Developing Insights in Cartilage Repair

Pieter J. Emans
Lars Peterson
Editors

ICRS
International Cartilage Repair Society

Springer

Developing Insights in Cartilage Repair

Pieter J. Emans • Lars Peterson
Editors

Developing Insights in Cartilage Repair

Springer

Editors
Pieter J. Emans, PhD, MD
Department of Orthopaedic Surgery
Maastricht University Medical Center
Maastricht
The Netherlands

Lars Peterson, MD, PhD
Department of Orthopedics
University of Gothenburg
Gothenburg
Sweden

ISBN 978-1-4471-5384-9 ISBN 978-1-4471-5385-6 (eBook)
DOI 10.1007/978-1-4471-5385-6
Springer London Heidelberg New York Dordrecht

Library of Congress Control Number: 2013951515

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Movement is essential for our health and quality of life. Healthy cartilage is paramount in allowing and maintaining this movement. Cartilage is not only present in articulating joints, but also in the rib-cage, ear, nose, bronchial tubes and intervertebral discs. It is essential for functions as breathing, hearing, articulation and locomotion. Hyaline articular cartilage is a truly remarkable material both structurally and functionally. Its principal function is to provide a smooth, lubricated surface for articulation, and is able to withstand an enormous amount of intensive and repetitive forces combined with low friction. These properties are unique and are found only in nature. Articular cartilage enables us, to move, walk, sport, etc. Unfortunately, once damaged cartilage does not heal.

The International Cartilage Repair Society (ICRS) was founded by a mixture of clinical researchers, fundamental researchers, and clinicians. As such the ICRS is a unique forum for international collaboration in cartilaginous tissue research by bringing together clinicians, clinical researchers and basic scientists, engaged or interested in the field of cartilage biology, imaging, cartilaginous tissue engineering and translational clinical approaches to treatment of cartilage pathologies. Although most of the treatment and research involves articular cartilage, it is the aim of the ICRS to study and learn more about cartilage from "the tip of the nose to the big toe".

The link between laboratory work and the daily treatments of patients in a clinical setting is extremely important to the ICRS. This book is an example that the ICRS is a platform for young and energetic clinicians and (basic) scientists to present their work and start inter-disciplinary fruitful collaborations. An inspiring example of a fruitful collaboration between fundamental researchers and clinicians is the chapter entitled "The Genesis of Autologous Chondrocyte Transplantation/ Implantation: From a Hypothesis via an Animal Model to a Clinical Reality".

Furthermore, the society supports research projects and together with the industry the ICRS organizes scholarships and fellowships to stimulate clinicians and researcher to fuel the field with new ideas.

This book is the first of a series, and its chapters are contributed by the Genzyme/ Sanofi and Stryker travelling fellows and gives an overview of existing knowledge

and presents novel findings in basic and clinical research. The book illustrates the progress made in search for the solution in cartilage related problems. Fundamental research, biomaterials, bioreactors, imaging, existing cartilage repair strategies, and emerging cartilage repair techniques are described in this book. The editors also realize that several cartilage repair techniques and other aspects of cartilage (repair) are not described. These issues may be subject in future books of the ICRS book series.

Editors
Prof. dr. L. Peterson, PhD, MD
Gothenburg, Sweden
Dr. P.J. Emans, MD, PhD
Maastricht, The Netherlands

Series Editors
Prof. dr. A. Hollander (ICRS president)
Prof. dr. D. Saris (ICRS past-president)

Content

Contributors

Samuel B. Adams, MD Department of Orthopaedic Surgery, Duke University Medical Center, Durham, NC, USA

Luca Andriolo Biomechanics Lab. and III Orthopaedic Clinic, Rizzoli Orthopaedic Institute, Bologna, Italy

Tamas Bardos, MD, PhD Department of Orthopedics, University of Pecs, Pecs, Hungary

Emersons Green NHS Treatment Centre, Bristol, UK

Robert H. Brophy, MD Department of Orthopaedic Surgery, Washington University School of Medicine, Chesterfield, MO, USA

Roberto Emanuele Buda, MD I Clinic of Orthopaedics and Traumatology, Rizzoli Orthopaedic Institute, Bologna, Italy

Maria Chiara Bulzamini, MD I Clinic of Orthopaedics and Traumatology, Rizzoli Orthopaedic Institute, Bologna, Italy

Marjolein M.J. Caron Department of Orthopaedic Surgery, CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, Maastricht, The Netherlands

Marco Cavallo, MD I Clinic of Orthopaedics and Traumatology, Rizzoli Orthopaedic Institute, Bologna, Italy

Diego H. Zanolli de Solminihac, MD Department of Orthopaedic Surgery, Clinica Alemana, Santiago, Chile

Marco K. Demange Hospital das Clínicas, University of São Paulo, São Paulo, SP, Brazil

Department of Orthopedic Surgery - Cartilage Repair Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Aad A.M. Dhollander Department of Orthopaedic Surgery and Traumatology, Ghent University Hospital, Ghent, Belgium

Mark E. Easley, MD Department of Orthopaedic Surgery, Duke University School of Medicine, Durham, NC, USA

Pieter J. Emans, PhD, MD Department of Orthopaedic Surgery, CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, Maastricht, The Netherlands

Christopher H. Evans Department of Orthopaedic Surgery, Center for Advanced Orthopaedic Studies, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Giuseppe Filardo Nano-biotechnology Lab, Rizzoli Orthopaedic Institute, Bologna, Italy

Sandro Giannini, MD I Clinic of Orthopaedics and Traumatology, Rizzoli Orthopaedic Institute, Bologna, Italy

Andreas H. Gomoll Department of Orthopedic Surgery - Cartilage Repair Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Simon Görtz, MD Department of Orthopaedic Surgery, University of California at San Diego, San Diego, CA, USA

Friedrich F. Hennig Department of Trauma Surgery, University Hospital of Erlangen, Erlangen, Germany

Damir Hudetz Department of Orthopaedic Surgery, St. Catherine's Hospital, Zabok, Croatia

Department of Orthopaedic Surgery, University Hospital Sveti Duh, Zagreb, Croatia

Clemente Ibarra, MD Orthopedic Sports Medicine and Arthroscopy Division and Tissue Engineering Cell Therapy and Regenerative Medicine Unit, National Institute of Rehabilitation, Mexico City, Mexico

Alan Ivkovic Department of Orthopaedic Surgery, St. Catherine's Hospital, Zabok, Croatia

Department of Orthopaedic Surgery, University Hospital Sveti Duh, Zagreb, Croatia

Department of Biotechnology, University of Rijeka, Rijeka, Croatia

Aldo Izaguirre, MD Orthopedic Sports Medicine and Arthroscopy Division, National Institute of Rehabilitation, Mexico City, Mexico

M. Jelic Department of Orthopaedic Surgery, Clinical Hospital Center Zagreb, School of Medicine, University of Zagreb, Zagreb, Croatia

Elizaveta Kon, MD Nano-biotechnology Lab, Rizzoli Orthopaedic Institute, Bologna, Italy

Evgeny E. Krynetskiy, MD Joint Implant Surgeons of Florida, Fort Myers, FL, USA

Ryosuke Kuroda Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Chuo-ku, Kobe, Japan

M.J. Limbourg Department of Medical and Scientific Affairs, TiGenix NV, Leuven, Belgium

F.P. Luyten Division of Rheumatology, University Hospitals KU Leuven, Leuven, Belgium

Maurilio Marcacci Biomechanics Lab. & III Orthopaedic Clinic, Rizzoli Orthopaedic Institute, Bologna, Italy

Takehiko Matsushita Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Chuo-ku, Kobe, Japan

Patrick C. McCulloch, MD Methodist Center for Orthopedic Surgery and Sports Medicine, Houston Methodist Hospital, Houston, TX, USA

Tom Minas Department of Orthopedic Surgery - Cartilage Repair Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Anell Olivos-Meza, MD Orthopedic Sports Medicine and Arthroscopy Division, National Institute of Rehabilitation, Mexico City, Mexico

Selene G. Parekh, MD, MBA Department of Orthopaedic Surgery, Duke University Medical Center, North Carolina Orthopaedic Clinic, Durham, USA

Marko Pecina Department of Orthopaedic Surgery, School of Medicine, University of Zagreb, Zagreb, Croatia

Lars Peterson, MD, PhD Department of Orthopedics, University of Gothenburg, Gothenburg, Sweden

Francesc Perdisa Biomechanics Lab. & III Orthopaedic Clinic, Rizzoli Orthopaedic Institute, Bologna, Italy

Ryan M. Porter Department of Orthopaedic Surgery, Center for Advanced Orthopaedic Studies, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Gian M. Salzmann, MD Department of Orthopaedic and Trauma Surgery, University Medical Center, Albert-Ludwigs University Freiburg, Freiburg, Germany

Lew C. Schon, MD Department of Orthopaedic Surgery, Duke University Medical Center, North Carolina Orthopaedic Clinic, Durham, USA

Martin J. Stoddart AO Research Institute Davos, Davos Platz, Switzerland

Thomas Tampere Department of Orthopaedic Surgery and Traumatology, Ghent University Hospital, Ghent, Belgium

Francesc Tentoni Biomechanics Lab. & III Orthopaedic Clinic, Rizzoli Orthopaedic Institute, Bologna, Italy

Siegfried Trattnig Department of Radiology, MR Center, Medical University of Vienna, Vienna, Austria

J. Vanlauwe Department of Orthopedics and Traumatology, University Hospital Brussels, Brussels, Belgium

Lodewijk W. van Rhijn Department of Orthopaedic Surgery, CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, Maastricht, The Netherlands

Francesca Vannini, MD, PhD I Clinic of Orthopaedics and Traumatology, Rizzoli Orthopaedic Institute, Bologna, Italy

Peter C.M. Verdonk Orthopaedic Center Antwerp, Monica Hospitals Antwerp, Antwerp, Belgium

Faculty of Medicine, Department of Physiotherapy and Orthopaedic Surgery, Ghent University, Ghent, Belgium

René Verdonk Department of Orthopaedic Surgery and Traumatology, Ghent University Hospital, Ghent, Belgium

Enrique Villalobos Jr, MD Orthopedic Sports Medicine and Arthroscopy Division, National Institute of Rehabilitation, Mexico City, Mexico

Andreja Vukasovic Department of Histology and Embryology, School of Medicine, University of Zagreb, Zagreb, Croatia

Goetz H. Welsch, MD Department of Trauma Surgery, University Hospital of Erlangen, Erlangen, Germany

Tim J.M. Welting Department of Orthopaedic Surgery, CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, Maastricht, The Netherlands

Part I
Introduction

Chapter 1
General Introduction

Pieter J. Emans and Lars Peterson

Abstract Hyaline cartilage enables us to move our joints even when exposed to high mechanical forces. Other types of cartilage can be found in tissues like the ear, nose, airway etc. In contrast to many other tissues only one type of cell is found in hyaline cartilage this cell is the chondrocyte. Since chondrocytes are capable to produce their own matrix, it is possible to generate cartilage in a laboratory setting. This approach applies to the ideas of tissue engineering. However aspects such as tissue architecture, integration to host tissue, and costs remain of concern when trying to repair and/or produce adequate hyaline cartilage capable to withstand high repetitive mechanical forces.

Keywords Cartilage repair • Collagen type 2 • Biological surgery • Tissue Engineering

Key Points
- Cartilage is essential for different functions found in the human body.
- Hyaline cartilage is found in joints and is capable to withstand high repetitive mechanical forces.
- Cartilage has a very limited capacity of self repair.
- The structure and architecture of healthy hyaline cartilage remain challenging to restore when cartilage repair is performed.

P.J. Emans, PhD, MD (✉)
Department of Orthopaedic Surgery, CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, 5800, Maastricht NL-6202 AZ, The Netherlands
e-mail: pj.emans@maastrichtuniversity.nl

L. Peterson, MD, PhD
Department of Orthopedics, University of Gothenburg, Gothenburg, Sweden

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_1, © Springer-Verlag London 2014

- Knowledge of (cel)biology, joint homeostasis, and biomechanical factors are essential for successful cartilage repair, this illustrates the need for a "biological" orientated surgeon.
- Defining riskprofiles for development of cartilage damage such as cartilage defects and even more for primary OA may boost preventative measures and development of therapies in early and asymptomatic patients at risk.

1.1 Introduction

Different types of cartilage can be found in the human body; (i) hyaline, (ii) elastic and (iii) fibro cartilage. Elastic cartilage is found in the ear and respiratory tract. The menisci and intervertebral discs contain fibrocartilage and hyaline cartilage is predominantly found in articular cartilage. Joint motion is possible by a truly remarkable material both structurally and functionally called hyaline cartilage [1–3]. Although one chapter describes the role of meniscal repair in relation to cartilage repair, the focus of this book is on articular cartilage. Since it is the aim of the International Cartilage Repair Society (ICRS) to bring together all cartilage researchers both clinicians, fundamental scientists, and clinical scientists. The book encapsulates fundamental cartilage biology aspects, biomaterials for cartilage repair, bioreactors, imaging of cartilage, cartilage repair techniques and future perspectives of cartilage repair. This first chapter gives an insight into cartilage, cartilage repair and challenges of cartilage repair.

1.1.1 Embryological Development

Chondrogenesis is a key event in developing limb buds beginning in the center of condensed mesenchyme. The earliest form of cartilage development is suggested to be 300 million years ago [4]. In humans the first rudiments develop during the 5th week of gestation. In the 8 week of the embryological life a relatively cell-poor intermediate zone begins to develop. This will form the joint cavity [5–7]. At the end of most bones articular joints are situated. The side where two bones form an articular joint, the ends of these bones are covered with hyaline cartilage. This articular cartilage is able to withstand very high mechanical forces with very low friction and thereby enables easy movement. A large number of bones are formed by a process called endochondral ossification. During this process a cartilage template is replaced by bone, in contrast with the cartilage in newly formed joints which remains cartilage. Both articular cartilage and bone mature and this leads to a well organised architecture and specialisation. The arcade-like architecture of cartilage is capable to withstand an enormous amount of intensive and repetitive forces during life. However, a British surgeon William Hunter made the now famous

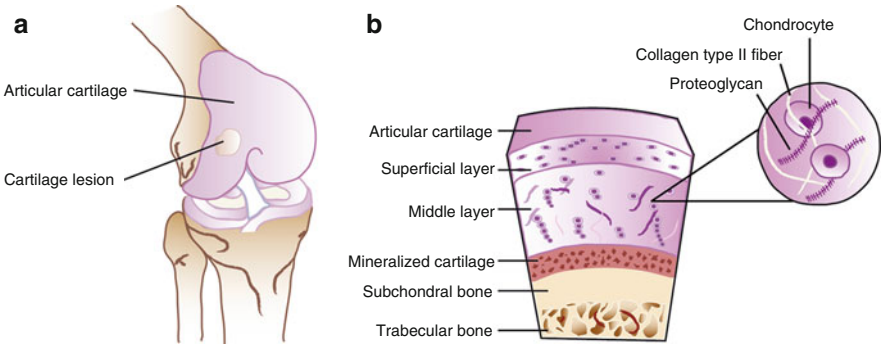

Fig. 1.1 Schematic representation of articular cartilage. (**a**) Normal view of articular cartilage of the knee with a cartilage lesion. (**b**) Magnified view of articular cartilage with specific zones indicated and a magnified view of the contents of the middle layer of articular cartilage (Reprints of PhD thesis of M. Caron, with permission M. Caron)

statement that "*From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and that once destroyed it is not repaired*" (Hunter 1743).

1.1.2 Cell

The chondrocyte is the only cell type found in articular cartilage (Fig. 1.1). In contrast to other tissues, the chondrocyte contributes to a relative low percentage of the cartilage matrix volume (1–5 %). In adults these chondrocytes lack cell-cell contact. Therefore communication between cells has to occur via the extracellular matrix (ECM). Cartilage is characterized by the absence of blood vessels, lymphatic vessels necessitates, and nerve fibers. Chondrocytes receive nutrients and oxygen via diffusion from the synovial fluid through the ECM and from the underlying bone. Their environment is therefore dominated by low oxygen levels and these cells have an anaerobic metabolism [8]. Each chondrocyte is a metabolically active unit which elaborates and maintains the ECM in its immediate vicinity [9]. A high content of proteoglycan aggregates and a relative absence of organized fibrillar collagens is found in the pericellular matrix. These aggregates are bound via hyaluronan to CD44 receptors of chondrocytes. The interaction between hyaluronan and its CD44 receptor is believed to protect the cell against "programmed cell dead" known as apoptosis [10–12].

1.1.3 Collagens

Collagen Type II is the most prominent collagen in cartilage which represents 90 % of collagens found in articular cartilage. Alternative splicing occurs in the Type II

Collagen gene. In the procollagen gene exon 2 encodes for a cysteine-rich domain in the amino-terminal propeptide which results in a type II A procollagen [13]. This type IIA is expressed by immature chondrocytes but is not expressed in mature cartilage. In Collagen type IIB, which is present in mature cartilage exon 2 is spliced out. Collagen type II in mature cartilage is composed of three identical polypeptide chains, α_1 (II), and belongs to the fibril forming class of collagens (class 1). Collagen Type II is synthesized and secreted as a procollagen precursor whose nonhelical extensions are removed by enzymes. During this process the large (35 kDa) chondrocalcin is released (The N-terminal propeptide). Hereafter the trimmed collagen is incorporated into the ECM where it is crosslinked. Within the deep layer of cartilage arcades of thick fibrils are formed whereas in the surface fine fibrils are arranged horizontally [8, 9] (Fig. 1.1).

Collagen type VI is only found in the pericellular matrix surrounding the chondrocyte [14–18]. The cell surrounded by hyaluronan and the layer of collagen type VI is called a "chondron". Chondrons harbor and protect the chondrocytes from mechanical forces [19–21]. Collagen Type IX belongs to the class 3 short-helix molecules which may function as a connector between Collagen Type II fibers [22–26]. Collagen type XI is a class1 (fibril forming) collagen and is coassembled in the heterotypic fibrils of articular cartilage [27]. The average half-life of collagens in articular cartilage is calculated to be 117 years while the average half-life of skin collagens is 15 years [28].

1.1.4 Other Important Matrix Components

Aggrecan is the name of an aggregating proteoglycan which consists of a central protein with multiple sulfated glycosaminoglycans (GAG's), especially Ketatan Sulfate and Chondroitine Sulfate, covalently attached to it. The average half live of aggrecan molecules, measured with aspartic acid racemization is approximately 2 years [29]. However aggrecan half live is different between the zones [30, 31].

Hyaluronan is a long polymer of repeating disaccharides (Fig. 1.2). Aggrecan is bound to this polymer and the bond is stabilized by link protein. The high concentration of anionic charge of the GAG's has important biomechanical features. The negative charges repel each other which results in an expanded state of these large molecules. This expanded state ensures that these matrix molecules remain "captured" in the collagen network.

1.2 Cartilage Repair

Treatment of damaged cartilage can be grouped to four concepts of principle; the four R's [32]. The joint surface can be; (i) resected, (ii) relieved, (iii) replaced or (iv) restored. A joint prosthesis is an example of joint replacement, joint distraction and

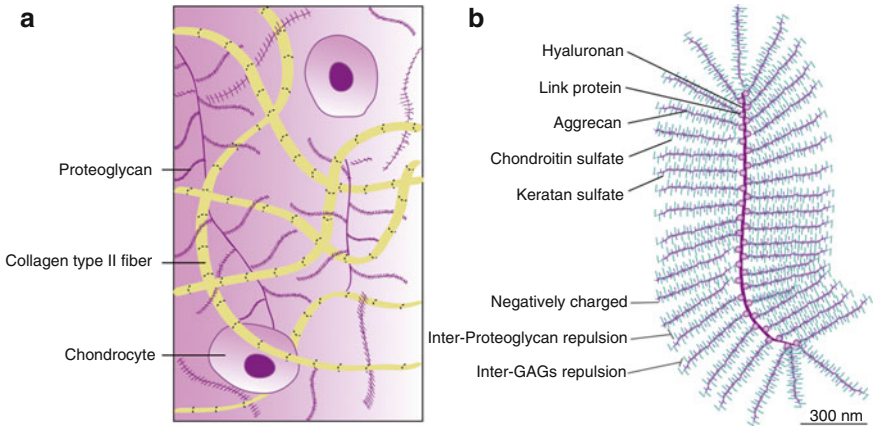

Fig. 1.2 Composition of the extracellular matrix of cartilage. (**a**) Schematic representation of different components of extracellular matrix of cartilage including the collagen fibril network and proteoglycans. (**b**) Proteoglycans have the appearance of "bottle brush" structure. They contain the glycosaminoglycan (GAG) sidechains of chondrotoitin and keratan sulfate and are linked to a hyaluronan backbone via link proteins (Reprints of PhD thesis of M. Caron, with permission of M. Caron)

osteotomies can induce joint relieve. Osteotomies are used to re-align the axis of loading in patients with a malalignment of the leg. By transferring the load to the less affected cartilage (e.g. previously less loaded/damaged cartilage) the damaged part is relieved. Arthodesis is an example of joint resection. For Tissue Engineering (TE) and Regenerative Medicine (RM) techniques the focus is on cartilage restoration.

Restoration implies methods to heal or regenerate the joint surface with or without the subchondral bone into healthy hyaline articular cartilage. Three strategies can be considered when attempts are made to heal or restore cartilage. (I) Subchondral Drilling, Abrasion, and Microfracture (Fig. 1.3) are techniques to allow penetration of bone marrow through the subchondral bone into the defect of damaged cartilage [33–42]. Abrasion is removal of a mm thin superficial layer of the subchondral bone plate aiming to allow a capillary bleeding surface in the defect [43]. These techniques may improve the clinical wellbeing of the patient and the joint surface defect may be healed to some extent. However the healing process is inadequate since no functional hyaline cartilage but fibrocartilage is formed [34, 42]. Nonetheless, these methods are cheap and easy to perform and are therefore seen as the currently best option to relieve the complaints. Other clinical studies have suggested that any beneficial effect is related to the arthroscopic procedure itself and the debridement of the damaged area. A nonspecific effect might be related to joint lavage rather than the penetration of the subchondral bone [44, 45]. In conclusion, these techniques may have some benefit with regard to small defects but no effect has been proved in relation to large defects, osteoarthritic joints or older patients [38].

(II) Implants vary from non-degradable and degradable implants, cells, periosteum or perichondrium, to Osteochondral Autograft Transfer System (OATS or

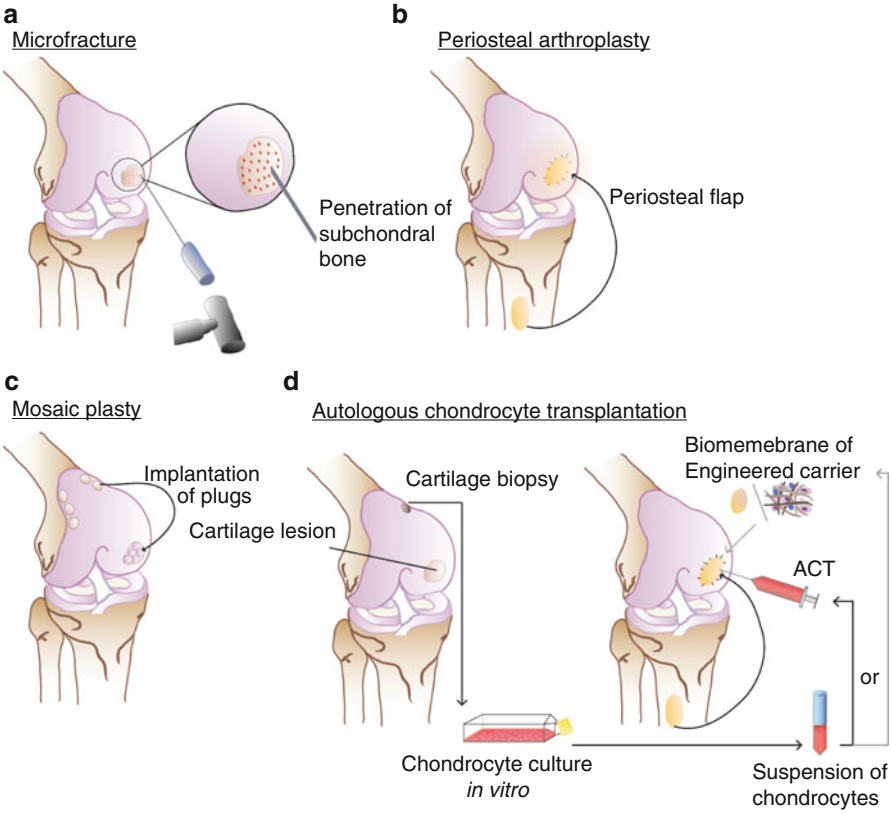

Fig. 1.3 Currentcartilage repair techniques. (**a**) Schematic view of the microfracture procedure. (**b**) Schematic view of the periosteal arthroplasty procedure. (**c**) Schematic view of the mosaic plasty procedure. (**d**) Schematic view of procedure of autologous chondrocyte transplantation (*ACT*) or chondrocyte transplantation with an engineered carrier (Reprints of PhD thesis of M. Caron, with permission of M. Caron)

Mosaicplasty) (Fig. 1.3) and Osteochondral Allografts [46–52]. The biomaterials and periosteum can be combined with cells or growth factors. Periosteal Arthroplasty is an interesting way of treating cartilage defects since many have reported the chondrogenic potential of periosteum, but initially promising short term results seem to deteriorate over time [53–62]. More than 90 % of collagen type II in the hyaline cartilage formed in the cartilage defects treated with periosteal grafts has been reported in rabbit models [63]. Perichondrial arthroplasty used for human cartilage repair was first described by Skoog et al. [64]. This technique has been reported to give an initial cartilage repair [65, 66]. On the long term poor results related to overgrowth of the graft and calcification as reported by Bouwmeester et al. [67]. These authors concluded that a better fixation of the graft might improve the results. In a study comparing periosteum with perichondrium, chondrogenesis was observed significantly more using periosteal grafts [68].

(III) Osteochondral Grafts can be divided in autologous and allogenic. Mosaicplasty or OATS involves harvesting one or more osteochondral plugs from a

relatively less weight-bearing region of the joint and subsequent implantation of this graft into an articular defect. Possible donor site morbidity is bypassed if osteochondral allografts are used [53–62]. However, when using allografts disease transfer from donor to recipient remain of concern.

1.2.1 Cartilage and Tissue Engineering

Cartilage was identified as a tissue for which it was thought to be possible to recreate them in a laboratory setting using the combination of cell isolation culture techniques and carrier materials. Combining technologies from material science, cell biology, and clinical needs has led to the rise of the field of TE and RM. In the 1960's researchers proposed the idea of creating tissues in a laboratory which may replace damaged or diseased tissues and cell biologist observed that cells could sort themselves *in vitro* to populations with tissue-like characteristics [69]. Adding a structure (material) such as a collagen gel to fibroblast cultures was shown to further resemble structural characteristics of skin. Later the work of Peterson and co-workers showed that chondrocytes could be cultured and successfully be transplanted for the repair of cartilage defects [70]. This technique is entitled Autologous Chondrocyte Transplantation or Implantation (ACT or ACI). In the beginning of ACT no artificial structures were used to maintain the chondrocytes in the cartilage defect as an autologous periosteal flap was used for that purpose (Fig. 1.3). Optimization of ACT has led to the introduction of collagen meshes to support and maintain chondrocytes which were transplanted into the defect. Already earlier in the mid-1980s, Langer and co-workers proposed that biodegradable polymers could serve as a scaffold for the organisation and maturation of cells into the desired tissues. As such it was proposed that this approach would enable engineering of thicker and hard tissues such as cartilage. The collaboration of scientists of different disciplines such as cell biology, biomaterials, biomechanics, engineering and translational medicine has already led to fruitful scientific achievements. And although cell therapies based on TE for skin and cartilage are commercially available, which apply to the definition of TE such as Carticel® and Epicel® of Genzyme, the initial expectations of TE and RM have not been met. Although some examples of successful treatment by engineered tissues such as bladder and trachea can be found in the clinic, engineering tissues is not performed on a large scale [71, 72].

1.2.2 Complete Engineering of Cartilage or Providing the Proper Impulses and Environment for Regeneration and/or Repair

In the approach to engineer tissues in a laboratory setting and subsequently transplanting them into the body lies the key question; "*until what level should we engineer tissue and when should nature take over*?". It is often the aim of many researchers to engineer a mature tissue which is directly able to take over the

function of the diseased tissue or organ. In nature a cascade of interactions occur during the process of tissue repair. During this process both the environment as well as the reparative tissue adapt to each other and the local biomechanical requirements. In such a manner both integration of repair tissue and tissue remodelling is achieved. The capacity of a mature TE tissue to adapt to the local needs such as integration, remodelling, etc. is lower than a relatively less mature tissue. In addition, in order to create a robust and thicker tissue, the use of scaffolds, growth factors and more differentiated cells may be inevitable. However it remains the question whether the local environment is able to adapt in an appropriate manner to all non-physiological stimuli which are introduced. Per example how does the normal tissue remodelling, repair and integration respond to a scaffold which alters local biomechanical stimuli which are known to be essential for tissue remodelling? How do transplanted and environmental cells respond to material properties such material surface, breakdown products, architecture etc.? How does the normal fine-tuned orchestra of tissue repair respond to transplanted cells which are normally not present at a certain phase of tissue repair? Since it is largely unknown what this local effect is and how these factors contribute in it, a clear shift is observed in the attempts to repair tissue. This shift includes more specific natural stimuli which trigger and enhance the regenerative capacity of the tissue itself. Injection of stem cells or progenitor cells (cell therapies), and the induction of regeneration by biologically active molecules can all be regarded as an example of Regenerative Medicine (RM). For both TE and RM it becomes more and more evident that studying the underlying natural and developmental processes of cartilage and bone can serve as a blueprint to identify important cell sources, biochemical, biomechanical, structural stimuli and timing thereof. It is expected that insight in these biological mechanisms will enhance the progress in the field of cartilage repair and perhaps also in prevention of cartilage degradation as can be seen in primary OA and post-traumatic, secondary OA.

1.3 Challenges in Cartilage Repair

1.3.1 Biological Surgery

Currently most of the orthopaedic surgery consists of replacing or supporting damaged tissues. This is often done by sutures, anchors, plates, screws, nails and artroplastic implants. The biological and mechanical properties of these materials are defined. In contrast when transplanting living tissue (e.g. cells with or without supporting scaffold, pieces of tissue, or whole bone/cartilage transplants), important factors of such (biological) age of the donor, (biological) age of the recipient, weight of the recipient, number of preceding procedures, leg alignment, size, containment, and location of the cartilage defect, status of the meniscus and the ligaments, and status of the subchondral bone all play an important role when making a decision if

cartilage repair is possible and which treatment is optimal for this particular patient. Rather than results of randomized clinical trials, it is due to the experience of large volume surgeons, results found in large cohorts, fine-tuning of techniques and indications that have led to a treatment algorithm [73]. Therefore the biological surgeon distinguishes him/herself from the "classic orthopaedic surgeon" in such a manner that the biological surgeon should have knowledge not only in the field of biomechanics, materials and surgical procedures but also should have an insight in the field of joint homeostasis and (cell)biology as well as time for repair and regeneration of different tissues involved. One of the first examples of biological surgery may be the Anterior Cruciate Ligament (ACL) reconstruction in which the (biological) age of the patient, type of graft, etc. play an important role in the success of this procedure. Since insight in joint biology, joint homeostasis, the interaction between cartilage and its subchondral bone are all essential for successful cartilage repair, cartilage repair is the ultimate example of biological surgery (see Chaps. 8, 9, 10, 11, and 12). The genesis of ACT and thus of biological surgery is illustrated by Chap. 10. It is the unique interaction between clinicians, biomechanical engineers, biologists, material experts that will lead to new therapies for both cartilage repair, meniscal repair and OA. This interaction can be found in the ICRS.

1.3.2 Recapitulating the Mechanical Properties of Cartilage

As mentioned before, during the rise of the field of TE, cartilage was indicated to be a tissue which was suitable to engineer in the laboratory. This expectation was due to the fact that cartilage exists of only one cell type which was thought to lack complex functions, different specialized cells, and different sub-specialized tissues which can be seen in other organs such as brain, liver, heart etc. The chondrocyte is the only cell type which produces its own matrix. The chondrocytes can be harvested and redifferentiate hereafter and produce their own matrix in monolayer, three dimensional culture systems and carriers such as different scaffolds. The same can be done with stemcells originating from different sources [74]. The role of stem-cells is discussed in Chaps. 3 and 17. The challenge is to produce cartilage with comparable mechanical properties as mature hyaline cartilage and that the newly produced cartilage in the end reach the same metabolic tissue turnover as the surrounding normal cartilage. Hyaline cartilage has a unique structure which to the best can be compared with the opera house of Sidney in which tension wires which hold the typical arch like structure. In cartilage these arches can also be found and the tension wires consist of collagen type II, the hydrostatic pressure to tension the collagen type II fibers is provided by the negative charges of aggrecans maintaining a high concentration of water. It seems that this unique structure is formed starting from embryology to adolescence [75]. During this period it appears that in the human the architecture is formed going from unloaded to partially loading in the period of embryology until taking the first steps as a baby to full loading but no impact during the age of 2–3 years where jumping and running are still difficult to

an increased loading in the following years. During this whole period the cartilage is also exposed to flexion and extension, combined with the intermitted hydrostatic loading. Finite element analysis has predicted that sliding indentation is the underlying loading regime which results in the architecture and thus mechanical properties of cartilage [76, 77]. This process of adaptation to mechanical forces is well known in bone but in contrast to bone the adaptive and regenerative capacity of cartilage is very limited and the formation of the proper architectural structure and mechanical properties remain troublesome. However it must be emphasized that cartilage and the subchondral plate and trabecular bone are developed together and act as a functional unit. Engineering cartilage which is comparable to native cartilage seems very difficult especially if one does not have insight in the importance of the bone as a part of cartilage function. Optimization of bioreactors may further optimize the mechanical properties of the engineered graft prior to implantation. An insight in the requirement and development of such bioreactors is given in Chap. 5. Except for osteochondral autograft (which often lack the desired surface radius) and osteochondral allografts (which are discussed in Chaps. 8 and 9), restoration of the biomechanical properties of native cartilage is essential for (long term) success. Since it seems hardly possible to create this tissue by TE principles in a laboratory, an adequate biomechanical environment after implantation in a defect seems essential. In that perspective the status of the meniscus and possible repair of the meniscus (see Chap. 12), the weight of the patient, the alignment of the leg, stability of the joint, and the post operative loading regime should all be evaluated and optimized. Novel development in the area of high resolution imaging techniques enable monitoring the repair process but also the possible differentiation in fibrous tissue and/or bone (interlesional osteophytes), and the development of the proper architecture. This will provide novel insights and further improvement in the field of cartilage repair. These emerging imaging techniques are discussed in Chap. 7. Reports indicate that the properties of repaired cartilage after repair by Matrix Assisted Chondrocyte Implantation are superior to microfracture [78].

1.3.3 The Need to Keep Cells in Their Desired Chondrogenic Lineage?

For both chondrocyte based therapies as well as stemcell based therapies formation of fibrotic tissue, fibrocartilage, formation of hypertrophic cartilage, and formation of bone(intralesional osteophytes) have been described [79]. It is therefore essential that cells do not (de)different into fibroblast like cells or hypertrophic chondrocytes both prior to implantation or after implantation or recruitment into the cartilage defect. Especially when using progenitor cells for cartilage repair, ossification of the repaired tissue may occur which in turn may impair clinical results. Due to the potential of being a one step procedure the use of cartilage grafts and/or minced

cartilage seem attractive. These techniques remain to be proven and are described in Chap. 18. Unwanted ossification resulting in interlesional osteophytes has also been described when minced cartilage is used for cartilage repair [79]. Examples of ossification and formation of interlesional osteophytes when applying stemcells are microfracture and periosteum or perichondrium plasty [53, 54]. These findings illustrate that maintaining differentiated progenitor cells in their chondrogenic lineage remains challenging in cartilage repair. It appears that more than chondrocytes, progenitor cells have the tendency to follow the different phases of endochondral ossification towards hypertrophy and mineralisation when triggered to differentiate into cartilage. However due to their potential the use of progenitor cells remains attractive to explore (see Chap. 17). As such keeping cells in their desired differentiation state is of the utmost importance when applying these cells for RM purposes.

1.3.3.1 Methods to Influence Post Transplantation Differentiation

Identification and selection of chondrocytes capable to redifferentiate and possibly maintain their chondrogenic phenotype may be a first step to prevent unwanted fibrous or hypertrophic differentiation. An example of such an identification and selection is described in Chap. 17. Using paracrine factors of cells may be another method to influence optimal differentiation and tissue formation. Findings of Hendriks and co-workers showing that chondrocytes stimulate bone marrow stem cells towards chondrogenesis when both cell types are co-cultured [80]. These findings were later bolstered by Fisher and coworkers showing that human articular cartilage-derived soluble factors and direct co-culture are potent means of improving chondrogenesis and suppressing the hypertrophic development of progenitor cells [81]. In this study and other work of the group of Richter the PTHrP is an important candidate soluble factor involved in this effect. PTHrP is primarily known as a key regulator in the process of endochondral ossification. Other (growth)factors have also been described as potential stimuli to optimize both the microenvironment of transplanted or invading cells and the joint homeostasis. These anabolic and catabolic factors are discussed in Chaps. 2, 3, and 4. It is recently shown that cyclooxygenases (COX) inhibitors are also able to decrease hypertrophy of chondrocytes [81]. Studying the process of endochondral ossification and further unravelling how and why articular chondrocytes maintain their phenotype and prevent from hypertrophy may enhance cartilage repair techniques by generating stable cartilage among which does not lead to intralesional osteophytes (Chap. 3). Another important factor may be the influence of the carrier material. Both its biochemical properties, biomechanical properties and breakdown products influence cell differentiation and tissue formation. Several biomaterials are discussed in Chap. 6. In summary optimizing cells, their microenvironment and the total joint homeostasis may further increase the outcome of cartilage repair techniques.

1.4 Prevention and Change of Diagnosis of Cartilage Defects

Since cartilage disorders are found in 63 % of all arthroscopic procedures [82] and in an even larger percentage of athletes cartilage lesion are found (see Chap. 14). This illustrates that a large percentage of the population probably has, to some extend, damaged cartilage. Since cartilage is avascular and does not contain nerves most of these patients remain undiagnosed until the damaged joint becomes painful or progresses to posttraumatic OA. In contrast to osteoporosis there is no riskprofile which can predict which patient has damaged cartilage, which patient has (asymptomatic) OA, which patient will develop OA, and what the progression of cartilage damage and/or OA will be. Studies which change the load of an OA knee such as an osteotomy or distraction, seem to illustrate that some form of tissue regeneration takes place [83]. If this tissue is (healthy) articular cartilage is still subject of ongoing studies. However, these findings together with the possibility to monitor cartilage with better imaging techniques (Chap. 7) challenge the field in; (i) defining riskprofiles for development of cartilage damage such as cartilage defects and even more for primary OA, and (ii) prevention and developing therapies in early and asymptomatic patients at risk. Since virtually only symptomatic cartilage damage is diagnosed, it is unknown if damage to cartilage may be reversible. Karsdal and coworkers showed that cartilage degradation is completely reversible in the presence of high levels of aggrecanase-mediated aggrecan degradation but that there is an impaired repair capacity after induction of MMP-mediated aggrecan and collagen type II degradation [84]. The change from diagnosing cartilage disorders in a rather late stage to diagnosing cartilage disorders in an early perhaps asymptomatic stage, together with novel insight in cartilage biology, stemcell biology, upcoming cartilage repair techniques, and established cartilage repair techniques may, to some extend, challenge the statement that “cartilage once damaged does not heal”.

1.5 Conclusion

Cartilage is a unique tissue which is essential for our physical and even mental health since cartilage is involved in functions such as breathing, hearing, articulation and locomotion. Since cartilage has no or a very limited capacity for self-repair it is paramount to improve current cartilage repair strategies and develop novel cartilage repair strategies. For this purpose it is important to combine the knowledge and input of clinicians, clinical researchers and basic scientists, engaged or interested in the field of cartilage biology, imaging, cartilaginous tissue engineering and translational clinical approaches to treatment of cartilage pathologies. All these aspects are described in this book.

References

1. Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. Clin Orthop Relat Res. 2001;(391 Suppl): S26–33.
2. Buckwalter J, Mankin H. Articular cartilage: tissue design and chondrocyte matrix interactions. Instr Course Lect. 1998;47:487–504.
3. Hasler EM, Herzog W, Wu JZ, Muller W, Wyss U. Articular cartilage biomechanics: theoretical models, material properties, and biosynthetic response. Crit Rev Biomed Eng. 1999; 27(6):415–88.
4. Urist M. Biogenesis of bone: calcium and phophorus in the skeleton and blood in vertebrate evolution. In: Greep R, Astwood E, editors. Handbook of physiology. Washington, DC: American Physiological Society; 1976. p. 183–213.
5. Aydelotte M, Kuettner K. Heterogeneity of articular chondrocytes and cartilage matrix. In: Woessner J, Howell DS, editors. Cartilage degradation: basic and clinical aspects. New York: Marcel Dekker; 1992. p. 37–65.
6. Anderson H. Histochemical studies of the human hip joint. Acta Anat. 1962;48:258–92.
7. Gray DJ, Gardner E. Prenatal development of the human knee and superior tibiofibular joints. Am J Anat. 1950;86(2):235–87.
8. Schenk R, Eggli P, Hunziker E. Articular cartilage morphology. In: Kuettner K, Schleyerbach R, Hascall V, editors. Articular cartilage biochemistry. New York: Raven Press; 1986. p. 3–22.
9. Aydelotte MB, Greenhill RR, Kuettner KE. Differences between sub-populations of cultured bovine articular chondrocytes. II. Proteoglycan metabolism. Connect Tissue Res. 1988;18(3): 223–34.
10. Noonan KJ, Stevens JW, Tammi R, Tammi M, Hernandez JA, Midura RJ. Spatial distribution of CD44 and hyaluronan in the proximal tibia of the growing rat. J Orthop Res. 1996;14(4): 573–81.
11. Lisignoli G, Grassi F, Zini N, Toneguzzi S, Piacentini A, Guidolin D, et al. Anti-Fas-induced apoptosis in chondrocytes reduced by hyaluronan: evidence for CD44 and CD54 (intercellular adhesion molecule 1) invovement. Arthritis Rheum. 2001;44(8):1800–7.
12. Fujita Y, Kitagawa M, Nakamura S, Azuma K, Ishii G, Higashi M, et al. CD44 signaling through focal adhesion kinase and its anti-apoptotic effect. FEBS Lett. 2002;528(1–3):101–8.
13. Ryan MC, Sandell LJ. Differential expression of a cysteine-rich domain in the amino-terminal propeptide of type II (cartilage) procollagen by alternative splicing of mRNA. J Biol Chem. 1990;265(18):10334–9.
14. Chang J, Nakajima H, Poole CA. Structural colocalisation of type VI collagen and fibronectin in agarose cultured chondrocytes and isolated chondrons extracted from adult canine tibial cartilage. J Anat. 1997;190(Pt 4):523–32.
15. Hambach L, Neureiter D, Zeiler G, Kirchner T, Aigner T. Severe disturbance of the distribution and expression of type VI collagen chains in osteoarthritic articular cartilage. Arthritis Rheum. 1998;41(6):986–96.
16. Poole CA, Ayad S, Schofield JR. Chondrons from articular cartilage: I. Immunolocalization of type VI collagen in the pericellular capsule of isolated canine tibial chondrons. J Cell Sci. 1988;90(Pt 4):635–43.
17. Poole CA, Flint MH, Beaumont BW. Chondrons extracted from canine tibial cartilage: preliminary report on their isolation and structure. J Orthop Res. 1988;6(3):408–19.
18. Poole CA, Ayad S, Gilbert RT. Chondrons from articular cartilage. V. Immunohistochemical evaluation of type VI collagen organisation in isolated chondrons by light, confocal and electron microscopy. J Cell Sci. 1992;103(Pt 4):1101–10.
19. Guilak F, Jones WR, Ting-Beall HP, Lee GM. The deformation behavior and mechanical properties of chondrocytes in articular cartilage. Osteoarthritis Cartilage. 1999;7(1):59–70.

20. Knight MM, Ross JM, Sherwin AF, Lee DA, Bader DL, Poole CA. Chondrocyte deformation within mechanically and enzymatically extracted chondrons compressed in agarose. Biochim Biophys Acta. 2001;1526(2):141–6.
21. Poole CA, Flint MH, Beaumont BW. Chondrons in cartilage: ultrastructural analysis of the pericellular microenvironment in adult human articular cartilages. J Orthop Res. 1987;5(4): 509–22.
22. Eyre DR, Apon S, Wu JJ, Ericsson LH, Walsh KA. Collagen type IX: evidence for covalent linkages to type II collagen in cartilage. FEBS Lett. 1987;220(2):337–41.
23. van der Rest M, Mayne R. Type IX collagen proteoglycan from cartilage is covalently cross-linked to type II collagen. J Biol Chem. 1988;263(4):1615–8.
24. Wu JJ, Eyre DR. Covalent interactions of type IX collagen in cartilage. Connect Tissue Res. 1989;20(1–4):241–6.
25. Muller-Glauser W, Humbel B, Glatt M, Strauli P, Winterhalter KH, Bruckner P. On the role of type IX collagen in the extracellular matrix of cartilage: type IX collagen is localized to intersections of collagen fibrils. J Cell Biol. 1986;102(5):1931–9.
26. Mankin H, Mow V, Buckwalter J. Articular cartilage structure, composition, and function. In: Buckwalter J, Einhorn T, Simon S, editors. Orthopaedic basic science. Rosemont: AAOS; 2000. p. 443–70.
27. Eyre D, Wu J, Woods P, et al. The cartilage-specific collagens: structural studies. In: Kuettner K, editor. Articular cartilage and osteoarthritis. New York: Raven Press; 1992. p. 119–31.
28. Verzijl N, DeGroot J, Thorpe SR, Bank RA, Shaw JN, Lyons TJ, et al. Effect of collagen turnover on the accumulation of advanced glycation end products. J Biol Chem. 2000;275(50): 39027–31.
29. Maroudas A, Uchitel N, Babyliss M, Gilav E. Racemization of aspartic acid in proteoglycans from human articular cartilage. Trans Orthop Res Soc. 1994;19:13.
30. Mok SS, Masuda K, Hauselmann HJ, Aydelotte MB, Thonar EJ. Aggrecan synthesized by mature bovine chondrocytes suspended in alginate. Identification of two distinct metabolic matrix pools. J Biol Chem. 1994;269(52):33021–7.
31. Hauselmann HJ, Fernandes RJ, Mok SS, Schmid TM, Block JA, Aydelotte MB, et al. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. J Cell Sci. 1994;107(Pt 1):17–27.
32. O'Driscoll SW. The healing and regeneration of articular cartilage. J Bone Joint Surg Am. 1998;80(12):1795–812.
33. Aglietti P, Buzzi R, Bassi PB, Fioriti M. Arthroscopic drilling in juvenile osteochondritis dissecans of the medial femoral condyle. Arthroscopy. 1994;10(3):286–91.
34. Altman RD, Kates J, Chun LE, Dean DD, Eyre D. Preliminary observations of chondral abrasion in a canine model. Ann Rheum Dis. 1992;51(9):1056–62.
35. Bradley J, Dandy DJ. Results of drilling osteochondritis dissecans before skeletal maturity. J Bone Joint Surg Br. 1989;71(4):642–4.
36. Furukawa T, Eyre DR, Koide S, Glimcher MJ. Biochemical studies on repair cartilage resurfacing experimental defects in the rabbit knee. J Bone Joint Surg Am. 1980;62(1):79–89.
37. Insall J. The Pridie debridement operation for osteoarthritis of the knee. Clin Orthop Relat Res. 1974;101:61–7.
38. Kim HK, Moran ME, Salter RB. The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits. J Bone Joint Surg Am. 1991;73(9):1301–15.
39. Meachim G, Roberts C. Repair of the joint surface from subarticular tissue in the rabbit knee. J Anat. 1971;109(2):317–27.
40. Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg Am. 1976;58(2):230–3.
41. Rae PJ, Noble J. Arthroscopic drilling of osteochondral lesions of the knee. J Bone Joint Surg Br. 1989;71(3):534.
42. Vachon A, Bramlage LR, Gabel AA, Weisbrode S. Evaluation of the repair process of cartilage defects of the equine third carpal bone with and without subchondral bone perforation. Am J Vet Res. 1986;47(12):2637–45.

43. Johnson LL. Arthroscopic abrasion arthroplasty historical and pathologic perspective: present status. Arthroscopy. 1986;2(1):54–69.
44. Jackson RW. The scope of arthroscopy. Clin Orthop Relat Res. 1986;208:69–71.
45. Ogilvie-Harris DJ, Fitsialos DP. Arthroscopic management of the degenerative knee. Arthroscopy. 1991;7(2):151–7.
46. Chu CR, Coutts RD, Yoshioka M, Harwood FL, Monosov AZ, Amiel D. Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): a tissue-engineering study. J Biomed Mater Res. 1995;29(9):1147–54.
47. Elford PR, Graeber M, Ohtsu H, Aeberhard M, Legendre B, Wishart WL, et al. Induction of swelling, synovial hyperplasia and cartilage proteoglycan loss upon intra-articular injection of transforming growth factor beta-2 in the rabbit. Cytokine. 1992;4(3):232–8.
48. Freed LE, Vunjak-Novakovic G, Langer R. Cultivation of cell-polymer cartilage implants in bioreactors. J Cell Biochem. 1993;51(3):257–64.
49. Grande DA, Halberstadt C, Naughton G, Schwartz R, Manji R. Evaluation of matrix scaffolds for tissue engineering of articular cartilage grafts. J Biomed Mater Res. 1997;34(2):211–20.
50. Hendrickson DA, Nixon AJ, Grande DA, Todhunter RJ, Minor RM, Erb H, et al. Chondrocyte-fibrin matrix transplants for resurfacing extensive articular cartilage defects. J Orthop Res. 1994;12(4):485–97.
51. Nixon AJ, Sams AE, Lust G, Grande D, Mohammed HO. Temporal matrix synthesis and histologic features of a chondrocyte-laden porous collagen cartilage analogue. Am J Vet Res. 1993;54(2):349–56.
52. Reddi AH. Symbiosis of biotechnology and biomaterials: applications in tissue engineering of bone and cartilage. J Cell Biochem. 1994;56(2):192–5.
53. Convery FR, Meyers MH, Akeson WH. Fresh osteochondral allografting of the femoral condyle. Clin Orthop Relat Res. 1991;273:139–45.
54. Czitrom AA, Keating S, Gross AE. The viability of articular cartilage in fresh osteochondral allografts after clinical transplantation. J Bone Joint Surg Am. 1990;72(4):574–81.
55. Garrett JC. Treatment of osteochondral defects of the distal femur with fresh osteochondral allografts: a preliminary report. Arthroscopy. 1986;2(4):222–6.
56. Garrett JC. Fresh osteochondral allografts for treatment of articular defects in osteochondritis dissecans of the lateral femoral condyle in adults. Clin Orthop Relat Res. 1994;303:33–7.
57. Garrett JC. Osteochondral allografts for reconstruction of articular defects of the knee. Instr Course Lect. 1998;47:517–22.
58. Ghazavi MT, Pritzker KP, Davis AM, Gross AE. Fresh osteochondral allografts for post-traumatic osteochondral defects of the knee. J Bone Joint Surg Br. 1997;79(6):1008–13.
59. Gross AE, Aubin P, Cheah HK, Davis AM, Ghazavi MT. A fresh osteochondral allograft alternative. J Arthroplasty. 2002;17(4 Suppl 1):50–3.
60. Gross AE, McKee NH, Pritzker KP, Langer F. Reconstruction of skeletal deficits at the knee. A comprehensive osteochondral transplant program. Clin Orthop Relat Res. 1983;174: 96–106.
61. Horas U, Schnettler R, Pelinkovic D, Herr G, Aigner T. Osteochondral transplantation versus autogenous chondrocyte transplantation. A prospective comparative clinical study. Chirurg. 2000;71(9):1090–7.
62. Bugbee W, Cavallo M, Giannini S. Osteochondral allograft transplantation in the knee. J Knee Surg. 2012;25(2):109–16.
63. O'Driscoll SW, Keeley FW, Salter RB. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion. An experimental investigation in the rabbit. J Bone Joint Surg Am. 1986;68(7):1017–35.
64. Skoog T, Johansson SH. The formation of articular cartilage from free perichondrial grafts. Plast Reconstr Surg. 1976;57(1):1–6.
65. Homminga GN, Bulstra SK, Bouwmeester PS, van der Linden AJ. Perichondral grafting for cartilage lesions of the knee. J Bone Joint Surg Br. 1990;72(6):1003–7.
66. Homminga GN, Bulstra SK, Kuijer R, van der Linden AJ. Repair of sheep articular cartilage defects with a rabbit costal perichondrial graft. Acta Orthop Scand. 1991;62(5):415–8.

