in mice with ACLT injury-induced OA, both the lentiviral vector encoding MIP-1γ small hairpin RNA (shRNA) and control vector were injected into the intra-articular knee joint. Neutralization of MIP1γ reduced the infiltration of immune cells and macrophages, reduced IL-1 cytokine expression. Importantly, there was a significant reduction in osteoclast number and cartilage damage, suggesting that MIP-1γ plays an

important role in the early progression of OA and may be a potential therapeutic target [53].

Surgically induced OA animal models include the destabilization of the medial meniscus model (DMM), a surgical model of injury that causes osteoarthritis in mice and rats [54]. The medial menisco-tibial ligament is transected, affecting the mechanical stability of the knee joint, whilst inducing cartilage damage, as described in humans with progressive OA [55]. Importantly, this model causes osteoarthritis to occur slowly with associated inflammation of the joint and includes validated pain endpoints similarly experienced by humans [54]. This model allows the progression of the disease to be monitored over time, from early to advanced osteoarthritis. The DMM model also shows changes in the subchondral bone, with an increase in sclerotic bone formation.

The DMM model has been used to analyse the early changes of articular cartilage and subchondral bone that occur in OA; a study by Fang et al. observed changes at 2, 5 and 10 weeks in this model, with cartilage degeneration and bone mineral density which increased after 5 weeks. This study also showed no evidence of subchondral bone resorption after 2 weeks, suggesting this occurs prior to this time point. Furthermore, TRAP staining in this model showed increased osteoclasts mainly within the osteophytes in the subchondral bone plate at 5 and 10 weeks post-surgery [54]. This suggests that osteoclasts may play an important role in the development of osteophytes, and in the later stages of OA progression.

The DMM model has been used to investigate the role of cytokines in OA. A study by Rowe et al. investigated the changes in macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine, which is elevated in patients with knee OA. MIF is produced by innate cells and plays an important role in regulating neutrophil migration and activating the innate immune pathway, as well as stimulating macrophage activation and phagocytosis. Knockdown of MIF in a mouse model protected aged mice from naturally developing OA, but this was not evident in young mice with surgically induced OA. This suggests that while macrophages are evident in both classifications of OA, the differences between young and old patients may be important in determining therapeutic targets. Macrophages in young adults with OA may be more active and more proficient at undergoing polarization from the M1 to M2 macrophage phenotype. Identification of the macrophage phenotype in OA has been determined in older patients, with a greater number of M1 to M2 macrophages in the synovial fluid of patients with severe knee OA (average age 58) [8]. Therefore, age as well as severity of the disease, may play important roles in identifying therapeutic targets. M2 macrophages and their role in repair are potentially key to preventing progression of the disease.

The DMM model of OA has also been used to investigate the role of PAR-1 and PAR-2 in OA. PAR-2 has been shown to be upregulated in the synovium of patients with OA [56], and both PAR-1/2 are important for the development of synovial inflammation and pain [57]. In the mouse DMM model, mice with PAR-2 deletion showed a significant reduction in synovial macrophage activation in the first-week post-surgery [58]. In a follow-up study, PAR-2 deletion reduced the development of osteophytes and osteosclerosis, and appeared to improve pain [59]. PAR-2 has also been shown to play a role in fracture healing, where deletion altered the callus morphology that occurs during fracture healing [60]. Therefore, PAR-2 may play an important role in regulating macrophage activity during the development of OA.

The DMM model, however, is not a perfect example of the development of OA as seen in humans. The loss of bone seen in early human OA has not been clarified in the DMM model, despite the observed changes in cartilage degradation, though as mentioned above subchondral bone resorption may occur in the very early stages in this model and is quickly resolved.

12.3 Human Osteoarthritis and Translational Research

Management of advanced OA often involves whole joint replacement surgery as a main therapy in managing both pain and patient motility. Whereas in young adults and patients with early-onset OA there are few treatments, and the disease is often managed through the administration of anti-inflammatory medications [61, 62]. This does not combat the development of OA, which can often deteriorate with age. Therapies that target inflammation, and bone and cartilage degradation have been investigated in order to reduce symptoms and potentially prevent disease progression. These include targeting pro-inflammatory cytokines that are produced by monocytes and macrophages, such as TNF and IL-1b, mentioned previously. These therapies have shown promise in reducing inflammation in humans and potentially preventing pain and structural degeneration [63]. Similarly, therapeutic targets that block osteoclast activity have been developed in order to reduce the subchondral bone remodelling that leads to the destruction of the bone observed in early OA. These therapies include targeting RANKL, the osteoclastogenic activity marker, and Cathepsin K which is a protease produced by the osteoclast which degrades collagen fibrils. These targets have been used in other bone diseases, such as osteoporosis, and show improved bone mineral density and reduced risk of fracture. Therefore, targeting myeloid lineage cells may provide potential effective treatments that combat the debilitating features that occur in the early stages of OA.

12.3.1 Identification of Myeloid Cells in Human OA Biopsies

Myeloid cell populations have been investigated in patients with OA. A study by Loukov et al. analysed the levels and expression of monocytes in patients with knee OA, and observed an overall decrease in the number of monocytes in the peripheral blood of women with knee OA, compared to the healthy patient control group [64]. This study also shows an increase in the expression of a key trafficking receptor, CCR2 in circulating monocytes, CCR2 is critical for monocyte trafficking and promotes infiltration of the synovium. However, the patients with OA showed a greater percentage of activated monocytes, with increased expression of CD16 which is associated with monocyte differentiation into osteoclasts, and increased expression of HLA-DR, an antigen-presenting molecule expressed by inflammatory monocytes to activate T cells. The activated circulating monocytes expressed increased levels of pro-inflammatory cytokines including TNFα and IL-1b compared to healthy controls [64], both cytokines play a key role in activating osteoclastogenesis. Furthermore, another study by Durand et al. showed that the monocytes derived from OA patient blood compared to healthy patients showed increased osteoclastogenic potential, with increased resorptive activity and decreased osteoclast apoptosis [65]. This suggests that there is a strong recruitment of activated inflammatory monocytes to the synovium, and the monocytes in circulation have a strong potential to become osteoclasts. The synovial fluid obtained in this study was from female patients over the age of 50, with age-matched controls. A study by Gómez-Aristizábal similarly showed an increased population of monocytes within the synovial fluid of patients with knee OA, with a high number of double-positive CD14+CD16+ pro-inflammatory monocytes [66]. The subsets of monocytes/macrophages, CD14+CD16+ and CD14+CD16neg, also correlated with patient-reported outcomes, including the Knee Injury and Osteoarthritis Injury Score (KOOS) questionnaire and the and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain, stiffness and function questionnaire. Therefore, this study suggests that monocyte populations in OA may be important as therapeutic targets and as potential biomarkers of symptomatic knee OA.

Another study showed a significantly larger population of CD14+ macrophages in the synovial membrane of patients with knee OA compared to patients with hip OA. Interestingly, the

number of activated macrophages in the synovial tissue of patients with knee OA correlated with the radiographic severity of the disease unlike the previous study, and also correlated with joint symptoms including pain, aching and stiffness [67]. This suggests that different sites of OA may play a role in targeting specific therapies.

Synovial macrophages have also been associated with the development of osteophytes, depletion of synovial macrophages in a murine model of induced arthritis showed a reduction in osteophyte formation, fibrosis and synovial activation. There was also a reduction in expression of key markers of bone formation TGFβ, BMP-2 and BMP-4 by synovial lining surface cells. This suggests that synovial macrophages play an important role in the excess bone formation that occurs in the later stages of OA [68]. This was confirmed in an animal model of OA by Van Lent et al., where TGFβ injections into murine knee joints stimulated the formation of osteophytes, and removal of the synovial lining macrophages prior to injection reduced osteophyte formation [45].

Therefore, the monocyte–macrophage population in OA play key roles in regulating inflammation as well as influencing pain and stiffness, inducing osteoclastogenesis, and potentially stimulating the formation of osteophytes. Targeting this cell population as therapeutic targets may be important for improving function and quality of life for patients with OA, and potentially preventing progression of the disease.

12.4 Targeting Myeloid Cells to Treat OA

The prevalence of OA in both the aging population and younger adults has led to the search of disease modifying OA drugs (DMOADs) that specifically inhibit both the symptomatic and structural changes that occur with the disease. Ongoing trials with potential therapeutics will optimistically improve joint function, prevent bone remodelling and provide pain relief for patients with OA, preferably at any stage of the disease.

OA therapies have been investigated in animal models and/or clinical trials which target pathways related to macrophages or osteoclasts, these are reviewed below.

12.4.1 Clinical Therapies Targeting Inflammatory Cytokines in OA

Inflammation involves the recruitment of immune cells to the targeted area, these cells include monocytes and macrophages, as described above. Both monocytes and macrophages produce inflammatory cytokines that stimulate the immune response, as well as activating osteoclastogenesis. Increased expression of specific cytokines has been identified in the joints of patients with OA, including TNFα, IL-1, IL-6 and MIP-1, as well as many others. These have become primary targets of OA therapies in an effort to reduce the inflammation and pain that is prevalent with the disease, as well as potentially reducing cartilage degradation and the dysregulated bone remodelling. A brief description of the current cytokine targeted therapies is summarized below.

12.4.1.1 Targeting Interleukin Pro-inflammatory Cytokines, IL-1 and IL-6

Evidence of upregulated interleukin cytokines have been detected in the synovial fluid of patients with OA, these cytokines also correlated with radiographic evidence of OA. Both IL-1 and IL-6 play important roles in regulating bone homeostasis by inducing differentiation of osteoclast precursors, and stimulating bone resorption [69]. Therefore, these cytokines have been investigated as therapeutic targets for OA.

Analysis of biopsies from patients with OA provided clinical evidence of increased IL-1 expression, including IL-1a and IL-1b, in the synovial fluid, synovial membrane as well as the cartilage and subchondral bone layer at elevated levels [70]. However, IL-1 antibodies investigated in both animal models and in clinical trials have yielded disappointing results.

The main IL-1 antibody was developed by Amgen in 1993 and is an IL-1 receptor antagonist (IL-1Ra), named Anakinra. Anakinra blocks the activity of both forms of IL-1 (IL-1α and IL-1β) and has been tested in vivo in animal models with promising results. In particular, the intra-articular injection of Anakinra in a canine ACLT model of OA reduced the presence of osteophytes and cartilage lesions, animals received either 2 or 4 mg injections for 4 weeks post-surgery [71].

Clinical testing of this antibody was pursued in a pilot study which presented improved function measurements and reduced pain with intra-articular injection [72]. However, the results of a larger multicentre randomized double-blinded placebo-controlled study investigating two doses of Anakinra were contrary, with no improvements compared to placebo treatment. The 12-week study included patients from a younger age group (34–82 years, mean age = 62); however, age groups were not separated in the analysis of the results [73]. The disappointing results from the larger study suggest this IL-1R antibody may not be a suitable target for the treatment of OA, despite showing some evidence of pain relief in patients treated with the higher dose.

Other IL-1 antibodies and inhibitors have been developed, including the human mAB AMG108. AMG108 is an IL-1 receptor-blocking antibody and has been tested in patients with rheumatoid arthritis, with similar effectiveness as Anakinra. AMG108 has been tested clinically in a double-blind, placebo-controlled study in patients with knee OA (NCT00110942) [74]. AMG108 was given subcutaneously to 80 randomized patients (300 mg) and compared to 80 patients who received the placebo control (average age = 60.1). The main study aim was to determine the clinical efficacy of AMG 108, by determining the change in WOMAC score (pain) from baseline, secondary aims were to determine the safety and pharmacokinetics of AMG 108. Unfortunately, in this study despite minor improvements in pain scores, the study outcomes were not statistically significant.

An IL-1β antibody therapy, Canakinumab, has shown promise of reducing OA-related adverse events and the incidence of total knee and total hip replacements in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) cohort study. In this trial, 10,061 participants (average age = 61) were given either placebo or canakinumab at either 50, 150 or 300 mg subcutaneously every 3 months for approx. 3.7 years. Patients in this cohort showed high levels of the high-sensitivity C-reactive protein (hs-CRP) and a history of myocardial infarction. Exploratory data from this trial show canakinumab reduces both the incidence of total knee or hip replacement and shows a reduction in osteoarthritis adverse event [75]. Canakinumab also been shown to reduce cardiovascular event rates in participants who were administered either 150 or 300 mg [76]. This study shows promising preliminary data for the use of this antibody in the treatment of osteoarthritis; however, more in-depth analysis is required to determine dose, treatment length and adverse events associated with this drug therapy. In particular, the protection of bone microarchitecture and the prevention of cartilage degeneration would be key in supporting canakinumab as a key treatment for both early and mature osteoarthritis.

Other IL antibodies investigated clinically include MABp1 (IL-1α), gevokizumab (IL-1β) and the fusion protein rilonacept (targeting IL-1a, IL-1b, and IL-1Ra), the majority of which have been shown to be ineffective therapies for treating patients with OA, or were not investigated further.

Another approach has been the development of a novel human dual variable domain immunoglobulin (DVD- Ig) which simultaneously binds and inhibits both IL-1a and IL-1b but does not interfere with the binding of the IL-1Ra or other IL-1 family members. Termed ABT-981, pre-clinical testing in the mouse DMM OA model showed ABT-981 inhibited the progression of OA induced in this model; however, the specific details of this are not mentioned in the published abstract. Testing of ABT-981 was performed in a human cohort, in a randomized, double-blind, multi-dose, placebo-controlled phase 1 study in patients with OA of the knee. Four doses of subcutaneously injected ABT-981

were investigated in patients (40–70 years old, mean age = 60) with radiographic evidence of knee OA. Cohort 1–3 received 4 SC injections of ABT-981 (0.3, 1 or 3 mg/kg) or placebo every other week (EOW) on days 1, 15, 29 and 43. Cohort 4 received 3 SC injections of ABT-981 (3 mg/kg) or placebo every 4 weeks (E4W) on days 1, 29 and 57. The primary outcomes of the study were to determine the safety, tolerability and pharmacokinetics of ABT-981. Secondary outcomes were the immunogenicity, pharmacodynamics and patient-reported outcomes of the treatment. The outcomes of this trial were positive, ABT-981 was well tolerated and dose-dependently reduced the serum expression of IL-1a, IL-1b, as well as levels of high-sensitivity C-reactive protein (CRP) and C1M, C3M, metabolites of type 1 and type 3 collagen, respectively [77]. Therefore, this provides evidence that ABT-981 reduced both tissue turnover markers and inflammatory markers in patients with OA and is, therefore, a promising therapeutic for patients with OA. Interesting, treatment with ABT-981 did not significantly reduce pain scores compared to placebo; however, the author makes note that longer studies with a larger population is needed as this study included low patient numbers with mild to moderate OA, suggesting reduction in pain may be observed in patients with more severe OA.

Of note, a common adverse effect observed with all IL-1 treatments was infection; the ABT-981 injection was associated with a 13% incidence of site erythema. Similarly, trials using canakinumab and anakinra both recorded higher infection rates compared to placebo control [77].

As mentioned above, the cytokine interleukin-6 was a predictive marker of early knee OA in humans, confirmed by radiography [16]; serum IL-6 levels correlated with decreased tibial cartilage volume in patients 50–79 years of age with radiographic knee OA, measured over 3 years [78]. Therefore, the development of an IL-6 antibody would potentially inhibit the progression of OA in patients. An IL-6 neutralizing antibody, MR16–1, was tested in the murine DMM model of OA. MR16–1 was given to mice (0.5 mg once a week) for 6 weeks and was shown to reduce cartilage lesions, osteophyte size and synovial inflammation. However, there was no effect on subchondral bone, with no change between surgical intervention or antibody treatment [79].

Another IL-6 receptor antagonist has been tested in a clinical trial that began in 2015, the trial tested the efficacy of Tocilizumab on patients with erosive hand OA in a double-blind, randomized placebo-controlled trial (NCT02477059). Patients were recruited between 40 and 85 years of age and given Tocilizumab (8 mg/kg) or placebo control twice for 4 weeks, with assessment after 6, 8 and 12 weeks. Primary outcomes were pain scales using the visual analogue scale (VAS), secondary outcomes were the assessment of painful, swollen joints, overall disability, stiffness and function measured by Dreiser's algofunctional index and Functional Cochin hand index. (Ref clinical trial: https://clinicaltrials.gov/ct2/show/NCT02477059).

Comparably, the IL-6 receptor antibody, MRA, has been tested for treatment of rheumatoid arthritis (RA) in a multicentre, double-blinded, placebo-controlled trial [80]. MRA is an anti-human IL-6R antibody that inhibits the binding of IL-6 to the receptor. Patients received either MRA (4 or 8 mg/kg body weight) or placebo intravenously every 4 weeks for 3 months. Patients treated with the antibody showed a reduction in inflammatory markers, including C-reactive protein levels and erythrocyte sedimentation rate. This suggests that this antibody may also be a valid treatment for OA.

12.4.1.2 JAK Inhibitors

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is a cytokine signalling pathway. This pathway is activated in patients with Rheumatoid arthritis and is associated with chronic inflammation. Therefore, JAK-selective small molecule inhibitors (SMIs) were developed, such as tofacitinib (JAK3), and baricitinib (JAK1/2), which inhibit the specific JAK activity and inhibit the JAK/STAT signaling pathway [81]. The JAK pathway

is likely activated in OA, as this pathway is regulated by growth factors and cytokines including IL-6 which as mentioned above is upregulated in OA [70].

Tofacitinib, the JAK3 inhibitor was tested in a rat model of inflammatory arthritis (RA), the rats were given an oral dose of Tofacitinib (6.2 mg/kg) every day. Treatment significant reduced edema, inflammatory cell infiltrates and numbers of osteoclasts within the medullary space of the distal tibia [65]. JAK inhibition also decreased the expression of IL-6 and IL-1 in the paw tissue. The inhibition of both inflammation and osteoclastogenesis in this model suggests JAK inhibitors may be a suitable therapy for patients with early OA.

JAK inhibitors have been predominantly tested for patients with Rheumatoid arthritis; however, several studies have identified adverse effects associated with treatment including the development of herpes zoster (shingles). Both Tofacitinib and baricitinib are current therapies used to treat RA and have been tested in phase III clinical trials, both inhibitors improve activity, function and patient-reported outcomes [82].

12.4.1.3 Targeting TNF

The pro-inflammatory cytokine TNF-α has been recognized as an upregulated marker of inflammation in patients with OA [70]. TNF-α is made in abundance by macrophages and has been detected in tissue-resident macrophages in the synovium of patients [83]. A human anti-TNF-a monoclonal antibody named Adalimumab has been developed as an anti-inflammation therapy due to the positive results from murine models of OA. For example, a surgically induced OA in a rat model treated with Adalimumab attenuated the subchondral bone loss that occurs with ACLT injury [84]. TNF antibodies have been investigated in human clinical trials, with a range of doses and treatment times; however, the results are conflicting, with few showing a reduction in pain (OARSI score) in patients with moderate to severe cases of OA [85]. Two previous clinical trials investigated the effects of Adalimumab in an erosive hand OA study, a 12 month RCT (NCT00296894) and a knee OA study, a small Phases I/II study of 17 patients with knee OA (NCT00686439), where the drug was ineffective in treating the disease. Currently, a phase II double-blinded, randomized multicentre clinical trial is ongoing in Canada, which will evaluate the clinical efficacy and safety of adalimumab versus placebo in patients diagnosed with osteoarthritis of the knee, to investigate the efficacy of the antibody against pain (NCT02471118). Interestingly, changes in bone resorption with anti-TNF therapies in patients with OA have not been investigated despite the evidence in animal models as mentioned above, and additionally in patients with rheumatoid arthritis who showed reduced bone resorption with a-TNF treatment [86]. Other TNF antibodies have been investigated as potential therapeutics for OA, such as infliximab which has mainly been studied as a therapy for erosive hand OA [87].

12.4.2 Osteoclast Targeted Therapies

Subchondral bone resorption occurs in the early development of OA, studies have shown that changes to the subchondral bone often precedes damage to the articular cartilage. Importantly, in early osteoarthritis, bone remodelling is 20-fold higher compared to normal bone with increased markers of osteoclast activity. Studies have shown evidence of activated osteoclastic remodelling occurs in women with progressive OA through the detection of markers of bone resorption, CTx and NTx. These markers were measured at three-time points over 2 years, in a cohort of postmenopausal women, aged from 45 to 62, and compared to patients with non-progressive OA [37]. Therefore, therapies that target the early bone resorption in OA may prevent the progression of the disease.

12.4.2.1 Bisphosphonates

Bisphosphonates were developed in the 1950s as a therapy to reduce the bone loss observed in

osteoporosis. The active compound in bisphosphonates are the phosphonate groups which have a high affinity to and bind to hydroxyapatite crystals, in an action similar to inorganic pyrophosphate (PPi) produced by the body [88]. Bisphosphonates are preferentially incorporated into the bone mineral matrix at active sites of bone remodelling. Release of bisphosphonates by osteoclastic resorption inadvertently disrupts osteoclast adherence, preventing the release of protons that stimulate bone resorption [89].

Bisphosphonates have been trialled as a therapeutic to combat the development of OA in animal models, including the naturally occurring OA guinea pig model mentioned previously. The guinea pigs were treated with the bisphosphonate, Alendronate, which resulted in an accrual of both subchondral cancellous and cortical bone. However, changes to the articular cartilage were also observed, with increased articular cartilage degeneration in the Alendronate treated group [90]. Alternatively, the use of Alendronate in a rat anterior cruciate ligament transection (ACLT) model showed protective chondrogenic effects, as well as reduced subchondral bone remodelling [91]. The major difference in these models is the naturally occurring OA in the guinea pig model versus post-traumatic OA incurred from ACLT injury. As post-traumatic OA is more likely to cause early OA in young patients, this suggests the results from the rat ACLT injury model may be more indicative of the human disease.

Bisphosphonates have been tested clinically in patients with OA, a meta-analysis investigating the efficacy of bisphosphonates in knee OA described the ineffectiveness of this therapy on reducing pain, functional improvement or prevention of radiographic progression of the disease [92]. The meta-analysis included seven randomized controlled trials, where patients had confirmed knee OA and were treated with either a bisphosphonate or placebo drug. The bisphosphonates included are risedronate, alendronate, zoledronic acid, clodronate and neridronate, at varying doses and treatment times.

The meta-analysis included results from the USA/Canada KOSTAR study, (Knee OA Structural ARthritis study), which was a 2-year multicentre double-blinded randomized study, including 1232 patients from 42 study areas (mean age = 60.5) [93]. The European KOSTAR study was also analysed, 1251 patients were recruited from 44 sites in Europe (average age = 63.6) [94]. The KOSTAR studies investigated the efficacy of risedronate with 3 varying concentrations, taken either daily (5, 15 mg) or weekly (35 or 50 mg). The primary outcome for both trials included WOMAC pain, WOMAC function and the percentage of patients experiencing radiographic progression (defined as 0.6 mm of joint space narrowing over 24 months). Data reported from both studies showed no change in radiographic progression with risedronate treatment, despite evidence of reduced cartilage degradation (reduced collagen degradation marker) [94].

Another clinical trial analysed in the meta-analysis includes the BRISK study (the British study of risedronate in structure and symptoms of knee OA), a 1-year prospective double-blinded study, which included 284 patients treated with risedronate (5 or 15 mg) or placebo for 12 months [95]. Patients were 40–80 years old (average age = 63.2), and patients had confirmed mild to moderate medial-compartment knee OA. The primary outcomes of the study were to detect differences in symptoms and function with risedronate treatment, including pain scores (WOMAC pain), WOMAC function and the mean change in joint space width. This study also investigated any adverse events that occurred during the 12-month study period. The results of this clinical trial showed the higher dose of risedronate reduced markers of cartilage degradation and bone resorption, and improved WOMAC function. The other study outcomes of pain and joint space width trended positively but did not reach significance. Therefore, the outcomes of this smaller, shorter time course likely emulate the KOSTAR study, with little long-term benefits of this bisphosphonate on ameliorating the effects of OA.

An additional study included in this meta-analysis included a randomized, double-blinded, placebo-controlled trial in patients with mild to moderate knee OA, treated with Alendronate for

6 months. Patients were a younger cohort (average age = 47), with grades I or II of knee osteoarthritis on the Kellgren–Lawrence scale. Patients received 70 mg of Alendronate orally, or placebo pill, once a week for 24 weeks (n = 19/20 patients per group). The primary aims of the study were to detect changes in pain, stiffness, and function with Alendronate treatment, unfortunately no significant changes were detected, despite an improvement in the total WOMAC score at 4 weeks [96].

A study conducted using Zoledronate was also included, the aim of this study was to determine the effect of a single dose of zoledronate on pain and bone marrow lesions (BML) (ACTRN 12609000399291). Patients were determined to have clinical knee OA as defined by the American College of Rheumatology criteria and had significant knee pain and a minimum of one BML. Patients were aged between 50 and 80 years and were given either zoledronate (5 mg in 100 mg of fluid) or placebo intravenous infusion (average age = 64.2 and 60.4 years consecutively). The primary outcomes were pain intensity and the maximal area of BML assessed by MRI at 6 months. Secondary outcomes of this study were further pain intensity analysis at both 3 and 12 months, outcomes of the knee injury and osteoarthritis outcome score (KOOS) questionnaire at 3, 6 and 12 months, and BML size at 12 months as well as safety outcomes. This study showed that a single infusion of zoledronate effectively reduced pain intensity and BML size after 6 months post-treatment; however, no further improvements were observed at the 12 month time point [97]. This suggests that zoledronate, despite having good efficacy in the short term with a single dose may only have limited effects on improving OA in patients. Further dosages may be more beneficial in reducing BMLs in patients with OA.

A study included in the meta-analysis investigated the use of Clodronate on patients with knee OA in a phase III trial (EUDRACT 2009-012956-26). Patients were between the ages of 50 and 75 (average age = 66, n = 80), with a radiographic Kellgren–Lawrence score > 2. Patients were given either 2 mg of clodronate or placebo by intra-articular injection every week for 4 weeks and outcomes were assessed after 12 weeks. The primary outcomes of the trial were pain assessment by visual analogue scale (VAS) measured at week 8. Secondary outcomes were changes in WOMAC score for pain, stiffness and function, changes in Lequesne index which measures pain, maximum distance walked and daily activities, acetaminophen consumption and patient assessed outcomes. This study showed clodronate treatment improved pain outcomes as measured by VAS, improved Lequesne index and WOMAC pain subscale. Patients also showed a reduction in acetominaphen consumption. This study shows clodronate may reduce symptoms of symptomatic OA over 12 weeks. Radiographic measurements were not included in this trial, and therefore changes of the joint were not measured [98].

The final study included in the meta-analysis was a trial for the clinical use of neridronate. Neridronate is an amino-bisphosphonate that has been shown as an effective treatment for other bone diseases such as osteogenesis imperfecta. Unlike other bisphosphonates, neridronate can be administered either intravenously or intramuscularly. The study by Varenna et al. investigated the effects of neridronate on a cohort of patients 50 years or older who had acute trauma to the knee, a radiographic Kellgren–Lawrence (KL) grading score greater than 2, continuous worsening of knee pain and a BML larger than 1 cm [99]. Patients (n = 68) were either given intravenous injections of neridronate (100 mg/8 mL) or placebo for 2 months, treatment was administered every third day starting from day 1 (first infusion) and ending on day 10 (fourth infusion). The average age of patients was 64 for the neridronate treated group and 67 for the placebo group. The primary outcomes of the study was the change in pain intensity as measured by the visual analogue scale, the secondary outcomes were the WOMAC pain questionnaire, the McGill pain questionnaire, the 36-Item Short Form Health Survey and change in BMLS evaluated by the whole-organ MRI score. The outcomes of the study were positive, with neridronate treatment reducing pain intensity scores and reduction in

the size of BMLs in patients with acute painful OA [99]. The results from this study suggest neridronate may be effective in reducing symptoms of OA, the change in BML lesion size suggests that this therapy may also be useful for patients with early OA by inhibiting subchondral bone resorption.

The results from the clinical trials discussed suggest that bisphosphonates have the potential to reduce pain and improve OA scores on the Kellgren–Lawrence scale, whilst reducing BMLs. However, not every trial measured the changes in joint space, or BML area, impacting the evidence that bisphosphonates improve the structural joint changes caused by OA. The meta-analysis concludes that bisphosphonates may be beneficial to certain subsets of patients with OA, who display high rates of subchondral bone turnover. Therefore, due to the changes that occur in progression of OA, with rapid subchondral bone resorption occurring in the initial stages of the disease, treatment with bisphosphonates is perhaps most effective in the early stage of OA, preventing the destruction of bone and potentially ameliorating cartilage degradation.

12.4.2.2 Cathepsin K Antibody

Cathepsin K is a protease actively made by osteoclasts during the resorptive phase in order to degrade the collagen fibrils in the bone mineral matrix. Cathepsin K is also made by chondrocytes where it cleaves components of the cartilage matrix, type II collagen and aggrecan [100]. A selection of Cathepsin K antibodies have been investigated as potential therapeutics for the treatment of OA.

A specific Cathepsin K antibody, L-006235, has been tested in rodent models of OA. In a rabbit ACLT model, L-006235 was given for 7 weeks post-surgery and significantly reduced collagen degradation products, which are markers for cartilage degradation and bone resorption [101]. In a collagen-induced arthritis model in mice, similar reduction of these markers was observed with prophylactic treatment [102]. Therefore, Cathepsin K showed promise as a potential target for OA therapeutics.

One such Cathepsin K antibody is Odanacatib developed by Merck & Co. Odanacatib was shown to selectively inhibit cathepsin K and inhibited bone resorption in both preclinical models of bone loss and phase I trials [101, 103, 104]. However, this drug has now been discontinued due to the severe adverse advents that occurred with administration during clinical trials, including stroke.

Another Cathepsin K inhibitor, named MIV-711, has been associated with reduced expression of bone resorptive biomarkers and cartilage loss. This inhibitor was used to treat OA in two different animal models, rabbits subjected to ACLT injury and dogs subjected to partial medial meniscectomy. In both animal models, treatment with MIV-711 reduced bone resorption biomarkers, subchondral bone loss and cartilage degradation, as measured by micro-CT and macroscopic scoring for cartilage degeneration [105]. Therefore, this led to the investigation of MIV-711 in clinical trials.

Results from a Phase IIa clinical trial using MIV-711 in patients with ACR Knee OA has recently been reported. In this trial, 164 patients received either 100 or 200 mg of MIV-711 daily for 28 days (n = 82/MIV-711 treatment, n = 80 placebo control). Preliminary results showed reduced knee OA structural progression, but no significant change to pain scores was measured compared to placebo over 6 months. These results suggest cathepsin K inhibitors are a favourable treatment for preventing the progression of bone loss and cartilage degeneration in OA but may not combat joint pain. The results for the completion of this trial will be important in influencing the future of cathepsin k inhibitors as potential OA therapeutics.

12.4.2.3 Denosumab

Subchondral bone resorption is a key feature of early OA. Regulating bone remodelling may be an important factor in reducing the bone resorption that occurs in the early stages of osteoarthritis, through targeting the activity and differentiation of osteoclasts. One such method is by inhibiting the key osteoclastogenic factor, RANKL. A RANKL antibody has been devel-

oped in order to target osteoclastic activity, called Denosumab. Denosumab is an IgG2a monoclonal antibody that specifically binds RANKL, preventing the activation of its receptor RANK, inhibiting osteoclastic resorption. Denosumab is approved as a treatment for osteoporosis and reduces bone turnover and fractures in postmenopausal women and patients with rheumatoid arthritis [106–108].

These results motivated at trial of Denosumab as a treatment in early osteoarthritis. A clinical study using Denosumab to treat patients with knee OA (aged 50 and older) is currently ongoing in the UK (ISRCTN96920058, Salford Royal Hospital and University of Manchester started 2016). Patients will receive a subcutaneous injection of 60 mg of Denosumab once for 6 months, with calcium and vitamin D supplementation. Additionally, a clinical trial using Denosumab to treat erosive hand OA is also ongoing at Ghent University Hospital (NCT02771860, Ghent University Hospital, started 2016). Patients (aged 30 or older) will be given a 60 mg subcutaneous injection of Denosumab every 12 weeks, with Calcium/vitamin D supplementation, for 48 weeks.

12.4.2.4 Strontium Ranelate.

Strontium ranelate has been investigated as an OA treatment as it is a regulator of bone homeostasis. Strontium ranelate induces bone formation and importantly, inhibits of bone resorption [81]. Various studies have investigated the effects of strontium ranelate in vitro on osteoblasts, osteoclast precursor cells and osteoclasts, providing evidence that strontium has strong inhibitory effects on bone resorption. This includes inhibiting monocyte differentiation into osteoclasts, decreasing osteoclast activity, increasing osteoclast apoptosis and inhibiting the production of pro-osteoclastogenic markers by osteoblasts [82]. A rat model of surgically induced OA (medial meniscal tear model) treated with strontium ranelate showed similar results in vivo, with the drug attenuating the articular cartilage damage caused by the MMT injury and preventing subchondral bone resorption [83]. However, another study in a DMM model of OA in guinea pigs showed strontium ranelate may cause an increase in the prevalence of bone marrow lesions (BML) [109]. The 3-month-old animals were given strontium ranelate every day for 12 weeks (625 mg/kg), post-surgery analysis of the joints showed increased BMD and bone volume, but with an increased number of BMLs.

Improvement in bone mineral density and bone volume has led to clinical trials that assessed the effect of strontium ranelate on knee OA. One such trial is the 3-year international double-blinded, randomized, placebo-controlled trial, named SEKOIA (ISRCTN41323372). This trial investigated the effects of two doses of strontium ranelate (1, 2 g per day, or placebo) on 1683 patients with grade 2 or 3 knee OA as determined by the Kellgren Lawrence grading, the average age of patients between groups was 62.4, 63.5, and 62.8 consecutively. The primary endpoint of the study was changes in joint space width determined by radiographical analysis. The study also assessed WOMAC pain and changes in CTX-II, and described a reduction in all three measures with strontium ranelate treatment regardless of dose [110]. The extended report of this trial showed a reduction in BML score in patients who showed a presence of lesions at the start of the trial [111]. This suggests a discrepancy between animal models of OA and humans, however, strontium ranelate treatment in patients with early OA may have an increased risk of developing BMLs, in comparison to patients with advanced OA that includes established lesions.

12.4.3 Additional Therapies with Actions on Myeloid Cells

12.4.3.1 Targeting NGF

Nerve growth factor (NGF) is a neurotrophin that regulates pain through the NGF-tropomyosin-related kinase (NGF-TrkA) pathway. NGF has been shown to be expressed by both monocytes and macrophages [112] and has

been shown to be involved in tissue healing in fracture healing models. A mouse fracture healing model with NGF transgenic mice showed increased levels of NGF within the callus and increased osteoclast formation [113]. Levels of NGF were found to be elevated in the blood and synovial fluid of patients with OA [114], leading to the development of anti-NGF therapies. NGF inhibitors have shown promise in combating both pain, cartilage degeneration and the subchondral bone remodelling that occurs in OA. Various antibodies that bind NGF have been tested in patients with OA, resulting in a range of effects, including a serious side effect of rapid progressive OA [115]. A NGF inhibitor, Pentosan (Pentosan Polysulphate Sodium (PPS)) tested in vitro showed inhibition of osteoclast transcription factors NFATc1 and cFos, reductions in TRAP+ve osteoclast numbers and inhibition osteoclast activity markers, Cathepsin K and MMP-9 [116]. Similarly, in a rat model of inflammatory arthritis, treatment with PPS (20 μg/g) suppressed osteoclast marker genes Cathepsin K and TRAP in the synovial membrane, and reduced expression of pro-inflammatory cytokines, IL-1b, and TNFα [117].

This has led to a single-centre, open-label clinical trial, testing the efficacy and activity of PPS in patients with OA, either grades 2 or 3 using the Kellgren–Lawrence Grading System. Pentosan was given to 20 patients, who received 6 weekly subcutaneous injections (2 mg/kg). The primary outcome of this trial was to assess the efficacy, safety and patient satisfaction of Pentosan treatment in patients with mild radiographic knee OA. The results of this small study were positive, with reduced pain scores possibly associated with reduced inflammation and decreased markers of cartilage degradation [118]. Similar results were observed in a randomized, double-blind, placebo-controlled pilot study with 114 patients treated with PPS (3 mg/kg) by injection into the gluteal muscle region of each patient each week for 4 weeks (PPS treated = 54, placebo control = 60; average age = 62.5 and 64.0 consecutively) [119]. Neither of these studies analysed the other markers of OA, including bone remodelling or inflammation.

Further studies have been performed using Tanezumab, a monoclonal antibody that binds and prevents NGF from activating TrkA receptors on nociceptive neurons. Various trials have been conducted with Tanezumab and have been discussed in two separate meta-analysis reviews regarding this use of Tanezumab for patients with knee OA, published in 2016 and 2017 [120, 121]. Clinical trials have shown that tanezumab improves both pain and function scores, but serious adverse events have also been recorded including rapidly progressive osteoarthritis and osteonecrosis. Both meta-analysis reviews suggest that Tanezumab requires further investigation on the long-term safety and efficacy of the drug. For example, a trial was conducted in Japan assessing the preliminary efficacy, safety and pharmacokinetic (PK) profile of tanezumab in patients with moderate to severe pain associated with knee OA (NCT00669409) [122]. Patients were recruited between 35 and 75 years of age ($n = 83$) with a clinical diagnosis of knee OA and radiographic confirmation (Kellgren–Lawrence score of 2 or higher). Patients received a single intravenous injection of Tanezumab (10, 25, 50, 100, 200 mg/kg), or placebo and were followed for 92 or 120 days. Patient age ranged from 57 to 60 years of age per treatment group. The primary outcomes of the trial were knee pain intensity based on the Western Ontario and McMaster Universities OA scale (WOMAC) and personal daily reports recorded by the patients. The secondary outcomes were the PK profile of Tanezumab and adverse events. The results from the study showed improved pain intensity and WOMAC scores with 25, 100 and 200 mg/kg of Tanezumab, however, doses 10 and 50 mg/kg showed no significant change compared to placebo. This variation may be due to the small number of participants per group ($n = 6 \pm 10$ per group).

A clinical trial is currently ongoing in Australia using PPS to treat patients with knee OA and the presence of bone marrow lesions (ACTRN:12617001311347). The trial is at Phase IIB and shows promise for reducing pain and the formation of bone marrow lesions. The double-blinded trial includes subcutaneous injection of

PPS (100 mg/mL) twice weekly for 6 weeks. Patients recruited were between 40 and 75 years of age and presented with radiographic OA, with Kellgren–Lawrence grade 2 or higher. The primary outcome of this study is the change in the Knee Injury and Osteoarthritis Injury Score (KOOS) pain score from baseline to day 53. Secondary outcomes are KOOS function, KOOS symptom, KOOS quality of life score, changes in Bone marrow lesion volume as assessed by MRI, adverse events, blood cell counts and coagulation parameters (blood clotting time APTT and INR). Results from this study will hopefully provide positive advancement for PPS as a therapy for patients with OA.

12.4.3.2 Targeting MMPs

Matrix metalloproteinases (MMPs) play important roles in degrading collagen, in both cartilage and bone. Production of MMPs is induced by proinflammatory cytokines TNFα and IL-1b, which are highly expressed in OA tissue, furthermore, MMP13 has been detected at high levels in patients with OA [123]. Inhibitors for MMPs have been investigated as potential therapeutics in order to prevent cartilage degradation and osteoclastic bone resorption. Knockdown of MMP13 in particular, in a murine model of surgically induced OA protected cartilage destruction, but did not show any effect on osteophyte development [124]. Additionally, global deletion of MMP13 in mice also caused other abnormalities, including bony union in growth plates and metaphyseal flaring. Over expression of MMP13 in a murine model induced both articular cartilage degeneration and focal lesions associated with OA [125].

MMP inhibitors have been trialled in patients with OA with disappointing results, a pan MMP inhibitor termed PG-116800 was tested in a randomized, double-blind, placebo-controlled, multicentre, parallel-group clinical trial. PG-116800 effectively inhibited MMPs including MMP13. Patients with mild to moderate knee OA were given an oral formation of PG-116800 (25 mg (n = 81), 50 mg (n = 80), 100 mg (n = 80), or 200 mg (n = 80)) or placebo twice daily for 1 year. Patients were 40–80 years of age (average age = 62). The primary outcomes of this trial were the progression of joint space narrowing and changes in pain and function scores (WOMAC). Unfortunately, treatment with PG-116800 at any dose did not improve any of the primary outcomes, and resulted in significant adverse effects in patients including arthralgia, decreased range of motion in the shoulder of 23% of patients, adverse hand events including oedema, palmar fibrosis, Dupuytren contracture, or persistent tendon thickness or nodules, increased shoulder stiffness and myalgia. Musculoskeletal toxicity, also ‘musculoskeletal syndrome’, has been reported to occur with a majority of MMP inhibitors, with similar adverse effects as described in this study. Therefore, this has led to a decline in the investigation of MMP inhibitors for treatment of musculoskeletal diseases.

However, the development of specific MMP inhibitors may prevent the myriad of adverse events associated with global MMP therapies. The positive results from animal models with selective MMP13 inhibitors show promise for improving the damage and pain that occurs with OA. One study investigated the use of a selective MMP13 inhibitor (ALS 1-0635), in a rat medial meniscal tear model of OA. This study showed positive results, with improved pain scores assessed by weightbearing by the animals, dose-dependent reductions in both cartilage and bone degradation scores with treatment and a reduction in osteophyte score [126], and no clinical signs or histologic changes characteristic of musculoskeletal syndrome. Therefore, specific MMP13 inhibitors may be a potential therapy for the treatment of OA in reducing cartilage damage and reducing pain, however, no specific inhibitors have been trialled thus far.

12.5 Conclusion

Synovitis is an increasingly recognized contribution to OA pathogenesis, and the role of monocytes, macrophages in initiating and sustaining joint inflammation in OA is an area of active investigation. Increased understanding of the role

of these innate immune cells in OA pathogenesis has the potential to lead to mechanism-targeted therapies to prevent OA progression. Similarly, a better understanding how these cells and their cousins, osteoclasts, contribute to joint damage through the production of inflammatory cytokines and degradative enzymes could identify additional targets for therapeutic intervention. While therapies targeting myeloid-derived cytokines, MMPs and osteoclasts have already been tested with mixed results, an improved understanding of the pathogenic role of myeloid cells in OA is needed to inform target selection and timing of therapy.

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13 Mesenchymal Stromal Cells and Extracellular Vesicles

Michelle L. Delco and Nikita Srivastava

13.1 Introduction

13.1.1 Early Osteoarthritis Pathogenesis

Primary OA is considered to be associated with aging and heredity, whereas secondary or post-traumatic osteoarthritis (PTOA) is a consequence of articular injury [1, 2]. Regardless of classification, the etiopathogenesis of osteoarthritis (OA) is complex and incompletely understood. However, both biological and mechanical processes are known to be involved, and our understanding of the relative importance, contribution, and interplay of these factors has evolved over time [3, 4].

The highly ordered structure of the cartilage extracellular matrix (ECM) is critical to the mechanical and functional properties of the tissue. The ECM is composed of type II collagen and proteoglycans, the most important being aggrecan. Chondrocytes, the sole cell type in articular cartilage, are primarily responsible for maintaining the integrity of the ECM through the synthesis of aggrecan and other ECM components. OA was initially considered to be non-inflammatory, and primarily the consequence of mechanical damage to articular cartilage [3]. However, advances in the field of molecular biology in the 1990s led to the recognition of ECM-degrading enzymes, including aggrecanases such as metalloproteinases (MMP)-3 and ADAMTS-5 and collagenases including MMP-13 in cartilage degeneration. Catabolic responses in chondrocytes were found to be mediated by soluble factors including cytokines and prostaglandins. Activation of alarmins and other innate immune signaling was implicated in a feed-forward catabolic cycle involving pathology in, and interplay between all joint tissues [5]. For example, activation of key transcription factors such as NFkB [6] results in the production of inflammatory mediators including TNFa, IL-1β, and IL-6 by cartilage, bone, and synovium [5, 7]. This leads to excessive reactive oxygen species generation and oxidative tissue damage, promoting further inflammation and cell senescence [8, 9]. Multiple studies have documented elevated cytokine levels [10] and complement components [11] in OA tissues and synovial fluid, as well as overwhelming evidence of synovitis in OA patients [12] and have to the recognition of synovial inflammation as a crucial feature of early OA pathology [3, 4]. Thus, the understand-

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ing of OA pathogenesis has evolved from a degenerative, non-inflammatory condition, to a disease wherein both the innate and adaptive immune responses play a central role, and OA progression is characterized by chronic, low-grade inflammation [13].

Mechanotransduction pathways have been increasingly recognized at the crux of mechanical and biological inputs into OA pathogenesis. Both physiologic and hyperphysiologic cartilage loading activates chondrocyte mechanoreceptors, including mechano-sensitive ion channels and integrins, leading to intracellular signaling cascades [14]. For example, TRPV4 and PIEZO1/2 channels on the chondrocyte plasma membrane generate electrical currents in response to mechanical forces [15]. TRPV4 is a Ca^{++} permeable, non-specific cation channel that is activated by physiologic (i.e., non-injurious) loading. TRPV4 initiates a mechano-osmotic transduction cascade to initiate anabolic responses in chondrocytes [16–18]. Conversely, injurious mechanical loading is transduced by PIEZO1 and PIEZO2 ion channels [17] which initiates catabolic signaling and downstream expression of cytokines, chemokines, and prostaglandins [19] including 1L-1β and TNFα [4, 20] promote cartilage degeneration and perpetuate local and inflammation via their action on chondrocytes, synoviocytes, and macrophages [19]. These findings call attention to early OA as a disease at the intersection of mechanics and biology, underscoring the importance of proactive intervention to preserve the mechanical integrity of cartilage.

13.1.2 Regenerative Medicine Approach to Early Osteoarthritis

No therapeutics are available to prevent OA [21, 22], and current treatment strategies are largely palliative, focused on managing pain, stiffness, and inflammation. Common approaches include anti-inflammatory drugs (NSAIDs), intra-articular (IA) corticosteroid injections, low-impact exercise, and physical therapy to improve quality of life and joint function. However, no pharmacologic or biologic treatments have been shown to protect articular cartilage or prevent OA progression [23–25]. Increasing evidence suggests that the earliest events in OA pathogenesis must be targeted in order to make progress toward disease-modifying therapies. Furthermore, clinical interventions including regenerative therapies must be implemented early in the course of disease, arguably prior to the development of overt clinical symptoms. This approach requires the development of more sensitive diagnostics including reliable imaging, biochemical, and/or molecular markers of early disease to determine which patients are at risk of progressing to symptomatic OA and require proactive intervention.

A fundamental challenge is that articular cartilage has limited intrinsic healing capacity. Chondrocytes are difficult to target for pharmaceutical intervention because cartilage is avascular, and the dense and highly charged ECM can be difficult or impossible for therapeutics to penetrate [3, 26–28]. Due to the lack of effective treatment strategies, MSCs and MSC-derived extracellular vesicle (EV)-based therapies have generated excitement in the field of regenerative orthobiologics.

Although MSC-based therapies have been used to treat joint injury and early OA for years, and multiple studies have provided evidence that MSCs can improve functional outcomes and preserve articular cartilage, the overall results of clinical trials have been variable [29] (Pas, et al., BMJ 2017). Moreover, the quality of evidence provided by individual studies is inconsistent [30]. A recent meta-analysis concluded that few clinical studies provide satisfactory levels of evidence, and the quality of data cannot be assessed according to *The Journal of Bone and Joint Surgery's* (JBJS) levels of evidence rating scale, which helps inform clinical decision-making [31]. Therefore, an undeniable need exists for more high-quality pre-clinical and clinical studies to guide the development of new cell-based regenerative therapies. Nonetheless, MSC and EVs are increasingly emerging as strategies with high therapeutic potential. The following is a

review of current knowledge regarding MSCs and MSC-derived EVs, focusing on the biological characteristics, likely mechanisms of action, and the therapeutic rationale for these emerging regenerative therapies in the context of early OA.

13.2 Mesenchymal Stromal Cells

13.2.1 Definition and Classification of MSCs

Mesenchymal stromal cells (MSCs) are characterized by their ability to self-renew and differentiate into multiple cell lineages including chondroblasts, osteoblasts, and adipocytes [32, 33]. Lineage-committed progenitors are generated via symmetric and asymmetric division and can then differentiate into tissue-specific cells [34]. Debate exists over MSC nomenclature regarding usage of the term "stem" versus "stromal." The term mesenchymal stem cell was introduced by Caplan et al. in the 1990s after generating cartilage and bone from ex vivo culture of mesenchymal tissue [35]. The subsequent use of differing terminology in pre-clinical and clinical studies and associated assumptions have led to conflated descriptions of bone marrow-derived "stem" cells with "stromal" cells from different tissue sources [35, 36]. In order to standardize nomenclature, the International Society for Cell and Gene Therapy (ISCT) recommended defining "mesenchymal stem cells" as bone-marrow-derived, self-renewing cells with in vivo multipotency. This is distinct from "mesenchymal stromal cells," which are progenitors derived from various tissues and demonstrate multipotency via in vitro differentiation assays [37]. The term "Medicinal Signaling Cells" has also been recently proposed to highlight the trophic and immunomodulatory effects of MSCs, without reference to their differentiation potential [36].

A major challenge in MSC biology has been the identification of markers that can be reliably used to differentiate a purified population of MSCs with unique functional properties. There is no evidence of a single cell surface marker for the identification of MSCs [26, 27]; however, the ISCT has identified the following four minimum criteria to define human MSCs: (1) adherence to plastic (i.e., cell culture plates) and fibroblast-like morphology, (2) expression of "positive" cell surface markers including CD105, CD90, CD73, CD44 (≥95% expression), (3) non-expression of hematopoietic "negative" markers such as CD34, CD45, CD14 or CD11b, CD79a or CD19, and HLA-DR (≤2% expression), (4) trilineage potential, or the ability to differentiate into osteoblasts, adipocytes, and chondrocytes in vitro [32, 38–40].

Variation exists in the expression of cell surface markers related to MSCs identification criteria, and markers are classified into two broad categories: sole and stemness markers. Sole markers, including Stro-1, SSEA-4, CD271, and CD146 are directly linked to the potency, growth capacity, and quality of MSCs [41, 42]. Generally, these markers are highly expressed surface proteins, indicative of plastic adherence, and are hence used to differentiate MSC-like cells from other cells in the in vivo environment. On the other hand, stemness markers are moderately expressed and facilitate the identification of MSCs with abundant fibroblastic colony-forming units (CFU-Fs) and multipotency. Expression of these stemness markers by isolated MSCs varies depending on their tissue of origin. Notably, Stro-1 and CD271 are not universally expressed in MSCs from all tissues; CD271 is highly expressed in bone marrow- and adipose-derived MSCs, but low or no expression is present in MSCs from synovial membrane, umbilical cord, and peripheral blood [41]. This underscores that MSCs derived from different tissues are not equivalent, and extrapolating findings and comparing results between studies using MSCs from different sources can be problematic [43].

Although the ISCT guidelines were meant to promote uniformity, this system of defining MSCs has increasingly been called into question, as the criteria may not adequately reflect the phenotypic, biochemical, and functional diversity exhibited by MSCs of various lineages. One concern is the ISCT criteria are based on characteristics of MSCs expanded in vitro, and which may not reflect MSC populations in vivo. Further, the

majority of markers were identified in bone marrow-derived cells, so the extent to which these markers are applicable to MSCs from other tissues is not clear [41]. These markers are also known to be sensitive to culture and processing techniques, a prominent example of which is the sensitivity of surface antigens to reagents used during cell passaging; the positive markers CD44, CD105, and CD73 are lost from the plasma membrane within 30 min of lifting MSCs with trypsin (Tsuji et al., cell transplant 2017). The lack of standardized protocols across basic, preclinical, and clinical MSC research is one obstacle to clinical advancement. Finally, many in vitro and in vivo studies have utilized MSCs which meet the tri-lineage differentiation and plastic-adherence criteria, but not cell surface marker criteria [33]. This inconsistency again limits the ability to interpret data and compare results across studies.

13.2.2 MSC Tissue Source

MSCs can be isolated from many tissues including bone marrow, adipose tissue, synovium, peripheral blood, skeletal muscles, and dental pulp [26, 27, 31, 41] MSCs were first identified in bone marrow by Friedenstein et al. in 1966, and evidence of chondrogenic, osteogenic, and muscular differentiation potential was demonstrated in 1970 [44]. The first successful clinical application of MSCs was reported in 2001 using autologous bone-marrow MSCs to repair bone defects [45]. Tissue of origin can influence MSC characteristics and function, therefore, tissue source is an important consideration when interpreting published literature, developing new MSC-based regenerative therapies, or selecting cell populations for clinical use. Tissue-specific differences include protein expression, cytokine profile, yield, and differentiation potential [46].