67. Bouwmeester SJ, Beckers JM, Kuijer R, van der Linden AJ, Bulstra SK. Long-term results of rib perichondrial grafts for repair of cartilage defects in the human knee. Int Orthop. 1997;21(5):313–7.
68. Vachon A, McIlwraith CW, Trotter GW, Norrdin RW, Powers BE. Neochondrogenesis in free intra-articular, periosteal, and perichondrial autografts in horses. Am J Vet Res. 1989;50(10): 1787–94.
69. Steinberg MS. Mechanism of tissue reconstruction by dissociated cells. II. Time-course of events. Science. 1962;137:762–3.
70. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331(14):889–95.
71. Oberpenning F, Meng J, Yoo JJ, Atala A. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat Biotechnol. 1999;17(2):149–55.
72. Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, et al. Clinical transplantation of a tissue-engineered airway. Lancet. 2008;372(9655):2023–30.
73. Cole BJ, Pascual-Garrido C, Grumet RC. Surgical management of articular cartilage defects in the knee. J Bone Joint Surg Am. 2009;91(7):1778–90.
74. Caplan AI. New era of cell-based orthopedic therapies. Tissue Eng Part B Rev. 2009;15(2): 195–200.
75. Hunziker EB, Kapfinger E, Geiss J. The structural architecture of adult mammalian articular cartilage evolves by a synchronized process of tissue resorption and neoformation during postnatal development. Osteoarthritis Cartilage. 2007;15(4):403–13.
76. Khoshgoftar M, van Donkelaar CC, Ito K. Mechanical stimulation to stimulate formation of a physiological collagen architecture in tissue-engineered cartilage: a numerical study. Comput Methods Biomech Biomed Engin. 2011;14(2):135–44.
77. Kock LM, Ravetto A, van Donkelaar CC, Foolen J, Emans PJ, Ito K. Tuning the differentiation of periosteum-derived cartilage using biochemical and mechanical stimulations. Osteoarthritis Cartilage. 2010;18(11):1528–35.
78. Welsch GH, Mamisch TC, Domayer SE, Dorotka R, Kutscha-Lissberg F, Marlovits S, et al. Cartilage T2 assessment at 3-T MR imaging: in vivo differentiation of normal hyaline cartilage from reparative tissue after two cartilage repair procedures–initial experience. Radiology. 2008;247(1):154–61.
79. Cole BJ, Farr J, Winalski CS, Hosea T, Richmond J, Mandelbaum B, et al. Outcomes after a single-stage procedure for cell-based cartilage repair: a prospective clinical safety trial with 2-year follow-up. Am J Sports Med. 2011;39(6):1170–9.
80. Hendriks J, Riesle J, van Blitterswijk CA. Co-culture in cartilage tissue engineering. J Tissue Eng Regen Med. 2007;1(3):170–8.
81. Welting TJ, Caron MM, Emans PJ, Janssen MP, Sanen K, Coolsen MM, et al. Inhibition of cyclooxygenase-2 impacts chondrocyte hypertrophic differentiation during endochondral ossification. Eur Cell Mater. 2011;22:420–36; discussion 436–7.
82. Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. Arthroscopy. 2002;18(7):730–4.
83. Intema F, Van Roermund PM, Marijnissen AC, Cotofana S, Eckstein F, Castelein RM, et al. Tissue structure modification in knee osteoarthritis by use of joint distraction: an open 1-year pilot study. Ann Rheum Dis. 2011;70(8):1441–6.
84. Karsdal MA, Madsen SH, Christiansen C, Henriksen K, Fosang AJ, Sondergaard BC. Cartilage degradation is fully reversible in the presence of aggrecanase but not matrix metalloproteinase activity. Arthritis Res Ther. 2008;10(3):R63.

Part II
Fundamental Research, Biomaterials and Bioreactor Involved in Cartilage Repair

Chapter 2
Gene Therapy in Articular Cartilage Repair

Alan Ivkovic, Andreja Vukasovic, Ryan M. Porter, Damir Hudetz, Marko Pecina, and Christopher H. Evans

Abstract The restoration of damaged articular cartilage remains one of the biggest challenges in modern clinical orthopaedics. There is no pharmacological treatment that promotes the repair of cartilage, and non-operative treatment inevitably leads to the development of premature osteoarthritis. Current treatment modalities include microfracture, transplantation of osteochondral grafts and autologous chondrocyte implantation (ACI), each having its own benefits and shortcomings. New biological approaches to cartilage repair that are based on the use of cells and molecules that promote chondrogenesis and/or inhibit cartilage breakdown offer a promising alternative to current treatment options. Chondrogenesis is a precisely orchestrated process which involves many growth factors and signaling molecules, and by modifying the local cellular environment, it is possible to enhance formation of more natural cartilage tissue within the defect. These bioactive molecules are difficult to administer effectively. For those that are proteins or RNA molecules, gene transfer has emerged as an attractive option for their sustained synthesis at the site of repair. To accomplish this task,

A. Ivkovic (✉) • D. Hudetz
Department of Orthopaedic Surgery, St. Catherine's Hospital, Bracak 8, Zabok 49240, Croatia

Department of Orthopaedic Surgery, University Hospital Sveti Duh, Zagreb, Croatia

Department of Biotechnology, University of Rijeka, Rijeka, Croatia
e-mail: alan.ivkovic@gmail.com

A. Vukasovic
Department of Histology and Embryology, School of Medicine, University of Zagreb, Zagreb, Croatia

R.M. Porter • C.H. Evans
Department of Orthopaedic Surgery, Center for Advanced Orthopaedic Studies, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

M. Pecina
Department of Orthopaedic Surgery, School of Medicine, University of Zagreb, Zagreb, Croatia

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_2,

two main strategies have been explored. The direct or *in vivo* approach delivers exogenous DNA directly into the joint. In this case synovial lining cells are the main site of gene transfer; depending on the vector, cells around or within the defect may also be genetically modified. During indirect or *ex vivo* delivery, cells are recovered, genetically manipulated outside the body, and then returned to the defect. Delivery of the genetic material to the living cell can be accomplished by use of either viral or non-viral vectors. While viral vectors are much more effective, they raise several safety concerns. Numerous preclinical animal studies have confirmed the effectiveness of these approaches in joints, and several phase I and II clinical gene therapy studies in the local treatment of arthritis provide reason for cautious optimism. This chapter will provide insight into the field of gene therapy in cartilage repair, and its potential for safe and effective clinical translation.

Keywords Cartilage defects • Gene therapy • Vectors • Growth factors

Key Points

- Although the lack of a natural repair process in cartilage is not due to a single, recessive gene, regeneration may be stimulated by gene transfer.
- There is plethora of possible candidate genes for promoting chondrogenesis, cell proliferation, maturation and matrix synthesis along with the inhibition of cartilage degradation.
- Gene therapy requires a dependable and safe delivery system to carry the therapeutic gene(s) into the target cells where they will be expressed.
- Transduction is application of viral vectors while the use of non-viral vectors is called transfection.
- There are two main strategies for gene delivery to joint cells: a direct, or *in vivo*, and an indirect, or *ex vivo*, approach.
- The duration and level of gene expression are important aspects of gene therapy. Cartilage repair would likely require modest levels of transgene expression for limited periods of time, which is more easily achieved than long-term expression.
- Articular chondrocytes and mesenchymal stem cells (MSCs) are currently the two most promising cell types for transplantation approaches.
- When speculating on the possible vector system to be used in clinical translation, recombinant adeno-associated viruses (AAV) seem like the most likely candidate.

2.1 Introduction

Gene therapy is based on the premise that it is possible to compensate for a defective gene in a recessive Mendelian disease by the delivery and expression of a functional one. The first successful gene therapy clinical trial took place in the United States in 1990 involving two patients who suffered from a rare immune disorder called adenosine deaminase severe combined immunodeficiency (ADA-SCID). By using retrovirally-mediated transfer of wild-type adenosine deaminase (ADA) cDNA into the T cells of the patients, it was possible to normalize the number of blood T cells, as well as to improve and normalize many cellular and humoral immune responses [1]. Gene therapy for ADA-SCID and X-linked SCID has now become the standard of care for these diseases. Promising clinical data have recently been published for hemophilia, β-thalessemia, Leber congenital amaurosis, and lipoprotein lipase deficiency [2–6]. These successes validate the concept of using gene therapy for monogenetic, recessive diseases where a single, defined gene is defective. But it is difficult to apply to cartilage repair because its lack of a natural repair process is not due to a single, recessive gene and there is no obvious candidate, single therapeutic gene.

For a very long time articular cartilage was thought of as a quiscent tissue with no possibility of regeneration after injury. The realization that cartilage is a metabolically active tissue, with various matrix components continually being turned over at different rates, has created the paradigm shift of using biological approaches to repair cartilage. Tissue remodelling involves co-ordinated production of matrix metalloproteinases (MMPs) and the ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs) family of proteinases, coupled to the synthesis of new proteoglycans and proteins. Molecules involved in cartilage matrix breakdown include MMP-1 (collagenase-1), MMP-3 (stromelysin- 1), MMP-9 (gelatinase 92 kD), and MMP-13 (collagenase- 3). The activity of these proteinases is restrained by the action of tissue inhibitors of metalloproteinases (TIMPs). Many factors are involved in regulation of cartilage turnover (Table 2.1). These include humoral factors such as insulin-like growth factor-1 (IGF-I) and cytokines including interleukin-1 (IL-1), tumor necrosis factor (TNF), and transforming growth factor β (beta) (TGF-β (beta)), which are produced by chondrocytes, synovial cells and other cells found within joints. With age, articular chondrocytes loose their function; their anabolic and mitotic activities decline, expression of senescence-associated enzymes increases and telomere length decreases; aggrecans decrease in size and aggregation, and collagen cross linking increases [7–12]. These are associated with structural changes such as fibrillation and thinning of cartilage and decline of surface repair.

Spontaneous repair of chondral defects is very limited while osteochondral defects involving underlying bone fill with bone marrow that clots, leading to healing with fibrous tissue, or, at best, fibrocartilaginous tissue. Such articular defects predispose to osteoarthritis (OA). There is no pharmacological treatment for cartilage defects, and current surgical modalities include microfracture, transplantation

Table 2.1 Mechanisms of action and candidate genes for cartilage repair

Mechanism of action	Candidate gene	References
Anabolic factors	Chondrogenic transcription factors: SOX5, SOX6, SOX9	[13, 23–31]
	Growth factors:	[10, 32–36]
	IGF-1	[37–40]
	BMP-2, -4, -7	[32, 41–48]
	TGF-β	[49, 50]
	FGF-2	
Anticatabolic factors	Inhibition of proinflammatory cytokines:	[21, 51, 52]
	IL-1Ra	[53–55]
	sIL-1R	
	sTNFR	
Cytoprotection/ Proliferation factors	Inhibition of apoptosis: bcl-2	[67]
	Heat shock proteins:	[24, 26]
	HSP70, GRP78	[69, 70]
	Telomerase: hTERT	[63]
	Cell cycle regulator: p21	

of ostechondral grafts and various cell-based options (ACI being the most common) with or without a scaffold, each having its own benefits and shortcomings [13]. If chosen wisely, each of these techniques may yield good clinical results in terms of pain reduction and improvement of joint function. However, to date none of the proposed techniques results in production of fully matured hyaline cartilage, and there is a continuing need for new and innovative approaches to treat cartilage defects.

Biological approaches that are based on the use of cells and molecules that promote chondrogenesis and/or inhibit cartilage breakdown offer promising, novel treatment options and form a good basis for the application of gene therapy in articular cartilage repair. Stimulation of chondrogenesis, cell proliferation, maturation and synthesis of an authentic extracellular matrix, along with the inhibition of cartilage degradation, are the main strategies employed to accomplish this task. All of these processes are complex, being regulated by a number of different molecules; hence there is a plethora of possible candidate genes for promoting cartilage repair. Selecting the appropriate gene for this purpose is a major challenge to using gene therapy for repairing cartilage.

2.2 General Principles of Gene Therapy

Gene therapy requires a dependable and safe delivery system to carry the therapeutic gene(s) into the target cells where they will be expressed. Commonly used vectors can be viral or non-viral (Table 2.2). When viral vectors are applied, gene delivery is called transduction; the use of non-viral vectors is called transfection. Transfection can occur through natural processes, such as endocytosis, and

Table 2.2 Properties of the main viral vectors used in gene therapy

Virus	Key properties of wild-type virus	Advantages	Disadvantages
Adenovirus	Double stranded genome	Straightforward production at high titers	Inflammatory and antigenic
	~35 kb long	Transducing non-dividing cells	
	Non-enveloped	Wide choice of serotypes	
	Over 50 serotypes		
	~100 nm in size		
	Genome remains episomal		
Herpes simplex virus (HSV)	Double stranded DNA genome	Very efficient transduction of dividing and non-dividing cells	Complex genome – difficult to produce
	~150 kb long	Has natural latency in neurons	Cytotoxic
	Enveloped	Very large carrying capacity	
	~200 nm in size		
	Genome remains episomal		
Adeno-associated virus (AAV)	Single-stranded DNA genome	Perceived to be safe (wild-type virus cause no known disease)	Difficult to produce
	4.8 kb long	Transduces non-dividing cells	Carrying capacity is insufficient for certain applications
	Non-enveloped	Thought to have low immunogenicity, but this is being re-evaluated	
	Growing number of serotypes identified		
	~20 nm in size		
Oncoretrovirus	RNA genome	Straightforward production of vectors at moderate titers	Risk of insertional mutagenesis
	~8–10 kb long	Pseudotyped vectors have wide host range	Require host-cell division
	Enveloped		
	~100 nm in size		
Lentivirus	RNA genome	Straightforward production of vectors at moderate titers	Risk of insertional mutagenesis, but nonintegrating vectors are being developed, and have proved effective in animal models
	~8–10 kb long	Pseudotyped vectors have wide host range and are often very efficient	
	Enveloped	Transduces non-dividing cells	
	~100 nm in size		

efficiency can be enhanced by physical methods, such as electroporation, the use of a gene gun, and liposomes [14–19]. While non-viral vectors are perceived to be safer and easier to manufacture, viral vectors are much more efficient.

Recombinant retroviruses, such as those derived from Moloney murine leukemia virus, were the first to be used in human gene therapy clinical trials. Even though they ensure persistence of the transgene within transduced cells, they infect only dividing cells [20]. Another important property is the random integration of retroviral genetic material into the host genome, an event that might lead to insertional mutagenesis and the activation of tumor genes [21]. Lentivirus, a specific class of retrovirus that includes Human Immunodeficiency Virus, does not require host cell division for efficient transduction. These vectors transduce synovium very effectively after intra-articular injection but, like other retroviruses, pose the risk of insertional mutagenesis [22, 23]. Non-integrating lentiviral vectors have been developed to overcome this concern.

Recombinant adenoviruses have been the vectors most commonly used in clinical trials. They deliver their genomes as episomes, infect both dividing and non-dividing cells, and have large carrying capacity. However, they tend to excite a strong immune response and normally this leads to short-term transgene expression because transduced cells are cleared by the immune system.

Recombinant AAV present several advantages as gene delivery vehicles. Because wild-type AAV produces no known human diseases, they are perceived to be safe, and their DNA is maintained in a stable, episomal form in the nuclei of cells they transduce. Various serotypes of AAV have been shown to transduce chondrocytes, MSCs and synoviocytes. New technologies have enabled easier production of AAV [24–28].

Regardless of the vector used, there are two main strategies for gene delivery to joint cells: a direct, or *in vivo*, and an indirect, or *ex vivo*, approach. *In vivo* delivery is a simpler, less costly, one-step procedure in which vectors are delivered straight into the joint and can modify all available cells. A disadvantage of this strategy is that vectors are introduced into the patient where their subsequent activity cannot be easily controlled. Using *ex vivo* approaches allows for better control of gene transfer. Cells are genetically modified outside the body and then introduced into the joint. Although nominally safer, this approach is more complex and expensive than the *in vivo* approach. The choice of delivery method is based on a number of considerations including the type of the vector to be used, the transgene and the target cells. Delivery of growth factors might be more effective and safer when limited to the defect itself; implantation of cells that have been genetically modified outside of the body might better accomplish such localized delivery.

The duration and level of gene expression are additional, important aspects of gene therapy, which are defined by the therapeutic application. For example, certain monogenic diseases such as lysosomal storage diseases (e.g., Gaucher's disease) or osteogenesis imperfecta may require life-long expression of corrected gene in order to produce sustained clinical improvement [29]. On the other hand, treatment of malignant diseases may require very large amounts of transgene expression for very limited periods of time, in order to eliminate tumor cells without causing significant adverse effects [30]. Along these lines, cartilage repair would likely require modest levels of transgene expression for limited periods of time, which is more easily

achieved than long-term expression [31–33]; indeed, this may already be achievable using current technology.

Depending on the application, regulation of transgene expression may be important. One option is to use exogenous molecules to control transgene expression. Tetracycline-controlled activation of transgene expression is the most commonly used system in eukaryotic cells [34]. For this system, transcription is reversibly turned either on or off (Tet-On or Tet-Off) in the presence of the antibiotic tetracycline or one of its derivatives (e.g. doxycycline). An alternative strategy relies on the natural responsiveness of selected promoters to endogenous stimuli, such as pro-inflammatory cytokines. In theory, such systems could be activated in the presence of certain pathophysiological events e.g. exacerbation of OA or rheumatoid arthritis (RA) [35].

2.3 Candidate Genes for Therapeutic Intervention

A vast number of bioactive cues are known to be involved in the process of chondrogenesis and the maintenance of cartilage homeostasis. Although these signals are pleiotrophic, interactive and redundant, here they will be described according to their principal mechanism of action (Table 2.1).

2.3.1 Anabolic Factors

Chondrogenic transcription factors (*Sex determining region Y- box 5*, *6*, *9* (*SOX5*, *6*, *9*)). A variety of transcription factors have been enlisted in attempts to stimulate anabolic pathways in cartilage. With regard to anabolic transcription factors, most of the focus has been placed on targeting the SOX genes, SOX9 and co-factors SOX5 and SOX6, which are essential for chondrocyte differentiation and cartilage formation. During embryogenesis, SOX9 is expressed in all chondroprogenitor cells and its expression coincides with expression of collagen II [36–38]. Human chondrocytes from OA cartilage have been successfully transduced with retro-, lenti-, adeno- and AAV carrying SOX genes. *In situ* overexpression of SOX9 in normal and OA articular cartilage stimulated proteoglycan and type II collagen synthesis in a dose-dependent manner. These effects were not associated with changes in chondrocyte proliferation. These effects of SOX genes have been shown in MSCs derived from bone marrow and adipose tissue [19, 39–44].

Growth factors (*IGF-I*, *bone morphogenic proteins* (*BMPs*), *TGF-β* (*beta*), *fibroblast growth factor – 2* (*FGF-2*), *growth differentiation factor–5* (*GDF-5*)). Numerous growth factors have been employed in attempts to stimulate anabolic pathways in cartilage. IGF-I is expressed in developing and mature cartilage; it stimulates both cell proliferation and synthesis of aggrecan and collagen type II. Also, IGF-I is a survival factor for chondrocytes. It cannot induce cartilage formation from MSCs, however, so its principal target cells are chondrocytes [45, 46]. Transfection of articular chondrocytes with a plasmid vector containing the cDNA

for human IGF-I and subsequent transplantation of transfected cells onto the surface of articular cartilage explants led to the formation of a new tissue layer on the cartilage explant surface. Subsequent analysis showed thicker cartilage, higher percentage of collagen type II, and increased DNA and glycosaminoglycan (GAG) synthesis in the underlying explants [47]. Allogeneic chondrocytes transfected with plasmid IGF-I and encapsulated in alginate have been transplanted into rabbit osteochondral defects, leading to improved articular cartilage repair and acceleration of the formation of the subchondral bone after 14 weeks [16]. Intra-articular injection of adenoviral vector expressing human IGF-I promoted proteoglycan synthesis without significantly affecting inflammation or cartilage breakdown in rabbits. In addition, no adverse effects were observed 7 days after the treatment [48]. Recombinant AAV-mediated overexpression of IGF-I proved to have long term anabolic effects on chondrocyte cultures from human OA cartilage [49].

Chondrogenic differentiation, maturation and maintenance feature among the wide-ranging biological activities of BMPs. BMP-2, -4 and -7 have been mostly investigated in the context of cartilage regeneration. Both chondrocytes and MSCs of different origins have been successfully transduced with these genes. For example, chondrocytes modified with adenovirus carrying BMP-7 were transplanted onto cartilage explants and maintained *in vitro*. After 3 weeks, thicker neotissue was formed, positive for type II collagen and proteoglycan but negative for type X collagen [50]. When this method was used *in vivo* in an equine model, early post-treatment results were very positive, similar to those found in an *ex vivo* model. However, 8 months later the results were disappointing. Few implanted cells persisted and there was no difference between repair tissue in controls and BMP-7 treated animals [51]. With the use of MSCs retrovirally transduced to express BMP-4 in a rat model, cartilage repair was better than in controls after 6 months [52]. Side effects of BMP gene transfer to joints include osteophyte formation as a result of BMP-transfected cells engaging the synovium, and causing the differentiation of MSCs towards hypertrophic chondrocytes and osteoblasts [53].

All three isoforms of TGF-β (beta) have potent chondrogenic properties. They stimulate matrix synthesis and mitosis of chondrocytes and induce MSC differentiation into chondrocytes [45]. Adenoviruses, retroviruses, AAV and plasmids have been successfully employed in the genetic modification of chondrocytes and MSCs with TGF-β (beta). Chondrocytes modified to overexpress TGF-β(beta)1 increase their hyaline extracellular matrix synthesis in culture [54, 55]. Repair of cartilage was achieved *in vivo* when bone marrow MSCs modified with adenovirus and plasmid to express TGF-β(beta)1 were implanted into chondral and ostechondral defects [56, 57]. Moreover, retrovirally transduced allogenic chondrocytes expressing TGF-β(beta)1 were successully introduced into the joints of patients with OA in a clinical trial [58]. However, TGF-β(beta)1, either applied directly as a protein or via local overexpression, is not suitable for direct intraarticular application as it triggers adverse synovial reactions [59–61].

FGF-2 is a potent chondrocyte mitogen. *In vitro* and *in vivo* studies have shown that FGF-2 gene transfer may be applicable for the treatment of articular cartilage disorders in which cellular repopulation is a therapeutic goal. This beneficial effect

is mediated primarily through fibroblast growth factor receptor 3 (FGFR3), while some anti-anabolic effects observed are mediated primarily through fibroblast growth factor receptor 1 (FGFR1) [62]. Combined transfection of other anabolic factors with FGF-2 improved cartilage healing *in vivo* and less degenerative changes were observed in adjacent cartilage tissue [63].

GDF-5, (BMP-14 or cartilage derived morphogenic protein – 1(CDMP-1)) is known to be an important regulatory factor during the embryologic development of the appendicular skeleton and has been shown to be involved in chondrogenesis [64–66]. It promotes aggregation of mesenchymal cells and enhances chondrocyte differentiation during develeopment and in adult MSCs [67–70]. Bone derived MSCs transfectes with GDF-5 gene enhanced the repair of osteochondral defects [18]. Two different studies successfully injected adenovirus particles carrying the GDF-5 gene in rat tendons and mice degenerated discs respectfully, causing healing in terms of higher collagen II and GAG content [71, 72].

2.3.2 Anticatabolic Factors

Inhibition of proinflammatory cytokines (*interleukin-1 receptor antagonist* (*IL-1Ra*), *soluble interleukin-1 receptor* (*sIL-1R*), *soluble tumor necrosis factor receptor* (*sTNFR*)). IL-1Ra was the first gene used in a clinical trial for gene therapy in joint diseases, paving the path for other genes to follow [73, 74]. Inflammatory cytokines are highly expressed in RA and their role in OA is increasingly appreciated. Their activities have been successfully reduced in animal models of OA and RA, by transfer of genes encoding IL-1Ra, sIL-1R, sTNFR, mostly by delivering them directly into the joint using different viral and non-viral vectors [27, 75–79]. This protected hyaline matrix synthesis, thereby promoting cartilage repair. Since enhanced matrix breakdown may result from both biological and biomechanical signaling, the most effective control of degradation could be achieved by increase of downstream regulators such as TIMPs. Up-regulation of the gene for TIMP is a logical approach to the inhibition of MMP-mediated cartilage degradation. Certain members of the ADAMTS family are also inhibited by TIMPs. Chondrocytes and synovial fibroblasts have been transduced *in vitro* with the TIMP-1 gene and inhibitor IkappaBalpha respectively which resulted in the decreased activity of several MMPs [80, 81].

2.3.3 Cytoprotection/Proliferation Factors

Additional genes of recent interest for improving cartilage repair are those that affect the senescence and life cycle of chondrocytes, protecting these cells from stressful stimuli and apoptosis. Chondrocytes have been successfully modified with B-cell lymphoma 2 (BCL-2) [82], 70 kDa heat shock protein (HSP70) [83], human

telomerase reverse transcriptase (hTERT) and 78 kDa glucose-regulated protein (GRP78) [84, 85] target genes *in vitro*. HSP70 has also been evaluated *in vivo*. This therapy resulted in cytoprotection and better extracellular matrix synthesis. Synoviocytes, adenovirally transduced with cyclin dependent kinase inhibitor 1 (p21), down regulate expression of several inflammatory cytokines including IL-1β (beta), as well as MMP-1 and -3 [86]. There are numerous studies showing that the best anabolic response can be achieved with combinations of genes encoding different factors [63, 87–89].

2.3.4 Post Transcriptional Gene Regulation: MicroRNAs

MicroRNAs are the focus of emerging novel therapeutic strategies, including for cartilage repair. MicroRNAs form a class of non-coding, single strand RNAs that regulate gene expression at the post-transcriptional level by binding to specific sequences within target transcripts. MicroRNAs can act as both positive and negative factors in cartilage homeostasis [90, 91]. Studies on human chondrocytes and in animal models have shown that microRNAs have roles in chondrogenensis and both the anabolic and catabolic events of articular metabolism. During chondrogenesis, microRNA-140 expression in MSC cultures increases in parallel with the expression of SOX9 and collagen type II, alpha 1 (COL2A1). Normal human articular cartilage express microRNA-140, but this expression is significantly reduced in OA tissue. *In vitro* treatment of chondrocytes with IL-1β (beta) suppresses microRNA-140 expression. Conversely, transfection of chondrocytes with microRNA-140 down-regulates IL-1β (beta)-induced ADAMTS5 expression [92–95].

MicroRNA-145 has shown to affect differentiation of MSCs by acting directly on SOX9. Overexpression of microRNA-145 in MSCs decreases the expression of COL2A1, aggrecan (AGC1), cartilage oligomeric matrix protein (COMP), collagen type IX, alpha 2 (COL9A2), and collagen type XI, alpha 1 (COL11A1), and reduces GAG contents synthesis. In contrast, the inhibition of microRNA-145 significantly enhances the mRNA expression of the aforementioned genes and increases GAG production [96, 97]. MicroRNA-145 acts as a direct SOX9 repressor in normal healthy human articular chondrocytes. Experimentally increased microRNA-145 levels cause greatly reduced expression of tissue-specific microRNAs (microRNA-675 and microRNA-140), while increasing levels of the hypertrophic markers Runt related transcription factor 2 (RUNX2) and MMP13, characteristic of the changes occurring in OA [98].

Additional microRNAs have begun to be identified as having a role in cartilage homeostasis. Yamasaki et al. [99] have shown that microRNA-146a is intensely expressed in low grade OA cartilage but less in high grade OA, and its expression decreases in accordance with the level of MMP-13 expression. The expression of microRNA-146 was markedly elevated by IL-1β stimulation in human chondrocytes *in vitro*. Nakasa et al. [100] showed that microRNA-146a inhibits

osteoclastogenesis and has some anticatabolic properties in collagen-induced arthritic joints in mice. The overexpression of microRNA-9, microRNA-98 and microRNA-146 in human chondrocytes can reduce IL-1β (beta) yet increase TNF-α (alpha) mRNA. MicroRNA-9, upregulated in OA tissue, inhibits the secretion of metalloproteinase MMP-13 by isolated human chondrocytes. In addition, the inhibition or overexpression of microRNA-9 can regulate MMP-13 and type II collagen content [101]. MicroRNA-27a reduced MMP-1 and Insulin-like growth factor-binding protein 5 (IGFBP-5) synthesis, controlling arthritis in an indirect way. IL-1β (beta)-induced apoptosis was significantly reduced in rabbit chondrocytes when microRNA-34a was silenced [91, 97, 102].

2.4 Gene Therapy Strategies for Articular Cartilage Repair

2.4.1 Gene Delivery to the Synovium

When delivering genes directly to synovium, possible responding cells are synoviocytes and chondrocytes exposed to transgene products diffusing from synovial cells. Synovium has a large surface area compared to cartilage and is more amenable to gene delivery. Chondrocytes are present at low density and are lodged inside a dense matrix which makes them less accessible to vectors. When attempting to influence cartilage metabolism via gene delivery to synovium, it makes most sense to deliver cDNAs encoding secreted factors, such as IL-1Ra or IGF-1. Direct gene delivery to synovium has been mostly used for treating patients with RA [79, 81, 103]. *Ex vivo* approach using synovial fibroblasts in Phase I clinical trial was successfully initiated in 1996 [73, 74].

2.4.2 Gene Delivery to Chondrocytes

Despite the success of procedures such as ACI, using autologous chondrocytes as target cells for gene delivery requires additional procedures to retrieve the cells from the joint, and cause additional damage to the joint. An alternative strategy could involve taking chondrocytes from different locations in the body, such as the cartilage of nasal septum or ribs [104]. Other issues to be addressed regarding chondrocytes are time span in culture, potential dedifferentiation of cells that can occur with extended culture, and mode of application. One strategy is to load and implant the cells on matrices or use some kind of glue to keep them in place [63, 105]. Recently promising results of a phase I clinical trial have been published in which allogenic chondrocytes retrovirally transduced to express TGF-β(beta)1 were delivered to the knee joints of subjects with advanced OA [58].

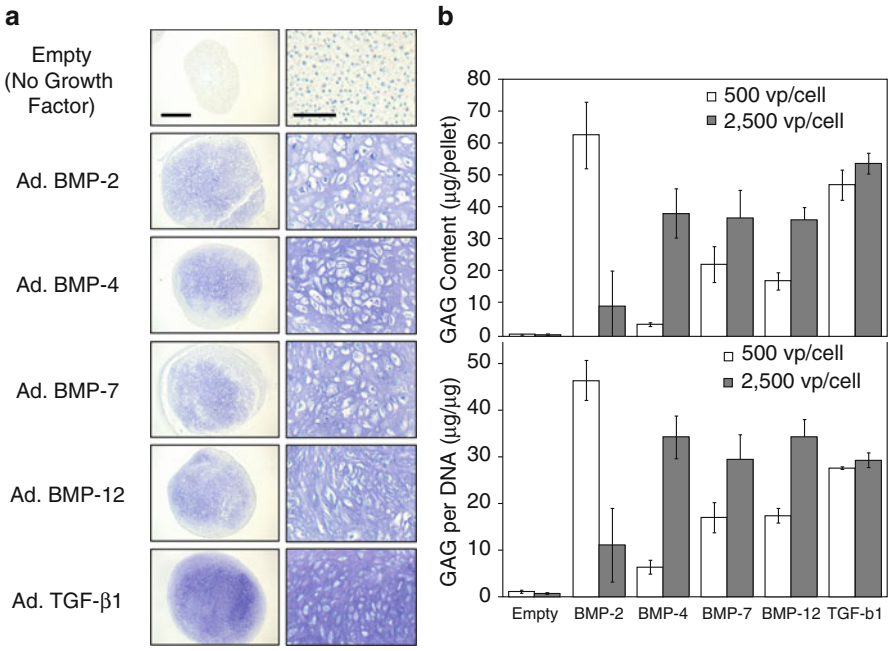

Fig. 2.1 Chondrogenic induction of human bone marrow-derived MSCs by growth factor overexpression: comparison of different BMPs with TGF-β (beta) 1. Human MSCs were transduced with adenoviral vectors encoding either no transgene (empty) or one of several known chondrogenic growth factors, then were cultured as cell aggregates for 4 weeks under standard chondrogenic conditions. (**a**) The resulting cell pellets were sectioned and stained with Toluidine Blue, which binds with sulfated glycosaminoglycan (GAG) chains. The two columns show representative pellets for the most chondrogenic dose of each virus at different magnifications (*left* scale bar = 500 μm; *right* scale bar = 100 μm). While all the presented BMPs induced chondrogenesis and proteoglycan deposition by human MSCs, they generally led to a more hypertrophic phenotype than did TGF-β (beta) 1 overexpression. (**b**) GAG levels within digested pellets were measured quantitatively by dimethylmethylene blue binding. Total GAG content per pellet (*upper panel*) and deposition relative to DNA content (*lower panel*) are shown for the same growth factors in (**a**) delivered using either 500 or 2,500 viral particles (vp)/cell. The GAG deposition response to BMP overexpression was highly dependent on the amount of viral vector and the corresponding level of BMP secretion. Cells were less sensitive to TGF-β (beta) 1 secretion levels. TGF-β (beta) 1 overexpression promoted higher cell density within pellets after 4 weeks, so that maximum GAG/DNA levels were relatively lower than for BMP-overexpressing groups

2.4.3 Gene Delivery to Mesenchymal Progenitors

MSCs offer an attractive alternative to chondrocytes as vehicles for *ex vivo* gene delivery to sites of cartilage damage. These cells can easily be retrieved from bone marrow, periosteum, synovium, adipose tissue, or skeletal muscle and differentiated into chondrocytes in tissue culture [106, 107] (Fig. 2.1). MSCs in combination with

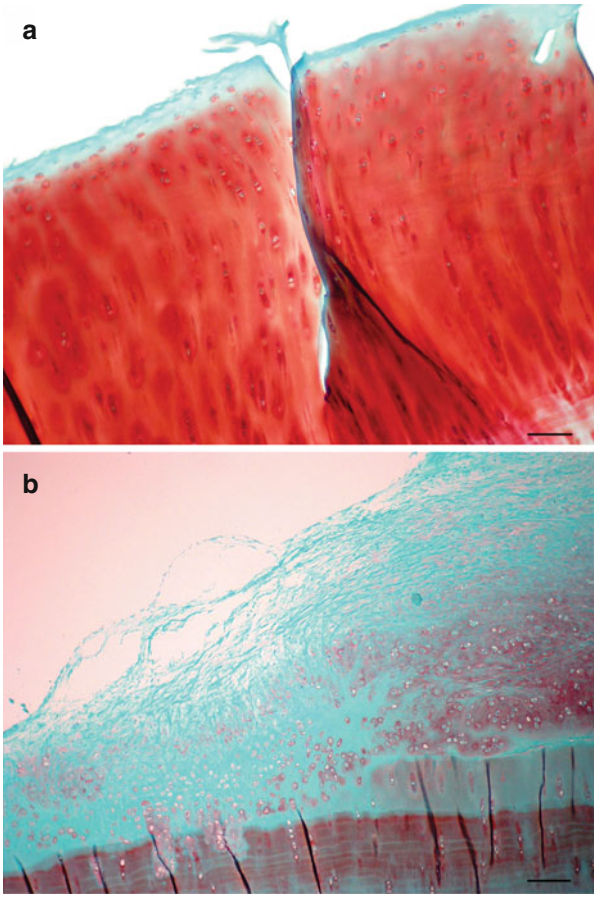

Fig. 2.2 Hyaline nature of cartilage healing after treatment with bone marrow clot adenovirally transduced with TGF**β**(beta)1 compared to healing after no treatment. Critical-size full-thickness cartilage defects in sheep were treated either with bone marrow clot genetically modified to secrete TGF**β**(beta)1 (**a**) or left untreated (**b**). After 6 months histology revealed formation of hyaline cartilage (*stained red*) in genetically treated defects and mixture of fibrous tissue (*stained green*) and fibrocartilage in untreated defects. Staining: Safranin O; magnification: 100×; scale bar = 100 **μ**m

different scaffolds have been successfully employed to treat chondral and osteochondral defects in animal models *in vivo* [18, 56, 57, 108, 109] (Fig. 2.2). One potential challenge for using MSCs concerns the prevention of hypertrophic maturation that is typically associated with their chondrogenic differentiation [110]. According to the literature, MSCs from synovium form chondrocytes without undergoing hypertrophic differentiation. One way of delivering MSCs form bone marrow to the cartilage defects is clot technology designed by Pascher et al. [109]. After aspiration, bone marrow is transduced with vector carrying certain gene and left briefly at room temperature to clot. Clot is then placed into the defect without any fixation.

As an alternative to MSCs, recent progress has been made regarding the use of induced pluripotent stem cells (iPSCs) for treatment of cartilage defects. Wei et al. formed iPSCs from OA cartilage and then differentiated them into chondrocytes using lentiviral transduction of TGF-β (beta)1 in an alginate matrix [111].

2.5 Challenges for the Clinical Application of Gene Therapy to Promote Articular Cartilage Repair

Even though gene transfer to joints is local, there is still concern about systemic effects and safety issues are important impediments to successful clinical translation. The whole field of gene therapy carries the perception of being risky, unsafe and difficult to deliver. However, thorough review of available data suggests that this perception is partially exaggerated. There have been over 1 700 clinical trials worldwide with more than 10,000 patients being treated, and only few fatalities have been unequivocally connected with gene therapy itself [112]. However, each of these events has been seized by media, creating a negative perception of the whole field. This was highlighted by the 2007 death of a subject in an arthritis gene therapy trial [113]. Although subsequent investigation exonerated locally administered gene therapy from being responsible for this death, it was a huge step back in efforts to translate gene therapy into clinical practice for treating joint conditions. It also emphasizes the importance of selecting not only an appropriate gene vector system, target gene and delivery method, but also suitable subjects for these trials. Thus, the *ex vivo* approach, although has some drawbacks compared to *in vivo*, might represent a safer option for cartilage repair. Additionally, some genes when applied *in vivo* had significant local side effects compared to *ex vivo* approach. Adenovirally mediated delivery of TGF- β(beta)1 or BMP-2 to the synovial lining, for instance, was found to generate joint fibrosis, extreme swelling, osteophytes and cartilage degeneration [59, 108, 114, 115]. Regarding the choice of a proper target gene, it may be more suitable to target anti-inflammatory genes into the synovium where they can have more general intra-articular effect, while use of growth factors should be localized to chondrocytes.

Several critical questions must be answered in order to select an effective gene therapeutic strategy. What are the optimal treatment modalities for different types of cartilage damage? For example, how are large defects treated relative to small defects? Which cells should be used or targeted? Which vectors best target these cells? What is the transgene of choice? Moreover, should gene therapy strategies be modified to reflect cartilage anisotropy? Articular cartilage is organized in 4 layers (superficial, intermediate, deep and mineralized cartilage); the most significant structural and molecular difference is between deep layer and mineralized cartilage. Type II collagen is present in the deep zone, while type type X collagen predominates in mineralized cartilage; additionally hypertrophic chondrocytes express alkaline phosphatase. Would cartilage implants constructed so that lower layer cells are modified or stimulated to preferentially express type X collagen and alkaline phosphatase, while upper layer cells express collagen type II, constitute the most appropriate therapy? This would definitely be a more scientifically and technologically challenging, laborious and costly approach.

Articular chondrocytes and MSCs are currently the two most promising cell types for transplantation approaches. Since it has been shown that MSCs exhibit

immunosuppressive properties, they may survive when transplanted into allogeneic hosts, although this is controversial. Allografting would make clinical translation much easier, since this approach avoids damaging already impaired joints to obtain autologous cells. It is not difficult to envision commercially available, genetically modified MSCs ready to be transplanted into localized cartilage defects with one-step, minimally invasive surgical procedures.

When speculating on the possible vector system to be used in clinical translation, AAV seems like the most likely candidate [25]. AAV causes no known human disease, has appropriate packaging capabilities, and transduces non-dividing cells. It has been thought to have low immunogenicity, but this is being re-evaluated. Another drawback is its complicated and costly production (see Table 2.1).

Use of scaffolds in cartilage repair is one the most exciting areas in orthopaedic research, both for scientists and clinicians [116]. Most current research is focused on resorbable scaffolds whose main function is to provide temporary, three-dimensional templates on which cells can adhere and synthesize extracellular matrix (ECM). As the scaffold resorbs, it is progressively replaced by newly formed, functional tissue. This approach, termed matrix-assisted chondrocyte transplantation is currently used in clinical practice to treat localized cartilage defects [117]. Autologous chondrocytes are attached to different types of matrices (e.g. collagen, hyaluronic acid etc.) and transplanted into the defect. Combining genetically modified cells with tissue engineered matrix might be a more effective strategy than cell delivery alone [88]. This would allow complete filling of the defect and three-dimensional orientation of genetically modified cells, thus ensuring more natural environment for production of ECM. Furthermore, optimal mechanical properties of the scaffold would make handling easier and surgical procedures more convenient to perform.

2.6 Conclusion

Translating gene therapy for treating cartilage lesions into clinical practice is not easy. Cartilage defects are not life threatening diseases, and treatment modalities developed so far serve their main purpose – minimizing the pain and improving the quality of life. Most of these biological approaches provide either cells alone, or construct made of cells and temporary scaffolds. Gene therapy has emerged as a feasible option to add as the final ingredient in this system, providing sustainable local expression of bioactive cue(s). A key challenge for translating this into clinical practice will depend on the development of safe and effective gene delivery systems with long-lasting expression of therapeutic transgenes, the identification of effective yet safe combinations of therapeutic genes, identification of the ideal target cell(s) (chondrocytes, MSCs or synoviocytes) and identifying the most appropriate carriers which better support the chondrogenic process within the defect. The regulatory issues, timelines and costs should not be underestimated as barriers to translation.

References

1. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, et al. T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. Science. 1995;270(5235):475–80.
2. Nathwani AC, Tuddenham EG, Rangarajan S, Rosales C, McIntosh J, Linch DC, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N Engl J Med. 2011;365(25):2357–65.
3. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature. 2010;467(7313):318–22.
4. Simonelli F, Maguire AM, Testa F, Pierce EA, Mingozzi F, Bennicelli JL, et al. Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration. Mol Ther. 2010;18(3):643–50.
5. Stroes ES, Nierman MC, Meulenberg JJ, Franssen R, Twisk J, Henny CP, et al. Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. Arterioscler Thromb Vasc Biol. 2008;28(12):2303–4.
6. Carpentier AC, Frisch F, Labbe SM, Gagnon R, de Wal J, Greentree S, et al. Effect of alipogene tiparvovec (AAV1-LPL(S447X)) on postprandial chylomicron metabolism in lipoprotein lipase-deficient patients. J Clin Endocrinol Metab. 2012;97(5):1635–44.
7. Martin JA, Buckwalter JA. Aging, articular cartilage chondrocyte senescence and osteoarthritis. Biogerontology. 2002;3(5):257–64.
8. Martin JA, Buckwalter JA. Human chondrocyte senescence and osteoarthritis. Biorheology. 2002;39(1–2):145–52.
9. Martin JA, Buckwalter JA. The role of chondrocyte senescence in the pathogenesis of osteoarthritis and in limiting cartilage repair. J Bone Joint Surg Am. 2003;85-A Suppl 2:106–10.
10. Skaalure SC, Milligan IL, Bryant SJ. Age impacts extracellular matrix metabolism in chondrocytes encapsulated in degradable hydrogels. Biomed Mater. 2012;7(2):024111.
11. Bader DL, Salter DM, Chowdhury TT. Biomechanical influence of cartilage homeostasis in health and disease. Arthritis. 2011;2011:979032.
12. Evans CH, Georgescu HI. Observations on the senescence of cells derived from articular cartilage. Mech Ageing Dev. 1983;22(2):179–91.
13. Gomoll AH, Farr J, Gillogly SD, Kercher JS, Minas T. Surgical management of articular cartilage defects of the knee. Instr Course Lect. 2011;60:461–83.
14. Conwell CC, Huang L. Recent advances in non-viral gene delivery. Adv Genet. 2005; 53:3–18.
15. Oligino TJ, Yao Q, Ghivizzani SC, Robbins P. Vector systems for gene transfer to joints. Clin Orthop Relat Res. 2000;379(Suppl):S17–30.
16. Madry H, Kaul G, Cucchiarini M, Stein U, Zurakowski D, Remberger K, et al. Enhanced repair of articular cartilage defects in vivo by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I). Gene Ther. 2005;12(15):1171–9.
17. Kaul G, Cucchiarini M, Arntzen D, Zurakowski D, Menger MD, Kohn D, et al. Local stimulation of articular cartilage repair by transplantation of encapsulated chondrocytes overexpressing human fibroblast growth factor 2 (FGF-2) in vivo. J Gene Med. 2006;8(1):100–11.
18. Katayama R, Wakitani S, Tsumaki N, Morita Y, Matsushita I, Gejo R, et al. Repair of articular cartilage defects in rabbits using CDMP1 gene-transfected autologous mesenchymal cells derived from bone marrow. Rheumatology (Oxford). 2004;43(8):980–5.
19. Kim HJ, Im GI. Electroporation-mediated transfer of SOX trio genes (SOX-5, SOX-6, and SOX-9) to enhance the chondrogenesis of mesenchymal stem cells. Stem Cells Dev. 2011; 20(12):2103–14.
20. Cucchiarini M, Madry H. Gene therapy for cartilage defects. J Gene Med. 2005;7(12):1495–509.
21. Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med. 2003;348(3):255–6.

22. Gouze E, Pawliuk R, Gouze JN, Pilapil C, Fleet C, Palmer GD, et al. Lentiviral-mediated gene delivery to synovium: potent intra-articular expression with amplification by inflammation. Mol Ther. 2003;7(4):460–6.
23. Gouze E, Pawliuk R, Pilapil C, Gouze JN, Fleet C, Palmer GD, et al. In vivo gene delivery to synovium by lentiviral vectors. Mol Ther. 2002;5(4):397–404.
24. Xiao X, Li J, Samulski RJ. Efficient long-term gene transfer into muscle tissue of immunocompetent mice by adeno-associated virus vector. J Virol. 1996;70(11):8098–108.
25. Grieger JC, Choi VW, Samulski RJ. Production and characterization of adeno-associated viral vectors. Nat Protoc. 2006;1(3):1412–28.
26. Toh ML, Hong SS, van de Loo F, Franqueville L, Lindholm L, van den Berg W, et al. Enhancement of adenovirus-mediated gene delivery to rheumatoid arthritis synoviocytes and synovium by fiber modifications: role of arginine-glycine-aspartic acid (RGD)- and non-RGD-binding integrins. J Immunol. 2005;175(11):7687–98.
27. Kay JD, Gouze E, Oligino TJ, Gouze JN, Watson RS, Levings PP, et al. Intra-articular gene delivery and expression of interleukin-1Ra mediated by self-complementary adeno-associated virus. J Gene Med. 2009;11(7):605–14.
28. Shi S, Mercer S, Trippel SB. Effect of transfection strategy on growth factor overexpression by articular chondrocytes. J Orthop Res. 2010;28(1):103–9.
29. Marijanovic I, Radnic M, Matic I, Erceg-Ivkosic I. Osteogenesis imperfecta and achievements in cell and gene therapy. Acta Med Croatica. 2010;64(3):191–200.
30. Mohr L, Geissler M, Blum HE. Gene therapy for malignant liver disease. Expert Opin Biol Ther. 2002;2(2):163–75.
31. Morisset S, Frisbie DD, Robbins PD, Nixon AJ, McIlwraith CW. IL-1ra/IGF-1 gene therapy modulates repair of microfractured chondral defects. Clin Orthop Relat Res. 2007;462:221–8.
32. Nixon AJ, Brower-Toland BD, Bent SJ, Saxer RA, Wilke MJ, Robbins PD, et al. Insulinlike growth factor-I gene therapy applications for cartilage repair. Clin Orthop Relat Res. 2000;379(Suppl):201–13.
33. Nixon AJ, Haupt JL, Frisbie DD, Morisset SS, McIlwraith CW, Robbins PD, et al. Gene-mediated restoration of cartilage matrix by combination insulin-like growth factor-I/interleukin-1 receptor antagonist therapy. Gene Ther. 2005;12(2):177–86.
34. Sun Y, Chen X, Xiao D. Tetracycline-inducible expression systems: new strategies and practices in the transgenic mouse modeling. Acta Biochim Biophys Sin (Shanghai). 2007;39(4):235–46.
35. Varley AW, Munford RS. Physiologically responsive gene therapy. Mol Med Today. 1998;4(10):445–51.
36. Wheatley S, Wright E, Jeske Y, MCCormack A, Bowles J, Koopman P. Aetiology of the skeletal dysmorphology syndrome campomelic dysplasia: expression of the Sox9 gene during chondrogenesis in mouse embryos. Ann N Y Acad Sci. 1996;785:350–2.
37. Lefebvre V, Behringer RR, de Crombrugghe B. L-Sox5, Sox6 and Sox9 control essential steps of the chondrocyte differentiation pathway. Osteoarthritis Cartilage. 2001;9(Suppl A):S69–75.
38. Leung VY, Gao B, Leung KK, Melhado IG, Wynn SL, Au TY, et al. SOX9 governs differentiation stage-specific gene expression in growth plate chondrocytes via direct concomitant transactivation and repression. PLoS Genet. 2011;7(11):e1002356.
39. Li Y, Tew SR, Russell AM, Gonzalez KR, Hardingham TE, Hawkins RE. Transduction of passaged human articular chondrocytes with adenoviral, retroviral, and lentiviral vectors and the effects of enhanced expression of SOX9. Tissue Eng. 2004;10(3–4):575–84.
40. Cucchiarini M, Thurn T, Weimer A, Kohn D, Terwilliger EF, Madry H. Restoration of the extracellular matrix in human osteoarthritic articular cartilage by overexpression of the transcription factor SOX9. Arthritis Rheum. 2007;56(1):158–67.
41. Tew SR, Li Y, Pothacharoen P, Tweats LM, Hawkins RE, Hardingham TE. Retroviral transduction with SOX9 enhances re-expression of the chondrocyte phenotype in passaged osteoarthritic human articular chondrocytes. Osteoarthritis Cartilage. 2005;13(1):80–9.

42. Yang HN, Park JS, Woo DG, Jeon SY, Do HJ, Lim HY, et al. Chondrogenesis of mesenchymal stem cells and dedifferentiated chondrocytes by transfection with SOX Trio genes. Biomaterials. 2011;32(30):7695–704.
43. Im GI, Kim HJ, Lee JH. Chondrogenesis of adipose stem cells in a porous PLGA scaffold impregnated with plasmid DNA containing SOX trio (SOX-5,-6 and -9) genes. Biomaterials. 2011;32(19):4385–92.
44. Ikeda T, Kamekura S, Mabuchi A, Kou I, Seki S, Takato T, et al. The combination of SOX5, SOX6, and SOX9 (the SOX trio) provides signals sufficient for induction of permanent cartilage. Arthritis Rheum. 2004;50(11):3561–73.
45. Ivkovic A, Marijanovic I, Hudetz D, Porter RM, Pecina M, Evans CH. Regenerative medicine and tissue engineering in orthopaedic surgery. Front Biosci (Elite Ed). 2011;3:923–44.
46. Huang YL, Qiu RF, Mai WY, Kuang J, Cai XY, Dong YG, et al. Effects of insulin-like growth factor-1 on the properties of mesenchymal stem cells in vitro. J Zhejiang Univ Sci B. 2012;13(1):20–8.
47. Madry H, Zurakowski D, Trippel SB. Overexpression of human insulin-like growth factor-I promotes new tissue formation in an ex vivo model of articular chondrocyte transplantation. Gene Ther. 2001;8(19):1443–9.
48. Mi Z, Ghivizzani SC, Lechman ER, Jaffurs D, Glorioso JC, Evans CH, et al. Adenovirus-mediated gene transfer of insulin-like growth factor 1 stimulates proteoglycan synthesis in rabbit joints. Arthritis Rheum. 2000;43(11):2563–70.
49. Weimer A, Madry H, Venkatesan JK, Schmitt G, Frisch J, Wezel A, et al. Benefits of rAAV-mediated IGF-I overexpression for the long-term reconstruction of human osteoarthritic cartilage by modulation of the IGF-I axis. Mol Med. 2012;18:346–58.
50. Hidaka C, Quitoriano M, Warren RF, Crystal RG. Enhanced matrix synthesis and in vitro formation of cartilage-like tissue by genetically modified chondrocytes expressing BMP-7. J Orthop Res. 2001;19(5):751–8.
51. Hidaka C, Goodrich LR, Chen CT, Warren RF, Crystal RG, Nixon AJ. Acceleration of cartilage repair by genetically modified chondrocytes over expressing bone morphogenetic protein-7. J Orthop Res. 2003;21(4):573–83.
52. Kuroda R, Usas A, Kubo S, Corsi K, Peng H, Rose T, et al. Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. Arthritis Rheum. 2006;54(2):433–42.
53. Steinert AF, Proffen B, Kunz M, Hendrich C, Ghivizzani SC, Noth U, et al. Hypertrophy is induced during the in vitro chondrogenic differentiation of human mesenchymal stem cells by bone morphogenetic protein-2 and bone morphogenetic protein-4 gene transfer. Arthritis Res Ther. 2009;11(5):R148.
54. Shuler FD, Georgescu HI, Niyibizi C, Studer RK, Mi Z, Johnstone B, et al. Increased matrix synthesis following adenoviral transfer of a transforming growth factor beta1 gene into articular chondrocytes. J Orthop Res. 2000;18(4):585–92.
55. Moller HD, Fu FH, Niyibizi C, Studer RK, Georgescu HJ, Robbins PD, et al. TGF-beta-1 gene transfer in joint cartilage cells. Stimulating effect in extracellular matrix synthesis. Orthopade. 2000;29(2):75–9.
56. Ivkovic A, Pascher A, Hudetz D, Maticic D, Jelic M, Dickinson S, et al. Articular cartilage repair by genetically modified bone marrow aspirate in sheep. Gene Ther. 2010;17(6): 779–89.
57. Wang W, Li B, Li Y, Jiang Y, Ouyang H, Gao C. In vivo restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with fibrin gel, bone marrow mesenchymal stem cells and DNA complexes. Biomaterials. 2010;31(23):5953–65.
58. Ha CW, Noh MJ, Choi KB, Lee KH. Initial phase I safety of retrovirally transduced human chondrocytes expressing transforming growth factor-beta-1 in degenerative arthritis patients. Cytotherapy. 2012;14(2):247–56.
59. Mi Z, Ghivizzani SC, Lechman E, Glorioso JC, Evans CH, Robbins PD. Adverse effects of adenovirus-mediated gene transfer of human transforming growth factor beta 1 into rabbit knees. Arthritis Res Ther. 2003;5(3):R132–9.

60. Watson RS, Gouze E, Levings PP, Bush ML, Kay JD, Jorgensen MS, et al. Gene delivery of TGF-beta1 induces arthrofibrosis and chondrometaplasia of synovium in vivo. Lab Invest. 2010;90(11):1615–27.
61. van Beuningen HM, van der Kraan PM, Arntz OJ, van den Berg WB. Transforming growth factor-beta 1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. Lab Invest. 1994;71(2):279–90.
62. Vincent TL. Fibroblast growth factor 2: good or bad guy in the joint? Arthritis Res Ther. 2011;13(5):127.
63. Orth P, Kaul G, Cucchiarini M, Zurakowski D, Menger MD, Kohn D, et al. Transplanted articular chondrocytes co-overexpressing IGF-I and FGF-2 stimulate cartilage repair in vivo. Knee Surg Sports Traumatol Arthrosc. 2011;19(12):2119–30.
64. Chang SC, Hoang B, Thomas JT, Vukicevic S, Luyten FP, Ryba NJ, et al. Cartilage-derived morphogenetic proteins. New members of the transforming growth factor-beta superfamily predominantly expressed in long bones during human embryonic development. J Biol Chem. 1994;269(45):28227–34.
65. Luyten FP. Cartilage-derived morphogenetic protein-1. Int J Biochem Cell Biol. 1997;29(11): 1241–4.
66. Storm EE, Kingsley DM. GDF5 coordinates bone and joint formation during digit development. Dev Biol. 1999;209(1):11–27.
67. Hotten GC, Matsumoto T, Kimura M, Bechtold RF, Kron R, Ohara T, et al. Recombinant human growth/differentiation factor 5 stimulates mesenchyme aggregation and chondrogenesis responsible for the skeletal development of limbs. Growth Factors. 1996;13(1–2): 65–74.
68. Tsumaki N, Tanaka K, Arikawa-Hirasawa E, Nakase T, Kimura T, Thomas JT, et al. Role of CDMP-1 in skeletal morphogenesis: promotion of mesenchymal cell recruitment and chondrocyte differentiation. J Cell Biol. 1999;144(1):161–73.
69. Bai X, Xiao Z, Pan Y, Hu J, Pohl J, Wen J, et al. Cartilage-derived morphogenetic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes. Biochem Biophys Res Commun. 2004;325(2):453–60.
70. Feng G, Wan Y, Balian G, Laurencin CT, Li X. Adenovirus-mediated expression of growth and differentiation factor-5 promotes chondrogenesis of adipose stem cells. Growth Factors. 2008;26(3):132–42.
71. Rickert M, Wang H, Wieloch P, Lorenz H, Steck E, Sabo D, et al. Adenovirus-mediated gene transfer of growth and differentiation factor-5 into tenocytes and the healing rat Achilles tendon. Connect Tissue Res. 2005;46(4–5):175–83.
72. Liang H, Ma SY, Feng G, Shen FH, Joshua LX. Therapeutic effects of adenovirus-mediated growth and differentiation factor-5 in a mice disc degeneration model induced by annulus needle puncture. Spine J. 2010;10(1):32–41.
73. Evans CH, Robbins PD, Ghivizzani SC, Herndon JH, Kang R, Bahnson AB, et al. Clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis. Hum Gene Ther. 1996;7(10):1261–80.
74. Evans CH, Robbins PD, Ghivizzani SC, Wasko MC, Tomaino MM, Kang R, et al. Gene transfer to human joints: progress toward a gene therapy of arthritis. Proc Natl Acad Sci U S A. 2005;102(24):8698–703.
75. Fernandes J, Tardif G, Martel-Pelletier J, Lascau-Coman V, Dupuis M, Moldovan F, et al. In vivo transfer of interleukin-1 receptor antagonist gene in osteoarthritic rabbit knee joints: prevention of osteoarthritis progression. Am J Pathol. 1999;154(4):1159–69.
76. Roessler BJ, Hartman JW, Vallance DK, Latta JM, Janich SL, Davidson BL. Inhibition of interleukin-1-induced effects in synoviocytes transduced with the human IL-1 receptor antagonist cDNA using an adenoviral vector. Hum Gene Ther. 1995;6(3):307–16.
77. Zhang HG, Xie J, Yang P, Wang Y, Xu L, Liu D, et al. Adeno-associated virus production of soluble tumor necrosis factor receptor neutralizes tumor necrosis factor alpha and reduces arthritis. Hum Gene Ther. 2000;11(17):2431–42.

78. Gould D, Yousaf N, Fatah R, Subang MC, Chernajovsky Y. Gene therapy with an improved doxycycline-regulated plasmid encoding a tumour necrosis factor-alpha inhibitor in experimental arthritis. Arthritis Res Ther. 2007;9(1):R7.
79. Adriaansen J, Khoury M, de Cortie CJ, Fallaux FJ, Bigey P, Scherman D, et al. Reduction of arthritis following intra-articular administration of an adeno-associated virus serotype 5 expressing a disease-inducible TNF-blocking agent. Ann Rheum Dis. 2007;66(9):1143–50.
80. Kafienah W, Al-Fayez F, Hollander AP, Barker MD. Inhibition of cartilage degradation: a combined tissue engineering and gene therapy approach. Arthritis Rheum. 2003;48(3): 709–18.
81. Bondeson J, Lauder S, Wainwright S, Amos N, Evans A, Hughes C, et al. Adenoviral gene transfer of the endogenous inhibitor IkappaBalpha into human osteoarthritis synovial fibroblasts demonstrates that several matrix metalloproteinases and aggrecanases are nuclear factor-kappaB-dependent. J Rheumatol. 2007;34(3):523–33.
82. Surendran S, Kim SH, Jee BK, Ahn SH, Gopinathan P, Han CW. Anti-apoptotic Bcl-2 gene transfection of human articular chondrocytes protects against nitric oxide-induced apoptosis. J Bone Joint Surg Br. 2006;88(12):1660–5.
83. Grossin L, Cournil-Henrionnet C, Pinzano A, Gaborit N, Dumas D, Etienne S, et al. Gene transfer with HSP 70 in rat chondrocytes confers cytoprotection in vitro and during experimental osteoarthritis. FASEB J. 2006;20(1):65–75.
84. Piera-Velazquez S, Jimenez SA, Stokes D. Increased life span of human osteoarthritic chondrocytes by exogenous expression of telomerase. Arthritis Rheum. 2002;46(3):683–93.
85. Sato M, Shin-Ya K, Lee JI, Ishihara M, Nagai T, Kaneshiro N, et al. Human telomerase reverse transcriptase and glucose-regulated protein 78 increase the life span of articular chondrocytes and their repair potential. BMC Musculoskelet Disord. 2012;13(1):51.
86. Nonomura Y, Kohsaka H, Nagasaka K, Miyasaka N. Gene transfer of a cell cycle modulator exerts anti-inflammatory effects in the treatment of arthritis. J Immunol. 2003;171(9): 4913–9.
87. Shi S, Mercer S, Eckert GJ, Trippel SB. Regulation of articular chondrocyte aggrecan and collagen gene expression by multiple growth factor gene transfer. J Orthop Res. 2012;30: 1026–31.
88. Chen B, Qin J, Wang H, Magdalou J, Chen L. Effects of adenovirus-mediated bFGF, IL-1Ra and IGF-1 gene transfer on human osteoarthritic chondrocytes and osteoarthritis in rabbits. Exp Mol Med. 2010;42(10):684–95.
89. Madry H, Orth P, Kaul G, Zurakowski D, Menger MD, Kohn D, et al. Acceleration of articular cartilage repair by combined gene transfer of human insulin-like growth factor I and fibroblast growth factor-2 in vivo. Arch Orthop Trauma Surg. 2010;130(10):1311–22.
90. Ambros V. The functions of animal microRNAs. Nature. 2004;431(7006):350–5.
91. Dong S, Yang B, Guo H, Kang F. MicroRNAs regulate osteogenesis and chondrogenesis. Biochem Biophys Res Commun. 2012;418(4):587–91.
92. Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum. 2009;60(9):2723–30.
93. Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, et al. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev. 2010;24(11):1173–85.
94. Araldi E, Schipani E. MicroRNA-140 and the silencing of osteoarthritis. Genes Dev. 2010;24(11):1075–80.
95. Tardif G, Hum D, Pelletier JP, Duval N, Martel-Pelletier J. Regulation of the IGFBP-5 and MMP-13 genes by the microRNAs miR-140 and miR-27a in human osteoarthritic chondrocytes. BMC Musculoskelet Disord. 2009;10:148.
96. Yang B, Guo H, Zhang Y, Dong S, Ying D. The microRNA expression profiles of mouse mesenchymal stem cell during chondrogenic differentiation. BMB Rep. 2011;44(1):28–33.
97. Yang B, Guo H, Zhang Y, Chen L, Ying D, Dong S. MicroRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. PLoS One. 2011;6(7):e21679.

98. Martinez-Sanchez A, Dudek KA, Murphy CL. Regulation of human chondrocyte function through direct inhibition of cartilage master regulator SOX9 by microRNA-145 (miRNA-145). J Biol Chem. 2012;287(2):916–24.
99. Yamasaki K, Nakasa T, Miyaki S, Ishikawa M, Deie M, Adachi N, et al. Expression of microRNA-146a in osteoarthritis cartilage. Arthritis Rheum. 2009;60(4):1035–41.
100. Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M. The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis. Arthritis Rheum. 2011;63(6):1582–90.
101. Jones SW, Watkins G, Le Good N, Roberts S, Murphy CL, Brockbank SM, et al. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. Osteoarthritis Cartilage. 2009;17(4):464–72.
102. Abouheif MM, Nakasa T, Shibuya H, Niimoto T, Kongcharoensombat W, Ochi M. Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model in vitro. Rheumatology (Oxford). 2010;49(11):2054–60.
103. Wehling P, Reinecke J, Baltzer AW, Granrath M, Schulitz KP, Schultz C, et al. Clinical responses to gene therapy in joints of two subjects with rheumatoid arthritis. Hum Gene Ther. 2009;20(2):97–101.
104. Kafienah W, Jakob M, Demarteau O, Frazer A, Barker MD, Martin I, et al. Three-dimensional tissue engineering of hyaline cartilage: comparison of adult nasal and articular chondrocytes. Tissue Eng. 2002;8(5):817–26.
105. Cucchiarini M, Madry H, Ma C, Thurn T, Zurakowski D, Menger MD, et al. Improved tissue repair in articular cartilage defects in vivo by rAAV-mediated overexpression of human fibroblast growth factor 2. Mol Ther. 2005;12(2):229–38.
106. Seo S, Na K. Mesenchymal stem cell-based tissue engineering for chondrogenesis. J Biomed Biotechnol. 2011;2011:806891.
107. Park J, Gelse K, Frank S, von der Mark K, Aigner T, Schneider H. Transgene-activated mesenchymal cells for articular cartilage repair: a comparison of primary bone marrow-, perichondrium/periosteum- and fat-derived cells. J Gene Med. 2006;8(1):112–25.
108. Gelse K, von der Mark K, Aigner T, Park J, Schneider H. Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. Arthritis Rheum. 2003;48(2):430–41.
109. Pascher A, Palmer GD, Steinert A, Oligino T, Gouze E, Gouze JN, et al. Gene delivery to cartilage defects using coagulated bone marrow aspirate. Gene Ther. 2004;11(2):133–41.
110. Pelttari K, Steck E, Richter W. The use of mesenchymal stem cells for chondrogenesis. Injury. 2008;39 Suppl 1:S58–65.
111. Wei Y, Zeng W, Wan R, Wang J, Zhou Q, Qiu S, et al. Chondrogenic differentiation of induced pluripotent stem cells from osteoarthritic chondrocytes in alginate matrix. Eur Cell Mater. 2012;23:1–12.
112. Evans CH, Ghivizzani SC, Robbins PD. Orthopedic gene therapy–lost in translation? J Cell Physiol. 2012;227(2):416–20.
113. Evans CH, Ghivizzani SC, Robbins PD. Arthritis gene therapy's first death. Arthritis Res Ther. 2008;10(3):110.
114. Bakker AC, Joosten LA, Arntz OJ, Helsen MM, Bendele AM, van de Loo FA, et al. Prevention of murine collagen-induced arthritis in the knee and ipsilateral paw by local expression of human interleukin-1 receptor antagonist protein in the knee. Arthritis Rheum. 1997;40(5):893–900.
115. Gelse K, Jiang QJ, Aigner T, Ritter T, Wagner K, Poschl E, et al. Fibroblast-mediated delivery of growth factor complementary DNA into mouse joints induces chondrogenesis but avoids the disadvantages of direct viral gene transfer. Arthritis Rheum. 2001;44(8):1943–53.
116. Kon E, Delcogliano M, Filardo G, Busacca M, Di Martino A, Marcacci M. Novel nanocomposite multilayered biomaterial for osteochondral regeneration: a pilot clinical trial. Am J Sports Med. 2011;39(6):1180–90.
117. Ebert JR, Fallon M, Ackland TR, Wood DJ, Janes GC. Arthroscopic matrix-induced autologous chondrocyte implantation: 2-year outcomes. Arthroscopy. 2012;28:952–64.

Chapter 3
Targeting Inflammatory Processes for Optimization of Cartilage Homeostasis and Repair Techniques

Marjolein M.J. Caron, Tim J.M. Welting, Lodewijk W. van Rhijn, and Pieter J. Emans

Abstract The outcome of cartilage repair techniques is often hampered by unwanted ossification (e.g. intralesional osteophytes) at the site of the repaired cartilage. Furthermore, stimulating progenitor cells towards chondrocytes and locking them in their desired state is another important hinge point in cartilage repair techniques. Studying the cartilage formation process by endochondral ossification may provide important clues which further enhance cartilage repair techniques in general and may provide crucial information to prevent unwanted ossification in particular. During endochondral ossification mesenchymal progenitors differentiate into proliferative chondrocytes which gradually further differentiate into hypertrophic chondrocytes and finally die by apoptosis; the remaining scaffold is mineralised towards bone. This process takes place in growth plates, during fracture healing and in part during development of articular cartilage, where the endochondral ossification halts at the chondrogenic phase. While inflammation is generally regarded as a negative factor for joint homeostasis and cartilage development, it is also known that inflammation is the first and essential phase of tissue repair in general and bone fracture healing via endochondral ossifcation indeed also depends on haematoma formation and subsequent inflammatory microenvironment. Recently, a growing body of experimental evidence has been published, showing that inflammatory molecules (e.g. NF-κB, COX-2, iNOS, TNFα,

M.M.J. Caron • T.J.M. Welting • L.W. van Rhijn
Department of Orthopaedic Surgery, CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, Maastricht, The Netherlands

P.J. Emans, PhD, MD (✉)
Department of Orthopaedic Surgery, CAPHRI School for Public Health and Primary Care, Maastricht University Medical Center, 5800, Maastricht NL-6202 AZ, The Netherlands
e-mail: pj.emans@maastrichtuniversity.nl

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_3, © Springer-Verlag London 2014

interleukins) and their down-stream pathways are not only associated with cartilage degeneration, but are also crucially involved in the initiation of the chondrogenic differentiation process and regulation of cartilage hypertrophy and mineralization. The data described in these reports suggest that one could use these inflammatory pathways for cartilage regenerative medicine, as the initiation of chondrogenic differentiation is a crucial moment for progenitor cell-based cartilage repair techniques. Furthermore, targeting inflammatory mediators may also provide a potential pharmacological approach to prevent or decrease chondrocyte hypertrophic differentiation and subsequent bone formation (e.g. intralesional osteophytes) in cartilage repair techniques.

This chapter describes important characteristics of hyaline articular cartilage, drawbacks of current cartilage repair techniques, the process of endochondral ossification and how inflammation related molecules are involved in different phases of endochondral ossification. In addition, this chapter discusses how better insight into these pathways may provide novel molecular tools to modulate chondrogenesis in cartilage regenerative medicine.

Keywords Cartilage repair • Intralesional osteophyte • Inflammation • Chondrogenesis • Progenitor cells • NF-κB • COX-2

Key Points

- The outcome of cartilage repair techniques is often hampered by unwanted ossification (e.g. intralesional osteophytes) at the site of the repaired cartilage.
- Studying the cartilage formation process by endochondral ossification may provide important clues which further enhance cartilage repair techniques in general and may provide crucial information to prevent unwanted ossification in particular.
- While inflammation is generally seen as a negative factor for joint homeostasis and cartilage development, it is also known that inflammation is the first and essential phase of tissue repair in general.
- One may implement these inflammatory pathways for cartilage regenerative medicine, as the initiation of chondrogenic differentiation is a crucial moment for progenitor cell-based cartilage repair techniques.

3.1 Introduction: Cartilage

Motion in articular joints is possible by a truly remarkable material both structurally and functionally, named hyaline articular cartilage [1–4]. This articular cartilage is able to withstand an enormous amount of intensive and repetitive forces combined with low friction and thereby allows easy movement. The extracellular matrix (ECM) of cartilage determines these cartilage-specific functions and is mainly

composed of water (65–80 %), collagens (12–21 %), proteoglycans(6–10 %) and other glycoproteins (2–3, 5 %) [5]. Only 1–5% of the articular cartilage volume consists of chondrocytes, the main cell type found in articular cartilage [6]. Furthermore, cartilage is characterized by the absence of blood vessels, lymphatics and nerve fibers. This implicates that cartilage is mainly hypoxic and chondrocytes have to receive their nutrients and oxygen via diffusion from the synovial fluid, through the surrounding extracellular matrix and from the underlying subchondral bone [7]. Cartilage defects can arise due to trauma or cartilage degeneration, but are generally difficult to diagnose [8, 9]. Since cartilage has no nerve fibres, cartilage lesions often present with only (minor) effusion of the affected joint or without symptoms at all. Symptoms as joint pain, locking phenomena and reduced or disturbed joint-function may arise from other tissues or structures likely to be damaged upon trauma (e.g. subchondral bone, ligaments or menisci). Although progenitor cells are found in the superficial layer of articular cartilage [10, 11], cartilage has a limited ability for self-repair [12, 13]. This was already recognized in 1743 when the British surgeon William Hunter made the now famous statement: "*From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and that once destroyed it is not repaired*" [14]. This observation is one of the main reasons for clinicians and researchers to explore ways for cartilage repair. Because, when left untreated, the joint surface will deteriorate even further, ultimately leading to osteoarthritis (OA).

3.2 Calcification in Cartilage Repair Techniques

Cartilage restoration implies methods to heal or regenerate the joint surface, with or without the subchondral bone, into healthy hyaline articular cartilage to restore joint functioning. To date there are multiple fruitful cartilage repair techniques; however, the ultimate cartilage repair technique has not been found yet. One of the main drawbacks is unwanted ossification (and formation of intralesional osteophytes) at the site of the repaired cartilage [15, 16].

As described above, the properties of the (hyaline) cartilage matrix are essential to withstand the repetitive compressive forces which are put on the joints, allowing easy movement. Hypertrophic cartilage or even mineralized cartilage in the articular surface has inferior properties concerning resisting repetitive mechanical loading to that of hyaline cartilage and will thereby result in the further destruction of the joint cartilage and can act as a source of pain [17]. Chondrocyte hypertrophic differentiation is thus of concern in cartilage repair techniques but also in the onset of osteoarthritis, as e.g. markers for hypertrophic differentiation are specifically expressed at early stages of OA [18–20]. In addition to formation of hypertrophic cartilage, stimulating progenitor cells towards extracellular matrix-producing chondrocytes and keeping them in their desired differentiation state is another important factor to consider in cartilage repair techniques [15, 16].

Bone marrow stimulating techniques such as microfracture, abrasion and subchondral drilling are easy applicable, cheap and reliable methods to attempt

the functional repair of cartilage defects. These techniques are based on the penetration of the subchondral bone allowing ingress of bone marrow stem cells into the site of the damaged cartilage [21–31]. These cells are thought to differentiate into the chondrogenic lineage and become functional ECM-producing chondrocytes which replace the damaged cartilage. However, formation of fibrocartilage and calcification of repaired tissue hampers clinical outcome on the long term [16, 30]. Another source of chondro-progenitor cells can be found in the cambium layer of the periosteum and in the perichondrium. These cells have been described to have a chondrogenic potential as well [12, 32–41]. Covering cartilage defects with periosteum-derived grafts (periosteal arthroplasty) is therefore an explored strategy to treat cartilage defects [42–50]. On short term, results were found to be quite promising in giving initial cartilage repair [43–46, 48]. Unfortunately, on the long term results were poor and failure was related to overgrowth and calcification of the graft [42].

Other techniques imply the transplantation of adult chondrocytes or cartilage such as mosaicplasty (Osteochondral Autograft Transfer System; OATS), allografts and Autologous Chondrocyte Transplantation (ACT), which may overcome these drawbacks. Mosaicplasty or OATS involves harvesting osteochondral plugs from a relatively less weight-bearing region of the joint and subsequent implantation of these plugs into the articular defect [51–54]. The use of allografts can overcome possible donor site morbidity [52, 53, 55–61] or shortage of graft material. ACT refers to a cell- based cartilage repair procedure, where cartilage is harvested arthroscopically from a less weight-bearing region of the joint and transferred to a specialized laboratory where the chondrocytes are enzymatically released from their matrix and expanded *in vitro*. The patient then undergoes a second operation where the *in vitro* expanded chondrocytes are re-implanted at the damaged site of the articular cartilage, in combination with a covering membrane (periosteum of biomembrane) [62–64] or pre-seeded in a matrix (Matrix Assisted Chondrocyte Transplantation; MACT) [65]. Nevertheless, the use of these techniques is restricted due to a limited availability of autologous cartilage (mosaicplasty) or donors, possible disease transfer (allografts), or expensive and time consuming logistics and culture methods (ACT). Furthermore, cartilage hypertrophy is also seen after ACT, albeit more in the periosteum-covered ACT than in de matrix-assisted ACT [66, 67].

The use of progenitor cells for cartilage repair remains of interest. When applied for cartilage repair, stem cells have a natural tendency to differentiate into the chondrogenic lineage, via a process called endochondral ossification, forming cartilaginous tissue in the damaged area which gives initial cartilage repair. However, on the long term, progenitor-based grafts tend to calcify as a natural result of the endochondral ossification process. Microfracture and periosteum or perichondrium plasty [42, 68], are good examples here of, all showing adverse ossification and/or formation of interlesional osteophytes. Recently these osteophytes have also been described when articular cartilage was transplanted into a defect [69].

Beside appropriate induction of differentiation, maintaining these progenitor cells in the desired differentiation state and preventing them from further

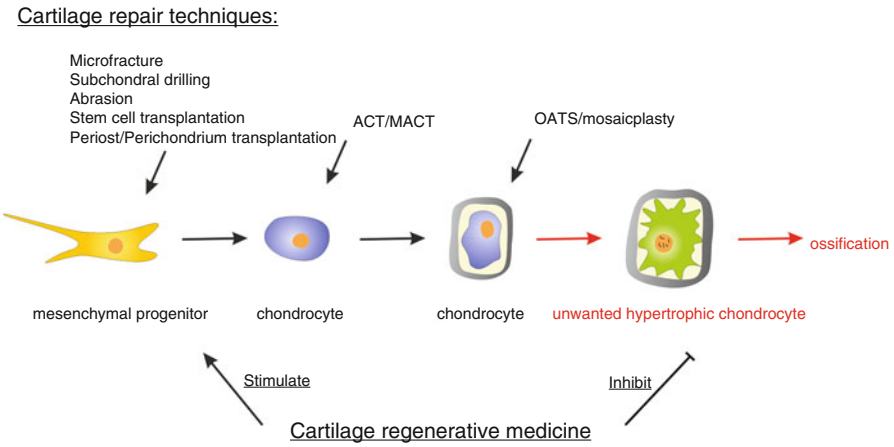

Fig. 3.1 Different phases of chondrogenic differentiation and targets of cartilage regenerative techniques

hypertrophic differentiation is therefore a major challenge for stemcell-based cartilage repair strategies [15]. Studying the process of endochondral ossification and further unraveling how and why articular chondrocytes maintain their phenotype and are saved from hypertrophy may enhance cartilage repair techniques by generating stable cartilage. A suggestion in which stage during the endochondral process the different cartilage repair techniques are positioned is given in Fig. 3.1.

3.3 Chondrogenic Phase of Endochondral Ossification

Chondrogenic differentiation encompasses the commitment and differentiation of chondro-progenitor cells towards chondrocytes (see Fig. 3.1). *In vivo*, chondrogenic differentiation is almost exclusively initiated from local mesenchymal progenitor cells that reside in cartilaginous tissue (growth plate resting zone or the articular cartilage superficial layer [70, 71]) or in surrounding fibrous tissues (e.g. periosteum [37, 72]). *Ex vivo* (*in vitro*), however, chrondrogenic differentiation has been reported from various primary (mesenchymal) progenitor cell sources including synovial fluid/membrane, adipose tissue, induced pluripotent stem cells (iPS [73]), bone marrow and many more [74].

In addition to providing articulating joint surfaces with functional cartilage and maintaining cartilage integrity, chondrogenic differentiation also plays an essential role during endochondral ossification (Fig. 3.1). Endochondral ossification underlies skeletogenesis and bone fracture healing and is a developmental process during which cartilaginous primordia are gradually replaced by bone tissue. Growth plate chondrocytes originating from the resting zone or fracture callus chondrocytes originating from mesenchymal progenitors gradually proliferate, produce a

Differentiation step	Extracellular martrix markers	Regulatory markers	Growth and differentiation factors
Chondrogenic progenitor cells (mesenchymal cells)	Col1a1	Sox9, Runx2	Shh, TGF-β
Prechondrocytes	Ncam1, Tnc	Sox9, L-Sox5, Sox6	TGF-β FGF-2, BMP-2,4,7 Wnt, PTHrP
Early chondrocytes	Col2a1, Acan, Crtl1	Sox9, L-Sox5, Sox6 Nkx3.2, Atf2, Creb, Fgfr3	TGF-β FGF-2, BMP-2,4,7 Wnt, PTHrP
Chondrocytes (columnar)	Col2a1,Col9a1, Col11a1, Acan, Crtl1, Comp, Matnl GAGs	Sox9, L-Sox5, Sox6 Nkx3.2, Atf2, Creb, Fgfr3	TGF-β FGF-2, BMP-2,4,7, IGF-1 Wnt, PTHrP
Prehypertrophic chondrocytes	Col2a1,Col9a1, Col11a1, Col10a1 Acan, Crtl1, Comp, Matnl GAGs	Runx2, Runx3, Ihh, Pthr1	Wnt/β-catenin, BMP-2,7, TGF-β
Hypertrophic chondrocytes	Co110a1	Runx2, Runx3, Mef2c	VEGF, Wnt/β-catenin, BMP-2,7
Terminal chondrocytes	MMP13, Alp, Opn	Runx2, c-Maf	VEGF, Wnt/β-catenin

Fig. 3.2 Markers for chondrogenic differentiation. Schematic representation of successive steps of chondrogenic differentiation during endochondral ossification with schematic representation of the cells, major extracellular matrix markers, regulatory markers and growth and differentiation factors expressed at each step

cartilaginous matrix and further differentiate into mineralized hypertrophic chondrocytes which finally die by apoptosis. The remaining mineralized extracellular matrix provides a molecular scaffold for infiltrating osteoblasts and osteoclasts to adhere to and remodel, setting the stage for *de novo* bone deposition [75, 76].

Notably, chondrocytes in articular cartilage retain their chondrocyte phenotype and, except for chondrocytes near the tidemark, normally do not further differentiate into hypertrophic chondrocytes, probably due to the local microenvironment. Unfortunately, as a natural result of this endochondral ossification process, *in vitro* chondrogenic differentiation of progenitor cells for cartilage regenerative purposes tends to progress into hypertrophic differentiating chondrocytes.

3.3.1 Molecular Factors in Chondrogenic Differentiation

Different phases of chondrogenic differentiation can be characterized by different functional marker molecules (Fig. 3.2). Chondrogenic differentiation starts when mesenchymal progenitor cells are triggered to differentiate into the chondrogenic lineage. Chondrogenic progenitor cells express typical ECM and cell adhesion molecules like tenascin c (Tnc), syndecan 3 (Sdc3), N-cadherin (Ncad) and Ncam1 (neural cell adhesion molecule 1). One of the first key important chondrogenic differentiation regulatory events is activation of the Sox-trio transcription factors; Sox9 (SRY-(sex determining region Y)-box9) in combination with L-Sox5 and Sox6 are responsible for commitment and differentiation in the chondrogenic lineage [77–79]. Together they drive the transcription of the important ECM genes collagen type II (Col2a1) and the main proteoglycan aggrecan (Acan) [78, 80–83]. Other ECM genes have also been shown to be under transcriptional control of Sox9,

of which collagen type IX (Col9a1), collagen type XXVII (Col27a1) and matrilin 1 (Matn1) are important ones [84–87].

Eventually the (hyaline articular) cartilage ECM consists of a collagen network which is comprised of primarily Col2a1, and additionally of Col9a1 and collagen type XI (Col11a1) which help to form and stabilize the collagen type II fibril network [88–91]. Minor quantities of Col6a1, Col12a1, Col14a1 and Col27a1 are also found in cartilage [92]. This collagen network is surrounded by a highly hydrated aggregation of proteoglycans and other glycoproteins. Glycoproteins and proteoglycans as COMP (cartilage oligomeric protein), Matrilin1 (Matn1/Crtm), perlecan (Hspg2), versican (Vcan), decorin (Dcn), biglycan (Bgn) and fibromodulin (Fmod) are characterized by their ability to interact with and support the collagen fibril network and retention and transport of growth factors [79, 93]. Aggrecan (Acan) is the main proteoglycan and forms macromolecular complexes by binding to hyaluronan via link proteins and binding of glycosaminoglycans (GAGs), such as chondroitin sulfate and keratan sulfate. The glycosaminoglycan side chains of the proteoglycans are composed of repeating disaccharide units carrying negatively charged sulphate and carboxyl groups. The resulting fixed negative charge density attracts mobile cations and water into the ECM and thus provides in the elastic properties of the tissue [94, 95]. In addition to resisting compressive forces and providing lubrication during movement, the high water retention capacity of hyaline cartilage also supports in distributing nutrients to chondrocytes. The proteoglycan aggregations, together with the quality of the collagen network determine the strength and flexibility of the cartilage tissue and ability to withstand repetitive compressive forces for which articular cartilage has been designed to [2, 4, 96, 97]. For articular chondrocytes, the differentiation process stops here and cells provide maintenance of the articular surface for life. It is important to realize that in articular cartilage the ratio of cells to ECM, and composition of the ECM are important for proper joint functioning. These are therefore factors to take into account for cartilage regenerative techniques. Based on collagen type II orientation and chondrocyte shape and distribution, four zones can be distinguished in articular cartilage [1, 3, 4]. In the superficial zone, chondrocytes are flattened and are surrounded by a thin layer of ECM, mainly composed of collagen-fibres. The fibres are oriented parallel to the articular surface and are supported by a relatively low content of proteoglycans, which results in high tensile stiffness and the ability to distribute load over the surface and protecting the deeper layers. In the transitional zone the cells and collagen fibres appear dispersed randomly [98, 99] and in this zone high concentrations of proteoglycans enable the tissue to bear compressive forces. In the deep zone, chondrocytes are grouped radially in columns and the thicker collagen fibres are arranged perpendicular to the articular surface, providing the greatest resistance to compressive forces In the calcified zone, (hypertrophic) chondrocytes are distributed sparsely and are surrounded by a calcified matrix. The calcified layer plays an integral role in securing the cartilage layer to the subchondral bone by anchoring the collagen fibrils to the subchondral bone tissue. The junction between uncalcified and calcified cartilage is called the “tidemark”. At the tidemark shear stresses are converted into compressive forces which are in turn transmitted to the subchondral bone [100].

Thus, for optimizing progenitor cell-based cartilage repair techniques it is thus of importance to not only create cells which produce enough ECM, but also that this ECM has the right composition.

3.3.2 Molecular Factors in Chondrocyte Hypertrophy

In contrast to articular chondrocytes, the (proliferative) chondrocytes in growth plates, or involved in fracture healing, further differentiate into hypertrophic chondrocytes which subsequently undergo a remodeling of their extracellular matrix (Fig. 3.2). These chondrocytes then exit the cell cycle and increase in cell volume up to ten times [101]. There is an increase in expression of Runx2 (Runt-related transcription factor 2) and Mef2c (Myocyte-specific enhancer factor 2C), which are important transcription factors for collagen type X (Col10a1), the main collagen found in hypertrophic chondrocytes [102–105]. Furthermore, under stimulation of Runx2 and Mef2c, hypertrophic chondrocytes also express vascular endothelial growth factor (VEGF) to stimulate vascular ingrowth [79, 106]. Also several MMPs (matrix metalloproteins) and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) for breakdown of the ECM are syhthesized [79, 107]. At the final stage of hypertrophic differentiation several mineralization proteins are expressed, such as Alp (alkaline phosphatase) and osteopontin (also known as bone sialoprotein I), which mineralize the extracellular matrix [79, 106–108]. Finally, the hypertrophic chondrocytes die by apoptosis, leaving their mineralized extracellular matrix behind for osteoblasts to adhere, which will eventually remodel the matrix into bone tissue.

3.3.3 Growth Factors and Paracrine Regulators in Chondrogenic Differentiation

In growth plate development as well as in the development and homeostasis of articular cartilage several signaling pathways are interacting or shared between the different tissues. Indian hedgehog (Ihh) and parathyroid hormone related peptide (PTHrP) coordinate chondrocyte proliferation and differentiation in the paracrine PTHrP-Ihh feedback loop [76]. PTHrP is synthesized by proliferating chondrocytes and perichondrial cells [76] and maintains chondrocyte proliferation by activating Cyclin D1 [109] and prevents premature hypertrophy by inducing Cyclin D1-mediated degradation of Runx2 [110]. Proliferating chondrocytes located at a sufficient distance from the PTHrP source stop proliferating and become hypertrophic, Ihh synthesizing cells [111]. Ihh is expressed by prehypertrophic chondrocytes and accelerates the (hypertrophic) differentiation of proliferative chondrocytes and additionally it increases the expression of PTHrP, resulting in a feedback loop that controls the pace of chondrocyte proliferation and maturation [112–114]. Next

to the PTHrP-Ihh loop, fibroblast growth factors (FGFs) crucially regulate chondrocyte proliferation and differentiation possibly by stimulating Sox9 expression and inhibiting proliferation and Ihh expression [76]. FGF signaling is balanced by bone morphogenic protein (BMP)- signaling [115]. BMPs are described to have multiple roles during bone and cartilage formation, as well as growth plate development [116]. Interestingly; BMPs were initially discovered because of their remarkable ability to ectopically induce endochondral bone formation [117]. In a cartilage context, BMPs are involved in stimulating early chondrogenesis, cartilage maintenance and hypertrophic differentiation [116]. Especially BMP-2, BMP-4 and BMP-7 (OP-1) have been demonstrated to promote chondrogenic differentiation *in vitro* [116]. BMPs belong to the transforming growth factor beta (TGF-β) superfamily, which are important regulators of differentiation, proliferation, tissue homeostasis and -repair in general. TGF-β isoforms (TGF-β1, TGF-β2 and TGF-β3) support the differentiation of mesenchymal progenitor cells into the chondrogenic lineage [118–123]. The TGF-β isoforms mainly signal through phosporylated R-Smads, which in combination with co-(transcriptional) factors regulate specific target-gene expression [124, 125]. Related to its chondrogenic properties, TGF-β signalling is also involved in the formation of osteophytes during OA [126–129]. Another important regulator of chondrogenic differentiation is the canonical Wnt (wingless-type MMTV integration site family)/β-catenin signalling pathway. Upon binding of a Wnt ligand to its receptor (Frizzled), cytosolic β-catenin translocates to the nucleus where it forms complexes with transcription factors such as the TCF/LEF (transcription factor/lymphoid enhancer-binding factor) family and thereby regulates downstream target-gene expression. In absence of the Wnt signal cytosolic β-catenin is phosphorylated by GSK-3β (glycogen synthase kinase 3β) and subsequently degraded [130–132]. Members of the canonical Wnt/β-catenin signalling pathway are generally expressed during hypertrophy and accordingly also promote chondrocyte hypertrophy, presumably via the TCF/LEF binding site in the promoter region of the Runx2 gene [133–135]. In early chondrogenic differentiation Sox9 interacts with β-catenin and promotes its phosphorylation and thereby degradation thereby preventing osteoblastic or hypertrophic differentiation [133, 136–139].

In conclusion the process of endochondral ossification is dictated by spatiotemporal expression and function of variable transcription factors, ECM molecules and interacting regulatory molecules.

3.4 Importance of Cartilage Homeostasis in Outcome of Cartilage Repair

To maintain hyaline cartilage and prevent repaired cartilage from hypertrophic differentiation and as such further optimize cartilage repair approaches, local environmental factors need to be optimized. Such environmental factors are part of a healthy joint homeostasis which also enables hyaline cartilage to maintain its desired chondrogenic phenotype and prevent it from hypertrophic differentiation.

Joint homeostasis is described to be essential during cartilage repair, but methods for improving joint homeostasis in cartilage repair techniques are hardly addressed [140, 141]. An improved microenvironment may not only be the key to a new generation of bone marrow-based techniques to regenerate hyaline cartilage [142], but may also be a key factor for other progenitor cell based strategies and even cartilage repair in general. While inflammation is generally seen as a negative factor for joint homeostasis and are contributing factors in OA and rheumatoid arthritis (RA), it is also known to be the first and essential phase of tissue repair in general. Moreover, bone fracture healing depends on haematoma formation [143–145]. This suggests that inflammatory processes could be relevant pathways for addressing cartilage tissue repair. Supporting data for this notion is found in bone fracture healing processes where haematoma formation and injury-induced inflammatory responses are essential for fracture healing and its accompanying chondrogenic differentiation / endochondral ossification [143–145]. This essential inflammatory response induces local expression of extracellular signalling molecules like TGF-β1, BMPs, insulin-like growth factor (IGF)-1 and platelet derived growth factor (PDGF), which regulate chondrogenic differentiation processes [146, 147]. In addition, several inflammatory cytokines and chemokines (e.g. interleukin-1 (IL-1), IL-6, tumor necrosis factor alpha (TNFα), prostaglandin E_2 (PGE_2) and nitric oxide (NO)) are essential for bone fracture repair as well [144, 147–149].

3.4.1 Inflammatory Molecules and Chondrogenic Differentiation

The general understanding on the role of inflammatory molecules in articular cartilage development, maintenance and osteoarthritic degradation is a katabolic one. Inflammatory processes that initiate and/or maintain the osteoarthritic status in an OA joint are thought to mainly originate from the synovium possibly reacting to cartilage breakdown products. Here synoviocytes produce inflammatory mediators that attack the cartilage matrix, causing infiltration of immune cells and finally affect cartilage viability and function. Important inflammatory molecules in the OA progression are e.g. NF-κB (nuclear factor kappa-light-chain-enhancer of activated B-cells), TNFα, interleukins and cyclooxygenases [150, 151]. Interestingly, despite the overall katabolic environment in an OA joint, osteoarthritis often induces osteophyte formation. Basically, these are ossifying and isolated ectopic cartilaginous tissues near the synovial membrane, which are committed to follow the process of endochondral ossification [152, 153]. The formation of cartilaginous osteophytes is in contradiction with the overall katabolic environment in the OA joint and it is therefore hypothesized in literature that precursor cells from the synovial or periosteal tissue are activated to undergo chondrogenic differentiation by mechanisms that are not fully understood yet [153], but do require TGFβ's for their induction [154, 155]. Recent reports show that the inflammation related NF-κB subunit p65 is an essential transcription factor for Sox9 and BMP-2 [156, 157]. Transcriptional

induction of BMP-2 by p65 was found to be essential for longitudinal bone growth via endochondral ossification [158]. Similarly, TNFα was found to induce expression of BMP-2 as well [159, 160]. Essentially, these previous reports for the first time explored the connection between inflammatory pathways and chondrogenic differentiation in an anabolic way, instead of the classic degenerative connection only. Further support for this new dogma was found by Aung and colleagues, who recently published that OA cartilage-conditioned medium is able to induce chondrogenesis of human bone marrow stem cells [161]. Chen *et al* confirmed this phenomenon *in vivo* by subcutaneous implantation of fibrin glue mixed with bone marrow stem cells (BMSCs) and osteoarthritic cartilage fragments [162]. It was found that, specifically in the presence of OA cartilage, BMSCs are induced to differentiate in the chondrogenic lineage. In addition, it was shown that human mesenchymal stem cells produce growth factors after stimulation with LPS or TNFα in a NF-kB dependent manner [163]. Together these reports suggest that OA chondrocytes excrete factors that induce chondrogenic differentiation of mesenchymal progenitor cells. Finally, recent work by the authors confirms the hypothesis that indeed external inflammatory factors (LPS, TNFα, etc.) are able to induce chondrogenic differentiation of progenitor cells, even without the addition of well-known chondrogenic growth factors (e.g. TGFβs or insulin) [164]. P65, COX-2 and iNOS are specifically expressed in the resting zone chondrocytes of the developing growth plate, indicating that an inflammatory process is involved in early chondrogenic differentiation. Furthermore, activated p65 was found to be a crucial factor in the induction of inflammatory molecule-driven chondrogenic differentiation, by initiating an early transient induction of Sox9. In addition to the classic Sox9 function in cartilaginous matrix synthesis, this novel Sox9 characteristic somehow relates to the very early initiation of chondrogenic differentiation via mechanisms that are still unknown. Taken together there is a recently growing body of experimental evidence, showing that inflammatory molecules and their down-stream pathways are not only associated with cartilage degeneration, but are also crucially involved in the initiation of the chondrogenic differentiation process. However, it is important to realize that this a very mild and temporarily action and takes place very early in differentiation and these same mediators could have very different, katabolic, actions later in chondrogenic differentiation/cartilage maintenance. These data suggest that for cartilage regenerative medicine one might make use of these inflammatory properties, as the initiation of chondrogenic differentiation is a crucial event for progenitor cell-based cartilage repair techniques.

3.4.2 Inflammatory Molecules and Cartilage Hypertrophic Differentiation

Another interesting inflammatory phenomenon in the development of OA is being explored. For the articular cartilage component, osteoarthritis is in many ways similar to endochondral ossification, as in OA articular chondrocytes start to

differentiate into hypertrophic chondrocytes for reasons that are not yet completely understood [18–20]. Notably, the OA associated inflammatory factors and accompanying cell stress are known to be involved in chondrocyte hypertrophic differentiation in the growth plate and may explain why articular cartilage is terminally differentiating in OA [165]. Stress-related pathways that are activated in growth plate chondrocyte hypertrophic differentiation involve ER-stress/unfolded protein response, oxidative stress [166–168], advanced glycation end product formation (AGEs) [169–173], DNA damage and others [165]. In the growth plate these pathways are activated due to the rapid cell proliferation in the proliferative zone, reoxygenation of hypertrophic chondrocytes from the subchondral bone marrow, vast extra cellular matrix protein synthesis, etc [165]. Moreover, as a result of hypertrophic differentiation, these cells also start to express inflammatory molecules (COX-1, COX-2 [174], iNOS [175–177], p65 [158, 178, 179] and others (our unpublished data)), which are thought to enhance the intrinsic cellular capacity for hypertrophic differentiation. The message that should be taken from these observations is that failure of cartilage reparative and regenerative techniques due to formation of interlesional osteophytes, hypertrophic differentiation and calcification of cartilage grafts, may originate from similar processes. The pathways and phenomena stated above are therefore expected to be promising targets for avoiding failure due to terminal differentiation of the cartilage graft in the clinic.

We recently found that pharmacological inhibition of the key inflammatory enzyme cyclooxygenase-2 by e.g. Celecoxib decreases the level of chondrocyte hypertrophic differentiation, even in BMP-2 induced chondrocyte hypertrophy [174]. This may provide a potential pharmacological approach to prevent or decrease chondrocyte hypertrophic differentiation in cartilage repair techniques. Other authors have identified anti-oxidative components that decrease inflammatory signaling or chondrocyte hypertrophic differentiation. These components include N-acetyl cysteine [180–182], resveratrol [183–185], and even mechanical loading [165, 186]. Similarly, parathyroid hormone related peptide (PTHrP) is known for its capacity to keep proliferating articular chondrocytes in their chondrocyte state and prevent them for further developing into hypertrophic chondrocytes [15, 187]. In conclusion, targeting inflammatory mediators and stress related pathways may thus provide a potential pharmacological approach to prevent or decrease chondrocyte hypertrophic differentiation in cartilage repair techniques.

3.5 Conclusion

In summary, it now becomes clear that inflammatory signaling is not only involved in cartilage degradation, but is also indispensable for initiating the differentiation of chondrocytes from progenitor cells on, albeit it in a very mild and temporarily action. Thereby this brings a whole new view on the role of inflammatory mediators and their link to cartilage in general. Especially for progenitor cell based repair technologies, these new insights could be employed to increase the differentiation

potential of progenitor cells toward engineered cartilaginous tissue *in vitro and in vivo*. Furthermore, part of the failure of cartilage repair techniques originates from calcification or hypertrophic differentiation of the cartilage graft, as well due to the development of interlesional osteophytes. The authors believe that part of these adverse effects might be avoided when joint homeostasis which is ideal for the different phases of regeneration or repair is also taken into account as an important factor in the post-operative treatment strategy after cartilage repair. A synovial fluid environment supplemented with the aforementioned factors might contribute to the success rate on an anti-hypertrophic basis possibly for both the chondrogenically differentiating cells as well as the subchondral bone. Additionally it could also be envisioned that any joint homeostasis-disturbing intervention could benefit from an approach where joint homeostasis which is optimal for cartilage repair is recognized as a prerequisite for success. For these, anti-oxidative and anti-hypertrophic agents could play an important role to achieve this goal as well. In addition, since hypertrophy and ossification are also believed to be essential underlying processes in the process of OA these findings may also be of concern in the process of OA.

Acknowledgements This work is financially supported by the Dutch Arthritis Association (grant LLP14) (http://www.reumafonds.nl) and the Dutch Stichting Annafonds|NOREF (grants 07/07 and 08/42) (http://www.annafonds.nl). The authors also thank D.A.M Surtel and A. Cremers.

References

1. Buckwalter J, Mankin H. Articular cartilage: tissue design and chondrocyte matrix interactions. Instr Cours Lect. 1998;47:487–504.
2. Hasler EM, Herzog W, Wu JZ, et al. Articular cartilage biomechanics: theoretical models, material properties, and biosynthetic response. Crit Rev Biomed Eng. 1999;27:415–88.
3. Mankin H, Mow V, Buckwalter J. Articular cartilage structure, composition, and functioned. Rosemont: AAOS; 2000.
4. Poole AR, Kojima T, Yasuda T, et al. Composition and structure of articular cartilage: a template for tissue repair. Clin Orthop Relat Res. 2001;(391 Suppl):S26–33.
5. Moreira-Teixeira LS, Georgi N, Leijten J, et al. Cartilage tissue engineering. Endocr Dev. 2011;21:102–15.
6. Aydelotte MB, Greenhill RR, Kuettner KE. Differences between sub-populations of cultured bovine articular chondrocytes. II. Proteoglycan metabolism. Connect Tissue Res. 1988;18:223–34.
7. Schenk R, Eggli P, Hunziker E. Articular cartilage morphologyed. New York: Raven Press; 1986.
8. Curl WW, Krome J, Gordon ES, et al. Cartilage injuries: a review of 31,516 knee arthroscopies. Arthroscopy. 1997;13:456–60.
9. Hjelle K, Solheim E, Strand T, et al. Articular cartilage defects in 1,000 knee arthroscopies. Arthroscopy. 2002;18:730–4.
10. Dowthwaite GP, Bishop JC, Redman SN, et al. The surface of articular cartilage contains a progenitor cell population. J Cell Sci. 2004;117:889–97.
11. Park Y, Sugimoto M, Watrin A, et al. BMP-2 induces the expression of chondrocyte-specific genes in bovine synovium-derived progenitor cells cultured in three-dimensional alginate hydrogel. Osteoarthritis Cartilage. 2005;13:527–36.