Bone-marrow-derived MSCs (BM-MSCs) have been the most widely utilized for preclinical and clinical studies, followed by adipose-tissue-derived MSCs (A-MSCs) [41, 43]. Although MSCs from both these tissues have been used to treat OA in animals and humans [47–49], there is conflicting evidence regarding the differences between BM- and A-MSCs with respect to cell yield, growth kinetics, and differentiation capacity [50, 51]. BM-MSCs are often selected for therapeutic applications due to ease of acquisition, rapid proliferation in vitro, low surface expression of MHC antigens, relatively high yields, and some evidence of longer-term persistence in the recipient site [31]. Some studies suggest BM-MSCs have higher chondrogenic potential than A-MSCs [52], and BM-MSCs can be readily expanded in culture and induced to varying levels of differentiation prior to treatment [53]. One limitation of BM-MSCs is the finding that increased donor age and extended in vitro culture can decrease proliferation and differentiation potential, and increase the proportion of senescent cells [54, 55]. Another drawback of BM-MSCs for clinical use is that the bone marrow aspiration harvest procedure of can be painful [43]. Therefore, alternative sources of MSCs have been pursued.

Adipose tissue-derived MSCs (A-MSCs) can differentiate into cartilage, bone, tendon, skeletal muscle, and fat and have several advantages for the purposes of therapeutic development [56, 57]. A-MSCs are abundant and can be harvested in a minimally invasive manner from lipoaspirates [51, 53]. In addition, the stromal vascular fraction of adipose may contain as much as a 500-fold higher yield of MSCs than the bone-marrow [58]. Some evidence suggests that the regenerative potential of A-MSCs may not be adversely affected by age [59, 60]. However, several comparative studies suggest A-MSCs have lower chondrogenic potential, lower cartilage-specific ECM protein production including lower expression of collagen type II compared to BM-MSCs [61].

The synovial membrane represents another promising source of MSCs in the context of early OA, as synovial-derived MSCs (S-MSCs) can be harvested via minimally invasive arthroscopic surgery with few complications [62]. Some evidence suggests S-MSCs have superior chondro-

genic potential and higher cell yields compared to other tissue sources [62]. S-MSCs also possess lower osteogenic potential compared to BM- and periosteal-MSCs which, in theory could minimize the risk of dystrophic calcification [63]. However, there is still limited clinical evidence for the efficacy of S-MSCs, with data being limited to preclinical studies [53].

Peripheral blood has also been identified a potential source of MSCs but as yet, limited evidence supports this approach [64]. Peripheral blood-derived MSCs (PB-MSCs) hold the promise of the least invasive harvest technique [65]; however, isolation of PB-SCs from blood via apheresis is somewhat complicated, and patient stimulation is required to increase MSC yield [53, 64]. A further limitation is that PB-MSCs may display phenotypic MSC markers only in hypoxic conditions [66], making identification and study more difficult [67]. Recently, many niches with the skeletal system have been found to harbor distinct populations of progenitor cells [68], and these sources will undoubtedly continue to be pursued for their therapeutic potential.

13.2.3 Autologous Versus Allogeneic MSCs

Self-derived, or autologous MSCs have been most commonly been reported in the clinical and preclinical literature, and have proven safe in multiple clinical studies [69]. MSCs demonstrate low immunogenicity, and the risk is even lower with autologous MSCs, since they are derived from the patients' own tissue. However, there are disadvantages associated with using autologous sources, including the cost of individual harvest, isolation, expansion, and banking of each patient's cells [70]. Further, recent evidence suggests that factors including genetics, age, and medical history of the MSC donor can significantly effect of the quality of MSCs [71]. For example, increasing patient age was associated with decreased yields of BM-MSC [72], and MSCs obtained from obese individuals had impaired differentiation and proliferation potential.

Although the status of MSCs as "non-immunogenic" has recently been challenged, the preponderance of evidence indicates low or no expression of MHC class II and low expression of MHC I surface antigens by MSCs, even after differentiation into chondrocytes, adipocytes, and osteocytes [73]. Furthermore, few studies have reported adverse immune responses in vitro and in vivo [74]. Allogeneic MSCs can be derived from various tissues, but are mostly placenta-derived in the United States [72]. Advantages of allogeneic MSCs include decreased lag time (i.e., delay in administration) and cost, improved quality assurance, and potential for commercialization. Therefore, allo-MSCs can be considered a less invasive and logistically convenient alternative to autologous MSCs, and have been pursued as potential "off the shelf" products, which would allow a larger patient population access to regenerative therapy [70, 75]. However, there are concerns regarding the safety, viability, and function of allo-MSC. Risk of blood-borne pathogen transmission highlights the need for careful donor screening [72]. Despite low incidence of acute rejection, repeated allo-MSC injections can result in a memory immune response. Formation of donor-specific antibodies leads to accelerated MSC clearance and decreased efficacy [72, 76, 77], even in the absence of an overt adverse immune response in the recipient. Recent in vivo evidence indicates that MHC crossmatching can prevent the recipient antibody-mediated clearance of donor MSCs, suggesting this step is likely necessary for future therapeutic development of allogeneic MSC.

In summary, there remains a paucity of clinical studies clearly establishing the efficacy of MSCs in early OA, let alone data comparing MSC sources. Therefore, no definitive recommendations can be made regarding clinical use of one sub-type of MSC over another. Large-scale, high quality trials are needed. However, there have been few reports of safety concerns when using either autologous and allogeneic MSCs [2, 78].

13.2.4 Heterogeneity Within MSCs Populations

Increasing evidence suggests that, in addition to variability *between* MSCs populations, phenotypic and functional heterogeneity *within* MSC populations can impact the efficacy of MSC therapies, as well as patient outcomes ([33, 79]; McLeod, et al. ECM 2017). MSC cultures are comprised of distinct sub-populations with varying characteristics, including gene expression and lineage potential [33, 80]. Even single cell-derived colonies of human BM-MSCs contain at least three morphologically different sub-populations with varying differentiation potency: small, rapidly self-renewing cells, elongated spindle-shaped fibroblast cells, and slow-replicating large, cuboidal, or flattened cells [81, 82]. In addition, the percentage of human bone-marrow CFU-Fs with osteogenic potential was shown to be higher than those adipogenic potential, in an in vitro culture [83]. Therefore, increased research efforts have focused on methods to identify and select MSC sub-populations with specific functional and therapeutic advantages, and this trend will likely continue.

13.3 MSC-Based Strategies for the Treatment of Early OA

Many in vivo pre-clinical studies in both small and large animal models [84], and an ever-increasing number of clinical trials have explored MSC-based therapies for the treatment of early OA, with the goal of restoring joint homeostasis, preserving articular cartilage, and slowing or preventing progression to a late-stage disease characterized by chronic pain and joint dysfunction [69, 85, 86]. There are two main MSC-based strategies to treat early OA; Intra-articular injection and repair of focal chondral lesions.

13.3.1 Intra-articular Injection of MSCs

Intra-articular (IA) injection is a simple, minimally invasive, and efficient delivery option for MSCs [78]. The first preclinical study of MSC treatment for OA employed IA delivery of autologous BM-MSCs 6 weeks after meniscectomy and ACL transection in a caprine model. Treatment resulted in meniscal repair and chondro-protection at 6 months, with no evidence of the injected MSCs in healing tissue [87] This work led, in part, to the hypothesis that MSCs promote repair through paracrine and trophic mechanisms rather than engraftment and differentiation [88].

IA MSC therapy has been investigated in a limited number of clinical trials [89]. In one study, IA injection of AD-MSCs in knee OA patients led to improved function, decreased pain scores, and reduction in cartilage defects with evidence of hyaline-like articular cartilage regeneration [90]. Overall, however, the evidence supporting the regeneration of articular cartilage has been variable [70, 89]. Combining IA injection of MSCs with a bioactive carrier such as platelet-rich plasma, fibrin gel, or hyaluronic acid scaffolds may reduce chondrocyte apoptosis and improve chondrogenic differentiation [70, 84]. For example, the combination of platelet rich plasma (PRP) with BM-MSCs and AD-MSCs for OA treatment promoted cartilage matrix synthesis and improved the therapeutic benefit of MSCs [91, 92]. Studies have demonstrated a dose-response effect with IA MSC injection, however, the appropriate number of cells for clinical use, as well as the optimal timing and frequency of treatment is unknown [90]. Although, IA injection of MSCs is likely most beneficial when applied as early as possible in the development of OA, to preserve cartilage and restore joint homeostasis [93, 94]. Although clinical evidence remains limited and long-tern studies are needed, results of preclinical studies suggest that IA MSC treatment has a disease-modifying effect when applied early after joint injury. IA adipose-derived, α10 integrin-selected MSCs, administered 4 days after articular injury prevented early PTOA progression, including cartilage degeneration and subchondral bone changes in a large animal talus impact model [95].

13.3.2 MSC-Based Repair of Focal Cartilage Lesions

In the procedure of autologous chondrocyte implantation (ACI), chondrocytes are extracted from the non-weight-bearing region of a patient's joint, expanded in culture, and then transplanted into a focal cartilage lesion [96]. Although this procedure is often considered the gold standard cell therapy for OA [97], and some studies report good long-term function, overall results have been modest. Notably, structural outcomes are variable, with the presence of disorganized, fibrocartilaginous repair tissue and poor integration of the grafted defect with surrounding cartilage. Disadvantages of ACI include variable chondrocyte health and morbidity at the cartilage harvest site, poor chondrocyte proliferation, and low chondrogenic capacity after in vitro expansion [68]. In an attempt to overcome these challenges, the procedure was modified, replacing chondrocytes with autologous BM-MSCs, due to their improved availability, minimal donor site morbidity compared to surgical chondrocyte harvest, and rapid MSC proliferation in culture [28]. Preclinical and clinical studies support the use of BM-MSCs in ACI-like and other similar procedures, although with variable structural outcomes, ranging from hyaline-like cartilage to fibrous repair tissue [23]. Many promising and innovative therapies are currently in development, which combine MSCs and other biologic and tissue engineering approaches, such as bioactive carriers, matrices, and 3D printed scaffolds are being developed, and are currently in preclinical testing although limited clinical evidence exists at this time [68].

13.4 Proposed Therapeutic Mechanism of MSCs

Several clinical studies have provided convincing evidence that MSCs can improve OA symptoms and prevent cartilage degeneration; however, the exact mechanism(s) of action are still incompletely understood. In vitro and ex vivo research suggests several broad therapeutic mechanisms of MSCS, including direct engraftment and differentiation, immunomodulation via paracrine activity of the MSC secretome, and newer paradigms such as the delivery of MSC-derived extracellular vesicles, and intercellular mitochondrial transfer.

13.4.1 Direct Engraftment and Differentiation

It was initially presumed that the administration of MSCs would promote damaged tissue regeneration and repair directly: MSCs would home to sites of tissue damage, engraft, and differentiate into site-specific functional tissue. However, results from several early animal studies contradicted this hypothesis. Despite evidence of tissue repair/regeneration after treatment, there was little evidence of long-term engraftment of implanted cells [87, 98–101]. Many subsequent homing and engraftment studies have similarly shown minimal long-term engraftment [43], and one study in mice revealed less than 1% of MSCs persist for longer than a week after systemic administration [76, 100]. Nonetheless, limited evidence of MSCs incorporation into repair tissue does exist. In one large animal model of OA, intra-articular injection of autologous MSCs resulted in apparent cartilage healing and MSC engraftment at sites of cartilage damage [102]. Therefore, although MSCs may contribute to repair tissue under certain circumstances, it is now generally accepted that the ability of exogenous MSCs to engraft and differentiate is not required, nor is it the predominant mechanism by which MSCs provide therapeutic benefit. The preponderance of evidence implicates the MSC "secretome" whereby trophic factors and soluble mediators secreted by MSCs act in a paracrine fashion to modulate the immunologic environment and promote endogenous tissue repair.

13.4.2 The MSC Secretome

The MSC secretome is defined as the set of MSC-derived bioactive factors, including soluble proteins, nucleic acids, lipids, and extracellular vesicles (Fig. 13.1), which have shown therapeu-

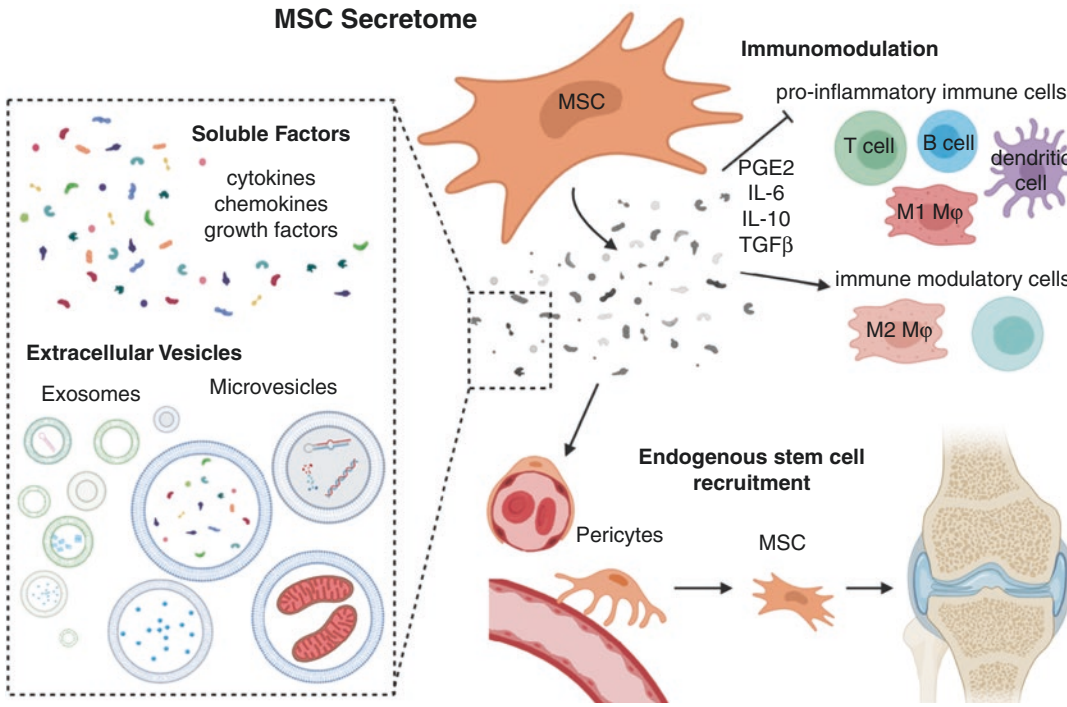

Fig. 13.1 Illustration of the MSC Secretome

tic benefits similar to those observed after MSC transplantation [26, 27]. The specific paracrine mechanism by which components of the MSC secretome promote repair and regeneration remain an area of active investigation [103], but the major effects include immunomodulation and recruitment of endogenous stem cells.

13.4.3 Immunomodulation

Many studies have documented the immunomodulatory (or anti-inflammatory) effects that MSCs can exert either via direct cell–cell contact or by the paracrine activity of the MSC secretome [43, 104, 105]. However, the underlying cellular and molecular mechanisms are not completely clear. One study revealed that MSCs increased expression of COL2A1 and ACAN in chondrocytes, and decreased the expression of MMP-13 and NF-κB [106]. MSCs can also suppress the activity of immune cells via the secretion of anti-inflammatory prostaglandin E2 (PGE2) and other bioactive factors like NO and IDO (Fig. 13.1) [84, 107].

Evidence suggest MSCs modulate the activity of macrophages by inhibiting the activity of inflammatory M1-type macrophages and promoting their conversion to anti-inflammatory M2-like phenotype. Intraarticular MSCs may suppress joint inflammation by blocking activation of inflammatory CD4+ Th1 cells, and promote production of immunosuppressive CD4+ T regulatory cells (Tregs) [26, 27, 104]. Based on mixed lymphocyte reaction assays, several studies indicate MSC inhibit T cell proliferation and shift the ratio of T helper cells from a pro-inflammatory Th1 to an anti-inflammatory Th2 subtype. MSCs production of transforming growth factor-beta (TGFβ) and hepatocyte growth factor (HGF) may also drive differentiation of naïve T cells into regulatory T cells that promote immune tolerance, with increased production of the anti-inflammatory cytokine IL-10 and reduced TNFα and IL12 [104] (Fig. 13.1).

In addition to the paracrine mechanism, direct cell–cell contact by MSCs can also regulate immune cell function. Proliferation of activated T cells was inhibited via the interaction between the molecule programmed death 1 (PD-1) on MSCs with ligands PD-L1 and PD-L2 on effector T cells [108]. Furthermore, MSCs can render T cells anergic [74] during their interaction due to their lack of surface expression of CD80 and CD86, important co-stimulatory molecules in T cell activation. MSCs can also inhibit the proliferation of B cells by modifying the activation of extracellular signal-regulated kinases (ERK)1/2 and the p38 mitogen-activated protein kinase (MAPK) pathways [109]. Besides regulating T cell, B cell, and macrophage activity, MSCs can also exert an inhibitory effect on other immune cells like dendritic cells (DCs) and natural killer (NK) cells. The migration and maturation of DCs is suppressed by MSCs [110], and mature DCs co-cultured with MSCs had decreased TNF-α and increased IL-10 secretion [111]. Similarly, after co-culture with MSCs, NK cells display inhibited cytotoxicity and cytokine production [105]. Surprisingly, evidence suggests that even apoptotic, metabolically inactivated, or fragmented MSCs can exert immunomodulatory effects [112–114]. This suggests non-viable cells or cellular components could replace live MSC therapies, avoiding certain safety concerns.

13.4.4 MSC Priming/Licensing

Cellular pre-conditioning by cytokine stimulation or hypoxic priming is known to change to the composition of the MSC secretome [26, 27, 115]. Evidence suggests that in vivo, MSCs are found dormant as pericytes that are activated in response to tissue damaged in a phenomenon referred to as "licensing." Licensed MSCs are then capable of modulating the local immune response [116]. Some evidence suggest inflammatory priming can improve the survival of MSC after implantation into inhospitable in vivo environments [117]. In OA-related studies, double TNFa/IFNg priming of MSCs results in a superior anti-inflammatory and immunomodulatory secretory profile [118, 119]. Another strategy, hypoxic priming decreases cellular stress and early senescence of MSCs during culture expansion. However, optimal priming protocols for various MSCs populations and therapeutic applications are unknown [120]. Despite promising experimental evidence, the clinical potential of MSCs priming has yet to be explored [118].

13.4.5 Mitochondrial Transfer

Recent work has revealed that mitochondrial (MT) dysfunction is one of the very earliest responses of chondrocytes to injury and may mediate downstream catabolic signaling cascades that drive cartilage degeneration and OA [121–123]. Further, direct mitoprotective therapy prevents MT dysfunction, chondrocyte death, and cartilage matrix degeneration after articular cartilage injury [121, 123]. These findings provide support for pursuing new strategies to target MT function for the prevention and treatment of early OA.

Surprisingly, recent evidence suggests that injured and dysfunctional cells recruit help from MSCs in the form of whole-organelle donation [124]. Mitochondrial transfer by MSCs has been identified as a mechanism of damaged cell repair in cells with impaired mitochondrial function [125–127]. Although the specific mechanisms are still largely unknown, cells undergoing MT dysfunction can accept healthy mitochondria from MSCs.

Several potential mechanisms of intercellular mitochondrial transfer from MSCs have been identified, including tunneling nanotubes, gap junction-medicated micro-vesicle transfer, and cell–cell fusion [127, 128]. Moreover, mitochondrial transfer from MSCs has been found to restore recipient cell function, preserve viability, and improve healing in several tissues including myocardium, cortical neurons, renal tubular epithelium, and lung epithelial cells [127–131]. Mitochondrial transfer has not previously been reported in cartilage. Very recent work (in submission) provides the first evidence that bone-marrow-derived MSCs donate MT to

chondrocytes undergoing MT dysfunction in several in vitro and ex vivo models. The concepts of mitochondrial dysfunction and mitochondrial transfer represent novel paradigms in the field of regenerative medicine which have not been extensively explored but represent one of the many possible future directions in regenerative medicine to combat early OA.

13.5 Extracellular Vesicles

Initial research into the MSC secretome focused on cytokines, chemokines, and growth factors as key therapeutic factors of the secretome. However, increasing evidence implicates extracellular vesicles, including micro-vesicles and exosomes as the active therapeutic component of the MSC secretome [28].

13.5.1 Biology of Extracellular Vesicles

Extracellular vesicles (EVs) are a class of cell-secreted, membrane-bound, nanoscale particles released by most or all cell types, that deliver molecular cargo to facilitate intercellular communication and signaling. EVs represent a heterogenous population of particles, made up of distinct sizes, cargo, membrane composition, biogenesis, and functions [132]. EV membrane composition and cargoes are highly dependent on their cell of origin [133, 134]. Three main sub-groups of EVs have been classified: exosomes, microvesicles (sometimes referred to as microparticles or ectosomes), and apoptotic bodies (Fig. 13.2). These EV sub-groups are defined by their size, biogenesis, and expression of membrane markers [135]. However, there remains a lack of universally accepted criteria to distinguish each sub-group [136, 137].

Exosomes are the smallest EVs in diameter, ranging from about 30 to 150 nm. They are generated by the fusion of endosomal multivesicular bodies with the plasma membrane through the process of exocytosis. Microvesicles are larger, generally having a diameter of 100–1000 nm and are released from cells through the process of outward budding from the cell membrane [138]. Apoptotic bodies range from 500 to 5000 nm in diameter [139]. Apoptotic bodies are released as blebs from the plasma membrane during apoptosis [97]. Thus far, exosomes have attracted the most attention for possible therapeutic development and have been more widely studied than microvesicles and apoptotic bodies [140, 141].

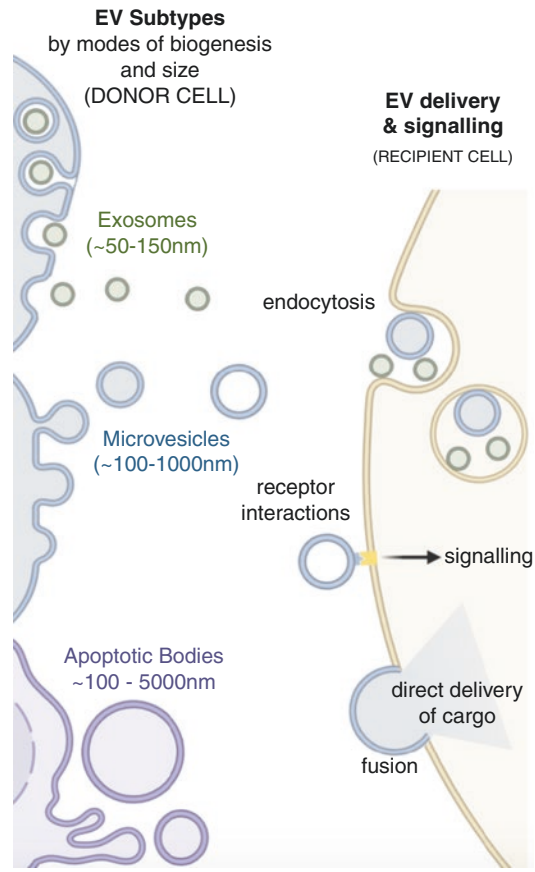

Fig. 13.2 Illustration of the different EV Subtypes

EVs can interact with and elicit responses in target cells in several ways (Fig. 13.2). EV membrane proteins, such as MHC I and II [142, 143], transferrin receptors [144], and integrins can participate in receptor-ligand interactions and activate downstream signaling pathways, for example, via calcium signaling [145] or mitogen-activated protein kinase (MAPK) activation

[144], in the target cell. These interactions may or may not result in the internalization of the EV and delivery of its cargo to the target cell via the endocytic pathway. Alternatively, EVs can fuse with the recipient cell membrane to deliver cargos directly [141].

EVs cargos often include biologically active signaling molecules which can influence a wide array of cellular functions [97]. EVs have been referred to as the "snail mail" of intercellular communication due to their ability to transfer lipids, nucleic acids (DNA, mRNAs, and miRNAs), and proteins from the donor to the recipient cells. Past research efforts have largely explored the function of EVs in intercellular communication in the context of pathological processes such as cancer and autoimmune diseases, the EV-mediated regulation of cell, and tissue homeostasis has been less well studied [146].

13.5.2 EVs and Immunomodulation

Depending on their membrane composition and cargoes, EVs can act on the innate immune system as either pro- or anti-inflammatory mediators [146]. For example, microparticles released by neutrophils were shown to increase the release of TGFβ1, and down-regulate the activation of macrophages [147]. EVs also modulate the acquired immune response by acting as a source of antigens for antigen presenting cells (APCs). APCs, including dendritic cells, macrophages, and B cells, serve as a link between the innate and adaptive immune responses. Antigens are captured and processed by APCs, followed by presentation to T cells via MHC molecules and co-stimulatory signaling. EVs can modulate the immune responses by providing stimulatory or down-regulatory signals upon capture by APCs [146]. For example, exosomes were internalized and processed by immature dendritic cells for presentation to CD4 (+) T cells [148]. Moreover, EVs possessing MHC or co-stimulatory molecules on their lipid membrane can directly engage in antigen presentation or co-stimulation [149–151].

13.5.3 EVs in OA Pathogenesis

In the context of OA, the mechanical degradation of articular cartilage is associated with changes in EVs populations with synovial fluid [152]. One hallmark of OA is an imbalance between the synthesis and degradation of the extracellular matrix (ECM), such that the loss of matrix structural integrity cannot be compensated by synthesis [153]. Microvesicles have been identified as communication channels between chondrocytes and fibroblast-like synoviocytes (FLS), regulating the disease process of OA. This was evidenced by a threefold increase in FLS MMP-13 production when treated with EVs derived from IL-1β stimulated chondrocytes [152]. Exosomes derived from IL-1β stimulated FLS have also produced OA-like changes in in vitro and ex vivo models by upregulating MMP-13 and ADAMTS-5, and downregulating COL2A1 and ACAN expression in articular chondrocytes [154]. Furthermore, miRNA profiles of EVs derived from synovial fluid of OA patients revealed differential expression of miRNAs in a gender-specific manner. In particular, certain miRNAs such as the estrogen-responsive miR-26a were downregulated in female patients. Moreover, a greater number of miRNAs were differentially regulated for female OA group versus the male OA group. This may help to explain the higher incidence of OA in females than in males [138, 155]. EVs isolated from synovial fluid of patients with end-stage knee osteoarthrosis are capable of inciting a pro-inflammatory response via upregulation of IL-1β, IL-6 and IL-8, and MMP-3 [156].

Flow cytometric assessment of EVs has revealed that synovial fluid has a distinct EV signature [157]. Hence, the differences in the content of synovial fluid and plasma EVs could potentially be used as biomarkers for disease development and progression. For example, EV cargo profiling revealed a 2.5-fold increase in miR-200C in EVs isolated from OA patients. This miRNA has been associated with chondroprotective mechanism and can IL6-mediated inflammation and increase synthesis of type II collagen [152].

13.6 MSC-Derived Extracellular Vesicles

Stem-cell derived EVs are potent intercellular messengers that can participate in maintaining tissue homeostasis [146, 158] EVs derived from multiple cell types have been explored as regenerative therapies [159–161]; however, MSCs are the most commonly reported cell source. There is evidence that MSC-derived EVs (MSC-EVs) may possess the same anti-inflammatory and trophic properties as their cells of origin [135, 158]. Similar to their parent MSCs, human MSC-EVs have been shown to exert anti-inflammatory and anti-catabolic effects when incorporated into OA chondrocytes, by downregulating the production of inflammatory cytokines including IL-1α, IL-1β, IL-6, IL-8, IL-17, and COX2, as well as promoting chondrocyte proliferation to regenerate cartilage in vitro [162]. MSC-EVs promote angiogenesis, inhibit apoptosis, and promote cell proliferation [115, 158, 163–166]. In another study, treatment of IL-1β stimulated chondrocytes with human A-MSC derived EVs resulted in decreased production of TNFα, IL-6, MMPs, NO, and PGE2 secondary to inhibition of the transcription factors NFκB and activator protein-1. In addition, treatment increased the expression of IL-10 and type-II collagen [164, 167]. One study revealed MSC-derived exosomes were associated with bone and cartilage regeneration in vitro and in vivo by interacting with the bone microenvironment [139]. Various miRNA cargos, such as miR23b and miR-92a, carried by MSC-derived exosomes have been shown to promote cartilage regeneration [168, 169] in several OA models, including collagenase-induced and destabilization of medial meniscus models [170]. Taken together, these findings suggest MSC-EVs are not only chondroprotective but may in fact promote cartilage regeneration.

Therapeutic development of EVs in place of cell therapy is attractive because non-cellular therapies avoid some safety concerns associated with viable stem cells, such as possible tumorigenesis, risk of emboli, and immune targeting resulting in low cell survival [171, 172]. Potential advantages of EVs include their small size, low immunogenicity allowing allogeneic therapy, and potential for manipulation to improve delivery and function [158, 173]. EVs would also avoid the time and cost of MSC collection and expansion [159]. In the context of OA, signals from MSC-derived EVs should be more stable than MSCs, without the concern of adopting a pro-inflammatory phenotype or undergo senescence when placed into the pathological joint environment [158].

13.6.1 Bioengineered Vesicles

Extracellular vesicles, particularly exosomes, have been investigated as a delivery system for drugs, microRNAs, and proteins [171], and engineered EVs are emerging as a promising cell-free therapeutic strategy, due to their biocompatibility and physicochemical stability [172, 174–176]. EVs are considered the ideal candidate for bioengineering due to their ability to directly interact with target cells and avoiding risks of toxicity, rapid clearance, and side effects that are associated with other delivery formulations. Engineered biomaterials such as a hydrogels to encapsulate EVs or binding EVs to a scaffold could be used for targeted therapy and sustained delivery of EVs to the injured joint, in order to reduce the dose and frequency of administration [158, 171]. Future directions include exosomes being engineered via nanoparticle-based technology to carry specific cargo (proteins or surface markers) to regulate intercellular communication in a targeted manner. Technology and engineering approaches are necessary to scale technology for clinical feasibility [177, 178].

13.7 Challenges to the Development of MSC-EV Therapies

Despite the clear potential of EV-based therapy, there are many challenges on the path to translation of MSC-EVs for the treatment of early OA. The same caveats mentioned for MSC therapies apply to MSC-EVs, except that the EV field

is even younger than cellular regenerative therapies. The following issues represent the first set of hurdles in this burgeoning field.

13.7.1 EV Characterization

Certain proteins are considered pan-EV markers and are common to most EVs [146]. These include cytoskeletal, cytosolic, heat shock, plasma membrane proteins, and proteins involved in vesicle trafficking. Proteins often used as markers include tetraspanins including CD9, CD63, CD81 and CD82, 14-3-3 proteins, MHC molecules, cytosolic proteins, such as specific stress proteins (heat shock proteins; HSPs), Tsg101, the endosomal sorting complex required for transport (ESCRT-3), and the binding protein Alix [179, 180]. While different proteomic profiles are associated with EV subgroups, no single marker can uniquely identify EVs [146]. Furthermore, there is a lack of widely accepted markers to distinguish between the different EV subgroups. Finally, biodistribution of EVs after intra-articular delivery is unknown and may be subject to rapid clearance [97].

13.7.2 EV Isolation and Storage

Although guidelines have been established by the International Society for Extracellular Vesicles (ISEV), no consensus for standard methods of EV isolation currently exist [132]. There are considerable difficulties associated with EV isolation and purification methods such as low yield, contamination, and incomplete isolation of EV fractions, resulting in mixed populations of vesicles [158, 181]. The most common isolation technique for exosomes is based on differential centrifugation of MSC-conditioned media to remove cells and debris, followed by ultracentrifugation on a sucrose density gradient to remove contamination in the EV pellet, such as protein aggregates [171]. Other methods of EV isolation include size exclusion, polymeric precipitation, and microfluidic devices [180]. Sub-fractionations of EV subgroups can also be achieved by affinity chromatography techniques, employing antibodies against specific known or suspected EV surface markers [182, 183], or using ligands such as heparin, which are reactive with EV surfaces [184]. Other techniques for isolating EV subtypes include differential ultracentrifugation, density gradient centrifugation (sucrose or iodixanol gradients), filtration [146], isoelectric focusing (charge separation) [185, 186], or separation according to size by field-flow fractionation techniques [187]. However, different methods produce EVs and EV fractions with variable homogeneity, and also affect the proteomic profiles, hence making it difficult to compare findings from different proteomic studies. The maintenance of various biological activities of EVs during preservation and storage is largely unknown, and optimal techniques will need to be developed for EVs as biologic therapies as well as engineered EVs used as drug carriers (Jeyaram and Jay).

As with cell therapies, inconsistency between EVs study protocols results in difficulty comparing data the few pre-clinical and clinical studies that are currently available. Therefore, considerable challenges must be overcome before EVs can be considered for use in therapeutic applications. The role of EVs in normal tissue maintenance and repair, and how they may be involved in age-related degeneration of tissues leading to disease progression is still largely unexplored, and more research in this area will lead to the development of innovative therapeutic strategies in the future [138].

13.8 Summary and Conclusion

MSC therapy for early OA has proven safe, but limited evidence supports efficacy in reducing pain, improving patient function, preserving cartilage structure, and delaying end-stage disease. Conclusions that can be drawn from early positive clinical reports are limited due to methodological limitations and lack of standardization [188], which highlights the need for large, high-quality, long-term, clinical trials.

The number of studies exploring MSC-derived EVs for the treatment of OA is still small [158]. Furthermore, significant work is required to standardize MSC and MSC-derived product manufacturing procedures, determine potency measures, optimize frequency and methods of delivery, identify specific populations of cells/vesicles for different disease stages and subtypes, enable meaningful comparison of functional outcomes from clinical studies, and validate efficacy, among others [97].

The path to clinical translation is fraught with many unanswered questions and challenges. However, given the dearth of effective treatment options, the field of regenerative medicine and specifically emerging MSCs and MSC-EVs therapies represent promising avenues to combat the unmet clinical needs of OA.

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Part III

Treatment of Early OA

14 Role of Injection Therapy in Early Osteoarthritis: Cortisone, Viscosupplement, PRP?

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14.1 Introduction

Injection therapy plays a major role in the treatment strategies for osteoarthritis (OA). A wide spectrum of injective solutions is available for the local minimally invasive delivery of various substances such as corticosteroids, hyaluronic acid (HA), and platelet-rich plasma (PRP). Overall, these approaches showed the potential to positively affect joint homeostasis, reducing symptoms and improving joint function and quality of life, allowing to postpone the need for more sacrificing procedures [1]. This is particularly true for earlier degrees of degeneration, where the articular environment might better respond to the injection of a biologically active substance. In this light, there has been an increasing attention to identify patients affected by OA at the early stages. In particular, the definition of early knee OA sparked great interest among researchers in the last years, although no commonly accepted definition is available yet. In 2012, an European Society of Sports Traumatology, Knee Surgery and Arthroscopy (ESSKA) consensus [2] defined classification criteria of symptomatic early knee OA implying the combination of symptoms, signs, and structural changes. Accordingly, a patient can be classified as having early OA of the knee if three criteria are fulfilled: knee pain with at least two episodes for 10 days in the last year; standard radiographs Kellgren and Lawrence grade 0 or 1 or 2 (osteophytes only); arthroscopic findings of cartilage lesions and/or magnetic resonance imaging (MRI) findings demonstrating articular cartilage degeneration and/or meniscal degeneration, and/or subchondral bone marrow lesions. Most recently, a further ESSKA consensus definition proposal has been published, further detailing that early OA might be divided into a more diffuse, more ill-defined OA affecting the whole joint, or a focal pattern, concentrating around a focal cartilage lesion and the involved compartment [2]. The idea to distinguish these two entities is to favor the adoption of the most suitable treatment in this crucial early OA period when it could be still possible to effectively influence the disease progression.

In fact, the definition of an early phase is paramount to address the disease progression more

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effectively, possibly with disease-modifying effects before the joint reaches the point of no return of more severe OA. A too advanced tissue damage might negatively influence the potential benefit since knee joint tissues and their biological response could be already compromised by anatomical structural changes not addressable with simple injective procedures. In this light, it would be important to test each specific treatment in the subpopulation of early OA patients, to quantify the potential benefit in terms of clinical outcome and disease progression. Unfortunately, while the scientific discussion is still running on the best way to identify and classify early OA, the literature on cartilage and OA treatments rarely adopt this focus when documenting the benefit of either conservative or surgical approaches [3, 4]**.** Thus, the evidence on their potential in early OA cases can be derived only indirectly from the available literature on populations affected by different OA stages.

This is particularly important when dealing with injective treatments, where the proper timing could maximize their positive effects. In the initial stage of the disease, with no clear lesions or associated abnormalities requiring to be addressed surgically, local injective treatments might have a higher potential to positively influence joint microenvironment and lead to a clinical improvement. In this light, specific studies are needed to properly investigate the benefits of intra-articular treatments of corticosteroids, HA, and PRP for early knee OA. Keeping in mind these limits of the current literature, the following paragraphs will summarize the evidence to explain the rationale, as well as the potential and limitations of the main injectable treatments, which can be used to address early knee OA.

14.2 Corticosteroids

Intra-articular corticosteroid injections represent one of the most common conservative therapies for OA. They were first introduced in the 1950s to take advantage of the anti-inflammatory properties of corticosteroids and guarantee a great local concentration and action, simultaneously avoiding the possible complications of their systemic administration [5]. Indeed, the role of inflammation in OA development and progression is well demonstrated, and the use of corticosteroids has been recommended for the treatment of knee OA, especially in the presence of an inflammatory flare-up [6, 7]. Interestingly, early OA shows an enhanced inflammatory microenvironment: compared to late OA, increased concentrations of CD4+ and CD68+ proinflammatory cells secreting inflammatory molecules such as TNFα and IL1β, and an incremented new vessel formation [8]. Accordingly, corticosteroids may be useful to tackle the inflammatory process and, consequently, not only to reduce symptoms, but also to counteract the negative effects of inflammation for the evolution of the disease.

Unfortunately, a literature specifically focused on early knee OA is still lacking, while several studies documented the effectiveness of corticosteroids in patients with all degrees of OA, underlying a partial and short-term symptom improvement [9, 10]. In particular, the responders' rate at short-term follow-up is more than 70%, being the presence of joint tenderness a predictor of response [11]. While different products have been proposed, none of the available corticosteroids used for intra-articular injections (i.e., triamcinolone, betamethasone, and methylprednisolone) seems to be more effective than the others [12, 13]. It should be stressed that these positive results are not long-lasting: at the 6-month follow-up, the advantages over placebo are not confirmed and the responders' rate falls to 20% [9, 11]. Thus, to guarantee a longer lasting benefit to OA patients, corticosteroid injections can be repeated, although not earlier than 3 months from the previous injection. In fact, corticosteroids may cause dangerous changes in the joint, facilitating tissue atrophy, joint destruction, and cartilage degeneration [14]. The repeated injections have been correlated with cartilage loss, which could be particularly detrimental in early OA, without offering a significant long-term symptom improvement [15]. The deleterious consequences of repeated injections could be even enhanced in the subjects suffering from early OA due to their younger age and the consequent greater number of years they have to

cope with the disease. Moreover, corticosteroids have a documented direct immunosuppressive effect, and an increased risk of joint infections has been reported after repeated corticosteroid injections, especially in high-risk subjects, such as immunosuppressed subjects or patients requiring prosthesis in the near future [16].

Accordingly, the real benefit of intra-articular corticosteroids is now considered limited, especially in light of the harmful consequences of long-term corticosteroid administration and the short-term benefits [17]. Even though intra-articular corticosteroids are still used by a large number of physicians, due to the low cost and availability, other options are now considered as intra-articular treatment for OA, aiming at achieving longer lasting results with a more favorable risk profile [7, 18].

14.3 Viscosupplementation

HA is a glycosaminoglycan, which plays an important role within the knee joint, where it provides joint lubrication and shock absorbency and acts as the backbone for the proteoglycans of the extracellular matrix. In normal adult knees, HA concentration ranges from 2.5 to 4.0 mg/mL, but, during the course of OA, the synovial fluid undergoes degradation similarly to other tissues of the joint, with a decrease of average molecular weight (MW) and HA concentration by 33–50% [19]. Interestingly, these changes have been correlated with joint pain and functional impairment [20]. In this light, intra-articular delivery of HA into the joint (or viscosupplementation) has been proposed as a therapy for knee OA already in 1960 and applied in the first human trial for OA treatment in 1974, with the aim to restore the natural protective functions of HA by increasing synovial fluid elasticity and viscosity [21].

Currently, there are more than 80 different HA preparations on the market [22]. They present several different features, such as origin (natural or bacterial fermentation), sterilization process (heat or ultrafiltration), concentration (0.8–30 mg/mL), volume of injection (0.5–6.0 mL), posology, molecular structure (linear, cross-linked, and a mix of both), and MW [22]. Some preparations also include additives, such as mannitol, sorbitol, or chondroitin sulfate [22]. All these factors could theoretically have an additional impact on the effect of the viscosupplementation treatment, but the paucity of literature on many of these aspects cannot allow clear conclusions about the superiority of one characteristic over another. Published reports were mainly centered on origin, number of injections, and MW. Regarding the HA origin, products derived from biological fermentation demonstrated to provide a safer profile compared to avian-derived products, which reported injection site flare-ups due to avian-derived proteins [23], while a better efficacy between different products has not been proved [24]. The number of injections may vary from a single injection to a series of 5 weekly injections [25], but, once again, the superiority of one strategy over another remains controversial. A recent systematic review compared single- with multiple-injection formulations of HA for the treatment of knee OA, but no consistent difference was found on patient-reported outcomes [26]. This finding is in contrast with a previous meta-analysis that showed a better pain relief with multiple injections and supports a recent clinical trend toward the adoption of less invasive solutions requiring a lower number of injections [25].

MW is the main studied aspect so far. According to MW, three different categories can be identified: low (500–800 kDa), intermediate (800–2000 kDa), and high (2000–6000 kDa) MW, this one including cross-linked formulations of HA [22]. Preclinical studies suggest different properties according to MW. More precisely, higher MW HAs could provide superior chondroprotective, proteoglycan/glycosaminoglycan synthesis, anti-inflammatory, mechanical, and analgesic effects [20]. On the other hand, lower MW HA has been suggested to better penetrate the extracellular matrix of the synovium and cell membranes, thus maximizing its concentration and facilitating its influence on the synovial cells [27]. This interaction could represent the mechanism through which intra-articular HA may lead to its disease modifying

and long-lasting effects. The average residence time of intra-articular HA in the joint is only 2–3 days, but prolonged effects lasting several weeks post-injection have been observed, suggesting other mechanisms of action to be at work [28]. Intra-articular HA has been found to have positive effects on the endogenous synthesis of HA and extracellular matrix components by stimulating synovial fibroblasts; it is also responsible for a chondroprotective effect by mitigating proteoglycan loss in cartilage and apoptosis of chondrocytes; nonetheless, it is also involved in the reduction of HA degradation by decreasing the production of proinflammatory cytokines. All these mechanisms could explain the observed clinical effects exceeding the mere intra-articular HA duration [20]. However, these findings have been shown only in preclinical studies. In this light, the current literature cannot provide clear conclusions on the real joint effects in vivo, as well as on the different action and efficacy of low and high MW HA [29, 30]. In fact, even though some comparative studies and systematic reviews suggested greater therapeutic effect of high MW HA in the treatment of knee OA in comparison with other formulations, a real consensus on this issue has been not reached yet [23].

Despite these controversies, the recent literature focusing on intra-articular HA injections for knee OA supported beneficial effects on pain, function, and patient global assessment. An updated literature analysis recently focused on 17 meta-analyses, finding an overall positive effect for the use of intra-articular HA versus placebo [28]: 13 of these were in favor of intra-articular HA treatment [28]. Interestingly, current literature has also demonstrated that intra-articular HA positive effects were shown in the earlier stages of knee OA [31]. Accordingly, another recent systematic review and meta-analysis demonstrated a statistically significant pain relief only for patients with early-moderate knee OA of intra-articular HA when compared with saline, while no effects were shown in the late OA subgroup [32]. Evidence supporting HA efficacy was proved once again when the latter was compared with the administration of intra-articular corticosteroids. The literature showed a longer lasting benefit than intra-articular corticosteroids exerting residual detectable effects up to 6 months [33]. More precisely, corticosteroids showed better clinical results at 1-month follow-up, where no differences could be pointed out at 3 months. On the contrary, HA was proved to be more effective than corticosteroids at 6 months [34]. Corticosteroids might still have a role, as different injectable might have different indications. In fact, differently from corticosteroids, synovitis can impair the efficacy of HA. In fact, enzymes and oxidants (hyaluronidases and free radicals) can degrade HA chains, and effusion fluid can dilute HA concentration. In this light, viscosupplementation cannot be performed with severe effusion, and acute episodes should be treated before with either nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids [33], while HA should be postponed to a less inflammatory phase [33].

Overall, intra-articular HA remains a common intervention for knee OA, with a good safety profile [35], and is often adopted in the clinical practice as safe alternative to oral NSAIDs and opioids for knee OA. In addition, intra-articular HA can be as safe as paracetamol itself, which is widely prescribed as a first-line therapy for OA even though its effect on pain is small and with no effect on physical function and stiffness in knee OA patients [36, 37]. Still, even though intra-articular HA seems to offer a good benefit/risk balance among the various pharmacologic treatments to ameliorate knee OA patients' symptoms [38–41], recommendations for this therapeutic strategy vary among national and international guidelines [42–47].

Whereas the Osteoarthritis Research Society International (OARSI) guidelines recommended intra-articular HA as potentially useful in patients with knee OA, the American Academy of Orthopaedic Surgeons (AAOS) clinical practice guidelines determined that the evidence was inconclusive, and a recommendation could not be made for or against the use of intra-articular HA. Similarly, the American College of Rheumatology (ACR) recommendations do not advocate the use of intra-articular HA for the initial management of knee OA. Still, despite the

lukewarm recommendations or even the recommendations against, many rheumatologists, orthopedic surgeons, and other clinicians worldwide continue to offer this treatment to facilitate the control of symptoms and delay the need for surgical interventions [48, 49].

This contributed to boost research on this subject, with the latest evidence suggesting interesting and promising results.

However, a better assessment of the utility of intra-articular HA and specifically designed high-level studies are needed to improve the understanding of the most suitable indications for this treatment option. As such, a proper selection of patients seems to be of paramount importance as shown in a recent cost analysis, where the use of intra-articular HA demonstrated cost-effectiveness superiority when compared to other treatments (oral NSAIDs, braces and orthosis, and physical therapy), especially in the earlier stages of knee OA, while the cost-effectiveness in patients with later stage was not shown [50]. In this light, patients presenting less articular damage and a potentially higher biological response could represent the proper target for intra-articular HA, as in the early knee OA stages.

14.4 Platelet-Rich Plasma

Platelet-rich plasma (PRP) has been introduced in the musculoskeletal field less than 20 years ago. The opportunity to obtain a concentrate of growth factors directly from the patient blood made PRP a possible treatment solution for all the diseases in which the regeneration process might play a role. Among these, OA is probably the musculoskeletal disease, where more clinical trials on PRP have been performed. OA is characterized by a degenerative microenvironment affecting the cartilage and the influence of growth factors on cartilage regeneration is well established [51]. Platelets' alpha granules constitute a reservoir of critical growth factors, as well as cytokines, chemokines, and many other proteins, which showed to take part in the homeostasis of articular cartilage, being involved in both healing process and immunoregulation. These biologically active proteins seem to be able to influence and promote a favorable joint environment, favoring the restoration of a homeostatic balance in degenerative joints [52]**.** In vitro studies suggested that PRP can theoretically influence mesenchymal stromal cells, chondrocytes, synovial cells, meniscal cells, and newly attracted cells to act synergistically toward an anti-inflammatory and tissue healing profile [53, 54]. This positive influence may result in an improvement of cartilage quality and decrease of synovial tissue inflammation, as suggested by studies using qualitative MRI.

PRP can be obtained by centrifugation or filtration of the whole blood to concentrate or isolate platelets to a level higher than normal plasma levels [55]. Several preparation methods of PRP can yield products with different composition and characteristics in terms of platelet and leukocyte concentrations, volume of whole blood harvested, final volume, storage procedures, platelet activation method, and formation of a fibrin matrix [56] (Fig. 14.1).

This heterogeneity makes it very difficult to compare clinical results of different studies and to gain a full understanding of the potential and limitations of the different PRPs for the treatment of knee OA. Thus, the overall benefits of PRP reported in meta-analyses, although substantial, are a result of a simplification of the field, and further studies are needed to clarify which formulation provides the best results for early knee OA [57]. Among other factors, leukocyte concentration is one of the most relevant and controllable factors and some authors advocated better results for leukocyte-poor PRP. However, these results are based only on an indirect comparison of groups from different studies and a study directly comparing PRP with or without leukocytes actually documented similar clinical results up to 12 months of follow-up, although patients who received a leukocyte-rich PRP were more likely to experience pain and swelling (self-limiting) after the injections [58]**.** The number of injections could also influence the effectiveness of PRP. There is no consensus on the most effective approach regarding the posology of PRP injections for OA, although there are preliminary

Fig. 14.1 Platelet-rich plasma (PRP) preparation process

data suggesting the benefit of a three-injection scheme for early OA [59]. These clinical findings have been recently confirmed by an in vivo study demonstrating that three intra-articular PRP injections provided better inflammation reduction of the synovium and more durable results than a single PRP injection [60]. Due to the paucity of the evidence, specific data on the different platelet concentrates for early stage OA are still lacking, and no clear conclusions regarding the posology can be drawn besides an overall benefit.

Despite the controversies regarding the best formulation and administration regimen, intra-articular PRP injections are gaining a large use in the clinical practice, thanks to the safety, low cost, and the simple preparation technique to exploit blood biological potential [61]. Starting from the first clinical studies on PRP injections for knee OA, suggesting promising results in terms of safety and symptoms improvement [62, 63], several studies supported this injective strategy, providing a significant reduction of pain and functional improvement up to 12 months [64–67]**.** In addition, some evidence suggested that the clinical improvement provided by PRP can be perceived by some patients also beyond 24 months, with a subsequent gradual reduction over time [68]. Nevertheless, the effectiveness of PRP was questioned since a high influence of the placebo effect was reported [69, 70]. However, the results of several meta-analyses converge in indicating that intra-articular PRP injections may have more benefit in terms of pain relief and functional improvement than a mere placebo, without increasing the risk of adverse events [57, 66, 71–73]. Moreover, a recent meta-analysis showed that, according to the existing literature, PRP overcomes not only the placebo effect but also other treatment options, such as steroids and HA, with results exceeding the minimal clinically important difference [57]. PRP advantages compared with viscosupplementation increase over time and are clinically significant after 12 months [57].

PRP documented benefits could be even higher in early OA, where the degenerative process is still at its early phases with the inflammatory microenvironment playing an important role [8, 69]. In fact, the evidence on the effectiveness of PRP in the management of early OA is growing with clinical trials showing positive results [74, 75]. Remarkably, the advantages of PRP over HA and placebo seem to be more pronounced in the patients with an early stage disease [59]. However, despite the large number of clinical trials on the intra-articular use of PRP, this product is not yet recommended by international societies. The last AAOS and ACR guidelines on intra-articular treatment of knee OA still opposed the use of PRP [18] due to the low level of the evidence supporting this approach, while OARSI did. However, the evidence on this topic is growing rapidly, and the effectiveness of PRP, taking into account the limitations due to the

differences among its formulations, seems to be confirmed [57]. The studies specifically focused on the earlier stages of the disease are still limited, but current results support PRP as a promising solution to address early OA and postpone more invasive solutions.

14.5 Take Home Message

Intra-articular injections are performed daily by physicians, but their real benefit in knee OA is often questioned. The theoretical benefit on the inflammatory and degenerative microenvironment, which found a support in the preclinical studies, is not always confirmed in the high-level clinical trials. A possible explanation of this apparent contradiction may be due to the poor ability to identify the correct target for these treatment options and to the attempt to address too advanced OA stages. In this light, the definition of early OA as a pathological target may help musculoskeletal specialists to identify a subset of patients that could benefit the most from intra-articular injections. In particular, as suggested by preliminary evidence, corticosteroids can provide short-term benefit reducing joint inflammation, while viscosupplementation and PRP may be suitable options for the conservative management of early OA.