12. Emans PJ, Surtel DA, Frings EJ, et al. In vivo generation of cartilage from periosteum. Tissue Eng. 2005;11:369–77.
13. Mankin H, Mow V, Buckwalter J. Articular cartilage repair and osteoarthritised. Rosemont: American Academy of Orthopaedic Surgeons; 2000.
14. Hunter W. Of the structure and disease of articulating cartilages. 1743. Clin Orthop Relat Res. 1995;317:3–6.
15. Dickhut A, Pelttari K, Janicki P, et al. Calcification or dedifferentiation: requirement to lock mesenchymal stem cells in a desired differentiation stage. J Cell Physiol. 2009;219:219–26.
16. van Osch GJ, Brittberg M, Dennis JE, et al. Cartilage repair: past and future–lessons for regenerative medicine. J Cell Mol Med. 2009;13:792–810.
17. van der Kraan PM, van den Berg WB. Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? Osteoarthritis Cartilage. 2012;20: 223–32.
18. Kamekura S, Kawasaki Y, Hoshi K, et al. Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. Arthritis Rheum. 2006;54:2462–70.
19. Kawaguchi H. Endochondral ossification signals in cartilage degradation during osteoarthritis progression in experimental mouse models. Mol Cells. 2008;25:1–6.
20. Saito T, Fukai A, Mabuchi A, et al. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. Nat Med. 2010;16: 678–86.
21. Aglietti P, Buzzi R, Bassi PB, et al. Arthroscopic drilling in juvenile osteochondritis dissecans of the medial femoral condyle. Arthroscopy. 1994;10:286–91.
22. Altman RD, Kates J, Chun LE, et al. Preliminary observations of chondral abrasion in a canine model. Ann Rheum Dis. 1992;51:1056–62.
23. Bradley J, Dandy DJ. Results of drilling osteochondritis dissecans before skeletal maturity. J Bone Joint Surg Br. 1989;71:642–4.
24. Furukawa T, Eyre DR, Koide S, et al. Biochemical studies on repair cartilage resurfacing experimental defects in the rabbit knee. J Bone Joint Surg Am. 1980;62:79–89.
25. Insall J. The Pridie debridement operation for osteoarthritis of the knee. Clin Orthop Relat Res. 1974;101:61–7.
26. Kim HK, Moran ME, Salter RB. The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits. J Bone Joint Surg Am. 1991;73:1301–15.
27. Meachim G, Roberts C. Repair of the joint surface from subarticular tissue in the rabbit knee. J Anat. 1971;109:317–27.
28. Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg Am. 1976;58:230–3.
29. Rae PJ, Noble J. Arthroscopic drilling of osteochondral lesions of the knee. J Bone Joint Surg Br. 1989;71:534.
30. Steinwachs MR, Guggi T, Kreuz PC. Marrow stimulation techniques. Injury. 2008;39 Suppl 1:S26–31.
31. Vachon A, Bramlage LR, Gabel AA, et al. Evaluation of the repair process of cartilage defects of the equine third carpal bone with and without subchondral bone perforation. Am J Vet Res. 1986;47:2637–45.
32. Emans PJ, Pieper J, Hulsbosch MM, et al. Differential cell viability of chondrocytes and progenitor cells in tissue-engineered constructs following implantation into osteochondral defects. Tissue Eng. 2006;12:1699–709.
33. Gallay SH, Miura Y, Commisso CN, et al. Relationship of donor site to chondrogenic potential of periosteum in vitro. J Orthop Res. 1994;12:515–25.
34. Iwasaki M, Nakahara H, Nakase T, et al. Bone morphogenetic protein 2 stimulates osteogenesis but does not affect chondrogenesis in osteochondrogenic differentiation of periosteum-derived cells. J Bone Miner Res. 1994;9:1195–204.

35. Iwasaki M, Nakahara H, Nakata K, et al. Regulation of proliferation and osteochondrogenic differentiation of periosteum-derived cells by transforming growth factor-beta and basic fibroblast growth factor. J Bone Joint Surg Am. 1995;77:543–54.
36. Iwasaki M, Nakata K, Nakahara H, et al. Transforming growth factor-beta 1 stimulates chondrogenesis and inhibits osteogenesis in high density culture of periosteum-derived cells. Endocrinology. 1993;132:1603–8.
37. Nakahara H, Bruder SP, Goldberg VM, et al. In vivo osteochondrogenic potential of cultured cells derived from the periosteum. Clin Orthop Relat Res. 1990;259:223–32.
38. Nakahara H, Dennis JE, Bruder SP, et al. In vitro differentiation of bone and hypertrophic cartilage from periosteal-derived cells. Exp Cell Res. 1991;195:492–503.
39. Nakahara H, Goldberg VM, Caplan AI. Culture-expanded human periosteal-derived cells exhibit osteochondral potential in vivo. J Orthop Res. 1991;9:465–76.
40. Nakata K, Nakahara H, Kimura T, et al. Collagen gene expression during chondrogenesis from chick periosteum-derived cells. FEBS Lett. 1992;299:278–82.
41. O'Driscoll SW, Recklies AD, Poole AR. Chondrogenesis in periosteal explants. An organ culture model for in vitro study. J Bone Joint Surg Am. 1994;76:1042–51.
42. Bouwmeester SJ, Beckers JM, Kuijer R, et al. Long-term results of rib perichondrial grafts for repair of cartilage defects in the human knee. Int Orthop. 1997;21:313–7.
43. Homminga GN, Bulstra SK, Bouwmeester PS, et al. Perichondral grafting for cartilage lesions of the knee. J Bone Joint Surg Br. 1990;72:1003–7.
44. Homminga GN, Bulstra SK, Kuijer R, et al. Repair of sheep articular cartilage defects with a rabbit costal perichondrial graft. Acta Orthop Scand. 1991;62:415–8.
45. O'Driscoll SW, Keeley FW, Salter RB. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion. An experimental investigation in the rabbit. J Bone Joint Surg Am. 1986;68:1017–35.
46. O'Driscoll SW, Keeley FW, Salter RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion. A follow-up report at one year. J Bone Joint Surg Am. 1988;70:595–606.
47. O'Driscoll SW, Saris DB, Ito Y, et al. The chondrogenic potential of periosteum decreases with age. J Orthop Res. 2001;19:95–103.
48. Skoog T, Johansson SH. The formation of articular cartilage from free perichondrial grafts. Plast Reconstr Surg. 1976;57:1–6.
49. Vachon A, McIlwraith CW, Trotter GW, et al. Neochondrogenesis in free intra-articular, periosteal, and perichondrial autografts in horses. Am J Vet Res. 1989;50:1787–94.
50. Zarnett R, Salter RB. Periosteal neochondrogenesis for biologically resurfacing joints: its cellular origin. Can J Surg. 1989;32:171–4.
51. Easley ME, Scranton Jr PE. Osteochondral autologous transfer system. Foot Ankle Clin. 2003;8:275–90.
52. Gross AE, McKee NH, Pritzker KP, et al. Reconstruction of skeletal deficits at the knee. A comprehensive osteochondral transplant program. Clin Orthop Relat Res. 1983;174:96–106.
53. Horas U, Schnettler R, Pelinkovic D, et al. Osteochondral transplantation versus autogenous chondrocyte transplantation. A prospective comparative clinical study. Chirurg. 2000;71: 1090–7.
54. Onstott AT, Moczo A, Harris NL. Osteochondral autotransfer–newer treatment for chondral defects. AORN J. 2000;71(843–845):848–51.
55. Convery FR, Meyers MH, Akeson WH. Fresh osteochondral allografting of the femoral condyle. Clin Orthop Relat Res. 1991;273:139–45.
56. Czitrom AA, Keating S, Gross AE. The viability of articular cartilage in fresh osteochondral allografts after clinical transplantation. J Bone Joint Surg Am. 1990;72:574–81.
57. Garrett JC. Treatment of osteochondral defects of the distal femur with fresh osteochondral allografts: a preliminary report. Arthroscopy. 1986;2:222–6.

58. Garrett JC. Fresh osteochondral allografts for treatment of articular defects in osteochondritis dissecans of the lateral femoral condyle in adults. Clin Orthop Relat Res. 1994;303:33–7.
59. Garrett JC. Osteochondral allografts for reconstruction of articular defects of the knee. Instr Course Lect. 1998;47:517–22.
60. Ghazavi MT, Pritzker KP, Davis AM, et al. Fresh osteochondral allografts for post-traumatic osteochondral defects of the knee. J Bone Joint Surg Br. 1997;79:1008–13.
61. Gross AE, Aubin P, Cheah HK, et al. A fresh osteochondral allograft alternative. J Arthroplasty. 2002;17:50–3.
62. Brittberg M. Autologous chondrocyte implantation–technique and long-term follow-up. Injury. 2008;39 Suppl 1:S40–9.
63. Brittberg M, Lindahl A, Nilsson A, et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331:889–95.
64. Brittberg M, Peterson L, Sjogren-Jansson E, et al. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. J Bone Joint Surg Am. 2003;85-A Suppl 3:109–15.
65. Bartlett W, Skinner JA, Gooding CR, et al. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. J Bone Joint Surg Br. 2005;87:640–5.
66. Harris JD, Siston RA, Brophy RH, et al. Failures, re-operations, and complications after autologous chondrocyte implantation–a systematic review. Osteoarthritis Cartilage. 2011;19: 779–91.
67. Pietschmann MF, Niethammer TR, Horng A, et al. The incidence and clinical relevance of graft hypertrophy after matrix-based autologous chondrocyte implantation. Am J Sports Med. 2012;40:68–74.
68. Cole BJ, Farr J, Winalski CS, et al. Outcomes after a single-stage procedure for cell-based cartilage repair: a prospective clinical safety trial with 2-year follow-up. Am J Sports Med. 2011;39:1170–9.
69. Vasiliadis HS, Danielson B, Ljungberg M, et al. Autologous chondrocyte implantation in cartilage lesions of the knee: long-term evaluation with magnetic resonance imaging and delayed gadolinium-enhanced magnetic resonance imaging technique. Am J Sports Med. 2010;38: 943–9.
70. Abad V, Meyers JL, Weise M, et al. The role of the resting zone in growth plate chondrogenesis. Endocrinology. 2002;143:1851–7.
71. Karlsson C, Lindahl A. Articular cartilage stem cell signalling. Arthritis Res Ther. 2009; 11:121.
72. Emans PJ, Caron MMJ, van Rhijn LW, et al. Cartilage tissue engineering; lessons learned from periosteum. Tissue Sci Eng. 2011;S2:002.
73. Medvedev SP, Grigor'eva EV, Shevchenko AI, et al. Human induced pluripotent stem cells derived from fetal neural stem cells successfully undergo directed differentiation into cartilage. Stem Cells Dev. 2011;20:1099–112.
74. Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol. 2004;36:568–84.
75. Erlebacher A, Filvaroff EH, Gitelman SE, et al. Toward a molecular understanding of skeletal development. Cell. 1995;80:371–8.
76. Kronenberg HM. Developmental regulation of the growth plate. Nature. 2003;423:332–6.
77. de Crombrugghe B, Lefebvre V, Nakashima K. Regulatory mechanisms in the pathways of cartilage and bone formation. Curr Opin Cell Biol. 2001;13:721–7.
78. Lefebvre V, Behringer RR, de Crombrugghe B. L-Sox5, Sox6 and Sox9 control essential steps of the chondrocyte differentiation pathway. Osteoarthritis Cartilage. 2001;9(Suppl A):S69–75.
79. Lefebvre V, Smits P. Transcriptional control of chondrocyte fate and differentiation. Birth Defects Res C Embryo Today. 2005;75:200–12.
80. Akiyama H, Chaboissier MC, Martin JF, et al. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes Dev. 2002;16:2813–28.

81. Han Y, Lefebvre V. L-Sox5 and Sox6 drive expression of the aggrecan gene in cartilage by securing binding of Sox9 to a far-upstream enhancer. Mol Cell Biol. 2008;28: 4999–5013.
82. Lefebvre V, Huang W, Harley VR, et al. SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1(II) collagen gene. Mol Cell Biol. 1997;17:2336–46.
83. Lefebvre V, Li P, de Crombrugghe B. A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. EMBO J. 1998;17:5718–33.
84. Genzer MA, Bridgewater LC. A Col9a1 enhancer element activated by two interdependent SOX9 dimers. Nucleic Acids Res. 2007;35:1178–86.
85. Jenkins E, Moss JB, Pace JM, et al. The new collagen gene COL27A1 contains SOX9-responsive enhancer elements. Matrix Biol. 2005;24:177–84.
86. Oh CD, Maity SN, Lu JF, et al. Identification of SOX9 interaction sites in the genome of chondrocytes. PLoS One. 2010;5:e10113.
87. Rentsendorj O, Nagy A, Sinko I, et al. Highly conserved proximal promoter element harbouring paired Sox9-binding sites contributes to the tissue- and developmental stage-specific activity of the matrilin-1 gene. Biochem J. 2005;389:705–16.
88. Fassler R, Schnegelsberg PN, Dausman J, et al. Mice lacking alpha 1 (IX) collagen develop noninflammatory degenerative joint disease. Proc Natl Acad Sci USA. 1994;91:5070–4.
89. Li Y, Lacerda DA, Warman ML, et al. A fibrillar collagen gene, Col11a1, is essential for skeletal morphogenesis. Cell. 1995;80:423–30.
90. Nakata K, Ono K, Miyazaki J, et al. Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing alpha 1(IX) collagen chains with a central deletion. Proc Natl Acad Sci USA. 1993;90:2870–4.
91. Vikkula M, Mariman EC, Lui VC, et al. Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. Cell. 1995;80:431–7.
92. Eyre D. Collagen of articular cartilage. Arthritis Res. 2002;4:30–5.
93. Knudson CB, Knudson W. Cartilage proteoglycans. Semin Cell Dev Biol. 2001;12:69–78.
94. Lesperance LM, Gray ML, Burstein D. Determination of fixed charge density in cartilage using nuclear magnetic resonance. J Orthop Res. 1992;10:1–13.
95. Venn M, Maroudas A. Chemical composition and swelling of normal and osteoarthrotic femoral head cartilage. I. Chemical composition. Ann Rheum Dis. 1977;36:121–9.
96. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect. 1998;47:477–86.
97. Lin Z, Willers C, Xu J, et al. The chondrocyte: biology and clinical application. Tissue Eng. 2006;12:1971–84.
98. Hunziker E. Articular cartilage structure in humans and experimental animalsed. New York: Raven Press; 1992.
99. Aydelotte M, Kuettner K. Heterogeneity of articular chondrocytes and cartilage matrixed. New York: Marcel Dekker; 1992.
100. Radin EL, Martin RB, Burr DB, et al. Effects of mechanical loading on the tissues of the rabbit knee. J Orthop Res. 1984;2:221–34.
101. Hunziker EB. Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes. Microsc Res Tech. 1994;28:505–19.
102. Arnold MA, Kim Y, Czubryt MP, et al. MEF2C transcription factor controls chondrocyte hypertrophy and bone development. Dev Cell. 2007;12:377–89.
103. Drissi MH, Li X, Sheu TJ, et al. Runx2/Cbfa1 stimulation by retinoic acid is potentiated by BMP2 signaling through interaction with Smad1 on the collagen X promoter in chondrocytes. J Cell Biochem. 2003;90:1287–98.
104. Linsenmayer TF, Eavey RD, Schmid TM. Type X collagen: a hypertrophic cartilage-specific molecule. Pathol Immunopathol Res. 1988;7:14–9.
105. Zheng Q, Zhou G, Morello R, et al. Type X collagen gene regulation by Runx2 contributes directly to its hypertrophic chondrocyte-specific expression in vivo. J Cell Biol. 2003;162: 833–42.

106. Gerber HP, Vu TH, Ryan AM, et al. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med. 1999;5: 623–8.
107. Hess J, Porte D, Munz C, et al. AP-1 and Cbfa/runt physically interact and regulate parathyroid hormone-dependent MMP13 expression in osteoblasts through a new osteoblast-specific element 2/AP-1 composite element. J Biol Chem. 2001;276:20029–38.
108. Sato M, Morii E, Komori T, et al. Transcriptional regulation of osteopontin gene in vivo by PEBP2alphaA/CBFA1 and ETS1 in the skeletal tissues. Oncogene. 1998;17:1517–25.
109. Beier F, Ali Z, Mok D, et al. TGFbeta and PTHrP control chondrocyte proliferation by activating cyclin D1 expression. Mol Biol Cell. 2001;12:3852–63.
110. Zhang M, Xie R, Hou W, et al. PTHrP prevents chondrocyte premature hypertrophy by inducing cyclin-D1-dependent Runx2 and Runx3 phosphorylation, ubiquitylation and proteasomal degradation. J Cell Sci. 2009;122:1382–9.
111. St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes Dev. 1999;13:2072–86.
112. Bitgood MJ, McMahon AP. Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. Dev Biol. 1995;172:126–38.
113. Long F, Zhang XM, Karp S, et al. Genetic manipulation of hedgehog signaling in the endochondral skeleton reveals a direct role in the regulation of chondrocyte proliferation. Development. 2001;128:5099–108.
114. Vortkamp A, Lee K, Lanske B, et al. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science. 1996;273:613–22.
115. Yoon BS, Pogue R, Ovchinnikov DA, et al. BMPs regulate multiple aspects of growth-plate chondrogenesis through opposing actions on FGF pathways. Development. 2006;133: 4667–78.
116. Yoon BS, Lyons KM. Multiple functions of BMPs in chondrogenesis. J Cell Biochem. 2004; 93:93–103.
117. Urist MR. Bone: formation by autoinduction. Science. 1965;150:893–9.
118. Chimal-Monroy J, Bravo-Ruiz MT. Diaz de Leon L. Regulation of chondrocyte differentiation by transforming growth factors beta 1, beta 2, beta 3, and beta 5. Ann N Y Acad Sci. 1996;785:241–4.
119. Chimal-Monroy J, Diaz de Leon L. Differential effects of transforming growth factors beta 1, beta 2, beta 3 and beta 5 on chondrogenesis in mouse limb bud mesenchymal cells. Int J Dev Biol. 1997;41:91–102.
120. Ferguson CM, Schwarz EM, Puzas JE, et al. Transforming growth factor-beta1 induced alteration of skeletal morphogenesis in vivo. J Orthop Res. 2004;22:687–96.
121. Ferguson CM, Schwarz EM, Reynolds PR, et al. Smad2 and 3 mediate transforming growth factor-beta1-induced inhibition of chondrocyte maturation. Endocrinology. 2000;141: 4728–35.
122. Johnstone B, Hering TM, Caplan AI, et al. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res. 1998;238:265–72.
123. Lorda-Diez CI, Montero JA, Martinez-Cue C, et al. Transforming growth factors beta coordinate cartilage and tendon differentiation in the developing limb mesenchyme. J Biol Chem. 2009;284:29988–96.
124. Feng XH, Zhang Y, Wu RY, et al. The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for smad3 in TGF-beta-induced transcriptional activation. Genes Dev. 1998;12:2153–63.
125. Lagna G, Hata A, Hemmati-Brivanlou A, et al. Partnership between DPC4 and SMAD proteins in TGF-beta signalling pathways. Nature. 1996;383:832–6.
126. Elford PR, Graeber M, Ohtsu H, et al. Induction of swelling, synovial hyperplasia and cartilage proteoglycan loss upon intra-articular injection of transforming growth factor beta-2 in the rabbit. Cytokine. 1992;4:232–8.

127. van Beuningen HM, van der Kraan PM, Arntz OJ, et al. Does TGF-beta protect articular cartilage in vivo? Agents Actions Suppl. 1993;39:127–31.
128. van Beuningen HM, van der Kraan PM, Arntz OJ, et al. Transforming growth factor-beta 1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. Lab Invest. 1994;71:279–90.
129. Hunziker EB. Growth-factor-induced healing of partial-thickness defects in adult articular cartilage. Osteoarthritis Cartilage. 2001;9:22–32.
130. Aberle H, Bauer A, Stappert J, et al. Beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. 1997;16:3797–804.
131. Behrens J, von Kries JP, Kuhl M, et al. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature. 1996;382:638–42.
132. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005;434:843–50.
133. Day TF, Guo X, Garrett-Beal L, et al. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005;8:739–50.
134. Dong YF, Soung do Y, Schwarz EM, et al. Wnt induction of chondrocyte hypertrophy through the Runx2 transcription factor. J Cell Physiol. 2006;208:77–86.
135. Hill TP, Spater D, Taketo MM, et al. Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell. 2005;8:727–38.
136. Akiyama H. Control of chondrogenesis by the transcription factor Sox9. Mod Rheumatol. 2008;18:213–9.
137. Akiyama H, Chaboissier MC, Behringer RR, et al. Essential role of Sox9 in the pathway that controls formation of cardiac valves and septa. Proc Natl Acad Sci USA. 2004;101:6502–7.
138. Ryu JH, Kim SJ, Kim SH, et al. Regulation of the chondrocyte phenotype by beta-catenin. Development. 2002;129:5541–50.
139. Topol L, Chen W, Song H, et al. Sox9 inhibits Wnt signaling by promoting beta-catenin phosphorylation in the nucleus. J Biol Chem. 2009;284:3323–33.
140. Yang KG, Saris DB, Verbout AJ, et al. The effect of synovial fluid from injured knee joints on in vitro chondrogenesis. Tissue Eng. 2006;12:2957–64.
141. Saris DB, Dhert WJ, Verbout AJ. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair. J Bone Joint Surg Br. 2003;85:1067–76.
142. Richter W. Mesenchymal stem cells and cartilage in situ regeneration. J Intern Med. 2009;266:390–405.
143. Einhorn TA. The science of fracture healing. J Orthop Trauma. 2005;19:S4–6.
144. Gerstenfeld LC, Cullinane DM, Barnes GL, et al. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. J Cell Biochem. 2003;88:873–84.
145. Grundnes O, Reikeras O. The importance of the hematoma for fracture healing in rats. Acta Orthop Scand. 1993;64:340–2.
146. Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone. Biology and clinical applications. J Bone Joint Surg Am. 2002;84-A:1032–44.
147. Mountziaris PM, Mikos AG. Modulation of the inflammatory response for enhanced bone tissue regeneration. Tissue Eng Part B Rev. 2008;14(2):179–86.
148. Einhorn TA, Majeska RJ, Rush EB, et al. The expression of cytokine activity by fracture callus. J Bone Miner Res. 1995;10:1272–81.
149. Rundle CH, Wang H, Yu H, et al. Microarray analysis of gene expression during the inflammation and endochondral bone formation stages of rat femur fracture repair. Bone. 2006;38:521–9.
150. Pelletier JP, Martel-Pelletier J. The Novartis-ILAR Rheumatology Prize 2001 Osteoarthritis: from molecule to man. Arthritis Res. 2002;4:13–9.
151. Sofat N. Analysing the role of endogenous matrix molecules in the development of osteoarthritis. Int J Exp Pathol. 2009;90:463–79.

152. Gelse K, Soder S, Eger W, et al. Osteophyte development–molecular characterization of differentiation stages. Osteoarthritis Cartilage. 2003;11:141–8.
153. van der Kraan PM, van den Berg WB. Osteophytes: relevance and biology. Osteoarthritis Cartilage. 2007;15:237–44.
154. Blaney Davidson EN, Vitters EL, van Beuningen HM, et al. Resemblance of osteophytes in experimental osteoarthritis to transforming growth factor beta-induced osteophytes: limited role of bone morphogenetic protein in early osteoarthritic osteophyte formation. Arthritis Rheum. 2007;56:4065–73.
155. van den Berg WB, van Osch GJ, van der Kraan PM, et al. Cartilage destruction and osteophytes in instability-induced murine osteoarthritis: role of TGF beta in osteophyte formation? Agents Actions. 1993;40:215–9.
156. Feng JQ, Xing L, Zhang JH, et al. NF-kappaB specifically activates BMP-2 gene expression in growth plate chondrocytes in vivo and in a chondrocyte cell line in vitro. J Biol Chem. 2003;278:29130–5.
157. Ushita M, Saito T, Ikeda T, et al. Transcriptional induction of SOX9 by NF-kappaB family member RelA in chondrogenic cells. Osteoarthritis Cartilage. 2009;17:1065–75.
158. Wu S, Flint JK, Rezvani G, et al. Nuclear factor-kappaB p65 facilitates longitudinal bone growth by inducing growth plate chondrocyte proliferation and differentiation and by preventing apoptosis. J Biol Chem. 2007;282:33698–706.
159. Fukui N, Ikeda Y, Ohnuki T, et al. Pro-inflammatory cytokine tumor necrosis factor-alpha induces bone morphogenetic protein-2 in chondrocytes via mRNA stabilization and transcriptional up-regulation. J Biol Chem. 2006;281:27229–41.
160. Fukui N, Zhu Y, Maloney WJ, et al. Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF-alpha in normal and osteoarthritic chondrocytes. J Bone Joint Surg Am. 2003;85-A Suppl 3:59–66.
161. Aung A, Gupta G, Majid G, et al. Osteoarthritic chondrocyte-secreted morphogens induce chondrogenic differentiation of human mesenchymal stem cells. Arthritis Rheum. 2011;63: 148–58.
162. Chen CC, Liao CH, Wang YH, et al. Cartilage fragments from osteoarthritic knee promote chondrogenesis of mesenchymal stem cells without exogenous growth factor induction. J Orthop Res. 2012;30:393–400.
163. Crisostomo PR, Wang Y, Markel TA, et al. Human mesenchymal stem cells stimulated by TNF-alpha, LPS, or hypoxia produce growth factors by an NF kappa B- but not JNK-dependent mechanism. Am J Physiol. 2008;294:C675–82.
164. Caron MM, Emans PJ, Surtel DA, et al. Activation of NF-kappaB/p65 facilitates early chondrogenic differentiation during endochondral ossification. PLoS One. 2012;7:e33467.
165. Zuscik MJ, Hilton MJ, Zhang X, et al. Regulation of chondrogenesis and chondrocyte differentiation by stress. J Clin Invest. 2008;118:429–38.
166. Henrotin Y, Kurz B, Aigner T. Oxygen and reactive oxygen species in cartilage degradation: friends or foes? Osteoarthritis Cartilage. 2005;13:643–54.
167. Jallali N, Ridha H, Thrasivoulou C, et al. Modulation of intracellular reactive oxygen species level in chondrocytes by IGF-1, FGF, and TGF-beta1. Connect Tissue Res. 2007;48: 149–58.
168. Morita K, Miyamoto T, Fujita N, et al. Reactive oxygen species induce chondrocyte hypertrophy in endochondral ossification. J Exp Med. 2007;204:1613–23.
169. Cecil DL, Johnson K, Rediske J, et al. Inflammation-induced chondrocyte hypertrophy is driven by receptor for advanced glycation end products. J Immunol. 2005;175:8296–302.
170. Handl M, Filova E, Kubala M, et al. Fluorescent advanced glycation end products in the detection of factual stages of cartilage degeneration. Physiol Res. 2007;56:235–42.
171. Huang CY, Hung LF, Liang CC, et al. COX-2 and iNOS are critical in advanced glycation end product-activated chondrocytes in vitro. Eur J Clin Invest. 2009;39:417–28.
172. Kume S, Kato S, Yamagishi S, et al. Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. J Bone Miner Res. 2005;20:1647–58.

173. Nah SS, Choi IY, Lee CK, et al. Effects of advanced glycation end products on the expression of COX-2, PGE2 and NO in human osteoarthritic chondrocytes. Rheumatology (Oxford). 2008;47:425–31.
174. Welting TJ, Caron MM, Emans PJ, et al. Inhibition of cyclooxygenase-2 impacts chondrocyte hypertrophic differentiation during endochondral ossification. Eur Cell Mater. 2011;22: 420–36; discussion 436–27.
175. Arasapam G, Scherer M, Cool JC, et al. Roles of COX-2 and iNOS in the bony repair of the injured growth plate cartilage. J Cell Biochem. 2006;99:450–61.
176. Baldik Y, Diwan AD, Appleyard RC, et al. Deletion of iNOS gene impairs mouse fracture healing. Bone. 2005;37:32–6.
177. Mais A, Klein T, Ullrich V, et al. Prostanoid pattern and iNOS expression during chondrogenic differentiation of human mesenchymal stem cells. J Cell Biochem. 2006;98:798–809.
178. Itoh SS, Ushita M, Ikeda T, Yano F, Ogata N, Chung U, Nakamura K, Kawaguchi H. NF-kappa B family member RelA/p65, a transcription factor of Sox9, is essential for chondrogenic differentiation and skeletal growth. Osteoarthritis Cartilage. 2009;17:S12–3.
179. Wu S, Fadoju D, Rezvani G, et al. Stimulatory effects of insulin-like growth factor-I on growth plate chondrogenesis are mediated by nuclear factor-kappaB p65. J Biol Chem. 2008;283:34037–44.
180. Kishimoto H, Akagi M, Zushi S, et al. Induction of hypertrophic chondrocyte-like phenotypes by oxidized LDL in cultured bovine articular chondrocytes through increase in oxidative stress. Osteoarthritis Cartilage. 2010;18:1284–90.
181. Nakagawa S, Arai Y, Mazda O, et al. N-acetylcysteine prevents nitric oxide-induced chondrocyte apoptosis and cartilage degeneration in an experimental model of osteoarthritis. J Orthop Res. 2010;28:156–63.
182. Roman-Blas JA, Contreras-Blasco MA, Largo R, et al. Differential effects of the antioxidant n-acetylcysteine on the production of catabolic mediators in IL-1beta-stimulated human osteoarthritic synoviocytes and chondrocytes. Eur J Pharmacol. 2009;623:125–31.
183. Csaki C, Keshishzadeh N, Fischer K, et al. Regulation of inflammation signalling by resveratrol in human chondrocytes in vitro. Biochem Pharmacol. 2008;75:677–87.
184. Liu FC, Hung LF, Wu WL, et al. Chondroprotective effects and mechanisms of resveratrol in advanced glycation end products-stimulated chondrocytes. Arthritis Res Ther. 2010;12:R167.
185. Shakibaei M, Csaki C, Nebrich S, et al. Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochem Pharmacol. 2008;76: 1426–39.
186. Chowdhury TT, Bader DL, Lee DA. Dynamic compression counteracts IL-1beta induced iNOS and COX-2 activity by human chondrocytes cultured in agarose constructs. Biorheology. 2006;43:413–29.
187. Fischer J, Dickhut A, Rickert M, et al. Human articular chondrocytes secrete parathyroid hormone-related protein and inhibit hypertrophy of mesenchymal stem cells in coculture during chondrogenesis. Arthritis Rheum. 2010;62:2696–706.

Chapter 4
Osteoarthritis: Molecular Mechanisms and Treatments

Takehiko Matsushita and Ryosuke Kuroda

Abstract Osteoarthritis (OA) is an aging-associated joint disease with degeneration of articular cartilage. OA is also caused or accelerated by trauma and joint injuries. Pathological features of OA are characterized by articular cartilage breakdown with inflammation in synovium, osteophyte formation and changes in subchondral bone, followed by eventual joint destruction. During development OA, Joint homeostasis, entire environment of joint that is necessary for maintaining the joint in a healthy condition, is altered and such global alterations seem to affect chondrocyte metabolisms and cartilage reparatory capacity. Recent several reports also indicated that osteoarthritic conditions in the joint affect the clinical outcomes of the cartilage repair treatments and treating joints with OA still remains challenging. Therefore to understand the molecular mechanisms of OA is an essential step to treat OA and to obtain better clinical outcomes after cartilage repair procedures. One of the trends in recent research is development or discovery of disease modifying osteoarthritis drugs (DMOADs) which can counteract against causative factors for OA. DMODAs are expected to alleviate patient's symptoms and slow down the progression of OA or prevent OA. Some pharmacological agents and growth factors are being investigated in clinical trials. In the future, DMOADs can be introduced as a new therapeutic approach for treatment of OA and possibly some of the DMOADs could be combined with cartilage repair techniques.

Keywords Osteoarthritis • Molecular mechanisms • Pathological conditions • Joint homeostasis • DMOADs

T. Matsushita (✉) • R. Kuroda
Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
e-mail: matsushi@med.kobe-u.ac.jp

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_4,

Key Points

- Osteoarthritis; Osteoarthritis (OA) is characterized degeneration of cartilage associated with global deteriorations in the joint.
- Molecular mechanisms; Molecular mechanisms for development of OA have been examined using animal models and human chondrocytes, leading to identification of some causative factors.
- Pathological conditions; Pathological conditions of OA is characterized by decreased anabolic activity and increased catabolic factors in the whole joint.
- Joint homeostasis; Joint homeostasis is an entire environment of joint that is necessary for maintaining the joint in a healthy condition.
- DMOADs; DMOADs is disease modifying osteoarthritis drugs that slow down the progression of OA or prevent OA.

4.1 Introduction

4.1.1 Osteoarthritis and Joint Homeostasis

Osteoarthritis (OA) increases during aging in association with degeneration of articular cartilage. Although OA is an aging-associated joint disease, OA is also caused or accelerated by trauma and joint injuries. Characteristic features of OA are degeneration of articular cartilage and break down of articular cartilage in association with subchondral bone changes and increased inflammatory reactions in synovium and cartilage. Joint homeostasis is balanced between anabolic factors and catabolic factors. Increased catabolic activity over anabolic activities is observed during development of OA. Although new surgical techniques and biomaterials to treat cartilage defects and osteoarthritic joints have been developed, overall clinical outcomes of the application of the solo technique were moderate mostly in a short-time period. Filardo et al. reported the clinical outcomes of autologous chondrocyte implantation (ACI) in patients with isolated degenerative cartilage lesions. Although the ACI procedure significantly improved symptoms, the overall results were lower with respect to the outcome reported in different study populations and the number of failures was higher [1]. Therefore, treating patients with degenerative cartilage is more challenging than patients with non-degenerative cartilage. Interestingly the efficacy of cartilage repair by periosteum transplantation was examined in a goat cartilage defect model with different surgical timing points. The early-treated group showed a better repair than the late-treated group [2]. This study suggests that degenerating cartilage has lower reparative capacity or the entire environment of damaged joint is less favorable for cartilage repair and restoring "joint homeostasis" is important. How joint homeostasis after a defect relates to joint homeostasis in the process of (idiopathic) OA is unknown. Therefore to understand the molecular mechanisms of OA is an essential step to treat joints with OA and to obtain better clinical outcomes after cartilage repair procedures. In this chapter, OA pathology and therapeutic approaches for OA are described.

4.2 Pathological Conditions in OA

4.2.1 Inflammation and Cytokines

Inflammation of OA is characterized by inflammation in synovium and increased inflammatory responses in cartilage [3]. A variety of inflammatory cytokines are produced by synovium and chondrocytes [4, 5]. Among such inflammatory cytokines, interleukin (IL) family members, tumor necrosis factor (TNF), have been implicated in the pathological states of OA joints. In addition, Nitric oxide (NO) has been also implicated as a causative factor [6].

4.2.2 Cartilage Degrading Enzymes

A number of studies have shown that Metalloproteinases (MMPs) [7] and A Disintegrin-like and Metalloproteinases with Thrombospondin Motifs (ADAMTS), especially ADAMTS-4 and 5 play major roles in pathogenesis of OA [8–10].

4.2.3 Subchondral Bone Changes

A radiographic assessment showed that both subchondral cortical plate and subjacent horizontal trabeculae increased in thickness in early OA phase, prior to joint space narrowing [11]. In a rat anterior cruciate ligament transection (ACLT) model, subchondral bone loss was observed within 2 weeks after surgery followed by significant increased subchondral bone volume compared with control sham knees [12]. Similarly in a canine ALCT-model, thinning of subchondral plate was observed in medial tibial metaphyses where cartilage damage was severe while it was not evident in lateral side where cartilage damage was mild, suggesting strong correlation of cartilage damage and subchondral bone changes [13].

4.3 Disease Modifying Osteoarthritis Drugs (DMOADS)

While a growing number of studies have revealed pathological conditions and numerous factors contributing to pathogenesis of OA, research in development or discovery of disease modifying osteoarthritis drugs (DMOADs) have been progressed. DMOADS target causative factors for OA, such as increased inflammation, abnormal chondrocyte hypertrophic differentiation, increased cartilage degrading enzymes, increased catalytic activities over anabolic activities in chondrocytes and subchondral bone changes. DMOADs include biochemical compounds, natural products, anti-inflammatory drugs, anti-resorptive drugs and growth factors.

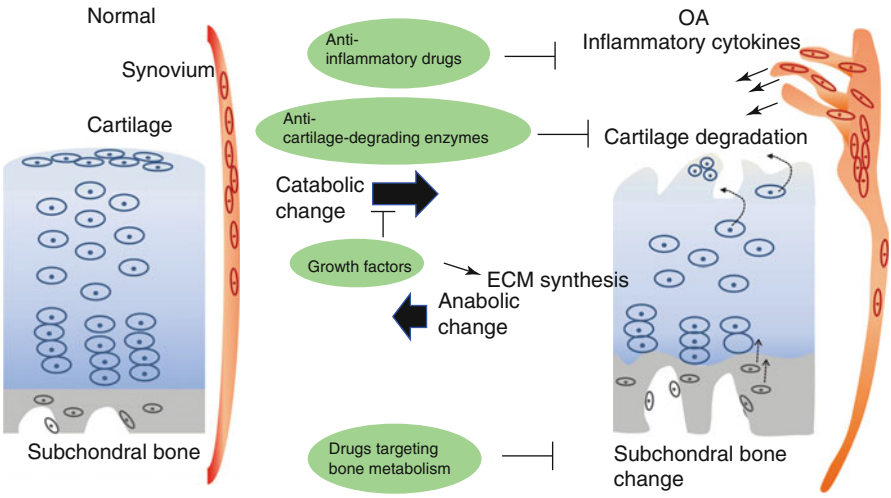

Fig. 4.1 A schema showing pathological changes during osteoarthritis (*OA*) progression and disease modifying osteoarthritis drugs (DMOADs). During OA progression, catabolic factors such as inflammation, cartilage-degrading enzymes increase while anabolic activity such as production of extracelluar matrix (*ECM*) decreases. OA is also associated with abnormal subchondral bone changes. Drugs targeting these causative factors have been being investigated as DMOADs

Those DMODAs are expected to alleviate patient's symptoms and slow down the progression of OA or prevent OA. Some pharmacological agents and growth factors are being investigated in clinical trials. In this section, some DMOADS are discussed Fig. 4.1.

4.3.1 Inhibitors of Degrading Enzymes and Inflammation

4.3.1.1 MMP Inhibitors

MMPs are believed to be a major contributor for cartilage degradation, and inhibition of MMPs is rationally attractive treatment for OA. PG-116800, a matrix-metalloproteinase (MMP) inhibitor for MMP-2, -3, -8, -9, -11, -13, and -14 decreased symptoms in patients with mild-to-moderate knee OA [14]. However the MMP inhibitor PG-116800 has also induced side effects in musculoskeletal system [15]. In addition, King et al. reported musculoskeletal side effects in 28 of 35 patients with colorectal hepatic metastases upon treatment with the MMP inhibitor, Marimastat [16]. To avoid musculoskeletal side effects, new drugs that more selectively inhibits MMP-13 were investigated. Oral administration of ALS 1-0635; a selective inhibitor of MMP-13 reduced cartilage degradation in a rat medial menisectomy model without apparent musculoskeletal toxicity [17]. More recently, new MMP-13 inhibitor, that is effective and suitable for intra-articular injection, has been reported [18]. Clinical data will follow.

4.3.1.2 Aggrecanase Inhibitors

Despite important roles of aggrecanases in the pathogenesis of OA that have been reported, there are only a few reports about aggrecanase inhibitors in OA. The oral administration of aggrecanase inhibitor, AGC-523 reduced aggrecan fragment in a rat meniscal tear model, suggested a potential preventive role in progression of OA [19] and the efficacy of AGC-523 has been now being investigated in a clinical trial.

4.3.1.3 IL1 Inhibitors

A pro-inflammatory IL1β (beta) is activated by an enzyme, IL-1β (beta) converting enzyme, before being secreted as a mature form. Pralnacasan, a non-peptide inhibitor of IL-1β (beta) converting enzyme, was tested in a collagenase-induced mouse OA model and STR/1N mice, the latter develop OA spontaneously. Pralnacasan reduced joint damage in the two experimental models of OA [20]. However a clinical trial of Pralnacasan was stopped because of its toxicity.

IL-1 receptor antagonist, anakinra has been reported to be moderately effective in patients with active RA when used as monotherapy or in combination with methotrexate [21]. To evaluate the effect of anakinra, a randomized, double-blind, placebo-controlled study was conducted. However a single intra-articular injection of anakinra did not significantly improve symptoms of the patient of OA [22]. Recently effects of a single intra-articular injection of anakinra were tested on patients with acute ACL tear in the randomized control study. Intra-articular injection of anakinra reduced knee pain and improved function over a 2-week interval compared with injection of placebo [23]. Although anakinra might play a beneficial role in reducing inflammation and pain, its effect in OA needs more research.

4.3.1.4 TNF Antagonists

As in patients with RA, efficacy of TNF antagonists was examined in patients with OA. TNFα (alpha)-antagonists infliximab and etanercept suppressed TNFα (alpha)-induced NO production from human cartilage [24]. In a clinical trial, the efficacy of infliximab was examined in 10 women with bilateral hand erosive OA. Treatment with monthly intra-articular injections of infliximab in each affected proximal and distal interphalangeal joint of the hand significantly reduced pain at the 1-year follow-up. There was a tendency that the treatment with infliximab slows worsening of the radiological score in the hand although it failed to reach statistically significant difference at the 12-month follow-up [25]. In an open-label pilot trial, 12 patients with erosive hand OA received adalimumab 40 mg every other week for 12 weeks. The treatment with adalimumab did not significantly improve the symptoms although modest improvements were observed [26]. Recently results of another randomized double-blind were reported. In the clinical trial, 60 patients with erosive hand OA received 40 mg adalimumab or placebo subcutaneously every 2 weeks

during a 12-month. The treatment with adalimumab significantly delayed the progression of joint damage compared to placebo [27].

4.3.1.5 iNOS Inhibitor

Nitric oxide has been suggested to play important role in pathogenesis of OA In nitric oxide synthase (NOS2)-deficient mice, cartilage proteoglycan depletion induced by the intra-articular injection of Zymosan was markedly reduced [28]. In an experimental dog OA model, administration of N-iminoethyl-L-lysine (L-NIL), a selective inhibitor of inducible nitric oxide synthase (iNOS), orally as a liquid solution decreased the size of the cartilage lesions and reducing the activity of metalloproteases in cartilage and the production of IL-1β (beta) by synovium [29, 30]. The inhibitory effect of the iNOS inhibitor appears to be partially through reduction in production of major catabolic factors such as MMP, IL-1β (beta), peroxynitrite and cyclooxgenase (COX)-2 expression [31]. A clinical trial to examine the disease modifying efficacy of iNOS inhibitor, SD-6010, in overweight and obese subjects with knee OA has been recently completed [32]. The treatment of SD-6010 significantly reduced the rate of joint space narrowing in the obese patients with mild knee OA during the first 48 weeks. However the effect of the treatment with SD-6010 was not significant at 96 weeks and neither in the obese patients with severe OA [33]. Although it may have an effect for short time, its effect appears to be limited in obese patients.

4.3.2 Growth Factors

4.3.2.1 Fibroblast Growth Factor (FGF) -18

FGF-18 exerts anabolic effects in human articular chondrocytes, increasing matrix formation [34]. Interestingly, in a rat OA model in which OA was surgically induced by medial meniscus injury, bi-weekly intra-articular injections of FGF18 for 3 weeks increased cartilage thickness of articular surface and the joint periphery, resulted in significant reductions in cartilage degeneration scores [35]. Clinical trials of treatment of OA by intra-articular injection of FGF18 have been recently completed [36, 37]. Results of the studies are not yet available and needs to be awaited.

4.3.2.2 Bone Morphogenetic Protein (BMP)-7

BMP-7 has been reported to have potent anabolic effects on chondrocytes. Recombinant human (rh)BMP -7 stimulated proteoglycan synthesis and collagen synthesis in human chondrocytes and counteracted against the down-regulation of

proteoglycan synthesis induced by low doses of IL-1β (beta) [38, 39]. In addition, stimulatory effects of BMP-7 on cartilage repair have been reported [40]. RhBMP-7 promoted repair of full-thickness osteochondral defects in a dog [41] and in a goat [42] model. Furthermore the efficacy of intra-articular injection of BMP-7 was examined in a sheep impact cartilage injury model. Sheep knee joints that received rhBMP-7 immediately after and 3 weeks after injury exhibited less cartilage damage compared with the non-injected group [43]. Similarly weekly intra-articular injection BMP-7 prevented OA progression in a rabbit ACLT model without obvious adverse effects on the joint [44] and inhibited OA progression induced by excessive treadmill running in rats [45]. In a phase I clinical trial, double-blind, randomized, placebo-controlled, was conducted to examine the safety and efficacy of BMP-7 for treatment of patients with knee OA. In the phase I clinical trial, no major adverse events was observed and injection of 0.1 and 0.3 mg BMP-7 more improved the symptoms of the patients than injection of placebo [46]. A phase II clinical trial to further examine the efficacy of BMP-7 in OA has been also completed [47].although the results have not yet been reported. Currently intra-articular injection of BMP-7 appears to be promising for treatment of OA, but it needs more research to determine whether the injection of BMP-7 can delay progression of OA and long term effect of the injection.

4.3.2.3 Platelet-Rich Plasma (PRP)

Platelets are known to contain a variety of growth factors, such as FGF-2, insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), Tansforming Growth Factor (TGF) β (beta), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF). PRP is made from patient's peripheral blood followed by centrifuge, producing platelet concentrates. Because PRP is relatively safe and easy to prepare, PRP has gained popularity in orthopaedic field for enhancing healing of bone, ligament injury, muscle injury and tendinopathy [48, 49].

The efficacy of PRP injection in treatment for patients with cartilage degeneration and OA was also examined. Intra-articular injection of PRP reduced pain and improved clinical scores over the 6 month-period although its effect decreased at the longer follow-up [50, 51]. In addition, PRP injections more improved clinical symptoms than hyaluronic acid injections [52, 53]. Although PRP can be effective treatment for OA, its disease modifying effect of OA has not been clarified. The effect of PRP might be through anti-inflammatory effects [54, 55] rather than anabolic effect on cartilage. In a pilot study, PRP was added to the autologous matrix-induced chondrogenesis (AMIC) technique to treat cartilage defect in the patella. However the addition of PRP to AMIC did not produce obvious beneficial effects [56]. One of the important issues in research of PRP is methodological variations in preparation of PRP. Currently no standardized technique exists and the differences in preparation techniques, making it difficult to obtain generalized valid data. Further detailed basic studies about efficacy of PRP in cartilage regeneration and OA are required.

4.3.3 Drugs Targeting Subchondral Bone Changes

4.3.3.1 Calcitonin

Calcitonin has been widely used for treatment for osteoporosis it has also been introduced for OA treatment based on the hypothesis that normalization of subchondral bone could prevent OA progression. In a dog ACLT model, reduced bone mineral density (BMD) was observed and the treatment of calcitonin by nasal spray prevented the reduction of BMD and reduced cartilage break down caused by the ACLT [57, 58]. A randomized, double blind, placebo-controlled clinical trial was conducted in 152 postmenopausal women. Oral intake of the 1.0 mg salmon calcitonin reduced urinary excretion of C-terminal telopeptide of collagen type II (CTX-II), suggested an effect of calcitonin in prevention of OA progression [59]. Similarly, in clinical trials including 41 patients, oral intake of salmon calcitonin for 84 days significantly decreased in the levels of CTX-II, C2C, and MMP-13 [60]. The effect of the calcitonin could exert through not only on changes in BMD but also direct effect on cartilage [61, 62]. Calcitonin receptor was expressed in chondrocytes and the treatment of calcitonin stimulated the production of proteoglycan and pro-peptides of collagen type II in human OA cartilage explants [63]. Very recently, Sondergaard et al. reported that OA progression induced by destabilization of medial meniscus was significantly reduced in transgenic mice over-expressing salmon calcitonin compared with in wild type mice [64]. A clinical trial examining efficacy and safety of oral Salmon calcitonin in patients with knee has been recently completed [65].

4.3.3.2 Bisphosophonates

Bisphosphonates have been also used for osteoporosis and they were expected to improve the quality of subchondral bone in OA. In a rat ACLT model, treatment of alendronate subcutaneouslly suppressed subchondral bone resorption and reduced the incidence and area of osteophyte formation [66]. Similarly in a rabbit ACLT model, alendronate reduced subchondral bone resorption and delayed the cartilage degeneration [67, 68]. One study shows that among 818 postmenopausal women who received alendronate and estrogen had less subchondral bone attrition and bone marrow edema-like abnormalities in the knee [69]. In addition, the treatment with Alendronate significantly reduced knee pain assessed by WOMAC scores in the elderly women [69]. In a double-blind placebo-controlled clinical study including 231 patients with mild to moderate knee OA, treatment with 15 mg risedronate improved the WOMAC index. In addition, there was a trend that risedronate delays joint space narrowing [70]. In a relatively large study in which 2,483 patients with medial compartment knee were enrolled, risedronate reduced symptoms of OA and reduced C-terminal crosslinking telopeptide of type II collagen, a cartilage degradation marker. However the treatments with risedronate did not significantly reduce

joint space narrowing assessed by radiography [71]. Very recently it has been reported that the intravenous infusion of zoledronic acid reduced knee pain and the size of the bone marrow lesion are in patients with knee OA [72].

4.4 Conclusions

Recent growing number of basic OA research has provided new insights into pathogenesis of OA and also has led to clinical trials for treatment of OA. Despite numerous pharmacological interventions for treatment of OA have been tried, to date none of pharmacological drugs has shown definite disease modifying effect. Therefore currently early diagnosis and intervention for preventing progression of OA before causing global alterations in joint homeostasis, seems to be an important approach for producing successful results. More research is required to discover new therapeutic approach and effective treatments for OA.

References

1. Filardo G, Kon E, Di Martino A, Patella S, Altadonna G, Balboni F, Bragonzoni L, Visani A, Marcacci M. Second-generation arthroscopic autologous chondrocyte implantation for the treatment of degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2012;20(9):1704–13.
2. Saris DB, Dhert WJ, Verbout AJ. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair. J Bone Joint Surg Br. 2003;85:1067–76.
3. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol. 2010;6:625–35.
4. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 2011;7:33–42.
5. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol. 2011;23:471–8.
6. Abramson SB. Osteoarthritis and nitric oxide. Osteoarthritis Cartilage. 2008;16 Suppl 2:S15–20.
7. Little CB, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, et al. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis Rheum. 2009;60:3723–33.
8. Stanton H, Rogerson FM, East CJ, Golub SB, Lawlor KE, Meeker CT, et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. Nature. 2005;434:648–52.
9. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature. 2005;434:644–8.
10. Little CB, Meeker CT, Golub SB, Lawlor KE, Farmer PJ, Smith SM, et al. Blocking aggrecanase cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair. J Clin Invest. 2007;117:1627–36.
11. Buckland-Wright C. Subchondral bone changes in hand and knee osteoarthritis detected by radiography. Osteoarthritis Cartilage. 2004;12(Suppl A):S10–9.
12. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, le Duong T. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. Bone. 2006;38:234–43.

13. Intema F, Hazewinkel HA, Gouwens D, Bijlsma JW, Weinans H, Lafeber FP, Mastbergen SC. In early OA, thinning of the subchondral plate is directly related to cartilage damage: results from a canine ACLT-meniscectomy model. Osteoarthritis Cartilage. 2010;18:691–8.
14. Krzeski P, Buckland-Wright C, Bálint G, Cline GA, Stoner K, Lyon R, Beary J, Aronstein WS, Spector TD. Development of musculoskeletal toxicity without clear benefit after administration of PG-116800, a matrix metalloproteinase inhibitor, to patients with knee osteoarthritis: a randomized, 12-month, double-blind, placebo-controlled study. Arthritis Res Ther. 2007;9: R109.
15. Hudson MP, Armstrong PW, Ruzyllo W, Brum J, Cusmano L, Krzeski P, Lyon R, Quinones M, Theroux P, Sydlowski D, Kim HE, Garcia MJ, Jaber WA, Weaver WD. Effects of selective matrix metalloproteinase inhibitor (PG-116800) to prevent ventricular remodeling after myocardial infarction: results of the PREMIER (Prevention of Myocardial Infarction Early Remodeling) trial. J Am Coll Cardiol. 2006;48:15–20.
16. King J, Zhao J, Clingan P, Morris D. Randomised double blind placebo control study of adjuvant treatment with the metalloproteinase inhibitor, Marimastat in patients with inoperable colorectal hepatic metastases: significant survival advantage in patients with musculoskeletal side-effects. Anticancer Res. 2003;23(1B):639–45.
17. Baragi VM, Becher G, Bendele AM, Biesinger R, Bluhm H, Boer J, Deng H, Dodd R, Essers M, Feuerstein T, Gallagher Jr BM, Gege C, Hochgürtel M, Hofmann M, Jaworski A, Jin L, Kiely A, Korniski B, Kroth H, Nix D, Nolte B, Piecha D, Powers TS, Richter F, Schneider M, Steeneck C, Sucholeiki I, Taveras A, Timmermann A, Van Veldhuizen J, Weik J, Wu X, Xia B. A new class of potent matrix metalloproteinase 13 inhibitors for potential treatment of osteoarthritis: evidence of histologic and clinical efficacy without musculoskeletal toxicity in rat models. Arthritis Rheum. 2009;60:2008–18.
18. Gege C, Bao B, Bluhm H, Boer J, Gallagher BM, Korniski B, Powers TS, Steeneck C, Taveras AG, Baragi VM. Discovery and evaluation of a non-Zn chelating, selective matrix metalloproteinase 13 (MMP-13) inhibitor for potential intra-articular treatment of osteoarthritis. J Med Chem. 2012;55:709–16.
19. Chockalingam PS, Sun W, Rivera-Bermudez MA, Zeng W, Dufield DR, Larsson S, Lohmander LS, Flannery CR, Glasson SS, Georgiadis KE, Morris EA. Elevated aggrecanase activity in a rat model of joint injury is attenuated by an aggrecanase specific inhibitor. Osteoarthritis Cartilage. 2011;19:315–23.
20. Rudolphi K, Gerwin N, Verzijl N, van der Kraan P, van den Berg W. Pralnacasan, an inhibitor of interleukin-1beta converting enzyme, reduces joint damage in two murine models of osteoarthritis. Osteoarthritis Cartilage. 2003;11:738–46.
21. Furst DE. Anakinra: review of recombinant human interleukin-I receptor antagonist in the treatment of rheumatoid arthritis. Clin Ther. 2004;26:1960–75.
22. Chevalier X, Goupille P, Beaulieu AD, Burch FX, Bensen WG, Conrozier T, Loeuille D, Kivitz AJ, Silver D, Appleton BE. Intraarticular injection of anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-controlled study. Arthritis Rheum. 2009;61:344–52.
23. Kraus VB, Birmingham J, Stabler TV, Feng S, Taylor DC, Moorman 3rd CT, Garrett WE, Toth AP. Effects of intraarticular IL1-Ra for acute anterior cruciate ligament knee injury: a randomized controlled pilot trial (NCT00332254). Osteoarthritis Cartilage. 2012;20(4):271–8.
24. Vuolteenaho K, Moilanen T, Hämäläinen M, Moilanen E. Effects of TNFalpha-antagonists on nitric oxide production in human cartilage. Osteoarthritis Cartilage. 2002;10:327–32.
25. Fioravanti A, Fabbroni M, Cerase A, Galeazzi M. Treatment of erosive osteoarthritis of the hands by intra-articular infliximab injections: a pilot study. Rheumatol Int. 2009;29:961–5.
26. Magnano MD, Chakravarty EF, Broudy C, Chung L, Kelman A, Hillygus J, Genovese MC. A pilot study of tumor necrosis factor inhibition in erosive/inflammatory osteoarthritis of the hands. J Rheumatol. 2007;34:1323–7.
27. Verbruggen G, Wittoek R, Cruyssen BV, Elewaut D. Tumour necrosis factor blockade for the treatment of erosive osteoarthritis of the interphalangeal finger joints: a double blind, randomised trial on structure modification. Ann Rheum Dis. 2012;71(6):891–8.

28. van den Berg WB, van de Loo F, Joosten LA, Arntz OJ. Animal models of arthritis in NOS2-deficient mice. Osteoarthritis Cartilage. 1999;7:413–5.
29. Pelletier JP, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, Di Battista JA, Martel-Pelletier J. Reduced progression of experimental osteoarthritis in vivo by selective inhibition of inducible nitric oxide synthase. Arthritis Rheum. 1998;41:1275–86.
30. Pelletier J, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, Martel-Pelletier J. Reduction in the structural changes of experimental osteoarthritis by a nitric oxide inhibitor. Osteoarthritis Cartilage. 1999;7:416–8.
31. Pelletier JP, Lascau-Coman V, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, Martel-Pelletier J. Selective inhibition of inducible nitric oxide synthase in experimental osteoarthritis is associated with reduction in tissue levels of catabolic factors. J Rheumatol. 1999;26:2002–14.
32. A long-term, placebo-controlled X-ray study investigating the safety and efficacy of SD-6010 in subjects with osteoarthritis of the knee (ITIC). ClinicalTrials.gov identifier: NCT00565812. Available from. http://clinicaltrials.gov/ct2/show/NCT00565812.
33. Hellio le Graverand MP, Clemmer RS, Redifer P, Brunell RM, Hayes CW, Brandt KD, Abramson SB, Manning PT, Miller CG, Vignon E. A 2-year randomised, double-blind, placebo-controlled, multicentre study of oral selective iNOS inhibitor, cindunistat (SD-6010), in patients with symptomatic osteoarthritis of the knee. Ann Rheum Dis. 2013;72:187–95.
34. Ellman MB, An HS, Muddasani P, Im HJ. Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene. 2008;420:82–9.
35. Moore EE, et al. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. Osteoarthritis Cartilage. 2005;13:623–31.
36. A multicenter study of rhFGF 18 in patients with knee osteoarthritis not requiring surgery. ClinicalTrials.gov identifier: NCT01033994. Available from. http://clinicaltrials.gov/ct2/show/NCT01033994.
37. Study of AS902330 (rhFGF-18) administered intra-articularly in patients with knee primary osteoarthritis who are candidates for total knee replacement. ClinicalTrials.gov identifier: NCT00911469. Available from. http://clinicaltrials.gov/ct2/show/study/NCT00911469.
38. Flechtenmacher J, Huch K, Thonar EJ, Mollenhauer JA, Davies SR, Schmid TM, Puhl W, Sampath TK, Aydelotte MB, Kuettner KE. Recombinant human osteogenic protein 1 is a potent stimulator of the synthesis of cartilage proteoglycans and collagens by human articular chondrocytes. Arthritis Rheum. 1996;39:1896–904.
39. Huch K, Wilbrink B, Flechtenmacher J, Koepp HE, Aydelotte MB, Sampath TK, Kuettner KE, Mollenhauer J, Thonar EJ. Effects of recombinant human osteogenic protein 1 on the production of proteoglycan, prostaglandin E2, and interleukin-1 receptor antagonist by human articular chondrocytes cultured in the presence of interleukin-1beta. Arthritis Rheum. 1997;40:2157–61.
40. Chubinskaya S, Hurtig M, Rueger DC. OP-1/BMP-7 in cartilage repair. Int Orthop. 2007;31:773–81.
41. Cook SD, Patron LP, Salkeld SL, Rueger DC. Repair of articular cartilage defects with osteogenic protein-1 (BMP-7) in dogs. J Bone Joint Surg Am. 2003;85-A Suppl 3:116–23.
42. Louwerse RT, Heyligers IC, Klein-Nulend J, Sugihara S, van Kampen GP, Semeins CM, Goei SW, de Koning MH, Wuisman PI, Burger EH. Use of recombinant human osteogenic protein-1 for the repair of subchondral defects in articular cartilage in goats. J Biomed Mater Res. 2000;49:506–16.
43. Hurtig M, Chubinskaya S, Dickey J. Rueger D.BMP-7 protects against progression of cartilage degeneration after impact injury. J Orthop Res. 2009;27:602–11.
44. Hayashi M, Muneta T, Takahashi T, Ju YJ, Tsuji K, Sekiya I. Intra-articular injections of bone morphogenetic protein-7 retard progression of existing cartilage degeneration. J Orthop Res. 2010;28:1502–6.
45. Sekiya I, Tang T, Hayashi M, Morito T, Ju YJ, Mochizuki T, Muneta T. Periodic knee injections of BMP-7 delay cartilage degeneration induced by excessive running in rats. J Orthop Res. 2009;27:1088–92.

46. Hunter DJ, Pike MC, Jonas BL, Kissin E, Krop J, McAlindon T. Phase 1 safety and tolerability study of BMP-7 in symptomatic knee osteoarthritis. BMC Musculoskelet Disord. 2010;11:232.
47. Dose finding study of bone morphogenetic protein 7 (BMP-7) in subjects with osteoarthritis (OA) of the knee. ClinicalTrials.gov identifier: NCT01111045. Available from. http://clinicaltrials.gov/ct2/show/NCT01111045.
48. Mishra A, Woodall Jr J, Vieira A. Treatment of tendon and muscle using platelet-rich plasma. Clin Sports Med. 2009;28:113–25.
49. Lopez-Vidriero E, Goulding KA, Simon DA, Sanchez M, Johnson DH. The use of platelet-rich plasma in arthroscopy and sports medicine: optimizing the healing environment. Arthroscopy. 2010;26:269–78.
50. Kon E, Buda R, Filardo G, Di Martino A, Timoncini A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2010;18:472–9.
51. Filardo G, Kon E, Buda R, Timoncini A, Di Martino A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2011;19:528–35.
52. Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, Fornasari PM, Giannini S, Marcacci M. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. Arthroscopy. 2011;27:1490–501.
53. Spaková T, Rosocha J, Lacko M, Harvanová D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. Am J Phys Med Rehabil. 2012;91(5):411–7.
54. van Buul GM, Koevoet WL, Kops N, Bos PK, Verhaar JA, Weinans H, Bernsen MR, van Osch GJ. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. Am J Sports Med. 2011;39:2362–70.
55. Lippross S, Moeller B, Haas H, Tohidnezhad M, Steubesand N, Wruck CJ, Kurz B, Seekamp A, Pufe T, Varoga D. Intraarticular injection of platelet-rich plasma reduces inflammation in a pig model of rheumatoid arthritis of the knee joint. Arthritis Rheum. 2011;63:3344–53.
56. Dhollander AA, De Neve F, Almqvist KF, Verdonk R, Lambrecht S, Elewaut D, Verbruggen G. Verdonk PCAutologous matrix-induced chondrogenesis combined with platelet-rich plasma gel: technical description and a five pilot patients report. Knee Surg Sports Traumatol Arthrosc. 2011;19:536–42.
57. Behets C, Williams JM, Chappard D, Devogelaer JP, Manicourt DH. Effects of calcitonin on subchondral trabecular bone changes and on osteoarthritic cartilage lesions after acute anterior cruciate ligament deficiency. J Bone Miner Res. 2004;19:1821–6.
58. El Hajjaji H, Williams JM, Devogelaer JP, Lenz ME, Thonar EJ, Manicourt DH. Treatment with calcitonin prevents the net loss of collagen, hyaluronan and proteoglycan aggregates from cartilage in the early stages of canine experimental osteoarthritis. Osteoarthritis Cartilage. 2004;12:904–11.
59. Bagger YZ, Tankó LB, Alexandersen P, Karsdal MA, Olson M, Mindeholm L, Azria M, Christiansen C. Oral salmon calcitonin induced suppression of urinary collagen type II degradation in postmenopausal women: a new potential treatment of osteoarthritis. Bone. 2005;37:425–30.
60. Manicourt DH, Azria M, Mindeholm L, Thonar EJ, Devogelaer JP. Oral salmon calcitonin reduces Lequesne's algofunctional index scores and decreases urinary and serum levels of biomarkers of joint metabolism in knee osteoarthritis. Arthritis Rheum. 2006;54:3205–11.
61. Karsdal MA, Sondergaard BC, Arnold M, Christiansen C. Calcitonin affects both bone and cartilage: a dual action treatment for osteoarthritis? Ann N Y Acad Sci. 2007;1117:181–95.
62. Karsdal MA, Leeming DJ, Dam EB, Henriksen K, Alexandersen P, Pastoureau P, Altman RD, Christiansen C. Should subchondral bone turnover be targeted when treating osteoarthritis? Osteoarthritis Cartilage. 2008;16:638–46.

63. Sondergaard BC, Madsen SH, Segovia-Silvestre T, Paulsen SJ, Christiansen T, Pedersen C, Bay-Jensen AC, Karsdal MA. Investigation of the direct effects of salmon calcitonin on human osteoarthritic chondrocytes. BMC Musculoskelet Disord. 2010;11:62.
64. Sondergaard BC, Catala-Lehnen P, Huebner AK, Bay-Jensen AC, Schinke T, Henriksen K, Schilling S, Haberland M, Nielsen RH, Amling M, Karsdal MA. Mice over-expressing salmon calcitonin have strongly attenuated osteoarthritic histopathological changes after destabilization of the medial meniscus. Osteoarthritis Cartilage. 2012;20:136–43.
65. Efficacy and safety of oral salmon calcitonin in patients with knee osteoarthritis (OA 2 study). ClinicalTrials.gov identifier: NCT00704847. Available from. http://clinicaltrials.gov/ct2/show/NCT00704847.
66. Hayami T, Pickarski M, Wesolowski GA, McLane J, Bone A, Destefano J, Rodan GA, le Duong T. The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. Arthritis Rheum. 2004;50:1193–206.
67. Zhang L, Hu H, Tian F, Song H, Zhang Y. Enhancement of subchondral bone quality by alendronate administration for the reduction of cartilage degeneration in the early phase of experimental osteoarthritis. Clin Exp Med. 2011;11:235–43.
68. Shirai T, Kobayashi M, Nishitani K, Satake T, Kuroki H, Nakagawa Y, Nakamura T. Chondroprotective effect of alendronate in a rabbit model of osteoarthritis. J Orthop Res. 2011;29:1572–7.
69. Carbone LD, Nevitt MC, Wildy K, Barrow KD, Harris F, Felson D, Peterfy C, Visser M, Harris TB, Wang BW, Kritchevsky SB, Health Aging and Body Composition Study. The relationship of antiresorptive drug use to structural findings and symptoms of knee osteoarthritis. Arthritis Rheum. 2004;50:3516–25.
70. Spector TD, Conaghan PG, Buckland-Wright JC, Garnero P, Cline GA, Beary JF, Valent DJ, Meyer JM. Effect of risedronate on joint structure and symptoms of knee osteoarthritis: results of the BRISK randomized, controlled trial [ISRCTN01928173]. Arthritis Res Ther. 2005;7:R625–33.
71. Bingham 3rd CO, Buckland-Wright JC, Garnero P, Cohen SB, Dougados M, Adami S, Clauw DJ, Spector TD, Pelletier JP, Raynauld JP, Strand V, Simon LS, Meyer JM, Cline GA, Beary JF. Risedronate decreases biochemical markers of cartilage degradation but does not decrease symptoms or slow radiographic progression in patients with medial compartment osteoarthritis of the knee: results of the two-year multinational knee osteoarthritis structural arthritis study. Arthritis Rheum. 2006;54:3494–507.
72. Laslett LL, Doré DA, Quinn SJ, Boon P, Ryan E, Winzenberg TM, Jones G. Zoledronic acid reduces knee pain and bone marrow lesions over 1 year: a randomised controlled trial. Ann Rheum Dis. 2012;71(8):1322–8.

Chapter 5
Bioreactor Tissue Engineering for Cartilage Repair

Gian M. Salzmann and Martin J. Stoddart

Abstract Already *in utero* developing articular cartilage is exposed to, and is as well dependent of, a certain degree of mechanical stimulation (Brommer et al., Equine Vet J 37(2):148–154, 2005). Likewise, adult hyaline cartilage is strongly regulated by a frequent input of dynamic load. It is now clear that articular chondrocytes and mesenchymal stem cells clearly benefit from physical stimuli *in vitro* (Grad et al., Clin Orthop Relat Res 469(10):2764–2772, 2011). The term preconditioning has evolved in the field of cartilage tissue engineering, roughly describing an enhanced *in vitro* chondrogenesis by application of different stimuli which aims to generate more functional constructs for implantation. Physical stimulation is one way to precondition cells and is commonly realized by the use of bioreactors. Bioreactor systems can closely reproduce the *in vivo* environment, and can provoke a highly efficient chondrogenesis. They offer the possibility to evaluate novel therapeutic approaches while avoiding ethically challenging animal models. Mechanical load can be applied by tension, hydrostatic pressure, compression, shear, and any combination of these stimuli. In particular, the combination of compression and shear very closely resembles a human joint situation (Grad et al., Tissue Eng 12(11):3171–3179, 2006). Physical stimulation of articular chondrocytes and mesenchymal stem cells can result in an upregulation of the classical chondrogenic markers such as collagen 2, proteoglycan-4 and aggrecan. Furthermore it has been shown that cell-matrix constructs that have been subjected to physical loading highlighted an organized cell-matrix alignment in the direction of the mechanical stimulation, when compared to free-

G.M. Salzmann, MD (✉)
Department of Orthopaedic and Trauma Surgery,
University Medical Center, Albert-Ludwigs University Freiburg,
Hugstetter Strasse 55, Freiburg 79106, Germany
e-mail: gian.salzmann@uniklinik-freiburg.de

M.J. Stoddart
AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_5,

swelling cell-matrix constructs (Salzmann et al., Tissue Eng Part A 15(9): 2513–2524, 2009). Significantly increased mechanical properties have also been reported following mechanical stimulation *in vitro*. However, an effective chondrogenesis can only be generated when the stimulus is correctly applied in terms of modulus, frequency, duration and force. Furthermore, subjected cells have to be embedded within a 3-D environment which provides a sufficient mechanical backbone to withstand and transmit mechanical loads while in parallel still permitting effective chondrogenesis. Novel bioreactor tissue engineering approaches aiming for articular cartilage repair may focus on stem cell chondrogenesis combining physical with chemical stimuli, which have been shown to be very efficient in promoting *in vitro* chondrogenesis (Li et al., J Cell Physiol 227(5): 2003–2012, 2011).

Keywords Bioreactor • Tissue Engineering • Cartilage • Chondrocyte • Stem cells • Knee • Osteoarthritis • Biomechanical Stimuli • Preconditioning

Key Points

- Articular cartilage serves a predominantly biomechanical function.
- Hyaline cartilage is dependent on mechanical input to maintain function and integrity.
- Dynamic shear, compression, fluid flow and hydrostatic pressure are the biomechanical hallmarks within articulating joints.
- During gait the human walking cadence is normally at the range of 1 Hz by which articular cartilage experiences stresses between 3 and 10 Mpa with a strain of 10–15 %.
- Articular chondrocytes mainly increase collagen type 2, 6, 9, 11, aggrecan, COMP, PRG-4 and glycosaminoglycan expression as a physiological response to mechanical load.
- Bioreactors are devices to culture tissue by provision of a controllable, mechanically active environment.
- Bioreactors can be operated in order to improve, but also study, the structure, properties and integration of tissue.
- Bioreactors are capable of improving tissue construct size, cellularity and molecular composition of tissue such as cartilage by biomechanical modulation.
- Flow perfusion, hydrostatic pressure, rotating wall, spinner flask, compression, shear or combined stimuli are the most common modes of bioreactor stimulation.
- Bioreactor, functional tissue engineering is a rapidly increasing experimental and also early clinical field to study and precondition articular cartilage.

5.1 Introduction

Tissue Engineering is a field at the interface of engineering and biology which aims to repair or replace injured or diseased tissues and organs, such as articular cartilage. Since articular cartilage serves a predominantly biomechanical function, tailored tissue engineering principles are required. To meet this challenge a new paradigm termed functional tissue engineering is emphasizing biomechanical considerations during design and development of cell-scaffold constructs [1]. Bioreactors are devices to culture tissue by provision of a controllable, mechanically active environment which can be operated in order to improve, but also study, the structure, properties and integration of tissue. Bioreactors are capable of improving tissue construct size, cellularity and molecular composition.

It is becoming increasingly apparent that cartilage defects are frequently osteochondral lesions. The osteochondral junction represents the important backbone for the overlying hyaline cartilage. Effective articular cartilage repair can only be achieved when there is also healthy underlying bone [2]. Not only osteochondral lesions, but as well bone diseases such as infection, fractures, osteoarthritis or osteoporosis are becoming a major medical and socioeconomic problem. In this context, *ex vivo* tissue engineering strategies for *de novo* generation of bone tissue is also a major field of interest. The use of autologous bone-forming cells and three-dimensional porous scaffold materials are, comparable to cartilage tissue engineering, the two main components to realize bone tissue engineering [3]. Furthermore, different tissue engineering protocols have already realized tissue engineering production of osteochondral regenerates [4, 5]. However, this chapter is focussing on bioreactor tissue engineering with regard to articular cartilage. Nevertheless, there are many similarities concerning basic bioreactor principles, as well as typical bioreactor associated drawbacks such as insufficient nutrient and oxygen transport and removal of waste products from the cells at the interior of the scaffold.

An intra-articular environment can be regarded as harsh in terms of mechanical and chemical provocation. In particular, hyaline cartilage, which is covering the ends of long bones, is subjected to multiple repetitive load cycles and yet often produces a lifetime of pain free motion and weightbearing. It is a prerequisite for mammals to move and survive without lasting damage. Therefore, articular cartilage is a highly developed tissue in order to fulfil this task and withstand endless cycles of near frictionless motion during locomotion. The sacrifice associated with this specialization is a minimal ability to heal when damaged in post-puberty. Current cartilage repair procedures regularly fail to completely restore existing defects. Hyaline cartilage lesions are very frequent among adult subjects [6]. Furthermore, they often remain clinically silent initially, while morphologically progressing [7]. Synchronous to that, the well-balanced joint homeostasis can turn into an unstable equilibrium [8]. Both play a part in avicious circle, which may be initiated following blunt trauma, cruciate ligament ruptures, meniscal lesions or patella dislocation, but remain unnoticed over decades [9]. Only efficient cartilage repair techniques may arrest the progression of continuous degeneration before other means of

osteoarthritis prevention become available [10, 11]. Although current cartilage repair procedures often struggle to result in a truly efficient repair, tissue engineering applications are on the threshold of clinical implementation in order to improve *in vivo* transplant performance. These applications may remain close to currently available cartilage repair techniques, such as chondrocyte transplantation.

5.2 Cartilage Repair

Already in the 20th century a great variety of surgical techniques have been proposed in order to address existing articular cartilage lesions. Until today, three basically different surgical options have evolved and are in frequent worldwide use. However, there remains controversy on how and when which surgical technique should be applied. A worldwide accepted standard guideline does not exist [12]. Arthroscopic microfracturing aims for *in situ* repair of the cartilage defect. Bone marrow stem cells (BMSCs) migrate into the defect and settle within the debrided lesion. It is anticipated that these cells differentiate into chondrocytes leading to a phenotypically correct repair of the lesion. Osteochondral transplantation, which can be achieved using open, mini-open or arthroscopic techniques, is aiming to replace damaged tissue immediately. Not only the chondral surface, but the underlying bone is also extracted using hollow cutters and consecutively replaced by autologous or allogenous osteochondral cylinders, which have the same dimensions. Defective cartilage is immediately replaced by hyaline cartilage, while at the same time addressing the underlying bone. Autologous chondrocyte implantation (ACI) aims to regenerate the cartilage defect by using isolated autologous chondrocytes, which have been previously obtained from the joint and expanded *in vitro*. The surgical techniques all have their specific advantages and disadvantages. On any account, they have in common that *restitutio ad integrum* commonly does not occur following the post-operative intervention [13]. While early randomized controlled trials comparing operative techniques against each other remained mostly inconclusive [14–16], current evidence has shown superiority of cell-based methods, autologous chondrocyte transplantation, in comparison to microfracturing [17] as well when comparing with osteochondral transplantation [18]. These aspects become particularly true when horizontally large (above 3–4 cm^2) defects are concerned. Furthermore, a correlation between the quality of the repair tissue and clinical symptoms have been described. When mostly hyaline and hyaline-like tissue evolves at the defective site, the likelihood of a satisfying clinical outcome is clearly increased [19]. It was shown during clinical ACI that initial strong collagen type 2 and CD-44 expression within the chondrocytes is significantly correlated with an improved clinical outcome [20]. Tissue quality can be regarded as one major aspect when articular cartilage repair is concerned. Morphologically and thus functionally well-developed tissue following cartilage repair procedures is more likely correlated with a satisfying long-term clinical outcome than the opposite [21, 22]. The mechanical properties of articular cartilage are clearly related to its well-balanced

composition of collagens and proteoglycans. Those are maintained by the only co-existing compound within articular cartilage, the chondrocyte. This simple two-component structure was initially considered to be easy to reproduce and has been considered a perfect target for tissue engineering applications. However, hyaline cartilage is constantly dependent on a wide array of biomechanical und biochemical input in order to maintain its structure and integrity. It has been shown in an ankle fracture model that, when knee joint cartilage is not mechanically stimulated, it is suffering from atrophy when compared to loaded control [23]. It also has been shown that certain cartilage specific growth factors are upregulated following the postoperative course of surgically induced cartilage repair [24]. Proinflammatory cytokines, such as interleukin beta 1 and tumor necrosis factor alpha, play a major role within a functioning intra-articular environment [9]. Maintenance of correctly operating hyaline cartilage is owing to a required constant physicobiochemical input in order to provide a well-balanced joint homeostasis. Bearing this in mind, the postoperative rehabilitation following articular cartilage intervention is critical. However, it is required that the respective transplanttraverses certain stages of maturation [25]. It may take up 2–3 years post-implantation until a transplant can be regarded morphologically mature and fully ready to use [26]. It was shown during laboratory analysis of different clinical ACI products that the collagen 2/collagen 1 ratio was fardistant from that of native tissue at the time of transplantation [27]. This may be expected since the biopsies that are taken prior to ACI are usually small and contain few cells. Therefore, *in vitro* expansion procedures are required to increase cell numbers and it is known that proliferation is antagonistic to differentiation. Significant chondrocyte dedifferentiation occurs, with a concomitant increase in collagen I expression, as cell numbers and time in 2D monolayer, increase [28]. Progressive rehabilitation schemes have shown an improved outcome when comparing with traditional schemes following Matrix-assisted Chondrocyte Implantation (m-ACI) [29], but currently there is not enough evidence to constitute exact time points when a patient/a transplant is completely recovered. Related to failed complete restitution of articular cartilage, the transplantation of very immature tissue and consequently long patient rehabilitation tissue engineering principles are required for future cartilage defect repair.

5.3 Tissue Engineering

Tissue engineering aims to overcome limitations of traditional therapies by repairing or replacing damaged tissue with a *de novo* tissue that resembles the native tissue. These principles clearly aim for improved tissue quality at the time of transplantation in order to enhance the respective performance *in vivo*. Furthermore, patient recovery can be accelerated when more mature constructs are being implanted which require less time until full maturation. The term preconditioning has emerged to describe *in vitro* procedures that better prepare transplants for natural *in vivo* environments.

Among the large number of different ways to fulfil such principles, bioreactor tissue engineering defines a major aspect when articular cartilage repair is concerned. Bioreactors follow the goal to mimic (mostly biomechanical) natural joint surroundings. In that way cells behave *in vitro* as if they were *in vivo* [30]. In response to a biomechanical input, chondrocytes produce matrix as a natural response leading to protection and preparation for future mechanical stimulation; in the way of "form follows function." Following this concept, potential transplants can be trained/preconditioned prior to re-implantation. A major challenge to overcome is the inverse relationship between tissue maturation and its potential to integrate and adapt to the healthy tissue surrounding the defect.

Various bioreactors have been developed which can apply any combination of mechanical, chemical, electrical or magnetic stimulation to enhance mass transfer and nutrient transport within seeded cells, facilitating the correct tissue development. During musculoskeletal tissue engineering, they are applied for growth of three dimensional tissues, such as cartilage, prior to implantation. *In vivo* articular cartilage is affected by different biomechanical forces, such as direct compression, tensile and shear forces, the generation of hydrostatic pressure, cyclic osmotic changes, electric gradients as well as changes in the pH. There are a multitude of bioreactor systems available of varying complexity. However, an ideal system would allow a precise control of the physiological environment of the culture. Temperature, oxygen concentration, pH value, nutrients, media flow rate, metabolite concentration and eventually as well specific tissue markers have to be kept within close limits. The culture of tissue is a non-steady state process in which parameters constantly change. Bioreactor culture has to provide nutrients and gases as the respective tissue is accustomed to *in vivo*. A bioreactor mechanical stimulus should be of a dynamic and intermittent character rather than being static pressure in order to induce chondrogenesis. There should be an adequate fluid exchange within the cultured constructs to provide every cell with nutrients. Applied biomechanical load should be physiological. While low levels of stress has been shown to remain unanswered by the cells, too strong mechanical stimulation can even result in apoptotic processes being initiated. Furthermore the importance of scaffold binding sites to transmit mechanical signals to seeded chondrocytes during the initial moments of bioreactor culture has been reported.

There are currently different options to stimulate chondrogenic cells. Certain basic principles are by now familiar. The cellular response to load is specific to the type of load applied, and this has been shown to be true across the knee joint [31]. During the same loading cycle, the lateral tibial plateau has a greater cartilage contact deformation, but lower cartilage contact area when compared with the medial compartment. Both compartments demonstrate a cartilage contact deformation of between 10 and 15 %. The rotation of the femur with respect to the tibia also varies during gait [32]. A physiological response of articular chondrocytes can be identified by the production of typical markers of hyaline cartilage. Those are generally collagen type 2, 6, 9 and 11, aggrecan as well as the different glycosaminoglycans that are attached to the protein backbone. Furthermore, a healthy response to mechanical stimulation can be detected when the cells are expressing lubricin

(proteoglycan 4, PRG-4) and cartilage oligomeric protein (COMP). Also, histological and mechanical properties have to resemble mature cartilage in order to achieve functioning transplants [33].

Human hyaline cartilage can be described as being viscoelastic, resulting from its structural and chemical properties. The dynamic equilibrium of articular cartilage is related to its biphasic system. The solid phase is represented by porous and permeable parts of the ECM consistent of a collagen mesh, non-collagenous proteins and the non-covalently bound proteoglycans. The other phase is represented by the interstitial fluid along with ions solubilized within. The fluid phase can be separated into water and ions to have three different phases within articular cartilage. Negatively charged proteoglycans are capable of binding positively charged ions and thus water (fluid phase) along the osmotic gradient. This fluid influx, and the resulting cartilage swelling, is limited by external compressive forces and the resisting tensile collagenous network to reach a steady state. The complex orchestration of the cartilaginous network only works when the ECM and chondrocytes are well-balanced. Accordingly, it is the general goal during cartilage tissue engineering procedures to simultaneously proliferate and correctly differentiate chondrocytes, which are commonly cultured three-dimensionally. Human articular cartilage is composed of 60–80 % water, 10–20 % collagen type 2, 5–7 % aggregating proteoglycans, the rest being chondrocytes. This structure develops slowly and is dependent of mechanical forces during embryonic organogenesis. The tissue constantly remodels during the lifetime of the organism, emphasising the constant need for the correct biomechanical signals to be applied.

In such, synovial joints are the constant subject of several combining physical factors resulting in reactive change of volume, pressure gradient and fluid flow. Articular cartilage is typically exposed to stresses between 3 and 10 MPa with potential peaks up to 20 MPa at the hip joint. The human walking cadence is normally at the range of 1 Hz, which increases or decreases depending on speed of locomotion. Deformation of human cartilage without pathology is commonly at around 10–15 % strain. These are the cornerstones to which bioreactor tissue engineering principles are adjusting to in order to provoke a physiological tissue response. Hence, those values are true for mature human cartilage which has previously undergone complex differentiation processes during development and therefore have to be adapted for early *in vitro* tissue engineering processes. During *in vitro* tissue engineering it has been shown that chondrocytes are capable of reacting on biomechanical stimulations and converting them into intracellular signals which are essential for the maintenance of the entire tissue. While there is still a lack of knowledge, it is known that deformation of the chondrocyte itself may take part in a mechanical signal transduction pathway. Chondrocytes can react on shifting of currents and resulting electrical fields induced by mechanical forces. Furthermore mechanosensors such as integrins have been reported to reside on the extracellular membrane which can provide direct contact with the intracellular ECM. Moreover, mechanical stimulation can result in the activation of ion channels via shifting of the membrane potential [34, 35]. The exact mechanism of load sensing is unknown, and may be dependent on the cell type used (chondrocyte versus MSC), the scaffold material in which the cells are embedded, whether the matrix permits cell

attachments and through which membrane binding proteins. It has previously been shown that the mechanoregulation of chondrocytes in agarose gels requires the cells themselves to produce extracellular matrix prior to being responsive [1]. Whether this is hydrogel specific remains to be seen.

5.4 Bioreactor Systems

There are a variety of different bioreactor-induced ways to apply mechanical load. Following uniaxial compression the tissue hydrostatic pressure is increasing related to the resistance of the negative charges within the solid phase. Hydrostatic pressure, mostly related to the fixed charged density of the proteoglycans, is increasing to prevent tissue deformation. The collagenous network does not have major effects during these processes. However, when the compression is maintained as with static compression, more fluid is extravasated off the respective construct and pressure upon the collagens is constantly increased. In parallel, less fluid can pass through the construct resulting in a more rigid solid phase. These processes take place in order to protect rigid parts of the ECM from higher load peaks. In contrast to direct compression, hydrostatic pressure does not result in macroscopic deformation of cartilage. Since the solid matrix phase of cartilage is intrinsically incompressible, no tissue deformation will occur under an external hydrostatic load. However, hydrostatic pressure is considered as one of the most important forms of loading to act on cartilage *in vivo*. In contrast to direct compression, hydrostatic pressure is commonly not capable of harming the exposed tissue. Following the external application of hydrostatic pressure, hydrostatic pressure will increase inside a subjected cell-scaffold-construct. Pressurization results in only 10 % of the load remaining as direct compression on the solid phase. Hydrostatic pressure may result in only minimal strain at the cellular level. Thus, hydrostatic pressure may have direct effects on cell membrane ion channels with a pressure-dependent change in intracellular ion concentrations. Alterations in intracellular ion concentrations lead to changes in cellular gene expression, protein production and eventually biomechanical properties. During locomotion, and thus joint movement, intermittent shear stress is applied on to the tissue surface. In response, articular cartilage does not reduce volume, but deforms its structure. Shear stress is therefore mostly affecting the upper layers of cartilage or cell-scaffold constructs. Hence, chondrocytes within the upper layer of cartilage are commonly horizontally orientated, while those in deeper layers are usually found in vertical columns. But also cells within the less flexible deeper layers are thus strongly affected by shear stress, which results in an increased collagen content. There are bioreactors capable of exposing subjected constructs to isolated stimuli, while there are also devices that are capable of a combinatory mechanical input. The latter more closely resembles the human articulating joint and thus may generate a more tissue specific response. During bioreactor tissue engineering, one can adjust the magnitude, frequency, onset, and duration of load application.

A physiological biomechanical stimulus closely resembles joint motion of a human knee joint. The interaction between subchondral bone and cartilage which is a important for load transmission and maintenance of both tissues. This interaction is hard to mimic in a bioreactor. Within which, cartilage is recognizing stimuli as a rolling movement of direct compression in concert with a generation of shear and tensile forces and high hydrostatic pressure [36].

The multitude of bioreactor systems currently used is further complicated by a lack of standardisation and validation. Most systems are custom built, making comparisons between devices difficult. Additionally the various groups use different cells (age, species, origin, expanded versus non-expanded, different culture media) and the scaffolds in which the cells are embedded can also be radically different, leading to a varying degree of load transmission potentially through differing mechanisms. Even taking these differences into account, certain trends have become apparent.

5.4.1 Static Culture and Tension

When 3-D cultured chondrocytes are cultured *in vitro* under static free-swelling conditions, which is the current practice to realize matrix-assisted chondrocyte transplantation, it has been shown that little benefit is observed when chondrogenesis is concerned [27]. Static culture results in a non-homogenous cell distribution that does not resemble the native tissue [37]. Extracellular matrix production is not enhanced by isolated 3-D surroundings and chondrocytes have been shown to even downregulate typical markers for chondrogenic differentiation such as collagen type 2 or aggrecan under such growth conditions [38]. Furthermore the failure of static cultures to recreate the mechanical environment of *in vivo* tissue and to achieve mass transport of nutrients into large scaffolds result in the preferential growth of cells at the periphery of the scaffold which lacks the biomechanical and histological properties of native tissue- it has been previously termed as an edging effect [39].

Similar to static culture, tensile loading is not a typical stimulus within human joint surroundings and thus in isolation is not truly physiologically relevant for articular cartilage. Thereby, experimental studies mostly described detrimental effects following tension bioreactor tissue engineering. Yet, inhibitory effects such as down-regulation of proteoglycan production have been reported [40].

5.4.2 Bioreactors- Increasing Fluid Exchange

5.4.2.1 Flow Perfusion

Interstitial flow in articular cartilage is secondary to shear and compressive deformations during locomotion [41]. It is linked with the well-characterized

heterogeneity in structure and composition of its extracellular matrix. During flow perfusion bioreactor tissue engineering, cell culture medium is pumped continuously through a cell-matrix construct without internal transport limitations. Hereby the local nutrient supply, mass transfer is higher when comparing to rotating wall or spinner flask systems [42]. Homogenous cell distribution and higher cell seeding effectiveness are resultant. Also biomechanical input is placed onto the cells, which has been shown to enhance mechanical properties but as well to enhance the expression of the osteoblastic phenotype [43]. Flow perfusion may serve to provide every construct-cultured chondrocyte with nutrients in order to avoid edging effects. However, simple flow perfusion does not propose a relevant and adequate stimulus for articular chondrocytes to establish a functioning ECM as under these conditions it is not associated with a concurrent change in osmolarity within the tissue.

5.4.2.2 Rotating Wall

The principle of a rotating wall bioreactor is following basic rules of gravity. Cell-seeded scaffolds are cultured within medium-filled culture flasks. These are constantly rotated and thus kept from descending to the bottom of the flask. Thereby a dynamic laminar flow with a definitive shearing force evolves at the construct surface which is provoking an even cellular distribution and enhanced biomechanical properties. The rotating wall bioreactor is kept within a standard CO_2 incubator and to enable proper gaseous exchange, one side of the bioreactor chamber is a semi-permeable membrane. It was also shown that the rotating wall principle as well is applicable for isolated cells [44]. As with perfusion bioreactors, the rotating wall bioreactor allows for a larger construct to be cultured with a more even cell distribution, but does not apply mechanical stimulation more associated with an articulating joint.

5.4.2.3 Spinner Flask

During spinner flask bioreactor tissue engineering, cell-seeded scaffolds are attached to e.g. needles and are suspended in a flask of culture medium. A magnetic stir bar is constantly mixing the medium from the bottom of the flask and thus providing with a turbulent mixing of medium nutrients to the respective constructs. Thereby, the mechanical properties of the resulting tissue are enhanced. Drawbacks are to be found within the fact that related to the turbulent medium supply, application spikes appear that may result in mechanical cell death and unbalanced nutrient supply. Also, a fibrous capsule may generate around the constructs with biomechanically weak tissue within [37]. It was furthermore shown that alkaline phosphatase activity and osteocalcin secretion was higher in cells that were previously cultured within a spinner flask when comparing to static or rotating wall bioreactors indicating osteogenetic processes.

5.4.3 Bioreactors- Applying Mechanical Loads

5.4.3.1 Hydrostatic Pressure

During physiological locomotion, synovial joint fluid is kept within the cartilage ECM, which is increasing hydrostatic pressure. It is related to the negatively charged proteoglycans and limited by the collagenous network. When hydrostatic pressure is applied intermittently at physiological levels ECM production is promoted. The opposite is setting in when the stimulus is static. Parkkinen and colleagues already in 1993 reported an increased glycosaminoglycan synthesis during hydrostatic pressure bioreactor tissue engineering in cartilage explant culture. This effect could not be provoked within monolayer culture [45]. In contrast, when cartilage cells are cultured three-dimensionally within a scaffold and furthermore subjected to static hydrostatic pressure the production of external matrix is upregulated. One has to note that when the applied external artificial stimulus is non-physiological, e.g. too high, apoptosis may be induced within the cells. Generally hydrostatic pressure is one very attractive mechanical stimulus in order to increase chondrogenesis and thus ECM production and modulation. It works best when applied dynamically within physiological limits of 7–10 MPa. Within this range it has also been demonstrated that hydrostatic pressure has the potential to enhance chondrogenesis of both bone marrow [46] and infrapatellar fat pad derived MSCs [47].

5.4.3.2 Compression

Uniaxial compression as being observed within human joints is one of the most heavily studied modes of mechanical stimulation. If a compressive force is applied statically over time it is now accepted that in the majority of cases, a detrimental effect to the tissue will arise. Down-regulation of the typical markers collagen type 2 and 6, aggrecan and glycosaminoglycans are the result. Also, when cultured over short periods, dynamic loading of 15 % strain at 1 Hz did not result in a significantly upregulated hyaline-like ECM expression when comparing with static culturing conditions [48]. Numerous studies involving dynamic compression suggest a beneficial effect of load for chondrogenesis within chondrocytes, which is identified by the upregulation of collagen type 2 and aggrecan [1, 49]. Of studies involving compression alone, mostly a frequency of 1 Hz and either 10 % or 15 % compression has been applied [1, 49, 50]. Similar magnitudes led to the greatest increase in chondrogenic gene expression and GAG synthesis in MSCs [51–53]. Interestingly, it was also shown that dynamic modulation of chondrocytes can also to an extent counteract the usually detrimental expression of interleukin 1 beta [54, 55]. The effect of uniaxial compression appears to be dependent on the extent of matrix that is present around the cells [1]. It has also been proposed that it shows differing effects depending on whether chondrocytes or MSCs are stimulated. While there is little doubt that uniaxial load stimulates matrix synthesis in mature chondrocytes,

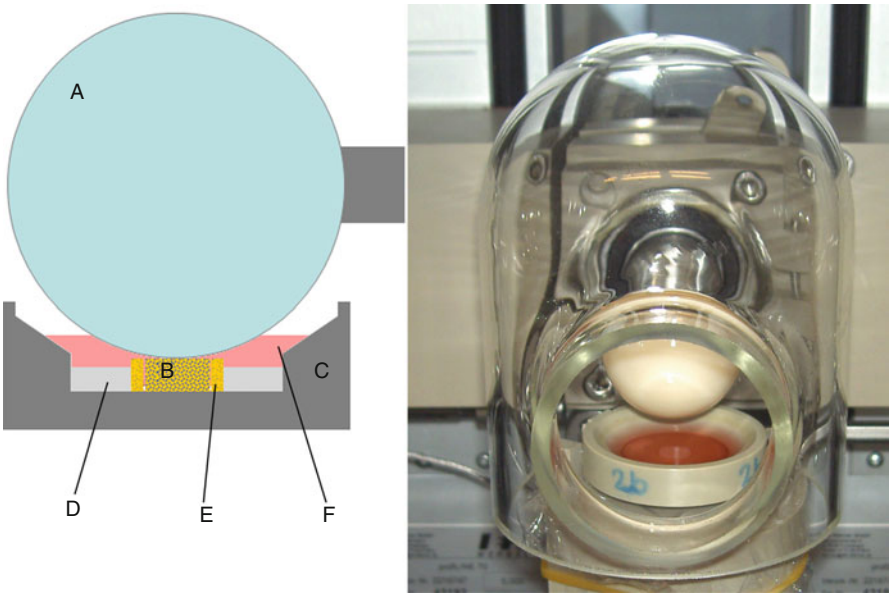

Fig. 5.1 *Left*: Cross sectional schematic of the scaffold with its holder. The ceramic hip ball (*A*) is pressed against the cell-seeded scaffold (*B*). The scaffold (*B*) is held in place within the main holder (*C*) by means of a circular PEEK (poly(ether ether ketone)) ring (*D*). A final ring of cell free fibrin/ PU scaffold (*E*) provides a final structural support to hold the sample in place. *Right*: Sample in holder. Both the sample and ball are housed in a glass bell to increase sterility (Reproduced from Schätti et al. [56] with kind permission from eCM journal)

uniaxial load alone does not appear to be able to induce chondrogenesis in BMSCs. Either a shear component is required [56] or a pre-stimulation with TGF-β is required to first induce chondrogenesis, resulting a in cell phenotype responsive to compression alone [57].

5.4.4 Combined Stimuli

Bioreactors have been developed which are capable of applying shear superimposed over compression [30, 58]. An example of such a device can be seen in Fig. 5.1. Within this device, shear, compression or a combination of the two can be independently controlled and regulated. Related to the fact that human articular chondrocytes are the subject of combined mechanical stimuli [59] it has been shown during *in vitro* experiments that a combined mechanical stimulus can be more efficient in generating chondrogenesis when compared to isolated stimulation [60]. The implementation of motion patterns which approximate the kinematics of physiological joint motion can lead to the development of a tissue with properties similar to native

articular cartilage. It has been shown that responses can be detected at the mRNA level within hours of the load being applied [58]. Studies have shown that dynamic compression and sliding surface motion, applied by a ceramic ball, improves the gene expression and the synthesis of cartilage specific matrix molecules in chondrocyte-scaffold constructs [61]. In such combined bioreactors one is capable to recognize differences in reacting ECM production. Sliding surface motion will more strongly result in the expression of Lubricin. Dynamic compression will more strongly evoke the expression of collagen type 2 and aggrecan. These different types of matrix expression are reminiscent of those appearing *in vivo* where chondrocytes are adapted to their biomechanical input [62]. It has been demonstrated that shear, superimposed over compression, is able to induce chondrogenesis of human MSCs in the absence of exogenous TGF-β [63] and as expected the response is dependent on the amplitude and frequency applied [64]. It has been proposed that shear is required for chondrogenic induction of MSCs [56].

5.4.5 Synergistic Processes

The synovial joint cavity is the host of a great variety of different growth factors, cytokines and other proteins. They interact heavily with mechanical stimuli in order to orchestrate an equilibrium within the articular cartilage. It is known that growth factors work synergistically with mechanical stimuli [65]. Such synergism was demonstrated when bovine articular chondrocytes overexpressing bone-morphogenetic protein-2 (BMP-2) were subjected to dynamic compression, shear and fluid flow within a bioreactor. When comparing to the isolated stimuli it was discovered that singular BMP-2 influence was more effective to induce the expression of typical chondrogenic markers when compared to a singular mechanical input. However, when those stimuli were combined clear synergistic effects were detected that were higher than the sum of the individual treatments for the expression GAG/DNA, collagen type 2, and cartilage oligomeric protein (COMP). Histology revealed a functional organization in combined groups including an intense safranin O staining. Also, immunostaining for collagen II and aggrecan was well detected with most intense expression within combined groups [38]. Paralleling growth factors or cytokines, as well hypoxia has been shown to result in an improved chondrogenesis over control in terms of stabilization of the chondrogenic phenotype [39].

Similar effects have been seen during the promotion of chondrogenesis in MSCs. Adipose derived cells transduced with IGF1 have been shown to lead to a chondrogenic response [66]. The chondrogenic response of human MSCs under multiaxial load can be further enhanced when the cells are transduced with adenoviral Sox9 [67] and such systems can be used to dissect the different regulation pathways of chondrogenic genes.

5.5 Conclusion

For the application of bioreactor tissue engineering, cells, either matrix-associated or condensed, are required for mechanical stimulation. When articular cartilage repair is concerned, chondrocytes are already involved with the maintenance of cartilage tissue and thus tailored for tissue engineering applications [68]. Hence, current clinical tissue engineering principles concentrate on the application of autologous chondrocytes. Yet, only one clinical bioreactor tissue engineering product is using autologous chondrocytes for knee joint cartilage repair, which have been mechanically stimulated within a bioreactor in beforehand, is currently in use [69]. Chondrocytes are expanded and seeded into a bovine type I collagen 3-dimensional honey- comb matrix. The seeded scaffold is then processed in a bioreactor in which culture conditions, including hydrostatic pressure, seeks to induce the chondrocytes to synthesize cartilage glycoproteins. On line, quality control is becoming a novel issue in cartilage repair. In particular tissue construct mechanical properties may be one significant part to support tissue quality and consecutive *in vivo* resilience. Notably, the Food and Drug Administration (FDA) requested mechanical data for all articular cartilage repair products in their guidance for "Repair or Replace Knee Cartilage", which additionally emphasizes the importance of mechanical characterization of cartilage constructs.

Related to the fact that the realization of ACI still requires two full operations and is adjunctive with a potential harmful donor-site morbidity [70], alternative cell sources are being robustly investigated [71, 72]. Almqvist and colleagues have shown satisfying midterm results when applying allogenic chondrocytes for knee cartilage defect repair among 21 subjects [73]. However, allogenic material may be ethical challenging, has potential disease transmission and immunological rejection risks and moreover might not be accessible in every country. Mesenchymal stem cells are capable of differentiating into different tissues such as bone and cartilage. Complex differentiation processes are actually required when aiming for true and foremost lasting chondrogenic differentiation [74]. Though, clinical studies have shown a similar outcome when comparing the effects of autologous chondrocyte transplantation versus autologous stem cell transplantation for the treatment of knee cartilage defects [75]. However, chondrogenic differentiation of stem cells is a difficult task, while current methods tend to induce an inadequate, hypertrophic differentiation cascade reminiscent of endochondral bone formation [76]. While MSCs have been found in numerous tissues, the detection of a progenitor like cell within cartilage itself which does not appear to be hypertrophic [77] may lead to new potential therapies. Effective bioreactor tissue engineering, potentially combining mechanical with physical stimuli, may be very attractive for future cartilage repair procedures when using mesenchymal stem cells [56].

Although progress has been achieved, there are still some significant hurdles to overcome before preconditioned tissue engineered cartilage repair can become a clinical reality. Not only do the optimal culture conditions need to be found, but they

need to be implemented into systems which are good manufacturing process (GMP) compliant. This requires that each culture vessel is independent and there is no potential for cross contamination between patients. The tracking and record keeping required for GMP also increases costs, meaning that the treatment must be demonstrably an improvement on current treatments. Also the logistics of the system need to be considered. If a central manufacturing plant is established then reliable transportation to and from the hospital may be required and this might involve shipment of live human products across international borders. Otherwise the facilities need to be on-site and economies of scale need to be considered. In either case, suitable quality control and tracking is required.

Once overcome, the economic advantages of a reliable treatment for articular defects are vast. In the future, cartilage defects may be treated by use of bioreactor preconditioned *de novo* cell-scaffold constructs, which are able to still integrate, provide with high quality repair tissue and severely reduce the time required for patient rehabilitation. When efficient, the high initial tissue engineering cost may be offset by highly effective osteoarthritis prevention which is all for the benefit of the patient.