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The Current Role of Stem Cell Therapy and iPS Cells

15

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15.1 Introduction

Over the last decade, there has been a great deal of interest in mesenchymal stem cells (MSCs) and their potential role to play in the treatment of osteoarthritis (OA). The burden of OA has seen an exponential increase, with the World Health Organization reporting 10% of men and 18% of women over the age of 60 years suffering from symptoms of OA [1]. This burden is expected to increase with the universal rising geriatric population [2–4]. The exact aetiology of OA remains uncertain, but literature has revealed age, obesity, trauma, genetics, infection and primary orthopaedic pathologies, together have a multifactorial role in contributing to the biochemical and biomechanical alterations in joint homeostasis to initiate or progress to OA [5, 6]. In the past, OA treatment strategies consisted of pain alleviation with drugs or interventions such as platelet-rich plasma [7], corticosteroid injections, viscosupplementation [8] and finally surgical interventions such as microfracture [9, 10], osteotomies [11] and finally arthroplasty [12, 13]. Recently, with further clarity on the pathophysiology of OA, research has shown numerous cytokines and free radicals having a significant role in increasing pro-inflammatory pathways leading to matrix degradation and onset of OA [14]. This has resulted in a keen interest in biological approaches for treatments using stem cells such as mesenchymal stem cells (MSCs), which have proven immunomodulatory and anti-inflammatory roles [15–18]. It has been suggested that some of these roles are fulfilled by paracrine signalling by employment of exosomes shed from MSCs, allowing for the regeneration and upregulation of endogenous chondrocytes [19, 20]. Recent studies also indicate that there may be subsets of MSCs that perform different functions [21]; MSC may, therefore, have multiple roles to play in cartilage repair. In the past, the most commonly used source of MSCs was bone marrow, but over time, literature has revealed that this source contributes inadequate cell numbers and is inferior when compared to other sources such as adipose and synovium [22–25]. Another source of stem cell is embryonic stem cells (ESCs), which are known to be superior in pluripotency but pose many ethical issues regarding their clinical and experimental

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use [26]. A more recent breakthrough cell source was discovered by Yamanaka et al. [27], where these authors were able to reprogramme mouse and human adult fibroblasts to become pluripotent cells, which exhibited embryonic stem cell morphology and growth properties [28]. These induced pluripotent stem (iPS) cells showed differentiation capacities into all three germ cell layers similar to ESCs, making them an additional potential cell source for all regenerative cell therapies. Table 15.1 summarizes the major differences between MSCs, ESCs and iPS cells, with modifications from a table compiled by Lin et al. [35].

Clinical improvement with the use of stem cell therapies has been shown in a number of pre-clinical trials, but the objective outcome data have not been consistent, and this limitation remains a limitation for clinical trials [36–39]. Overall, with the use of MSC therapies being deemed safe in either autologous or allogeneic form, much research has been focused on identifying cell based therapies to retard OA progression and reverse the disease-associated catabolic pathways [40–42]. In this chapter, we discuss the current roles of stem cells and the current research on iPS cells in OA management.

15.2 Mesenchymal Stem Cells in OA

As mentioned above, various types of stem cells exist, and depending on their tissue of origin, they possess different advantages and disadvantages. MSCs may be harvested from bone marrow [43], synovium, adipose [44], dental pulp [45], umbilical cord blood [46], peripheral blood [43, 46], placenta [47], muscle [48], skin [49] and periosteum [50]. Figure 15.1 illustrates a few of the many sources of MSCs in the human body. MSCs are characterized by their fibroblast-like shape, adherence to plastic, trilineage differentiation capacity, and immunophenotypes [51]. At present, there remains no consensus on the ideal cell source for MSCs, and considerations include harvest cell number, donor site, differentiation capacity, and proliferative potential. MSCs have been applied for chondral repair as they have the potential to differentiate into chondral, adipose, and bone tissue [51, 52]. Due to their lack of human leukocyte antigen class II, these cells exhibit low immunogenicity making allogeneic cell use possible [53]. MSCs have been postulated to function by either acting as a precursor to chondrocytes or to mediate joint regeneration via enhanced secretion of trophic factors by endogenous cells [54], thus supporting the possibility of multiple subsets [21]. The paracrine effects of MSCs, mediated by the shedding of exosomes and secretion of bioactive molecules, have been identified to be a key feature allowing for immunomodulation and facilitated tissue regeneration [19, 54–56]. These trophic factors increase cellular migration and differentiation while regulating prostaglandin and inflammatory molecule production [57].

Table 15.1 Comparing the major differences and similarities between MSCs, iPSCs and ESCs

	Morphology	Differentiation/ proliferative potential	Phenotype	Clinical application	Tumourgenicity	Ethical issues
MSCs	Fibroblastic-like	Mesodermal/Finite [29]	CD29+, CD44+, CD73+, CD90+, CD105+, CD166+, CD14−, CD31−, CD45−, CD34− [30]	Induction not necessary	−	−
IPSCs	Embryonic stem cell-like	All three germ layers/infinite [27, 31]	OCT4+, NANOG+, SOX2+, SSEA1+, SSEA3+, SSEA4+, TRA1-60+, TRA1-81+, ALP+ [32]	Requires induction	+	+
ESCs	Embryonic stem cell-like	All three germ layers + extraembryonic tissue/infinite [33]	SSEA-1,3,4+, CD324,90,117,326,9,24,59,133,31,49f, TRA-160,1-81+, AP+, Fzd 1-10, TDGF-1+ [34]	Requires induction	+	+

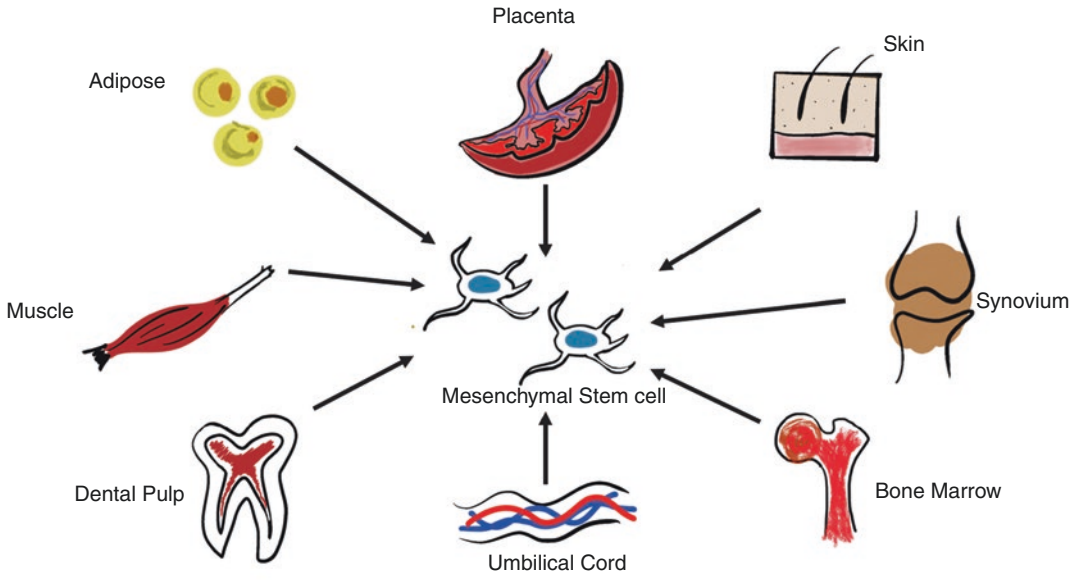

Fig. 15.1 Illustrating a few of the various sources of mesenchymal stem cells in the human body

MSC-based OA therapies have been investigated in both pre-clinical studies and clinical trials environments with cells from different tissue sources and various formulations. They are commonly isolated and expanded in culture before administration through direct intra-articular injection, or in combination with a tissue engineering strategy on a scaffold. One step injection protocols have been popular, with the earliest being simple bone marrow aspirate injections, although yields contained low MSC numbers [58]. Newer injections protocols have now been developed, such as using a stromal vascular fraction (SVF) [59–61] derived from adipose tissue and micro-fragmented adipose tissue [62]. These newer approaches utilizing adipose tissue have shown to yield higher number of MSCs [60, 63]. However, expansion of MSCs in vitro before administration as a treatment is not possible in many clinical settings due to regulatory restrictions imposed by government agencies.

15.3 Embryonic Stem Cells in OA

ESCs originate from the embryo, more specifically from the inner cell mass of the blastocyst depicted in Fig. 15.2. These cells are totipotent with the ability to differentiate into any cell type that would make up a fully developed human. ESCs are also highly proliferative and do not undergo differentiation like other cells. The fact that these cells are extremely young and possess the ability for self-renewal and differentiation into ectodermal, endodermal and mesodermal cells theoretically makes them the most superior stem cell source available for stem cell therapies. However, experiments with these cells have demonstrated teratoma formation, a finding that raises concerns about the use of ESCs in clinical cell therapies. The main challenges with ESCs have been related to ethical approvals and regulations, which do not allow for the harvest of the cells from the blastocyst. Therefore, despite the many positive reports on the potential of ESCs, the efficacy of such treatments and more importantly safety are yet to be determined.

15.4 Induced Pluripotent Stem Cells in OA

iPS cells were first generated using murine fibroblasts [27], soon after which they were created using human fibroblasts [28, 31]. In the murine models, the fibroblast cells were transduced

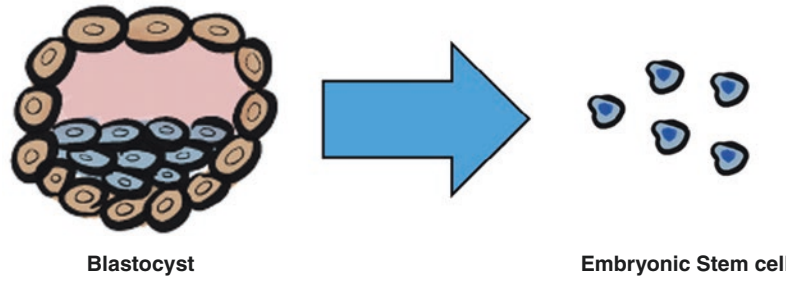

Fig. 15.2 Embryonic stem cells are harvested from the inner cell mass of the blastocyst

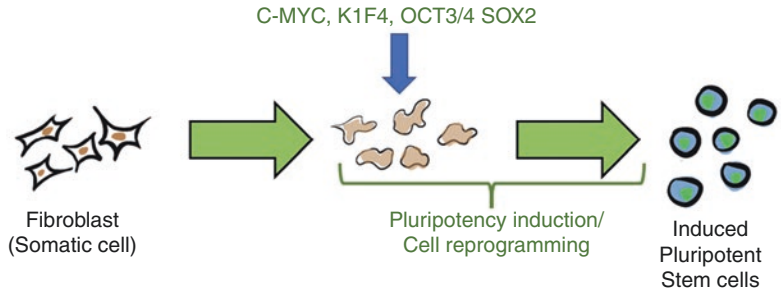

Fig. 15.3 Somatic cells are reprogrammed using four factors; c-Myc, K1f4, Oct3/4 and Sox2, which result in the production of induced pluripotent cells

using four factors; c-Myc, K1f4, Oct3/4, and Sox2, allowing for somatic cell reprogramming [27] illustrated in Fig. 15.3. These cells formed teratomas when transplanted into immunodeficient mice, where the production of hyaline cartilage was also noted [64]. iPS cells have been used in a variety of regenerative modalities with the hope of possible disease modification. Owing to their indefinite proliferative potential, ability to become any desired cell type, and abundance, iPS cells eliminate many shortcomings associated with the previously discussed stem cell therapies, making them an attractive option in current experimental trials [65]. Previous ethical concerns prevented the use of embryonic cells in such trials; however, iPS cells do not evoke such concerns. Another important advantage of iPS cells over bone marrow MSCs (BMMSCs) is that in vitro BMMSCs are primed towards endochondral ossification [66–68] resulting in the tissue produced being hypertrophic, expressing large amounts of collagen I and markers of calcifying cartilage [66, 69]. iPS cells could also allow for larger amounts of in vitro hyaline cartilage generation [68]. With respect to cartilage regeneration and OA, the use of iPS cells would allow for the generation of autologous cells with just the use of a skin fragment and initial dermal fibroblast cultures. After which, the cells can be induced to form iPS cells and then subsequently subjected to chondrogenic differentiation [70, 71]. Four induction methods have been studied for the conversion of iPS cells to chondrocytes [72, 73]. The first is with primary chondrocyte co-culture, where various secretory factors from the chondrocytes can stimulate the iPS cells towards chondrogenic differentiation. The second study method involves the use of growth factors for chondrocyte differentiation. A third method employing chondrogenic supplementation can be used similar to the method used for MSC chondrocyte differentiation. Finally, differentiation can be regulated by specific media changes mimicking that of normal developmental cell differentiation processes. This latter method appears to be the most successful method for the production of stable hyaline cartilage [74–76].

iPS cell therapies do have some challenges to overcome, and making them available for cell-based therapies is a major hurdle at this point in time. Although any cell can be reprogrammed to form an iPS cell, all cells appear to undergo some element of genetic mutation during their lifetime.

This risk could affect the reprogrammed cells negatively and enhance the potential for tumourigenicity [77]. Using embryonic cells from cord blood for iPS cell generation is, therefore, preferred in view of their low genetic modification rate [78, 79], but this option is not available in many cases. Cord blood banking has been a popular trend in recent times, but generating iPS cells from cord blood cells and subsequently banking them for a large population is an extensive and difficult task. Therefore, an allogeneic system may be more practical, and much research has been focused on making this a reality through iPS cell banks [80, 81]. Another concern is the unlimited proliferative potential of iPS cells, while being one of their major initial advantages, upon implantation, they theoretically could proliferate indeterminately and result in tumours. In view of such concerns, iPS cells cannot be implanted without prior differentiation, and exclusion of all iPS non-differentiated cells must be confirmed prior to the initiation of the therapy [68]. Numerous strategies to decrease such risks have been proposed, including the inclusion of suicide genes to destroy the cells in the event of an adverse effect [82]. There is no doubt regarding the value and potential of these universal cell sources to eliminate the shortcomings of previous cell-based therapies, especially with regard to the field of cartilage regeneration.

Clinical trials have not begun for cartilage repair treatments with iPS cells, but the ongoing pre-clinical research appears positive, along with the development of iPS cell banks. Pre-clinical studies aim to identify the safety of iPS cell treatments, including the ideal sources of these cells and optimal culture conditions. Yamashita et al. [74] performed a study with cultured human iPS cells in a chondrogenic medium containing specific growth factors resulting in chondrocytes, which were then transplanted into immunodeficient mice and mini-pigs. They found the presence of bone morphogenic protein 2 (BMP2), transforming growth factor b1 (TGF-b1), and GDF5 to be essential for chondrogenic differentiation of human iPS cells. They reported that transplantation of the cells into mice and mini-pigs showed good integration with the surrounding native cartilage. This is a positive finding, as when mature chondrocytes have been transplanted they do not exhibit such good integration. iPS cells being in very early phases of differentiation can mimic the normal developmental pathway allowing for better chondral maturation and, therefore, improved integration with the mature native cartilage tissue. These authors also did not report any teratomas or tumour formation in the in vivo studies, a major concern with the use of iPS cells. Various other studies have also used growth factors for chondrogenic differentiation of iPS cells and reported encouraging results [83, 84]. Other protocols to stimulate chondrogenic differentiation have involved MSC-like populations [70, 85], chondrocyte co-cultures [86], and embryoid body formation [87]. The progenitor cell for iPS cells has also been a topic of study, and neural crest cells were thought to be a good candidate given their ability to differentiate into osteochondral tissues. iPS cells derived from neural crest cells have been studied and shown to have good chondrogenic differentiation capacity under in vitro conditions; however, the cells did not achieve adequate defect filling when implanted to in vivo chondral defect sites. Again, there was no teratoma formation or tumour growth detected [88]. Other progenitor cell sources from which iPS cells have been derived and studied for differentiation into chondrocytes include umbilical cord blood [89], peripheral blood, [90, 91] and dermal fibroblasts [92]. Research is still experimenting with the ideal cell source and methods leading to chondrogenesis concerning iPS cells. For some, peripheral blood and umbilical cord blood have become preferred sources of iPS cells because of easy harvest and effective reprogramming [93]. Optimal methods for induction of chondrogenesis are under investigation, with each protocol having its own advantages. Suchorska et al. recently suggested the most direct, fast, and cost-effective methods to be monolayer cultures with growth factors or a medium conditioned with human chondrocytes [92]. These pre-clinical studies should lead to movement in the direction of further in vivo studies, and in time, clinical trials once they have achieved more efficient cell reprogramming and chondrogenesis protocols.

15.5 Review of Clinical Trials Using MSCs in OA

Several clinical trials have been reported using varying numbers of MSCs from different sources. These studies also report on different delivery modalities ranging from intra-articular injections to tissue engineered approaches. These variable approaches are discussed in the sub-sections that follow.

15.5.1 Intra-articular Injections

A systematic review conducted by Chahla et al. [94] investigated the use MSCs in the treatment of OA and concluded that they were unable to perform a meta-analysis due to the high heterogenicity between trials. Their review included 6 studies, of which 3 focused on MSC therapies in OA across 124 knees. Of the three studies reporting on OA, two utilized autologous adipose derived mesenchymal stem cells (ADMSCs) and one BMMSCs, which were expanded to passage 3. They noted overall positive clinical improvement in the selected studies and reported the therapies to be safe, but could not rule out a placebo effect. They concluded that literature quality is poor owing to lack of blinded trials, cell population definition, standardization, and quantitative metrics to define cell populations.

Kim et al. [95] analysed five randomized control trials (RCTs) (level II), where four trials employed BMMSCs and one ADMSCs. Their cumulative pain score assessment revealed significant improvement in clinical outcome scores [96–99]; however, the MRI evaluations from three of the selected studies showed no evidence for improvement [96–98]. They too concluded that the optimal cell concentration needed to be determined, along with better standardized trials and that, currently, despite the encouraging results, MSC injections in OA should be investigational based on the available literature.

A larger systematic review was performed by Ha et al. [100], where 17 level I–III studies were included. Their mean follow-up was to 28 months, and cell study sources included bone marrow, adipose, SVF, and umbilical cord blood. Of the 17 studies, all but 2 reported clinical improvement. Only seven studies compared the experimental arm to a control group, where four reported significantly better results in the MSC treated group [97–99, 101]. Eleven of 17 studies reported MRI evaluation, of which only two reported no change in cartilage status [96, 102]. The last two assessed outcomes were second look arthroscopy and histology. Of six studies, one reported no improvement at arthroscopy, [102] and out of four studies, one demonstrated osteoarthritic chondrocytes [102]. Their principal finding was similar to other reports in that they concluded there is limited evidence for the use of MSCs in knee osteoarthritis. Although several studies reported clinical benefit, the RCTs reported controversial results.

Jevotovsky et al. [103] performed a review to evaluate MSC use in OA, in relation to study quality and procedural specifics. Their conclusion was similar to the other discussed reviews in that MSC therapies alleviated symptoms of OA, but due to inconsistencies in study methodology, MSC preparations and protocol design, it is difficult to draw definite conclusions regarding the therapeutic benefits of MSC treatments. Most reviews regarding intra-articular therapies have reported MSC injections to be safe overall; however, a few adverse effects such as synovitis [96], pain and swelling have been reported, but such reactions were also found in study control groups, indicating that they could be associated with any injection [101]. The literature also remains inconclusive regarding the optimal MSC cell count in the intervention, as well as the number of doses, with some studies reporting higher cell number and multiple doses being more beneficial [39, 99, 104, 105].

15.5.2 Tissue Engineering Approaches

Tissue engineering utilizing cell-based strategies has aimed to take things further than simple injections, by programming the stem cells to differentiate towards specific target tissues [106, 107]. Studies have employed specific growth fac-

tors and scaffolds made of various biomaterials, all to provide the cells with an effective microenvironment to promote differentiation into chondral tissue [108]. Most clinical studies in this area have used MSCs in combination with a scaffold or an adjunct technique such as autologous chondrocyte implantation or microfracture. The most popular tissue source for clinical MSC tissue engineering treatments has been bone marrow, usually in the form of an autologous bone marrow aspirate concentrate. However, several of the other above-mentioned sources have also been used. MSCs have been combined as an adjunct to existing techniques such as augmented autologous matrix-induced chondrogenesis [109], as well as to microfracture [110], to improve the outcomes of already utilized techniques. MSCs have also been combined with scaffolds such as a collagen matrix [111–114], polyglycolic acid [115], polylactic acid, [116] and hyaluronan [117]. These studies have mostly reported clinical improvement and reasonable chondral defect fill; however, the quality of the repair tissue has been at best hyaline-like cartilage, which is still imperfect. MSCs appear to improve tissue quality and outcomes, but further research is required to generate repair tissue that is actual tissue regeneration. Isolation and quality control of MSCs remains the major challenge as, currently, the resultant cell populations are very heterogeneous with regard to proliferation, lineage differentiation, and molecular response patterns. This can lead to variable results in terms of chondrogenic differentiation efficiency [118]. Recently, a scaffold-free tissue engineering technique has been introduced using synovial MSCs in a high-density monolayer culture, which results in the formation of a three-dimensional tissue engineered construct (TEC) [119]. TEC implantation has shown favourable pre-clinical results demonstrating hyaline cartilage repair, which has both biological and mechanical properties similar to that of native cartilage [120]. With the excellent pre-clinical data, a clinical study was conducted using TEC in five patients with knee chondral defects. At 24-month follow up, patients had significantly improved clinical outcome scores, second-look arthroscopy demonstrated complete defect fill, and histology of a repair tissue biopsy showed the presence of hyaline cartilage [121]. The same group is currently performing a randomized control trial. Table 15.2 summarizes the results with each MSC tissue source and the resultant clinical outcomes.

Table 15.2 Summary of the differentiation capacities of bone marrow, adipose and synovium tissue and the clinical results for each MSC source

MSC sources	Differentiation capacity			Clinical applicability	Clinical results
	Osteogenic	Chondrogenic	Adipogenic		
Bone marrow	+++	+++	++	Harvest under L/A, ↓cell yield, painful	Direct use of bone marrow without cell expansion results in very low MSC yield despite concentration (0.01–0.02% of TCV) [122]. BMMSC therapies appear to improve clinical symptoms and are safe. Despite defect fill being adequate on MRI and second look arthroscopy, histology has shown a hyaline-like regenerate at best [58].
Adipose	+	+	+++	↑cell yield, ↑tissue requirement	Adipose tissue harvest results in a high number of MSCs (1 g tissue = 2000–20,000 ASCs) [59, 63]. This can overcome the need for cell expansion which results in loss of stem cell homing effects [123]. ASC and SVF therapies have shown significant clinical improvements and radiological outcomes along with good defect fill when compared to patients who did not undergo any treatment [124].

(continued)

Table 15.2 (continued)

MSC sources	Differentiation capacity			Clinical applicability	Clinical results
	Osteogenic	Chondrogenic	Adipogenic		
Synovium	+++	+++	+++	Painless, staged surgery, cell expansion required. Minimal tissue requirement	Synovial cells have demonstrated good proliferative potential and superior differentiation capacity, however, require expansion [22]. TEC have exhibited excellent chondral repair tissue quality, as well as having other favourable features such as adhesion and malleability without the need for additional fixation. Clinical histological trials have shown excellent clinical results. Second look arthroscopy and biopsy have shown complete defect filling and hyaline cartilage repair [121]. The regenerate has also demonstrated mechanical properties similar to that of normal cartilage tissue [119].

L/A local anaesthesia, *MSC* mesenchymal stem cell, *TCV* total cell volume, *BMMSC* bone marrow mesenchymal stem cell, *MRI* magnetic resonance imaging, *ASC* adipose-derived stem cell, *SVF* stromal vascular fraction, *TEC* tissue engineered construct

15.6 Conclusion

Currently, available literature on MSC therapies in osteoarthritis is voluminous, and despite this, it is difficult to deduce precise inferences regarding the effects of MSC therapies for OA treatment and chondral regeneration. The heterogeneity and inferior quality of clinical trials have instigated misperceptions and unregulated non-standardized use of what may be a valuable clinical solution for OA. The iPS cell has in pre-clinical studies shown immense potential and superiority over MSC treatments but has also exhibited possible tumourigenic risks. Without extensive pre-clinical studies and steps to mitigate such risks, as well as ascertain the detailed behaviour of these cells, clinical trials should be delayed. It is hoped that with the introduction of MSC therapy definitions, and the development of superior isolation and quality control protocols, better standardized clinical trials and indications will be published allowing for higher quality analysis of level I data. At present, stem cell therapies for OA should be investigational, and clinicians using them should be encouraged to collect outcome data in the form of high-quality RCTs defining their cell source and specifics of preparation so as to contribute to the standardization of protocols and evaluation of optimized procedures.

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Fat-Derived Stem Cells

16

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16.1 Mesenchymal Stem Cells: New Biological Insights to Face Early OA Degenerative Environment

Despite the symptomatic treatments available based on drugs, proteins, and antibodies, there is not any effective therapy that can reverse or slow down the progressive degeneration of OA yet. In this context, the interest of the scientific committee is focusing on cell-based therapies, with particular reference to mesenchymal stromal cells (MSCs) and their ability to secrete and release bioactive molecules with antiapoptotic, antiscarring, trophic, and immunomodulatory activities that indeed could represent a promising tool in the treatment of early OA [1].

Inflammation is a feature of early OA and might be responsible for the initiation of the degenerative cascades that result in long-term joint destruction. Infiltration of synovial tissue, associated with angiogenesis [2], by immune cells, mainly macrophages [3], is crucial in initial joint destruction, leading especially to the formation of the chronic inflammatory microenvironment typical of the initial stages of OA. Therefore, inhibiting the proinflammatory mechanisms during early OA could represent a promising strategy of new therapies such as cell therapy.

MSCs have been termed as "pericytes" as cells belonging to a perivascular niche [4–6] and have demonstrated to possess the ability to interact or "crosstalk" with resident cells, thus giving a new awareness about their action in response to an injury and radically changing the interpretation of their therapeutic role. The traditional use of MSCs as a "cell replacement tool" due to their ability to differentiate into chondrocyte-like cells has been now enriched by a new vision of MSCs as "sensing cells." They can manage the healing process by interacting with resident cells through a paracrine action, meant as secretion of bioactive molecules, which stimulates the innate potential of the tissue in the repair and modulation of inflammatory and immune reactions [7].

The rationale behind the use of MSCs as therapeutic tool in treating early OA relies on their immunomodulatory potential able to promote a shift from the OA inflammatory microenvironment toward a proregenerative microenvironment and tissue homeostasis.

In fact, MSCs can modulate the function of adaptive immune system typical of synovial inflammation, inhibiting T cells proliferation, suppressing B cells, attracting regulatory T cells, and inducing anti-inflammatory factors [8].

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Moreover, MSCs promote the transition from proinflammatory M1 to anti-inflammatory M2 macrophage phenotype through an anti-inflammatory factor such as prostaglandin E2. This secreted prostaglandin by interacting with the EP4 receptor present on the surface of stimulated M1 macrophages inhibits their secretion of cytokines with a strong proinflammatory attitude, mainly tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and increases the expression of anti-inflammatory mediators such as IL-10 by M2 macrophages [9]. Consequently, these MSC abilities avoid the cartilage degeneration mediated by macrophages in the early OA by preventing both the activation of matrix degrading enzymes such as matrix metalloproteinases (MMPs) and aggrecanases and the inhibition of the synthesis of matrix proteins by chondrocytes [2].

MSCs are also immune-evasive since expressing low levels of MHC class I molecules and completely lacks the expression of the MHC class II ones. This feature makes them scarcely immunogenic and offers some advantages in terms of clinical applications [10] as it is possible to use both autologous and allogeneic MSC-based therapies [11].

16.2 Features of ASCs: Adipose Tissue (Fat) Stem Cells

Given their nature as a subset of pericytes, MSCs can be found in all the vascularized tissues.

Rich in vascularization, subcutaneous adipose tissue, represents therefore a plentiful source of the MSC/pericytes and is recognized as a promising tool for clinical applications [4, 12–14].

Traditionally, bone marrow has been used as a source of MSCs (BMSCs) for clinical use, and most of the current MSC literature is about the cells of this origin. However, BMSCs constitute only a small proportion of the whole bone marrow cell population, and their number, together with their performances, negatively correlates with the age of donors. Moreover, the invasiveness of the harvesting procedure has pushed to look for alternative sources of MSCs having similar features [15]. MSCs have been identified in adipose tissue first in 2001, and because of their close similarity to BMSCs, they were described as an adequate cell pool for regenerative medicine approaches [16]. Adipose tissue is generally found in abundant quantity in most of the people and can be easily accessed and harvested with a less invasive surgical procedure without ethical issues [17]. It is constituted predominantly of mature adipocytes (67.6%), but it also contains other cell types such as adipose stem cells (ASCs), pericytes, leukocytes, fibroblasts, macrophages, and preadipocytes, which are embedded in a rich vascular network and constitute the "stroma" or "stromal vascular fraction" (SVF) [18–21]. The SVF generally acts as a reservoir system exerting a hypertrophic and hyperplastic action and providing suitable supplements to the growth of the tissue and the differentiation of preadipocytes. ASCs represent 5% of nucleated cells in the SVF, and therefore, their frequency within the source tissue is much higher than those of from BMSCs that indeed represent only 0.01–0.0001% of the whole bone marrow cells [22].

Subcutaneous adipose tissue is the most frequent source for regenerative approaches. It is located directly beneath the skin and is easily accessible and available in high quantity by liposuction, usually performed under local anesthesia. Another source of ASCs is the infrapatellar fat pad (Hoffa's fat pad) that can be accessed during knee surgery. Interestingly, ASCs isolated from this source show higher in vitro chondrogenic differentiation potential in comparison with ASCs from subcutaneous [23, 24]. Furthermore, the application of this tissue would overcome the regulatory hurdles generally encountered with the subcutaneous adipose tissue, since it allows a homologous application of ASCs. However, given the small amount of Hoffa's pad, its clinical application would necessary require in vitro expansion to guarantee an adequate number of cells.

Regardless of their origin, ASCs possess similar features to BMSCs such as antiapoptotic, immunomodulatory, trophic, angiogenic properties, and immunophenotypes. Indeed, they both

highly express the typical stem cell markers CD73, CD90, and CD105 but poorly express the hematopoietic markers CD45, CD235a, CD31, and HLA-DR [22, 25]. ASCs possess a good differentiation potential, with a higher adipogenic but lower chondrogenic and osteogenic potential with respect to BMSCs [22].

Moreover, given their high proliferation rate, ASCs can be easily collected in high quantities and banked for future clinical application.

16.3 Fat-Derived Cell Products in the Treatment of Early OA

ASCs have been demonstrated to exert both an anti-inflammatory and a chondroprotective activity in several studies regarding OA [10]. The immunomodulatory properties of these cells occur throughout their direct contact with many components of the innate immune system, including macrophages, or by the secretion of paracrine factors [26]. ASCs exert a strong secretory activity [27], producing several bioactive molecules that possess the ability to deliver protective and supportive factors which may reduce apoptosis, fibrosis, and inflammation [28], thus contributing to the treatment of the early OA.

While isolating in vitro cultured cells is a more traditional way to administer cell therapy, more recently, intraoperative solutions not involving cell expansion have been proposed as one-step approach to match clinical effectiveness and feasibility. As for untreated adipose tissue, this contains a clinically relevant number of ASCs that makes the use of this approach increasingly frequent. Obvious advantages of this approach are lower costs as well as a higher patients' compliance.

One-step procedures usually refer to minimally manipulated products that do not require substantial tissue and cell processing that may alter their original relevant characteristics. Lists of substantial manipulations have been provided by each country's regulatory agencies and include but are not limited to enzymatic dissociation, in vitro expansion, sterilization, irradiation, and cryopreservation. In minimally manipulated products derived from adipose tissue, SVF is isolated from the rest of the tissue components, usually through mechanical digestion followed by a centrifugation step [29]. Alternatively, adipose tissue can be used at the point of care in form of microfragmented fat (microfat). In this case, adipose tissue is purified, in some cases washed off of blood and oil residual, and resized through the use of filters to allow a fat size that allows it to pass through an injection needle [30]. The main difference between SVF and microfat is that SVF is a suspension of different cell populations without the presence of tissue matrix (or just in a very limited amount), whereas microfat consists of purified and resized adipose tissue, therefore keeping the stem cell niches intact. This technical and biological difference has not been demonstrated yet to influence the clinical outcomes since no direct comparative studies have been carried out so far.

In general, one-step procedures using the regenerative potential of adipose tissue, although convenient in term of time and cost saving, lack in accuracy to select MSC population only [31], as well as they do not allow to obtain a higher number of proregenerative cells. In fact, the number of SVF cells contained in 1 g of fat is about 1×10^5 [32]. Nevertheless, the current trend seems to show, supported by the encouraging results of the first clinical trials, an increase in the use of this approach.

In the following paragraphs, the term ASCs will refer to expanded cells, whereas SVF and microfat to minimally manipulated cell products (Fig. 16.1).

16.3.1 Preclinical Findings

The strong regenerative and immunomodulatory properties of ASCs have been assessed in several preclinical models of OA [33] in different species, such as rabbits [34], dogs [35, 36], goats [37], mice [26], horses [38], and rats [39], all resembling some of the features of the human disease. Beyond the animal species, the studies also differ in terms of many other parameters (Table 16.1).

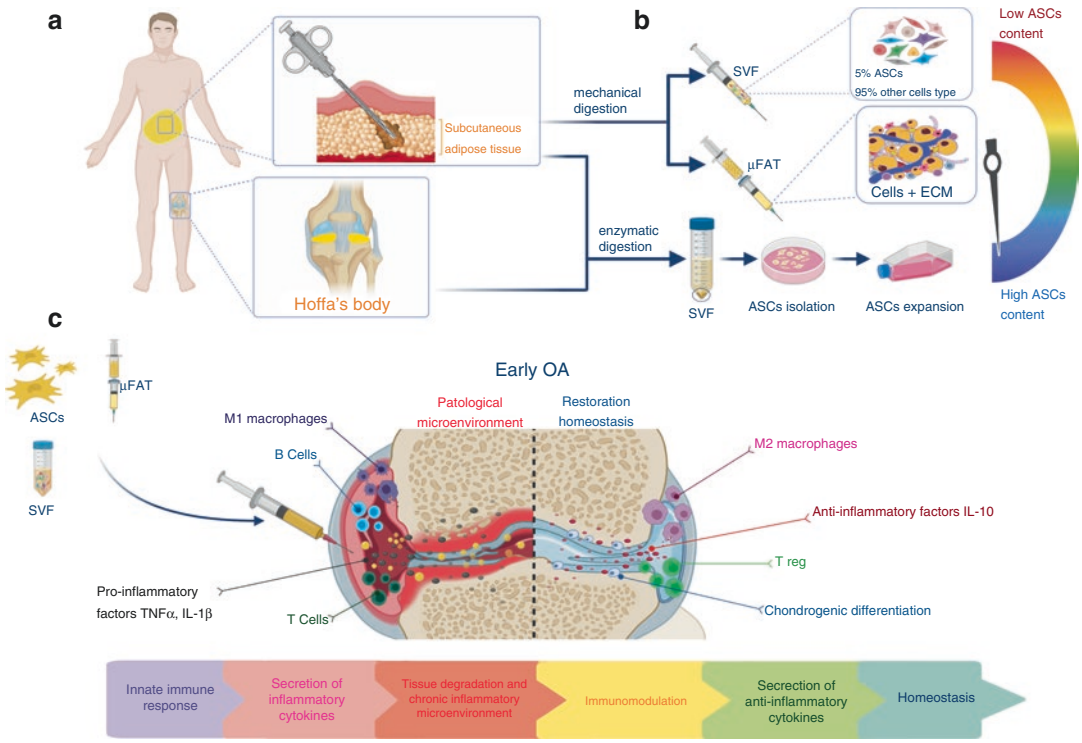

Fig. 16.1 Cell therapy approaches based on adipose tissue (fat) stem cells (ASCs) for the treatment of early OA. (**a**) Sources of fat stem cells. (**b**) Processing types and fat-derived cell products. (**c**) Injection of ASCs, SVF, or μFAT and their immunomodulatory activity in the early OA joint

In some cases, OA is induced surgically through anterior cruciate ligament transection or meniscectomy, in others using chemicals such as collagenase [40]. While the first method attempts to mimic more closely the OA onset, the latter is supposed to be more adequate to study the early changes of the tissue and the effects of early therapeutic interventions. However, although timing of cell therapy treatment would be a crucial aspect to investigate, very often, the animal studies do not report the stage of the disease, making it difficult to provide a correct evaluation of the outcomes. Another aspect to consider is the way of cell delivery. In preclinical studies, ASCs were suspended either in cell culture medium [34], phosphate buffered saline solution [39], or animal serum [26, 41]. In other cases, they were combined with platelet-rich plasma (PRP) [42], which given its content in growth factors stimulates the proliferation of ASCs and induces differentiation toward the chondrogenic lineage [37]. In other cases, cells were delivered seeded on resorbable scaffolds and 3D constructs [43]. In this context, polymeric scaffolds are aimed to facilitate the maintenance of the cells at the defect site and to provide them a suitable support for proliferation and chondrogenic differentiation [44]. However, given the clinical features of early OA, injective treatments are much more frequent that the use of cell-scaffold constructs.

Overall, the preclinical application of fat stem cells has proven to be safe in the majority of the studies performed: neither local adverse reactions, such as swelling or joint stiffness, nor systemic ones such as changes in weight and behavior and in the function of other organs were reported [45]. This safety profile was also confirmed in case of allogeneic ASCs [39] and xenografts [37], proving their low immunogenicity.

Another very relevant feature that differs among the studies is the use of nonexpanded or expanded cells. While the former allows for a

Table 16.1 Preclinical models of OA

References	Animal species	OA induction type	Experimental group	Timing	Administration method	Study design
Jurjens [43]	Goat	Medial parapatellar incision	ASCs expanded and SVF non-expanded Autologous	Same day of induction	Implantation surgery with scaffold	ASCs (5×10^5) or SVF (5×10^6 total cells) + collagen type I/III scaffold
Huurne [26]	Mouse	Chemical with collagenase IV	ASCs expanded Autologous	7–5 days or 24 h after OA induction	Intra-articular injection	ASCs (2×10^4) + mouse serum with mouse albumin 4%
Toghraie [34]	Rabbit	ACTL	ASCs expanded Allogeneic (rabbit)	12 weeks from OA induction	Intra-articular injection	ASCs (1×10^6) + 1 mL medium
Desando [41]	Rabbit	ACTL	ASCs expanded Autologous	8 weeks from OA induction	Intra-articular injection	ASCs (2×10^6 or 6×10^6) + 4% RSA
Mei [39]	Rat	ACTL	ASCs expanded Allogeneic (rat)	4 weeks from OA induction	Intra-articular injection	ASCs (1×10^6) + PBS
Ko [37]	Goat	Medial meniscectomy	ASCs expanded Allogeneic (human)	9 weeks from OA induction	Intra-articular injection	ASCs (7×10^6) + medium (DMEM/F12)
Zeira [46]	Dogs	Spontaneous OA	Non expanded MFAT Autologous	N.A.	Intra-articular injection	MFAT (0.5–4 mL) + physiological solution

ASCs adipose stem cells, *ACTL* anterior cruciate ligament transection, *DMEM/F12* Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12, *MFAT* microfragmented adipose tissue, *N.A.* not assessed, *OA* osteoarthritis, *PBS* phosphate-buffered saline, *RSA* rabbit serum albumin, *SVF* stromal vascular fraction

heterogeneous cell suspension in the form of SVF or microfat that can be applied in the one-step procedure, the latter implies in vitro culture of ASCs leading to a larger number of homogeneous cells. However, this approach requires a two-step procedure.

16.3.1.1 Expanded ASCs

Most preclinical studies are about the use of expanded ASCs in two-step procedures. The dose of injected cells varies between one and seven million of cells, based on the animal model and anatomic site. Overall, the injection of cells in a dose within this range has provided good results. However, a study conducted in a rabbit model would suggest the use of a lower number of ASCs (two millions) rather than a higher dose (six millions) as a more effective approach in counteracting OA progression [41]. For what concern the timing of the treatment, in a murine model of OA, the injection of ASCs 7 days after the chemical induction of OA inhibits the synovial thickening, whereas the injection 14 days after OA induction shows no significant protection against synovial activation or joint degeneration [26]. About the method of action, the local intra-articular injection of autologous ASCs provided beneficial effects and improved the cartilage quality supposedly through their immunomodulatory and immunosuppressive activities on the synovial microenvironment [45]. They were demonstrated to provide a chondroprotective effect, mitigate the synovial membrane inflammation by inhibiting the thickening of the lining layer, and reduce the expression of TNFα, IL-1β, and MMPs in OA animal models [39, 41]. The localization of the injected GFP-labeled ASCs within the synovium, just below the lining layer in OA knee joints,

emphasizes this aspect. The interaction between ASCs and macrophages within the intimal layer was observed 5 days after the injection, suggesting that the beneficial effects of ASCs on the synovium are activated already since the early phases of the treatment [26].

16.3.1.2 Nonexpanded (Minimally Manipulated) Adipose-Derived Cell Products

While the majority of the clinical trials study is about one-step procedures involving the use of SVF or microfat, most preclinical studies focus on the use of expanded ASCs. To the best of our knowledge, the literature about the use of SVF or microfat in animal studies is very limited. In a recent study in dogs with spontaneous osteoarthritis, variable quantities of microfat have been injected into the joint. Microfragmented adipose tissue (μFAT) administration significantly improved joint function and reduced pain and symptoms, for at least 6 months, with a trend of steady increase during time, probably by acting through a strong analgesic, anti-inflammatory, and trophic activities [46]. Moreover, μFAT administration determined the formation of a tissue that certainly displays neither the same mechanical properties of normal hyaline cartilage nor the same durability. This suggests that the reparative action promoted by MSCs is mainly not by their differentiation but by their long-lasting promotion of a regenerative microenvironment [46].

Another interesting paper, although focused in experimental focal osteochondral defects, compared expanded ASCs with SVFs [43]. The authors showed that the cartilage regeneration process after SVF injection occurred in 4 months compared to 4 weeks when expanded ASCs were used. This finding may be due to the initial lower number of ASCs delivered in the SVF group (1.0×10^5 to 3.6×10^5 ASCs) compared with the standardized 5×10^5 cultured in the ASC group. Probably, the lack of difference at 4 months between the two groups confirms that the heterogeneous SVF cell populations can still guide an efficient regenerative process through synergistic effect [43]. However, further studies are mandatory to identify the most reliable animal model to standardize the best preclinical methodology for using these cells in the early stages of the disease and establish the most advantageous between one- and two-step methods.

16.4 Clinical Trials

Most of the clinical trials have been conducted in knee joint, although there are some few preliminary results in other joints [47–49]. Regardless of the target joint, the substantial differences in terms of treatment protocols do not allow us to drive straightforward conclusions on the clinical efficacy of therapies based on the use of fat-derived products for the treatment of early OA [50]. Nevertheless, in this paragraph, we will attempt to provide some relevant information retrieved by the literature that is increasingly rich in papers about these joint regeneration approaches.

The selection of the ideal candidate for these treatments is a critical issue [50]. Often, in fact, the studies are carried out in small heterogeneous cohorts that include patients presenting both initial and late stage chondral degeneration. This makes it difficult to correlate results to the severity of the pathology and, thus, to reach strong guidelines for early OA patients. Prospective trials on larger cohorts of selected early OA patients or with a study design that involves a careful randomization of the disease stage are needed. A meta-analysis only including nine randomized controlled trials was conducted to assess the therapeutic efficacy of expanded ASCs and BMSCs for knee OA at 6-, 12-, and 24-month follow-up [51]. The clinical outcomes were evaluated by using the most diffused patient-reported outcome measures, such as visual analog scale (VAS), Western Ontario McMaster Universities Osteoarthritis Index (WOMAC), Lysholm knee, and Tegner activity scales. ASCs treatment determined an improvement of VAS and WOMAC scores at any follow-up time, with better results than those obtained by using BMSCs. Nevertheless, all the four phase II studies included in this meta-analysis reported on small

groups of patients (from 10 to 26) with a Kellgren–Lawrence (KL) grade ranging from I–II to IV [52–55]. Moreover, the treatment protocols were very heterogeneous in terms of cell source (subcutaneous adipose tissue and infrapatellar fat pad), number of administered cells or procedures, different follow-ups, and concomitant procedures (debridement, PRP, and others).

A systematic review [10] analyzed the results of injective mesenchymal stem cell-based treatments for knee osteoarthritis, paying attention to avoid studies implying arthroscopic debridement not to include a further bias when evaluating the clinical efficacy of expanded ASCs and SVF. Three of the studies included in the systematic analysis about patients with early OA (KL I-II) showed the safety and effectiveness of high doses of expanded ASC for intra-articular injection [56–58]. They showed that a dose of $50–100 \times 10^6$ cells was crucial to achieve relevant clinical benefits maintained up to 2 years follow-up [56–58]. Moreover, it was demonstrated that two repeated ASC injections at 3 and 6 weeks, followed by an additional one at 48 weeks, allowed for significant improvements in terms of pain, knee function, and cartilage volume [57, 58]. Other three studies conducted on a low number of patients (from 6 to 17) focused on the use of SVF or microfat for the treatment of early OA [59–61]. SVF alone [60] or combined with PRP [59] lead to functional improvement and pain relief up to 2 years in early OA patients, whereas the use of microfat promoted significant improvements in terms of pain and cartilage quality up to 1 year [61].

In a retrospective study performed in a larger cohort of patients, a direct comparison of intra-articular injection of ASCs (42 patients, 59 knees) or SVF (38 patients, 69 knees) for the treatment of knee OA (KL II-IV) was performed, with particular attention to the patient's response to the treatment in relation to the baseline KL grade [31]. In the ASC group, a more rapid and greater improvement in pain and symptoms was showed along with a slightly higher proportion of responders according to Outcome Measures in Rheumatology–Osteoarthritis Research Society International (OMERACT-OARSI) criteria. At 6-month follow-up, in the same group, the proportion of responders was 100% in patients with KL II, decreasing as the OA severity increased.

Two studies of the same research group evaluated the efficacy of the use of MSCs derived from the Hoffa's fat pad for the treatment of KL III-IV OA [54, 62]. In a group of 18 knee OA patients, these expanded cells injected together with PRP were effective in reducing pain and improving knee function, with positive outcomes related to the number of cells injected. Interestingly, the results at 2-year follow-up were better than short-term ones [62]. In a second study, 25 knee OA patients and a matched control group were treated with PRP combined with a selected dose of MSCs derived from Hoffa's fat pad or PRP alone, respectively. Even in this case, the results showed pain reduction and functional improvement in all the patients belonging to both groups. The comparison with control group showed that, although the preoperative functional scores of the study group were significantly poorer than those of the control group, the clinical results at the last follow-up were not significantly different between the groups [54]. However, given these promising results obtained in patients with mild to severe OA, studies on patients presenting lower grade of cartilage degeneration are needed to verify the clinical efficacy of the treatment for the early OA with MSCs derived from the infrapatellar fat pad.

Overall, the majority of the results reported in the literature refer to clinical trials conducted using ASCs and identify these cells as promising for the treatment of early OA. Specific protocols for ASC-based therapy have been tested and verified in long-term follow-up, and also given the paucity of studies about SVF and microfat in OA patients, currently, the efficacy of expanded ASCs seems to be superior to that of SVF.

16.5 Conclusion

ASCs perfectly respect the ideal criteria that a stem cell must possess in order to be used in regenerative medicine, thus representing a valid alternative to BMSCs for the treatment of cartilage lesions. In particular, their regenerative,

immunomodulatory, and immunosuppressive potential makes them an ideal candidate to treat the initial degenerative and inflammatory condition characterizing the early OA stages. Indeed, as demonstrated by basic and preclinical studies, ASCs-based therapies have been proven to be safe and potentially effective in counteracting OA progression, modulating the inflammatory microenvironment in the early stages of the disease. However, the heterogeneity of the preclinical studies negatively influences the comparison of the results and the possibility to draw a solid conclusion about their effectiveness.

Clinical trials confirmed the ability of ASCs to counteract the early OA and allowed to develop specific and effective treatment protocols. Although promising evidence concerning the algo-functional improvements after treatments reported in few studies, the clinical efficacy of the use of MSCs derived from the infrapatellar fat pad as long as of microfat and SVF need to be further confirmed. In particular, studies concerning the effectiveness of these minimally manipulated biologics would be a great boost for intra-articular regenerative medicine as they would allow us to bypass the complex regulatory concerns and costs associated with cell expansion.

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17 Bone Marrow Aspirate Concentrate for the Treatment of Early Osteoarthritis

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17.1 Introduction

Osteoarthritis (OA) is a chronic degenerative disease, which affects articular cartilage. The prevalence of osteoarthritis ranges from 14% to 18% of the adult population aged over 60 years old, of which knee OA is the most prevalent, followed by hip and hand OA [1, 2]. Current conventional treatments for early osteoarthritis include medications such as nonsteroidal anti-inflammatory drugs, steroids, and supplements, which focus on managing pain and inflammation. The recent advancement to the use of orthobiologics such as platelet-rich plasma (PRP), mesenchymal stem cells (MSCs), and bone marrow aspirate concentrate (BMAC) aims to prevent disease progression by altering tissue homeostasis. As disease-modifying treatments are limited in clinical late-stage osteoarthritis, early intervention with biologics such as BMAC can be critical in preventing disease progression.

Bone marrow (BM)-derived cells are one of the commonly used biologics for the treatment of osteoarthritis. Bone marrow MSCs (BM MSCs) are a progenitor stem cell population found in the bone marrow that appear to be promising for the treatment of OA upon intra-articular injection [3, 4]. They act by three different mechanisms: (a) differentiation of MSCs into specific cell lineages, (b) secretion of exosomes and cytokines by MSCs to modulate inflammation, cell growth, and survival, and (c) direct MSC contact with host cells to modulate function [5]. However, since they need to be culture expanded before implantation, they are more than "minimally manipulated" and, as such, subject to regulatory approval. The clinical use of BM MSC therapies is currently not approved by the Food and Drug Administration (FDA) [6]. The use of BMAC is, thus, one of the few methods, by which progenitor cells such as BM MSCs can be implanted clinically, as it is currently approved by many regulatory bodies around the world, including the FDA. The processing of BMAC is typically done

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at the point of care in an entirely closed system, making it one of the safest and most feasible ways to implant bone-marrow-derived progenitors and growth factors.

Autologous BMAC has been used clinically in many studies for the treatment of early osteoarthritis. In this chapter, we discuss the methods and equipment used for the harvest and processing of BMAC, its cellular and growth factor components, and possible mechanisms of action. We review published clinical studies that have applied BMAC for the treatment of early osteoarthritis and discuss their findings, including the various factors that may affect treatment outcomes.

17.2 Harvest and Processing of BMAC

Bone marrow aspirate (BMA) is typically harvested from the iliac crest, femur, or tibia. The posterior iliac crest is the most common aspiration site, as it gives a better yield of BM MSCs compared to other sites such as the anterior iliac crest, femur, or tibia [7, 8]. However, the percentage of BM MSCs in BMA is extremely low, between 0.001% and 0.01% [9], and the delivery of large volumes of BMA to the treatment site is not feasible. The centrifugation of BMA overcomes this issue by achieving the concentration of most cell types and growth factors found in BMA into a small volume that can be directly implanted. The BMA is typically concentrated at the point of care using commercially available centrifuges to create BMAC. Most commercial systems utilize density gradient centrifugation to isolate and concentrate the mononuclear cell (MNC) or total nucleated cell (TNC) fraction along with platelets, which is separated from the red blood cells (RBCs) and plasma. Nearly all the supernatant plasma is then removed, and the total nucleated cell fraction and platelets are resuspended in the remaining plasma, resulting in a concentrated mixture of cells and growth factors (Fig. 17.1). A stepwise method for the harvest and processing of BMAC is described by Chahla et al. [10].

There are multiple commercial systems available today to achieve the concentration of bone marrow aspirate at the point of care. These include the Harvest Smart Prep system (Terumo BCT), the BioCUE (Zimmer Biomet), the Magellan (Isto Biologics), the Angel Bone Marrow Processing System (Arthrex), the Pure BMC device (Angel Corporation), the ART BMC device (Celling Biosciences), and Accelerate BMC (Exactech). The technical features and quality parameters of many of these point-of-care devices are reviewed in [11]. One prospective study compared the Harvest, Magellan, and BioCUE systems and found that the Harvest system achieved a significantly higher number and concentration of MSCs, after centrifugation, compared to the Biomet and Magellan systems [12]. This may indicate that the Harvest system achieves more efficient concentration compared to the other two systems studied. Another study that compared the Biomet, Harvest, and Arthrex systems noted that the Harvest system concentrated white blood cells (WBCs) more consistently than the Arthrex system. The Harvest system recovered the highest percentage of colony-forming units (CFU-Fs), indicating MSCs, CD34+ hematopoietic stem cells (HSCs), and WBCs, while the Biomet system recovered the highest percentage of platelets [13]. Thus, it seems that BMACs processed in different commercial systems show differences in cellular composition, which may lead to differences in clinical outcomes. Each system holds an advantage for the concentration of a particular cell type, indicating the clinical significance of the system used.