References

1. Mauck RL, Soltz MA, Wang CC, Wong DD, Chao PH, Valhmu WB, et al. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. J Biomech Eng. 2000;122(3):252–60.
2. Madry H. The subchondral bone: a new frontier in articular cartilage repair. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):417–8.
3. Rauh J, Milan F, Gunther KP, Stiehler M. Bioreactor systems for bone tissue engineering. Tissue Eng Part B Rev. 2011;17(4):263–80.
4. Deng T, Lv J, Pang J, Liu B, Ke J. Construction of tissue-engineered osteochondral composites and repair of large joint defects in rabbit. J Tissue Eng Regen Med. 2012 Jul 9. doi:10.1002/term.1556. [Epub ahead of print].
5. Schaefer D, Martin I, Jundt G, Seidel J, Heberer M, Grodzinsky A, et al. Tissue-engineered composites for the repair of large osteochondral defects. Arthritis Rheum. 2002;46(9):2524–34.
6. Curl WW, Krome J, Gordon ES, Rushing J, Smith BP, Poehling GG. Cartilage injuries: a review of 31,516 knee arthroscopies. Arthroscopy. 1997;13(4):456–60.
7. Buckwalter JA, Mankin HJ, Grodzinsky AJ. Articular cartilage and osteoarthritis. Instr Course Lect. 2005;54:465–80.
8. Lotz MK, Carames B. Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA. Nat Rev Rheumatol. 2011;7(10):579–87.
9. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 2011;7(1):33–42.
10. Ding C, Cicuttini F, Blizzard L, Jones G. Genetic mechanisms of knee osteoarthritis: a population-based longitudinal study. Arthritis Res Ther. 2006;8(1):R8.
11. Ding C, Cicuttini F, Scott F, Cooley H, Boon C, Jones G. Natural history of knee cartilage defects and factors affecting change. Arch Intern Med. 2006;166(6):651–8.
12. Farr J, Cole B, Dhawan A, Kercher J, Sherman S. Clinical cartilage restoration: evolution and overview. Clin Orthop Relat Res. 2011;469(10):2696–705.

13. Loken S, Heir S, Holme I, Engebretsen L, Aroen A. 6-year follow-up of 84 patients with cartilage defects in the knee. Knee scores improved but recovery was incomplete. Acta Orthop. 2010;81(5):611–8.
14. Vasiliadis HS, Wasiak J. Autologous chondrocyte implantation for full thickness articular cartilage defects of the knee. Cochrane Database Syst Rev. 2010;10:CD003323.
15. Knutsen G, Drogset JO, Engebretsen L, Grontvedt T, Isaksen V, Ludvigsen TC, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. J Bone Joint Surg Am. 2007;89(10):2105–12.
16. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg Am. 2004;86-A(3):455–64.
17. Saris DB, Vanlauwe J, Victor J, Almqvist KF, Verdonk R, Bellemans J, et al. Treatment of symptomatic cartilage defects of the knee: characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. Am J Sports Med. 2009;37 Suppl 1:10S–9.
18. Bentley G, Biant LC, Vijayan S, Macmull S, Skinner JA, Carrington RW. Minimum ten-year results of a prospective randomised study of autologous chondrocyte implantation versus mosaicplasty for symptomatic articular cartilage lesions of the knee. J Bone Joint Surg Br. 2012;94(4):504–9.
19. Brun P, Dickinson SC, Zavan B, Cortivo R, Hollander AP, Abatangelo G. Characteristics of repair tissue in second-look and third-look biopsies from patients treated with engineered cartilage: relationship to symptomatology and time after implantation. Arthritis Res Ther. 2008;10(6):R132.
20. Niemeyer P, Pestka JM, Salzmann GM, Sudkamp NP, Schmal H. Influence of cell quality on clinical outcome after autologous chondrocyte implantation. Am J Sports Med. 2012;40(3): 556–61.
21. Vasiliadis HS, Danielson B, Ljungberg M, McKeon B, Lindahl A, Peterson L. Autologous chondrocyte implantation in cartilage lesions of the knee: long-term evaluation with magnetic resonance imaging and delayed gadolinium-enhanced magnetic resonance imaging technique. Am J Sports Med. 2010;38(5):943–9.
22. Vavken P, Samartzis D. Effectiveness of autologous chondrocyte implantation in cartilage repair of the knee: a systematic review of controlled trials. Osteoarthritis Cartilage. 2010; 18(6):857–63.
23. Hinterwimmer S, Krammer M, Krotz M, Glaser C, Baumgart R, Reiser M, et al. Cartilage atrophy in the knees of patients after seven weeks of partial load bearing. Arthritis Rheum. 2004;50(8):2516–20.
24. Schmal H, Niemeyer P, Zwingmann J, Stoffel F, Sudkamp NP, Mehlhorn AT. Association between expression of the bone morphogenetic proteins 2 and 7 in the repair of circumscribed cartilage lesions with clinical outcome. BMC Musculoskelet Disord. 2010;11:170.
25. Hirschmuller A, Baur H, Braun S, Kreuz PC, Sudkamp NP, Niemeyer P. Rehabilitation after autologous chondrocyte implantation for isolated cartilage defects of the knee. Am J Sports Med. 2011;39(12):2686–96.
26. Cerynik DL, Lewullis GE, Joves BC, Palmer MP, Tom JA. Outcomes of microfracture in professional basketball players. Knee Surg Sports Traumatol Arthrosc. 2009;17(9):1135–9.
27. Albrecht C, Tichy B, Nurnberger S, Hosiner S, Zak L, Aldrian S, et al. Gene expression and cell differentiation in matrix-associated chondrocyte transplantation grafts: a comparative study. Osteoarthritis Cartilage. 2011;19(10):1219–27.
28. Schnabel M, Marlovits S, Eckhoff G, Fichtel I, Gotzen L, Vecsei V, et al. Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. Osteoarthritis Cartilage. 2002;10(1):62–70.
29. Ebert JR, Robertson WB, Lloyd DG, Zheng MH, Wood DJ, Ackland T. Traditional vs accelerated approaches to post-operative rehabilitation following matrix-induced autologous chondrocyte implantation (MACI): comparison of clinical, biomechanical and radiographic outcomes. Osteoarthritis Cartilage. 2008;16(10):1131–40.

30. Wimmer MA, Grad S, Kaup T, Hanni M, Schneider E, Gogolewski S, et al. Tribology approach to the engineering and study of articular cartilage. Tissue Eng. 2004;10(9–10):1436–45.
31. Salzmann GM, Buchberger MS, Stoddart MJ, Grad S, Milz S, Niemyer P, et al. Varying regional topology within knee articular chondrocytes under simulated in vivo conditions. Tissue Eng Part A. 2011;17(3–4):451–61.
32. Hosseini A, Van de Velde SK, Kozanek M, Gill TJ, Grodzinsky AJ, Rubash HE, et al. In-vivo time-dependent articular cartilage contact behavior of the tibiofemoral joint. Osteoarthritis Cartilage. 2010;18(7):909–16.
33. Bian L, Fong JV, Lima EG, Stoker AM, Ateshian GA, Cook JL, et al. Dynamic mechanical loading enhances functional properties of tissue-engineered cartilage using mature canine chondrocytes. Tissue Eng Part A. 2010;16(5):1781–90.
34. Millward-Sadler SJ, Wright MO, Davies LW, Nuki G, Salter DM. Mechanotransduction via integrins and interleukin-4 results in altered aggrecan and matrix metalloproteinase 3 gene expression in normal, but not osteoarthritic, human articular chondrocytes. Arthritis Rheum. 2000;43(9):2091–9.
35. Millward-Sadler SJ, Wright MO, Flatman PW, Salter DM. ATP in the mechanotransduction pathway of normal human chondrocytes. Biorheology. 2004;41(3–4):567–75.
36. Heath CA, Magari SR. Mini-review: mechanical factors affecting cartilage regeneration in vitro. Biotechnol Bioeng. 1996;50(4):430–7.
37. Vunjak-Novakovic G, Martin I, Obradovic B, Treppo S, Grodzinsky AJ, Langer R, et al. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. J Orthop Res. 1999;17(1):130–8.
38. Salzmann GM, Nuernberger B, Schmitz P, Anton M, Stoddart MJ, Grad S, et al. Physicobiochemical synergism through gene therapy and functional tissue engineering for in vitro chondrogenesis. Tissue Eng Part A. 2009;15(9):2513–24.
39. Wernike E, Li Z, Alini M, Grad S. Effect of reduced oxygen tension and long-term mechanical stimulation on chondrocyte-polymer constructs. Cell Tissue Res. 2008;331(2):473–83.
40. Connelly JT, Vanderploeg EJ, Levenston ME. The influence of cyclic tension amplitude on chondrocyte matrix synthesis: experimental and finite element analyses. Biorheology. 2004; 41(3–4):377–87.
41. Olivares AL, Marsal E, Planell JA, Lacroix D. Finite element study of scaffold architecture design and culture conditions for tissue engineering. Biomaterials. 2009;30(30):6142–9.
42. Elder BD, Athanasiou KA. Hydrostatic pressure in articular cartilage tissue engineering: from chondrocytes to tissue regeneration. Tissue Eng Part B Rev. 2009;15(1):43–53.
43. Yeatts AB, Fisher JP. Bone tissue engineering bioreactors: dynamic culture and the influence of shear stress. Bone. 2011;48(2):171–81.
44. Marlovits S, Tichy B, Truppe M, Gruber D, Vecsei V. Chondrogenesis of aged human articular cartilage in a scaffold-free bioreactor. Tissue Eng. 2003;9(6):1215–26.
45. Parkkinen JJ, Ikonen J, Lammi MJ, Laakkonen J, Tammi M, Helminen HJ. Effects of cyclic hydrostatic pressure on proteoglycan synthesis in cultured chondrocytes and articular cartilage explants. Arch Biochem Biophys. 1993;300(1):458–65.
46. Miyanishi K, Trindade MC, Lindsey DP, Beaupre GS, Carter DR, Goodman SB, et al. Dose- and time-dependent effects of cyclic hydrostatic pressure on transforming growth factor-beta3-induced chondrogenesis by adult human mesenchymal stem cells in vitro. Tissue Eng. 2006;12(8):2253–62.
47. Vinardell T, Rolfe RA, Buckley CT, Meyer EG, Ahearne M, Murphy P, et al. Hydrostatic pressure acts to stabilise a chondrogenic phenotype in porcine joint tissue derived stem cells. Eur Cell Mater. 2012;23:121–34.
48. Nicodemus GD, Bryant SJ. The role of hydrogel structure and dynamic loading on chondrocyte gene expression and matrix formation. J Biomech. 2008;41(7):1528–36.
49. Chowdhury TT, Bader DL, Shelton JC, Lee DA. Temporal regulation of chondrocyte metabolism in agarose constructs subjected to dynamic compression. Arch Biochem Biophys. 2003;417(1):105–11.

50. Mauck RL, Wang CC, Oswald ES, Ateshian GA, Hung CT. The role of cell seeding density and nutrient supply for articular cartilage tissue engineering with deformational loading. Osteoarthritis Cartilage. 2003;11(12):879–90.
51. Campbell JJ, Lee DA, Bader DL. Dynamic compressive strain influences chondrogenic gene expression in human mesenchymal stem cells. Biorheology. 2006;43(3–4):455–70.
52. Huang CY, Hagar KL, Frost LE, Sun Y, Cheung HS. Effects of cyclic compressive loading on chondrogenesis of rabbit bone-marrow derived mesenchymal stem cells. Stem Cells. 2004;22(3):313–23.
53. Terraciano V, Hwang N, Moroni L, Park HB, Zhang Z, Mizrahi J, et al. Differential response of adult and embryonic mesenchymal progenitor cells to mechanical compression in hydrogels. Stem Cells. 2007;25(11):2730–8.
54. Chowdhury TT, Bader DL, Lee DA. Dynamic compression counteracts IL-1 beta-induced release of nitric oxide and PGE2 by superficial zone chondrocytes cultured in agarose constructs. Osteoarthritis Cartilage. 2003;11(9):688–96.
55. Chowdhury TT, Bader DL, Lee DA. Dynamic compression counteracts IL-1beta induced iNOS and COX-2 activity by human chondrocytes cultured in agarose constructs. Biorheology. 2006;43(3–4):413–29.
56. Schatti O, Grad S, Goldhahn J, Salzmann G, Li Z, Alini M, et al. A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells. Eur Cell Mater. 2011;22:214–25.
57. Thorpe SD, Buckley CT, Vinardell T, O'Brien FJ, Campbell VA, Kelly DJ. The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-beta3 induced chondrogenic differentiation. Ann Biomed Eng. 2010;38(9):2896–909.
58. Stoddart MJ, Ettinger L, Hauselmann HJ. Enhanced matrix synthesis in de novo, scaffold free cartilage-like tissue subjected to compression and shear. Biotechnol Bioeng. 2006;95(6):1043–51.
59. Brommer H, Brama PA, Laasanen MS, Helminen HJ, van Weeren PR, Jurvelin JS. Functional adaptation of articular cartilage from birth to maturity under the influence of loading: a biomechanical analysis. Equine Vet J. 2005;37(2):148–54.
60. Grad S, Gogolewski S, Alini M, Wimmer MA. Effects of simple and complex motion patterns on gene expression of chondrocytes seeded in 3D scaffolds. Tissue Eng. 2006;12(11):3171–9.
61. Grad S, Loparic M, Peter R, Stolz M, Aebi U, Alini M. Sliding motion modulates stiffness and friction coefficient at the surface of tissue engineered cartilage. Osteoarthritis Cartilage. 2012;20(4):288–95.
62. Li Z, Yao S, Alini M, Grad S. Different response of articular chondrocyte subpopulations to surface motion. Osteoarthritis Cartilage. 2007;15(9):1034–41.
63. Li Z, Kupcsik L, Yao SJ, Alini M, Stoddart MJ. Mechanical load modulates chondrogenesis of human mesenchymal stem cells through the TGF-beta pathway. J Cell Mol Med. 2010;14(6A):1338–46.
64. Li Z, Yao SJ, Alini M, Stoddart MJ. Chondrogenesis of human bone marrow mesenchymal stem cells in fibrin-polyurethane composites is modulated by frequency and amplitude of dynamic compression and shear stress. Tissue Eng Part A. 2010;16(2):575–84.
65. Mauck RL, Nicoll SB, Seyhan SL, Ateshian GA, Hung CT. Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering. Tissue Eng. 2003;9(4):597–611.
66. Li J, Zhao Q, Wang E, Zhang C, Wang G, Yuan Q. Dynamic compression of rabbit adipose-derived stem cells transfected with insulin-like growth factor 1 in chitosan/gelatin scaffolds induces chondrogenesis and matrix biosynthesis. J Cell Physiol. 2012;227(5):2003–12.
67. Kupcsik L, Stoddart MJ, Li Z, Benneker LM, Alini M. Improving chondrogenesis: potential and limitations of SOX9 gene transfer and mechanical stimulation for cartilage tissue engineering. Tissue Eng Part A. 2010;16(6):1845–55.
68. Grad S, Eglin D, Alini M, Stoddart MJ. Physical stimulation of chondrogenic cells in vitro: a review. Clin Orthop Relat Res. 2011;469(10):2764–72.
69. Crawford DC, Heveran CM, Cannon Jr WD, Foo LF, Potter HG. An autologous cartilage tissue implant NeoCart for treatment of grade III chondral injury to the distal femur: prospective clinical safety trial at 2 years. Am J Sports Med. 2009;37(7):1334–43.

70. Matricali GA, Dereymaeker GP, Luyten FP. Donor site morbidity after articular cartilage repair procedures: a review. Acta Orthop Belg. 2010;76(5):669–74.
71. Stoddart MJ, Grad S, Eglin D, Alini M. Cells and biomaterials in cartilage tissue engineering. Regen Med. 2009;4(1):81–98.
72. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol. 2007;213(2):341–7.
73. Dhollander AA, Verdonk PC, Lambrecht S, Verdonk R, Elewaut D, Verbruggen G, et al. Midterm results of the treatment of cartilage defects in the knee using alginate beads containing human mature allogenic chondrocytes. Am J Sports Med. 2012;40(1):75–82.
74. Goldring MB, Tsuchimochi K, Ijiri K. The control of chondrogenesis. J Cell Biochem. 2006;97(1):33–44.
75. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. Am J Sports Med. 2010;38(6):1110–6.
76. Weiss S, Hennig T, Bock R, Steck E, Richter W. Impact of growth factors and PTHrP on early and late chondrogenic differentiation of human mesenchymal stem cells. J Cell Physiol. 2010;223(1):84–93.
77. Williams R, Khan IM, Richardson K, Nelson L, McCarthy HE, Analbelsi T, et al. Identification and clonal characterisation of a progenitor cell sub-population in normal human articular cartilage. PLoS One. 2010;5(10):e13246.

Chapter 6
Biomaterials for Osteochondral Reconstruction

Elizaveta Kon, Francesco Perdisa, Giuseppe Filardo, Luca Andriolo, Francesco Tentoni, and Maurilio Marcacci

Abstract The modern regenerative procedures demonstrated to offer the replacement of the articular surface with a hyaline-like tissue, but the properties of the healthy cartilage tissue are still unmatched by any available substitute. Moreover, the treatment of osteochondral lesions is even more biologically challenging since two different tissues are involved (bone and articular cartilage) with a distinctly different intrinsic healing capacity. For the repair of the entire osteochondral unit, several authors have highlighted the need for biphasic scaffolds, to reproduce the different biological and functional requirements for guiding the growth of the two tissues, and different specific scaffolds have been developed for the treatment of large chondral or osteochondral articular defects.

At the time being, among these only two scaffolds used for osteochondral regeneration are commercialized for clinical application. One is a bilayer porous PLGA-calcium-sulphate biopolymer. The second osteochondral scaffold is a nanostructured biomimetic HA-collagen scaffold with a porous 3-D tri-layer composite structure, mimicking the whole osteochondral anatomy. Other osteochondral scaffolds are still under preclinical investigation. In this chapter we focus on reviewing the available evidence on the clinical outcome of these osteochondral scaffolds, as well as on reporting the new biomaterials developed and tested in preclinical studies that show to be promising for osteochondral regeneration.

Keywords Biomaterial • Scaffold • Osteochondral reconstruction

E. Kon, MD (✉) • G. Filardo
Nano-biotechnology Lab, Rizzoli Orthopaedic Institute,
Via di Barbiano n. 1/10, Bologna, Italy
e-mail: e.kon@biomec.ior.it

F. Perdisa • L. Andriolo • F. Tentoni • M. Marcacci
Biomechanics Lab. & III Orthopaedic Clinic, Rizzoli Orthopaedic Institute,
Bologna, Italy

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_6, © Springer-Verlag London 2014

Key Points

- Tissue engineering promises not only to repair, but to regenerate human damaged tissues, thus it becomes a "hot" topic also for cartilage repair.
- Despite that, cartilage regeneration is still an issue for the orthopaedic surgeon, due to its unmatched properties.
- The different properties of bone and cartilage make the treatment of osteochondral lesions even more difficult, requesting poliphasic scaffolds composed of different biomaterials.
- Currently, several biomaterials are developed and tested as scaffold for osteochondral repair, mainly at a preclinical level.
- Only for two of them have been reported results of human use: a biphasic and a triphasic polymeric scaffold.

6.1 Introduction

Articular cartilage has limited healing potential, reflecting its peculiar anatomical structure and functional characteristics [1], whose changes may produce an accelerated loss of articular surface, leading to end-stage arthritis. In fact, it's proven that even small and single chondral defects represent a risk to more extensive joint damage [2].

The role of the subchondral bone in this etiopathogenetic process has been recently extensively studied: in fact, it may be involved not only primarily in case of osteochondritis dissecans (OCD), osteonecrosis or trauma [3–5], but even focal chondral defects, if left untreated, may involve secondarily the underlying subchondral bone, either with overgrowth or bone loss [6, 7]. Thus, the surgeon should consider both cartilage and bone reconstruction when approaching to treat osteochondral lesions [2].

The treatment of the entire osteochondral unit is more challenging than the chondral layer alone, due to the different healing capacity of cartilage and bone. In recent years, the new achievements in tissue engineering allowed the development of biomaterials to manage even such complex lesions, where previous treatments were not indicated.

This chapter is focused on the biomaterials used nowadays for osteochondral reconstruction, either developed and tested in preclinical studies or already being applied in clinical practice.

6.2 Biomaterials for Cartilage Regeneration

In the last decades, tissue engineering offered a promising and concrete alternative to traditional techniques aiming to help and guide tissue regeneration. These regenerative procedures aim at recreating a hyaline-like tissue in order to restore the

articular surface as similar as possible to the physiological one, both biologically and biomechanically, and overcoming the limits of the previously available treatment options.

The rationale of using biomaterials as a scaffold is to obtain a temporary three-dimensional structure of biodegradable polymers for the *in vitro* growth of living cells and their subsequent implantation into the lesion area. An ideal scaffold should mimic biology, architecture and structural properties of the native tissue, thus facilitating cell infiltration, attachment, proliferation, and differentiation. Moreover, it should be biocompatible and biodegradable through safe biochemical pathways at suitable time intervals to support the first phases of tissue formation and gradually be replaced by the regenerating tissue. Three-dimensional scaffolds showed that they can help chondrocyte to maintain their differentiated phenotype [8], and also promote a homogeneous distribution while avoiding the risk of chondrocyte leakage.

Several scaffolds have been developed and applied to tissue engineering in the attempt to fulfil better the requirements of cartilage regeneration process, with substantial differences regarding the materials chosen and their physical forms (fibers, meshes, gels) [9]. Solid scaffolds provide a substrate that cells can adhere to, whereas gel scaffolds physically entrap the cells.

Each of the various materials tested in orthopaedics to form these three-dimensional structures must have some basic requirements, such as the capability to support, both *in vitro* and *in vivo*, the necessary cell activity for regeneration (i.e. attachment, proliferation and differentiation) then *in vivo* to provide a mechanical support and biological stimuli to the regenerating tissue [10].

Natural biomaterials can be proteins or polysaccharides of the extracellular matrix, thus containing sites for cellular adhesion, they are usually fully biocompatible, whereas the source and the purity of the material used become important to avoid immune response of the host. On the other hand, natural materials have usually a limited range of mechanical properties [11]. Among these, protein-based biomaterials, such as collagen, fibrin or silk, as well as polysaccharide-based biomaterials, including agarose, alginate, hyaluronian and chitosan, have been widely tested as 3-D scaffold to guide and support tissue regeneration [12, 13].

Synthetic biomaterials offer the advantages of a defined chemical composition to better control their mechanical properties, degradation rate and shape [10]. They possibly present lack of sites for cell adhesions and can potentially product toxic substances after degradation [14], but innovations in the chemistry of these materials have improved their biocharacteristics and biocompatibility [9].

Most commonly synthetic materials used as matrices include poly(alfa-hydroxy) acids: mainly poly lactic acid (PLA) and poly glycolic acid (PGA) and their derivated co-polymers (PLGA), poly epsilon-caprolactone (PCL), poly propylene fumarate (PPF), poly dioxanone (PDO) [15].

Finally bioactive glasses and ceramics, such as hydroxyapatite or tricalcium phosphate (TCP), have shown good attitude to promote the formation of a bone-like apatite layer [16, 17], thus in the last years they have been commonly used for the treatment of bone loss, also in association with stem cells [18].

The increasing interest of research on osteochondral articular defects favored the development of specific scaffolds with distinctly different intrinsic healing capacity. Biphasic scaffolds aim at treating the entire osteochondral unit, by reproducing the different biological and functional requirements in order to guide the growth of these two different tissues [19–21], trying to combine the benefits of both synthetic and natural materials.

Stiff biomaterials, capable to support cell expansion and vascularization, in addition to the production of collagen type I and HA matrix seems to be more appropriated for bone regeneration; whereas hyaline cartilage has an avascular structure consisting of proteoglycan hydrogel embedded into a collagen type II network.

6.2.1 Preclinical Experience: In Vivo *Studies*

There are several concerns about biomaterials for osteochondral repair, with regards to the different biomaterials and their related properties and, depending on that, to the possibility to use the scaffold alone or in association with endogenous chondroinductive molecules or specific cell sources, such as chondrocytes or, more recently, mesenchymal stem cells (MSC).

Among the many preclinical experiments performed nowadays, only a few *in vivo* studies have shown good results for biomimetic ostechondral grafts; here the most promising are reported.

A controlled chondrocyte and osteoblast culture has been respectively shown by a agarose hydrogel and sintered microspheres of PLGA-glass composite scaffold [22]. Three distinct yet continuous regions were observed: cartilage, calcified cartilage and bone-like matrices. Moreover, a higher cell density enhanced chondrogenesis, improving graft mechanical properties over time. The authors are currently focusing on scaffold optimization and *in vivo* studies for this kind of stratified scaffold.

A scaffold combining hyaluronate and atelocollagene (chondral layer) and HA and beta-TCP (bone layer) showed good results in a porcine knee animal model [23]. The scaffold was implanted alone or either seeded with cells, while other groups underwent to autologous chondroctyte implantation (ACI) or filling with osteochondral fragments alone into the defect. In control group the defect was left empty. International Cartilage Repair Society (ICRS) macroscopic grading [24] was similar for the first 3 groups and lower for both ACI and control groups. Best histological ICRS visual assessment score was observed for the scaffold alone group, while indentation study showed comparable results for the both scaffold groups and also ACI group.

A different bilayered scaffold made of poly vinyl alcohol/gelatin-nanohydroxyapatite/polyamide6 (PVA-n-HA/PA6) has been implanted into rabbit muscles, in association with induced bone marrow stem cells (BMSCs), showing the production at 12 weeks of ectopic neocartilage in the PVA layer and reconstitution of the subchondral bone in the deeper layer [25].

A composite scaffold consisting of PCL and HA has been implanted into rabbit's condyle in addition to transforming growth factor (TGF)-beta3, producing full

coverage of hyaline cartilage at the surface and 130 % more chondrocytes compared to the scaffold alone [26].

A hydrogel bilayered scaffold of polymer oligo(polyethylene glycol) fumarate has been tested, loaded with TGF-beta1 into gelatin microparticles in its superficial layer, with positive effects on the quality of regenerating tissue in the rabbit [27].

Another study compared the release of Bone Morphogenetic Proteins (BMP)-2 and insulin-like growth factor (IGF)-1 incorporated in PLGA and silk fibroin microspheres or alginate gel, seeded with bone marrow mesenchymal stem cells (BMSCs). The silk microspheres exhibited more osteogenic and chondrogenic differentiation along the concentration gradients, due to a better delivery of BMP-2 [28].

Finally, a new scaffolding approach has very recently been reported for osteochondral defects in rabbit femoral condyle. Bioactive microspheres of poly(D,L-LACTIC-CO-glycolic) acid were developed with a continuous gradient transition between cartilage-promoting and bone-promoting growth factors. Results after 6 and 12 weeks suggested that the gradient in bioactive signaling may have been beneficial for both bone and cartilage regeneration compared to the control, as confirmed by histology. Moreover, in this study additional benefits were showed after preseeding the scaffolds with umbilical cord mesenchymal stromal cells (UCMSCs) [29].

6.2.2 Clinical Experience

Currently, only a few scaffolds for osteochondral regeneration are suitable for clinical application, among these, only two have been already reported in literature.

One is a bilayer porous PLGA-calcium-solfate biopolymer (TruFit®, Smith & Nephew, Andover, MA; Fig. 6.1). Results after implantation of this osteochondral graft substitute are controversial, still with no available information on long-term durability [30, 31].

Carmont et al. [32] suggested that, although an intermediate postoperative interval can be associated with unfavourable MRI images, the plug appearance may significantly improve at further follow-up. Thus, they recommended perseverance, reporting a delayed incorporation and maturation of articular cartilage but good clinical results in an 18-year old footballer at 2 years. Bedi et al. reported [33] the good outcome in 26 patients who underwent to OAT at the knee, where the donor site was filled with this scaffold: they also noticed a slow improvement of the implant site in MRI appearance.

Conversely, Barber et al. didn't document with CT scans signs of maturation, osteoconduction, or ossification of the scaffold in any of the 9 patients evaluated [34].

Finally, a prospective study on 20 patients evaluated at 6 and 12 months, both clinically and with MRI, has been very recently reported. The results in the short term appeared to be modest, but with no signs of deterioration of the repair tissue. Three patients (20 %) underwent to revision surgery with autologous bone grafts during the follow-up period and biopsies showed fibrous vascularized repair tissue for each of them [35].

Fig. 6.1 Trufit® bilayered scaffold. Both cartilage and bone layer are visible. The cylindrical shape mimic a mosaic-style ostechondral plug and different diamaters are available

The second osteochondral scaffold is a nanostructured biomimetic scaffold (Maioregen®: Fin-Ceramica S.p.A., Faenza, Italy; Fig. 6.2) with a porous 3-D three-layer composite structure, mimicking the whole osteochondral anatomy: the cartilaginous Type I collagen layer has a smooth surface, the intermediate tide-mark-like layer consists of a combination of Type I collagen (60 %) and HA (40 %), whereas the lower layer consists of a mineralized blend of Type I collagen (30 %) and HA (70 %) reproducing the subchondral bone. This scaffold was introduced into clinical practice as a cell-free approach after animal studies showed good results in terms of both cartilage and bone tissue formation: it provided similar macroscopic, histological and radiographic results when implanting scaffold loaded with autologous chondrocytes or scaffold alone, probably inducing an in situ regeneration through stem cells coming from the surrounding bone marrow [36].

An analysis at early post-operative time, 4–8 weeks, was performed for 13 patients (15 lesions) to attest clinical and MRI outcome and at the same time evaluate the mechanical stability of the implanted graft [37]. Thirteen of the implantation sites had complete attachment and adherence, while in two patients partial

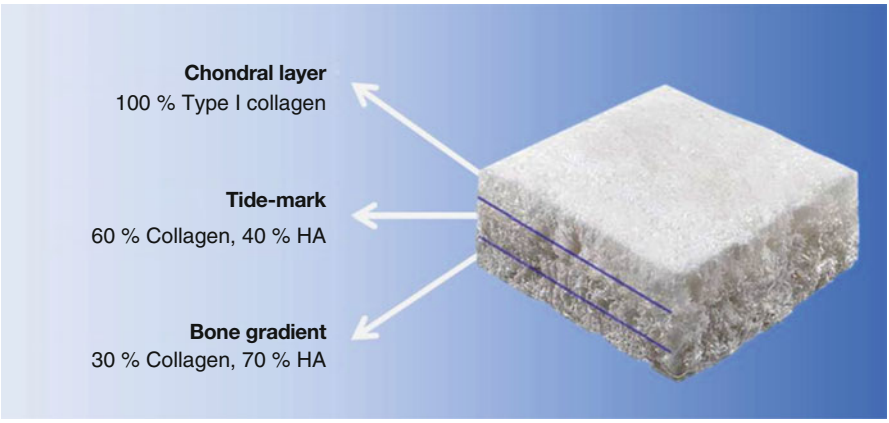

Fig. 6.2 Maioregen® scaffold with the three different gradient layers. Its wide size (35×35 mm) allows the surgeon to cut the scaffold into appropriate size and shape to better fit the lesion

attachment was found: the weak mechanical fixation was probably due to inadequate surgical technique with insufficient shoulder coverage of the prepared implantation site. Further analysis at 6 months revealed partial reabsorption of the graft in one case with an incomplete cartilage layer and a complete subchondral structure, while in the other case an inhomogeneous tissue filled in the entire treated.

Promising preliminary results of a pilot study on 28 patients affected by chondral and osteochondral lesions have been recently reported [38]. A slower recovery was observed in older, less active patients who experienced adverse events, or in patellar lesions. However, at 2 years of follow-up good results were reported in all patients with both clinical and MRI evaluations, showing the potential of this osteochondral one-step procedure also for the treatment of complex salvage lesions, as testified also by the case of an active 46 years old patient, who previously underwent to anterior cruciate ligament (ACL) reconstruction, and treated with 3 scaffold implants for degenerative lesions of medial femoral condyle (MFC), trochlea and patella, with associated closing wedge high tibial osteotomy (HTO) [39]. At 1 year of follow-up, the patient didn't complain anymore about pain and had a complete ROM, while his sport activity level was only slightly inferior than the pre-injury one. MRI evaluation showed at 6 months a satisfactory resurfacing of the lesions, with hyaline-like signal and minimal subchondral oedema, which was totally reabsorbed at the 12 months' evaluation.

Currently a multicenter clinical trial is ongoing on 150 patients over Europe [40].

6.3 Discussion and Conclusion

The ultimate goal of tissue-engineering is "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use". Tissue engineering, involving the use of a biocompatible, structurally and

mechanically stable scaffolds, incorporating specific cell sources and bioactive molecules, has shown promising results in bone and cartilage tissue repair. The ideal biomaterial to use as scaffold should assemble the positive quality of both natural and synthetic biomaterials, thus providing a structural support for migration, adhesion and differentiation of the desired individual cell type and at the same time being biodegradable, biocompatible and offering a stable fixation.

During the last decades several scaffolds have been developed to deliver growth factors and/or cells to the site of tissue trauma in order to guide tissue repair or regeneration. When tissue engineering is applied to the treatment of osteochondral defects, bi or multilayered scaffolds seems to be the way to address the different anatomical and functional characteristics of these tissues.

Some researchers propose that the main function of biomaterials as a scaffold is to carry cell elements or bioactive signals in the lesion site, while others believe in the potential of the scaffold material itself to promote chondral or osteochondral regeneration by harnessing and guiding the body's self-regenerative potential, but there is no agreement yet and research regarding biomaterials, scaffold techniques and biological or biophysical enhancements is extensively going on.

For example the use of superparamagnetic nanoparticles (MNPs) has been increasing in medicine in the last years to improve the performances of the tissue regeneration offered by the scaffold approach, but a very few studies have been reported of this tissue engineering approach for ostechondral regeneration [41, 42]. On the other hand great hopes are worldwide addressed in stem cells therapies: actually, trials on the different sources and cell-type are going hand in hand with testing different biomaterials to be applied [43–48].

The recent achievements in tissue engineering represent a promising and fascinating alternative to traditional treatments in every field of regenerative medicine, including osteochondral repair. Researchers are currently focusing on developing new biomaterials and also testing possible improvements with a multidisciplinary approach, that appear to be unavoidable for the orthopaedic surgeon, promising that exciting developments are forthcoming.

References

1. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect. 1998;47:477–86.
2. Pape D, Filardo G, Kon E, van Dijk CN, Madry H. Disease-specific clinical problems associated with the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):448–62. Epub 2010 Feb 12. Review.
3. Kocher MS, Tucker R, Ganley TJ, Flynn JM. Management of osteochondritis dissecans of the knee: current concepts review. Am J Sports Med. 2006;34(7):1181–91.
4. Ahuja SC, Bullough PG. Osteonecrosis of the knee: a clinicopathological study in twenty-eights patients. J Bone Joint Surg Am. 1978;60(2):191–7.
5. Kon E, Vannini F, Buda R, Filardo G, Cavallo M, Ruffilli A, Nanni M, Di Martino A, Marcacci M, Giannini S. How to treat osteochondritis dissecans of the knee: surgical techniques and new trends: AAOS exhibit selection. J Bone Joint Surg Am. 2012; 94(1):e1(1–8).

6. Gratz KR, Wong BL, Bae WC, Sah RL. The effects of focal articular defects on cartilage contact mechanism. J Orthop Res. 2009;27(5):584–92.
7. Henderson IJ, La Valette DP. Subchondral bone overgrowth in the presence of full-thickness cartilage defects in the knee. Knee. 2005;12(6):435–40. Epub 2005 Sep 8.
8. Grigolo B, Lisignoli G, Piacentini A, et al. Evidence for redifferentiation of human chondrocytes grown on a hyaluronan-based biomaterial (HYAff 11): molecular, immunohistochemical and ultrastructural analysis. Biomaterials. 2002;23(4):1187–95.
9. Kon E, Verdonk P, Condello V, Delcogliano M, Dhollander A, Filardo G, et al. Matrix-assisted autologous chondrocyte transplantation for the repair of cartilage defects of the knee: systematic clinical data review and study quality analysis. Am J Sports Med. 2009;37 Suppl 1:156S–66. Epub 2009 Oct 27.
10. Capito RM, Spector M. Scaffold-based articular cartilage repair. IEEE Eng Med Biol Mag. 2003;22(5):42–50.
11. Lee SH, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. Adv Drug Deliv Rev. 2007;59(4–5):339–59. Epub 2007 Apr 12.
12. Ochi M, Uchio Y, Tobita M, Kuriwaka M. Current concepts in tissue engineering technique for repair of cartilage defect. Artif Organs. 2001;25(3):172–9.
13. Pabbruwe MB, Esfandiari E, Kafienah W, Tarlton JF, Hollander AP. Induction of cartilage integration by a chondrocyte/collagen-scaffold implant. Biomaterials. 2009;30(26):4277–86. Epub 2009 Jun 17.
14. Getgood A, Brooks R, Fortier L, Rushton N. Articular cartilage tissue engineering: today's research, tomorrow's practice? J Bone Joint Surg Br. 2009;91(5):565–76.
15. Mano JF, Reis RL. Osteochondral defects: present situation and tissue engineering approaches. J Tissue Eng Regen Med. 2007;1(4):261–73.
16. Bernhardt A, Lode A, Boxberger S, Pompe W, Gelinsky M. Mineralised collagen – an artificial, extracellular bone matrix – improves osteogenic differentiation of bone marrow stromal cells. J Mater Sci Mater Med. 2008;19(1):269–75. Epub 2007 Jun 28.
17. Mastrogiacomo M, Muraglia A, Komlev V, Peyrin F, Rustichelli F, Crovace A, Cancedda R. Tissue engineering of bone: search for a better scaffold. Orthod Craniofac Res. 2005;8(4):277–84.
18. Marcacci M, Kon E, Moukhachev V, Lavroukov A, Kutepov S, Quarto R, Mastrogiacomo M, Cancedda R. Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue Eng. 2007;13(5):947–55.
19. Keeney M, et al. The osteochondral junction and its repair via bi-phasic tissue engineering scaffolds. Tissue Eng Part B Rev. 2009;15(1):55–73.
20. Martin I, Miot S, Barbero A, Jakob M, Wendt D. Osteochondral tissue engineering. J Biomech. 2007;40(4):750–65. Epub 2006 May 26.
21. Tampieri A, Celotti G, Landi E, Sandri M, Roveri N, Falini G. Biologically inspired synthesis of bone-like composite: self-assembled collagen fibers/hydroxyapatite nanocrystals. J Biomed Mater Res A. 2003;67(2):618–25.
22. Jiang J, Tang A, Ateshian GA, Guo XE, Hung CT, Lu HH. Bioactive stratified polymer ceramic-hydrogel scaffold for integrative osteochondral repair. Ann Biomed Eng. 2010;38(6):2183–96. Epub 2010 Apr 22.
23. Im GI, Ahn JH, Kim SY, Choi BS, Lee SW. A hyaluronate-atelocollagen/beta-tricalcium phosphate-hydroxyapatite biphasic scaffold for the repair of osteochondral defects: a porcine study. Tissue Eng Part A. 2010;16(4):1189–200.
24. International Cartilage Repair Society. Cartilage injury evaluation package, 2000. Available at: http://www.cartilage.org/_files/contentmanagement/ICRS_evaluation.pdf. Accessed on 2013 July 22.
25. Redman SN, Oldfield SF, Archer CW. Current strategies for articular cartilage repair. Eur Cell Mater. 2005;9:23–32.
26. Lee CH, Cook JL, Mendelson A, Moioli EK, Yao H, Mao JJ. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. Lancet. 2010;376:440–8.
27. Holland TA, Bodde EW, Baggett LS, Tabata Y, Mikos AG, Jansen JA. Osteochondral repair in the rabbit model utilizing bilayered, degradable oligo (poly (ethylene glycol) fumarate) hydrogel scaffolds. J Biomed Mater Res A. 2005;75(1):156–67.

28. Wang X, Wenk E, Zhang X, Meinel L, Vunjak-Novakovic G, Kaplan DL. Growth factor gradients via microsphere delivery in biopolymer scaffolds for osteochondral tissue engineering. J Control Release. 2009;134(2):81–90.
29. Dormer NH, Singh M, Zhao L, Mohan N, Berkland CJ, Detamore MS. Osteochondral interface regeneration of the rabbit knee with macroscopic gradients of bioactive signals. J Biomed Mater Res A. 2012;100(1):162–70. doi:10.1002/jbm.a.33225. Epub 2011 Oct 19.
30. Williams RJ, Gamradt SC. Articular cartilage repair using a resorbable matrix scaffold. Instr Course Lect. 2008;57:563–71.
31. Melton JT, Wilson AJ, Chapman-Sheath P, Cossey AJ. TruFit CB® bone plug: chondral repair, scaffold design, surgical technique and early experiences. Expert Rev Med Devices. 2010;7(3):333–41.
32. Carmont MR, Carey-Smith R, Saithna A, Dhillon M, Thompson P, Spalding T. Delayed incorporation of a TruFit plug: perseverance is recommended. Arthroscopy. 2009;25(7):810–4.
33. Bedi A, et al. The maturation of synthetic scaffolds for osteochondral donor sites of the knee: an MRI and T2-mapping analysis. Cartilage. 2010;1(1):20–8.
34. Barber FA, et al. A computed tomography scan assessment of synthetic multiphase polymer scaffolds used for osteochondral defect repair. Arthroscopy. 2011;27(1):60–4. Epub 2010 Oct 16.
35. Dhollander AA, Liekens K, Almqvist KF, Verdonk R, Lambrecht S, Elewault D, Verbruggen G, Verdonk PC. A pilot study of the use of an osteochondral scaffold plug for cartilage repair in the knee and how to deal with early clinical failures. Arthroscopy. 2012;28(2):225–33. Epub 2011 Oct 20.
36. Kon E, Delcogliano M, Filardo G, Fini M, Giavaresi G, Francioli S, Martin I, Pressato D, Arcangeli E, Quarto R, Sandri M, Marcacci M. Orderly ostechondral regeneration in a sheep model using a novel nano-composite multilayered biomaterial. J Orthop Res. 2010;28(1):116–24.
37. Kon E, Delcogliano M, Filardo G, Pressato D, Busacca M, Grigolo B, Desando G, Marcacci M. A novel nano-composite multi-layered biomaterial for treatment of osteochondral lesions: technique note and an early stability pilot clinical trial. Injury. 2010;41:693–701.
38. Kon E, Delcogliano M, Filardo G, Busacca M, Di Martino A, Marcacci M. Novel nano-composite multilayered biomaterial for osteochondral regeneration: a pilot clinical trial. Am J Sports Med. XX(X):1–11.
39. Kon E, Delcogliano M, Filardo G, Altadonna G, Marcacci M. Novel nano-composite multilayered biomaterial for the treatment of multifocal degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2009;17(11):1312–5. Epub 2009 May 26.
40. Detailed information about the multicenter clinical trial "Study for the Treatment of Knee Chondral and Osteochondral Lesions" available at: http://clinicaltrials.gov/ct2/show/NCT01282034. Accessed on 2013 July 22.
41. Bock N, Riminucci A, Dionigi C, Russo A, Tampieri A, Landi E, Goranov VA, Marcacci M, Dediu V. A novel route in bone tissue engineering: magnetic biomimetic scaffolds. Acta Biomater. 2010;6(3):786–96.
42. Tampieri A, Landi E, Valentini F, Sandri M, D'Alessandro T, Dediu V, Marcacci M. A conceptually new type of biohybrid scaffold for bone regeneration. Nanotechnology. 2011;22(1):015104.
43. Natesan S, Baer DG, Walters TJ, Babu M, Christy RJ. Adipose-derived stem cell delivery into collagen gels using chitosan microspheres. Tissue Eng Part A. 2010;16(4):1369–84.
44. Kurth T, Hedbom E, Shintani N, Sugimoto M, Chen FH, Haspl M, Martinovic S, Hunziker EB. Chondrogenic potential of human synovial mesenchymal stem cells in alginate. Osteoarthritis Cartilage. 2007;15(10):1178–89.
45. Iwasa J, Engebretsen L, Shima Y, Ochi M. Clinical application of scaffolds for cartilage tissue engineering. Knee Surg Sports Traumatol Arthrosc. 2009;17(6):561–77.
46. Erisken C, Kalyon D, Wang H, Ornek C, Xu J. Osteochondral tissue formation through adipose-derived stromal cell differentiation on biomimetic polycaprolactone nanofibrous scaffolds with graded insulin and beta-glycerol phosphate concentrations. Tissue Eng Part A. 2011;17(9–10):1239–52.
47. Gao J, Yao JQ, Caplan AI. Stem cells for tissue engineering of articular cartilage. Proc Inst Mech Eng H. 2007;221(5):441–50.
48. Hao W, Dong J, Jiang M, Wu J, Cui F, Zhou D. Enhanced bone formation in large segmental radial defects by combining adipose-derived stem cells expressing bone morphogenetic protein 2 with nHA/RHLC/PLA scaffold. Int Orthop. 2010;34(8):1341–9.

Part III
Imaging of Cartilage

Chapter 7
Advanced Magnetic Resonance Imaging of Cartilage Repair

Goetz H. Welsch, Friedrich F. Hennig, and Siegfried Trattnig

Abstract Articular cartilage injuries are common findings within different joints and patients benefit from optimal diagnosis and treatment. Magnetic resonance imaging (MRI) has become the method of choice for diagnosis of chondral injuries and for the follow-up of patients after cartilage repair surgery. Thus, early and precise diagnosis together with surgical treatment options may offer a possibility for patients with cartilage defects to avoid OA or to delay the progression of OA. Therefore, widespread cartilage repair techniques, including arthroscopic or open surgical approaches as well as marrow-stimulation techniques, osteochondral grafting, and chondrocyte implantation/transplantation, require knowledgeable and high quality follow-up.

The present chapter provides an overview of the current state of the art of MRI in patients with cartilage injuries or after cartilage repair. Initially an overview about the pre-requirements of high quality MR imaging of articular cartilage and its repair will be provided. Then cartilage sensitive MR protocols will be introduced and described including basic MR sequences and new three-dimensional isotropic approaches. Morphological post-operative cartilage repair MR imaging will be provided, again based on standard and advanced MR techniques. Special focus will be given on post-operative scoring with the MR observation of cartilage repair tissue (MOCART) score. Furthermore the ultra-structure of the repair tissue and the surrounding cartilage can be assessed

G.H. Welsch, MD (✉) • F.F. Hennig
Department of Trauma Surgery, University Hospital of Erlangen,
Maximiliansplatz 1, Erlangen 91054, Germany
e-mail: goetz.welsch@uk-erlangen.de

S. Trattnig
Department of Radiology, MR Center, Medical University of Vienna,
Vienna, Austria

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_7,

non-invasively by means of biochemical MRI techniques such as delayed Gadolinium enhanced MRI of cartilage (dGEMRIC), T2 mapping, T1 rho, diffusion weighted imaging or other techniques. These new MR methodologies as well as their sensitivity to specific components of articular cartilage and the repair tissue will be provided and discussed. Additionally examples of other advanced or future options will be given, such as biomechanical MRI approaches and MRI of animal models after cartilage repair. Concluding the differences of the various cartilage repair techniques with respect to their imaging appearance will be presented and discussed. Bringing these advanced MR imaging methods into the diagnosis and treatment of cartilage injuries and repair, new insight will be given non-invasively which can be used in clinical routine, scientific studies and during clinical trials.

Keywords MRI • Cartilage Repair • 3D Imaging • Isotropic • T2 Mapping • dGEMRIC • MOCART

Key Points

- MRI is the gold standard in diagnostic imaging of cartilage and cartilage repair.
- For diagnosis of cartilage injuries or chronic cartilage changes, specific MR sequences with specific resolution and other parameters are needed.
- For initial diagnosis as well as for post-operative MRI, the sequences protocol has to cover the "whole organ" (e.g. all structures within the knee joint).
- Advanced isotropic 3D MR sequences have the possibility to visualize the joint in 3D, where cartilage and other changes can be assessed in more detail.
- After cartilage repair procedures the MOCART (magnetic resonance observation of cartilage repair tissue) score is able to assess the state of the repair tissue (Table 7.1).
- Biochemical MR methods are able to visualize the ultra-structure of the repair tissue non-invasively as an "virtual biopsy".
- Delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) and other techniques are able to quantify the glycosaminoglycan (GAG) content of the cartilage repair tissue.
- T2 Mapping and other techniques are able to quantify the hydration of the repair tissue and provide an insight in the collagen network.
- For biochemical MRI it is of outmost importance to do a zonal assessment of the cartilage (e.g. to differentiate a deep and superficial cartilage layer).

Table 7.1 New (advanced) magnetic resonance observation of cartilage repair tissue (MOCART) score which can be accomplished by standard MR sequences or an isotropic 3D MR sequence

Variables
1. Defect fill (degreee of defect repair and filling of the defect in relation to the adjacent cartilage)
○ 0 %
○ 0 - 25 %
○ 25 - 50 %
○ 50 - 75 %
○ 75 - 100 %
○ 100 %
○ 100 - 125 %
○ 125 - 150 %
○ 150 - 200 %
○ > 200 %
Localization
○ Whole area of cartilage repair ○ > 50 % ○ < 50 %
○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing
2. Cartilage Interface (integration with adjacent cartialge to border zone in two planes)
Sagittal (Femur, Patella, Trochlea, Tibia)
○ Complete
○ Demarcating border visible (split-like)
○ Defect visible <50 %
○ Defect visible >50 %
Coronal (Femur, Tibia); Axial (Patella, Trochlea)
○ Complete
○ Demarcating border visible (split-like)
○ Defect visible <50 %
○ Defect visible >50 %
Localization
○ Whole area of cartilage repair ○ > 50 % ○ < 50 %
○ Weight-bearing ○ Non weight-bearing
3. Bone interface (integration of the transplant to the subchondrol bone; integration of a possible periosteal flap)
○ Complete
○ Partial delamination
○ Complete delamination
○ Delamination of periosteal flap
Localization
○ Weight-bearing ○ Non weight-bearing

(continued)

Table 7.1 (continued)

Variables

4. Surface (constitution of the surface of the repair tissue)

- ○ Surface intact
- ○ Surface damaged <50% of depth
- ○ Surface damaged >50% of depth
- ○ Adhesions

Localization

○ Whole area of cartilage repair ○ > 50 % ○ < 50 %
○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing

5. Structure (constitution of the repari tissue)

- ○ Homogeneous
- ○ Inhomogeneous or cleft formation

Localization

○ Whole area of cartilage repair ○ > 50 % ○ < 50 %
○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing

6. Signal intensity (Intensity of MR signal in of the repair tissue in comparison to the adjacent cartilage)

- ○ Normal (identical to adjacent cartilage)
- ○ Nearly normal (slight areas of signal alternation)
- ○ Abnormal (large areas of signal alteration)

Localization

○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing

7. Subchondral lamina (Constitution of the subchondral lamina)

- ○ Intact
- ○ Not intact

Localization

○ Whole area of cartilage repair ○ > 50 % ○ < 50 %
○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing

8. Chondral osteophytes (Osteophytes within the cartilage repair area)

- ○ Absent
- ○ Osteophytes < 50 % of the thickness of the cartilage transplant
- ○ Osteophytes > 50 % of the thickness of the cartilage transplant

Localization

Size: _____ mm (plane: _____) x _____ mm (plane: _____)
○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing

9. Bone marrow edema (Maximum size and localization in relation to the cartilage repair tissue and other alterations assessed in the 3D MOCART score).

- ○ Absent
- ○ Small (< 1cm)
- ○ Medium (< 2cm)
- ○ Large (< 4cm)
- ○ Diffuse

Localization

Size: _____ mm (plane: _____) x _____ mm (plane: _____)
○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing
○ Relation to other alterations within this score of variable No. _____ .

Table 7.1 (continued)

Variables
10. Subchondral bone (Constitution of the subchondral bone) ○ Intact ○ Granulation tissue ○ Cyst ○ Sclerosis Localization ○ Whole area of cartilage repair ○ > 50 % ○ < 50 % ○ Central ○ Peripheral ○ Weight-bearing ○ Non weight-bearing
11. Effusion (Approx. size of joint effusion visualized in all planes) ○ Absent ○ Small ○ Medium ○ Large

Variables 1-11 for 3D MOCART score; subcategories “localization” optional

7.1 Introduction

Magnetic resonance imaging (MRI) is intensively used to assess the cartilage injuries as well as structural changes in the cartilage repair tissue and the adjacent structures post-operatively. Due to its strength to visualize all different tissues, MRI has been recognized as an excellent tool to define the exact conditions in the joint. Besides the cartilage and the repair tissue, the menisci, the ligaments and tendons, the synovial and synovialis as well as the bone can be assessed. Concerning cartilage repair, with the given tissue contrast and sensitivity to tissue composition, MRI has a very high potential in the description of joints before and after different cartilage repair procedures. Specifically it may (a) help estimating the size, nature and location of lesions preoperatively, in order to optimize surgical planning, (b) provide *in vivo* data on the mechanical strains in the target environment that the repair tissue needs to withstand, (c) help to evaluate the quality and success of tissue repair processes after surgical treatment and (d) allow to monitor degenerative changes in the whole joint after cartilage repair.

Significant advances have been made in characterizing, quantifying and standardizing the specific morphological as well as biochemical changes in patients before and after cartilage repair. Besides the exact evaluation of the cartilage defect respectively the cartilage repair tissue, also the surrounding tissues can be assessed in best possible fashion non invasively [1–3]. Concerning bony irregularities, MRI depicts calcified bone as a signal void, comparably to radiography; furthermore structural changes, especially bone marrow edema can be assessed very precisely [4]. The role of the subchondral bone plate before and after different cartilage repair surgeries is of enormous interest. Pre-operatively the specific surgical treatment option is not only based on the character and size of the cartilage defect but even more dependent on the formation of the underlying bone. Comparably in the follow-up after cartilage repair surgery, the characterization of

the subchondral bone is of utmost importance to measure the success of the specific surgical technique. Besides the evaluation of cartilage and bone, all other structures within the joint have to be visualized and to be taken into consideration. Hence the joint where cartilage repair takes place has to be seen as a "whole organ" and whole organ scores will be important not only in osteoarthritis but also in cartilage repair [5].

Concerning articular cartilage, MRI can visualize morphological alterations such as reduction in cartilage volume, cartilage contour irregularities, fissures and cartilage thinning [6]. As structural cartilage damage is preceded by biochemical alterations such as proteoglycan loss, or changes in the collagen matrix, there is a substantial interest in detecting such changes in the course of cartilage disease/injury or after cartilage repair [7–9].

The biochemical MRI techniques most often reported to visualise cartilage ultra-structure are delayed Gadolinium-Enhanced MRI of Cartilage (dGEMRIC) and T2 mapping [10, 11]. Using dGEMRIC, biochemical MRI has the ability to quantify functionally relevant macromolecules within articular cartilage such as glycosamnioglycans (GAG). GAG are the main source of fixed charge density in cartilage, which are often decreased in the early stages of cartilage degeneration and are considered as a key factor in the progression of cartilage damage. The role of GAG is comparably important in the follow-up after cartilage repair procedures where hyaline like repair tissue with a normal or nearly normal amount of proteoglycans has been described to have a positive predictive values [12]. T2 relaxation time mapping reflects the interaction of water and the extracellular matrix. Changes in hydration as well as collagen anisotropy, reported to be early indicators of cartilage deterioration, can be visualized by T2 relaxation time mapping. In cartilage repair, quantitative T2 mapping is able to assess the zonal structure of the repair tissue, and hence the maturation of the repair tissue over time [13].

These recent advances in MR sequences together with the implementation of high-resolution MRI due to high-field MR systems as well as sophisticated coil technology have overcome existing limitations and led to promising *in-vivo* approaches in morphological and biochemical MRI in cartilage repair [14–16].

The aim of this chapter was to review the current literature and present own ideas of our working group, on MRI in cartilage repair with the focus on advanced morphological and biochemical MR imaging techniques. Hence the existing cartilage defect/injury can be assessed in detail, enabling for decision making in the specific therapeutical pathway. After cartilage repair the repair tissue as well as the surrounding structures can be assessed non-invasively and possible complications can be depicted.

7.2 Pre-requirements for Cartilage Imaging

When working on an optimal protocol for cartilage imaging, the first question is which MR system to use. Commonly available systems are of different vendors and have field strengths of 1.0, 1.5 or 3.0 T. There are different studies available to

compare different field strengths in there ability to diagnose knee pathologies as well as providing information on the benefits of higher field strengths [17–19].

Although at 1.0 or 1.5 T, MRI is able to detect cartilage irregularities in high quality, the 3.0 T examinations provided a better visibility especially of smaller structures and cartilage was better delineated [19]. This is usually based on an increased average signal-to-noise and contrast-to-noise ratio at higher fields. Concluding, at 3.0 T, imaging of the knee is faster and/or a higher visibility (and resolution) of anatomic structures can be reached [18, 19]. In cartilage injury or repair, the highest available field strength should be used to provide the best available quality of the MR protocol.

Besides the field strength however, the selection of a dedicated, multi-channel coils is possibly even more important [18, 19]. Most available MR scanner today come with an 8 or 15 channel knee coil. To use these coils in cartilage patients will improve the image quality and provides also in 1.0 or 1.5 T the ability to end up in high-resolution MR protocols in an acceptable acquisition time. The benefit of multi-channel coils lies (in parts) in the possibility of parallel-imaging where basically more information can be acquired in less time. In a study by Zou et al. it was demonstrated that parallel imaging can be applied to current knee cartilage protocols with an acceleration factor of two (reduces acquisition time by 50 %) without degrading measurement accuracy and good reproducibility [20].

The pre-requirement of an optimal MR scan is using the right sequences and plan the sequences on the localizer (initial landmark scan of the knee) in the right direction. Hence in a standard 2D MR evaluation, to gain high-quality, high-resolution images, the anatomical curvature and localisation of e.g. the femoral condyles have to be taken into consideration. This is especially important after cartilage repair when the area where the repair has taken place is known. By e.g. adapting the sequence-slab (orientation) exactly on the respective femoral condyle, the repair tissue and the adjacent structures can be assessed in best possible quality and resolution.

7.3 Diagnosis of Cartilage Injury: Pre-operative MRI

The quality of the diagnosis is naturally one of the most important parts when treating patients. When a cartilage injury is diagnosed, besides the age of the patient, the activity level, the symptoms and other clinical findings, the suspected size of the cartilage defect is one of the most important things when planning surgery. Hence the pre-operative MRI needs to be of high quality, especially as existing studies show that radiologic reports based on standard morphological MRI frequently underestimate the actual size of a lesion (which were then found intra-operatively) [21, 22]. In the study of Gomoll and co-workers, cartilage lesions where underestimated up to 300 % in the patello-femoral joint [22]. Based on a high quality MRI, this should not be the case and cartilage lesions should be graded better. For sure it will never be possible that a 100 % match is reached between non-invasive diagnosis and the following surgery, nevertheless for

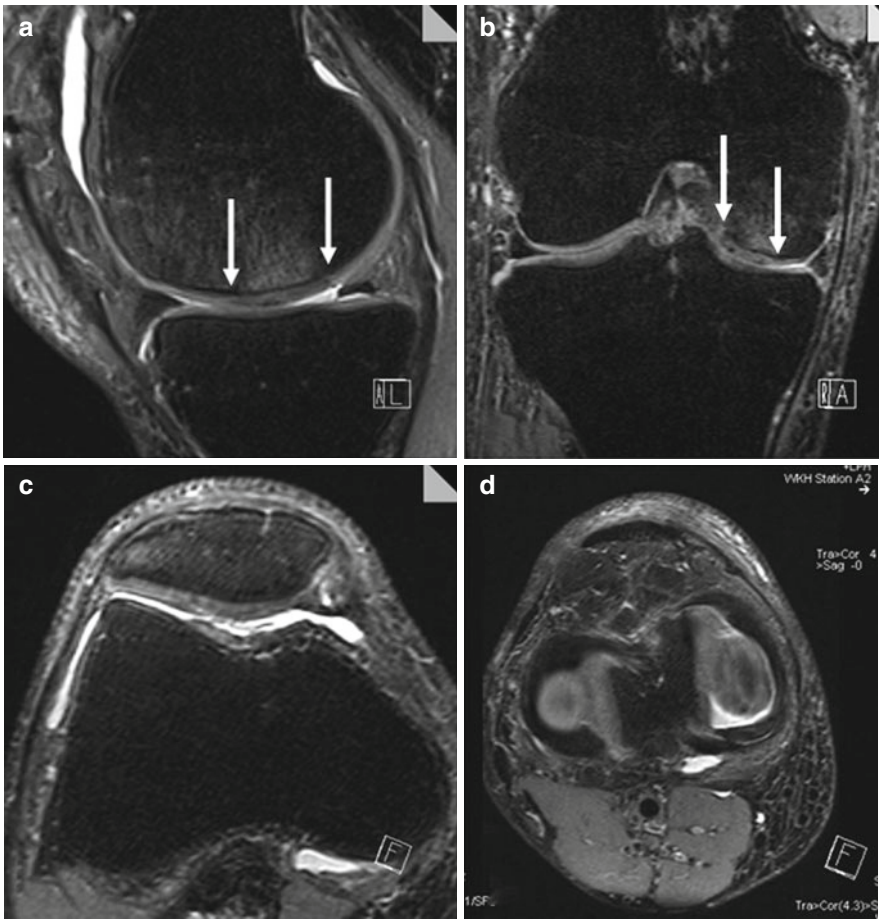

Fig. 7.1 An isotropic 3D data set of a PD SPACE sequence visualizes in different multi-planar reconstructions (MPR) a patient after cartilage repair of the femoral condyle. Besides the cartilage repair tissue in two planes (marked by *arrows*, **a**, **b**) the patello-femoral joint (**c**) and the menisci (**d**) are reconstructed

preparing a tailored surgical approach, the match has to be in the range of the real defect. Reasons for the pre-operative underestimation of the cartilage lesion are based on different reasons. First a standard MRI usually consists of 2D sequences with a slice thickness in between 3 and 5 mm and an existing interslice gap. Hence the borders of the cartilage defect are not exactly depicted. Furthermore there are regions in the knee (e.g. the trochlea) where the assessment of the anatomy is nearly not possible by 2D MR sequences. Possible better results can be reached by exploiting isotropic MR sequences [16, 23]. With these sequences, a 3D data set can be acquired (e.g. $0.5 \times 0.5 \times 0.5$ mm) without any gap between the slices. Using 3D viewing tools the observer can navigate three-dimensionally within the knee joint and all anatomical regions can be graded adequately. Figure 7.1 visualizes

such a data set in different multi-planar reconstructions (MPR) in a patient with after cartilage repair. Cartilage sensitive isotropic sequences as well as 3D viewing tools are available by every vendor and not dependent on the field strength.

Besides morphological MRI, also biochemical MR sequences, such as dGEMRIC, T2 mapping or others, can be used in pre-operative imaging. Although a full thickness cartilage defect cannot be evaluated, biochemical MRI is a very promising tool to (i) assess the borders of the cartilage defect regarding to their quality, to (ii) assess the cartilage defect itself if there is not a full-thickness defect, and (iii) to assess the cartilage quality of the surrounding tissue. Although nearly no studies are available on this topic, all given examples will be topics of future research and will help in clinical decision making. To assess the "real" border of the deteriorated cartilage is very important and although this decision is done intra-operatively, as mentioned above, more knowledge has to be acquired pre-operatively that a better planning of the surgical procedure is possible. To evaluate a more chronic and not full-thickness cartilage defect in its quality of the thin remaining cartilage layer is another possible option for the pre-operative use of biochemical MRI. Hence the biochemical and biomechanical quality of these cartilage areas can be assessed. This is roughly comparable to the evaluation of overall cartilage quality of the joint. Especially in older patients, based on these possibilities, it might be easier to know in advance if a patient will benefit from a surgical cartilage repair procedure or not. Comparable data is available in joint preserving hip surgery where the possible success is based on the pre-operative cartilage quality as measured by dGEMRIC [24]. By including biomechanical MRI, initial studies showed that early cartilage changes can be quantified and detected [25, 26].

Concluding pre-operative MRI (respectively optimal cartilage diagnosis) should contain of a set of cartilage sensitive MR sequences, and if possible a 3D-isotropic MR sequence and as well as (if possible) a biochemical MR sequence. Moreover the rest of the joint has to be diagnosed in comparably high quality.

7.4 Post-operative MRI

An optimal MRI protocol after a cartilage repair procedure, should in principle contain the same set of sequences than the pre-operative MRI. However as the area where the repair procedure has taken place is now known, this area can be depicted in more detail in highest possible resolution. The planning of such a sequences slab is mentioned above; by exploiting high resolution in the limited area of cartilage repair, early changes like beginning delimitation, subtle split like lesions, or underlying bony changes can be diagnosed and possibly treated with the aim to prevent the patient from a failure of the repair procedure.

In the post operative follow-up, the magnetic resonance observation of cartilage repair tissue (MOCART) scoring system is claimed to allow subtle and suitable assessment of the articular cartilage repair tissue [27, 28]. This MR assessment of the MOCART score is based on standard 2D MR sequences,

depending on the locality of the area of cartilage repair, the MR evaluation of the cartilage repair tissue is performed on sagittal, axial or coronal planes using high spatial resolution together with a slice thickness of 2–4 mm. However, also this MOCART scoring system can now be performed in more detail and with additive variables, enabling for a more precise depiction of the repair tissue as well as the surrounding structures. This new "3D" MOCART score [16] can be still assessed by 2D standard MR sequences, however also the new and above mentioned 3D isotropic MR sequences can be used and their potential benefits are incorporated into this new score. In recent literature, this score seems to be reproducible and can be achieved by different MR protocols and in different joints besides the knee joint [16, 23]. A scoring sheet for the new MOCART score is presented in Table 7.1.

7.5 Morphological MR Sequences

Recommendations for the specific sequences protocol in cartilage repair are hard to make as there is an existing regional variation. Widely used MRI techniques are intermediate-weighted fast spin-echo (FSE) and three-dimensional (3D), fat-suppressed gradient-echo (GRE) acquisitions [41–45]. Whereas the GRE sequence visualizes cartilage defects attributable to T1 differences between cartilage and fluid, the FSE sequence uses differences in T2 weighting. Compared to fluid, cartilage is higher in signal intensity on fat-suppressed T1-weighting and lower on intermediate or T2-weighting. While the 3D-GRE sequence with fat suppression is suitable for visualization of the thickness and surface of cartilage and allows 3D volume measurements, the FSE sequence is sensitive for the assessment of the internal cartilage structure as well [41, 42, 44]. The subchondral bone also displays high signal intensity, due to fatty marrow, which remains relatively hyperintense on FSE T2 sequences. Intra-chondral cartilage matrix alterations, surface changes, and fibrillation can thus be assessed. Another advantage of FSE sequences is the low sensitivity to magnetic susceptibility artifacts, which are suppressed by the multiple refocusing 180° pulses of the FSE, which facilitates reliable post-operative MRI assessment. Both sequences, the fat-suppressed 3D GRE and the T2-weighted FSE, have shown excellent results, with high sensitivity, specificity, and accuracy for detecting cartilage lesions in the knee [41, 42, 46]. Depending on the vendor however, the names of these sequences are different (Fast Spin Echo (FSE), Turbo Spin Echo (TSE), Rapid Acquisition with Refocusing Echoes (RARE)). To include or exclude fat saturation (FS) is another important issue. The differences in terms of FS are most relevant in the evaluation of the underlying bone. Whereas without FS the structure of the bone, e.g. after osteochondral grafting, can be assessed in more detail, only with present FS, bone marrow edemas can be seen and graded. In the last years, proton-density (PD) TSE or FSE sequences in different orientations (sagittal, coronal, axial) with and without FS have become the standard in orthopaedic as well as in cartilage imaging. The slice thickness is usually between 3 and 4 mm and the in-plane resolution

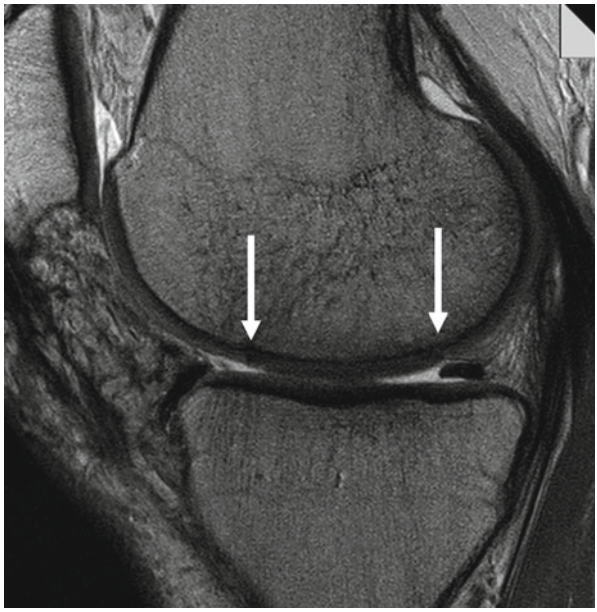

Fig. 7.2 A high-resolution PD-TSE sequence shows an exemplary patient after cartilage repair of the medial femoral condyle (marked by *arrows*). Besides the exact defect filling and the subchondral bone plate, especially the cartilage interface to the surrounding healthy cartilage is getting visible

should be about 0.5 × 0.5 mm to assess the cartilage in high enough resolution. To include a PD-TSE sequence with even higher in-plane resolution (~0.2–0.3 × 0.2–0.3 mm) and possibly lower slice thickness (~2 mm) where e.g. the medial femoral condyle or the patella can be assessed post-operatively after cartilage repair is strongly recommended. With such high resolution images of the area where the cartilage repair has taken place, the cartilage repair tissue and possibly early changes/pathologies can be analysed in more detail. An example of a patient after cartilage repair imaged with a high-resolution PD-TSE sequence please find in Fig. 7.2. These sequences can also be used for morphological assessment after cartilage repair using the magnetic resonance observation of cartilage repair tissue (MOCART) scoring system.

7.6 Advanced Isotropic 3D MR Sequences

The above mentioned isotropic MR sequences have the potential for high-resolution isotropic imaging, with a voxel size down to 0.4 mm [3], and can thus be reformatted in arbitrary planes. Such sequences are called SPGR (spoiled gradient echo), FLASH (Fast Low-Angle Shot), or VIBE (Volume Interpolated Breath-hold Examination), DESS (Double-Echo Steady-State), and SSFP (Steady-State Free Precession) or True-FISP (Fast Imaging with Steady-State Precession), among others. Furthermore, isotropic 3D fast spin-echo sequences have recently become available (called PD [proton-density] SPACE [Sampling Perfection with Application optimized Contrasts using different flip angle Evolutions] or 3D Fast Spin-Echo

(FSE) Extended Echo-Train Acquisition (XETA)), which provide the opportunity to characterize the constitution of cartilage, bone, menisci, ligaments, and the surrounding tissue within one clinically applicable sequence. Other available sequences are T2*-weighted gradient recalled-echo acquired in the steady-state (GRASS), gradient recalled-echo (GRE), and fast field-echo (FFE), always depending on the providing vendor. The classic cartilage (and cartilage segmentation) sequence is the FLASH sequence, a fat-suppressed, gradient-recalled-echo sequence with radiofrequency spoiling [29], showing high reproducibility in the segmentation of articular cartilage, and facilitates accurate evaluation of total cartilage volume and regional distribution. Nevertheless, also newer isotropic sequences enable for cartilage volume measurements.

In addition the 3D-DESS sequence was introduced as another MRI acquisition that could measure changes in cartilage thickness and volume in a longitudinal follow-up study of the OA initiative [30]. Another sequence with potential benefits because of substantially higher SNR and CNR, compared to the 3D-FLASH sequence, is the 3D-True-FISP sequence [31]. This advantage in signal might allow for higher spatial resolution, and thus, potential improvement of the accuracy of the segmentation process, especially at the articular surface [31]. With high-filed MRI, this advantage might also be used to perform isotropic MR measurements in a minimal amount of time. Comparing the performance of an 3D-SPGR sequence and two 3D-SSFP sequences at 1.5 and 3 T, Kornaat et al. [18] found SSFP-based techniques to show the highest increase in SNR and CNR efficiency at 3.0 T MRI. In recent articles by Duc et al., the True-FISP sequence as an SSFP-based sequence was studied in detail at 1.5 T and also showed promising results. Compared to a 3D-FLASH and a 3D-DESS sequence, the preoperative detection of cartilage defects is possible with similar sensitivity, specificity, and accuracy for the water-excitation True-FISP sequence; however, again, the SSFP-based sequences showed the highest SNR and CNR efficiency [32]. This enables– even at 1.5 T – the use of a dedicated, eight-channel knee coil to complete an isotropic (0.6 mm^3) 3D- True-FISP sequence in approximately 3 min with better performance in diagnosing cartilage defects, anterior cruciate ligament abnormalities, and meniscal tears, than a set of standard 2D sequences [33]. Promising results for the assessment of cartilage lesions, as well as other internal knee derangements, might be provided by 3D fast spin-echo sequences. Compared to 2D FSE sequences, an isotropic (0.7 mm^3) 3D-FSE XETA sequence provided isotropic data sets with the possibility of reformatting in arbitrary planes and high cartilage SNR [34]. An example of a fat-saturated isotropic 3D-PD SPACE image, reconstructed using a 3D viewing tool, is depicted in Fig. 7.1.

7.7 Biochemical MRI Methods

Especially in the post-operative follow-up after cartilage repair, biochemical MR sequences provide additional information on the ultra-structure and the composition of the cartilage repair tissue and the surrounding cartilage. T1 mapping using the

dGEMRIC technique, T2 mapping, T1 rho, diffusion weighted imaging and many other techniques are showing very promising results. Different repair tissues (e.g. MACT versus MFX) can be clearly distinguished, different matrices used for MACT can be assessed in the ability to produce hyaline like repair tissue and the maturation of the cartilage repair tissue after various techniques can be analysed and quantified.

7.7.1 T1 dGEMRIC and Other Proteoglycan Sensitive Techniques

One of the major macromolecules in cartilage and cartilage repair tissue, the glycosamnioglycans (GAG), can be quantified using T1-dGEMRIC. Intravenously administered gadolinium diethylenetriamine pentaacetate anion (Gd-DTPA^{2-}), penetrates the cartilage through both the articular surface and the subchondral bone. The contrast equilibrates in inverse relation to the fixed charge density (FCD), which is, in turn, directly related to the GAG concentration; therefore, T1, which is determined by the Gd-DTPA^{2-} concentration, becomes a specific measure of tissue GAG concentration, suggesting that Gd-DTPA^{2}-enhanced MRI has the potential for monitoring GAG content of cartilage *in vivo* [35]. Thus, T1 mapping enhanced by delayed administration of Gd-DTPA^{2-} (T1 dGEMRIC) can be considered the method of choice for detecting proteoglycan depletion in articular cartilage.

As differences in pre-contrast values between cartilage repair tissue and normal hyaline cartilage are larger compared to early cartilage degeneration, in cartilage repair tissue, the pre-contrast T1 values must be calculated, as well [36]. The concentration of GAG is represented by delta ΔR1, i.e., the difference in relaxation rate (R1 = 1/T1) between $T1_{precontrast}$ and $T1_{postcontrast.}$ Thus, the sequence must be performed twice, for pre-contrast and delayed post-contrast T1 mapping, which increases the total scan time for standard inversion recovery (IR) evaluation. For this reason, a new approach for fast T1 mapping has shown promising results and is increasing the clinical applicability of the dGEMRIC technique [7]. An example of a patient after MACT of the medial femoral condyle is presented in Fig. 7.3.

A study showed dGEMRIC to be able to differentiate between different cartilage repair tissues with higher delta ΔR1 values, and thus, lower GAG content for cartilage repair tissue after MFX compared to MACT [37]. Another recent study shows the ability to only use the post contrast T1-dGEMRIC mapping without any loss of information on the constitution of the repair tissue [38]. As the mapping of the GAG concentration is desirable for the diagnosis and monitoring of cartilage pathologies and the presented dGEMRIC technique has the limitation of contrast agent administration and a time delay before post-contrast MRI, a recently described technique for the assessment of GAG concentration *in vivo* by chemical exchange-dependent saturation transfer (CEST) may have potential in future applications on articular cartilage [39]. Furthermore T1rho is seen by different authors as a measure of GAG concentration [40, 41]. Although the specificity to directly quantify the proteoglycan content might be less pronounced compared to dGEMRIC and some authors see

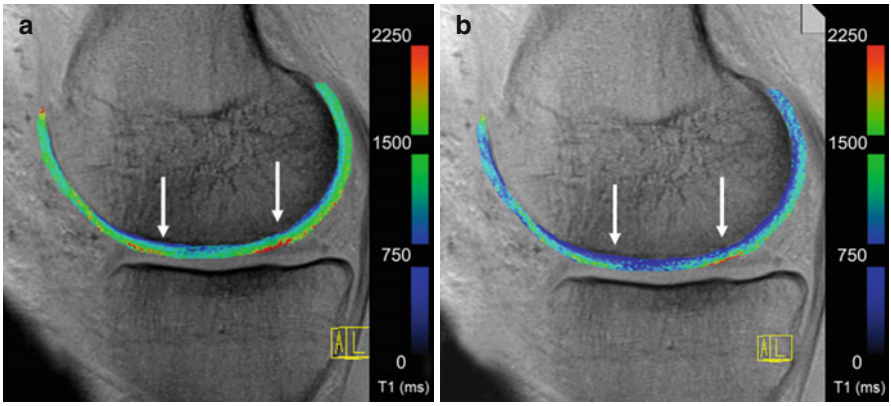

Fig. 7.3 A patient after MACT of the medial femoral condyle is presented pre (**a**) and post (**b**) the i.v. administration of Gadolinium resulting in the T1 mapping dGEMRIC technique. The slightly reduced glycosaminoglycan contant is getting visible in the post-contrast (**b**) image

clear correlations to collagen sensitive techniques [42], T1rho still is a very promising MR technique to image macromolecules.

7.7.2 T2 Mapping and Other Collagen Sensitive Techniques

Perhaps the most frequently implemented biochemical MR technique is the transverse relaxation time (T2) of cartilage as a sensitive parameter for the evaluation of changes in water and collagen content and tissue anisotropy [11]. Cartilage T2 reflects the interaction of water and the extracellular matrix on a molecular level. The collagen fiber orientation defines the layers of articular cartilage. Thus, the three-dimensional organization and curvature of the collagen network, influenced by water mobility, the proteoglycan orientation, and the resulting magic angle at 55° (with respect to the main magnetic field (B0)) influence the appearance of T2 [26]. In healthy articular cartilage, an increase in T2 values from deep to superficial cartilage layers can be observed. Histologically validated animal studies have shown this zonal increase in T2 values as a marker of hyaline or hyaline-like cartilage structure after cartilage repair procedures within the knee. To visualize this zonal variation *in vivo*, high spatial resolution is essential. In combination with a dedicated (multi-channel) coil nevertheless, T2 mapping in clinically applicable scan time could be achieved on most available (1.5 T and above) MR magnets. In cartilage repair tissue T2 values have shown an increase in the early post-operative follow-up, which enables for visualization of cartilage repair tissue maturation [20]. Furthermore it has been shown that a zonal T2 evaluation is able to differentiate cartilage repair tissue after MFX and MACT [9]. Whereas cartilage repair tissue after MFX–histologically seen as fibrocartilage–shows no clear zonal increase from deep to superficial cartilage aspects, repair tissue after MACT – histologically reported as hyaline-like–shows a significant stratification.

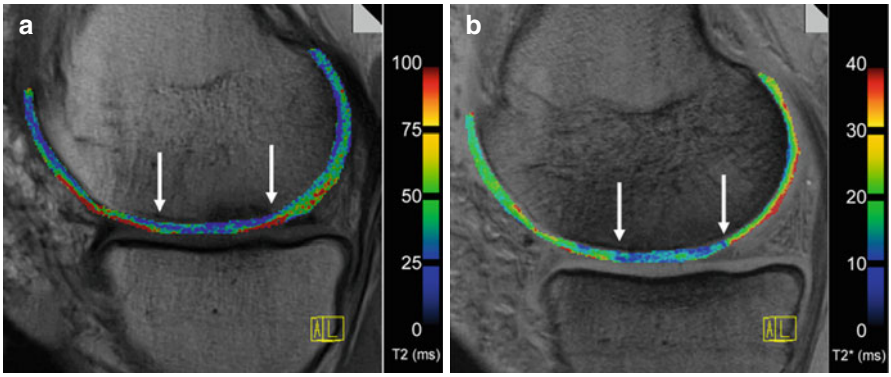

Fig. 7.4 The same patient (compared to Fig. 7.3) after MACT as assessed by T2 (**a**) and T2* (**b**) mapping. In both techniques lower T2 and T2* values are getting visible in the cartilage repair tissue reflecting an altered collagen network within the repair tissue (marked by *arrows*)

In addition to standard 2D multi-echo spin-echo T2 relaxation, T2*- weighted 3D gradient-echo articular cartilage imaging has shown reliable results in the evaluation of chondromalacia of the knee [43]. In recent studies, T2* mapping, with its potentially short scan times, was correlated to standard T2, and showed information comparable to that obtained for articular cartilage in the knee, but with overall lower T2* values (ms) [44, 45]. Furthermore, also for T2*, a clear zonal variation between deep and superficial cartilage layers was described for healthy cartilage; after cartilage repair using MFX, however, this stratification could not be found [44]. Thus, for standard T2, as well as for comparable techniques, zonal assessment of healthy and altered articular cartilage is crucial. En exemplary patient after MACT assessed by T2 and T2* mapping is visualized in Fig. 7.4.

In addition to T2 or T2 star mapping, magnetization transfer contrast has been shown reliable in the evaluation of the collagen organization and might be more sensitive to the collagen content and less dependent on the hydration of the tissue [46].

7.8 Conclusion

Over the last years MRI has become the gold standard in the diagnoses of cartilage injuries and in the follow-up after different cartilage repair procedures. Different cartilage sensitive MR sequences with high enough resolution to assess the relatively thin cartilage layers are available. Besides standard MR techniques, new isotropic MR sequences are becoming more and more available and have the possibility to assess all other knee derangements besides cartilage defects. Using these sequences after cartilage repair procedures, the MOCART (magnetic resonance observation of cartilage repair tissue) score is able to assess the state of the repair tissue as well as the adjacent structures. Besides these morphological MR techniques, so called biochemical MR methodologies (like T2 mapping or delayed

gadolinium enhanced MRI of cartilage (dGEMRIC)) are able to assess the collagen matrix respectively to quantify the glycosaminoglycan (GAG) content of native cartilage and cartilage repair tissue. In their combination, morphological and biochemical MR techniques are able to assess the repair tissue and the whole joint very precisely.

References

1. Potter HG, le Chong R, Sneag DB. Magnetic resonance imaging of cartilage repair. Sports Med Arthrosc. 2008;16(4):236–45.
2. Trattnig S, Millington SA, Szomolanyi P, Marlovits S. MR imaging of osteochondral grafts and autologous chondrocyte implantation. Eur Radiol. 2007;17(1):103–18.
3. Welsch GH, Mamisch TC, Hughes T, Domayer S, Marlovits S, Trattnig S. Advanced morphological and biochemical magnetic resonance imaging of cartilage repair procedures in the knee joint at 3 Tesla. Semin Musculoskelet Radiol. 2008;12(3):196–211.
4. Brittberg M, Winalski CS. Evaluation of cartilage injuries and repair. J Bone Joint Surg Am. 2003;85-A Suppl 2:58–69.
5. Hunter DJ, Guermazi A, Lo GH, Grainger AJ, Conaghan PG, Boudreau RM, et al. Evolution of semi-quantitative whole joint assessment of knee OA: MOAKS (MRI Osteoarthritis Knee Score). Osteoarthritis Cartilage. 2011;19:990–1002.
6. Recht M, Bobic V, Burstein D, Disler D, Gold G, Gray M, et al. Magnetic resonance imaging of articular cartilage. Clin Orthop Relat Res. 2001;391(Suppl):S379–96.
7. Trattnig S, Marlovits S, Gebetsroither S, Szomolanyi P, Welsch GH, Salomonowitz E, et al. Three-dimensional delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) for in vivo evaluation of reparative cartilage after matrix-associated autologous chondrocyte transplantation at 3.0T: preliminary results. J Magn Reson Imaging. 2007;26(4):974–82.
8. Watanabe A, Boesch C, Anderson SE, Brehm W, Mainil Varlet P. Ability of dGEMRIC and T2 mapping to evaluate cartilage repair after microfracture: a goat study. Osteoarthritis Cartilage. 2009;17(10):1341–9.
9. Welsch GH, Mamisch TC, Domayer SE, Dorotka R, Kutscha-Lissberg F, Marlovits S, et al. Cartilage T2 assessment at 3-T MR imaging: in vivo differentiation of normal hyaline cartilage from reparative tissue after two cartilage repair procedures–initial experience. Radiology. 2008;247(1):154–61.
10. Burstein D, Velyvis J, Scott KT, Stock KW, Kim YJ, Jaramillo D, et al. Protocol issues for delayed Gd(DTPA)(2-)-enhanced MRI: (dGEMRIC) for clinical evaluation of articular cartilage. Magn Reson Med. 2001;45(1):36–41.
11. Mosher TJ, Dardzinski BJ. Cartilage MRI T2 relaxation time mapping: overview and applications. Semin Musculoskelet Radiol. 2004;8(4):355–68.
12. Knutsen G, Drogset JO, Engebretsen L, Grontvedt T, Isaksen V, Ludvigsen TC, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. J Bone Joint Surg Am. 2007;89(10):2105–12.
13. Welsch GH, Mamisch TC, Marlovits S, Glaser C, Friedrich K, Hennig FF, et al. Quantitative T2 mapping during follow-up after matrix-associated autologous chondrocyte transplantation (MACT): full-thickness and zonal evaluation to visualize the maturation of cartilage repair tissue. J Orthop Res. 2009;27(7):957–63.
14. Potter HG, Black BR, le Chong R. New techniques in articular cartilage imaging. Clin Sports Med. 2009;28(1):77–94.
15. Welsch GH, Trattnig S, Domayer S, Marlovits S, White LM, Mamisch TC. Multimodal approach in the use of clinical scoring, morphological MRI and biochemical T2-mapping and diffusion-weighted imaging in their ability to assess differences between cartilage repair tissue after microfracture therapy and matrix-associated autologous chondrocyte transplantation: a pilot study. Osteoarthritis Cartilage. 2009;17:1219–27.

16. Welsch GH, Zak L, Mamisch TC, Resinger C, Marlovits S, Trattnig S. Three-dimensional magnetic resonance observation of cartilage repair tissue (MOCART) score assessed with an isotropic three-dimensional true fast imaging with steady-state precession sequence at 3.0 Tesla. Invest Radiol. 2009;44(9):603–12.
17. Bolog N, Nanz D, Weishaupt D. Muskuloskeletal MR imaging at 3.0 T: current status and future perspectives. Eur Radiol. 2006;16(6):1298–307.
18. Kornaat PR, Reeder SB, Koo S, Brittain JH, Yu H, Andriacchi TP, et al. MR imaging of articular cartilage at 1.5T and 3.0T: comparison of SPGR and SSFP sequences. Osteoarthritis Cartilage. 2005;13(4):338–44.
19. Schoth F, Kraemer N, Niendorf T, Hohl C, Gunther RW, Krombach GA. Comparison of image quality in magnetic resonance imaging of the knee at 1.5 and 3.0 Tesla using 32-channel receiver coils. Eur Radiol. 2008;18:2258–64.
20. Zuo J, Li X, Banerjee S, Han E, Majumdar S. Parallel imaging of knee cartilage at 3 Tesla. J Magn Reson Imaging. 2007;26(4):1001–9.
21. Azer NM, Winalski CS, Minas T. MR imaging for surgical planning and postoperative assessment in early osteoarthritis. Radiol Clin North Am. 2004;42(1):43–60.
22. Gomoll AH, Yoshioka H, Watanabe A, Dunn JC, Minas T. Preoperative measurement of cartilage defects by MRI underestimates lesion size. Cartilage. 2011;2:389–93.
23. Welsch GH, Zak L, Mamisch TC, Paul D, Lauer L, Mauerer A, et al. Advanced morphological 3D magnetic resonance observation of cartilage repair tissue (MOCART) scoring using a new isotropic 3D proton-density, turbo spin echo sequence with variable flip angle distribution (PD-SPACE) compared to an isotropic 3D steady-state free precession sequence (True-FISP) and standard 2D sequences. J Magn Reson Imaging. 2010;33(1):180–8.
24. Kim YJ, Jaramillo D, Millis MB, Gray ML, Burstein D. Assessment of early osteoarthritis in hip dysplasia with delayed gadolinium-enhanced magnetic resonance imaging of cartilage. J Bone Joint Surg Am. 2003;85A(10):1987–92.
25. Apprich S, Mamisch TC, Welsch GH, Stelzeneder D, Albers C, Totzke U, et al. Quantitative T2 mapping of the patella at 3.0T is sensitive to early cartilage degeneration, but also to loading of the knee. Eur J Radiol. 2012;81:e438–43.
26. Apprich S, Welsch GH, Mamisch TC, Szomolanyi P, Mayerhoefer M, Pinker K, et al. Detection of degenerative cartilage disease: comparison of high-resolution morphological MR and quantitative T2 mapping at 3.0 Tesla. Osteoarthritis Cartilage. 2011;18(9):1211–7.
27. Marlovits S, Singer P, Zeller P, Mandl I, Haller J, Trattnig S. Magnetic resonance observation of cartilage repair tissue (MOCART) for the evaluation of autologous chondrocyte transplantation: determination of interobserver variability and correlation to clinical outcome after 2 years. Eur J Radiol. 2006;57(1):16–23.
28. Marlovits S, Striessnig G, Resinger CT, Aldrian SM, Vecsei V, Imhof H, et al. Definition of pertinent parameters for the evaluation of articular cartilage repair tissue with high-resolution magnetic resonance imaging. Eur J Radiol. 2004;52(3):310–9.
29. Recht MP, Piraino DW, Paletta GA, Schils JP, Belhobek GH. Accuracy of fat-suppressed three-dimensional spoiled gradient-echo FLASH MR imaging in the detection of patellofemoral articular cartilage abnormalities. Radiology. 1996;198(1):209–12.
30. Eckstein F, Kunz M, Schutzer M, Hudelmaier M, Jackson RD, Yu J, et al. Two year longitudinal change and test-retest-precision of knee cartilage morphology in a pilot study for the osteoarthritis initiative. Osteoarthritis Cartilage. 2007;15(11):1326–32.
31. Weckbach S, Mendlik T, Horger W, Wagner S, Reiser MF, Glaser C. Quantitative assessment of patellar cartilage volume and thickness at 3.0 Tesla comparing a 3D-fast low angle shot versus a 3D-true fast imaging with steady-state precession sequence for reproducibility. Invest Radiol. 2006;41(2):189–97.
32. Duc SR, Pfirrmann CW, Schmid MR, Zanetti M, Koch PP, Kalberer F, et al. Articular cartilage defects detected with 3D water-excitation true FISP: prospective comparison with sequences commonly used for knee imaging. Radiology. 2007;245(1):216–23.
33. Duc SR, Pfirrmann CW, Koch PP, Zanetti M, Hodler J. Internal knee derangement assessed with 3-minute three-dimensional isovoxel true FISP MR sequence: preliminary study. Radiology. 2008;246(2):526–35.

34. Gold GE, Busse RF, Beehler C, Han E, Brau AC, Beatty PJ, et al. Isotropic MRI of the knee with 3D fast spin-echo extended echo-train acquisition (XETA): initial experience. AJR Am J Roentgenol. 2007;188(5):1287–93.
35. Bashir A, Gray ML, Boutin RD, Burstein D. Glycosaminoglycan in articular cartilage: in vivo assessment with delayed Gd(DTPA)(2-)-enhanced MR imaging. Radiology. 1997;205(2):551–8.
36. Watanabe A, Wada Y, Obata T, Ueda T, Tamura M, Ikehira H, et al. Delayed gadolinium-enhanced MR to determine glycosaminoglycan concentration in reparative cartilage after autologous chondrocyte implantation: preliminary results. Radiology. 2006;239(1):201–8.
37. Trattnig S, Mamisch TC, Pinker K, Domayer S, Szomolanyi P, Marlovits S, et al. Differentiating normal hyaline cartilage from post-surgical repair tissue using fast gradient echo imaging in delayed gadolinium-enhanced MRI (dGEMRIC) at 3 Tesla. Eur Radiol. 2008;18:1251–9.
38. Trattnig S, Burstein D, Pinker K, Szomolanyi P, Welsch GH, Mamisch TC. T1(Gd) gives comparable information as delta T1 relaxation rate in dGEMRIC evaluation of cartilage repair tissue. Invest Radiol. 2009;44:598–602.
39. Ling W, Regatte RR, Navon G, Jerschow A. Assessment of glycosaminoglycan concentration in vivo by chemical exchange-dependent saturation transfer (gagCEST). Proc Natl Acad Sci U S A. 2008;105(7):2266–70.
40. Regatte RR, Akella SV, Lonner JH, Kneeland JB, Reddy R. T1rho relaxation mapping in human osteoarthritis (OA) cartilage: comparison of T1rho with T2. J Magn Reson Imaging. 2006;23(4):547–53.
41. Borthakur A, Mellon E, Niyogi S, Witschey W, Kneeland JB, Reddy R. Sodium and T1rho MRI for molecular and diagnostic imaging of articular cartilage. NMR Biomed. 2006;19(7):781–821.
42. Mlynarik V, Szomolanyi P, Toffanin R, Vittur F, Trattnig S. Transverse relaxation mechanisms in articular cartilage. J Magn Reson. 2004;169(2):300–7.
43. Murphy BJ. Evaluation of grades 3 and 4 chondromalacia of the knee using T2*-weighted 3D gradient-echo articular cartilage imaging. Skeletal Radiol. 2001;30(6):305–11.
44. Hughes T, Welsch GH, Trattnig S, Brandi L, Domayer S, Mamisch TC. T2-star relaxation as a means to differentiate cartilage repair tissue after microfracturing therapy. Intern Soc Magn Reson Med. 2007;15:183.
45. Wietek B, Martirosian P, Machann J, Mueller-Horvath C, Claussen CD, Schick F. T2 and T2* mapping of the human femoral-tibial cartilage at 1.5 and 3 Tesla. Intern Soc Magn Reson Med. 2007;15:516.
46. Welsch GH, Trattnig S, Scheffler K, Szomonanyi P, Quirbach S, Marlovits S, et al. Magnetization transfer contrast and T2 mapping in the evaluation of cartilage repair tissue with 3T MRI. J Magn Reson Imaging. 2008;28(4):979–86.

Part IV
Cartilage Repair Techniques

Chapter 8
Osteochondral Allograft Transplantation: The Rationale and Basic Science

Patrick C. McCulloch and Simon Görtz

Abstract The use of allograft tissues for orthopedic reconstructions has seen a dramatic rise in the past decade. For example, allograft bone is widely used to fill bone voids, provide temporary structural support, and to induce local tissue repair. One application for the use of allograft tissues is to attempt to reconstruct the articular surface in patients with chondral or osteochondral lesions. As our understanding of the basic science of allograft tissues has grown, we have seen an evolution of its use from primarily a structural entity into one in which these tissues are implanted with the intent of preserving their biological activity and function, as well.

Articular cartilage is a particularly good target for allograft transplantation given that it is aneural, avascular, and relatively immuno-priveledged. Reports of allograft osteochondral reconstructions have been performed with excellent results. The procurement, preservation and storage of these allografts are critical factors in maintaining an appropriate balance of tissue availability and chondrocyte viability, while ensuring high standards for safety. Newer therapies which can provide an off-the-shelf option for the treatment of chondral defects have been developed and are available in some areas. More of these products or biologically-active devices can be expected in the years to come. An understanding of the potential benefits and limitations of the use of allograft tissues can help to direct research and clinical applications which optimize their role in articular reconstruction.

Keywords Allograft • Articular cartilage • Osteochondral • Transplantation

P.C. McCulloch, MD (✉)
Methodist Center for Orthopedic Surgery and Sports Medicine,
Houston Methodist Hospital,
6550 Fannin Street, Smith Tower Suite #2500, Houston, TX 77030, USA
e-mail: pcmcculloch@tmhs.org

S. Görtz, MD
Department of Orthopaedic Surgery, University of California at San Diego, UCSD,
350 Dickinson Street, San Diego, CA 92103-8894, USA

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_8,

Key Points

- Articular cartilage is a particularly good target for allograft transplantation given that it is aneural, avascular, and relatively immuno-priveledged.
- Improvements in tissue processing and storage, increased graft availability, and greater patient acceptance have led to increased use of osteochondral allografts for articular reconstruction.
- Benefits of using osteochondral allografts include: mature hyaline cartilage, preserved 3-dimensional structure, ability to manage both chondral and bone defects, ability to reconstruct large defects, and lack of donor site morbidity.
- Concerns regarding osteochondral allografts include: possibility of disease transmission or immunologic reaction, limited availability, and cost.
- Surgeons are encouraged to use accredited tissue banks with stringent criteria for donor screening and graft processing.

8.1 Background

Injuries to the articular surface present a difficult clinical problem. They have limited capacity for spontaneous healing, can become symptomatic, and may progress to osteoarthritis [1, 2]. Buckwalter noted that large chondral lesions will progress with time if not treated early with the appropriate treatment method, but which treatment is best for a given patient remains controversial [3]. There are many treatments for chondral and osteochondral defects including microfracture, autologous cell implantation (ACI), autograft transfer (OAT), and the use of engineered cartilage products and scaffolds. While each of these techniques has a role, they also have their limitations. At this time, only osteochondral allograft transplantation has the ability to reconstruct large areas of bone and cartilage with mature, layered, viable tissue in a single–stage procedure and without donor site morbidity.

The use of allograft tissues to reconstruct the articular surface of damaged joints is not a new concept [4, 5]. With the advancement in organ transplantation in the 1970s, the use of fresh allograft tissue to reconstruct articular surfaces gained popularity in North America [6]. It is now considered an established reconstruction technique in the US and grafts are commercially available from a number of different tissue banks. In 2010, 2,285 osteochondral allografts were sold in the US alone, which represented an 8 % increase over the previous year. According to market data, that trend of increased utilization is expected to continue [7]. One prominent US tissue bank, Musculoskeletal Transplant Foundation (MTF), now receives requests for approximately 700 fresh osteochondral allografts per year. This represents an increased of approximately 500 % since 2005. The most common request is for the femoral condyles of the knee (70 %), followed by talus (10 %), with the remainder being a mix of other tissues such as patella and humeral head (Musculoskeletal Transplant Foundation, 2012, Internal company data, personal

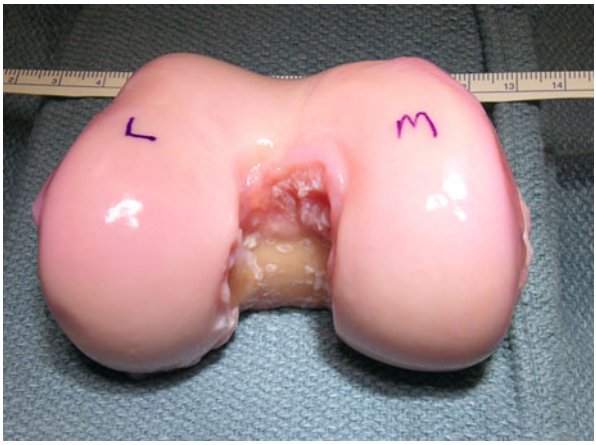

Fig. 8.1 A fresh whole distal femur allograft ready for transplantation

communication with the author). Allograft transplantation continues to gain interest in Europe and worldwide (Fig. 8.1).

8.2 Cartilage as a Transplant Tissue

Some of the same reasons why articular surface lesions show limited healing make them good candidates for allograft transplantation. Articular cartilage is avascular, aneural and alymphatic. Compared to whole organ transplantation where success requires re-establishing blood flow through tedious microsurgical vascular anastamoses, the articular surface does not require its own blood supply as it derives its nutrition through diffusion from the synovial fluid [8]. Furthermore, the chondrocytes are embedded in a thick and dense extra-cellular matrix which makes them relatively protected from host immunogenic cells, thereby obviating the need for donor-host immunologic matching [9]. Articular cartilage is aneural, and does not need a nerve supply to function normally. In fact, denervation of the articular segment may in fact be responsible for the pain-relief obtained in patients following osteochondral transplantation [10, 11]. These unique characteristics make articular cartilage truly an ideal tissue for transplantation.

8.3 Benefits of Allograft Tissue

Improvements in tissue processing and storage, increased graft availability, and greater patient acceptance have fueled interest in using osteochondral allografts for articular reconstruction. The use of allografts in orthopaedic surgical reconstructions has increased dramatically in the past decade. From 1996 to 2003, the number of allograft tissues distributed by the American Association of Tissue Banks (AATB)

increased 400 % [12]. The following describes several significant advantages that allografts have over other available treatment options.

With respect to articular reconstruction, the goal is to transplant mature hyaline articular cartilage with preservation of the normal architecture and living chondrocytes capable of surviving and maintaining the cartilage matrix. The "holy grail" in scaffold development is a three-dimensional composite graft that has sufficient biomechanical strength to support the articular segment, achieve adequate fixation, and allow for living chondrocyte in their native columnar orientation [13]. While osteochondral allografts do require bony integration, the surface is already mature. It preserves the 3-dimensional layered structure from the articular surface down to the subchondral bone. It is therefore truly a hyaline surface, rather than a "hyaline-like" surface as is often the cases with reparative techniques. One way to think of allograft transplantation is that it is not a reparative procedure, but rather a restorative one [14].

Furthermore, size-matched allografts can allow for accurate restoration of the contour of the joint surface with improved congruity. Matching the contour is very challenging with most techniques, especially for larger lesions or those with significant bone loss. Differences of even 1–2 mm may be the difference between success and failure [15]. While osteochondral autografts do work well for smaller defects, it is difficult to obtain the correct radius of curvature when taking a graft from a dissimilar area such as the trochlea and implanting it on the femoral condyle. This is especially true for larger defects which may require multiple autograft plugs, also known as mosaicplasty. The thickness of the articular cartilage and subchondral bone is also different throughout the knee. For example, using a trochlear autograft plug on the patella will result in relatively thin cartilage and incongruence of the subchondral bone layer, which may have significant biomechanical implications [16]. The use of allografts allows for more anatomic reconstruction where the lesion can be replaced with tissue from the same joint location, which is known as an orthotopic transplantation.

Osteochondral allografts can address both the cartilage and the subchondral bone. In the case of an osteochondral defect, the benefit is obvious. However, in the case of a purely chondral defect, if the cartilage is aneural, what causes the lesion to be painful? It has been postulated that the pain is mediated by the underlying subchondral bone. One of the reasons why osteochondral procedures provide good pain relief may be through denervation of the transplanted subchondral bone. This can explain why a painful OCD lesion or area of AVN can be replaced with an osteochondral allograft, which also contains nonviable bone, and still results in reliable pain relief [17, 18]. It is felt that lesions with significant bone marrow edema may portend a worse outcome with chondral resurfacing procedures compared to those with limited marrow edema. The results of ACI once the subchondral architecture has been altered by microfracture are diminished compared to those without previous microfracture [19]. These findings have led to a shift in thinking of these lesions as an articular segment problem, rather than just a cartilage problem. Allografts are able to remove potentially involved bone and provide cartilage that is firmly attached to bone at its base.

Perhaps the greatest advantage of osteochondral allografts is that there is no donor site morbidity to the patient. The size of the defect is the single greatest limitation of osteochondral autograft transfer (OATs), where larger or multiple plugs may be needed. However, with an allograft there is an essentially unlimited supply of tissue that can be used to match the defect or even multiple defects. Furthermore, this can be performed in a single-stage operation, which is a significant advantage over other procedures require an initial operation for tissue harvesting or bone grafting [20]. A single operation and rehabilitation limits the amount of time that patients need to take out of other activities such as work or school.

8.4 Disadvantages of Allografts

The most concerning disadvantages to osteochondral allograft transplantation are the potential risks of infection and immunologic reaction. When using fresh tissue, while the risks are small, they are real and must be discussed in the informed consent process with any patient considering this procedure. They are each discussed in greater detail later in this chapter.