Some studies continue to utilize Ficoll–Paque-based density gradient centrifugation to isolate and concentrate the bone marrow mononuclear cell (BM MNC) fraction. This method eliminates platelets and granulocytes as well as red blood cells, leading to higher concentrations of uncommitted stem cells [14]. However, it has been shown that Ficoll–Paque density gradient centrifugation can compromise BM MNC yield [15], and that the use of a BMAC device improved total nucleated cell (TNC) count to 2.4 times that of the Ficoll method [16]. The Ficoll method is

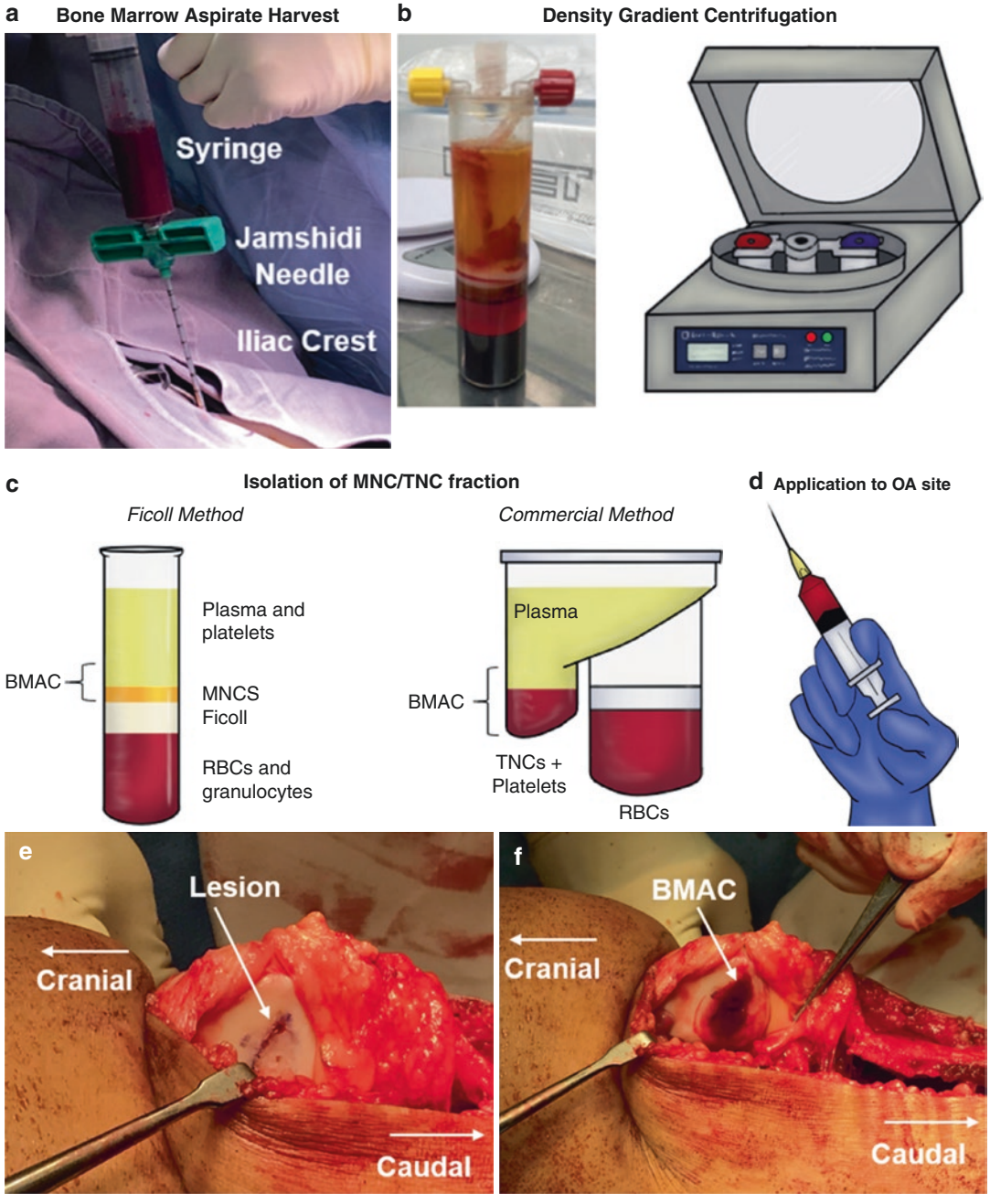


Fig. 17.1 Harvest, processing, and delivery of bone marrow aspirate concentrate (BMAC). (**a**) Bone marrow aspirate is harvested typically from iliac crest bone, (**b**) undergoes centrifugation either using a commercially available BMAC device or a Ficoll–Paque density gradient procedure to isolate (**c**) the mononuclear cell (MNC) or the total nucleated cell (TNC) fraction, which is then (**d**) applied to the osteoarthritic (OA) site for treatment. Images from our clinic showing a patellar cartilage lesion (**e**) before and (**f**) after application of BMAC treatment

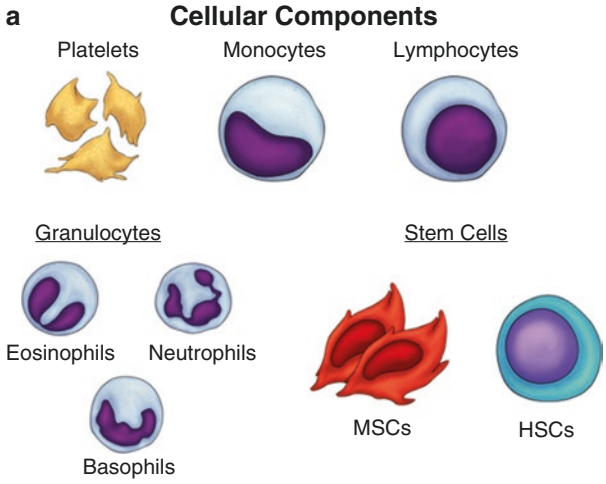
also not an entirely closed system unlike many commercial BMAC devices. It requires careful manual layering of the BMA over the Ficoll solution, making it investigator dependent, time-consuming, and requiring the use of a GMP facility.

17.3 BMAC Components and Possible Mechanism of Action

BMAC contains concentrated cells, including platelets, granulocytes, lymphocytes, monocytes, progenitor cells, and a small proportion of stem cells—MSCs and hematopoietic stem cells (HSCs) (Fig. 17.2a). A three- to fourfold increase in total nucleated cells was reported after bone marrow concentration compared to the same volume of bone marrow aspirate [16, 17], verifying that the systems used did concentrate nucleated cells. An increase of MSC concentration in BMAC compared to BMA has also been reported, with higher CD90+/CD73+/CD271+ MSC populations [18], and higher colony-forming unit (CFU) counts in BMAC [17–19]. MSCs have self-renewal capabilities and the ability to differentiate into osteocytes and chondrocytes upon implantation, to regenerate injured tissue. They also secrete a range of trophic factors, which can modulate inflammation, cell growth, and survival. CD34+ HSCs are also enriched in BMAC, making up 1–2% of cells [19, 20]. HSCs can promote angiogenesis and promote MSC osteogenesis [21, 22]. The platelet component of BMAC is rich in growth factors, which can aid in stem cell migration and provide stem cell adhesion sites [23].

BMAC also contains enriched levels of the growth factors such as platelet-derived growth factor-BB (PDGF-BB), vascular endothelial growth factor (VEGF), transforming growth factor- β1 (TGFβ1), bone morphogenic protein-2 (BMP2), and basic fibroblast growth factor (b-FGF) as well as cytokines such as interleukins (IL) IL-18 and IL-1β and the interleukin receptor antagonist IL-1ra (Fig. 17.2b). These growth factors can influence cell behavior upon implantation and promote MSC differentiation. TGFβ1 is known to promote MSC differentiation and chondrocyte proliferation [24, 25]. BMP-2 can have a synergistic effect along with TGFβ in promoting chondrogenesis [26]. PDGF functions to promote collagen synthesis and angiogenesis [27] and can suppress IL-1β cartilage degradation [28].

The growth factor and cellular components of BMAC differs significantly from those contained in other orthobiologics such as platelet-rich plasma (PRP), which is also commonly used in the treatment of OA. The most important distinc-



b Cytokines and Growth Factors

Growth Factors	Cytokines
PDGF-AB, AA, BB	IL-1ra
TGFβ1, 2	Il-8
VEGF	IL-1β
bFGF	
Activin A	
IGF-1	
BMP2	
EGF	

Fig. 17.2 The typical components of BMAC. (**a**) Cells including platelets, monocytes, lymphocytes, granulocytes, mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). (**b**) Growth factors and cytokines

tion is that BMAC contains MSCs, while PRP does not have any. While the number of platelets was similar in BMAC and PRP, WBCs were enriched 11-fold in BMAC compared to PRP [17]. BMAC also contained higher levels of bFGF than PRP, but similar levels of TGFβ1, PDGF-BB, VEGF, and BMP2 [17, 19]. BMAC contained higher levels of pro-inflammatory cytokines IL-1β and IL-8 than PRP, but also a clinically relevant concentration of IL-1ra [17]. The presence of IL-1β and IL-8 in BMAC may cause an unintended effect of neutrophil migration and monocyte stimulation at the injection site, leading to a more inflammatory phenotype. However, this is offset by the high levels of IL-1ra found in BMAC, which may lead to an overall anti-inflammatory effect via the prevention of IL-1 catabolism. Importantly, the ratio of IL-1ra/IL-1 in the BMAC needs to be considered, and this may vary based on the donor and the centrifugation system. When BMAC was processed using the Angel Arthrex system, the average ratios of IL-1ra/IL-1β were 193.54 at a 2% hematocrit setting and 720.62 at a 15% hematocrit setting, indicating that the BMAC would have significant anti-inflammatory effects [29]. Advantageously, the presence of other inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interferon gamma (IFNγ), and IL-6 was undetectable in BMAC [17].

17.4 Clinical Outcomes

In this review, our focus is on the use of BMAC for the treatment of early stage OA. Studies that included only early OA patients or those that included patients of all OA severity were reviewed and are summarized in Table 17.1. Studies that primarily focused on patients with severe Kellgren–Lawrence (KL) Scale Grade 3–4 OA or severe OA were omitted during review. The KL scale ranks the severity of knee OA based on AP knee radiographs through the identification of five hallmark radiological features of OA: formation of osteophytes on joint margins or tibial spines, periarticular ossicles associated with the distal and proximal interphalangeal joints, narrowing of the joint cartilage associated with sclerosis of subchondral bone, small pseudocystic areas with sclerotic walls situated usually in the subchondral bone, and finally the altered shape of the bone ends, in particular, the head of the femur [41]. Out of the 12 studies reviewed, nine focused on knee OA, one on hip OA, and one on both knee and hip OA. One study by Centeno et al. in 2015 focused on OA and/or rotator cuff tears of the shoulder [31]. Here, shoulder pathology was assessed through magnetic resonance imaging (MRI) and physical examinations.

Across the studies listed in the table, there is a general trend of improvement in outcome scores that mainly pertain to function and pain regardless of the method used to assess treatment outcome. These improvements can be seen as early as 1-month post-treatment with BMAC and the effect persists in subsequent follow-ups of up to 2 years [37, 38]. One of these studies compared the effect of BMAC alone to exercise therapy, concluding that the injection of BMAC showed more benefits [34]. All patients who received exercise therapy converted to BMAC injection after 3 months and showed results that were comparable to the initial BMAC injection group.

To note, the time of follow-up post-treatment is typical across all studies with the maximum data available being a 2-year follow-up. While it is evident that the short-term effects of BMAC are beneficial, the long-term effects of BMAC have yet to be elucidated. Therefore, continuous follow-up will be fruitful to determine if a single BMAC injection is sufficient in mitigating OA progression.

Apart from these measurement outcomes, which are reliant on patient response, two separate studies have reported MRI scores to objectively quantify the local effect of BMAC on the treated knee. Goncars and colleagues utilized the Whole Organ MRI Scoring (WORMS) method to determine the degree of abnormality within the affected region [35]. Of the 14 different features measured by WORMS, 3 were identified to have a significant improvement. These features are articular cartilage integrity, bone marrow abnormality and synovitis, all of which demonstrated-

Table 17.1 Overview of clinical studies that used bone marrow aspirate concentrate (BMAC) for early OA treatment

Study	OA pathology	Groups and number of patients	Additional factors	Marrow harvest	Dosage	Follow-up duration	Results	Safety
Centeno et al., 2014 [30]	KL grade 1–4, knee OA	Two treatment groups: 681 patients, 840 procedures	PRP and PL added to BMAC in both groups	Posterior superior iliac crest	1–3 mL BMAC produced, dosage unspecified	1, 3, 6 months, 1 year	Treatment using BMAC with adipose graft did not show an advantage over BMAC alone	No serious adverse events
		BMAC only 518 patients, 616 procedures, 397 M, 219 F Mean age: 54.3 ± 14.1		Density gradient centrifugation	For patients treated with additional adipose graft, 5–10 mL of BMC and adipose graft was injected		Significant improvements in self-rated functional and pain scores post-procedure	Minor complications: Swelling and pain post-treatment
		BMAC with adipose graft 163 patients, 224 procedures, 119 M, 105 F Mean age: 59.5 ± 10.3						
Centeno et al., 2014 [31]	KL grade 1–4, hip OA	216 patients, 124 M, 92 F	PRP and PL added to BMAC	Posterior superior iliac crest	1–4 mL of BMAC, 1 mL of PRP, and 1 mL of PL injectate solution	1, 3, 6 months, annually after treatment	Significant improvements in NPS and OHS. Patients ≤55 years old were likely to report greater improvements than older patients	No serious adverse events
		Mean age: 57 ± 10.6		Density gradient centrifugation				Minor complications: Swelling and pain post-treatment
Centeno et al., 2015 [32]	KL grade 1–4, knee OA	Two groups received BMAC with PRP and PL: 373 patients, 424 procedures (cell count data available for 409 procedures)	PRP and PL added to BMAC	Posterior superior iliac crest	1–3 mL BMAC produced	1, 3, 6 months, 1 year	Significant improvement in IKDC and LEFS scores	No serious adverse events
		Lower cell count 188 patients, 224 procedures, 145 M, 81 F Mean age: 54.5 ± 12.8		Density gradient centrifugation	Lower nucleated cell count in BMAC: $<4 \times 10^8$		Higher cell count group reported lower pain scores in comparison to the lower cell count group; however, there are no significant differences using other metrics	
		Higher cell count 170 patients, 185 procedures, 140 M, 45 F Mean age: 50.2 ± 15.6			Higher nucleated cell count in BMAC: $>4 \times 10^8$			

Centeno et al., 2015 [33]	Symptomatic OA at the glenohumeral joint and/or rotator cuff tear <1.5 cm	Two groups (102 patients, 115 shoulders):	PRP and PL added to BMAC	Posterior superior iliac crest	1–3 mL BMAC produced, dosage unspecified	1, 3, 6, 12 months, and annually post-treatment	Significant improvement in DASH and NPS scores	No serious adverse events
		OA only 34 patients, 27 M, 7 F Mean age: 52.1 ± 14.3		Density gradient centrifugation				
		Rotator cuff disorder 81 patients, 53 M, 28 F Mean age: 59.5 ± 11.9						
Centeno et al., 2018 [34]	KL grade 2–3, knee OA	Two treatment groups (48):	PRP and PL added to BMAC	Posterior superior iliac crest	5–7 mL injectate solution (75% by volume of BMAC, 12.5% by volume PRP, and 12.5% by volume PL)	6 weeks, 3, 6 months, 1, 2 years	Significant improvement in KSS-knee, KSS-function, SF-physical, LEAS, and ROM after BMAC treatment	No serious adverse events
		Exercise therapy control 22 patients Mean age: 57 ± 8.5 All 22 patients received BMAC treatment after 3 months (crossover group)		Density gradient centrifugation			Significant improvement in KSS-knee, SF-12 physical, and LEAS scores in crossover group compared to exercise therapy alone	Minor complications: Swelling and grinding with pain post-treatment
		BMAC with PRP and PL 26 patients Mean age: 54 ± 8.9						
Goncars et al., 2019 [35]	KL grade 2–3, knee OA	32 patients, 16 M, 16 F Mean age: 53.96 ± 14.15	Only MNC fraction of BMAC was used	Iliac crest	Up to 5 mL of MNC suspension was used	1, 3, 6, 12 months	Significant improvement in KOOS and WORMS score. Improvement in KSS score	No serious adverse events
		Two patients received BM-MNC injections in both knee joints		Density-gradient centrifugation	An average of $45.56 \pm 34.94 \times 10^6$ MNCs were injected			Minor complications: Swelling and pain post-treatment

(continued)

Table 17.1 (continued)

Study	OA pathology	Groups and number of patients	Additional factors	Marrow harvest	Dosage	Follow-up duration	Results	Safety
Kim et al., 2014 [36]	KL grade 1–4, knee OA	41 patients, 75 knees, 17 M, 24 F	Adipose tissue was added to BMAC	Anterior superior iliac crest or posterior superior iliac crest	7 mL of BMAC and 10 mL of adipose tissue to affected knee	3, 6, 12 months	Significant improvement in IKDC, KOOS, LKQ, SF-36, and VAS scores post-procedure	No serious adverse events
		Mean age: 60.7 (range 53–80)		SmartPReP2 platelet concentration system (harvest technology)	Estimated 2.4×10^5 adult stem cells and 1.8×10^9 MNCs			Minor complications: Swelling and pain post-treatment for up to 4 weeks
Oliver et al. 2015 [29]	KL grade 2–4, knee OA	70 (21 M, 49 F)	Lipoaspirate added to BMAC	Posterior superior iliac crest	3 mL of BMAC and 2 mL of lipoaspirate to affected joint; 1 mL of BMAC and 1 mL of lipoaspirate to medial joint capsule	90 days, 180 days, 1 year	Significant improvement in KOOS scores post-procedure	No serious adverse events
		Mean age: 60 (range 28–83)		Angel centrifugation system (Arthrex)				Minor complications: Transient pain and short-term swelling post-injection (<7 days)
Oliver et al., 2018 [37]	KL grade 2–4, knee OA	254 patients, 98 M, 156 F	Lipoaspirate added to BMAC	Posterior superior iliac crest	3 mL of BMAC to affected joint, 1 mL of BMAC and 1 mL of lipoaspirate to medial joint capsule, 1 mL of BMAC and 1 mL of lipoaspirate to other soft tissue injury to the knee	3, 6 months, 1, 2 years	Significant improvement in VAS, WOMAC pain and WOMAC stiffness scores post-procedure.	No serious adverse events
		Mean age: 60 (range 29–83)		Angel centrifugation system (Arthrex)				Minor complications: Increased pain, swelling, and experienced noise post-injection

Rodriguez-Fontan et al., 2018 [38]	KL grade 1–2, knee and hip OA	19 patients, 3 M, 16 F 25 joints: 10 knees, 15 hips Mean age: 58 ± 12.7 years	None	Anterior iliac crest BioCUE platelet concentration system (Zimmer Biomet)	12 mL of final BMAC, dosage unspecified	6, 12, 18, 24 months	Significant improvement in WOMAC scores post-procedure	No serious adverse events Minor complications (11 patients): Mild pain at BMC extraction site (24 h post-operation), pain 2 weeks post-injection, swelling
Shapiro et al., 2017; Shapiro et al., 2019 [39, 40]	KL grade 1–3, bilateral knee OA	Two treatment groups: 25 patients, 7 M, 18 F Mean age: 60 (42–68)	Platelet-poor plasma added to BMAC	Superior iliac crest	5 mL of BMAC with 10 mL platelet-poor bone marrow plasma to one knee	1 week, 3, 6 months	Significant improvement in OARSI ICOAP and VAS scores post-procedure	No serious adverse events.
		BMAC on right knee and placebo on left 13 patients *BMAC on left knee and placebo on right* 12 patients		Magellan autologous platelet separator system (Arteriocyte)	15 mL saline as placebo Estimated median of 56% mononuclear cells in 1 mL BMAC: 1.5×10^8 WBCs, 8.0×10^7 MNCs, 4.4×10^6 HSCs, and 3.4×10^4 MSCs	MRI only: 6, 12 months	No significant change was seen in quantitative T2 MRI mapping in second study	Minor complications: Effusions seen post-procedure, likely due to residual 15 mL product than inflammation

(continued)

Table 17.1 (continued)

Study	OA pathology	Groups and number of patients	Additional factors	Marrow harvest	Dosage	Follow-up duration	Results	Safety
Upadhyay et al., 2014 [14]	KL grade 0–1, knee OA	Two treatment groups: 50 patients, 12 M, 38 F *BMAC only* 25 patients, 7 M, 18 F Mean age: 56.12 ± 9.311 *Sodium hyaluronate control* 25 patients, 5 M, 20 F Mean age: 55.72 ± 7.673	BMAC was diluted in phosphate buffered saline	Posterior iliac crest Ficoll–Paque density gradient centrifugation	2 mL diluted BMAC or sodium hyaluronate	1, 3, 6 months	Significant improvement in WOMAC and VAS scores compared to controls at 6 months of follow up	No serious adverse events Minor complications: Transient pain at injection site (<2 days)

DASH Disabilities of the arm, shoulder, and hand, *IKDC* International Knee Documentation Committee, *KL* Kellgren–Lawrence, *KOOS* Knee Injury and Osteoarthritis Outcome Score, *KSS* Knee Society Score of Assessment and Function, *LEAS* Lower Extremity Activity Scale, *LKQ* Lysholm Knee Questionnaire, *NPS* Numeric Pain Score, *OARSI ICOAP* Osteoarthritis Research Society International Intermittent and Constant Osteoarthritis Pain Questionnaire, *OHS* Oxford Hip Scores, *PL* Platelet Lysate, *PRP* Platelet-Rich Plasma, *ROM* Range of Motion, *SF-12* Short Form-12 Scales, *VAS* Visual Analog Scale, *WOMAC* Western Ontario and McMaster Universities Arthritis Index, *WORMS* Whole Organ MRI Scoring

vdecreases in abnormalities after 6 months of treatment. Compared to the rest of the clinical studies we have identified, only the MNC fraction of BMAC was used in Goncars' study. The absence of other factors normally present within BMAC may alter treatment outcomes, indicating that the improvements seen in WORMS were due solely to the MNCs.

In contrast, Shapiro et al. used quantitative T2 MRI mapping to determine the regenerative capacity of BMAC in bilateral knee OA [39]. Each patient in the study received a BMAC injection to one knee and saline to the other, allowing the saline-injected knee to act as a placebo control. While the same short-term benefits of BMAC were evident, no significant changes that indicated cartilage regeneration due to BMAC were seen a year post-treatment. This led to the conclusion by the authors that BMAC failed to show regenerative potential as results were similar to the saline-injected knees. Interestingly, patients in the study reported improvements in the placebo knee, which may be indicative of the systemic effect of the MSCs originally injected to the treated knee or instead might be indicative of a placebo effect [40].

Of the 13 studies that have been listed in Table 17.1, 3 have reported the need for total knee or hip arthroplasty (TKA or THA) after BMAC injection. Patients receiving these interventions were those that did not respond positively to BMAC and typically had higher severity of OA compared to the rest of the group. Nevertheless, the need for knee or hip replacement makes up a minority in each study. In Centeno's 2018 study, before receiving BMAC treatment, 52% of the patients were candidates for TKA as they had KL Grade III OA. However, only 3 of the 48 patients received TKA during the follow-up period [34], perhaps indicating the success of the BMAC intervention. Rodriguez-Fontan reported that while 7 of the 19 patients in the study were unsatisfied with BMAC, only 2 received THA after 8 months post-treatment [38]. Of note, one of these patients was 65 and had pre-existing comorbidities such as diabetes, obesity, and osteoporosis, which would have contributed to the need for THA. Finally, in Kim's study, 22 of the 75 knees treated showed unfavorable results, but only four of these knees underwent additional interventions such as TKA, high tibial osteotomy, and unicondylar knee arthroplasty [36]. However, the medical history was not indicated for most patients that received these additional treatments, so it is unknown what the patient's OA severity was and what other confounding conditions they might have had. As the time of follow-up across all these studies is only available for up to 2 years, long-term data on the number of patients requiring TKA after early-stage BMAC treatment are needed to better determine the long-term efficacy of BMAC.

The severity of OA at the time of therapy could possibly be an important predictor of clinical outcomes. In one study by Centeno et al. in 2014, it was found that patients with lower OA severity (KL Grade 2) were significantly more likely (2.2 times) to report ≥50% improvement on the subjective reported outcome scale than KL Grade 3 patients [30]. However, this correlation did not extend to other outcomes such as the lower extremity functional questionnaire (LEFS) or the numeric pain scale (NPS). Similarly, Kim et al. reported that as KL grade increased, the response to BMAC injection was poorer, implying that patients with early OA benefitted greatly to the treatment compared to more severe OA [36]. Unlike the studies that we have listed in Table 17.1, the study by Kim and colleagues included additional treatment of PRP post-injection of up to 4 weeks for patients that experience pain and swelling at the joint site. This may lead to confounding effects of the initial treatment, masking the true effect of BMAC. Contrary to these studies, Oliver et al. showed that the severity of OA did not affect treatment outcomes, reporting that Visual Analog Scale (VAS), Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain, and WOMAC stiffness scores across KL Grades 2–4 showed similar improvements [29, 37].

Age may also be another important predictor of clinical outcomes. In 2014, Centeno et al. conducted a separate study on the efficacy of BMAC on hip OA. They found that patients younger than 55 years old were likely to answer

more favorably on the numeric pain scale (NPS) and Oxford Hip Scores (OHS) [31]. However, age did not have a strong correlation with positive treatment outcome. When grouping patients by age, Kim and colleagues found that older patients showed marginally inferior scores but were still statistically insignificant [36]. Thus, more investigation needs to be done to determine the effect of age on BMAC treatment efficacy. Moreover, other predictors such as gender and BMI were shown not to significantly affect outcome scores [29, 31–33].

Such variations in reporting especially with regard to whether KL grade correlates with favorable outcomes may suggest various possibilities. The method in which BMAC was processed and prepared to produce the final injectate may lead to differences in cellular composition that ultimately affect treatment outcomes. Another factor that is glaring across all the studies identified in Table 17.1 is the different measurement outcomes used to assess OA progression, leading to the inconsistencies across various studies. Furthermore, the follow-up surveys that are conducted in most studies are self-assessments by the patients. As these tests are subjective in nature, the response to and perception of these assessments, along with the degree of treatment satisfaction measured, will vary from patient to patient.

17.5 Perspectives

17.5.1 Augmentation of BMAC with Additional Factors

The combinatorial delivery of BMAC along with additional factors that could possibly enhance the therapeutic effects of BMAC has been trialled. In four studies, BMAC was injected along with PRP and platelet lysate (PL) [30, 32–34], while in one study, platelet-poor plasma was used [40]. In vitro, when added to culture medium, both PL and PRP improve MSC proliferation [42, 43] and differentiation into chondrocytes [43, 44]. Thus, they may improve the activity of MSCs when injected along with BMAC. Four studies reported the addition of adipose tissue (in the form of lipoaspirate) to the BMAC before injection. In one report, the addition of an adipose graft to BMAC did not produce any detectible benefits over the use of BMAC alone, upon intra-articular injection [30]. Other studies injected BMAC along with a few milliliters of lipoaspirate into involved soft tissue structures to utilize the inherent scaffolding properties of adipose tissue [29, 37]. However, as no control arm (BMAC without lipoaspirate) was included in these studies, the additional beneficial effects of lipoaspirate are unknown. Overall, it is undetermined whether the beneficial effects reported in these studies are due to the BMAC, the adjuvant factors, or a synergistic effect of the two. In the absence of further studies with appropriate controls, the optimal method to augment BMAC therapy remains undetermined.

17.5.2 Allogeneic or Autologous BMAC

All the studies reviewed in this chapter (Table 17.1) used autologous BMAC. While the use of autologous BMAC has obvious advantages due to the lack of immune response, it may not be as effective in older patients. The number of stem cells present in BMA and their efficacy is significantly lower in older patients than younger ones [45]. The use of allogeneic BMAC from younger patients may result in greater efficacy; however, there is currently a lack of studies utilizing allogeneic BMAC. This may be due to the potential safety concerns of graft versus host disease or secondary infection from the donor [46]. Until these concerns can be appropriately addressed, autologous BMAC may be the only suitable option for treatment as its benefits outweigh the risks that come with allogeneic BMAC.

17.5.3 Cellular Composition

The method of BMAC processing has a significant impact on the cellular composition of the BMAC (Fig. 17.1c). In two of the studies

reviewed, Ficoll–Paque density gradient centrifugation was used to concentrate BMA [14, 35]. Thus, in these studies, BMAC only contained the MNC fraction. In five studies by Centeno et al., the method of density gradient centrifugation (whether Ficoll–Paque or commercial device) was not mentioned. However, in these studies, BMAC was combined with PRP/PL before injection, introducing platelets, plasma, and growth factors. In the other five studies that used a commercial BMAC device, the BMAC included the TNC fraction, platelets, and a small amount of plasma. Varying efficacies of BMAC in these groups that used different methods could be due to the differences in cellular composition due to the BMAC processing method used and combination with other biologics.

17.5.4 Dosage

The amount of BMAC that was finally injected ranged from 1 to 12 mL in the studies reviewed (Table 17.1). However, only 4 of the 12 studies reviewed analyzed the cellular concentration of the BMAC product. It is probable that patient characteristics and the method of BMAC processing would have a significant effect on cellular concentration within a given amount of BMAC. Thus, it is difficult to compare dosages in different studies if the cellular concentration is not specified, even if the injection volumes were similar. The age of the patient may also affect the minimal effective dose of autologous BMAC. In older patients, larger volumes of BMA may need to be aspirated in order to achieve a similar stem cell yield as younger patients.

In one study where patients received either a low dose ($<4 \times 10^8$ nucleated cells) or a high dose ($>4 \times 10^8$ nucleated cells), both groups reported significant improvements in pain and function of the osteoarthritic knee joint. The only significantly improved outcome in the high cell dose group was a reported lower post-treatment pain scale value [32]. Although there were no differences in functional outcomes, the improved pain relief with a higher cell dose is an important finding. Another study that involved the injection of only BM MNCs used an average cell dose of $45.56 \pm 34.94 \times 10^6$ cells [35], which was effective in causing a significant improvement in Knee Injury and Osteoarthritis Outcome Score and WORMS scores after 6- and 12-month follow-ups.

Two studies estimated the number of MSCs injected—while one study injected a median of 4.4×10^6 HSCs and 3.4×10^4 MSCs (out of 8×10^7 total MNCs) [40], another estimated 2.4×10^5 adult stem cells and 1.8×10^9 MNCs were implanted when using a mixture of BMAC and adipose tissue [36]. These reported numbers of MSCs are far lower than the dosage of culture-expanded MSCs that appears to be effective for OA treatment [4]. This is expected, as other reports have noted that the amount of MSCs implanted using BMAC is several magnitudes lower than if culture-expanded MSCs are used [32, 47]. However, the presence of other cell types and concentrated growth factors in BMAC may lead to a combinatorial effect in the management of pain and inflammation, as well tissue regrowth. Overall, the dose of MNCs or TNCs required to achieve an effective clinical outcome for the treatment of OA is still unresolved.

More cell dose response studies are required in order to elucidate the appropriate BMAC dosage for the maximization of clinical outcomes. Future clinical studies should quantify cellular concentration in the BMAC before implantation. Understandably, there are difficulties in enumerating MSC numbers in BMAC by counting CFU-Fs or using flow cytometry, as these methods can be time consuming and require dedicated technical staff and equipment. However, the enumeration of nucleated cells within the BMAC using either a hematology analyzer or hemocytometer is both quick and feasible in a regular clinical setting.

17.5.5 Safety and Limitations of the BMAC Technique

Most of the clinical studies reviewed (Table 17.1) did not report any serious adverse effects after BMAC treatment. However, com-

mon minor adverse effects included short-term pain at the site of bone marrow harvest and transient swelling and pain at the site of injection up to 7-day post-injection. Centeno et al. reported 6% adverse events in the BMAC group (including two severe events) and 8.9% adverse effects in the BMAC + adipose graft group (including one severe event) [30]. However, they did not define what qualified as a severe adverse event. Overall, BMAC treatment appears to be a generally safe procedure, with few serious adverse events reported.

The invasive harvesting of autologous bone marrow aspirate from the iliac crest is a significant disadvantage of the BMAC technique, which can lead to pain at the harvest site. The presence of white blood cells such as monocytes and neutrophils in BMAC can cause the increased secretion of pro-inflammatory cytokines such as IL-1β and IL-8 [17], promoting inflammation at the injection site. However, the presence of IL-1β and IL-8 is offset by the high levels of IL-1ra found in BMAC, which may lead to an overall anti-inflammatory effect.

17.6 Conclusions

BMAC is one of the emerging orthobiologics that have shown promise for the treatment of early osteoarthritis. Several studies have evaluated BMAC as a treatment for OA, and it was found to be a generally safe treatment. Many studies have reported the reduction of pain and improved joint function after BMAC treatment. However, the regenerative effects of BMAC are still unresolved, and the varying efficacies of BMAC therapies reported indicate the need for the standardization of processing technique and dosage applied. The lack of information on cellular composition and concentration in many clinical studies makes it difficult to compare across studies and determine the true efficacy of BMAC, especially with regard to the minimum effective dose. Overall, longer term follow-up studies are required to determine the exact effects of BMAC treatment on disease progression.

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18 The Role of Alignment Correction With and Without Chondral Repair

Osama Aweid, Lachlan Batty, and Alan M. J. Getgood

18.1 Introduction

Focal osteochondral defects of the knee are common. In a study of over 30,000 arthroscopies, 5% of patients under the age of 40 were reported to have a grade IV lesion [1]. These defects can be responsible for progressive pain and disability and pose a treatment challenge in young, active patients, where arthroplasty options are inappropriate. Advancements in cartilage restoration procedures have expanded the orthopaedic surgeon's armamentarium in treating young patients with knee pain and joint surface defects by offering a more biologic solution. Concomitant surgeries such as realignment procedures which optimize the biomechanics are also important treatment tools and can synergistically influence the clinical outcome of cartilage restoration.

This chapter will review the association of coronal plane alignment, load distribution and cartilage health and describe the clinical indications for alignment correction as well as our preferred surgical techniques. It will review the current studies on outcomes of alignment correction with and without chondral repair as well as provide a treatment algorithm for patients presenting with early unicompartmental knee chondral pathology with concurrent coronal malalignment.

18.2 The Association of Load and Cartilage Health

18.2.1 Cartilage Strain and Activity

Articular cartilage (AC) is composed of individual chondrocytes bound together by an extracellular matrix (ECM). Its function is to support and distribute weight-bearing forces in diarthrodial joints and to reduce friction by providing a smooth lubricated surface. Nonetheless, AC has poor healing potential and is prone to both acute injury and degenerative conditions. This may be exacerbated by excessive load or maldistribution of load within the joint.

Magnetic resonance imaging in combination with high-speed dual-fluoroscopy has been used to characterise cartilage deformation during or after various activities. The results show that cartilage strains are influenced by activity type and location within the joint. A literature review by Sanchez-Adams et al. [2] has shown that during normal activities, diurnal strains range from 0% to 10%, post-activity strains range from 5% to 15%, and dynamic strains during activity, such as a short bout of running, range from 15% to 35% with greater strains seen on the medial side of the

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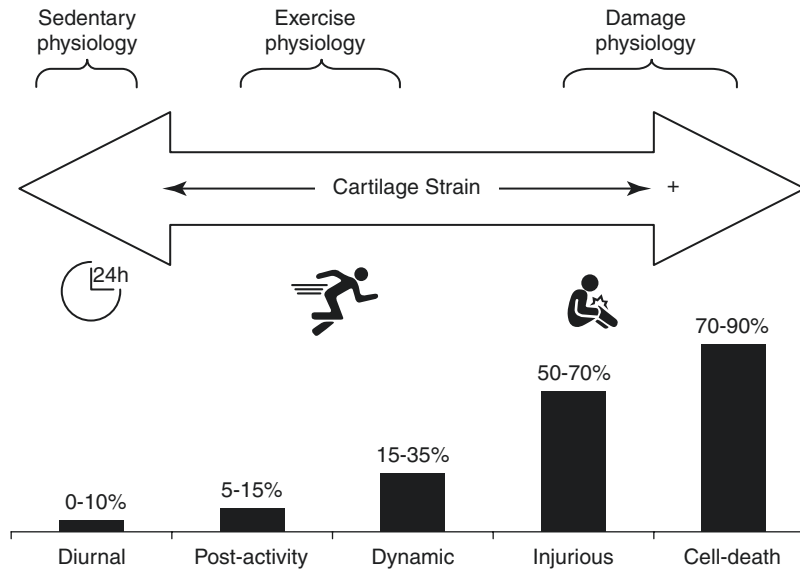

Fig. 18.1 Diurnal, post-activity, dynamic during activity, and damage inducing cartilage strains

joint (Fig. 18.1). Higher strain magnitudes of 50–70% can cause cartilage injury, whereas levels above 70% eventually induce cell death via necrosis and apoptosis.

18.2.2 Changes in Chondrocyte Function in Response to Loading

Mechanical loading of AC modulates chondrocyte behaviour through mechanotransduction, a process where cells convert a mechanical stimulus to electrochemical activity. This initially involves matrix and cell deformation due to mechanical load. This causes hydrostatic and osmotic pressure changes, altered matrix water content and changes in ion concentration. These changes are detected by osmomechanosensitive ion channels on the chondrocyte cell surface such as Transient receptor potential vanilloid 4 (TRPV4).

Physiologic dynamic strain of AC (15–35%) results in TRPV4-mediated Ca2+ signalling, which decreases expression of catabolic and pro-inflammatory genes and enhances synthesis of ECM components such as proteoglycans, collagens and cartilage oligomeric matrix protein (COMP) [3]. Higher strain magnitudes above physiological levels leads to direct cellular damage as well as pathologic loss of ECM constituents through the enhancement of catabolic enzymes, such as matrix metalloproteinases (MMPs) and aggrecanases [4]. Similarly, the absence of normal joint loading also leads to the inhibition of ECM synthesis. Animal models have shown that unloaded passive movements result in cartilage atrophy caused by increased MMPs and A Disintegrin and Metalloproteinase with Thrombospondin motifs [5]. The result is thinner and softer AC, which, in turn, is more susceptible to trauma and degenerative changes. These findings may explain the association of osteoarthritis (OA) with a more sedentary lifestyle and the lower occurrence of OA in recreational runners [6].

Natoli et al. [7] conducted an in vitro study to assess the more long-term impacts of impact loading on AC health. The results showed that cell death increased and tissue stiffness decreased 24 h following high impact, which persisted at 1 and 4 weeks. These findings suggest that an ongoing degenerative process is initiated following high impact loading and point to possible pathways to prevent or reverse AC loss, as discussed below.

18.3 Biomechanics of Gait and Coronal Plane Alignment

Due to the relationship between dynamic joint loading and cartilage health, epidemiological studies have demonstrated the importance of the knee adduction moment (KAM) in the pathogenesis of knee chondral disease. The KAM is a reliable indirect measure of medial knee load during walking and is calculated as the product of the ground reaction force (GRF) in the frontal plane and the perpendicular distance from the GRF to the knee joint centre of rotation, called the frontal plane lever arm [8] (Fig. 18.2).

In a systematic review by Foroughi et al. [9], the KAM was found to be higher in those with more severe OA than in those with less severe disease. Increased KAM was also related to increased knee pain and radiographic OA severity as well as faster OA progression. The latter is likely a consequence of the progressive increase in magnitude of KAM that results from worsening joint malalignment and laxity secondary to morphological changes in the affected compartment such as medial articular cartilage loss (Fig. 18.3).

To prevent the progression of AC loss and medial knee symptoms, various interventions have focussed on reducing the abnormal gait-related mechanical stresses on the knee joint resulting from an increased KAM. These treatments aim to manipulate either the GRF and or the lever arm that produces KAM. Conservative measures include weight loss and strengthening of the muscles around the knee. Gait retraining can also be effective and includes walking with an increased external and internal foot progression angle [10]. Surgically, valgus producing realignment osteotomy is a robust method of manipulating the lever arm and can be a powerful tool in reducing KAM (Fig. 18.2).

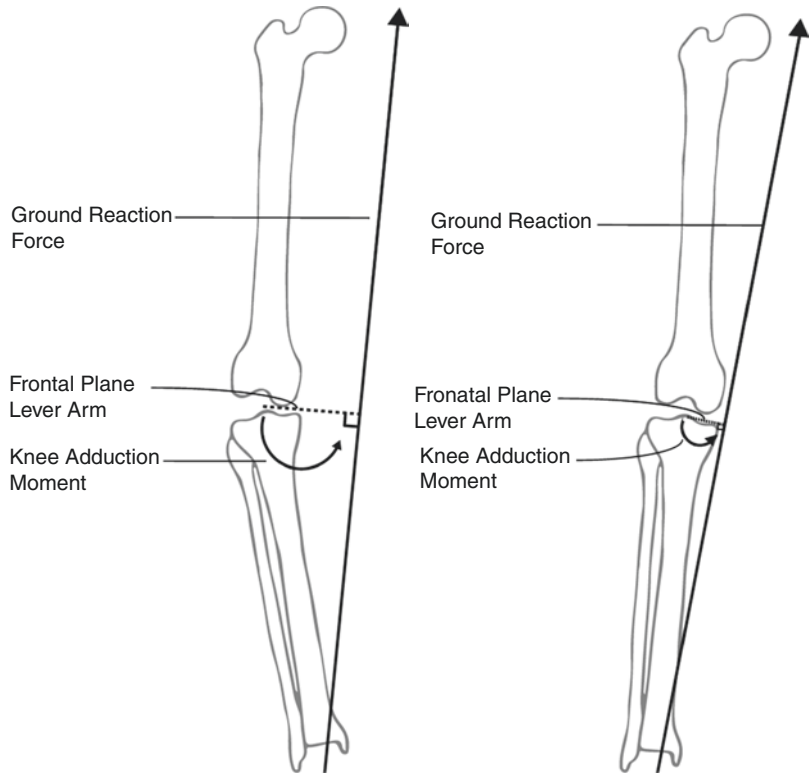

Fig. 18.2 The knee adduction moment during walking. This is higher in the presence of varus (left) versus valgus (right) alignment

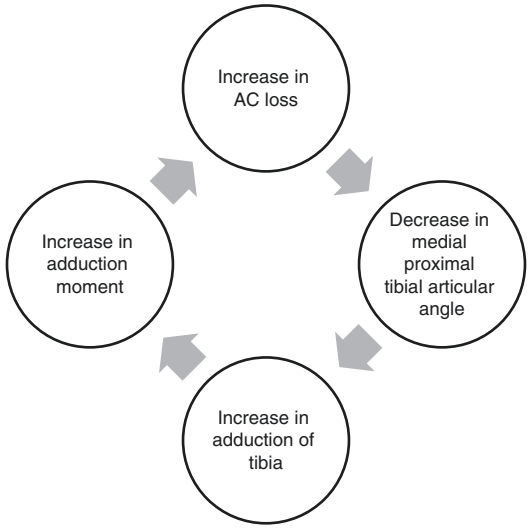

Fig. 18.3 Progressive increase in the magnitude of the knee adduction moment that results from continued medial articular cartilage loss

18.4 Realignment Osteotomy

18.4.1 Indications

In the context of knee pathology, osteotomy can be performed above or below the joint with opening or closing wedges most commonly performed, although many other types have been described. Originally described by Jackson and Waugh [11], the lateral closing wedge HTO was later popularized by Coventry [12]. These procedures subsequently fell out of favour for advanced degenerative disease with the advent of modern arthroplasty. However, there has been renewed interest in osteotomy with the development of joint preservation techniques, including cartilage regenerative procedures and meniscus allograft transplantation. Medial compartment overload in the presence of genu varum is the most common clinical scenario, where HTO is particularly attractive in the treatment of young, healthy patients who hope to maintain activity levels and postpone the need for joint arthroplasty. Our preferred surgical technique in this setting is the medial opening high tibial wedge osteotomy. Relative contraindications include individuals with severe medial compartment AC loss/advanced osteoarthritis, tricompartmental arthrosis, markedly decreased range of motion (ROM) and those aged >65 years [13].

18.4.2 Medial Opening High Tibial Wedge Osteotomy

Long-leg standing, weight-bearing radiographs are obtained pre-operatively for all patients undergoing a medial opening wedge HTO. We commonly plan the osteotomy size using a modification of the technique described by Dugdale [14] (Fig. 18.4). This involves drawing a line from the centre of the femoral head to and through the desired weight-bearing point of the knee (Line 1). This point lies slightly lateral to the tip of the lateral tibial spine. A second line is then drawn from this point to the centre of the tibiotalar joint (Line 2). Next, the intended osteotomy line is drawn from the medial cortex, around 4 cm below the joint line, to the lateral cortex towards the tip of the fibular head (Line 3). Line 2 is then extended superiorly to a length equal to the osteotomy line. Finally, the perpendicular distance from the superior extent of line 2 to Line 1 is measured to give the size of the osteotomy opening (line 4).

A knee arthroscopy can be performed on selected cases initially to assess associated chondral or meniscal pathologies. Following this, a medial approach to the proximal tibia is utilized whereby the superficial MCL is elevated with a Cobb elevator and cautery used to open up the anterior interval, and then the posterior recess in front of popliteus. The Cobb elevator is used again to elevate the muscle belly of popliteus off the posterior proximal tibia. With protective anterior and posterior retractors in place, a tibial guide pin is subsequently inserted in line with the planned osteotomy and the osteotomy completed with an oscillating saw and osteotomes. A spreading osteotome followed by a triangular wedge are introduced into the osteotomy and distraction performed to the templated width. A Tomofix plate (Depuy Synthes, Raynham, MA) is then applied to allow early weight-bearing as tolerated at 2 weeks post-operatively. Summary

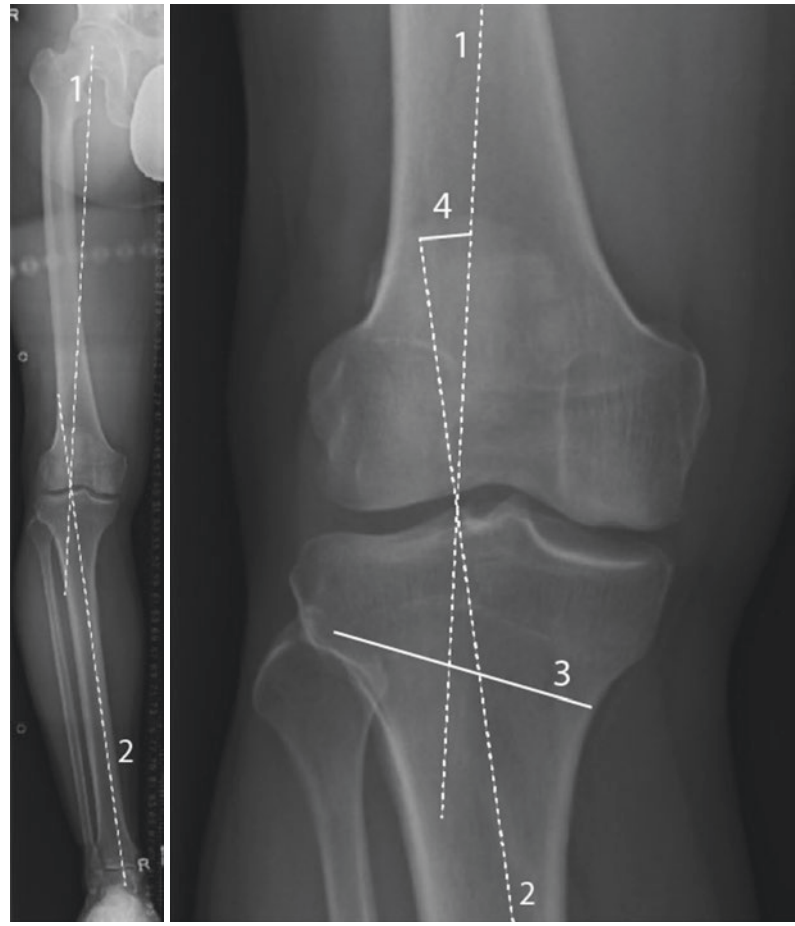

Fig. 18.4 Preoperative HTO templating using long-leg standing, weight-bearing radiographs. The osteotomy line [3] is drawn around 5 mm short of the lateral tibial cortex

Intraoperative of the procedure is presented in Fig. 18.5.

Having restored a near neutral or slight valgus mechanical alignment, dynamic loading of the damaged cartilage is reduced through a lowering of the frontal plane the lever arm that produces KAM (Fig. 18.2). Based on the studies relating load to AC health presented earlier, offloading the damaged cartilage should theoretically create a more suitable environment for the healing of medial compartment cartilage repair procedures. Our preference is to perform AC repair where indicated by extending the anteromedial skin incision and create an anteromedial arthrotomy to expose the medial femoral condyle (MFC), as shown in Fig. 18.6. Here, a 10-mm osteochondral autograft transfer (OAT) plug was taken from the non-weight-bearing part of the medial trochlea and successfully transplanted into an osteochondral defect over the medial femoral condyle. The remaining small defects were then drilled with a 2.4-mm pin to encourage bleeding and subsequent peripheral fibrocartilaginous healing.

18.4.3 Medial Closing Wedge Distal Femoral Osteotomy

To offload the lateral compartment of the knee, we typically employ a medial closing wedge distal femoral osteotomy (DFO). Templating is again performed using the Dugdale method; however, the tendency to overcorrect with a DFO should be noted given the more proximal position of the osteotomy; therefore, the angular change is effective over a longer distance compared to HTO. Arthroscopy can be performed initially if indicated. Following this, a medial sub-vastus

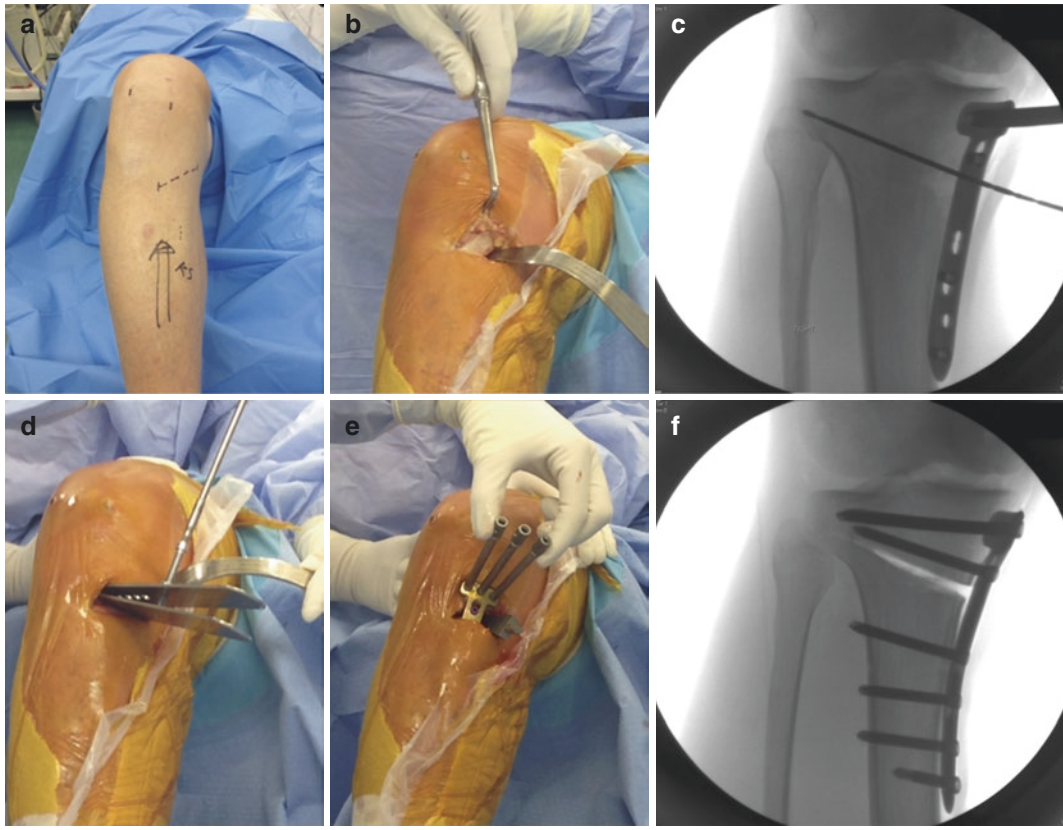

Fig. 18.5 (**a**, **b**) Medial opening wedge HTO approach. (**c**) Guide pin insertion in line with planned osteotomy (**d**, **e**) Spreading osteotome and plate application. (**f**) Final image intensifier image.

approach to the distal femur is used with elevation of the vastus medialis and the extensor mechanism. The femoral artery is in the proximal region of the approach (approximately 15 cm from the joint line) and care should be taken. Two converging guidewires are placed from the supracondylar region of the distal femur meeting at the desired hinge point (Fig. 18.7). If the guidewires are equidistant in length to the hinge point, there will be no cortical step off when the osteotomy is closed. The distance between the wires at the medial cortex corresponds to the pre-operative templating. We routinely use a biplane osteotomy with the addition of a coronal cut posterior to the trochlea to avoid intra articular extension. This cut also helps control rotation when the osteotomy is closed. With protective anterior and posterior retractors, the osteotomy is completed with an oscillating saw and osteotomes to remove the desired wedge. The lateral cortex hinge can be drilled with a K wire (controlled osteoclasis) to encourage plastic deformation and/or a wire placed across the hinge to reduce risk of fracture. The osteotomy is gently closed with manipulation of the leg. A pre-contoured locking plate is applied to allow for immediate weight bearing.

18.5 Clinical Outcomes of Realignment Osteotomy With and Without Chondral Repair

18.5.1 Osteotomy in Isolation

Surgery-induced reductions in KAM have been associated with improved clinical outcomes in the varus knee. Birmingham et al. prospectively evaluated Knee injury and Osteoarthritis Outcome Scores (KOOS) at 5-year post-op in

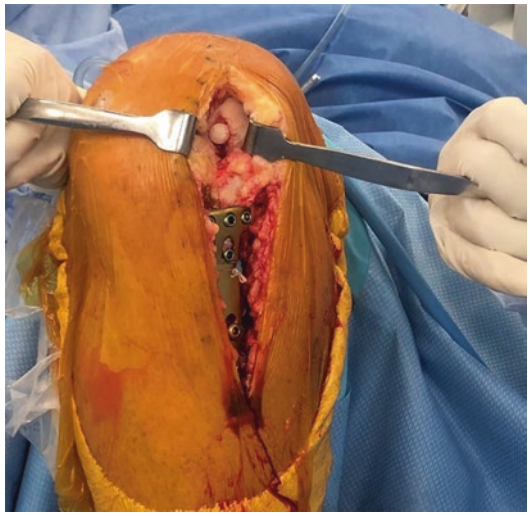

Fig. 18.6 Intra-operative photograph of a medial opening wedge HTO with a medial femoral condyle OATS procedure. A 10-mm OATS plug was taken from the non-weight-bearing part of the medial trochlea and successfully transplanted into an osteochondral defect over the medial femoral condyle. Of note, a medial meniscal posterior root repair was also performed in this case. To do this, ultrabraid sutures were placed through the meniscus in a luggage tag fashion and the free ends shuttled down through a tibial tunnel and tied over the plate (note sutures). (Source: Dhollander A., Getgood A. (2017) The Role of Alignment in Meniscal Tears and the Role of Osteotomy. In: LaPrade R., Arendt E., Getgood A., Faucett S. (eds) The Menisci. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-53792-3_11)

170 HTO patients and found that patients with reductions in knee adduction moment of 1.14–1.74% had highest 5-year KOOS scores [15]. In terms of survivorship (absence of conversion TKR), studies have reported between 30% [16] and 85% [17] at 20 years. The absence of conversion to TKR, however, is a crude measure as to the success of HTO and may not be reflective of symptomatology or patient-reported outcome measures.

Whilst the importance of alignment in chondral surgery has been established, the addition of chondral surgery to augment HTO remains controversial. Second look arthroscopic studies and MRI studies have demonstrated that chondral regeneration can occur after HTO in the absence of associated chondral interventions. Parker et al. noted a positive change in delayed gadolinium-enhanced magnetic resonance imaging of cartilage in the medial compartment of ten patients undergoing medial opening wedge HTO [18]. Jung et al. [19] assessed clinical outcomes and graded cartilage regeneration at a second-look arthroscopy in 159 knees at a mean of 2 years after medial opening wedge HTO. Some degree of fibrocartilage regeneration was seen on the medial femoral condyle in 92% of knees and in the medial tibial plateau in 69%. The authors concluded that cartilage of the medial femoral condyle and medial tibial plateau could be partially or entirely covered by newly regenerated cartilage at 2 years after adequate correction of varus deformity without cartilage regeneration strategies.

18.5.2 Non-Comparative Series of Osteotomy in Combination With Cartilage Surgery

Favourable results of HTO combined with microfracture [20], microfracture and abrasion therapy [21], autologous chondrocyte implantation (ACI) [22] and osteochondral allograft transplantation [23] have been demonstrated; however, the absence of a control group makes it difficult to interpret the effect of either treatment in isolation or establish if there is a cumulative or synergistic relationship. A 2017 systematic review by Kahlenberg et al. [24] reported on medium to long-term outcomes after cartilage restoration procedures performed with a concomitant HTO. The authors identified 18 eligible studies reporting on 839 knees. The most common cartilage preservation techniques included microfracture (22.2%), microfracture plus chondral abrasion or debridement (16.7%) and ACI (16.7%). Medial opening wedge HTO was performed in 83.3% of cases. Eleven studies reported on survivorship of 539 knees with a 6.8% conversion to knee arthroplasty at a mean follow-up of 6.2 years. Heterogeneous patient-reported outcome measures used across the studies showed improvements. The authors concluded that HTO with cartilage restoration procedures provides reliable improvement in functional status in the medium- to long-term period after surgery and

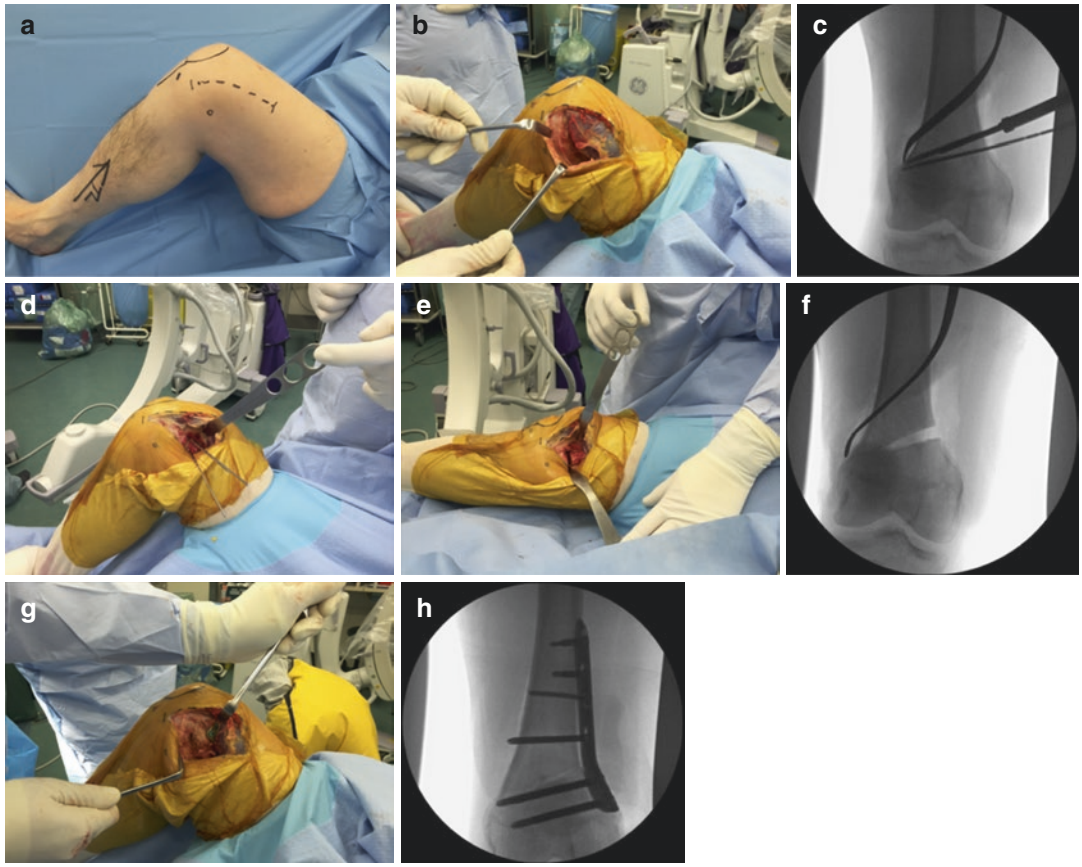

Fig. 18.7 (**a**, **b**) Medial closing wedge DFO approach. (**c**, **d**) Two converging guidewires aimed at the desired hinge point. (**e**, **f**) Desired wedge removed with addition of coronal cut posterior to the trochlea. (**g**, **h**) plate application and final image intensifier image. (Source: Dhollander A., Getgood A. (2017) The Role of Alignment in Meniscal Tears and the Role of Osteotomy. In: LaPrade R., Arendt E., Getgood A., Faucett S. (eds) The Menisci. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-53792-3_11)

has potential to delay or avoid the need for knee arthroplasty surgery. Further, the degree of malalignment where an osteotomy is indicated remains to be determined.

18.5.3 Comparative Series of Osteotomy With and Without a Chondral or Biologic Intervention

Feruzzi et al. [25] retrospectively reviewed 56 patients affected by medial osteoarthritis with varus alignment. Importantly, inclusion criteria included Kellgren–Lawrence grade 3 or 4 changes and ICRS grade 3 or 4 changes representing advanced chondral changes. A group of 20 patients treated by HTO in isolation were compared to 18 patients treated with HTO and concomitant ACI and 18 patients treated by HTO and concomitant microfracture. Groups were similar at baseline except for body mass index (BMI), which was higher in the microfracture group. At final follow-up 11 years, all treatment groups demonstrated improvements in Hospital for Special Surgery (HSS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores. However, isolated HTO and combined HTO + ACI demonstrated similar results, which were superior to HTO and microfracture. The effect of confounding by increased BMI in the microfracture group remains to be determined.

Wakitani [26] randomly allocated 24 patients to either HTO or HTO with transplantation of bone-marrow-aspirated and culture-expanded mesenchymal cells. At 42-week follow-up, there was no different in HSS scores between groups; however, the arthroscopic and histological grading score was better in the cell-transplanted group. Jung et al. [27] demonstrated equivalent clinical and histological outcomes following HTO with and without subchondral drilling in 61 patients with medial OA knees at 24 months. Similar results were reported by Schultz et al. [28] who found no difference in clinical outcome in a prospective study of 105 knees who underwent HTO in isolation, with diagnostic arthroscopy, with arthroscopic drilling or with abrasionplasty. Cartilage regeneration was thicker and more stable in association with the arthroscopic interventions.

A 2018 systematic review by Filardo et al. looked at the addition of cartilage treatment to HTO [29]. The authors noted the literature consisted of low-quality studies. Comparative studies showed that while surgical treatment targeting the cartilage layer led to an improvement in the treated tissue, this did not translate into clinical improvement. The authors concluded that there is 'no evidence to support the effectiveness of a cartilage treatment combined with HTO in patients affected by OA in misaligned joints'. Most studies included, however, were of older patients and had no long-term follow-up. As growth of repair cartilage progresses slowly and can take more than 5 years, clinically significant differences in comparative outcome may not be evident in the short term.

18.5.4 Comparative Series of Cartilage Surgery With and Without an Osteotomy

Correction of malalignment in cartilage repair surgery is important. In a review of consecutive failed cartilage repair surgical procedures, Krych et al. [30] found untreated malalignment was a contributory factor in 56% of cases. In a non-randomised case-control study, Bode et al. [31] reviewed 43 patients with an isolated cartilage defect of the medial femoral condyle (MFC) and subtle varus alignment (under 5°). A prospective cohort of 19 patients who were treated with HTO and MFC autologous chondrocyte implantation (ACI) was compared to a historical control cohort of 24 patients treated with MFC ACI in isolation. Mean patient age was 39 years; mean varus deformity was 2.84°. Groups were similar in terms of BMI, alignment and chondral lesion size. The mean post-operative valgus in the HTO group was 8.16°. At mean follow-up of 72 months, there were reduced re-interventions in the combined HTO and ACI group (10.5% versus 41.67%; $p = 0.023$). There was also a trend to increased KOOS and WOMAC scores in the combined HTO and ACI group; however, these did not reach statistical significance. It is possible that the low numbers in this study resulted in a type 2 error.

18.6 Treatment Algorithm

Before undertaking surgery, the clinician must take into consideration the characteristics of the chondral injury, the biomechanics of the lower limb and the physical condition and requirements of the patient. The patient's willingness to accept a prolonged rehabilitation protocol and the individual's expectation of the results will have an impact on treatment choice and success.

Evaluation begins with a full history including smoking status and previous trauma. History of trauma in conjunction with intermittent joint line pain at rest or at night, an effusion, mechanical symptoms (clicking, catching and locking) and localised joint line pain during weight-bearing can suggest a focal chondral defect though not specifically. Examination begins with an inspection of lower limb alignment, muscle bulk, signs of previous surgery and range of motion (ROM) followed by a gait assessment for abnormalities particularly a varus thrust. Palpation should also include the Pes Anserine bursa to rule out inflammation. Ligamentous, patellofemoral, neurovascular and meniscal status should be assessed for completion. Finally, relief of symptoms with an unloader brace may be predictive of a good outcome with realignment surgery.

Radiological assessment begins with bilateral long-leg standing weight-bearing, full extension knee anteroposterior (AP), 45° knee flexion weight-bearing AP (Rosenberg), lateral knee, and patella skyline views to identify the presence of malalignment and/or arthrosis. Where osteoarthritis is not diagnosed on plain radiograph, MRI is vital in evaluating the condition of the AC, menisci, bone marrow and ligamentous structures.

18.6.1 Our Decision-Making Process

All patients prior to being considered for surgery must have failed an adequately performed neuromuscular training rehabilitation program with the optional addition of intra-articular injection therapy to aid in the rehabilitation process by reducing pain and inflammation.

In the event of failing non-operative management, patients who are confirmed to have radiological malalignment, particularly in those whose alignment is asymmetric, may be offered realignment surgery as a primary procedure. The decision to perform a concomitant or staged articular cartilage restoration procedure is dependent on a number of factors, including the size and location of the defect and the status of subchondral bone (Fig. 18.8). Patient factors such as employment, activity goals, comorbidities, smoking status and

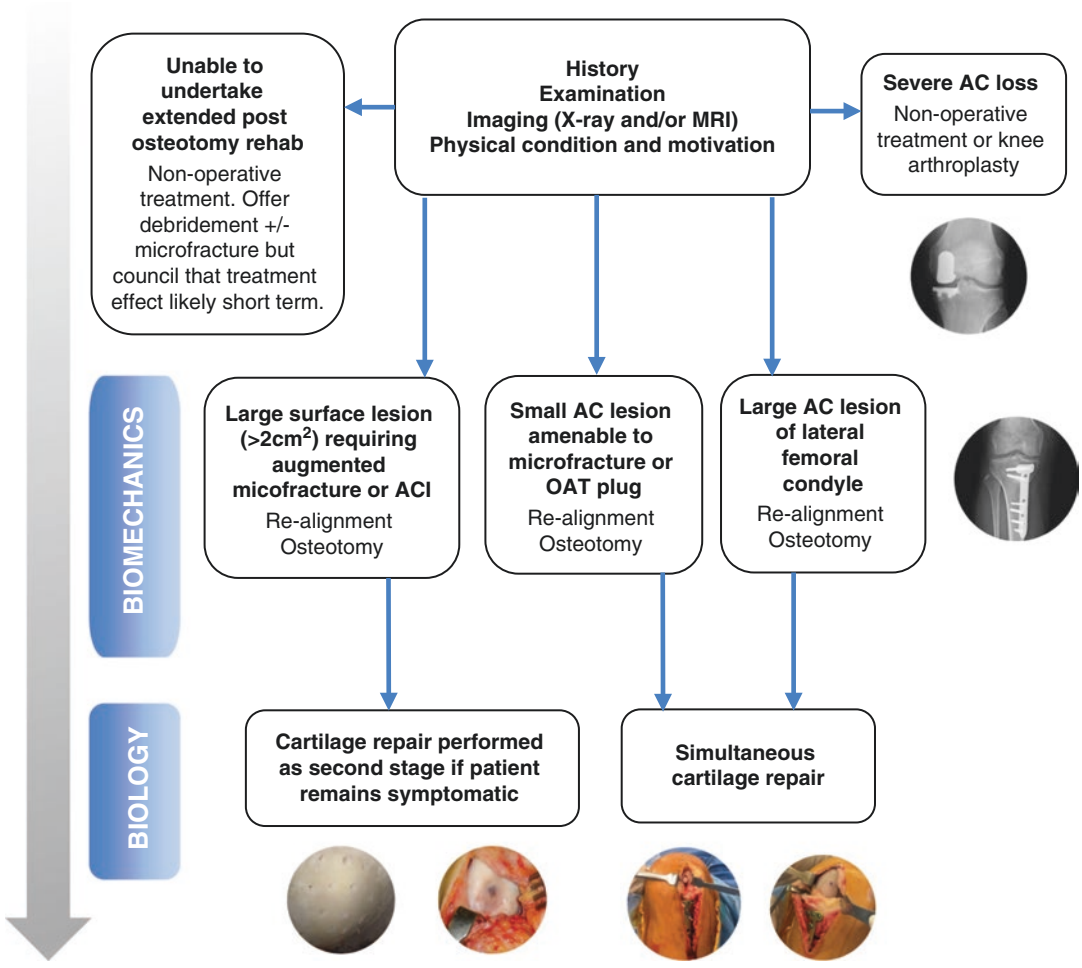

Fig. 18.8 Treatment algorithm for patients' articular cartilage pathology with concomitant coronal malalignment. Prior to surgery, patients must have failed an adequately performed neuromuscular training rehabilitation program with the optional addition of intra-articular injection. Ligament reconstruction and meniscus allograft transplantation where indicated are further treatments to optimize patient biomechanics

age should also be taken into consideration. Furthermore, access to cartilage treatment technology and associated costs should be taken into consideration, based upon geographical location of practice.

Scenarios that tend to push us towards performing realignment osteotomy and cartilage restoration as a simultaneous procedure include large osteochondral lesions of the lateral femoral condyle, in which the lack of bone stock in the condyle has a detrimental effect on the tibiofemoral articulation. Small lesions that can be treated either with a microfracture or OAT plug may also be performed simultaneously. Larger surface lesions (>2 cm^2), where an augmented microfracture may be indicated (or ACI-cell-based treatment), tend to be done in a staged fashion. The second stage is only performed in the event that the patient remains symptomatic.

18.7 Conclusion

Mechanical load has been shown to have a significant bearing on cartilage health. In order to successfully treat patients with knee pain and joint surface lesions, the surgeon must, therefore, adequately address the patient's biomechanical deficiencies and address appropriately. There is clear evidence that realignment osteotomy positively alters lower limb biomechanics and results in sustained improvements in patient centred outcomes. The addition of cartilage repair may act synergistically to further improve clinical outcomes and as further comparative studies with longer term follow-up are published, the use of biologic therapy in combination with realignment surgery will expand further into clinical practice.

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19 Meniscus Injury and Early Osteoarthritis

George Jacob, Kazunori Shimomura, David A. Hart, and Norimasa Nakamura

19.1 Introduction

The meniscus has proven to be an important structure within the knee joint whose true importance and function have been recognized early. The negative results of meniscectomy were described as early as 1923 [1]. A significant volume of literature has implicated meniscus loss after meniscectomy in joint degeneration and the onset of osteoarthritis [2–5]. Despite the convincing data and advancement in meniscal repair techniques, astonishingly, most meniscal injuries are still treated by partial or total meniscectomy [6, 7]. Current meniscus repair/replacement strategies have shown reasonable long-term results while long-term results after partial meniscectomy have shown to be rather disappointing [8–10]. With the growing affliction of osteoarthritis (OA) worldwide [11, 12], research has been focused on finding the predisposing factors contributing to OA. Some risk factors such as obesity or concomitant chondral injury [13, 14] appear to show more obvious correlations than genetics.

The meniscus plays a key role in providing stability, load distribution, and joint congruence. Post-injury, these roles are compromised and therefore can lead to a disruption of normal joint biomechanics [15–17] which in turn leads to the development and progression of OA [18, 19]. Due to the poor healing nature of the meniscal tissue, normal joint mechanics is certainly not restored in many situations leading to joint degeneration. Meniscal degeneration and tears are frequently encountered in patients with OA and most meniscal interventions are performed in settings of early OA [20]. Therefore, in some clinical scenarios, it remains uncertain whether the tear preceded the onset of OA or was a result of the OA [19]. Literature states that both chronologies are possible [21]. Despite the commencement of early OA in most meniscal interventions, restoring meniscus anatomy and function could reverse, retard, or prevent further joint degeneration. The current literature focuses on defining injury characteristics strongly related to OA along with the ideal intervention and timing of surgery. In this chapter, we aim to

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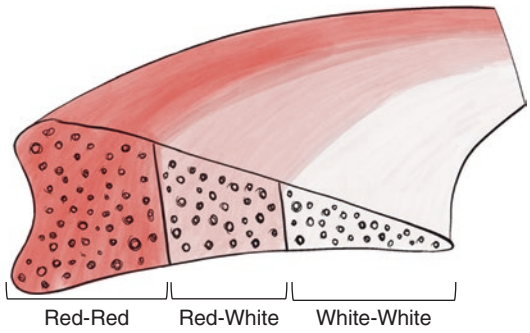

Fig. 19.1 Zones of the meniscus

discuss the relationship and characteristics of meniscal injury as a risk factor for OA development.

19.2 Meniscal Injury

Meniscal injuries usually constitute tears within the meniscal tissue as a result of a traumatic event (in younger patients) or degenerative tears (in older patients) [22]. Tears vary by their location, pattern, and zone of injury. These meniscal zones are divided into the red–red, red–white, and white–white zones based on their extent of vascularity, white–white having the least cellularity and blood supply, therefore, least healing potential [23, 24] (Fig. 19.1). Tear patterns have been classified into vertical–longitudinal, radial, oblique, horizontal, complex, and root tears [22]. Degenerative tears are commonly encountered in the setting of OA [19, 25] and data suggest that their incidence is higher than it was earlier understood as many remain asymptomatic [26–28]. Another important injury morphology is root tears which occur more commonly in the posterior horns of the menisci with a higher reported incidence in the medial meniscus [29]. Lateral meniscal root tears are more likely in association with anterior cruciate ligament (ACL) injury and medial meniscal root tears with degeneration [30]. Root tears lead to loss of anchorage of the meniscus leading to the displacement of the meniscal tissue known as extrusion (Fig. 19.2).

19.3 Biomechanics of the Meniscus

The functional biomechanics of the meniscus can be divided into load transmission, shock absorption, joint stabilization, and meniscal motion. During normal weight-bearing, the menisci are responsible for transmitting forces in the form of hoop stresses from the femur to the tibial joint surface [31]. Owing to the circumferential collagen fibre arrangements, the compressive axial forces are converted to horizontal tensile forces. Figure 19.3 illustrates the conversion of the vertical axial forces into horizontal meniscal hoop stresses. When the rounded femoral condyles bear down onto the menisci, they extrude somewhat [32] but return to their anatomical position owing to the root attachments and posterior meniscotibial ligaments [33]. Biomechanical studies have shown the variations in meniscal contact across the knee vary depending on the load applied. With no axial load, the majority of the contact is on the meniscus but with increasing load, the menisci cover less of the joint surface [34, 35]. As the menisci possess both a solid and fluid phase during compression, energy is absorbed by the tissue by joint fluid expulsion from the tissue. This is brought about due to the low permeability of the tissue matrix which results in a high frictional drag effectively dissipating the compressive forces and reducing their magnitude [36].The meniscus also plays a significant biomechanical role in joint stability. Owing to the structure of the menisci, they can increase joint congruency between the femoral and tibial condyles effectively adding to stability and efficient joint fluid circulation [37]. The medial meniscus also plays a role as an anteroposterior stabilizer secondary to the ACL and this has been convincingly proven in the literature [38–40]. The more immobile posterior horn of the medial meniscus has also been thought to provide a stop to prevent further posterior translation [41]. Meniscal motion is the final mechanical function of the meniscus. The dynamics of the meniscal tissue allow for it to maintain maximum joint congruency at all degrees of flexion and

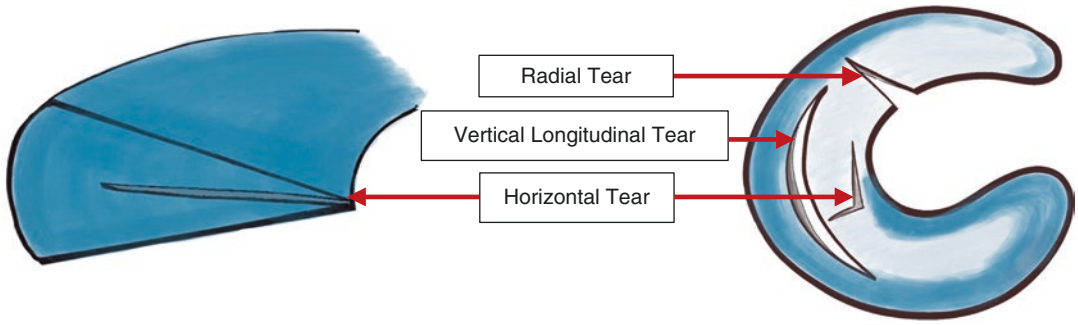

Fig. 19.2 Tear morphologies of the meniscus

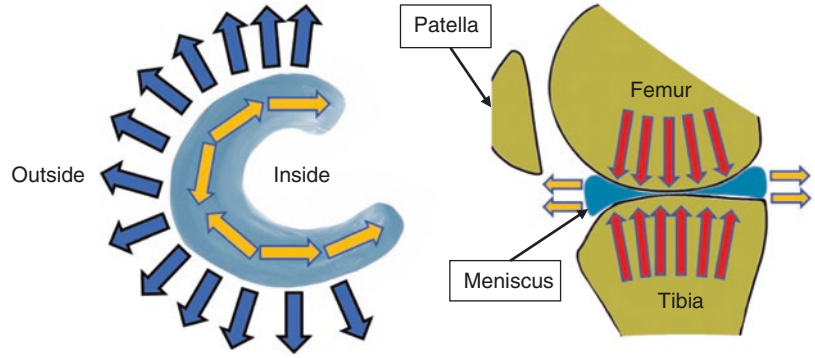

Fig. 19.3 Showing axial forces being converted into meniscal hoop stresses

load bearing of the femur on the tibia. Between the two menisci, the lateral has been shown to exhibit the most mobility with the posterior horn of the medial meniscus being the most fixed [42].

19.4 Biomechanics Post-Meniscal Injury

Meniscal injuries harm overall knee biomechanics with specific anatomical lesions affecting meniscal functions to varying degrees. Tears instantly alter the contact pressures and forces across the knee joint and several cadaveric studies have demonstrated this.

Load transmission after a meniscal injury is affected undesirably where the joint contact is significantly reduced. This, in turn, increases the peak local contact stress resulting in greater force transmission over a smaller area [43]. Depending on the extent of injury, the meniscus will then be ineffective in converting the axial forces into the earlier described hoop stresses. The shock absorptive function would be lost where the tissue is no longer able to function between its solid and fluid phase effectively. Larger tears and more specifically root tears affect meniscal motion, due to its loss in anchorage the tissue no longer maintains its anatomical position extruding to greater degrees during loading and then being unable to return to an anatomical position on the tibial plateau. Literature has also shown increased anterior tibial translation post-meniscectomy indicating that non-functioning meniscus would also considerably affect joint stability [38, 44].

19.5 Biochemical Changes Post-Meniscal Injury

Post-injury, there is a surge in pro-inflammatory mediators in the knee joint, e.g. interleukin-1β, tumour necrosis factor-α and catabolic enzymes, e.g. metalloproteases. This sudden increase in cytokines alters the normal joint homeostasis to imbalance the catabolic and

anabolic processes. This leads to increased extracellular matrix permeability resulting in more water within the articular cartilage altering its biochemical makeup and biomechanics [45]. As a result, the articular cartilage undergoes degeneration and neovascularization leading to early changes of OA. The subchondral bone remodels and there is osteophyte formation along with sclerosis. Osteoblasts within sclerotic bone are known to increase vascular endothelial growth factor production which contributes to the development of OA [46]. The articular cartilage fragments, and as it enters the synovial fluids, it attracts macrophages into the joint which causes further joint catabolism leading to the onset of OA.

19.6 Early OA

Recently, there has been discussion on the definition of early OA, and this has been to improve outcome assessments in studies but also to help diagnose OA before its radiological detection. Luyten et al. [47] described current treatment modalities and recommendations to be more 'reactive' than pre-emptive, where most patients diagnosed with OA already have a significant amount of joint destruction. More sensitive criteria for diagnosis may enable clinicians to intervene at an earlier stage of the disease. Their criteria proposed to combine the knee injury and osteoarthritis outcome score along with clinical examination and radiography. They postulate that this may help change the treatment strategy to a more 'proactive' one.

Regarding meniscal injuries, the onset of OA could be detected in its earliest phase where changes are still subtle and have not yet resulted in joint destruction perceivable on X-ray. The majority of current studies rely on radiography as an outcome to determine OA after meniscal injury, using more sensitive criteria for detection could improve the sensitivity of OA and therefore improve data quality and correlation.

19.7 Meniscal Injuries and Early OA

Meniscal injuries can be broadly classified into traumatic and degenerative tears. As mentioned earlier, there is debate on whether meniscal tears are a cause or consequence of OA given their coexisting nature, there is a certainty that they have a strong association. In the setting of a traumatic tear, the meniscus typically splits longitudinally, parallel to the collagen fibres or radially, perpendicular to the collagen fibres. Root tears are radial tears that occur within 1 centimetre of the meniscal root attachment site [48–50]. Radial and root tears impairs the function of the meniscus in a similar fashion leading to altered joint biomechanical and biochemical changes preceding the onset of OA. In degenerative tears, meniscal lesions are more often horizontal cleavage or complex macerated tears. This could be as a result of OA in the knee where the pathological biochemical and biomechanical changes associated with OA affect both the chondral surface and meniscal tissue [51]. A good example is the Framingham study where 82% of OA patients showed meniscal damage [52].

A study by Badlani et al. [53] using data from the OA initiative reported various meniscal tear patterns and their incidence of OA. The study included 32 patients who developed radiographic evidence of OA in a previously unaffected knee, matched against 64 patients with no evidence of OA. OA was diagnosed using anteroposterior radiographs and meniscal lesions characterized using magnetic resonance imaging (MRI). They concluded that meniscal extrusion, large radial tears, and complex tears could have the highest association with OA development. Englund et al. [54] conducted a similar case–control study and reported that comparable results in that meniscal injury did lead to a higher incidence of OA and that meniscal extrusion was a key factor suggesting root tears to be a major factor. These results are in agreement with the literature [55, 56] where extrusion impairs the function of the meniscus by reducing meniscal joint contact,

joint congruence and therefore increases chondral contact and force magnitude across the joint [57]. Tear size would also directly influence the progression of OA as there would be significantly reduced joint contact which is directly proportional to the size of the tear especially in radial tears [53]. Crema et al. [58] reported that intrasubstance tears of the meniscus did not have any association with OA; however, horizontal tears and meniscal maceration did show a close association with cartilage loss. It is worth noting that maceration of the meniscus could be a result of long-standing injury and OA, therefore, being effect rather than the cause.

Most literature does strongly link extrusions, large radial tears, and degenerative tears to OA due to pathological biomechanics; however, some pre-clinical and biochemical studies do show the possibility of triggering the OA cascade with any form of meniscal injury. Ogura et al. [59] compared levels of tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) between tissue biopsies from meniscal lesion sites, normal meniscal tissue, synovium near the lesion, and synovium from the opposite compartment. They reported increased levels of TNF-α and IL-6 at the injury site and local synovium when compared to the non-injured meniscus and non-adjacent synovium. This affirms there the strong presence of inflammatory mediators in the meniscal tissue and synovium which would contribute to OA progression in the knee joint. Another interesting pre-clinical study by Abusara et al. [60] demonstrated significantly increased chondrocyte death in meniscectomized mice knees within 240 min of cyclical loading when compared with control. This experimental model confirms that meniscal injury affects chondrocyte viability in an acute time frame and the biomechanical and chemical change has immediate consequences at a cellular level. Pre-clinical data from other studies have shown similar outcomes in that biochemical changes play a major role in joint health post-meniscal injury [61–63].

Time and age of the injury could also play a crucial role in the onset of OA and this was confirmed by Roos et al. [51]. The authors reported two important conclusions in this study. The first being both young and old patients who sustained meniscal injuries did have a higher incidence of OA. The second is that older patients (>30 years) developed OA at an average period of 5 years while younger patients (17–30 years) on average developed OA at 15 years post-injury. This does indicate that age and other variables could slow down the progression of OA post-meniscal injury. Table 19.1 summarises the review of literature on early OA in meniscal injury.

Table 19.1 Summary of literature on the incidence of early OA in the event of a meniscal injuries

Author/Journal	Study design/ Level of evidence	Conclusion
Badlani et al./ Am J Sports Med. 2013 [53]	Case control study/III	Meniscal tears with greater radial involvement and extrusion had higher incidence of radiographic OA
Englund et al./ Arthritis Rheum. 2009 [54]	Case–control study/III	Meniscal injury is a notable risk factor for development of radiologic OA
Bloecker et al./ Arthritis Care Res. 2015 [57]	Cohort study/ IV	Medial meniscus extrusion is associated with greater medial cartilage loss especially the external compartment due to pathological joint mechanics
Crema et al./ Osteoarthritis and Cartilage 2010 [58]	Observational study/IV	No significant association with intra-substance meniscal MRI signal changes and cartilage loss. Significant association between horizontal tears and meniscal maceration with cartilage loss

Abbreviations: *OA* Osteoarthritis

19.8 Meniscal Surgery and OA

With considerable literature supporting joint degeneration following meniscal injury, the question arises as to how to manage them. Meniscectomy and partial meniscectomy have

now been challenged by the recent trend of meniscal repair techniques. However, the outcomes and healing rates of meniscal repairs have not been predictable especially in inner white–white zone tears [64, 65].

When considering meniscectomy, several studies with long-term follow-up reported that despite patients demonstrating improved clinical and subjective outcomes scores [66], there was definite evidence for accelerated radiological degenerative changes [67]. Paradowski et al. [68] followed up patients post-meniscectomy for a mean period of 24.7 years and noted a higher incidence of both tibiofemoral and patellofemoral OA. They also compared the operated knee to the contralateral knee and noted a higher incidence of structural changes in the operated knee. Another more recent study assessed the radiographic changes post-arthroscopic partial meniscectomy and compared the patients' operated knee to the contralateral one. At an average follow-up of 8 years, they noted significant OA progression in the operated knee and a higher predisposition for OA in patients with higher body mass index and those sustaining degenerative tears [69]. This is contrary to literature that stated the type of lesion did not affect the outcomes of meniscectomy [70, 71].

Meniscal repair surgery has seen refinement and improved techniques have been developed when compared to earlier approaches. Some literature has suggested that tear chronicity did not affect the results of the meniscal repair [72, 73] and that a concomitantly performed ACL reconstruction results in better outcomes [74–76]. There are however studies that oppose these results stating that traumatic tears displayed better healing [77, 78] and that ACL reconstruction did not improve the healing rate [79, 80]. A meta-analysis by Xu et al. [81] reported on meniscal repairs with an average follow-up of 7 years and concluded that though the meniscal repairs had higher reoperations, patient reported outcomes and activity levels post-meniscal repair were higher. Persson et al. [82] studied the risk of symptomatic OA in subjects who had undergone meniscal repair versus meniscectomy versus the general population. Their study has a mean follow-up time of 10 years. Patients who have a meniscal repair had a 25%–50% less chance of consultation for symptomatic OA when compared to those who underwent arthroscopic partial meniscectomy. However, the risk of consultation for OA after the meniscal repair was still two times greater than that of the general population.

Meniscal root tears and extrusion have shown to be a major contributor to loss of meniscal function and leading to the development of OA [55, 56]. Meniscal root repairs have been addressed non-operatively, with meniscectomy, suture anchor repair, and with a transtibial pull-out repair [50]. Meniscal root repairs have been favoured as the results of meniscectomy and non-operative treatment have been poor [83]. Krych et al. [83] reported worse clinical outcomes and incidence of arthritis in patients treated non operatively for root tears and a higher rate of total knee arthroplasty at 5 year follow-up. Despite continued meniscal extrusion in many patients after meniscal root repair, the clinical and functional outcomes were better in those who underwent a root repair along with the reduced incidence of OA [56, 84]. Meniscal root repairs must be performed in such patients as it is evident if not done there is accelerated joint degeneration which results in worse outcomes and conversion to arthroplasty.

Biological augmentation and tissue engineering techniques to augment meniscal repairs have not become a standard or practice as of now, and despite a large volume of pre-clinical data, it has only been utilized in a few centres as an investigational method [85]. Methods to improve meniscal healing have utilized rasping of the lesion [86], fibrin clots [87], platelet-rich plasma, [88] and meniscal wrapping [89]. Microfracture in the femoral notch has also been employed in settings where concomitant ACL reconstruction is not being done in an attempt to potentiate healing by bringing marrow elements to the site of meniscal injury [90, 91]. Augmentation techniques are not yet backed by high-level evidence; however, they may be applied to more chronic and complex tears in individuals with a higher risk of an unsuccessful repair.

Patients who have sustained a meniscal injury are certainly at a higher risk for developing OA

in the knee joint and this can be a result of the biomechanical and biochemical changes that occur in association to the injury. Management options do have an important role in progression to OA. Meniscal repairs aim to preserve as much meniscal tissue as possible, despite higher failures and reoperation rates when compared to meniscectomy. This should not reflect the success of the surgery and state it is inferior to meniscectomy. Since meniscal repairs have better patient-reported outcomes and seemingly reduced risk for the development of OA, every effort should be made to repair meniscal tears. That being said, it is important to determine the tear profile and patient's risk factors to predict the failure of a repair and decide the ideal management option. Table 19.2 summarises the discussed literature of meniscal surgery results and incidence of OA. Figure 19.4 outlines a possible treatment algorithm for meniscal injuries.

Table 19.2 Summary of literature on meniscal surgery results and incidence of OA

Author/Journal	Study Design/ Level of Evidence	Surgery/Follow-up	Conclusion
Paradowski et al./ Osteoarthritis and Cartilage 2016	Cohort study/ III	Meniscectomy/24.7 years	Increased incidence of OA post-meniscectomy. Three in four patients developed tibiofemoral OA and one in four patellofemoral OA
Longo et al./J knee Surg 2018	Comparative study/III	APM/8.1 years	8 years following APM all patients had a significant progression of radiographic OA. Patients with greater BMI had a greater incidence of post-meniscectomy OA.
Tengrootenhuysen et al./ Knee Surg Sports TraumatolArthrosc 2011	Cohort Study/ III	Meniscal Repair/5.8 years	Meniscal tears repaired within younger patients within 6 weeks of injury had better results. Inside out repair techniques displayed better results Meniscal repairs in combination with ACLR had greater success
Xu, C., Zhao, J/Knee Surg Sports TraumatolArthrosc 2015	Metanalysis/ III	Meniscal Repair/7 years	Meniscal repairs have higher reoperation rates when compared to meniscectomy but demonstrate better patients reported outcomes
Persson et al./ Osteoarthritis and Cartilage 2017	Comparative study/III	Meniscus repair vs APM vs General Population/10 years	Patients seeking consultation for OA knee were higher in the meniscectomy group compared to the repair group however risk for OA consult significantly increased regardless of the type of surgery when compared to general population
Feucht et al. [84]/ Arthrosc 2015	Systematic Review/IV	Posterior root repair for Medial meniscus root tear/2.5 years	Root repair improved functional outcome significantly and reduced OA progression at short term follow-up. Healing and incidence of extrusion were less predictable
Chung et al. [55]/Knee Surg Sports Traumatol Arthrosc 2015	Metanalysis/ IV	Posterior root repair for Medial meniscus root tear	Medial meniscal posterior root repair demonstrated significant improvement in clinical outcomes. Meniscal extrusion was not reduced in the majority of cases and there was still progression of some degree of OA

Abbreviations: *OA* Osteoarthritis, *APM* Arthroscopic Partial Meniscectomy, *BMI* Body Mass Index, *ACLR* Anterior Cruciate Ligament Reconstruction

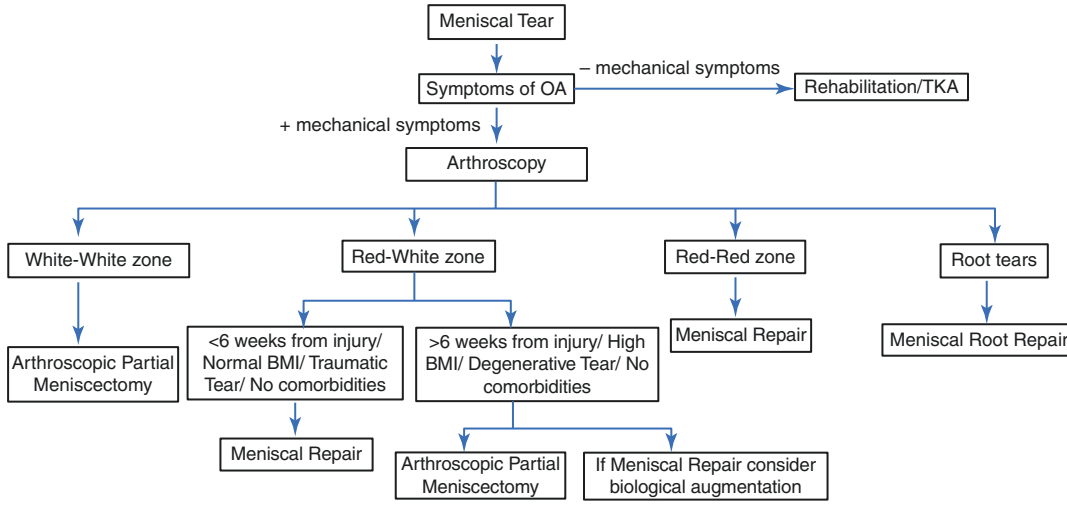

Fig. 19.4 Treatment algorithm for meniscal injuries. Abbreviations: *OA* Osteoarthritis, *TKA* Total Knee Arthroplasty

19.9 Conclusion

The relationship between meniscal injury and OA is plain to see with considerable literature reporting their strong association. It is important to recognize that the variations in the joint environment are immediate and the cycle of OA starts on a cellular and biochemical level followed by pathological biomechanics. The greater the damage to the meniscus, the more it is unable to perform its biomechanical functions leading to the onset of OA. Evidence points to meniscal extrusions and large radial tears to be the most significant contributor to the onset of OA but the smaller less obvious biochemical changes occurring in smaller longitudinal tears may be going unnoticed to some degree. We can be sure that patients with meniscal injuries should be considered for high risk of OA and treatments to restore the meniscus integrity and function will address pathological biomechanics. Meniscal surgeries whether being repair or meniscectomy have been proven to still be at risk for the development of OA when compared to normal subjects. Tears must be studied and the management executed depending on the patient's profile and tear morphology. A meniscal injury does predispose to OA and despite the currently available repair techniques complete restoration of meniscal biomechanics has not yet been achieved. However, a strategy to reverse the biochemical changes with OA modifying drugs may be worth considering with or without a meniscal repair and future research could hold the key in barricading the biochemical cascade in addition to the biomechanical changes leading to the onset of OA.

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20 The Role of Arthroscopic Debridement, Microfracture and Surface Procedures

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20.1 Introduction

20.1.1 Background

The rise in sport-related articular cartilage and meniscus injuries and the growing recognition of early osteoarthritic defects poses an increasing challenge for the sports surgeon. While severe osteoarthritis (OA) is easily recognizable and has reliable surgical treatment options such as joint replacement, early OA has more limited surgical options and indications for surgery vary from case to case. Traditional nonsurgical approaches to early OA, such as anti-inflammatory medications, braces, and physical therapy can have important benefits in providing symptom relief and slowing the progression of degenerative meniscal and cartilage disease. Yet, when nonoperative treatments fail, there may be a role for surgical management of early OA, especially when cartilage defects are focal in nature. Some of these surgical options include arthroscopic debridement, chondroplasty, and microfracture. A careful history and physical exam and the use of selective imaging are paramount in determining which patients meet the appropriate indications for surgical management.

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20.1.2 Clinical Evaluation

20.1.2.1 History

A detailed history should be obtained including the duration, onset, and type of knee symptoms and the occurrence of recent knee trauma. Special attention should be given to risk factors for OA including advanced age, obesity, prior knee injury, or surgery. Patients typically complain of insidious onset of pain localized to the affected knee compartment, stiffness, swelling, and vague reports of the knee not moving properly or exhibiting clicking, popping, or grinding. True "mechanical symptoms," such as catching or locking, are often thought to be the result of displaced meniscus tears. However, the sensation of catching and locking often described by patients is not specific for meniscus tears and can be equally prevalent in those with and without meniscus tears [1]. Other underlying pathologies often associated with these so-called mechanical symptoms include anterior cruciate ligament (ACL) insufficiency, synovitis, and degenerative changes associated with OA, such as uneven articular surface and bone marrow lesions [2–4]. Sudden severe onset of pain can often be associated with subchondral fracture of the distal femur or tibia [5].

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Patients with early degenerative joint disease and focal chondral defects often present with intermittent swelling as their only symptom. Pain may be exacerbated with weight bearing and activities of daily living such as prolonged sitting, standing, squatting, and climbing stairs, while pain with kneeling and going downstairs can be associated with patellofemoral lesions. It is important to assess a patient's activity level and expectations, including willingness and ability to comply with a prolonged post-operative rehabilitation regimen, which could include a six to eight week period of partial weight bearing in certain situations [6].

20.1.2.2 Exam

A thorough knee exam should be performed, with assessment for joint line tenderness, effusion, swelling, pain with range of motion, decreased motion or locking, malalignment, or ligamentous insufficiency. Examination of the lumbar spine and bilateral hips and contralateral knee are essential. Mechanical findings elicited with testing such as Thessaly, McMurray, and Apley compression tests do not necessarily imply meniscal pathology and may indicate underlying cartilage instability as well [1, 7]. Findings of femoral condyle or tibial plateau point tenderness are helpful, but not diagnostic. Pain elicited with the patellar grind test may be suggestive of a patellar or trochlear cartilage lesion while tenderness over the bony distal femur or proximal tibia may suggest bony stress injury or subchondral fracture. The presence of meniscal insufficiency and ligamentous instability may indicate the need for other concomitant procedures at the time of surgical intervention.

20.1.2.3 Imaging

While physical exam is useful, imaging is often necessary to accurately characterize the extent of chondral injury or degenerative change in the knee. Initial imaging should consist of standard weight-bearing radiographs including anteroposterior, lateral, and sunrise views. As degenerative changes often begin posteriorly on the distal femur and proximal tibia, a Rosenberg view of the knee (45° flexion weight-bearing radiograph) can be helpful for the identification of joint space narrowing [8]. In cases of suspected malalignment, a long-cassette mechanical axis view is also recommended [9]. Although early degenerative changes may not be readily visible on plain radiographs, images should be carefully evaluated for the presence of osteophytes and joint space narrowing. It is important to keep in mind that radiographic evidence of OA and degree of knee symptoms may not be directly related.

Magnetic resonance imaging (MRI) remains the gold standard with an accuracy of 90%–95% in detecting meniscus tears and cartilage abnormalities [10]. MRI allows for accurate assessment of cartilage thickness and morphological changes, including focal chondral defects, fibrillation, fragmentation, and diffuse thinning [11]. MRI can also aid in the diagnosis of subchondral fractures and osteonecrosis. Although MRI is important for determining the location of chondral defects during preoperative planning, it has been shown to underestimate the size of articular defects in up to 75% of lesions [12].

MRI should not be considered the first line imaging modality in elderly patients and in those with radiographic evidence of advanced OA. In these patients, there is a high likelihood of detecting incidental meniscus and cartilage degenerative changes that may not necessarily correlate with patient symptoms [13]. For this reason, the presence of meniscus tears or cartilage lesions on MRI should not guide surgical treatment in asymptomatic patients or in those with advanced OA. The decision to proceed with surgical intervention should be based on the full clinical picture and not imaging alone.

20.1.3 Surgical Management

The role of arthroscopic surgery in the setting of degenerative disease has historically been controversial. Early efforts at arthroscopy assumed a mechanical cause for pain in the degenerative knee and assumed that excision of degenerative tissue in the knee could provide relief. This thought process was fostered by high levels of initial patient satisfaction with knee arthroscopy.

Numerous studies, however, have shown that patients with generalized OA who undergo arthroscopic debridement do not perform better in the long term than those who are treated nonoperatively [14, 15]. Nonetheless, there remains a role for surgical treatment for degenerative meniscal tears and focal chondral defects.

In cases of failure of nonoperative treatment, surgical treatment options include arthroscopic lavage, chondroplasty, and microfracture. Surgical lavage helps to flush out cellular debris and degradative enzymes such as metalloproteinases, which are released upon chondrocyte injury and can incite synovial inflammation [16, 17]. Chondroplasty is a common knee procedure that facilitates debridement of damaged cartilage to create a more stable rim around the remaining defect and smoothens the remaining articular surface [18]. Arthroscopic debridement can also help reduce synovial inflammation and mechanical symptoms through removal of adhesions, loose bodies and loose cartilage fragments [18, 19]. Microfracture and other forms of marrow stimulation are surgical techniques employed in conjunction with chondroplasty in order to stimulate regeneration of cartilage lesions. Penetration of the underlying bony plate, either with mechanical chondroplasty or microfracture, helps to mount an inflammatory and vascular response that can aid in fibrocartilage formation and healing in avascular articular cartilage that otherwise has a limited intrinsic repair capacity [20].

20.2 Cartilage Injuries

20.2.1 Articular Cartilage and the Osteochondral Unit

Articular or hyaline cartilage provides a smooth surface for joint articulation and efficient load transmission with minimal friction. It is mostly composed of a dense extracellular matrix (ECM) of primarily type-II collagen fibers with glycosylated proteoglycans such as aggrecan [21]. Chondrocytes (2% total volume) are cells dispersed throughout the ECM and are responsible for its maintenance and repair [22]. Charge repulsion of the hydrophilic glycosylated proteins and resulting hydrostatic pressure helps retain a large amount of water (80% wet weight of cartilage). This is important for the ability of articular cartilage to resist large joint compressive loads several times larger than that of normal body weight despite an average thickness of 2–4 mm [23, 24]. The ECM is composed of a noncalcified and a calcified layer separated by a cartilage interface called the tidemark, below which hyaline cartilage transitions into subchondral bone (Fig. 20.1) [25].

Articular cartilage is dependent on diffusion for nutrition and is inherently avascular. No blood vessels or nerves are found within the matrix which leaves articular cartilage without an inherent healing capacity [26, 27]. Partial thickness cartilage injuries have minimal regenerative capacity, yet still result in cellular insults leading to metabolic disruptions. Altered proteoglycan compositions that result can lead to increased tissue hydration and collagen disorganization. This ultimately leads to increased force transmission to the underlying bone and to a cyclic degenerative process which may contribute to progression of OA.

When hyaline cartilage is disrupted down to bone, a healing response mounted by the intact underlying subchondral bone results in a layer of mature fibrocartilage covering the defect [28]. Contrary to hyaline cartilage, fibrocartilage is a tougher and more dense fibrous tissue found in menisci, tendons, and intervertebral discs. Mature fibrocartilage, predominantly composed of type-I collagen, has decreased durability and wear properties compared to articular cartilage [29, 30].

Within the osteochondral unit (Fig. 20.1), there is a dynamic relationship between cartilage and bone. Cartilage is connected through a calcified zone to the subchondral plate below it and is marked by a distinct histological boundary termed the tidemark. This subchondral cortical bone and the metaphyseal bone below are integral to cartilage health. Within the osteochondral unit, cartilage and bone exhibit a complex reciprocal relationship fostering the health of each component [31]. Biomechanically, articular cartilage is supported by the bony construct below.

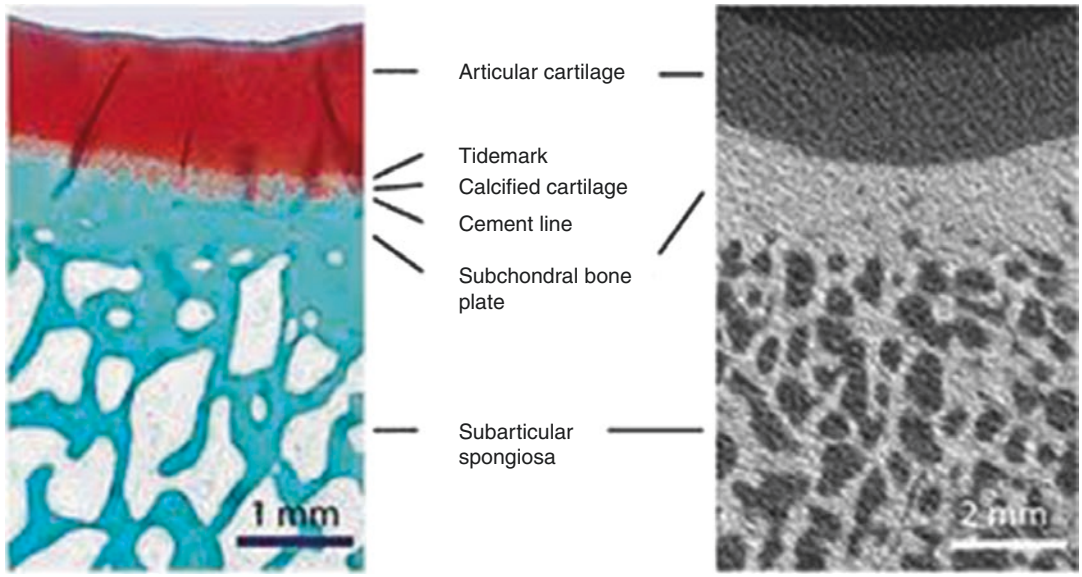

Fig. 20.1 The osteochondral unit: The tidemark is a distinct histological boundary that separates articular cartilage from the underlying subchondral bone. Within the osteochondral unit, cartilage and bone exhibit a complex and dynamic relationship. (From Olah T, Madry H. The Osteochondral Unit: The Importance of the Underlying Subchondral Bone in Farr J, Gomoll A eds. Cartilage Restoration Practical Clinical Applications. Second Edition. Springer International. 2018)

Damage to the cartilage can lead to bony changes and remodeling. In OA, inflammatory cytokines and osteoclast stimulation factors released by the synovium and cartilage influence the subchondral bone, and cytokines and prostaglandins involved in bone remodeling can affect the overlying cartilage [32]. Treatments for articular cartilage defects must therefore include considerations of the health and preservation of the entire osteochondral unit.

20.2.2 Prevalence and Natural History of Chondral Injuries

Chondral injuries can be acute or chronic and may result from mechanical, metabolic, vascular or genetic origins [20]. The resulting type of lesion can be classified as either a focal or degenerative lesion based on surface area and mechanism of injury. Focal lesions typically occur as a result of direct joint trauma or vascular disturbance such as in the case of osteochondritis dissecans. Degenerative lesions are typically more diffuse, resulting from progressive wear due to suboptimal joint mechanics, such as meniscus insufficiency, instability or malalignment [33].

Knee articular cartilage defects are common, with some studies estimating a 10%–12% prevalence in the general population [34]. The incidence of asymptomatic and symptomatic full-thickness cartilage injuries is higher in athletes, with an observed incidence of 59% and 36%, respectively [35]. A large study of 25,000 knee arthroscopies found chondral lesions in 60% of patients, 70% of which had associated pathologies such as meniscus and ACL tears [36]. The prevalence of knee OA, ranging from mild chondrosis to severe joint disease, is also quite high and is present in 60%–70% of adults 65 years and older [19, 20].

The natural history of articular cartilage lesions is progressive and permanent due to the limited intrinsic repair capacity of cartilage [16, 37]. Degenerative cartilage lesions begin with disruption of the chondral surface and initial fibrillation. Progressive damage to the surface of

the cartilage matrix and loss of proteoglycans leads to decreased hydrostatic pressure, resulting in a decreased ability to resist joint compressive forces. Chondrocytes have limited potential for replication, and their metabolism is dependent on an optimal chemical and mechanical microenvironment dependent on regular joint motion and dynamic loading. Dramatic alterations in chondrocyte metabolism, in which ECM degradation outweighs collagen synthesis, can lead to development of chondral degeneration and OA [38]. The avascular nature of articular cartilage further limits the capacity for intrinsic healing, while the complexity of articular cartilage structure complicates clinical efforts to restore chondral defects.

Development of cartilage flaps can result in pain and mechanical symptoms [37]. However, severity of clinical symptoms does not always directly correlate to amount of cartilage damage [39]. Focal chondral defects are thought to elicit painful symptoms, but much is still unknown about the natural history of cartilage lesions. There is a paucity of data on the progression of asymptomatic lesions. For instance, a study on 125 patients with asymptomatic chondral lesions at the time of ACL reconstruction revealed no significant difference in symptom severity and degree of radiographic changes compared to controls at eight years post-operatively [33]. Furthermore, lesion size did not directly correlate with symptom severity [33].

20.3 Arthroscopic Lavage, Debridement and Chondroplasty

20.3.1 Techniques

20.3.1.1 Arthroscopic Lavage and Debridement

Arthroscopic lavage allows for the egress of cellular debris and degradative enzymes such as metalloproteinases which are released upon chondrocyte injury and can incite synovial inflammation [16, 17]. In addition, arthroscopic debridement can remove inflamed or hypertrophied synovium, resect fibrinous scar, and smooth joint surfaces.

Early unblinded studies supported joint lavage as an effective surgical alternative to nonoperative treatment of early OA [40, 41]. One study of arthroscopic lavage demonstrated persistent pain relief in 37 knees with OA compared to nonoperative treatment at one year post-operatively [42]. Yet, larger retrospective studies and randomized trials have not demonstrated persistent improvement in pain and function after 3 months following arthroscopic lavage. A systematic review of over 500 patients across seven studies found only minimal improvement in pain and function provided by lavage compared to placebo or no intervention [43]. Moreover, a meta-analysis of six randomized controlled trials showed no significant benefit in pain or function with joint lavage compared with placebo at 3 months post-operatively and no difference between joint lavage combined with a steroid injection to arthroscopic lavage alone [44]. For this reason, arthroscopic lavage is thought to provide only transient symptomatic relief and is typically avoided as it does not offer a long-term solution, especially in athletes or active patients.

20.3.1.2 Arthroscopic Chondroplasty

Arthroscopic chondroplasty specifically refers to the removal of unstable or delaminating cartilage within the knee. Shavers, biters, curettes, knives, or gouges are used to incise unstable cartilage at its base to prevent further delamination. Unstable cartilage flaps of grade 2 or grade 3 chondral lesions are debrided down to a stable rim of healthy cartilage with care to avoid exposure of the subchondral bone (Fig. 20.2). Creation of vertical walls in full thickness lesions may reduce defect expansion over time [45]. Clinical improvement results from the removal of inciting unstable fragments of cartilage and may be significant enough to serve as definitive treatment for many lesions.

20.3.1.3 Abrasion Arthroplasty

Mechanical or abrasion arthroplasty calls for the arthroscopic removal of the superficial sclerotic bone using a mechanical rotary shaver and burr

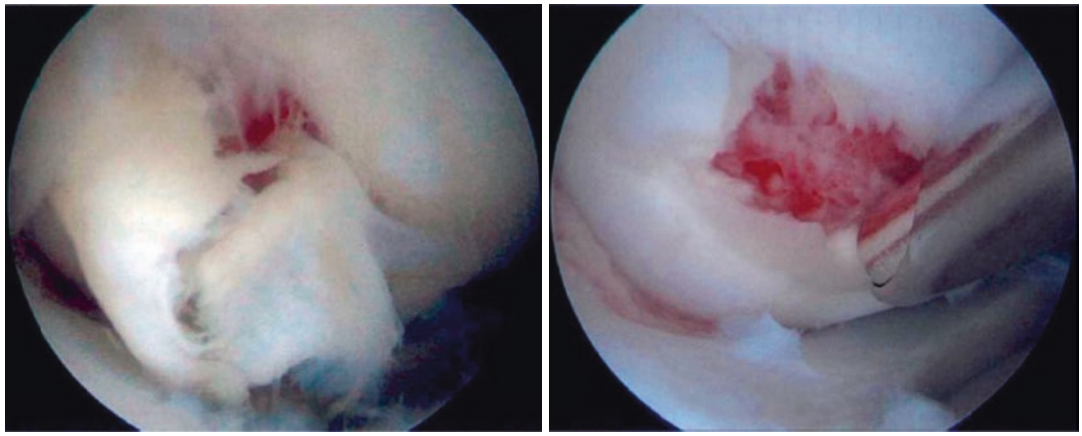

Fig. 20.2 Chondroplasty: Unstable cartilage flaps of grade 2 or grade 3 chondral lesions are debrided down to a stable rim of healthy cartilage with care to avoid exposure of the subchondral bone

[46]. Damaged cartilage is removed and the subchondral bone is debrided to a depth of 1–2 mm, exposing superficial vessels. Abrasion of the subchondral bone can facilitate the exudation of marrow elements onto the subchondral bone surface to encourage fibrocartilage development. However, disruption of the subchondral bone using the abrasion technique has the drawback of possibly destabilizing the surface bone. Studies comparing debridement with abrasion arthroplasty had typically demonstrated variable results [47, 48]. One study with limited numbers of patients, found 9 of 28 patients (32%) of the abrasion arthroplasty group were worse at 3-year follow-up, with 50% of the abrasion group requiring total knee arthroplasty during this same time period [48]. This unpredictability has largely led to abandonment of the use of this procedure.

20.3.1.4 Radiofrequency Chondroplasty

Radiofrequency chondroplasty uses a monopolar or bipolar wand to induce electron oscillations to create a "plasma field" that causes molecular reorganization of damaged cartilage matrix (coblation). The induced "plasma field" can facilitate smoothening of the articular surface and stabilization of the cartilage structure to protect against further progressive chondral damage [37, 49]. This potentially offers a benefit over mechanical chondroplasty which has been criticized for over resection of potentially healthy cartilage and leaving uneven surfaces [49].

Initial concerns with radiofrequency devices included risk of thermal injury, osteonecrosis, or chondrolysis seen by first generation radiofrequency devices and laser procedures [50]. Studies in humans, including several randomized controlled trials, however, have demonstrated longer time to revision, and better patient reported outcomes with coblation techniques over mechanical debridement [51, 52]. A large retrospective review of 824 patients showed no significant safety concerns by using bipolar radiofrequency ablation assuming the appropriate contact pressures and plasma settings [53].

Radiofrequency chondroplasty requires specialized equipment and meticulous attention to technique. Temperatures above 45 °C can result in cell death. Treatment is geared for grade 2 or grade 3 lesions, while the efficacy of this procedure for cartilage lesions which reach the subchondral plate remains unclear. Significant thermal damage can still occur with these devices (Fig. 20.3).

20.3.2 Post-operative Rehabilitation

Weight bearing is usually not restricted after arthroscopic debridement and chondroplasty, although ambulation can be limited for three to

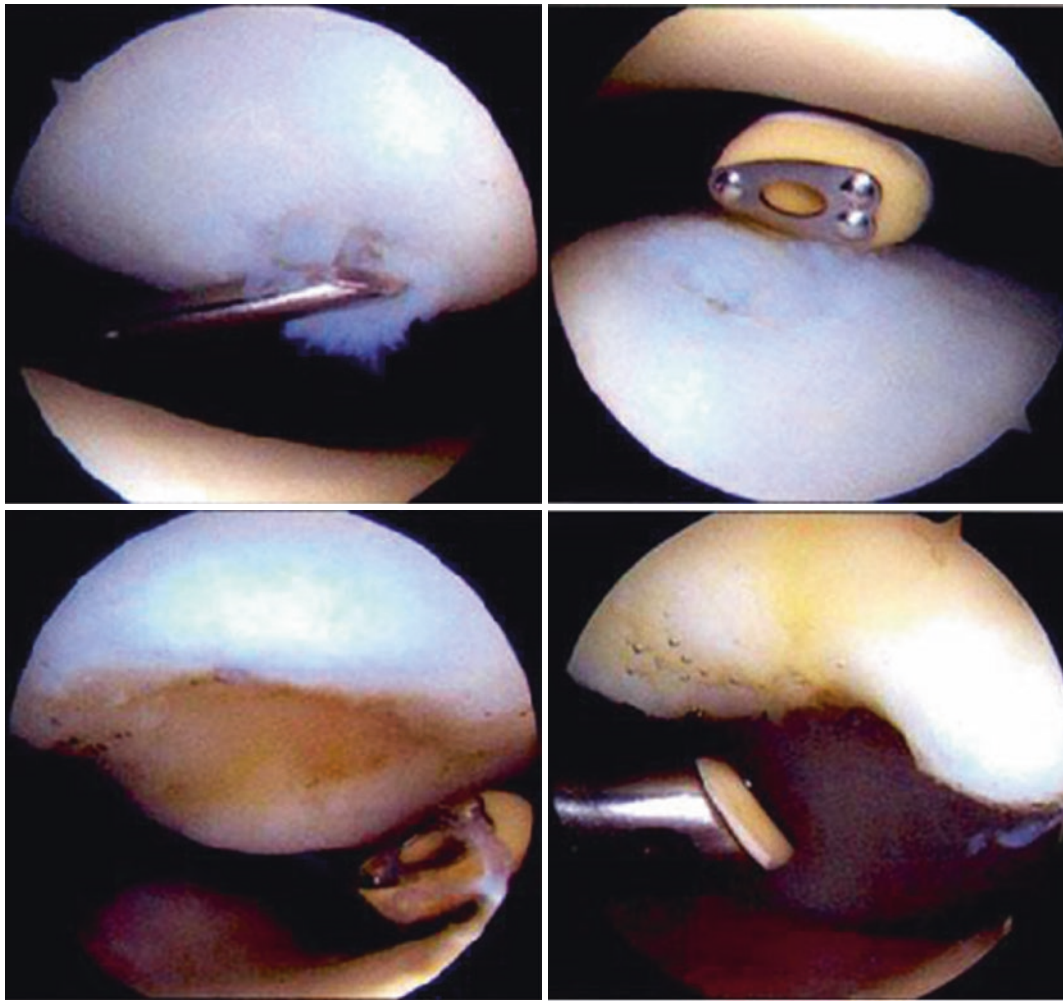

Fig. 20.3 Thermal damage from radiofrequency chondroplasty: In this example, radiofrequency ablation is performed on a focal chondral lesion with either inappropriate contact pressures or plasma settings. There is obvious physical and thermal damage to this cartilage lesion. Ultimately, further cartilage restorative procedures were necessary to treat this iatrogenic lesion

five days post-operatively in the setting of pain and quadriceps dysfunction [54]. Bracing is not generally recommended, but may be considered briefly for ambulation in cases with significant quadriceps dysfunction or to emphasize complete extension. Unrestricted passive range of motion exercises are initiated immediately post-operatively, with the objective of regaining full range of motion within two to three weeks. The use of a continuous passive motion machine (CPM) is surgeon dependent but is generally not required. Weight bearing and strengthening exercises are initiated one week post-operatively. Formal physical therapy is not required but can be prescribed in selected cases and in patients who are slow to progress. Attention is given to recovering range of motion, quadriceps strength, and normal gait. Full return to moderate impact activities is allowed beginning at six to eight weeks post-operatively [54].

20.3.3 Outcomes

Arthroscopic debridement and chondroplasty have demonstrated greater short-term improvements in patient-reported pain compared to lavage alone. One study showed that patients treated with arthroscopic debridement were five times more likely to report pain improvement at one and five years post-operatively compared to those treated with arthroscopic lavage [55]. Improved patient outcomes with debridement alone have been demonstrated with radiofrequency techniques [51–53].

Despite its reported success in short-term studies, the long-term benefits of arthroscopic debridement and chondroplasty are unclear. A large prospective randomized trial reported no significant benefit of lavage and debridement compared to a placebo procedure [15]. A more recent study identified significant short-term improvement in pain and physical function, but this difference was not significant at 2-year follow-up [56].

Moreover, arthroscopic debridement may not halt the radiographic progression of OA, with one study in young athletes reporting that 43% of patients showed significant joint space narrowing at 14-year follow-up [57]. However, these joint space changes were found to be mostly asymptomatic, with 80% of patients reporting good to excellent functional outcome scores and 75% of patients able to return to the same level of competitive sports following debridement [57].

Arthroscopic debridement and chondroplasty have demonstrated better clinical outcomes in early OA compared to later disease stages, with more than 90% of patients with mild arthrosis reporting improvement compared to only 49% of patients with moderate arthrosis and 12% of patients with severe arthrosis at 4–6 year follow-up. Therefore, arthroscopic debridement may afford the most clinical benefit for the treatment of early stage arthrosis [58]. While younger age and a smaller amount of chondral damage are associated with better patient reported outcomes, female sex and body mass index (BMI) greater than 30 have been associated with worse outcomes [14]. These observations reinforce the importance of appropriate patient selection to avoid failure of operative treatment.

20.4 Microfracture and Marrow Stimulation Techniques

20.4.1 Background

Perforation of the subchondral plate to allow fibrous ingrowth as a method to resurface osteoarthritic joints was first described by Pridie in 1959 [59]. Ficat et al. performed a modification of this procedure, termed spongialization, where all of the diseased cartilage and corresponding subchondral bone were removed to allow for marrow-induced fibrous healing from the exposed cancellous bed [60]. An arthroscopic technique, which involved abrasion of a sclerotic chondral lesion to allow for subsequent fibrocartilage formation, was introduced by Johnson [61]. Building off of these earlier procedures, the microfracture technique was popularized by Richard Steadman in the early 1980s for the treatment of full thickness chondral defects [11, 62]. Microfracture became a popular technique for the treatment of small chondral lesions as it was easily performed arthroscopically, could be performed as a single-stage procedure, and was inexpensive [63, 64].

The purpose of microfracture is to perforate the subchondral bone beneath a cartilage defect in order to expose cancellous bone and allow for release of marrow-derived growth factors and chemotactic cytokines for cartilage repair [65, 66]. Microfracture creates small channels ("microfractures") in the subchondral bone perpendicular to the bone surface which stimulate this marrow response. These "microfractures" allow access to marrow growth factors and pluripotent mesenchymal stem cells that can differentiate to produce fibrocartilaginous repair tissue [59, 62, 67]. The resulting fibrocartilage helps to fill the chondral defect and is theorized to help protect against secondary perifocal OA development which could occur if the defect is left untreated [68, 69]. This fibrocartilage is composed of type-I collagen, not the normal type-II collagen found in native hyaline cartilage, and

lacks the biomechanical properties of normal articular cartilage [63]. Specifically, this fibrocartilage has lower compressive stiffness, less resilience and poorer wear characteristics than native hyaline cartilage [70]. These histological changes following microfracture are due to endochondral ossification [71].

Historically, marrow stimulation techniques have been considered the gold standard for isolated chondral defects [11]. Despite the early popularity of the technique and reasonable short-term clinical success, long-term outcomes of microfracture have been underwhelming, with 25% or more of patients experiencing poor results at 10-year follow-up [72–76]. Increasing evidence has also shown that microfracture damages the microarchitecture of the underlying osteochondral unit leading to intralesional osteophytes and poorer outcomes of revision cartilage surface procedures [77]. As evidence emerges that microfracture may not lead to better outcomes than debridement alone, the status of microfracture as a gold standard has been debated with some authors even calling for the abandonment of the technique [78, 79].

20.4.2 Indications/Contraindications

Microfracture is indicated for the treatment of symptomatic small to midsize (<2–3 cm^2) articular cartilage lesions (Outerbridge classification grade III or IV) in active patients and slightly larger lesions (2–3 cm^2) in less demanding patients [80]. These lesions include full-thickness cartilage defects, unstable defects overlying subchondral bone, or partial thickness lesions that scrape down to the bone when probed [9, 11, 81]. In a systematic review, Mithoefer et al. analyzed over 3000 patients who underwent microfracture for cartilage defects and showed that age over 40 years, preoperative symptom duration of less than 1 year, up to 4 cm^2 lesion size in nonathletes, up to 2 cm^2 lesion size in athletes, and BMI less than 30 kg/m^2 were all factors associated with good outcomes after microfracture [82]. In a subsequent study, Goyal et al. showed that a large portion of patients who underwent microfracture for lesions greater than 4 cm^2 developed arthritis within 5 years of surgery [72]. Due to these studies as well as other reports, 4 cm^2 is often used as a maximum cutoff for chondral lesions that benefit most from microfracture in nonathletes while 2 cm^2 may be used in athletes [72, 82].

Contraindications to microfracture include advanced OA, inflammatory arthritis, chondral defects greater than 7–10 mm deep, and inability to participate in post-operative rehabilitation [11, 83, 84]. The height and sufficient thickness of the vertical rim surrounding the defect that holds the microfracture clot in place are also important factors [11, 84, 85]. Microfracture has been shown to be effective in patients older than 40 years of age; however, older patients may also experience greater difficulty performing required post-operative protected weight-bearing ambulation and the accompanying rehabilitation protocol [11]. Given the question of long-term viability of the fibrocartilage repair, use of microfracture in younger populations (especially with larger lesions) has been questioned [72–76].

Microfracture, along with other marrow-stimulating techniques, may cause damaging alterations in the underlying subchondral bone, leaving subsequent revision cartilage surface procedures including autologous chondrocyte implantation (ACI), less effective [77, 86]. For this reason, microfracture is usually contraindicated as a temporizing procedure if a more definitive cartilage surface procedure is to be considered later. In contrast to ACI, osteochondral allograft following prior microfracture is still associated with good survivorship and functional outcomes [87].

While one of the benefits of a first-line treatment like microfracture is its ease of application, the success of microfracture is certainly affected by mechanical alignment, meniscal status, and ligamentous stability [88]. Similar to preparations for other cartilage restorative procedures, long-standing mechanical axis radiographs are indicated and corrective osteotomies should be considered concomitantly or as a staged procedure to restore a neutral mechanical axis when needed. Similarly, a tibial tubercle osteotomy is considered for patellar defects. Uncorrected

ligamentous instability, meniscal deficiency, or malalignment remain relative contraindications for a microfracture.

20.4.3 Technique

The goal of microfracture is to "fill" the cartilage defect with a "superclot" of mesenchymal stem cells to stimulate fibrocartilage repair [11]. In the absence of concomitant procedures, this can be carried out during a single stage, minimally invasive procedure. A diagnostic knee arthroscopy is first performed with standard anterolateral and anteromedial portals. Concomitant intra-articular procedures, including partial meniscectomy, meniscus repair, or meniscal allografts are usually performed prior to creation of the microfracture holes to prevent visualization difficulties from the bleeding bone.

The first step in performing microfracture is assessment and preparation of the defect. Unstable cartilage flaps surrounding the lesion are debrided so that a stable, vertical edge of healthy cartilage is left surrounding the defect. Debridement of chronic lesions may be complicated by the presence of calcified cartilage and bony sclerosis [11, 89]. Care should be taken to remove the calcified cartilage layer without damaging the underlying subchondral plate [90]. Size, depth, and location of the cartilage lesion is re-assessed in order to confirm that microfracture is indicated.

Next, microfracture holes are created using a 30° or 45° microfracture awl or a 1.0 mm K-wire working from the periphery to the center and making sure that the instrument is perpendicular to the defect (Fig. 20.4). Care is taken to leave an adequate bone bridge between holes (1–2 mm or large enough so that the integrity of the subchondral bone is preserved in between holes). Small-diameter awls have been shown to lead to improved cartilage repair when compared with large-diameter awls [91, 92]. In a sheep model, Orth et al. compared small-diameter (1.0 mm) awls with large-diameter (1.2 mm) awls and showed that 1.0 mm awls were associated with improved histological quality of repair tissue, better surface grading, and decreased relative bone volume of the subarticular spongiosa 6 months after microfracture [92]. Small-diameter awls may be associated with less trabecular fragmentation of the subchondral bone and less compaction than large awls [93]. A 90° awl may be necessary when performing microfracture of the undersurface of the patella in order to achieve the necessary perpendicular angle. Holes are created 3–4 mm apart and at least 3–4 mm deep in order to avoid convergence. The depth of subchondral perforation influences outcome after microfracture. Using a rabbit model, Chen et al. showed that, compared with shallow perforation (2 mm), deeper perforation (6 mm) was associated with greater fill of the cartilage defect and greater hyaline character in the repair matrix [94].

Arthroscopic drilling may also be used for marrow stimulation in place of microfracture with awls. Similar to microfracture, deep drilling (6 mm) is associated with improved access to marrow stroma, an increase in mineralized bone and an enhanced cartilage repair compared with shallow drilling (2 mm) [95]. In a systematic review, Kraeutler et al. compared microfracture to arthroscopic drilling for the treatment of cartilage defects [95]. The authors identified seven basic science studies (two of which were performed in humans) and concluded that regardless of marrow stimulation technique, the quality of cartilage regeneration was poor and not that of hyaline cartilage in the native knee [95]. The authors also found that there is a lack of adequate basic science literature comparing these two techniques for focal cartilage lesions [95].

Subsequent reduction of irrigation fluid pump pressure can confirm appropriate depth of drilling and release fat droplets and blood from the marrow cavity into the defect. This forms what has been referred to as the "crimson duvet" (Fig. 20.5).

Sclerosis of the subchondral bone can diminish the ability to access the underlying marrow and can also lead to fissuring and compaction of bone, instead of marrow access [96]. Hoemann et al. studied ex-vivo microfracture of medial and lateral condyles from total knee arthroplasty

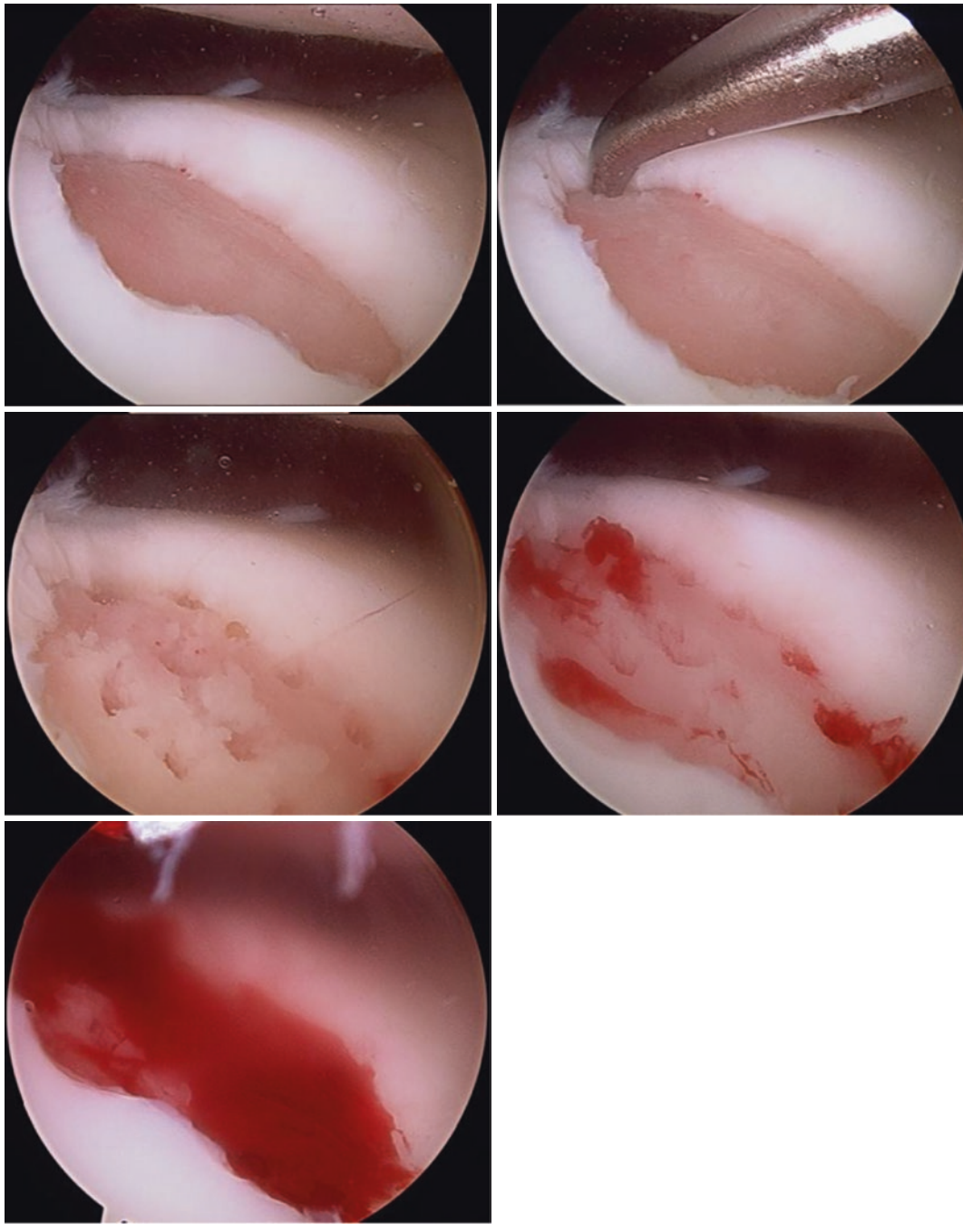

Fig. 20.4 Microfracture: Microfracture holes are created using a 45° microfracture awl working from the periphery to the center of the lesion and ensuring that the instrument is perpendicular to the defect

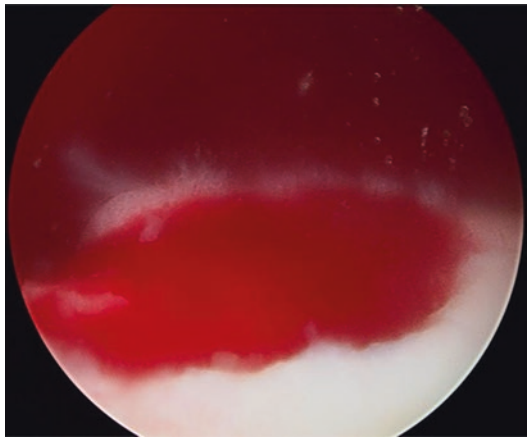

Fig. 20.5 Blood clot formation: Following microfracture, release of fat droplets and blood from the marrow cavity into the defect results in blood clot formation which has been referred to as "crimson duvet"

patients and showed that in extremely dense sclerotic bone, marrow access was only 40% following microfracture [96].

20.4.4 Postoperative Management and Rehabilitation

Postoperative rehabilitation is surgeon dependent and may vary depending on lesion size and location [11, 88]. Rehabilitation is important to establish an environment which promotes differentiation of recruited marrow progenitor cells into fibrocartilaginous repair tissue and protects the maturation process. In general, a period of protected weight bearing is recommended for 6–8 weeks for tibiofemoral joint lesions [61]. In contrast, patellofemoral lesions can often be treated with immediate flat foot weight bearing as tolerated, with progressive range of motion and physical therapy.

Range of motion is usually initiated immediately post-operatively and is considered essential to the nutrition of the growing fibrocartilage. Use of a CPM device is prevalent in multiple studies and is common in practice [88, 97]. In animal studies, CPM has strong evidence and appears to enhance nutrient delivery to the growing cartilage and promote growth of hyaline cartilage [98]. Clinical evidence for the necessity of CPM use however is lacking and has minimal high-level evidence [85, 98, 99]. While no one protocol exists for CPM use, one review cited a common regimen utilizing CPM with an initial range of motion from 0° to 30° and progressing as tolerated for 6–8 h daily over 6 weeks [100].

Physical therapy is often recommended with the initial goal of restoring full motion, patellar mobility, and starting isometric quadriceps strengthening. Full weight bearing is reintroduced at the 6-week mark. The second phase of therapy focuses on isokinetic quadriceps strengthening and resistance training. The patient is generally cleared for resumption of full-unrestricted activities at 4–6 months post-operatively.

20.4.5 Complications

Cartilage injuries involve the entire osteochondral unit. Microfracture and marrow stimulation techniques impact the architecture of the subchondral bone within the lesion. Subchondral bone overgrowth is frequently observed (62% of primary cases and 93% of revisions) and is associated with a 22% increase in failure rate at 6 years post-microfracture [101]. Related risk factors for poor outcomes include pre-operative bone overgrowth, high BMI, lesions located on the lateral femoral condyle, and excessive debridement. Patients may experience progression of cartilage degeneration with return of symptoms and progression to OA.

Microfracture can trigger a secondary center of ossification leading to the formation of intralesional osteophytes (Fig. 20.6) [102]. In one study, intralesional osteophytes were present in 54% of patients at 6 months and 70% of patients at 12 months [103]. The formation of bone cysts can also be present in up to 33% of patients [82].

Rarely, patients undergoing microfracture of trochlear defects may report catching or locking during the initial months of recovery as the patella rides over the trochlear defect [62]. These symptoms tend to improve as the defect populates with regenerative cells. Additionally, some patients report ongoing painless effusions that

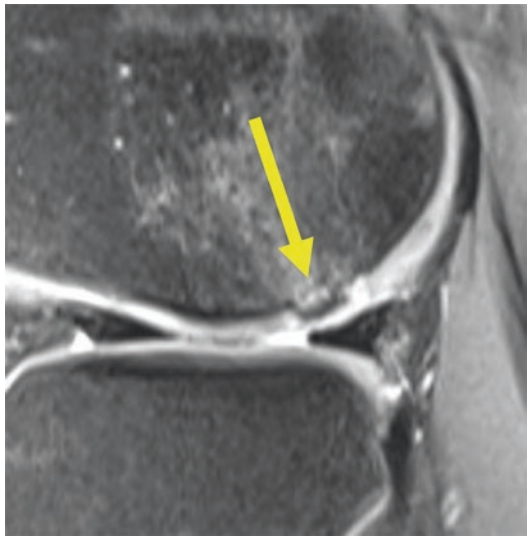

Fig. 20.6 Intralesional osteophyte: Microfracture can trigger a secondary center of ossification leading to the formation of intralesional osteophytes

can be treated conservatively [62]. Further research is required to determine if the rates of these complications can be decreased by improved drilling techniques or biological augmentation.

20.4.6 Outcomes

The efficacy of microfracture has been well documented in several short- and mid-term studies [72, 82, 104, 105]. Successful pain reduction and steady clinical improvements have been demonstrated for the first 2 years after surgery, with the greatest improvements reported in the first five years following microfracture. Long-term data has shown mixed results with one 15-year follow-up study demonstrating microfracture failure rate of 32.5% and radiographic analysis indicating that 50% of patients who had not experienced failure showed early OA. A study by Steadman et al. showed notable improvement at 11-year follow-up in patients younger than 45 years of age with full-thickness chondral defects without associated meniscus or ligament pathology [85].

In a large systematic review of over 3000 patients, Mithoefer et al. showed that improvement in knee function was consistent over the first 24 months after microfracture [82]. The authors noted that improvements in pain and function were seen in the early post-operative period and patient reported outcomes at 2 years after surgery were improved from preoperative scores, but only 67%–85% of patients noted continued improved outcomes between 2 and 5 years post-operatively [82]. Another systematic review validated previous results, showing that microfracture failure rates range between 11% and 27% at 5 years and 6%–32% at 10 years, with failure defined as the need for revision surgery or total knee arthroplasty [106]. A mid-term follow-up study of prospectively collected patient reported outcomes demonstrated clinically and statistically significant outcomes at 5.7 years and pointed to patient and defect-related characteristics that may influence outcomes after microfracture [107]. Treatment of isolated femoral defects outperformed microfracture of tibial lesions. Male patients, patients treated with multisite microfracture, and patients treated for lesions larger than 3.6 cm^2 showed less benefit. Combined offloading osteotomy and microfracture have demonstrated good mid-term results with 86% of patients avoiding conversion to total knee arthroplasty at 5-year follow-up [108]. In a study of 110 patients, Solheim et al. showed that 45% of patients converted to total knee arthroplasty or had poor functional scores at median follow-up of 12 years [76]. Poor outcomes were most commonly seen in those with degenerative articular changes, long duration of knee pain prior to surgery, poor preoperative functional scores, or who underwent partial meniscectomy at time of microfracture [76].

Outcomes following microfracture in athletes have also been studied. Gobbi et al. analyzed 61 athletes and showed improvement in patient reported outcomes at 2 years after surgery and a reoperation rate of 11% [73]. The authors also noted that patients treated for lesions of 4 cm^2 or less, had superior outcomes when compared to patients with larger lesions [73]. Other studies have identified defects in nonweight-bearing areas and duration of symptoms less than 1 year to be associated with

improved outcomes after microfracture in athletes [82, 109, 110]. Return to sport after microfracture has been demonstrated in athletes treated for lesions less than 2 cm^2 [110]. In a large meta-analysis of 2549 patients, 34% of whom underwent microfracture, Krych et al. showed a 58% return to play at 9.1 months following microfracture [111]. This return to play rate was lower than that of osteochondral allograft transplantation (88%), OATS (93%) and ACI (82%) demonstrated in the same analysis [111]. and slightly lower than return to play rates of 73%–95% from other reports of microfracture in high level athletes [110, 112, 113].

With the advent of cartilage restoration techniques, such as matrix-associated chondrocyte implantation (MACI), comparative studies have tried to redefine the contemporary indications for microfracture. A prospective study comparing these two techniques at two years for the treatment of symptomatic cartilage knee defects greater than 3 cm^2 in size showed statistically significant improvement at 2 years of follow-up with MACI [104]. Yet, a cost-effectiveness analysis of microfracture and autologous chondrocyte implantation at 5 years showed that microfracture is associated with both lower costs and lower cost per point increase in patient reported outcome measures [114]. A randomized controlled study comparing MACI with microfracture showed similar outcomes between both groups at 5-year follow-up [115]. Considering the relative technical simplicity and cost-effectiveness of microfracture and the lack of long-term data demonstrating a meaningful superiority of MACI over microfracture, there is likely still a role for microfracture in the treatment of smaller focal femoral chondral defects.

Several reasons for failure after microfracture have been proposed. Poor surgical technique or improper surgical indication may lead to failure especially in the case of large, uncontained cartilage lesions. Compaction of the subchondral layer or osseous overgrowth of subchondral bone has been associated with 93% of failures following microfracture in one report [101]. Other factors such as noncompliance with post-operative rehabilitation protocols may also lead to failure after microfracture [63]. Whereas it is commonplace to consider realignment procedures, meniscal transplants, and ligamentous reconstruction in conjunction with other types of cartilage restorative procedures, it is still less common to perform these corrective procedures combined with a microfracture. These pathologies, when left uncorrected, likely lead to increased failure of this technique.

20.4.7 Augmentation of Microfracture

Various augmentation techniques with biological adjuvants have been suggested in order to reduce failure rates and improve outcomes following microfracture. In a systematic review, Arshi et al. analyzed 18 articles with 625 patients and compared microfracture alone to microfracture with biological adjuvant [116]. The authors classified biological augments into either injectable or scaffold-based augmentation and showed that microfracture with injectable augmentation was associated with improved functional outcome scores compared with microfracture alone, while scaffolding-based augmentation trials showed similar post-operative improvements [116]. The authors highlight that the literature on biological augmentation is heterogenous and of limited quality evidence and therefore, make it difficult to draw conclusions on their use [116]. In a study assessing bone marrow stimulation for osteochondral lesions of the talus, concentrated bone marrow aspirate with bone marrow stimulation was associated with similar functional outcomes, improved integration of repair tissue, and less evidence of fissuring than marrow stimulation alone [117]. Platelet-rich plasma (PRP) has also been suggested as a possible adjuvant in the treatment of cartilage lesions. In a meta-analysis of seven studies, Boffa et al. showed that PRP did not provide a minimal clinically important difference when used as an adjuvant to microfracture for knee or ankle lesions [118]. Use of dehydrated allograft cartilage extracellular matrix scaffold within the microfractured defect has been reported to have higher percentages of

hyaline-like collagen in the repair tissue [119]. Due to heterogeneity in the literature and the low-level evidence currently available, more research is needed to better understand the effects of biological augmentation on outcomes following microfracture.

20.5 Conclusion

Focal chondral lesions in the setting of early OA are a common cause of pain and dysfunction. History, physical examination, and advanced imaging can help guide surgeons when selecting the appropriate treatment for these lesions. Surgical options for the treatment of chondral injuries include arthroscopic lavage, debridement, chondroplasty, and microfracture. Microfracture has been shown to have some short-term benefit in select patients, but many patients demonstrate continued pain and progression to radiographic OA at long-term follow-up. Studies assessing biological augmentation in the setting of microfracture are heterogeneous and comprised of low-level evidence. Further research assessing long term outcomes in appropriately selected may help to refine indications and improve techniques for these procedures.

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Osteochondral Allografts in Early Osteoarthritis

21

Eli T. Sayegh and Simon Görtz

21.1 Introduction

Chondral lesions of the knee exist on a continuum from isolated, focal defects to generalized osteoarthritis (OA), with intermediate disease states spanning these two conditions. These patients may have more subtle signs and symptoms that only manifest with higher-intensity, particularly impact activity [1, 2], and the prevalence of early OA may be higher than generally recognized. A study of over 25,000 young patients with a mean age of 39 years undergoing knee arthroscopy found chondral defects in 6 of 10 patients, with degenerative changes in 29% [3]. Other studies have likewise noted that 6 in 10 patients undergoing knee arthroscopy for any surgical indication exhibit chondral or osteochondral lesions [4], with full-thickness lesions in 10% of these cases [5]. These patients are often reluctant to accept the lifestyle limitations of conservative treatment or the activity restrictions and long-term survivorship concerns inherent to arthroplasty, while also exhibiting lower satisfaction and higher implant failure rates after undergoing arthroplasty [6]. Nonsurgical treatments such as physical therapy, nonsteroidal anti-inflammatory drugs, and injections may temporarily provide symptom palliation. Furthermore, knee arthroscopy with lavage, debridement, and chondroplasty does not reliably benefit this population of patients with early OA [7, 8].

Cartilage is known to diminish in function and quality with age, and the presence of focal chondral defects can precipitate progression to OA. The presence of an asymptomatic chondral defect doubles the rate of cartilage loss relative to healthy knees [9], and is associated with disease progression in over 80% of knees over a 2-year period [10]. The pathological cascade of OA may begin with a reversible pre-OA state followed by onset of early OA and finally established OA [11]. In early OA, fibrillation and vertical fissuring are seen within the articular cartilage, with progressive enlargement of the subchondral bone plate and subarticular spongiosa [12]. Early OA involves not only the cartilage but other soft-tissue structures including the menisci and synovium [12, 13]. It is not clear if or to which extent altered joint homeostasis in early OA interferes with the biological milieu necessary for successful cartilage repair. Although superficial chondral defects tend not to heal, they are less likely to be symptomatic than full-thickness defects, which generally heal with fibrocartilage [14]. Similarly, while smaller defects may be well-tolerated, larger and uncontained defects are

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more likely to disrupt joint homeostasis and set into motion the degenerative process.

While cartilage restoration procedures are an established part of the armamentarium for treating younger patients with focal chondral defects, a subset of the orthopaedic literature has investigated whether these surgical indications can be extended to patients with early OA. Multiple cartilage restoration techniques have been described including bone marrow stimulation, osteochondral autograft transfer, osteochondral allografting, and autologous chondrocyte implantation. These techniques variably address the cartilage defect and/or underlying subchondral bone. Of these, osteochondral allografting is the lone technique that restores mature orthotopic hyaline cartilage on a microscopic level, recapitulates the native anatomy on a macroscopic level, provides immediate (rather than progressive) defect fill, and avoids donor site morbidity or two-stage procedure innate to other techniques [14–17]. Unlike most other cartilage repair techniques, osteochondral allografting does not rely on lesion containment with "vertical walls" or an intact subchondral plate for success. It is also a denervating technique, replacing painful subchondral bone with an aneural scaffold, often providing immediate pain relief. Osteochondral allografts are particularly versatile in the treatment of large, complex, or multiple lesions as this is a size-independent technique. Osteochondral allografts can also be adapted to complex topographies such as the patellofemoral joint or lesions with bone loss or associated subchondral abnormalities [18]. Because of its versatility, this chapter will focus on the application of osteochondral allografting in the setting of early OA and complex chondral lesions.

21.2 Indications and Contraindications

The application of cartilage restoration techniques to early degenerative joints requires an appreciation for the adverse biomechanical and biochemical environment therein [19]. While most cartilage restoration techniques require well-shouldered lesions and/or vertical walls, joints with early OA are less likely to have healthy shouldering cartilage of consistent cartilage thickness [20]. Osteochondral allografting is most well-suited to large, high-grade (International Cartilage Repair Society [ICRS] score grades III/IV) osteochondral defects. Because it is a size-independent technique, osteochondral allografting can be used to treat lesions greater than 2 cm^2 in size, which generally is the upper limit for osteochondral autograft transfer. Its other advantages include the ability to restore bone stock, address diseased subchondral bone, and restore type II hyaline cartilage. Osteochondral allografts are also valuable in the revision setting after previous cartilage restoration procedures have failed. Disadvantages to osteochondral allografting include the relative scarcity of donor tissue, logistical challenges and costs of graft procurement and storage, and theoretical risk of disease transmission. Chondrocyte viability and matrix structural integrity are preserved during cold storage for up to 14 days but show signs of degradation after 28 days, while the hyaline matrix remains relatively intact [21]. As such, 28 days is used as an upper threshold for acceptable graft transplantation in current tissue banking practice.

Regardless of the technique employed, concomitant contributory malalignment, ligamentous, or meniscal deficiency should be addressed in staged or simultaneous fashion in order to protect the repaired cartilage. Contributory malalignment refers to the weightbearing axis of the lower extremity falling through the affected compartment of the knee. This should be addressed by unloading osteotomy in a staged or concomitant fashion, which has shown to be a safe and effective intervention to optimize outcomes of cartilage repair in general, and osteochondral allografting in particular [22, 23]. Contraindications to cartilage restoration procedures include uncorrected contributory malalignment, ligamentous and/or meniscal deficiency. Additional contraindications include inflammatory or crystalline arthropathy or advanced, multicompartmental OA, particularly in patients whose age and activity level are appropriate for arthroplasty solutions. Patient factors also

significantly impact the prognosis of cartilage repair, and should be considered when indicating a patient for surgery. These include (physiological) age, body mass index, activity level, prior surgeries, symptom duration, lesion chronicity, and tobacco use.

21.3 Preoperative Planning

A complete panel of standing radiographs should be obtained, including standing anteroposterior and posteroanterior 30 degree flexion (Rosenberg), true lateral, Merchant, and full-length limb alignment views. Magnetic resonance imaging (MRI) is also obtained to characterize both the chondral lesion and the degree of subchondral bone plate and marrow involvement beneath the ultimate repair site, in relation to the level of the physeal scar [24]. When considering lesion factors in preoperative decision-making, it should be noted that MRI classically underestimates lesion size, and that a staging arthroscopy can be helpful in establishing lesion characteristics and treatment options. The ICRS system allows cartilage lesion mapping using a standardized topographic grid based on involvement of specific regions of the femoral condyles, tibial plateau, trochlea, and/or patella (Fig. 21.1), and to assess overall disease burden [24]. Relative lesion size must be considered in relation to the size of the femoral condyle or overall compartment, as a 1-cm^2 lesion in a smaller patient, for instance, is not only more likely to be symptomatic [25] but also likely to cause greater disease burden and have more limited autograft donor options than the same lesion in a skeletally larger patient. Lesion location is another significant prognosticator. The femoral condyles are in general amenable to a wider range of cartilage restoration techniques, while the patellofemoral joint is more technically challenging because of the variable topography of the trochlea and patella. While allografts may be effectively size-matched, shape-matching in the patellofemoral joint is more challenging. Tibial plateau lesions should be carefully monitored as the native cartilage is thinner, and bipolar lesions are generally associated with poorer outcomes [25]. In our treatment algorithm, osteochondral allografts are the preferred treatment option in large lesions (particularly when presenting with a preeminent osseous deficiency), those with significant marrow signal change on MRI indicating disrupted subchondral plate, lack of shouldering/containment, and all revision situations, particularly after prior microfracture which might cause intralesional osteophyte formation influencing outcomes of cell-based repairs [26]. In rare cases, tibial osteochondral allografts with attached meniscus can be used to address (sub-) total meniscus deficiency in combination with a post-traumatic tibial (osteo-) chondral defect. This composite graft can address several of the key issues of meniscal allografting: the graft sizing/matching and fixation, which can reliably be achieved with extraarticular compression screws across the graft/host interface.

When osteochondral allografting is employed, the donor allograft is matched with the recipient primarily based on size, and no HLA- nor blood type-matching is performed. Although retrieval studies have not consistently shown evidence of immunologic response, development of anti-human leukocyte antigen class I cytotoxic antibodies has been observed in allograft recipients [27]. While this response does not appear to have overt clinical significance, overall bioburden of transplanted material remains a concern when considering maximal graft size. An anteroposterior radiograph with a sizing marker is used to measure the width of the tibial plateau just below the joint surface, aiming for a ± 2 mm match. With femoral condyle lesions, the diseased condyle is often larger, wider, and flatter and best suited to a larger donor allograft. An attempt is made to match the radius of curvature of the recipient site, but generally it is easier to match a larger graft to a smaller recipient than vice versa. Lastly, when axial malalignment is present, the surgeon may consider staging an adjacent osteotomy in order to avoid insult to the microvascularity of the recipient site.

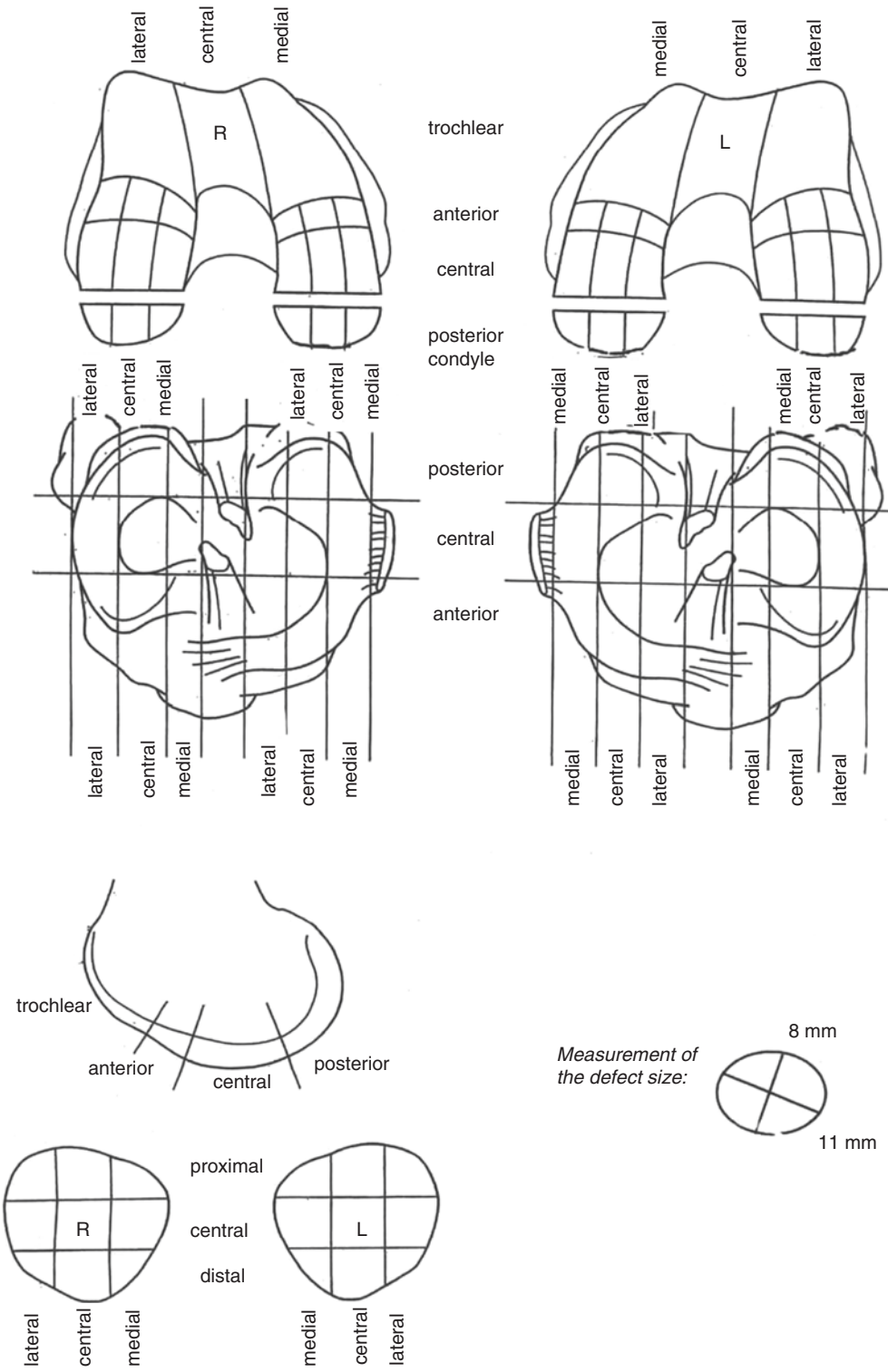

Fig. 21.1 The International Cartilage Research Society (ICRS) knee cartilage lesion mapping system. (Adapted from the ICRS Cartilage Injury Evaluation Package, With Kind Permission from ICRS)

21.4 Surgical Technique

The patient is positioned supine with optional use of a leg or foot holder, which can facilitate 70° to 130° of flexion as needed to expose the chondral lesion during the arthrotomy. A thigh tourniquet is generally employed, and use of tranexamic acid has gained popularity to optimize hemostasis and visualization. Commonly, patients have undergone prior staging arthroscopy to confirm the lesion extent and the size requirements of the graft. Fresh osteochondral allografting generally requires an open approach for lesion exposure and graft transplantation. Most femoral condyle lesions can be accessed without eversion of the patella but large complex lesions may require advanced approaches including quadriceps snip, tibial tubercle osteotomy, or collateral ligament release. In most situations, a standard midline incision is fashioned from the center of the patella to the tibial tubercle. A medial or lateral parapatellar arthrotomy is performed, depending on the lesion location, with dissection through the joint capsule, synovium, and fat pad (Fig. 21.2). Care is taken to avoid disrupting the anterior horn of the meniscus or the articular surfaces. For posterior or particularly large lesions, the meniscus may need to be detached and reflected, leaving a small cuff of anterior meniscal horn and root for later reattachment. Retractors are carefully inserted, while protecting the cruciate ligaments and chondral surfaces, and the knee is moved into flexion so that the lesion is exposed within the arthrotomy site. The lesion is then probed to assess its extent and stable margins.

The two main techniques for the preparation and transplantation of osteochondral allografts are the press-fit dowel and shell techniques. The press-fit dowel technique is technically similar to osteochondral autograft transfer. This technique is most suitable for contained femoral condylar lesions between 15 and 35 mm in diameter (Fig. 21.3). Fixation devices are not generally required in circumferentially contained grafts with a stable press-fit, unless the lesion involves the intercondylar notch or is otherwise uncontained (Fig. 21.4). Mechanical impaction of the graft should be avoided to minimize chondrocyte apoptosis [28]. Disadvantages of the dowel technique include poor applicability for far-posterior femoral and trochlear lesions due to the use of a circular coring system. In addition, ovoid-shaped and more complex geometric lesions require a greater amount of normal cartilage sacrifice at the recipient site to accommodate the circular donor plugs. Shell grafts are technically more difficult to perform and usually require fixation with bioabsorbable pins or small cannulated screws due to lack of containment. This powerful technique is suitable for complex defects requiring whole patella or trochlea transplantation. Depending on the method used, a smaller amount of native host cartilage may be sacrificed.

Regardless of technique, several common principles should be observed and applied: The amount of transplanted allogeneic bone should be minimized, this subchondral portion should be

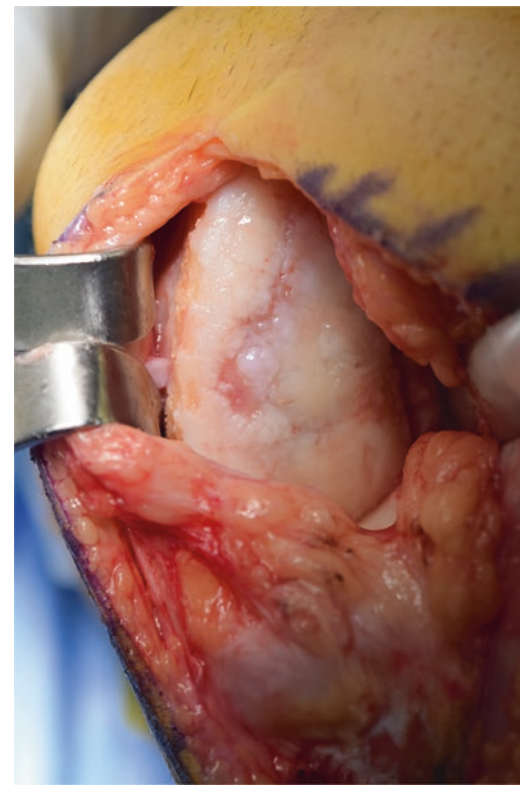

Fig. 21.2 Medial parapatellar arthrotomy demonstrating standard intraoperative exposure of a degenerative cartilage lesion of the medial femoral condyle

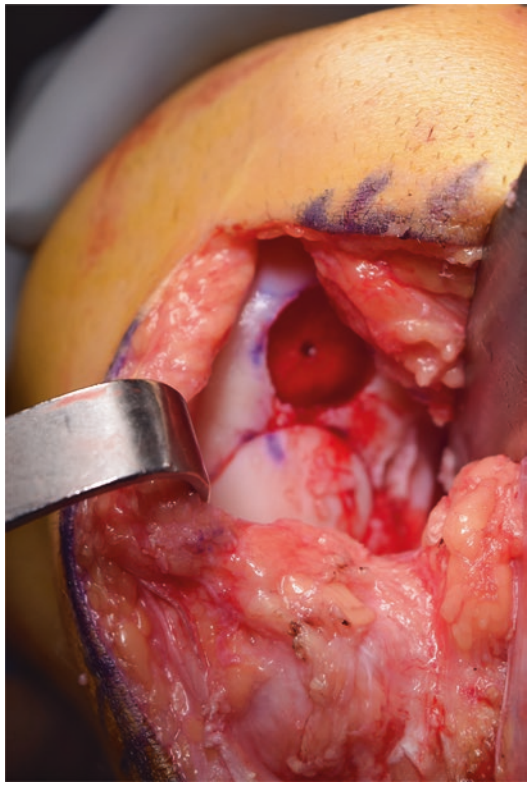

Fig. 21.3 Intraoperative view of above lesion being treated with multiple osteochondral allograft dowels. Please note minimal coring depth of the anterior recipient site extending just past the subchondral plate, to allow for bony ingrowth via creeping substitution

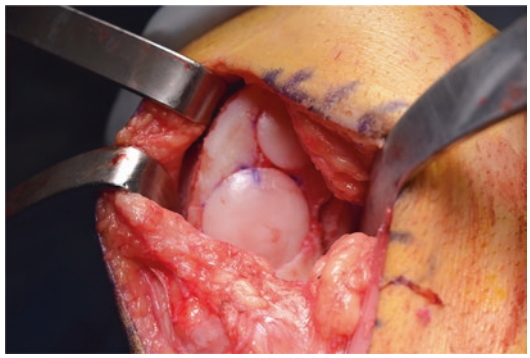

Fig. 21.4 Intraoperative view of same case, after placement of the second, anterior osteochondral allograft dowel. Please note stable press fit of both grafts not requiring additional fixation devices

copiously pulse lavaged to remove antigenic bone marrow elements, and stable fixation must be achieved. In dowel allografts, attempts should be made to limit total graft thickness to 10 mm or less. Deeper cystic subchondral lesion components can be curetted by hand and filled with autologous bone from the reaming or morselized allograft to minimize the amount of transplanted bone at the site, which is felt to be a rate limiting step in creeping substitution, remodelling, and ultimate integration of the osseous component of the graft. Conversely, patella or tibial shell grafts need a minimum thickness of 10 mm to avoid subchondral collapse and fracture. In any event, the subchondral portion of the composite allograft should be copiously pulse lavaged to remove marrow elements and debris to minimize bioburden of the graft and optimize osseous integration. Finally, grafts that are not inherently stable through a range of motion require secondary fixation to ensure stability of the repair construct.

21.5 Post-operative Management

Toe-touch weight-bearing with range of motion as tolerated is typically allowed immediately after surgery unless otherwise dictated by concomitant meniscal procedure, ligament reconstruction, or osteotomy. Bracing is seldom required except to protect concomitant procedures or in patellofemoral repairs, for which flexion is limited to less than 30° for the first 4–6 weeks, or in cases of bipolar tibiofemoral repairs in which an unloader brace is used to protect the compartment against excessive loads.

The rehabilitation program must factor in graft size and containment, fixation, and radiographic evidence of graft incorporation. Incorporation is routinely assessed by serial postoperative radiographs not unlike for fracture healing. Early rehabilitation emphasizes progressive ROM and quadriceps strengthening. At approximately 4 weeks, patients are allowed closed-chain exercises such as cycling.

Progressive weight bearing as tolerated can be allowed in the early post-operative period for smaller, well-contained lesions, or as late as 3 months for more complex repairs. Continuous passive motion (CPM) is not routinely employed unless there are patient-specific concerns for stiffness. After functional rehabilitation is complete, the patient is allowed to return to sports when functional rehabilitation allows, usually at approximately 6 months. Patients are cautioned against excessive impact loading of the allograft, particularly in the first year. The long-term outcome is inversely related to the time to treatment and overall disease burden in the affected joint. Whereas young patients with a focal lesion can reasonably be expected to return to normal impact-loading activities and preinjury function, the goals in a salvage situation are usually to delay or perhaps obviate the need for arthroplasty and allow return to activities of daily living and low-impact recreational activities.

21.5.1 Outcomes

Clinical outcomes after osteochondral allografting have been amply reported in the literature [29–46]. Gracitelli et al. [47] reported on 164 knees in 163 patients (mean age: 32.6 years) treated with OCA transplantation after prior subchondral marrow stimulation (SMS), osteochondral autograft transplantation (OAT), and autologous chondrocyte implantation (ACI). Mean allograft size was 8.5 ± 7.9 cm^2. Survivorship of osteochondral allografting in this study was 82% at 10 years and 74.9% at 15 years, with 89% of patients reporting being "extremely satisfied" or "satisfied." They showed that despite a high reoperation rate, OCA transplantation is a successful salvage surgical treatment after cartilage repair procedures, showing improved survivorship and functional outcomes of OCA transplantation after SMS, ACI, and OAT.

A systematic review of nine studies with generally low methodological quality with a total of 502 patients investigated the clinical outcomes of younger patients with early OA, as measured by clinical or radiological criteria, who underwent cartilage repair [48]. The majority of the included patients underwent autologous chondrocyte implantation. At follow-up to nine years, failure rates ranged from 8% to 27.3%, with 2.5% to 6.5% going on to require arthroplasty. These findings are also subject to high heterogeneity, with 46% to 100% of patients in those studies having undergone an index knee procedure and up to 67% undergoing concomitant procedures.

Chahal et al. [49] performed a systematic review of clinical outcomes of 19 eligible studies resulting in a total of 644 knees with a mean age was 37 years and mean follow-up of 58 months. With regard to etiology, the most common indications for transplantation included post-traumatic (38%), osteochondritis dissecans (30%), osteonecrosis from all causes (12%), and idiopathic (11%). Forty-six percent of patients had concomitant procedures, and the mean defect size across studies was 6.3 cm^2. The overall satisfaction rate was 86%. Sixty-five percent of patients (72 of 110) showed little to no arthritis at final follow-up. The reported short-term complication rate was 2.4%, and the overall failure rate was 18%.

21.6 Complications

Although all allografts are harvested and screened in accordance with the standards of the American Association of Tissue Banks, allograft-associated infections remain a concern. Like all joint preservation procedures, outcomes of osteochondral allografting can be limited by overall disease progression and degenerative burden in an arthritic joint organ. Allograft failure can occur due to nonunion or late fragmentation and collapse—although bone-to-bone healing reliably occurs in well-fixed grafts, revascularization of the graft is more variable. Fragmentation and collapse typically occur in unvascularized areas of the allograft, heralded by new pain or mechanical symptoms, often paradoxically during the delayed phase of creeping substitution after the actual graft/host interface appears consolidated on initial radiographs. However, subsequent imaging may show joint space narrowing and subchondral sclerosis or cyst formation. MRI can be useful for identify-

ing comorbid joint pathology. Mechanical allograft failure often is accompanied by foci of graft collapse on MRI, although interpretation of this modality is challenging even at baseline because increased fluid signal intensity may also accompany normal, well-functioning grafts. Depending on the degree of symptoms and objective joint function, treatment options include observation, selective removal of the fragmented portion of the graft, revision allograft transplantation, or conversion to arthroplasty.

21.7 Summary

Early knee OA is increasingly recognized as an intermediate stage on the continuum between isolated chondral lesions and generalized OA, and represents a potential timepoint for surgical intervention and disease modification in these younger patients. Younger patients with early knee OA represent a challenging demographic because they are poor candidates for conservative treatment or arthroplasty, but may potentially benefit from more versatile cartilage restoration techniques that address larger and more complex lesions. Osteochondral allografting restores osteochondral architecture and bone stock providing reliable osseous integration, as well as native tissue characteristics with viable type II hyaline cartilage. This versatile procedure has a long, successful clinical track record of durable biologic resurfacing for a wide spectrum of joint pathology [50, 51]. Unique limitations of osteochondral allografting include the logistical and financial aspects of their procurement, processing, and storage, although generally accepted as highly cost-effective [52]. The surgical technique for osteochondral allografting is reproducible, particularly so with the advent of specialized instrumentation. Despite a relatively high reoperation rate shared with other cartilage restoration procedures, patient satisfaction after osteochondral allografting is high and it remains a reasonable salvage option, particularly in patients of an age and activity level not optimally suited for prosthetic replacement.

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Cell-Based Procedures for Early Osteoarthritis

22

Gergo Merkely, Zgoda Molly, and Christian Lattermann

22.1 Introduction

Articular cartilage has very limited healing potential secondary to the poor regenerative capacity and avascular nature of cartilage and due to the presence of highly differentiated chondrocytes in the tissue [1]. Consequently, once articular cartilage is damaged, full recovery of its structure, function, and biomechanical properties is unlikely and is usually a step toward progression to osteoarthritis (OA) [2]. Articular cartilage injury comprises a spectrum of disease entities ranging from single, focal chondral defects to progressive degenerative disease, and end-stage OA. Consequently, the restoration of a symptomatic cartilage lesion is essential to avoid or slow down the OA progression.

Patients with early OA have been reported to have inferior outcomes with an increased prevalence of early failure after cartilage procedures [3, 4]. Both after cell-based procedures and after osteochondral autograft and allograft transplantation, the worst performing groups with regard to survival of the original graft are patients with early OA [4, 5]. The underlying reasons for this failure are unknown and likely multifactorial in nature. Contributing factors are age, duration of clinical symptoms (recurrent effusions and pain prior to chondral repair) [6, 7], the presence of severe preoperative muscular atrophy and deconditioning [8, 9], and joint environment (synovial inflammation, subchondral bone alterations).

Despite the aforementioned negative effects of early OA on the outcome of cartilage restoration procedures, symptomatic cartilage defects have to be restored in order to decrease the joint pain, improve function, and to avoid the development of chronic inflammation. Currently, neither cell-based procedures nor osteochondral autograft or allograft transplantation seems to be superior compared to one another in this patient population; however, cell-based procedures tend to be more sensitive to inflammatory changes within the joint [10]. For instance, IL-1b is known to cause matrix degradation in cartilage and elevated IL-1b levels seems to negatively influence the clinical outcomes following cell-based therapies [10, 11].

In our opinion, therefore, establishing more stages of early OA with strict inclusion and exclusion criteria would be necessary. Subsequently, clinical outcomes following different cartilage repair procedures could be compared which might yield a sufficient therapeutic algorithm for such patient population.

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As a first step on this road, the purpose of this chapter is to introduce different cell-based cartilage repair procedures that are currently used in patients with early OA.

22.2 Factors to Consider before Cell-Based Procedures

22.2.1 Importance of the Intact Subchondral Unit

The presence of articular cartilage within the knee joint is pivotal to the successful functioning of the knee itself. Not only does it provide a lubricated surface for the articulation of both the tibiofemoral and patellofemoral regions but also allows the transmission of loads with minimal friction [12]. Articular cartilage is a highly specialized tissue composed of the cell type chondrocytes. While these cells are exclusive to cartilage, they make up very little of its composition, with only 4% of cartilage wet weight contributed to the existence of chondrocytes [13]. Rather, articular cartilage is primarily composed of water which accounts for 65–85% of its total weight. Other major components include the extracellular matrix (ECM) comprised of type II collagen (15–20% of weight) and proteoglycans (PGs) (3–10% of weight) [2]. Examining the ECM further, it is composed of three primary types of macromolecules: fibers (collagen and elastic), proteoglycans, and glycoproteins. These components work together to retain water within the ECM, which is imperative to maintain its unique mechanical properties [12, 13]. The nature of the ECM is directly related to the volume and function of the chondrocytes as the cells are responsible for the synthesis and maintenance of ECM components [13]. As part of its specialized nature, articular cartilage has a highly organized structure of four distinct zones: the superficial (tangential) zone, middle (transitional) zone, deep (radial) zone, and calcified zone [13]. These different zones are characterized by the chondrocyte phenotype, cell shape, and ECM structure constituting that zone [14]. A notable feature within these zones is the tidemark—a thin basophilic line that separates the deep zone from the calcified zone. In other words, the tidemark designates the boundary between mineralized and unmineralized regions of articular cartilage and can be visualized on a slide stained with hematoxylin and eosin [13]. Beneath the calcified zone is the subchondral bone plate; their separation marked an evident landmark—the cement line. An interface of calcified cartilage securely anchors the articular cartilage to the subchondral bone. This attachment creates a complex of cartilage and bone referred to as the "osteochondral unit." It is important to mention this subchondral bone when discussing articular cartilage health, as their homeostasis is relevant to the discussion of joint health and potential treatment plants [15]. In practice, it is important to access the health and status of the subchondral bone prior to surgery using magnetic resonance imaging (MRI) (Fig. 22.1). The subchondral bone plays an essential role in supporting the articular cartilage in several various capacities. Mechanically, the subchondral bone is vital for the transfer of load as it attenuates the majority of the impact load felt by the knee joint (about 30%) in comparison to the load lessened by cartilage (1–3%) [16]. Furthermore, the subchondral bone is richly innervated by sensory and sympathetic nerve fibers, whereas the articular cartilage is not. These innervations have several functions including the facilitation of bone regeneration and remodeling, articular surface homeostasis, and the generation of pain [17]. Subchondral bone also differs from articular cartilage in its ample vascularization. The rich network of blood vessels within the subchondral bone are responsible for supplying at least 50% of key nutrients such as glucose, oxygen, and water to the cartilage by way of diffusion. The vessels are able to penetrate the calcified cartilage and support the deep layer metabolically in this way. Similarly, signaling molecules can also be transferred between bone, cartilage, and surrounding tissue via this extensive vascularization network [18, 19]. With the numerous functions and dependability of subchondral bone, it is easy to understand that any changes or damage to this area can potentially result in more significant injuries including

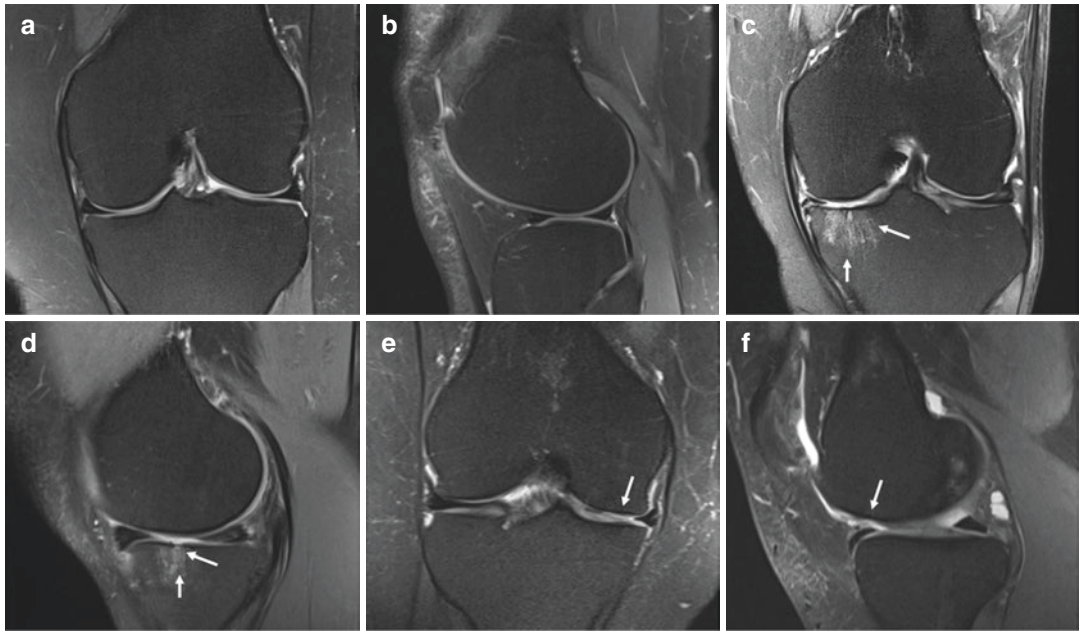

Fig. 22.1 Fat-suppressed intermediate-weighted magnetic resonance imaging (3 Tesla) of normal and full thickness cartilage lesions with and without bone marrow changes in the tibiofemoral compartment. (**a** and **b**) Coronal and sagittal images of normal cartilage in the tibiofemoral compartment. (**c** and **d**) Coronal and sagittal images of a full thickness cartilage lesion without underlying bone marrow changes. (**e** and **f**) Coronal and sagittal images of a full thickness cartilage lesion with underlying bone marrow changes

imbalanced osteochondral homeostasis between the various tissues and microenvironments of the joint. Some common clinical changes to the subchondral bone include bone marrow edema, underlying subchondral cystic change, and intralesional osteophyte and sclerosis. Bone marrow edema (BME) in particular is a stress response described as a nonspecific reaction of the bone to either an acute trauma or from chronic repetitive injury due to overload. BME may also represent nonhistological characteristics of tissue damage and repair including microtrabecular fracture and fracture healing [20–22]. Previous studies have shown that cartilage repair procedures are sensitive to the status of the underlying subchondral bone. In cases where a previous microfracture surface treatment resulted in subchondral bone changes such as bone marrow edema or subchondral sclerosis, there has been a considerable decline in long-term outcome results [22]. Furthermore, if the osteochondral unit is subject to repetitive microinjuries, there is a possibility of beginning a chronic repair mechanism. Ultimately, this mechanism leads to the formation of new, hardened bone just below the surface of cartilage (subchondral sclerosis), a common occurrence in patients following marrow stimulation techniques (MST) in the joint. This potentially provides the reason why in patients undergoing autologous chondrocyte implantation (ACI) with a history of MST, there is a three to eight times higher failure rate in addition to a decreased satisfaction rate [22–26].

22.2.2 Defect Characteristics

When a cartilage defect is discovered, there are several factors and elements that must be considered prior to treatment. Details about the defect size, location, containment, and chronicity must be indicated in order to determine if and how treatment can proceed [27].

The size and location of the cartilage defect have a significant impact on the surface treatment technique and plan chosen (Fig. 22.2). Smaller

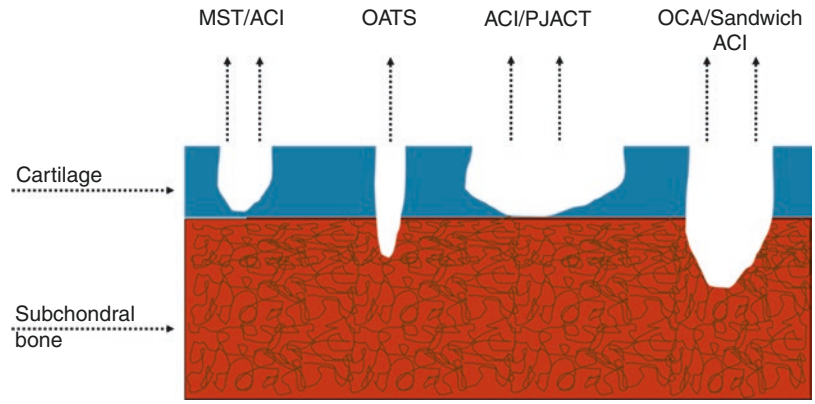

Fig. 22.2 Cartilage repair options for different cartilage defects

defects (<2–4 cm2) found on either the medial or lateral femoral condyle are primarily treated with marrow stimulation techniques (MST). These approaches seem to adequately fill small defects within this specific compartment of the knee joint however, it does have its disadvantages. MST leads to the formation of fibrocartilage repair tissue, an inferior replacement of the native hyaline cartilage. This substitution of repair cartilage poses a problem as the fibrocartilage does not reflect the same mechanical strength properties as its predecessor, leading to its inability to handle the repetitive load the knee joint consistently experiences [28–30]. In the event of larger lesions (>2–4 cm2) or any defects present in the patellofemoral compartment, MST is not sufficient treatment for neither the size nor location of the defect. Instead, cell-based procedures such as an autologous chondrocyte implantation (ACI) or particulated juvenile allograft transplantation (PJACT) are utilized with several studies reporting satisfactory outcome results most notable in long-term follow-up [4, 31–35]. ACI has also been shown to have good results in the treatment of multiple lesions and bipolar defects [36].

Another detail to mark about any cartilage defect is its containment, or the status of the cartilage surrounding the lesion. It is imperative that there are healthy cartilage shoulders around the defect for several reasons including containment of the MST clot as well as to minimize any potential damage to the graft due to disruptive loads. In the event that there are no cartilage shoulders and a defect is uncontained, there are certain approaches that can be utilized to contain an uncontained defect. An example is the placement of an osteochondral allograft (OCA) on the uncontained side of the lesion. Small holes can be drilled through the bone to be suture sites for graft placement or the graft may be sutured to the synovium. In any case of cartilage defect, it is generally more beneficial to leave a minimally chrondromalacic cartilage border as opposed to removing a border entirely leaving the defect uncontained.

The chronicity of the lesion must also be examined prior to treatment. More specifically, the presence and severity of any osteoarthritis (OA) in the joint. Previous data has shown that cartilage restoration or repair procedures result in unfavorable clinical outcomes when associated with advanced stage OA [4, 31–35, 37]. Due to this, patients with more than 50% of joint space narrowing are not recommended to undergo cartilage surface treatments as the risk for failure increases.

22.3 Technical Aspects of Cell-Based Treatment Options

22.3.1 Autologous Matrix-Induced Chondrogenesis (AMIC)

AMIC is a single-stage procedure which combines microfractures with the use of a porcine collagen type I/III bilayer matrix, to protect the blood clot that results from subchondral bone stimulation [38]. This technique can be

performed open or arthroscopically. AMIC results in overall superior short-term outcomes compared to microfracture alone [38, 39]. However, long-term durability is still controversial and has to be further evaluated. [39, 40]

22.3.2 Autologous Chondrocyte Implantation (ACI)

For larger lesions (>2–4 cm^2) and those in the patellofemoral compartment, the repair technique of choice is an autologous chondrocyte implantation (ACI). In the United States, the current generation of the ACI is termed MACI, for matrix associated chondrocyte implantation. Here, chondrocytes are first cultured onto a scaffold of Type I/III bilayer collagen prior to shipping. Previous studies report this cartilage repair procedure has a high satisfaction rate over long-term follow up, specifically showing good outcomes in more than 80% of those with femoral condyle defects and more than 70% of those with patellofemoral [4, 34]. Bipolar lesions, for example on the trochlea and patella, are also best treated with ACI [33, 41]. The primary advantage of ACI/MACI is that hyaline cartilage is produced within the defect as opposed to the replacement fibrocartilage resulting from MST [42].

The ACI/MACI is a two-stage procedure. The first stage requires the patient to undergo a diagnostic arthroscopy of the joint. In addition, a small cartilage biopsy of a nonweight-bearing area of the joint is also taken during this arthroscopic assessment for further analysis. The second stage of ACI/MACI procedure is the actual implantation of the membrane (Fig. 22.3). This is frequently performed via an arthrotomy, or an opening of the joint enough to clearly expose the cartilage defect. It is imperative to properly prepare the defect prior to placement of the scaffold. Defect preparation includes radical debridement of all fissured articular cartilage surrounding the full-thickness chondral injury. In surgery, small ring or closed curettes are utilized to debride the degenerated cartilage tissue to an adequate containment level. The goal of this debridement is to remove all fissured and degenerated tissue to eventually reach healthy contained cartilage while avoiding puncturing the subchondral bone.

Incomplete debridement of the defect area has correlated with early failure and poor patient outcomes following ACI/MACI surgery. Nondebrided fissured cartilage has the potential to poorly integrate adjacent cartilage which leads to the progression of disease or delamination of the repair tissue. Of equal importance to complete cartilage debridement is the preservation of an intact subchondral bone. In the event that the subchondral bone is perforated, the risk of bone bleeding becomes evident as well as the potential for mixed marrow cells populating the chondral defect. The latter case would not only introduce a variety of cells into an extrinsic location but also overload the defect area with foreign cells as well as those chondrocytes attempting to repair the area [43].

While ACI/MACI has shown immense potential for cartilage repair, there are certain cases that require special attention. For example, consider a patient that previously underwent an MST and has alterations of the subchondral bone such as sclerosis or intralesional osteophytes as a result. If an ACI/MACI still seems as the best technique for cartilage repair, the subchondral alterations must first be addressed before any enduring implantation is placed [44].

22.3.3 Particulated Juvenile Allograft Transplantation (PJACT)

In considering other cell-based procedures for cartilage repair, technology has made it possible to generate and use the prepackaged allograft, particulated juvenile allograft transplant (PJACT) [45]. The PJAC is prepared as 1 mm3 cubes of live juvenile chondrocytes in their native ECM from donors under the age of 13. A benefit of these younger chondrocyte cell is that their proteoglycan production is 100 xs more plentiful than their adult chondrocyte counterparts with no associated immunologic reaction [46]. The cubes are stored within blister packs contained in a storage medium

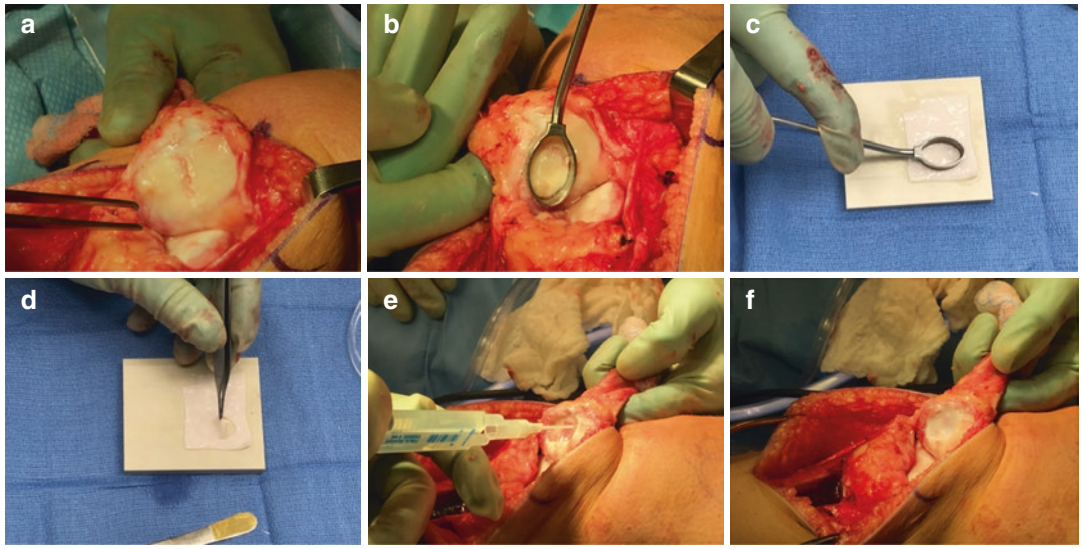

Fig. 22.3 Surgical images of an autologous chondrocyte implantation for a cartilage defect on the patella. (**a**) Monopolar focal patellar cartilage defect with friable fibrocartilage. (**b**) The cookie cutter (with defect size marked on the handles) is gently pushed down to the level of the calcified cartilage layer to create a contained defect with stable and vertical edges. (**c**, **d**) The cutter is used to cut the appropriate size graft from the MACI sheet. (**e**) The MACI is secured to the bone with glue and the periphery is sealed. (**f**) Glue is set and the MACI is stable

and one blister pack can cover up to a 2.5 cm^2 defect area [31]. This PJAC, much like ACI/MACI, can be used as a repair technique for contained, focal cartilage lesions in the joint. Moreover, in particular cases where either an osteochondral allograft transfer system (OATS) or osteochondral allograft transplantation (OCA) is used but does not cover the entire defect, a PJAC can be used in conjuncture to fill in the spaces. Multiple studies have reported increased functional outcomes, histologically filled hyaline-fibrocartilage, and near normal cartilage repair on MRI [35, 47].

The surgical procedure of PJAC implantation can be performed either open or arthroscopically. The initial step in each case is the same—the defect is identified and efficiently prepared prior to placement of the implant. This preparation includes extensive debridement of all fissured and undermined cartilage, confirmation of stable containment with vertical cartilage shoulders, and removal of the calcified layer without perforating the subchondral bone.

1. *Open technique:* Implantation of PJAC via an open surgical technique can be performed by two approaches:
   (a) In situ gluing: The PJAC is applied directly to the defect. Fibrin glue is then applied to the graft to fix it in place.
   (b) Ex situ gluing: A foil mold is first made of the defect itself. Then, the PJAC graft is distributed within the mold followed by fibrin glue. The glue is then allowed to cure or harden for approximately 3–10 min. The whole PJAC/glue complex is then removed from the mold. A fresh layer of fibrin glue added to the bottom of the complex which is then transported to the defect.

The first approach, in situ gluing, is often considered the better option. This is because there is less manipulation of the PJAC/glue complex and the possibility of better fixation and fuller coverage of the defect. For either approach in the open implantation of PJAC, it is important to keep in mind the uniform sizing that 1 package of graft covers 2.5 cm^2 of the defect. This implies that larger defects will need multiple grafts. Furthermore, the PJAC/glue construct must be thinner than the surrounding healthy cartilage shoulders. This is to minimize any potential for disruptive loads

that could result in the graft being damaged or destroyed.

2. *Arthroscopic technique*: In the case of an arthroscopic approach, the joint is first emptied of fluid. Then, the PJAC graft is loaded onto a 2.4–4 mm arthroscopic cannula and delivered onto the defect. A probe is used to smooth and shape the surface of the graft, and fibrin glue is injected into the defect area using a needle to fix the graft into place. Once the glue has been given time to set, the stability of the graft can be tested with passive movements of the joint. It is important that the joint is not filled with fluid for arthroscopy following the placement of the PJACT. The introduction of fluid increases the risk of the graft dislodging or dissociating from the defect. If there remains any concern for the graft's stability following the fixation of the fibrin glue, a collagen I–III membrane can be put in place to protect the PJAC.

Studies investigating the long-term follow-up and effects of the PJACT are continuing to examine any potential graft failures and their underlying causes. One such study has been recently completed by Tan et al. [48] as they evaluated four patients with failed PJACT. Of the four, two allografts failed to integrate into the surrounding cartilage and two had failures associated with impingement. Further histological inspection of patients with large lesions (>15 mm), the repair tissue from the allograft displayed Type I collagen with depleted PGs and Type II collagen. A common underlying theme that all studies have found is that an intact subchondral bone is necessary for a successful cartilage surface treatment.

22.4 Postoperative Rehabilitation After Cell-Based Procedures

A crucial element of a successful cell-based cartilage repair recovery process is the postoperative rehabilitation program. In general, the postoperative rehabilitation plan for cell-based procedures is very long and involved with a structured outline subjects should follow. This phased rehab program is very important as the graft needs the time and protection to mature and join with native tissue correctly. There are in general four postoperative recovery stages of an ACI/MACI rehabilitation that follows implantation. The first of these phases is the proliferative stage from surgery to up to 6 weeks following. Here, the chondrocytes continue to proliferate and the graft tissue begins to fill the defect. Next, the transition stage is from 6 to 12 weeks and soft, primitive tissue repair occurs. From 12 to 26 weeks is the early maturation stage, characterized by the repair beginning to solidify. Here, the ECM mainly consists of type-II collagen and aggrecan along with other matrix proteins. Lastly, the late maturation stage holds the fully matured chondrocytes and matrix occurring from 26 weeks to 3 years [4, 49]. Up until this last stage, the graft must be protected from too much weight bearing-load as is very sensitive and vulnerable, most particularly to shear forces. This is just one of the many reasons that the rehabilitation for this process is so lengthy and orderly.

Consequently, the rehabilitation plans have centered on the post-surgical phases of weight bearing status. A 2012 study by Ebert et al. [50] put forth an "accelerated" rehabilitation study where the patient would be allowed to become progressively weight bearing after 6–8 weeks of nonweight bearing. Instead, the following rehabilitation protocol outline in generally recommended. In the first 6 weeks following surgery, early motion is heavily emphasized. This is achieved by using continuous passive motion (CPM), active and isometric straight leg raises, and touchdown weight-bearing as tolerated. A stationary bike can be attempted by 3 weeks postsurgery. Any delay in the start of this early rehabilitation may potentially lead to limited knee motion and arthrofibrosis. Beginning week 7 and continuing until week 12, patients progress from partial to full weight bearing status. Prospectively, patients are weight-bearing as tolerated with the exception of two conditions—the lesion is very large (>8 cm^2) or any additional procedures, such as a meniscus repair or transplant, were completed that nullify any earlier weight bearing status. From 4 months on, functional activities such

as bicycle, treadmill, elliptical, outdoor walking, hiking, and swimming are permitted. Beginning at 12–14 months post-surgery, jogging is allowed under the condition of a normal knee examination and near normal MRI. Running is not permitted until 12–18 months while cutting sports such as football, basketball, or soccer are not allowed until after 18 months post-surgery. While this outline provides reliable guidance for the rehabilitation post cartilage repair, individual protocols must consider each patient's own surgical reconstruction, the maturation of the graft, and their previous activity level.

While it is safe to have a general outline for all cell-based procedures, the exact plan should be sculpted to the individual patient's needs and goals as they move through their rehabilitation program.

An indirect consequence of this surgery that effects the overall outcome is the recovery of muscle control and strength in the operative leg, particularly concerning the quadriceps muscle. The quadricep muscle, specifically, is so affected because after surgery the knee joint is immobilized causing the muscle to rapidly atrophy. Regaining strength takes a lot of time and effort through an intensive rehabilitation protocol [51]. Howard et al. [52] conducted a study to examine quadricep strength following ACI procedure. In analyzing both eccentric and concentric muscle strength, they found that the recovery of quadricep strength takes up to 1 year following surgery. Even at that time point, some quad-specific tasks such as sit-to-stand and step-up and over may not fully return to normal activity levels. It is apparent that this considerable loss of muscle function must be addressed when continuing rehabilitation. New therapies have recently come forward providing ways to possible overcome this muscle weakness. Such potential techniques moving forward to compact quadricep weakness are high intensity quadriceps training and blood flow restrictive therapy. In the latter, a cuff is placed on the operative limb to partially restrict incoming arterial blood flow to muscle but greatly restrict outgoing venous flow from the muscle itself [53, 54].

A study by Toonstra et al. [55] collected data from seven patients through open-ended semi-structured interviews. All the subjects had undergone ACI procedure and were presently at various points in their rehabilitation program. The study reported three major themes concerning the rehabilitation protocol itself and the subjects' attitudes toward it. Generally, the patients felt that their overall recovery was an emotionally and lengthy process that eventually makes them optimistic to reach their future goals. While the road to recovery is a large commitment in the case of cell-based cartilage repair procedures, the eventual outcomes show immense potential for improvement.

22.5 Definition of Partial and Complete Cartilage Repair Failure

As with any surgical case, there is always the risk of eventual treatment failure. In the instance of cell-based cartilage repair procedures, failure can be broadly characterized as recurrent pain symptoms and decrease in patient reported function and outcome as measured by patient reported outcome measure (PROM) instruments provided post-operatively.

The state of the graft can be accessed for any failure post-implantation by either MRI or an additional arthroscopic surgery. Potential failures of any cartilage repair graft include the inability of the graft to adequately fill the defect, any fibrous filling, or a partial or complete delamination of the graft itself. Both a partial and complete graft failure allude to an issue with the graft structure but to varying degrees of severity. A partial graft failure is defined as the removal of less than 25% of the graft area of less than 25% of the defect being underfilled. Conversely, a complete graft failure indicates more than 25% of the implanted graft being removed or the defect being underfilled. An unrelated but still important type of failure is progression of disease passed the status of the baseline. An example of this is the development of cartilage lesions within other compartments

of the knee joint than were initially affected. Furthermore, the appearance of any joint space narrowing or osteophyte formation which may suggest an increase in the severity of OA symptoms. Decision-making of which procedure to choose to revise a failed cartilage repair procedure is complicated and excides the scope of the current chapter. However, given the fact OA is a spectrum disease of entity, patients who underwent cartilage repair for symptomatic cartilage defect is highly likely to progress to definitive OA and undergo some sort of revision to cartilage repair surgery.

22.6 Conclusion

Articular cartilage is a key element of normal joint function. In early OA this may be one of the earliest tissues to be affected and it is often the focus of early treatment. While cartilage repair is no exact cure or defense against the inevitable presence of OA, there are several techniques to treat focal cartilage defects once they occur and therein providing better functional outcomes and hopefully delaying OA progression. Cartilage repair in early OA is of importance both with regard to the prevention of rapid progression but also with regard to the longevity and success of the cell-based cartilage repair. Previous studies have shown that in patients with early OA, cartilage repair treatment report poor outcomes and increased failure due to a multitude of reasons. For this reason, the establishment of defined set of guidelines, accurately describing the stages of early OA are immensely helpful to provide the best patient specific care possible.

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23 Coral-Based Bioscaffold for the Treatment of Osteochondral Lesions of the Knee

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23.1 Introduction

Chondral and osteochondral defects of the knee associated with joint degeneration are renowned for being challenging to address [1].

In the past years, different techniques have been adopted with the aim of treating joint surface lesions (JSL) associated to osteoarthritis (OA). Initial strategies such as microfracturing and arthroscopic debridement had not entirely satisfactory outcomes because of their inability to modify the course of the disease [5–9].

Another method approached for the treatment of lesions in joints with OA was matrix-assisted autologous chondrocyte implantation (MACI) which resulted in promising short-term results but worsening outcomes in the long term. Some of the issues of the MACI technique are the two-step surgical approach, issues with ex-vivo cultivation, and high costs of cell expansion [10–14].

Scaffolds have been proposed in order to keep the healing process of cartilage active and at the same time protect it from physical insults [2–4].

More recently, cell-free biomimetic scaffolds have been adopted to assist the regeneration of cartilage and the underlying subchondral bone in JSL. The advantages of this product are its availability and single-step surgical procedure. Nowadays, only a few scaffolds have been approved for clinical use, among those used there are monophasic and biphasic types [2, 15, 16].

Currently, there are several new technologies being developed for the treatment of osteochondral defects of weight bearing joints, among which, cell-based or acellular matrix-based technologies and biologic agents. Biomimetic scaffolds are being increasingly used, in particular it has been seen a tendency toward the development of multiphasic scaffolds able to promote regeneration of both the subchondral bone and cartilage layer.

Scaffold-based technologies have demonstrated to provide an environment for cell proliferation and differentiation into proper lineages capable of repairing the osteochondral defect. Properties of the ideal scaffold are still a subject of study, with the purpose of increasing the healing capacities of cells and signaling factors to obtain a superior tissue quality and, therefore, better clinical outcomes. The idea of creating a cell-free implant that is capable of providing the joint with appropriate stimuli that induce orderly and durable tissue regeneration is attractive, new biomaterials have been recently proposed to

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induce "in situ" regeneration after direct transplantation onto the defect site [17].

Tissue engineering aims to create 3D grafts by exploiting the patient's own stem cells and porous biomaterials as a template for tissue development [18]. To obtain better results in terms of tissue regeneration, the scaffold should mimic the biology, architecture, and the structural properties of native tissues in order to facilitate cellular migration, attachment, proliferation, and differentiation. Furthermore, important functional properties of a scaffold include biocompatibility and biodegradability through safe biochemical routes in order to avoid long-term complications due to the persisting presence of non-autologous material.

In case of osteochondral lesions of the knee, surgical treatment can be challenging. The difficulty arises since two different tissues are involved, bone and cartilage, with different ability to heal. A successful strategy to "engineer" osteochondral tissue is based on mimicking the natural contour of the articulating surface, achieving native mechanical properties and functional load-bearing ability, in order to lead to integration with the host cartilage and underlying subchondral bone. Surgical intervention that involves cartilage transplant has a downside, it is unable to protect chondrocytes that become overstressed and differentiate in fibroblasts, which ultimately leads to fibrous and weak cartilage with accumulation of type I collagen. As mentioned above, scaffolds are capable of protecting and microstimulating the chondrocytes simultaneously. This method will yield a more functional cartilage with the presence of aggrecan and collagen type II and avoid weakening and loss of function of the cartilage. In the case of acellular scaffolds, similar results are obtained with the same mechanism but with the addition of initial migration of neighboring chondrocytes in the scaffold area.

23.2 Chemical–Physical Composition of Agili-C

The scaffold can be defined as coral based implant, where the coralline skeletal material is composed of calcium carbonate in the crystalline form of aragonite. Corals are marine invertebrates from the Anthozoa class that include over 7000 species, 6 of which are used for medical applications: *Porites*, *Acropora*, *Lobophyllia*, *Goniopora*, *Polyphyllia*, and *Pocillopora.*

Coral exoskeletons (aragonite) are remarkably similar to human bone, including their 3D structure and pore interconnections in the crystalline form of calcium carbonate. These features, together with the high inter-connecter macroporosity required for vascular tissue ingrowth, make aragonite a suitable material for bone repair [19].

The calcium carbonate structures are gradually resorbed and replaced by functional bone tissue. Coral derivatives are commonly used as bone graft substitute and bone-void fillers.

These peculiar features allow bone marrow and synovial mesenchymal stem cells (MSC) adhesion, differentiation, and proliferation into chondrocytes, ultimately promoting articular cartilage restoration [20].

Agili-C scaffold consists of a porous, interconnected calcium carbonate (aragonite) derived from purified, inorganic coral exoskeleton: the lower part of the implant is composed of sole inorganic aragonite, while a square grid pattern of 2 mm deep-drilled channels is made in the top part of the scaffold.

Histology performed by an independent laboratory in a series of preclinical studies on the goat model (with evaluation performed at 6 and 12 months after implantation) confirmed the ability of Agili-C to regenerate hyaline cartilage, as demonstrated by the presence of collagen type II and aggrecan, and the lack of collagen type I in the repair tissue, alongside the reconstruction of the subchondral bone, with a gradual increase in tissue maturation over time. Further in-vitro analysis [21] revealed the potential of the chondral phase of Agili-C implant to recruit autologous chondrocytes from the surrounding healthy cartilage: these chondrocytes migrate inside the scaffold and contribute to the deposition of extracellular matrix (ECM) rich in collagen type II and aggrecan. Ultimately, the formation of a layer populated by progenitor-like cells on the surface of the implant was documented. Based on

Fig. 23.1 The Agili-C aragonite-based scaffold. The surface of the scaffold is represented by the micro-drilled layer, which is implanted 2 mm below the surrounding cartilage in a press-fit manner. (Reproduced with permission from Â© Cartiheal, Inc. All rights reserved)

the encouraging findings emerged from in vitro [21] and animal trials, a pilot clinical study on humans was performed [22] to confirm the safety of use and the potential to provide clinical improvement. The positive results from the pilot trial prompted a larger, multicenter observational study to be started.

Results of the application of Agili-C scaffold can be found in the following multicentric study of the duration of 2 years (Fig. 23.1).

23.3 Scaffold Preparation and Sterilization

The basic scaffold consists of coralline aragonite. Following a mechanical process, a square grid pattern of 1 to 2 mm deep channels is drilled in the chondral phase of the scaffold, using Bungard CCD, a CNC drilling, a routing machine, and an appropriate drill bit. This scaffold configuration was originally developed in the shape of cylinders. It is 10-mm high with variable diameters available, from 10 to 17.5 mm, to match the lesion size.

After extensive purification processes, needed to treat and remove trapped particles, debris and organic remnants, the implants are sterilized by 25 kGy gamma radiation.

23.4 Patient Selection and Evaluation

Eight European hospitals took part in the multicentric study. Patients' enrollment took place between 2016 and 2017, during which time all the patients were informed on the degenerative changes of their knee.

All the patients have been prospectively evaluated before the surgical procedure and during the follow-up visits at 6, 12, 18, and 24 months. During these visits, they have been clinically evaluated and interviewed to assess their symptomatology, actual physical status and knee functioning.

23.5 Surgical Technique and Rehabilitation Protocol

Standard knee arthroscopy is initially performed to ensure the patient's eligibility. A mini arthrotomy is performed to expose the lesion. CartiHeal surgical toolset is used to prepare the implantation site, creating a 12 mm deep cavity with perpendicular shoulders. The implant is then inserted through press-fit implantation in the opposite site, 2 mm below the articular cartilage. In case of multiscaffold implantation, it is important to keep at least a 5 mm bone bridge between the implants. Implant stability is ultimately tested with cyclic knee bending.

The rehabilitation protocol included toe-touch weight bearing using crutches for 4 weeks, followed by increasing partial weight bearing aimed at reaching full weight bearing after 6 weeks. Cryotherapy and continuous-passive-motion (CPM) are started during the first 2 days and continued for 3 weeks. During the first 48 h, cryotherapy in combination with a continuous-passive-motion (CPM) device are applied and continued for 3 weeks (Fig. 23.2).

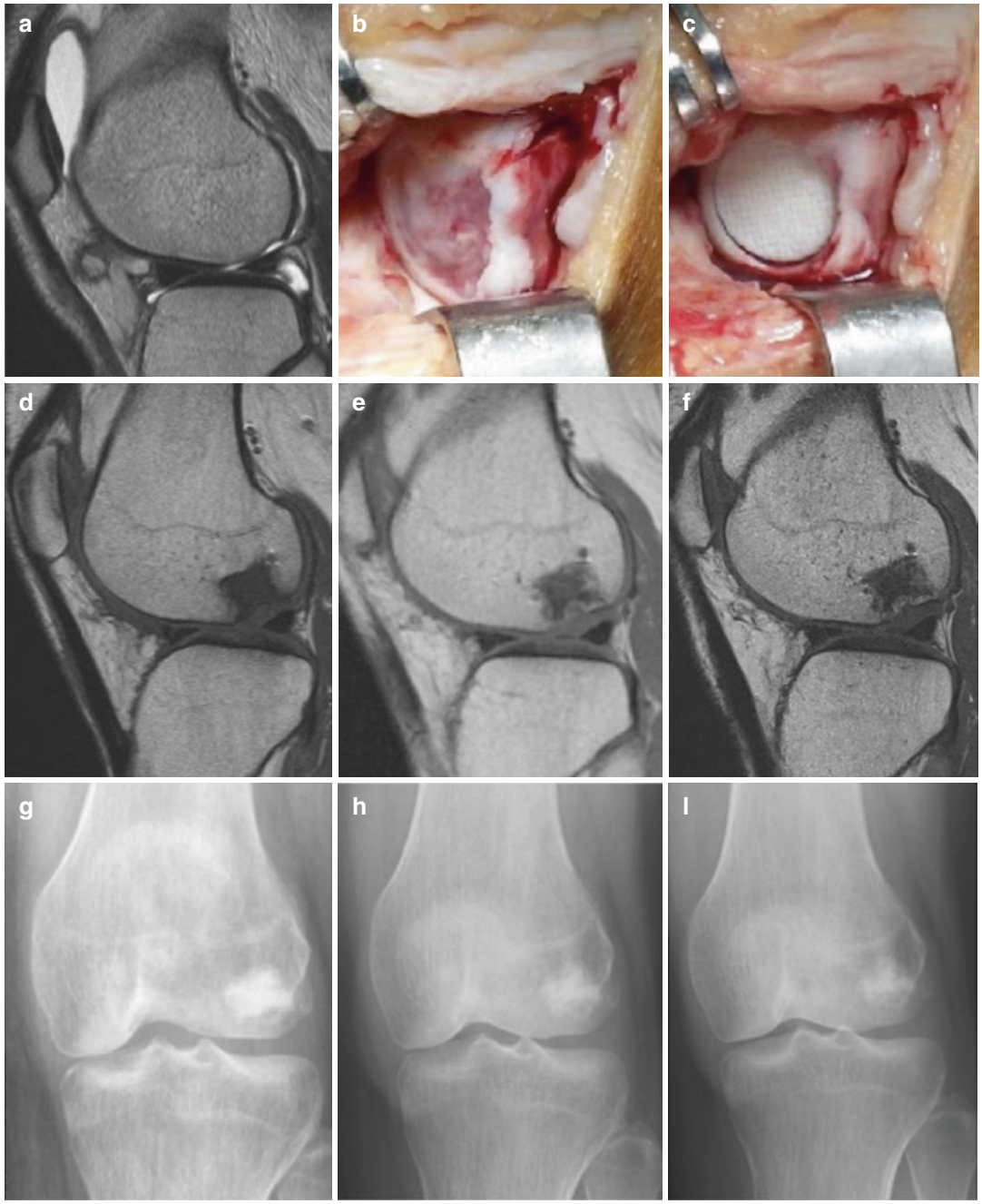

Fig. 23.2 A 32 year-old female with mild OA and an osteochondral defect on her LFC. Patient treated with a single aragonite-based implant. (**a**) Baseline MRI; (**b**) intraoperative view on the defect; (**c**) Agili-C implantation; (**d**) 6 months' MRI; (**e**) 12 months' MRI; (**f**) 24 months' MRI; (**g**) 6 months' X-ray; (**h**) 12 months' X-ray; (**i**) 24 months' X-ray. (Reproduced with permission from Â© Cartiheal, Inc. All rights reserved)

23.6 Histology

The following specimen was taken from a patient who underwent total knee reconstruction and was sent to an independent lab for CGP histological analysis. Newly formed cartilage was found on most of the surface of the scaffold, along with restoration and integration of the subchondral bone plate with the surrounding naive bone (Fig. 23.3).

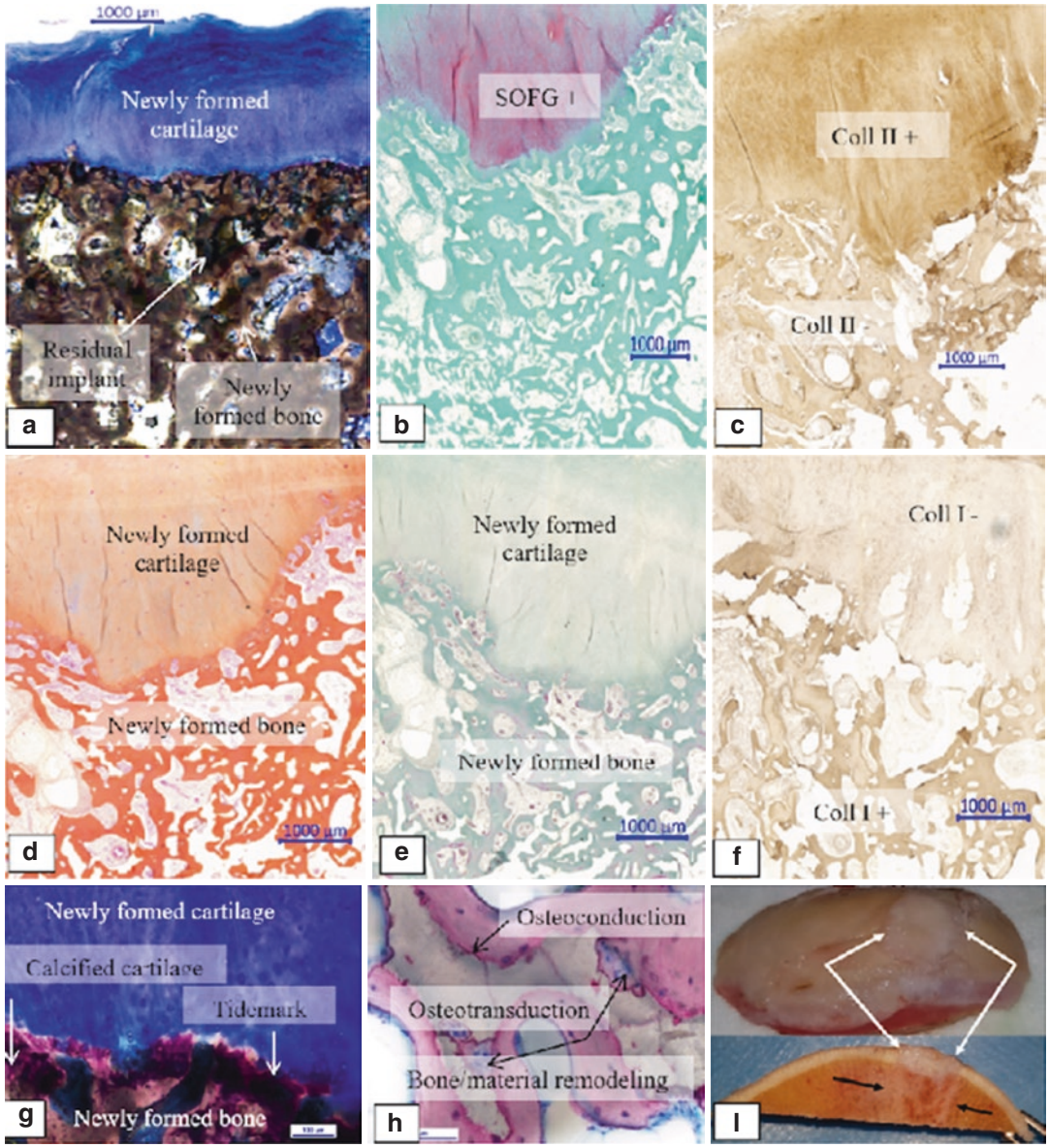

Fig. 23.3 Histologic evaluation of the explanted specimen. (**a**) Paragon stain; (**b**) Safranin-O-Fast Green stain; (**c**) Collagen type II marker; (**d**) Safranin Hematoxilin Eosin stain; (**e**) Masson trichrome; (**f**) Collagen type I marker; (**g**) Paragon stain; (**h**) Paragon stain; (**i**) The harvested condyle. (Reproduced with permission from Â© Cartiheal, Inc. All rights reserved)

23.7 Results

Significant improvement in all KOOS subscales was recorded (Pain: 49.6 ± 13.1, ADL: 56.1 ± 18.4, Sports: 22.8 ± 18.8, QoL: 23.5 ± 16.5, Symptoms: 55.4 ± 19.9) compared to the 24 months' follow-up (Pain: 79.5 ± 21.1, $p < 0.001$; ADL: 84.1 ± 21.4, $p < 0.001$; Sport: 60.8 ± 31.9, $p < 0.001$; QoL: 54.9 ± 30.4; $p < 0.001$; Symptoms: 77.7 ± 21.2, $p < 0.001$). IKDC-subjective score also improved from 37.8 ± 14.7 at baseline to 65.8 ± 23.5 at 24 months ($p < 0.001$). MRI evaluation showed a significant increase in defect filling over time, up to 78.7 ± 25.3% of surface coverage after 24 months. Treatment failure requiring revision surgery occurred in eight patients (9.3%).

Total number of patients	86
Age (mean ± SD)	37.4 ± 10.0
BMI (mean ± SD)	26.1 ± 3.5
Sex	60 M (69.8%)/26 F (30.2%)
Previous surgery in the affected knee	48 (55.8%)
ICRS grade	Grade 3: 21 (24.4%) Grade 4: 65 (75.6%)
Lesion size in cm^2 (mean ± SD)	3.0 ± 1.7
Lesion location	
Medial femoral condyle	44 (51.2%)
Lateral femoral condyle	15 (17.4%)
Trochlea	13 (15.1%)
Multiple sites	14 (16.3%)
K/L grade	Grade 2: 75 (87.2%) Grade 3: 11 (12.8%)
Concomitant procedures	19 pts. (22.1%) 2 HTO, 8 partial meniscectomy, 1 meniscal suture, 4 debridement of other superficial lesions (ICRS grade I or II), 3 loose body removal, 1 synovial plica removal

	Baseline	6 Months	12 Months	18 Months	24 Months	*p* (24 m vs. basal)
KOOS pain	49.6 ± 13.1	73.0 ± 21.1	77.5 ± 19.6	78.1 ± 21.1	79.5 ± 21.1	<0.001
KOOS ADL	56.1 ± 18.4	78.7 ± 20.9	82.5 ± 18.9	83.5 ± 20.3	84.1 ± 21.4	<0.001
KOOS sport	22.8 ± 18.8	48.1 ± 29.5	55.5 ± 29.9	56.0 ± 31.9	60.8 ± 31.9	<0.001
KOOS symptoms	55.4 ± 19.9	71.9 ± 21.7	75.9 ± 19.8	76.1 ± 22.0	77.7 ± 21.2	<0.001
KOOS QoL	23.5 ± 16.5	44.7 ± 27.6	48.7 ± 26.3	52.4 ± 27.7	54.9 ± 30.4	<0.001
KOOS overall (average of all 5 subscales)	41.5 ± 14.3	63.3 ± 21.7	68.0 ± 20.9	69.2 ± 22.8	71.4 ± 23.6	<0.001
IKDC	37.8 ± 14.7	55.4 ± 21.5	62.2 ± 20.6	63.6 ± 21.6	65.8 ± 23.5	<0.001

23.8 Adverse Events

- Fifteen patients were affected by knee swelling and pain.
- Three patients suffered knee stiffness.
- One patient experienced delayed surgical wound healing.
- Three patients experienced knee pain following physiotherapy sessions.
- Two patients had knee trauma during follow-up.
- One patient had a loose body removed that caused sporadic locking episodes.
- One patient experienced patellar tendinitis.

- One patient struggled with quadriceps weakness.
- One patient presented with synovial hypertrophy and exuberant intra-articular scar tissue.

23.9 Conclusion

The Agili-C scaffold has shown promising clinical and radiologic results at 2 year evaluation for the treatment of ICRS grade III and IV defects in knees affected by osteoarthritis, owing to its unique ability to promote osteoinduction and osteotrnansduction.

Despite the hostile joint environment, it has been shown that the scaffold may be able of enhancing the healing process of the osteochondral unit.

Taking into consideration the category of patients treated with the aragonite-based scaffold, the failure rate of 9.3% can be considered acceptable [20].

Further research should revolve around comparison between the randomized controlled studies with surgical standard of care to ascertain the superior treatment option.

Undoubtedly, longer term evaluation is essential to establish the longevity and soundness of the results.

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Potential Gene Therapy Options for Early OA

24

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24.1 Introduction

Osteoarthritis (OA) represents a high-burden noncommunicable disease (NCD) and is a principal cause of chronic disability in adults [1]. It is the most common inflammatory and degenerative disease of synovial joints. More than 500 million people worldwide suffer from its debilitating clinical symptoms and ~80% of the elderly population shows radiographic signs. The incidence of OA is further rising because of an aging population and the epidemic of obesity, especially in more economically developed countries [2]. The total age-standardized disability-adjusted life-years (DALY) rates considerably rose by 35% and age-standardized DALY rates by 4% between 1990 and 2015, [3] attesting to the ever-growing epidemiological and socioeconomic priority of OA.

The debilitating pain and the progressive loss of joint function are the two major clinical signs, leading to a considerably impaired quality of life. Irreparable degeneration of the articular cartilage is the major hallmark of early OA. However, OA is not a simple degenerative disease where the cartilage wears away with time. Rather, it is a progressive and complex derangement of the homeostasis resulting in an imbalance of the entire osteochondral unit and other tissues constituting a joint. Cartilage degradation is therefore not a discrete phenomenon, but part of a complex remodeling of joints. OA has a multifactorial etiology with risk factors including genetics, age, obesity, joint injury, knee malalignment, female sex, and joint loading. The recent failure of several clinical trials aiming to provide novel disease-modifying OA drugs (DMOADs) and biological interventions underscore the need for novel therapies. Especially the early phase of OA represents a valuable target for a molecular disease-modifying approach as a game-changing strategy for OA patients. Such early interventions are largely lacking, as most current therapeutic procedures aim at the advanced stage, including total joint replacement (TJR), while in early stages, unspecific conservative treatment options such as nonsteroidal anti-inflammatory drugs (NSAIDs) or opioids for pain relief, physiotherapy for the maintenance of a reduced joint function are applied, all aiming to delay TJR. However, they are frequently associated with adverse events especially in comorbid patients when used at long-term or high doses. Moreover, although TJR appears as an attractive "solution" for OA, it principally ignores the underlying causes and may lead to serious secondary problems, partly because of a disproportionate indication for younger patients. This chapter outlines potential gene therapy options for early OA.

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24.2 Principles of Gene Therapy for Early Osteoarthritis

The early phases of OA are probably the most relevant to be treated with gene therapy approaches since the structural destruction and erosion just begin and there is still cartilage remaining that can respond to a gene-based treatment. As early OA mainly affects the articular cartilage, this tissue is a key target for gene therapy in early OA. However, although cartilage damage itself is important, the altered cellular and humoral inflammatory and immunological patterns contribute to the progressive damage in early OA and thus gene therapy approaches may also target other tissues, most importantly the synovial membrane to interfere with the subsequent affection of the articular cartilage. For ex vivo approaches, articular chondrocytes are important and have been clinically used, although mesenchymal stromal cells (MSCs), fibroblasts, and bone marrow aspirates are also of interest.

24.3 The Normal Osteochondral Unit

24.3.1 Articular Cartilage

Hyaline articular cartilage is a connective tissue with a smooth, shiny, white surface, covering the ends of the articulating bone partners in diarthrodial joints [4, 5]. Its main function is to provide a smooth, low-friction surface for articulation and to facilitate load transmission to the underlying subchondral bone [5]. The extracellular matrix (ECM) of the articular cartilage is composed of a fluid phase (~80% of its total weight), mostly water, and a structure forming solid phase of collagens (75% of the dry weight), predominantly type-II collagen (>90% of all collagens), providing tensile strength, and proteoglycans (20–30% of the dry weight), most abundantly aggrecan, providing compressive resilience by entrapping large quantities of water through their hydrophilic glycosaminoglycan side chains [4, 6, 7]. The dense ECM encapsulates a small volume (~2%) of chondrocytes, the only cell type of this tissue, with an important role of producing and maintaining the ECM [5]. Based on the phenotype of the chondrocytes and the composition of the ECM, the articular cartilage can be divided into four zones: superficial (tangential), transitional (middle), deep (radial), and calcified zone [8]. Hyaline cartilage is an avascular, aneural, and alymphatic tissue [9, 10]. These characteristics with the limited motility and proliferative ability of mature chondrocytes result in an extremely low regenerative capability.

24.3.2 Subchondral Bone

Located below the articular cartilage, the subchondral bonetransmits loads, maintains the joint shape, and mechanically and metabolically supports the cartilage [11]. The two tissues are chiefly held together by three-dimensional interdigitation [12]. The subchondral bone has a dense vascularization and innervation [13]. Anatomically it can be divided into the subchondral bone plate (cortical bone) and the subarticular spongiosa (trabecular bone). The subarticular spongiosa is more porous and metabolically active than cortical bone, [7] and it is connected to the cartilage, across the subchondral plate by narrow canals and wider ampullae [13]. MSCs can be found in the bone marrow of the subarticular spongiosa, and if OA erosion exposes the subchondral bone plate, they migrate into the OA defect site and initiate an insufficient fibrocartilaginous repair response [14]. The subchondral bone and the articular cartilage, thus, form a tight functional association, termed as the osteochondral unit [15, 16].

24.4 Structural Changes to the Osteochondral Unit in Early OA

The course of OA begins with increased water content and swelling of the ECM, and increased metabolic activity of the chondrocytes, accompanied by surface fibrillations and primary osteoporotic changes of the subchondral bone [7, 10, 17,

18]. Disruption of the chondrocyte pericellular matrix exposes the cells to components of the interterritorial matrix, which deregulates chondrocyte function [6, 19]. Chondrocytes become apoptotic or form clusters, and in an attempt at repair, increase their synthetic activity, release catabolic enzymes including aggrecanases, which enhance matrix degradation and proinflammatory mediators, which contribute to synovial proliferation and vascular penetration, disrupting the tissue integrity and facilitating bone formation [1, 20]. The depletion of proteoglycans is followed by the erosion of the collagen network, resulting in deeper fissures, clefts, and delamination of the cartilage [6, 7, 18]. Hypertrophic changes of the cells result in expression of type-X collagen, expansion of the calcified zone, tidemark duplication, and reinitiation of endochondral ossification [1, 7, 21].

Parallel with the appearance of degenerative changes of the articular cartilage, OA structural changes develop in the subchondral bone too, beginning with an increased subchondral bone plate porosity and primary osteoporotic changes of the trabecular bone [17, 18]. Decreased mineralization and trabecular volume, number, and connectivity characterize this early stage of the disease [18]. The initial loss of subchondral bone is followed by subchondral sclerosis and an increasing subchondral trabecular volume and complexity, together with the formation of osteophytes which stabilize the joint [7, 18]. Later in the trajectory of OA, more pronounced subchondral bone changes develop including bone marrow lesions, cysts, and bone attrition [6].

24.5 Gene Delivery Methods to Articular Cartilage

The accessibility of different upgraded gene transfer techniques has successfully focused on the chondrocytes in vitro and in situ/in vivo when their impermeable ECM encompasses the cells. Such methods depend on either nonviral compounds or viral contaminant vehicles that utilize normal passage pathways in cells.

Gene therapy uses the delivery of deoxyribonucleic acid (DNA) into cells, which can be accomplished by several methods. The two major classes of methods are widely used (1) those that use naked DNA or DNA complexes (nonviral methods) and (2) those that use recombinant viruses (sometimes called biological nanoparticles or viral vectors). Generally, most viral vectors and nonviral vectors have been unable to transduce the articular chondrocytes surrounded by their local ECM directly. Interestingly, they have been an incredible possibility for an assortment of ex vivo gene transfer methods. Recombinant adeno-associated viral (rAAV) vectors are the only known class of gene vectors that can penetrate the dense extracellular cartilaginous matrix with high efficiency and make them particularly suited for approaches to improve the structural qualities of articular cartilage.

24.5.1 Viral Vectors

Different viral vectors have been employed to treat orthopedic lesions, including gene vehicles based on adenoviruses [22], retro/lentiviruses [23, 24], and the adeno-associated virus (AAV) [25–27]. Episomal adenoviral vectors are highly efficient to modify both in chondrocytes (~100% transduction efficiencies) and MSCs (~80% transduction efficiencies) but only over limited periods (some days to 1–2 weeks) while provoking detrimental host immune responses [28], as a primary concern to use in clinical applications, particularly in the treatment of OA. Additionally, intraarticular injection of such vectors is less available to the articular cartilage and mainly focuses on the synovial cells. The use of a helper-dependent adenovirus (HDAd) mediated intraarticular gene therapy approach for long-term expression of interleukin-1 receptor antagonist (IL-1Ra) as sustained symptomatic and disease-modifying therapy for OA was investigated in mouse and horse models, demonstrating safe symptomatic and disease-modifying effects [29].

Retroviral vectors have the advantage of integrating their DNA into the host genome, allowing them to maintain gene expression for

more extended periods. These retroviral vectors may cause insertional mutagenesis and actuation of oncogenes. Likewise, retroviral vectors transduce just dividing cells and may not appropriate with a limited host range and low efficiencies (<20% in MSCs before cell choice) [30]. Lentiviral vectors, a subclass of retroviruses, might be alternatives as they can integrate into the genome of nondividing cells (up to 95% in chondrocytes and 70% in MSCs), allowing for long-term transgene expression. However, again, there is a risk for insertional mutagenesis and safety regarding their typical origin [31, 32].

AAV is a nonpathogenic, replication-defective human parvovirus manipulated to generate small rAAV vector particles by completely replacing the viral sequences making them less immunogenic than adenoviral vectors. rAAV vectors are mostly maintained under stable episomal forms, allowing for long-term transgene expression (several months to years) and avoiding the risk of insertional mutagenesis. rAAV vectors can transduce both dividing (MSCs up to 65–92%) and nondividing (chondrocytes up to 95%) cells at relatively high efficiencies, favoring direct approaches in vivo. As some cell types remained relatively refractory to conventional rAAV vectors, intense research has been performed to generate hybrid, pseudotyped, chimeric, and self-complementary adeno associated viral vectors (scAAV). These vectors appeared to be capable of overcoming the rate-limiting step of conversion from single- to double-stranded DNA. The problem of the limited capacity of rAAV has been apprehended by taking advantage of the virus's ability to form circular concatamers. Consequently, rAAV became a preferred gene transfer method for cartilage repair [33–37]. Considerably, these vectors are already being engineered to treat experimental OA in vivo [38–40], for example, to overexpress insulin-like growth factor I (IGF-I) [41–43]. Delivery of rAAV vectors within biomaterials protects the AAV capsid epitopes from the action of neutralizing antibodies in vivo [44–46].

24.5.2 Nonviral Vectors

Although viral nucleic acid transfer systems have been more popular, significant effort has gone into enhancing the transfection efficiency of nonviral delivery, making nonviral approaches promising tools for further application in basic, translational, and clinical studies on OA; thus nonviral gene delivery technologies have the potential to transform the future development of disease-modifying therapeutics for OA [47].

Nonviral vectors involve complexing therapeutic DNA to various macromolecules, including plasmid cationic lipids and liposomes [48–50], polymers [51], polyamines and polyethyleneimine [52, 53], and nanoparticles [54]. Nonviral systems prevent the potential of acquiring replication competence inherent to viral vectors, can be repeatedly administered, carry large therapeutic genes, are relatively easy to produce on a large scale, have low immunogenicity, and are less expensive compared to viral gene transfection. Different industrially accessible procedures might be applied to focus on the chondrocytes or different OA cell populations, such as the lipid-based reagent FuGENE® 6 for chondrocytes [55] and a polyamine formulation for MSCs [56]. Despite having advantages, nonviral vectors have not yet replaced viral vectors due to relatively low efficiency on ex-vivo modified cells and the inadequacy to alter chondrocytes promptly in the cartilaginous ECM [57].

24.5.3 Strategies of Gene Therapy for Early OA

The major strategies of gene therapy for OA are in vivo and ex vivo approaches (Fig. 24.1). In vivo gene transfer directly applies the gene vector to the tissue of interest, which then produces the therapeutic gene product, while ex vivo gene transfer is based on providing a cell population that is overexpressing and producing the therapeutic gene product. Ex vivo gene transfer necessitates therefore the in vitro manipulation of the target cells.

24.6 Gene Transfer to Articular Chondrocytes In Vitro

Articular chondrocytes are in the major focus of gene therapy approaches for early OA, although also synovial cells, MSCs, meniscal fibrochondrocytes, tendon/ligament cells, muscle, and bone cells are of interest [58]. Numerous strategies based on the mechanisms involved in early OA have been followed. Of particular interest is the stimulation of chondrocyte proliferation and survival via transfer of inhibitors of apoptosis (bcl-2) [59], growth factors genes (e.g., IGF-I, bone morphogenetic proteins – BMPs, basic fibroblast growth factor – FGF-2,) [33, 60–66], telomerase (hTERT) [67, 68], but also heat shock protein 70 (HSP70) [69], superoxide dismutase (SOD) and catalase [70], siRNAs [71], IkappaBalpha [72], or Dickkopf (Dkk), Wnt inhibitory factor (WIF), β-catenin, or sclerostin [73–75]. Another logical interesting approach is the stimulation of anabolic pathways to return to normal levels of individual ECM molecules like type-II collagen and proteoglycans. Especially growth and transcription factors or signaling molecules have been tested, including IGF-I, FGF-2, BMPs, parathyroid hormone-related peptide—PTHrP, SOX factors [76], Indian Hedgehog—Ihh, zinc-finger protein 145—ZNF145 [16, 33, 34, 61, 64, 66, 68, 77–109].

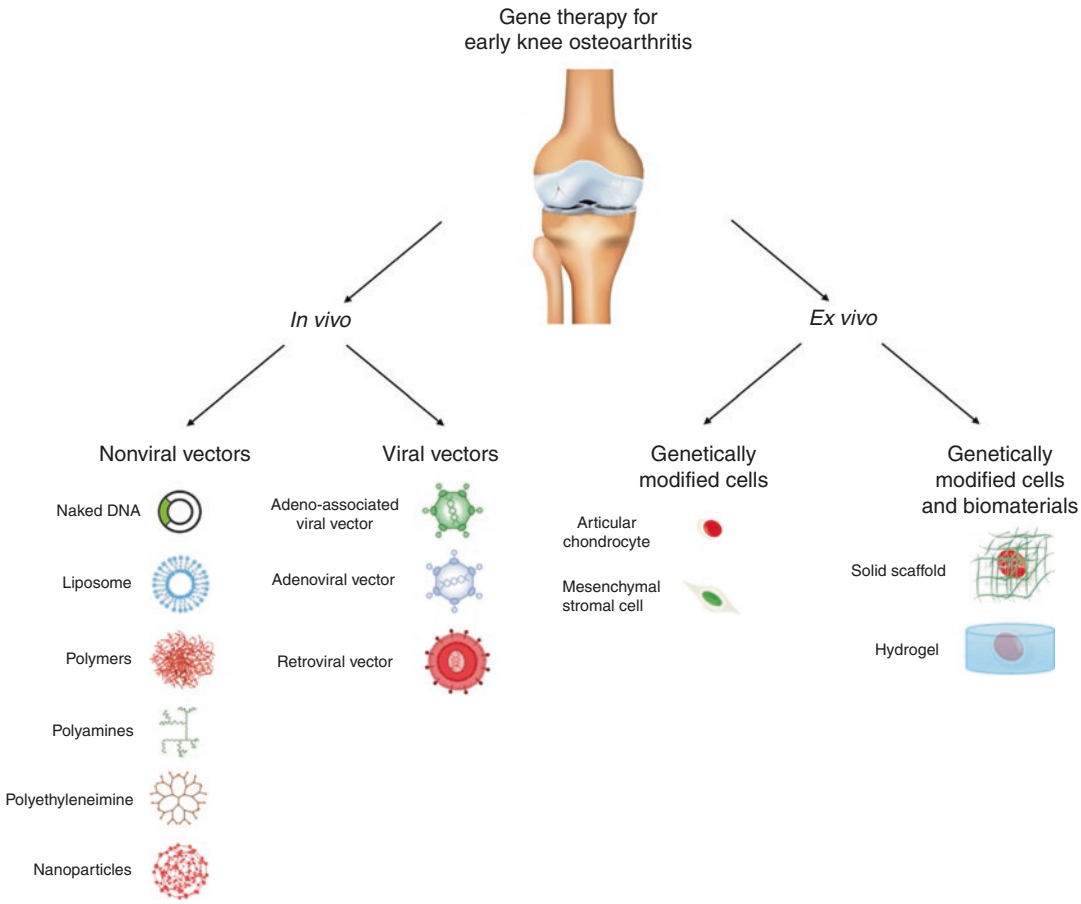

Fig. 24.1 Strategies of gene therapy for early OA. In vivo gene therapy approaches, including nonviral and viral vectors, directly transfer the gene vector into the tissue of interest in vivo, leading to the local overexpression and production of the therapeutic gene product. Ex vivo gene transfer, including genetically modified cells alone or in combination with biomaterials such as hydrogels, is based on providing in vivo and in vitro gene-manipulated cell population that is overexpressing and producing the therapeutic gene product

Particularly, relevant for early OA is also the inhibition of inflammatory and catabolic pathways that may be present, for example, by application of inhibitors of matrix-degrading enzymes [110–112] or of proinflammatory cytokines [37, 38, 40, 80, 85, 113–123], and also chondroprotective cytokines IL-4 and IL-10 [116, 124]. Many of these approaches have been also used in combined gene transfer tactics, for example, by coupling anabolic with anti-inflammatory strategies [79, 80, 85, 100, 101, 108, 120, 125–128].

24.7 Translational Investigations of Gene Therapy for Early OA In Vivo

Translational investigations of gene therapy for early OA in vivo examined various pathological aspects of OA [129–135]. Direct gene transfer strategies have been tested by either (1) systemic delivery or (2) intraarticular vector administration via intraarticular injection or direct application of the gene vector to cartilage following an arthrotomy. The classical approach of intraarticular injection achieves restricted and transient transduction of the synovium, being more adjustable inhibits inflammatory and catabolic pathways [96, 136, 137]. The small rAAV vectors are particularly useful as they can directly transduce the chondrocytes within their ECM. Direct gene transfer in experimental models of OA was performed using sequences to inhibit cartilage destruction like IL-1Ra [40, 116, 119, 138–140], IL-10 [116], HSP70 [69], gene silencers [141, 142], inhibitors of angiogenic and inflammatory pain processes [143, 144], antagonists of transforming growth factor-beta (TGF-β) and BMPs (to inhibit the formation of osteophytes [145, 146], antagonists of the canonical Wnt pathway [74], or kallistatin or angiogenic inhibitors [147, 148]. Recent approaches also use rAAV vectors overexpressing IL-1Ra as a secrete therapeutic products, showing elevated IL-1Ra levels in the synovial fluids of injected joints by >40-fold over endogenous levels sustained for at least 6 months [149]. A test of efficacy in an equine model using scAAV.IL-1Ra gene-delivery led to a 30–40% reduction in lameness and ~25% improvement in total joint pathology, which included reduced joint effusion and synovitis, and improved repair of osteochondral lesions [150].

However, it is unclear whether the loss of ECM and cells may be fully compensated although the breakdown of the cartilage could be contained, supporting the idea of a combined approach based on an additional stimulation of ECM synthesis by providing gene vectors coding for anabolic factors [79]. A current study showed that such combinatorial gene therapy protects against cartilage degeneration in post-traumatic OA. Combined delivery of helper-dependent adenoviruses expressing IL-1Ra and proteoglycan-4 (PRG4) preserved articular cartilage better than monotherapy in both models [151]. This improved protection was associated with increased expression of proanabolic and cartilage matrix genes together with decreased expression of catabolic genes and inflammatory mediators. In addition to improvements in joint tissues, this combinatorial gene therapy prolonged protection against thermal hyperalgesia compared to monotherapy, extending both physiological and functional protection in a model of post-traumatic OA [151].

Gene therapy for follistatin (FST) was recently shown to mitigate systemic metabolic inflammation and post-traumatic arthritis in high-fat diet-induced obesity. A single injection of rAAV-FST was administered in mice after several weeks of high-fat diet feeding [152]. The FST gene therapy mitigated the severity of OA following joint injury and improved muscle performance and decreased obesity-associated metabolic inflammation. As rAAV gene therapy shows an excellent safety profile and is currently in clinical trials for a number of conditions, such an approach may allow the development of therapeutic strategies not only for OA but also, more broadly, for obesity and associated metabolic conditions, including diseases of muscle wasting [152].

Targeting the articular inflammatory response in posttraumatic joints and thereby protecting cartilage from degradation and OA, a gene therapy approach using an rAAV vector to overexpress IL-10 in the joint was described [153].

Although intraarticular levels of IL-10 were 5- to 60-fold greater than control, and no perturbations of IL-10 in the systemic circulation were noted, further supporting the use of AAVs as gene therapy vectors for the treatment of joint disease [153].

Indirect gene transfer strategies via application of ex vivo modified cells can, in theory, not also provide a therapeutic protein but also a call population capable of resurfacing the damaged surface. Moreover, no free vector particles are introduced and the modified cells can be tightly controlled in vitro, although performing the additional in vitro steps also complicates testing, require relatively invasive techniques of preparation, with a limited supply and possibly impurities and/or changes in cell phenotype upon expansion [154, 155]. Synoviocytes were used for such ex vivo avenues in OA models by transporting inhibitors of inflammatory and catabolic processes (IL-1Ra alone or with IL-10) [118, 156]. Progenitor cells are more easily isolated and maintain multilineage potential and capability of differentiation and expansion [157, 158]. For example, intraarticular injection of muscle-derived stem cells that were modified via combined retroviral gene transfer of BMP-4 with sFlt-1 improved microstructural cartilage repair for up to 12 weeks in a rodent OA model [159].

Although the subchondral bone is involved in early OA [160], gene therapy approaches in this context have mainly been studied so far in focal osteochondral defect settings [57]. It is imaginable that a gene therapy approach targeting the subchondral bone is also of value for early OA, for example, as direct rAAV-mediated overexpression of human FGF-2 via improved the repair of the subchondral bone besides the articular cartilage in a lapine osteochondral defect model [16, 33, 34, 61, 64, 66, 68, 77–109]. Yet, elaborated gene therapy approaches do not always provide cartilage protection in OA models, but may instead result in increased ectopic bone formation [161]. For example, the anti-inflammatory and chondroprotective effects of tumor necrosis factor-inducible gene 6 (TSG-6) could not reduce cartilage damage compared to the controls when an adenoviral TSG-6 expression vector was injected into joints of C57BL/6 mice with collagenase-induced OA [161]. Instead, ectopic bone formation was found in the TSG-6 treated group, highlighting the importance of always considering also the bony compartment of the osteochondral unit [161]. Likewise, when rat stifle joints received a rAAV containing the Wnt-inhibitor Dkk-1 or a Wnt10b transgene, osteophytosis was decreased by Dkk-1, but unchanged by Wnt10b [162]. Destabilization of the joint negatively influenced bone architecture, increased osteophytosis, and decreased soft tissue integrity. Dkk-1 exacerbated the negative effects of destabilization, whereas Wnt10b had little effect on these parameters. Therefore, neither Wnt10b nor Dkk1 showed positive benefits on the progression of OA, suggesting that therapeutic strategies aimed at altering Wnt signaling in OA must consider the complexity of these effects [162].

24.8 Current Clinical Gene Therapy Trials for Osteoarthritis

Historically, the first human clinical gene therapy trial in the musculo-skeletal field was applied for rheumatoid arthritis (RA) of the metacarpo-phalangeal joints using the intraarticular injection of autologous synoviocytes transduced ex vivo via retroviral gene transfer of a cDNA of IL-1Ra into metacarpo-phalangeal joints of patients prior to open synovectomy [58, 163–165]. Local transgene expression was confirmed without undesired events, and even some clinical improvements in phase II studies were reported [58]. Since recombinant IL-1Ra was produced, such an approach has also potential value for early OA by inhibiting inflammation.

The first clinical trial for OA was performed in patients with end-stage (Kellgren and Lawrence grade 4) knee OA [166, 167], providing important lessons also for early OA approaches (Table 24.1). It was reported and repeatedly published [170, 171] that chondrocytes of a cell line established from human juvenile allogeneic chondrocytes (a single new-born donor with

Table 24.1 Clinical trials of currently tested gene therapy approaches in OA patients

Strategy	Route of application	Vector	Modified cells	Gene	ClinicalTrials.gov Identifier	References
In vivo	*i.a.*	Plasmid DNA	–	IL-10	NCT03477487	[168]
In vivo	*i.a.*	rAAV vector	–	IL-1Ra	NCT02790723	[58, 169]
Ex vivo	*i.a.*	Retroviral vector	A mixture of TGF-β1-transduced and irradiated human embryonic kidney 293 cells with normal human allogeneic articular chondrocytes	TGF-β1	NCT03203330	[170]

OA osteoarthritis, *i.a.* intraarticular, *IL-10* interleukin 10, *IL-1Ra* interleukin-1 receptor antagonist, *AAV* adeno-associated virus, *TGF-β1* transforming growth factor beta1, *NCT* national clinical trial

polydactyly) were modified via retroviral-mediated gene transfer to overexpress a cDNA encoding TGF-β1 [58]. As TGF-β1 stimulates chondrocyte proteoglycan synthesis [172], proliferation [173], and cartilage repair [174], this approach is also of value for early OA. TGF-β was released following retroviral transduction and overexpression [81] and the safety of the approach was reported to be confirmed in rabbit and goat models [175]. To avoid the risk of random integration into the genome and resulting carcinogenic effects distinctive of retroviral vectors, the transduced cells were reported to be irradiated prior to in vivo application [58]. These irradiated, TGF-β1-transduced chondrocytes were then were reported to be combined at a ratio of 1:3 with nontransduced, nonirradiated human juvenile allogeneic chondrocytes from the same cell line [58] and injected as a single intraarticular injection (total volume: 3.5 ml) into the knee subsequently to aspiration of the synovial fluid [167]. Initially, a multicenter, single-blind, phase IIa clinical trial was conducted (27 patients, late-stage knee OA [176]. Here, it was reported that patients received doses of either 6×10^6 or 1.8×10^7 TGF-β1-transduced allogeneic chondrocytes (1:1 ratio). It was published that both groups had improved clinical outcomes (pain, function, physical ability) without serious adverse events [176]. As high intraarticular levels of TGF-β1 may cause synovitis, pannus formation, cartilage erosion, joint effusion, and osteophyte development in animal models [177, 178], the safety of such an approach is of paramount importance. However, only minor side effects like swelling, effusion, and minor reactions localized to the injection site (edema, pain, warming sensation, or itching) were observed in an phase I safety trial in patients with advanced knee OA (full-thickness cartilage erosion) without serious and treatment-related adverse events [167]. Joint swelling and arthralgia resolved or were improving at the study end. An anaphylactic shock of 1 patient was ascribed to a severe hypersensitivity anaphylaxis to a cryopreservation medium (CS-10) [179]. Possibly the limited transgene expression of up to 2 weeks that was caused by the irradiation avoided major undesirable side effects [179]. A subsequent placebo-controlled randomized trial [179] (54 patients, mean age 58 years, body mass index <30, moderate knee OA of Kellgren and Lawrence grades 2–3) reported that at the final follow-up of 24 weeks after intraarticular injection, the treatment group showed significantly greater improvements (IKDC and VAS scores) compared to baseline. Patients who received the placebo were also reported to have improved IKDC and VAS scores, but significantly less compared with the gene treatment. The KOOS scores also significantly improved in both groups when compared to baseline without difference significant difference between the groups [179].

Based on the website of the company Kolon TissueGene [180], the composition of the cell-based product is rather based on a mixture of TGF-β1-transduced and irradiated 293 cells with normal allogeneic articular chondrocytes. 293 cells are more precisely referred to as human embryonic kidney (HEK) 293 cells. The cell line

was generated by exposing cultures of normal human embryonic kidney cells to sheared fragments of adenovirus type 5 (Ad5) DNA [181]. The basis of this cell transformation was the integration of ~4.3 kB of viral Ad5 DNA sequences into human chromosome 19 (19q13.2) [182]. HEK 293 cells are widely used for small-scale recombinant protein production and in viral vector propagation including the production of retroviral vectors and adenoviral vaccines [183]. When injected in immunocompromised mice, 293 cells were tumorigenic with varying frequency and size among different studies [184]. Probably because of inconsistencies with the reported source for the injectable genetically modified cells, the phase III clinical trial [170] in the United States started in 2018 (ClinicalTrials.gov Identifier: NCT03203330) was suspended for a while by the FDA [185]. On ClinicalTrials.gov, the recruitment status is currently posted as active, not recruiting (Last Update Posted: May 13, 2020) [186].

Another gene therapy trial [58] using a self-complementary AAV vector carrying an IL-1Ra transgene (sc-rAAV2.5IL-1Ra) (ClinicalTrials.gov Identifier: NCT02790723) [187] is currently recruiting participants [58, 169]. Nine participants with moderate knee OA in this nonrandomized phase I study evaluating the safety will receive sc-rAAV2.5IL-1Ra as an intraarticular injection. A previous preclinical safety and biodistribution study carrying a rat IL-1Ra transgene (sc-rAAV2.5rIL-1Ra) or human IL-1Ra transgene (sc-rAAV2.5hIL-1Ra) in Wistar rats with mono-iodoacetate (MIA)-induced OA showed that vector genomes persisted in the injected knees for up to 1 year with only limited vector leakage to systemic circulation and uptake in tissues outside the knee [188]. Low levels of IL-1Ra expression were observed in the vector-injected knees [188], and the gene therapy vector demonstrated an overall favorable safety profile [188].

Another phase I dose-escalation study (32 participants, 6 months follow-up) in subjects with severe knee OA was recently completed (ClinicalTrials.gov Identifier: NCT03477487) [189] to evaluate the safety, tolerability, and efficacy of a plasmid DNA encoding a variant of human IL-10 (XT-150). IL-10 is an anti-inflammatory cytokine that potently and broadly suppresses proinflammatory cytokine activity, and a 6-month toxicology study upon intraarticular injections of the plasmid vector in canine stifle joints was without pathologic findings [168]. Moreover, a translational, placebo-controlled study reduced pain measures in dogs without adverse findings [168].

24.9 Outlook

The field of gene therapy for OA has seen significant development over the past 10 years. Several gene therapy trials are now ongoing, using either ex vivo approaches to deliver TGF-β1 or the more potent in vivo strategies, applying either rAAV vectors encoding for IL-1Ra or plasmid DNA encoding for IL-10. These anti-inflammatory strategies are also applicable to early OA, especially post-traumatic OA, together with stimulation of classic anabolic pathways to stimulate chondrocyte proliferation and ECM production. rAAV vectors are particularly well adapted to target in a direct and durable manner, the articular chondrocytes affected in OA in situ for such approaches. Among the different growth factors, IGF-I represents a key candidate able to stop the loss of chondrocyte phenotypic stability in early OA because it is mitogenic for chondrocytes and simultaneously stimulates the expression of type-II collagen and proteoglycans, while the responsiveness to IGF-I is reduced in OA.

Ongoing clinical trials will lead to a better understanding of the mechanisms of repair induced by gene-based approaches. So far, there is no evidence that undesired events might occur as all clinical trials have shown a good safety profile. Placed in the context of the recent failure of clinical trials aiming to provide novel disease-modifying OA drugs, a gene-based approach will have to be measured by providing both quantitative structural improvements (e.g., reduction of cartilage loss) and clinical benefit for our patients Moreover, if pathological imbalances such as axial malalignment or an instable joint are present

no gene-based therapy may succeed if such underlying problems are not properly addressed.

For the future, we propose that ongoing and novel gene therapy approaches will be extended to the early phases of OA, targeting structural destruction and erosion at an early phase. Besides chondroanabolic treatments using growth or transcription factors, anti-inflammatory gene therapy approaches will also be developed to target pathological processes during the onset of early OA.

24.10 Competing Interest Statement

HM is Editor-in-Chief of *Osteoarthritis and Cartilage Open*. MC is a Board member of the *Orthopaedic Research Society*. All authors have no financial conflicts to declare in connection with this chapter.

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Surgical Management for Early Arthritis in the Shoulder

25

Jhillika Patel and Carolyn M. Hettrich

25.1 Introduction

Early arthritis of the shoulder can present a treatment dilemma for physicians, especially when the patient is young or has high activity requirements. Nonoperative management for the early Osteoarthritis (OA) will typically include physical therapy, NSAIDs, and glucosamine supplementation. If the patient remains unacceptably symptomatic, the next step in management can include ultrasound-guided corticosteroid injections or platelet-rich plasma (PRP) injections. When conservative management fails, surgical intervention can be considered. Surgical procedures traditionally have included debridement ± biceps tenotomy or shoulder arthroplasty. For patients who have focal cartilage defects, techniques such as microfracture, autologous chondrocyte implantation (ACI), and osteochondral autologous transfer system (OATS) can be considered.

The latter treatment options for the early osteoarthritis attempt to alter disease progression, and slow progression while restoring shoulder function and reducing pain. The hope is that these can delay shoulder arthroplasty, but this has not been shown to date. Clinical research is still being performed for these procedures, with some case series reporting positive outcomes and very few complications.

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25.2 Arthroscopic Debridement (Chondroplasty) and Biceps Tenotomy

Arthroscopic debridement provides a minimally invasive option to possibly achieve pain relief and restore motion in patients with shoulder arthritis. In most cases, the procedure provides short-term relief from symptoms [1]. This can be done in combination with the techniques discussed later. This procedure is most suited for the early stages of osteoarthritis for which the glenoid and the humeral head are concentric with visible joint space on a radiograph. When the joint incongruity is severe or for patients with large osteophytes, the procedure will not be effective [2]. Patients with a higher preoperative arthritis grade, chondral lesions greater than 2 cm^2, bipolar lesions, and a joint space narrowing of less than 2 mm were associated with arthroscopic failure and are then recommended for arthroplasty [3].

Bigliani et al. concluded that arthroscopic debridement was beneficial for treating early glenohumeral osteoarthritis of the shoulder. Concomitant procedures in this study included lavage of the glenohumeral joint, debridement of

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labral tears, partial synovectomy, and subacromial bursectomy [2]. About 80% of the patients in this study who underwent arthroscopy reported an excellent or good outcome. Ten of the 12 patients who indicated preoperative stiffness saw an improvement in range of motion [2]. A meta-analysis on arthroscopic debridement concluded that unipolar lesions of the glenoid or humeral head were associated with better Western Ontario Osteoarthritis of the Shoulder (WOOS), ASES, and SANE scores postoperatively than bipolar lesions [2].

The comprehensive arthroscopic management (CAM) procedure was developed by Millet et al. to address glenohumeral osteoarthritis in young, high-demand patients. The procedure involves starting with glenohumeral debridement followed by chondroplasty, synovectomy, loose body removal, osteoplasty, optionally capsular release (if needed), and axillary nerve neurolysis [4]. Depending on the patient's pathology subacromial decompression, subcoracoid decompression, and biceps tenodesis may be added to the CAM procedure [4]. A recent study of 38 patients who underwent CAM procedures with 5- and 10-year follow-up, demonstrated an improvement in ASES score from 63.3 to 89.6 after 5 years and to 80.6 after 10, concluding that patients experienced reduced pain and improved function [5].

Bicep injuries that lead to shoulder pain can also be relieved by surgical management through biceps tenotomy. Boileau et al. retrospective study on 68 patients treated arthroscopically with biceps tenotomy resulted in the mean constant score increase from 46.3 ± 11.9 to 66.5 ± 16.3 postoperatively [6]. Patients also had an improvement in the functional range of motion, with a significant increase in postoperative external rotation resulting in a higher degree of patient satisfaction (78%) [6]. A prospective randomized control trial involving 114 patients investigated the outcomes after biceps tenotomy and showed a significant improvement in ASES and WORC scores of 32.3% and 37.3%, respectively, at 24 months postoperatively [7].

25.3 Microfracture

Microfracture traditionally has been the preferred procedure to treat small unipolar humeral lesions on the cartilage. This cartilage damage does not heal easily because of its avascularity and the low migration of chondrocytes into these regions. As such, spontaneous healing of these sites is rare and can lead to further degeneration and osteoarthritis [8]. Microfracture involves penetrating the subchondral bone at the articular lesion site to stimulate clot formation. The pluripotent mesenchymal stem cells in the clot are derived from the bone marrow to produce fibrocartilage within this defect [9]. The advantage of the procedure is technically simple, easily available, has minimal morbidity, and is low cost, making it an attractive first-line treatment for articular cartilage injuries [9]. The downside is that it forms fibrocartilage and not hyaline cartilage.

An ideal patient for this treatment is <40 years old, with minimal damage to the cartilage, lesions isolated to the articular surface, no bone loss, and whose surrounding cartilage is intact [8]. Full-thickness symptomatic chondral defects in the articular portion of the glenohumeral joint are amenable to microfracture [8]. Better results are obtained for lesions less than 2 cm^2 in area. Microfracture is contraindicated if the underlying bone is also injured if it is a bipolar lesion, or there is significant osteoarthritis [10].

Millet et al. reported on 31 shoulders that had undergone microfracture. Six of these failed and underwent a subsequent surgery and were excluded from the data analysis. For the remaining 25 shoulders, there was on average a 20-point improvement in ASES score. When both the glenoid and the humerus were treated, the improvement in ASES score was 19 points, when only the glenoid was treated the improvement was 17 points, and when the only humerus was treated the improvement was 32 points. As the size of the lesion increased, the improvement on ASES score was lower indicating a negative relationship with size. The mean VAS scores decreased postoperatively from 3.8 to 1.6, with a significant improvement in activities of daily living, work, and sports [11].

Another study looked at 54 patients who either underwent humeral head resurfacing, or resurfacing of the humeral head with microfracture of the glenoid. The patients were followed for 10 years. As compared to a baseline relative Constant score of 58.1% for resurfacing alone, the mean relative Constant score for the humeral resurfacing with glenoid microfracture was significantly higher at 77.7% [12]. The pain scores improved for all patients.

Frank et al. studied the efficacy of microfracture for average glenoid defects of 1.66 cm^2 (range, 0.4–3.75 cm^2) and humeral defects of 5.07 cm^2 (range, 1.0–7.84 cm^2) [13]. At 2 year follow-up, there was a significant decrease in VAS pain scores (from 5.6 to 1.9) and statistically significant improvements in the ASES (44.3 to 86.3) and SST (5.7 to 10.3) scores were reported with 93% of the patients indicating satisfaction with the procedure [13].

25.4 Autologous Chondrocyte Implantation (ACI)

Both autologous chondrocyte implantation (ACI) and osteochondral autologous transfer systems (OATS) constitute restorative techniques that may present as options for the early osteoarthritis. Restorative procedures aim to re-establish the hyaline cartilage at the location of the chondral lesion. These procedures have been more thoroughly studied in the knee but may present as viable options for the shoulder. In the first step of ACI, the surgeon harvests chondrocytes from nonarticulating areas of a patient's shoulder or nonweight-bearing cartilage in the knee. These harvested cells are isolated and cultured for about 3–5 weeks to obtain sufficient quantities of chondrocytes, which are 5–ten million cells. During a second surgical procedure, the chondrocytes are injected into the cartilage lesion in the shoulder. These cells are held in place by a periosteal membrane which is secured by fibrin adhesive and sutures [14].

As discussed in other chapters, ACI in the knee has yielded favorable outcomes. Early studies in the shoulder are promising but limited in their significance due to small sample sizes and warrant additional investigation. Boehm reported on seven male patients who underwent ACI for symptomatic focal grade IV cartilage lesions of the humeral head who were followed for an average of 32 months. After ACI, the patients reported their subjective pain score on the VAS scale to be 0 at rest and 0 (0–2) during exercise, the median Constant score was 95 (80–100), and the median ASES score was 97 (90–100), demonstrating good outcomes. Subjects reported a significant improvement in median Subjective Shoulder Value, from an average of 60% preoperatively to 95% postoperatively [15]. Buchmann et al. report prospectively on four subjects undergoing ACI for humeral full-thickness chondral large defects or large symptomatic glenoid lesions. The mean VAS (0.3/10), the mean constant score (83.3 ± 9.9), and the mean ASES index (95.3 ± 8.1) represented satisfactory shoulder function [16]. All patients gained satisfactory shoulder function and had satisfactory coverage of the chondral lesion with fibrocartilaginous repair tissue formation on post-operative MRI [16]. Scheibel et al. followed eight patients prospectively who experienced symptomatic focal grade IV cartilage lesions of the humeral head and were treated with ACI. At a mean of 32.6 months postoperatively, all subjects demonstrated clinical improvement and only minor degenerative radiographic changes [17]. Clinical improvements were seen in these patients Constant scores which increased from 73.9 (57–89.6) to 88.7 (82.4–95.4) and ADL (activities of daily living) rate which increased from 12.9 (7–18) to 19.1 (18–20) [17]. While the authors concluded ACI to be a viable treatment option for focal cartilage lesions in the shoulder, they advised that it should be restricted to young, active, symptomatic patients [17].

25.5 Osteochondral Autologous Transfer System

Osteochondral autologous transfer system (OATS) can also treat focal cartilage lesions in the shoulder. Typically, shoulder arthroscopy is performed

first to measure the cartilage defects and to create recipient sites for press-fit autologous chondrocyte graft from the knee [18]. The autologous grafts are then typically harvested from the knee using lateral mini-arthrotomy from the outer edge of the lateral femoral condyle. These are then transferred to the prepared sites in the shoulder. The donor cavities in the knees can be left empty or backfilled with allograft plugs [18].

OATS is ideally suited for small lesions (100–250 mm^2) [14]. In addition, patients with a full-thickness osteochondral lesion of the glenohumeral joint are good candidates. This approach is suited to young adults (<40 years old) and active patients. In addition, OATS is an effective treatment option for patients who may have osteochondritis dissecans (OCD) of the humeral head [14].

Habermeyer et al. reported on patients who underwent OATS for treatment of chondral defects of the shoulder measuring 156 ± 60 mm^2 [19]. After 8–47 months, the Constant score increased to 89 ± 5 from a baseline of 74 ± 10, and good integration of osteochondral plugs with congruent tissue was observed on MRI [19]. At a mean follow-up of 8.75 years, the Constant score for these patients increased to 91 ± 5. The Samilson and Prieto grading for osteoarthritis showed an increase of at least one grade for all patients in the initial study, however, no significant change was observed at longer follow-up. All plugs showed full integration with the surrounding tissue on MRI, and there was no reported correlation between the size of the defect, the number of graft plugs, and the development of osteoarthritis [20].

In the reported studies, the osteochondral plugs were harvested from the knees. This is advantageous as it does not require two separate surgeries/anesthetics, but it does violate the knee joint, and there remains a risk of knee morbidity in a previously well-functioning knee [20].

25.6 Platelet Rich Plasma (PRP)

Platelet-rich plasma (PRP) is a common autologous biologic treatment used in musculoskeletal treatments. The PRP contains natural growth factors (transforming GF Beta, basic fibroblast GF, and platelet-derived GF), numerous cytokines, and anti-inflammatory mediators which can stimulate healing [21]. Depending on the tolerance for an inflammatory response either a leukocyte rich PRP (LR-PRP) or a leukocyte poor PRP may be chosen [21]. The PRP use varies between clinicians and given the variation in dosing, formulations, concentrations of growth factors, and the presence of leukocytes, it is difficult, if not impossible to make comparisons between studies.

Shoulder osteoarthritis leads to the gradual thinning of the articular cartilage with degeneration of the soft tissues. Oxidative stress-triggered angiogenesis and synovial matrix degradation induce joint inflammation in patients with osteoarthritis. TGF-β1, Ang-1, and Ang-2 are critical components in a PRP treatment to address this inflammation [22].

Kothari et al. compared PRP to corticosteroid and ultrasound therapy in 195 patients with shoulder arthritis. At 12 weeks, PRP showed statistically significant better VAS and QuickDash scores over corticosteroid and ultrasound therapy. Patients receiving PRP also had significantly better active and passive shoulder range of motion [23]. It is important to note that the PRP is palliative and is not regenerative.

25.7 Summary

25.7.1 Osteoarthritis in the Shoulder

Early arthritis of the shoulder can lead to debilitating pain and a decrease in patients' quality of life. Surgical procedures that can be considered for global OA include debridement, biceps tenotomy, and shoulder arthroplasty. For patients who have focal cartilage defects, techniques such as microfracture, autologous chondrocyte implantation (ACI), and osteochondral autologous transfer system (OATS) can be considered. In addition, biologic treatments such as platelet-rich plasma (PRP) can be used for possible palliative pain relief. As early work is promising, more investigative work should be done focusing on the shoulder to further evaluate these surgical techniques for improving function in patients experiencing OA.

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