Donor tissue availability is perhaps the most significant limitation, especially outside of the US. Patients may have to wait for up to several months for an appropriate sized graft to become available. Once tissue becomes available, there is a limited time for implantation, which means that the surgery cannot always be scheduled at a time that is optimal for both the surgeon and the patient. If the match is not readily available, that tissue will be wasted. MTF estimates that approximately 13 % of grafts are wasted due to lack patient availability, timing of surgery, or other factors such as insurance (Musculoskeletal Transplant Foundation, 2012, Internal company data, personal communication with the author). Furthermore, fresh osteochondral allografts have fallen victim to their own success. With increased popularity of these techniques, there is less availability of adequate grafts to each surgeon.

There are cultural and religious barriers to transplantation and patient acceptance may be a limitation in some areas. For example, the utilization of osteochondral allografts in Europe is significantly far behind North America. One of reasons posited for such geographical disparity is that European patients are generally averse to the use of any allograft tissue. The public may have developed an aversion to the idea after a series of high-profile failures of blood and tissue banks to adequately screen donors, resulting in disease transmission to patients who received blood transfusions and organ donations back in the 1980s [21]. The greater orthopedic community has benefited by European surgeons' search for alternatives, which made them leaders in other areas such as cell therapy and the development of scaffolds [20, 22, 23]. However, interest in the use of osteochondral and meniscal allografts is increasing at several centers in Europe [24].

Other disadvantages include that these can be technically demanding procedures both for the surgeon and the patient. Commercially available instrumentation sets have helped with the creation and implantation of certain types of grafts (Arthrex,

Naples, FL). These procedures are usually performed with open arthrotomies which can complicate rehabilitation. While mechanical failure of the grafts is uncommon, integration of the transplanted cartilage to the surrounding native cartilage is limited [1].

8.5 Graft Procurement

Most osteochondral allografts are obtained from either multi-organ donors or post-mortem donors [25]. Most tissue banks will accept an age range from approximately 15–40 years of age. This is primarily because younger donors are felt to have a lower incidence of cartilage pathology. The joint surface must pass a visual inspection for surface damage.

The preparation and storage of osteochondral allografts is different than for soft tissue grafts. The use of harsh chemicals for sterilization, irradiation and freezing techniques all result in poor chondrocyte viability. Therefore, these grafts are recovered aseptically in an operating room or similar facility. The American Association of Tissue Banks (AATB) requires that tissue be harvested within 15 h of asystole or within 24 h if the body has been refrigerated [26]. They are cleansed with a saline pulse lavage to remove as much of the marrow elements as possible [27]. They are then stored by the tissue bank in a proprietary storage medium. Conventional transplant storage medium, such as University of Wisconsin (UW) solution, appears to be better than storage in lactated Ringer's solution and the addition of bovine fetal calf serum has also been shown to be beneficial [28].

8.6 Graft Testing

The need for living chondrocytes precludes sterilization of the tissue. In the USA, the Food & Drug Administration (FDA) has oversight over tissue banks and has regulatory authority over donor selection and screening. Potential donors are screened by reviewing their medical records, interviews with the family, and a physical examination for outward signs of infection. Acceptable donors are then tested for HIV Types 1 & 2, HBV, HCV, and Syphilis. They are also screened for spongiform encephalopathies (such as "Mad Cow Disease"). In addition to governmental oversight, The American Association of Tissue Banks (AATB) provides more stringent protocols and testing requirements. The AATB is a nonprofit organization whose charter is to ensure that tissues are safe for transplantation, free of disease, of high quality and sufficient quantity to meet national needs. They provide accreditation to select tissue banks that agree to follow specific regulations and undergo periodic inspections of their facilities. For example, all accredited banks are required to test for the following: HIV and HCV by PCR nucleic acid testing (NAT) in addition to HIV Types 1 & 2 and HCV antibodies, HBV surface antigen and core

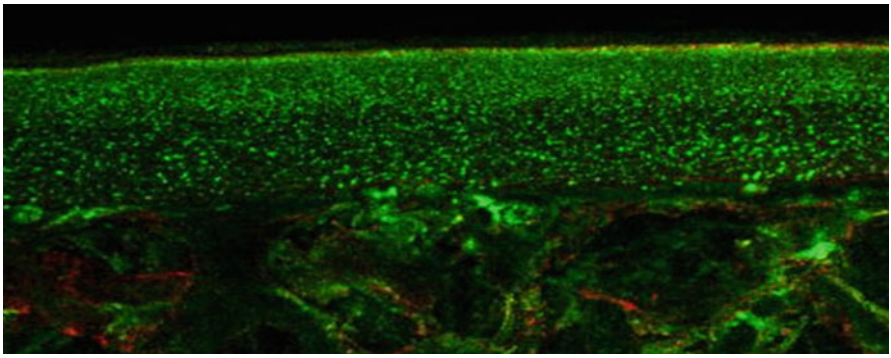

Fig. 8.2 A fresh articular segment which stains with green fluorescence (fluorescein diacetate) indicating a high level of viable chondrocytes

antibodies, HTLV-I/HTLV-II antibody, and syphilis assay [12]. PCR (NAT) testing has been shown to decrease the window from infection to positive test results from 6 weeks down to 10 days [29].

During processing, initial cultures are taken which are repeated at different time points. Grafts are often treated with an antibiotic solution. Any positive culture for Clostridium, Strep pyogenes or other high virulence organism results in immediate termination of the graft (AATB). The grafts are then held until all serologic and final culture data is completed. This may take up to 2 weeks in most tissue banks. MTF has among the most stringent release criteria. While they processed nearly 850 fresh grafts in 2011, they estimate that only about 60 % of these grafts were released for use either because of concerns about sterility, tissue quality, or donor factors such as a pending toxicology report that comes back positive (Musculoskeletal Transplant Foundation, 2012, Internal company data, personal communication with the author).

8.7 Graft Preservation and Storage

Biologically active grafts require living chondrocytes. For osteochondral allografts, immediate transplantation would provide the highest level of viable chondrocytes. Figure 8.2 shows a fresh cartilage which stains with green fluorescence (fluorescein diacetate) indicating a high level of viable chondrocytes (Fig. 8.2). However, currently accepted testing protocols require a holding period of approximately 15 days in order to ensure safety and limit the potential for disease transmission. This means that graft tissues need to be preserved for some period of time prior in transplantation. There are several methods of preserving and storing grafts including freezing, cryo-preservation, and refrigeration.

Fresh-frozen allografts are frozen down to −80 °C. While the freezing process may improve sterility and decrease immunogenicity, it results in loss of approximately >95 % of the chondrocytes [30–33]. Without active chondrocytes, the matrix

is not maintained and the cartilage deteriorates with time as evidenced by an increase in matrix metalloproteases (MMPs) [34]. Brighton and Jimenez studied fresh-frozen rabbit osteochondral allografts stored for 30 days. They found that up to 75 % showed degenerative changes such as fibrillation and proteoglycan loss [35, 36]. Therefore, this should not be thought of as a biologically active graft, but rather as a structural graft.

Cryopreservation is the process of treating tissues with glycerol and dimethyl sulfoxide (DMSO) to prevent ice crystal formation resulting in cellular swelling and lysis during subsequent freezing process. This can result in improved chondrocyte viability with a wide range from 20 to 70 % survival in multiple studies [37–39]. However, it is dependent on the depth of penetration of these reagents as the viable chondrocytes are generally seen on the surface but not in the deeper layers of the cartilage [40]. It appears that present protocols may not provide good integration [41]. However, continued research in cryopreservation could lead to improved shelf-life and therefore increased availability to patients.

"Transplantation" means that the tissue is alive, so the chondrocytes must survive and be able to maintain matrix production. Fresh allografts have the highest levels of chondrocyte viability [42, 43]. These grafts are stored in a lactated Ringer's solution or buffered culture medium and are refrigerated at 4 °C. Fresh grafts have been shown to be superior to frozen grafts in both histological and biomechanical testing [44]. Brighton showed that osteoarticular allografts could be stored in culture medium for up to 30 days with essentially normal biomechanical properties, with only minor swelling of the chondrocytes on histology and normal histochemistry [35]. When referring to bulk tissue grafts, these are known as "prolonged fresh" allografts. Chondrocytes can remain viable in storage up to 42 days, and this is the generally accepted shelf life [45–47].

However, many studies have found that the quality of the graft does deteriorate with increasing time from harvest to transplantation. There is a time-dependent deterioration of chondrocyte viability, cell density, and metabolic activity with relative preservation of the hyaline matrix [43, 47]. Grafts stored for greater than 14 days showed inferior chondrocyte viability and biomechanical properties compared those less than 14 days [48]. However, whether this affects the success of the procedure is not clear.

One study by Oates et al. compared transplantation of fresh allografts to ones stored in fetal calf serum at 4 °C for 14 days, and found no histological differences upon retrieval at the 12-week time point [49]. This appears to continue even after transplantation in grafts stored for longer than 15–20 days in one study using a non-human primate [50].

There have been several published series of prolonged fresh grafts [17, 51, 52]. In a series by McCulloch and another by Williams, the average number of days from harvest to transplantation was 28 and 30 days, respectively, with both studies showing good clinical results at minimum 2-year follow-up. There is likely a threshold for cell viability that is needed in order to maintain the matrix and prevent breakdown. However, accurate measurements of cell viability are difficult to assess [53]. To date, these studies have not shown a clear link between days in storage and

clinical outcomes. Ranawat showed no difference in fresh allografts that were stored for 1, 14 or 42 days [44]. The question of when a stored graft is no longer effective remains unanswered, but for optimal results it is believed that they should be implanted as soon as possible to maintain the maximal biologically activity.

8.8 Viability After Transplantation

Several retrieval studies have documented viable chondrocytes in the transplanted tissue [54–56]. Convery showed viable chondrocyte present in the graft at 8 years following transplantation. Czitrom found that chondrocyte viability ranged from 69 to 99 % in grafts 1–6 years after transplantation. Gross as shown that the articular cartilage can survive up to 25 years [57, 58]. Despite extensive analysis, most of these investigators were unable to determine if the viable chondrocytes originated from the donor or were the recipient's cells that had re-populated the graft. The process of host and donors cells both populating a tissue graft is known as "chimerism". Recently, one group was able to confirm living donor cells in an osteochondral graft retrieved 29 years after transplantation [59]. They used fluoroscine in situ hybridization (FISH) and genetic karyotype testing to confirm that the cells were indeed from the donor and showed no evidence of chimerism in the cartilage.

8.9 Risks of Disease Transmission

Bacterial infections are surprisingly rare with the use of allograft tissues. A recent paper reported 19 infections during which nearly five million allograft tissues had been distributed [60]. This corresponds to roughly 1 reported event per 200,000–250,000 grafts. In 2001, two patients developed a Clostridium infection after receiving allografts from the same donor. One of these patients, a 23 year-old man who had undergone a femoral osteochondral allograft transplantation died from the infection [61]. A Center for Disease Control (CDC) investigation found 14 other clostridial infections in patients who received allografts from this particular non-AATB-accredited tissue bank. In fact the donor had been refused by the local AATB facility prior to be sent to this facility which was found to have violated the AATB standards for donor recovery and processing. Other allograft-associated infections have been reported [62]. These examples highlight the importance of knowing the tissue bank providing your tissues.

Pre-implantations cultures by the surgeon have not been found to be an effective screening method. MTF followed 20 positive cultures performed in the OR and found no adverse outcomes in the recipient patients [63]. Other studies of allografts for ACL reconstruction found a 9–13.5 % incidence of positive cultures and no clinical infections. Due to the high false positive rate, intra-operative swab cultures are not recommended [64, 65].

Transmission of serious viral disease has been reported but is even less common. Four cases of Hepatitis C have been reported since 1992 [66–68]. Two cases of HIV transmission have also been reported as a result of allografts received back in 1984 and 1985 [69, 70]. These cases occurred prior to newer testing techniques which decrease the window between when donors may have been infected and antibodies first show up. With antibody testing alone, there is a window period of 1–6 months. Testing now involves the use of polymerase chain reaction (PCR) for nucleic acid testing which decreases that window period to approximately 1 week. The estimated current risk of HCV or HIV is felt to be around 1 in 421,000 [71]. Bugbee has noted that in their 20-year history encompassing more than 350 fresh allografts, they had no documented cases of disease transmission [72]. It is recommended that surgeons allograft tissues only from an AATB-accredited tissue bank, or outside of the US, one that follows similar guidelines for safety.

8.10 Immunologic Response

Transplanted osteochondral allografts do exhibit a variable immune response this is elicited by the major histocompatibility complex (MHC) antigen present on the surface of cells. The chondrocytes are embedded in the extra-cellular matrix which means that they have limited exposure to the host immunogenic cells. This environment has been termed "immuno-priviledged" [9]. Clinically, there has been a surprisingly low rate of significant immunologic reactions or "rejection" of osteochondral allografts. In laboratory studies, properly processed grafts show little or no immunologic response [31, 73]. Because rejection is uncommon, no human leukocyte antigen (HLA) or blood-type matching is required to achieve an acceptable clinical result. The only matching that is performed is for size and location.

The bone is the most immunogenic portion of an osteoarticular graft. While the cartilage is living, the bone is non-viable and must be replaced by invasion of vascularity from the recipient. This process is called "creeping substitution" and occurs with incorporation of bone graft and healing of AVN. When this occurs, the MHC Class I and II antigens in the bone are exposed and could potentially incite a cell-mediated immune response. It appears that the bone is the major cause of failure and this process of creeping substitution can lead graft failure and subsidence seen at 2–3 years following transplantation. Oakeshott looked at a series of failed allografts and found that the bone was dead, but 12 of 18 still had viable chondrocytes [73]. A common finding in failed allografts is mechanical instability with failure of integration of or cystic formation in the subchondral bone [58]. These may be indicative of bone rejection. Therefore, current techniques call for using the minimum depth of bone necessary for graft stability and the use of saline pulse lavage irrigation intraoperatively after the graft has been fashioned in an attempt to remove as much of the marrow elements as possible prior to insertion [18, 74] (Fig. 8.3).

While clinical success rates have been shown to be as high as 85 % [51, 52] not all osteoarticular allografts heal well. Errors in patient selection, technical errors such as over-zealous impaction during implantation, and failure to address other

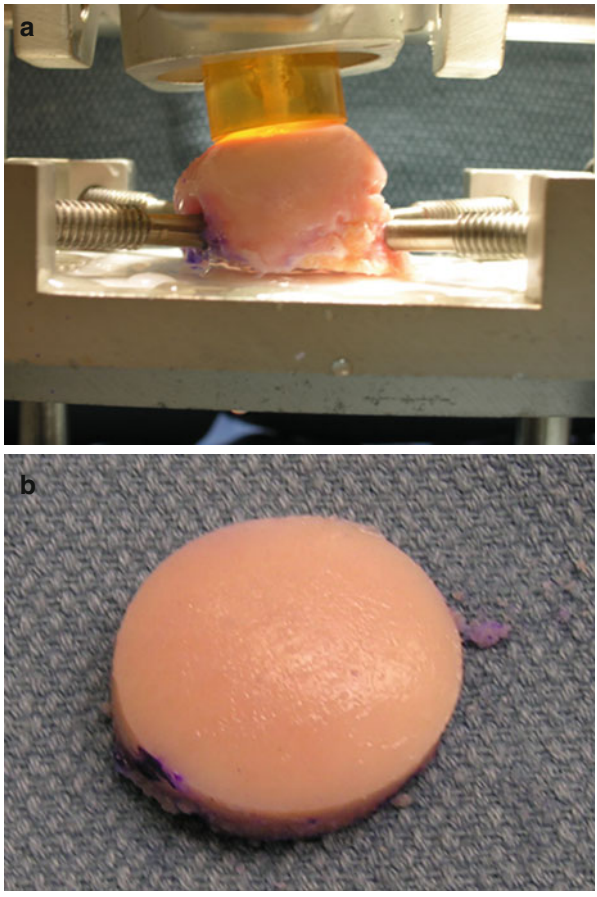

Fig. 8.3 A graft is fashioned from a fresh femoral condyle allograft using the minimal amount of bone and pulse lavage to remove marrow elements prior to press-fitting it into the recipient socket created at the defect site (**a**) Femoral hemicondyle secured into graft station with sizing dowel held in place, in preparation for core reaming. (**b**) Final dowel graft prior to implantation. Please note minimal osseous volume and ink mark for topographical orientation.

factors such as limb alignment have all been implicated. While the reason for such failures is likely multi-factorial, immune reaction may play a significant role. Lymphocytes do migrate to the area after allograft cartilage cell transplants [75]. A recent study has shown that patients who received fresh allografts can develop antibodies to antigens present in the donor [76, 77]. They found that 11 of 25 patients who received shell osteochondral allografts developed anti-HLA antibodies. The antibody-positive group showed inferior MRI characteristics with increased edema, bone marrow signal change, and a higher rate of surface collapse.

8.11 Future Directions

Improvement in tissue banking techniques will likely address some of the drawbacks of osteochondral transplantation. There is active research regarding the optimal methods of storage and preservation. Ways to maintain viability and extend storage times need to be developed. The addition of growth factors to the

storage medium, immune-modulation, and varying the storage temperature all show some promise.

Prolonged storage in a cold environment decreases metabolic activity of cells and adds risks. They may therefore be less able to maintain the matrix causing build up of free radicals, inflammatory mediators and cytokines. The re-warming process also leads to further nitric oxide production which can decrease proteoglycan synthesis. The use of gradual re-warming and nitric oxide synthase inhibitors may improve viability of these grafts [78].

Tumor necrosis factor alpha (TNFa) and interluikin-1 (IL-1) are modulators that have been implicated in this process and lead to apoptosis of the chondrocytes [79]. Adding blocking agents such as IGF-1 and caspases 1 & 3 inhibitors have been shown to prevent apoptosis of chondrocytes in culture [80, 81]. Etanercept is a drug that is used for rheumatologic diseases that has been shown to decrease TNF-alpha expression and lead to improved chondrocyte viability in storage up to 28 days [82]. Perhaps adding these modulators to the storage medium or during re-warming may be beneficial. Additional growth factors may help support the graft. Bovine fetal calf serum has been shown to be helpful in preserving osteochondral grafts and is commonly used. The use of allograft serum that is refreshed periodically may add patient specific growth factors that can improve upon present storage media and was shown to be effective in one study [83].

The bone is the most immunogenic part of the osteochondral allograft and one strategy to decrease the immunogenicity could arise from changing the way grafts are processed and stored. Chondrocytes are more resistant than osteoblasts to tissue culture at 37 °C. It is possible that pre-incubation of osteochondral allografts at 37 °C prior to storage at 4 °C may result in an optimized graft which contains viable cartilage and devitalized bone [84, 85].

To avoid issues with the bone, there are newer allograft techniques which use only cartilage. Mature allograft chondrocytes have been implanted on resorbable alginate beads, however there is concern about immunogenicity as the chondrocytes are not embedded in their matrix [75, 86]. More recently, minced allograft cartilage has come into use. DeNovo NT (Natural Tissue) (Zimmer, Inc., Warsaw, IN, and ISTO Technologies, Inc., St Louis, MO) is a commercially available minimally-modified allograft tissue obtained from juvenile donors. It is felt that the cartilage in skeletally immature donors may be superior as it is more metabolically active and the chondrocytes are further from cell senescence [87]. The cartilage is mechanically minced into small pieces which are transplanted into a defect and held in place with fibrin glue. Early results are just now becoming available and show some promise [88, 89]. A second generation product called DeNovo ET (Engineered Tissue) is now undergoing testing in the US. With this product, the minced juvenile allograft chondrocytes are isolated and expanded in culture. These cells can then be cryo-preserved meaning that more allograft tissue can become available from a limited supply of donors. Once requested, these allograft cells can be thawed and cultured whereby they create a matrix. The resulting product is similar to a large contact lens which improves tissue handling and can be fixed into a defect using fibrin glue. Tissue engineering strategies such as this could potentially lead to a

sustainable supply of allograft cartilage, and biologically active scaffolds which are available off-the-shelf for transplantation.

8.12 Summary

The use of osteochondral allograft transplantation for articular reconstruction is gaining popularity worldwide. Studies have shown that it is a relatively safe and effective treatment option for symptomatic chondral and osteochondral defects. It offers a solution to address even the largest and most complex defects, and therefore is particularly well-suited in salvage or revision cases. In the second part of this chapter, we will review the surgical techniques and clinical results. Further advances in tissue processing and storage are needed to maximize chondrocyte viability, minimize immunologic issues, and prolong the effective shelf life. Donor tissue is a limited resource and it is incumbent on surgeons and researchers to develop ways to utilize these tissues more efficiently in order to maximize the benefit to patients.

** "Osteochondral Allograft Transplantation of the Knee and Ankle: Technique and Results". By Simon Gortz and Patrick C. McCulloch

References

1. Buckwalter JA. Articular cartilage injuries. Clin Orthop Relat Res. 2002;402:21–37.
2. Mankin HJ. The response of articular cartilage to mechanical injury. J Bone Joint Surg Am. 1982;64(3):460–6.
3. Buckwalter JA. Evaluating methods of restoring cartilaginous articular surfaces. Clin Orthop Relat Res. 1999;367(Suppl):224–38.
4. Lexer EL. Joint transplantations and arthroplasty. Surg Gynecol Obstet. 1925;40:782.
5. Lexer EL. Substitution of whole or half joints from freshly amputated extremities by free plastic operation. Surg Gynecol Obstet. 1908;6:601.
6. Meyers MH, Akeson W, Convery FR. Resurfacing of the knee with fresh osteochondral allograft. J Bone Joint Surg Am. 1989;71:704–13.
7. iData Research Inc. Cartilage repair market. 2011. p. 110–30. Available at: http://www.idata-research.net/idata/index.php.
8. Lattermann CL, Romaine SE. Osteochondral allografts: state of the art. Clin Sports Med. 2009;28:285–301.
9. Langer F, Gross AE. Immunogenicity of allograft articular cartilage. J Bone Joint Surg Am. 1974;56A:297–304.
10. McDermott AG, Langer F, Pritzker KP, et al. Fresh small-fragment osteochondral allografts: long-term follow-up study on first 100 cases. Clin Orthop. 1985;197:96–102.
11. Bobic V. Arthroscopic osteochondral autograft reconstruction in anterior cruciate ligament reconstruction: a preliminary clinical study. Knee Surg Sports Traumatol Arthrosc. 1996; 3:262–4.
12. McAllister DR, Joyce MJ, Mann BJ, Vangsness Jr CT. Allograft update: the current status of tissue regulation, procurement, processing and sterilization. Am J Sports Med. 2007; 35(12):2148–58.

13. Revell CM, Athanasiou KA. Success rates and immunologic responses of autogenic, allogenic, and xenogenic treatments to repair articular cartilage defects. Tissue Eng. 2009;15(1):1–15.
14. Alford JW, Cole BJ. Cartilage restoration, part 1: basic science, historical perspective, patient evaluation, and treatment options. Am J Sports Med. 2005;33(2):295–306.
15. Feeley BT, Williams III RJ. Osteochondral allografts. In: Cole BJ, Gomoll A, editors. Biologic joint reconstruction. Thorofare: Slack Inc; 2009.
16. Robert H. Chondral repair of the knee joint using mosaicplasty. Orthop Traumatol Surg Res. 2011;97(4):418–29.
17. Gortz S, De Young AJ, Bugbee WD. Fresh osteochondral allografting for steroid-associated osteonecrosis of the femoral condyles. Clin Orthop Relat Res. 2010;468:1269–78.
18. Gortz S, Bugbee WD. Allografts in articular cartilage repair. Instr Course Lect. 2007;56:469–80.
19. Minas T, Gomoll AH, Rosenberger R, Royce RO, Bryant T. Increased failure rate of autologous chondrocyte implantation after previous treatment with marrow stimulation techniques. Am J Sports Med. 2009;37(5):902–8.
20. Jones DG, Peterson L. Autologous chondrocyte implantation. J Bone Joint Surg Am. 2006;88(11):2502–20.
21. Mueller MM, Seifried E. Blood transfusion in Europe: basic principles for initial and continuous training in transfusion medicine: an approach to an European harmonisation. Transfus Clin Biol. 2006;13(5):282–5.
22. Brittberg M. Cell carriers as the next generation of cell therapy for cartilage repair: a review of the matrix-induced autologous chondrocyte implantation procedure. Am J Sports Med. 2010;38(6):1259–71.
23. Kon E, Delcogliano M, Filardo G, et al. Novel nano-composite multilayered biomaterial for osteochondral regeneration: a pilot clinical trial. Am J Sports Med. 2011;39(6):1180–90.
24. Verdonk R, Almquist KF, Verdonk P. Logistics and European laws on allogeneic tissue transplantation. Orthopade. 2008;37(8):779–82.
25. Delloye C, Cornu O, Druez V, et al. Bone allografts: what they can offer and what they cannot. J Bone Joint Surg Br. 2007;89:574–80.
26. Vangsness Jr CT. Soft-tissue allograft processing controversies. J Knee Surg. 2006;19(3):215–9.
27. Gortz S, Bugbee WD. Fresh osteochondral allografts: graft processing and clinical implications. J Knee Surg. 2006;19(3):231–40.
28. Pennock AT, Wagner F, Robertson CM, et al. Prolonged storage of osteochondral allografts: does the addition of fetal bovine serum improve chondrocyte viability? J Knee Surg. 2006;19:265–72.
29. Caldwell III PE, Shelton WR. Indications for allografts. Orthop Clin North Am. 2005;26(4):459–67.
30. Enneking WF, Mindell ER. Observations on massive retrieved human allografts. J Bone Joint Surg Am. 1991;73:1123–42.
31. Enneking WF, Campanacci DA. Retrieved human allografts: a clinicopathological study. J Bone Joint Surg Am. 2001;83:971–86.
32. Rodrigo JJ, Thompson E, Travis C. Deep-freezing versus 4 degrees preservation of avascular osteocartilaginous shell allografts in rats. Clin Orthop Relat Res. 1987;218:268–75.
33. Tomford WH, Duff GP, Mankin HJ. Experimental freeze preservation of chondrocytes. Clin Orthop. 1985;197:11–4.
34. Acosta CA, Izal I, Ripalda P, et al. Cell viability and protein composition in cryo-preserved cartilage. Clin Orthop Relat Res. 2007;460:234–9.
35. Brighton CT, Shadle CA, Jimenez SA, Irwin JT, Lane JM, Lipton M. Articular cartilage preservation and storage. I: application of tissue culture techniques to the storage of viable articular cartilage. Arthritis Rheum. 1979;22:1093–101.
36. Jimenez SA, Brighton CT. Experimental studies on the fate of transplanted articular cartilage. In: Friedlaender GE, Mankin HJ, Sell KW, editors. Osteochondral allografts, banking and clinical applications. Boston: Brown; 1983. p. 73–9.

37. Schachar N, McAllister D, Sevenson M, et al. Metabolic and biochemical status of articular cartilage following cryopreservation and transplantation: a rabbit model. J Orthop Res. 1992;10:603–9.
38. Ohlendorf C, Tomford WM, Mankin HJ. Chondrocyte survival in cryo-preserved osteochondral articular cartilage. J Orthop Res. 1996;14:413–6.
39. Malinin TI, Wagner JL, Pita JC, et al. Hypothermic storage and cryopreservation of cartilage. Clin Orthop. 1985;187:15–26.
40. Judas F, Rosa S, Teixeira L, et al. Chondrocyte viability in fresh and frozen large human osteochondral allografts: effects of cryoprotective agents. Transplant Proc. 2007;39(8):2531–4.
41. Forriol F, Longo UG, Alvarez E, et al. Scanty integration of osteochondral allografts cryopreserved at low temperatures with dimethyl sulfoxide. Knee Surg Sports Trauma. 2011;19(7):1184–91.
42. Bugbee WD, Convery RF. Osteochondral allograft transplantation. Clin Sports Med. 1999;18:67–75.
43. Ball ST, Amiel D, Williams SK, et al. The effects of storage on fresh human osteochondral allografts. Clin Orthop Relat Res. 2004;418:246–52.
44. Ranawat AS, Vidal AF, Chen CT, et al. Material properties of fresh cold-stored allografts for osteochondral defects at 1 year. Clin Orthop Relat Res. 2008;466:1826–36.
45. Williams JM, Virdi AS, Pylawka TK, et al. Prolonged-fresh preservation of intact whole canine femoral condyles for the potential use as osteochondral allografts. J Orthop Res. 2005;23(4):831–7.
46. Williams 3rd RJ, Dreese JC, Chen CT. Chondrocyte survival and material properties of hypothermically stored cartilage: an evaluation of tissue used for osteochondral allograft transplantation. Am J Sports Med. 2004;32(1):132–9.
47. Williams SK, Amiel D, Ball ST, Allen RT, Wong VW, Chen AC, Sah RL, Bugbee WD. Prolonged storage effects on the articular cartilage of fresh human osteochondral allografts. J Bone Joint Surg Am. 2003;85-A(11):2111–20.
48. Pearsall IV AW, Tucker JA, Hester RB, et al. Chondrocyte viability in refrigerated osteochondral allografts used for transplantation within the knee. Am J Sports Med. 2004;32:125–31.
49. Oates KM, Chen AC, Young EP, Kwan MK, Amiel D, Convery FR. Effect of tissue storage on the in vivo survival of canine osteochondral allografts. J Orthop Res. 1995;13:562–9.
50. Malinin T, Temple HT, Buck BE. Transplantation of osteochondral allografts after cold storage. J Bone Joint Surg Am. 2006;88(4):762–70.
51. McCulloch PC, Kang RW, Sobhy MH, et al. Prospective evaluation of prolonged fresh osteochondral allograft transplantation of the femoral condyle: minimum 2-year follow-up. Am J Sports Med. 2007;35:411–20.
52. Williams III RJ, Ranawat AS, Potter HG, et al. Fresh stored allografts for the treatment of osteochondral defects of the knee. J Bone Joint Surg Am. 2007;89(4):718–26.
53. Lightfoot A, Martin J, Amendola A. Fluorescent viability stains overestimate chondrocyte viability in osteoarticular allografts. Am J Sports Med. 2007;35(11):1817–23.
54. Czitrom AA, Keating S, Gross AE. The viability of articular cartilage in fresh osteochondral allografts after clinical transplantation. J Bone Joint Surg Am. 1990;72:574–81.
55. McGoveran BM, Pritzker KP, Shasha N, et al. Long-term chondrocyte viability in a fresh osteochondral allograft. J Knee Surg. 2002;15:97–100.
56. Convery FR, Akeson WH, Amiel D, Meyers MH, Monosov A. Long-term survival of chondrocytes in osteochondral articular cartilage allograft. J Bone Joint Surg. 1996; 78A:1082–8.
57. Maury AC, Safir O, Heras FL, Pritzker KP, Gross AE. Twenty-five-year chondrocyte viability in fresh osteochondral allograft. A case report. J Bone Joint Surg Am. 2007;89(1):159–65.
58. Gross AE, Kim W, Las Heras F, et al. Fresh osteochondral allografts for post-traumatic knee defects: long-term follow-up. Clin Orthop Relat Res. 2008;466(8):1863–70.
59. Haudenschild DR, Hong E, Hatcher S, Jamali AA. Chondrogenic potential and homogeneity of cell populations of donor and recipient cells in a fresh osteochondral allograft. J Bone Joint Surg. 2012;94A(3):e17(1–7).

60. Yusaf MA, McAllister DR. Allograft processing and safety. In: Cole BJ, Gomoll A, editors. Biological joint reconstruction. Thorofare: Slack Inc; 2009.
61. Centers for Disease Control and Prevention. Notice to readers: unexplained deaths following knee surgery- Minnesota, November 2001. MMWR. 2001;50:1035–6.
62. Kainer MA, Linden JV, Whaley DN, et al. Clostridium infections associated with musculoskeletal allografts. N Engl J Med. 2004;350:2564–71.
63. Gocke DJ. Tissue donor selection and safety. Clin Orthop Relat Res. 2005;435:17–21.
64. Diaz-de-Rada P, Barriga A, Barroso JL, et al. Positive culture in allograft ACL-reconstruction: what to do? Knee Surg Sports Traumatol Arthrosc. 2003;11:219–22.
65. Guelich DR, Lowe WR, Wilson B. The routine culture of allograft tissue in anterior cruciate ligament reconstruction. Am J Sports Med. 2007;35(9):1495–9.
66. Centers for Disease Control and Prevention. Hepatitis C virus transmission from an antibody-negative organ and tissue donor. United States 2000–2002. MMWR. 2003;52:273–5.
67. Conrad EU, Gretch DR, Obermeyer KR, et al. Transmission of the hepatitis-C virus by tissue transplantation. J Bone Joint Surg Am. 1995;77A:221–4.
68. Eggen BM, Nordbo SA. Transmission of HCV by organ transplantation. N Engl J Med. 1992;326:411–3.
69. Centers for Disease Control and Prevention. Transmission of HIV through bone transplantation: case report and public health recommendations. MMWR. 1988;37:597–9.
70. Simonds RJ, Holmberg SD, Hurwitz RL, et al. Transmission of human immunodeficiency virus type 1 from a seronegative organ and tissue donor. N Engl J Med. 1992;326:726–32.
71. Zou S, Dodd RY, Stramer SL, Strong DM. Tissue Safety Group. Probability of viremia with HBV, HCV, HIV and HTLV among tissue donors in the United States. N Engl J Med. 2004;351:751–9.
72. Bugbee WD. Osteochondral allograft transplantation. In: Cole BJ, Gomoll A, editors. Biological joint reconstruction. Thorofare: Slack Inc; 2009.
73. Oakeshott RD, Farine I, Pritzker KP, et al. A clinical and histologic analysis of failed fresh osteochondral allografts. Clin Orthop. 1988;233:283–94.
74. Tomford WW. Transmission of disease through transplantation of musculoskeletal allografts. J Bone Joint Surg Am. 1995;77:1742–54.
75. Moskalewski S, Hyc A, Osiecka-Iwan A. Immune response by host after allogeneic chondrocyte transplantation to the cartilage. Microsc Res Tech. 2002;58:3–13.
76. Phipatanakul WP, VandeVord PJ, Teitge RA, et al. Immune response in patients receiving fresh osteochondral allografts. Am J Orthop. 2004;33:345–8.
77. Sirlin CB, Brossmann J, Boutin RD, et al. Shell osteochondral allografts of the knee: comparison of MR imaging findings and immunologic responses. Radiology. 2001;219:35–43.
78. Pylawka TK, Virdi AS, Cole BJ, et al. Reversal of suppressed metabolism in prolonged cold preserved cartilage. J Orthop Res. 2008;26(2):247–54.
79. Kim HT, Teng MS, Dang AC. Chondrocyte apoptosis: implications for osteochondral allograft transplantation. Clin Orthop Relat Res. 2008;466:1819–25.
80. Nuttall ME, Nadeau DP, Fisher PW, et al. Inhibition of caspase-3-like activity prevents apoptosis while retaining functionality of human chondrocytes in vitro. J Orthop Res. 2000;18(3):356–63.
81. Teng MS, Yuen AS, Kim HT. Enhancing osteochondral allograft viability: effects of storage media composition. Clin Orthop Relat Res. 2008;466:1804–9.
82. Linn MS, Chase DC, Healey RM, et al. Etanercept enhances preservation of osteochondral allograft viability. Am J Sports Med. 2011;39(7):1494–9.
83. Onuma K, Urabe K, Naruse K, et al. Allogenic serum improves cold preservation of osteochondral allografts. Clin Orthop Relat Res. 2012;470:2905–14.
84. Bastian JD, Egli RJ, Ganz R, et al. Chondrocytes within osteochondral grafts are more resistant than osteoblasts to tissue culture at 37 degrees C. J Invest Surg. 2011;24(1):28–34.
85. Pallante AL, Bae WC, Chen AC, et al. Chondrocyte viability is higher after prolonged storage at 37 degrees C than at 4 degrees C for osteochondral grafts. Am J Sports Med. 2009;37(S1):24S–32.

86. Almqvist KF, Dhollander AAM, Verdonk PCM. Treatment of chondral defects in the knee using alginate beads containing human mature allogenic chondrocytes. Am J Sports Med. 2009;37(10):1920–9.
87. Adkisson 4th HD, Martin JA, Amendola RL, et al. The potential of human allogeneic juvenile chondrocytes for restoration of articular cartilage. Am J Sports Med. 2010;38(7):1324–33.
88. Farr J, Yao JQ. Chondral defect repair with particulated juvenile cartilage allograft. Cartilage. 2011;2(4):346–53.
89. Kruse DL, Ng A, Paden M, Stone PA. Arthroscopic De Novo NT: juvenile allograft cartilage implantation in the talus: a case presentation. J Foot Ankle Surg. 2012;51:218–21.

Chapter 9
Osteochondral Allograft Transplantation: Surgical Technique and Results

Simon Görtz and Patrick C. McCulloch

Abstract Hyaline articular cartilage is an avascular and insensate tissue with a distinct structural organization, which provides a low-friction and wear-resistant interface for weight-bearing surface articulation in diarthrodial joints. Ideally, articular cartilage is maintained in homeostasis over the lifetime of an individual, with its biomechanical properties inherently suited to transmit a wide variety of physiologic loads through a functional range of motion. Although its viscoelastic characteristics make it ideally suited to transmit a wide variety of physiologic loads through a functional range of motion while maintaining homeostasis, it also displays an intrinsic inability to heal when injured in the skeletally mature individual. Thus, articular cartilage lesions commonly lead to significant disability, joint dysfunction and ultimately osteoarthritis. Current treatment options are limited and often ineffective at restoring healthy articular cartilage, especially in complex cartilage defects involving large areas of damage and associated subchondral bone loss. While several options for repair of articular cartilage defects do exist, fresh osteochondralallografting currently remains the only technique that restores anatomically appropriate, mature hyaline cartilage in large articular defects. Osteochondralallografting is a valuable and uniquely versatile cartilage restoration technique that can address even complex or multiple lesions in topographically challenging environments by restoring the anatomy of the native joint both

S. Görtz, MD (✉)
Department of Orthopaedic Surgery, University of California,
San Diego, 350 Dickinson Street, San Diego,
CA, 92103-8894, USA
e-mail: sgortz@ucsd.edu

P.C. McCulloch, MD
Methodist Center for Orthopedic Surgery and Sports Medicine,
Houston Methodist Hospital, 6550 Fannin Street,
Smith Tower Suite #2500, Houston,
TX 77030, USA
e-mail: pcmcculloch@tmhs.org

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_9, © Springer-Verlag London 2014

macroscopically and microscopically with a solid orthotopic replacement. As a result, osteochondralallografts have emerged to play an increasingly vital role in the clinical algorithm of cartilage restoration.

Keywords Fresh osteochondral allograft • Cartilage transplantation • Allografting

Key Points
- Assess and optimize the biological and mechanical environment of the joint.
- Minimize the osseous portion of the allograft to 3–6 mm except on tibial grafts, and where predicated by lesion topography.
- Remove all residual soft tissue and perform pressurized lavage of osseous graft portion to remove marrow elements prior to insertion.
- Avoid excessively impacting the graft during insertion.
- Ensure adequate stability; utilize adjunctive fixation where necessary.

9.1 Indications

Due to their compound osteoarticular nature, fresh osteochondral allografts are uniquely suited to address a wide spectrum of articular cartilage pathology, especially in disease entities that present with an osseous deficiency. Primary treatment can be considered for purely chondral defects whose size poses a relative contraindication for other treatments, and especially those that present with a loss of containment or bone involvement exceeding a depth of 6–10 mm. Allografts have also proven valuable in the salvage of knees that have failed other cartilage resurfacing procedures such as microfracture, autologous chondrocyte implantation, and osteochondral autologous plug transfer [1].

Specific conditions most amenable to allografting include osteochondritis dissecans (OCD) [2], osteonecrosis [3], and posttraumatic defects [4]. Other indications for allografting of the knee include select cases of multifocal or bipolar lesions as encountered in isolated, unicompartmental patellofemoral or tibiofemoral osteoarthritis in patients of an age and activity level that is not optimally suited for partial or total knee arthroplasty. In case of an absent meniscus, this can be implanted as part of a compound graft attached to its correlating tibial plateau, avoiding many of the size match and fixation pitfalls associated with isolated meniscal allograft transplantation. The advantage of an allogenous graft source is that even large and complex lesions can be resurfaced by reintroducing orthotopically appropriate, mature hyaline cartilage without inducing donor site morbidity and, with the fixation issue predictably relegated to bone-to-bone healing.

9.2 Contraindications

Although bipolar and multi-compartmental allografting have been moderately successful in the younger individual, allografting should not be considered an alternative to prosthetic arthroplasty in patients with advanced multi-compartment arthrosis and of an age and activity level suitable for prosthetic replacement. Likewise, the presence of open physes in the skeletally immature individual is a relative contraindication. Other relative contraindications to the allografting procedure include uncorrected ligamentous instability, meniscal insufficiency or contributory axial malalignment of the limb, which should be addressed prior or concomitantly to optimize the biomechanical environment. The presence of inflammatory disease, crystal-induced arthropathy or unexplained global synovitis generally represents a contraindication to cartilage repair procedures.

9.3 Alternative Treatments

Focal small to medium sized osteochondral lesions may be amenable to autologous grafting techniques or autologous chondrocyte implantation (ACI, see Chaps. 10 and 11), which has shown good outcomes in well-contained, unipolar lesions. Although a "sandwich" modification to the ACI procedure has been postulated to address significant bony deficiencies, results of this technique have not been individually reported. Overall, lesions that meet inclusion criteria for osteochondral allografting are often poorly suited for other cartilage restoration procedures, especially in the revision situation. None of these restorative procedures should be considered an alternative to prosthetic arthroplasty in an individual with symptoms, age and activity level that is appropriate for prosthetic replacement.

When considering realigning osteotomy in addition to an osteochondral allograft to address axial malalignment, staging the procedure is advised when the osteotomy site is juxtaposed to the allograft site as not to jeopardize the microvascularity of the recipient bone bed. Patients gaining satisfactory symptomatic relief from an isolated osteotomy alone may not require further surgical intervention but should be followed closely for signs of disease progression.

9.4 Results

The use of osteochondral transplants in biologic reconstruction of the knee joint has a long-standing clinical history internationally, and has evolved into a mainstay of clinical practice in the United States over the last quarter century. Traditionally, the allograft outcomes literature has been compounded by a high contingent of salvage cases owing to the lack of suitable treatment alternatives. However, the results of osteochondral

Table 9.1 Selected outcomes – osteochondral allografting in the knee

Author	Site of lesion	Diagnosis/ indication	Number of patients	Mean follow up (years)	Successful outcome
Chu [5]	Knee	Multiple	55	6.2	84 % G/E
Krych [6]	Knee	Multiple	43	2.5	88 % RTS
McDermott [7]	Knee	Trauma	50	3.8	76 % SCS
Ghazavi [8]	Knee	Trauma	126	7.5	85 % SVS
Beaver [9]	Knee	Trauma	92	14.0	63 % SVS
McCulloch [10]	Femur	Multiple	25	3.9	84 % SCS
Williams [11]	Femur	Multiple	19	4.0	79 % SCS
LaPrade [12]	Femur	Multiple	23	3.0	91 % G/E
Gross [4]	Femur	Trauma	60	10.0	85 % SVS
Garrett [13]	Femur	OCD	17	2–9	94 % G/E
Emmerson [2]	Femur	OCD	69	5.2	80 % G/E

SCS successful, *SVS* survivorship, *G/E* good/excellent, *RTS* return to sports

allografting compare favorably to those of other cartilage restoration procedures in matched indications, with consistent reports of good to excellent outcomes in excess of 80 % of cases at a mean follow up of up to 10 years (Table 9.1). Retrieval studies have demonstrated that viable chondrocytes are present and mechanical properties of the collagen matrix are maintained many years after transplantation [14, 15].

9.5 Surgical Technique

9.5.1 Femoral Condyle [16]

The patient is positioned supine with a proximal thigh tourniquet. A leg or foot holder is helpful in accessing the lesion by positioning and maintaining the leg in between 70° and 100° of flexion. A standard midline incision is made from the center of the patella to the tip of the tibial tubercle. For most femoral condyle lesions, a minimal anterior approach is sufficient, and eversion of the patella is not necessary. This skin incision is elevated subcutaneously, either medially or laterally to the patellar tendon, ipsilateral to the location of the lesion. A retinacular incision is then made from the superior aspect of the patella inferiorly, incising the fat pad without disrupting the anterior horn of the meniscus or damaging the articular surface. Once the joint capsule and synovium have been incised and the joint has been entered, retractors are placed medially and laterally, taking care to protect the cruciate ligaments and articular cartilage in the notch. The knee is then flexed or extended to the proper degree of flexion that presents the lesion to be treated into the arthrotomy site (Fig. 9.1). Excessive degrees of flexion limit the ability to mobilize the patella. The lesion then is inspected and palpated with a probe, to determine the extent, margins, and maximum size. In some cases where the lesion is posterior or very large, the meniscus may have to be detached and reflected, leaving a small cuff of tissue adjacent to the anterior attachment of the meniscus for reattachment at closure.

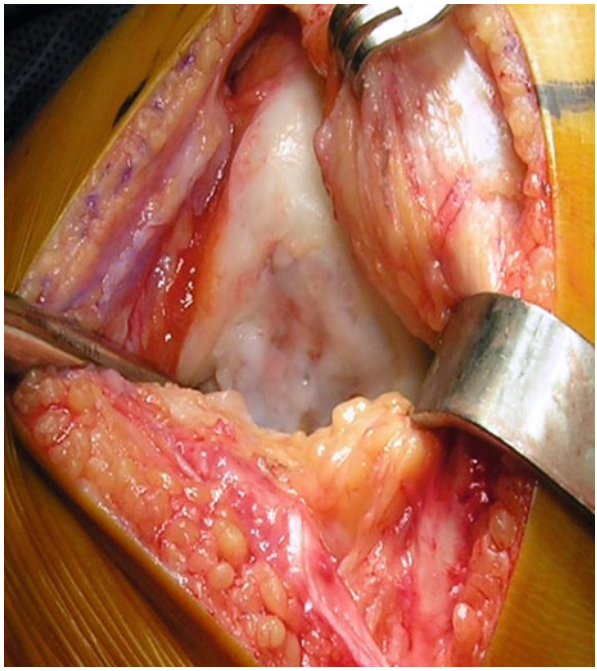

Fig. 9.1 Intraoperative photograph demonstrating an osteochondritisdissecans lesion in typical location on the lateral aspect of the medial femoral condyle, towards the intercondylar notch

The two commonly used techniques for the preparation and implantation of osteochondral allografts are the dowel technique and the shell graft technique. Each technique has advantages and disadvantages. The dowel technique is a similar technique in principle to autologous osteochondral transfer systems [refer to chapter]. This technique is optimal for contained condylar lesions between 15 and 35 mm in diameter. Fixation is generally not required in circumferentially contained lesions due to the stability achieved with the press fit of the dowel. Disadvantages include the fact that many lesions are not conducive to the use of a circular coring system, such as very posterior femoral, tibial, patellar, and trochlear lesions. Additionally, more ovoid a lesion in shape require more normal cartilage to be sacrificed at the recipient site in order to accommodate the circular donor plug. Shell grafts are technically more difficult to perform and typically require fixation. However, depending on the technique employed, less normal cartilage may need to be sacrificed. Also, certain lesions are more amenable to shell allografts due to their location.

9.5.2 Dowel Allograft

There are several similar proprietary instrumentation systems that are currently available for the preparation and implantation of dowel allografts up to 35 mm in diameter. After a size determination is made using a sizing guide dowel (Fig. 9.2), a guide wire is driven into the center of the lesion, perpendicular to the curvature of the articular surface. The size of the proposed graft then is determined, utilizing

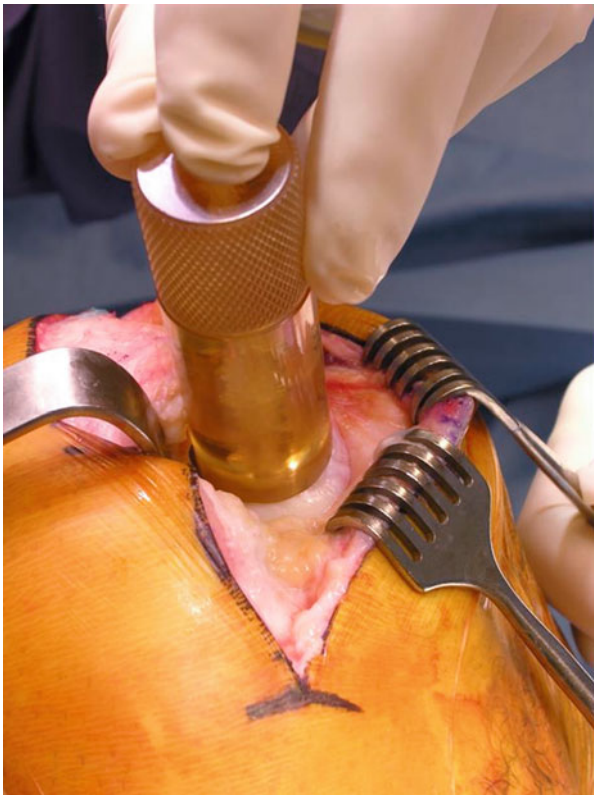

Fig. 9.2 The same lesion as shown in Fig. 9.1, being sized with a sizing dowel

sizing dowels, remembering that overlapping dowels (in a "snowman" configuration) can possibly deliver the best area coverage. The remaining articular cartilage is scored circumferentially, and a core reamer is used to remove the remaining articular cartilage and at least 3–4 mm of subchondral bone (Fig. 9.3). In deeper lesions, fibrous and sclerotic bone is removed to a healthy, bleeding osseous base. More extensive lesions should be manually curetted and packed with morselized autologous bone graft to fill these more extensive osseous defects. The guide pin then is removed, and circumferential depth measurements of the prepared recipient site are made and recorded.

The corresponding orthotopic location of the recipient site then is identified on the graft. The graft is placed into a graft holder (Fig. 9.4) (or alternately, held securely with bone-holding forceps). A saw guide then is placed in the appropriate position and alignment, again perpendicular to the articular surface; and an appropriate sized tube saw is used to core out the graft under continuous irrigation. Prior to removing the graft dowel from the condyle, an identifying mark is made to ensure proper orientation upon implantation. Once the graft cylinder is amputated using an oscillating saw and removed, depth measurements, which were taken from the recipient, are transferred to the bony portion of the graft (Fig. 9.5). This graft then

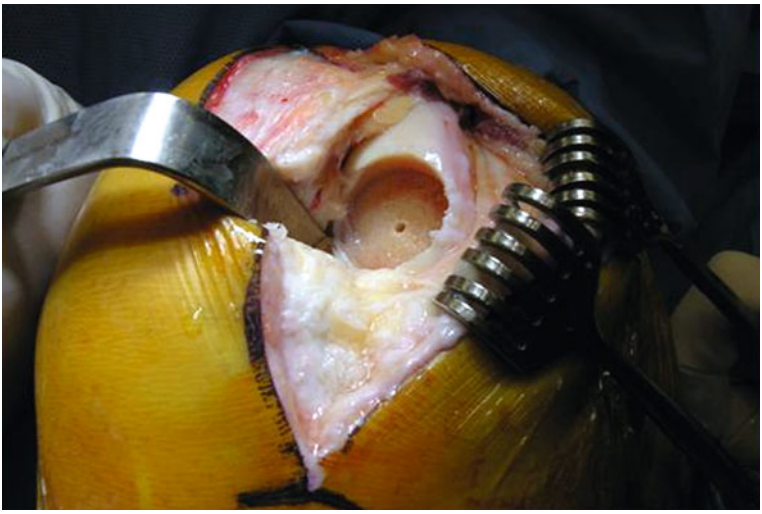

Fig. 9.3 Appearance of the lesion after core reaming of the osseous defect. Note the central hole marking the position of the guide pin, which has been removed

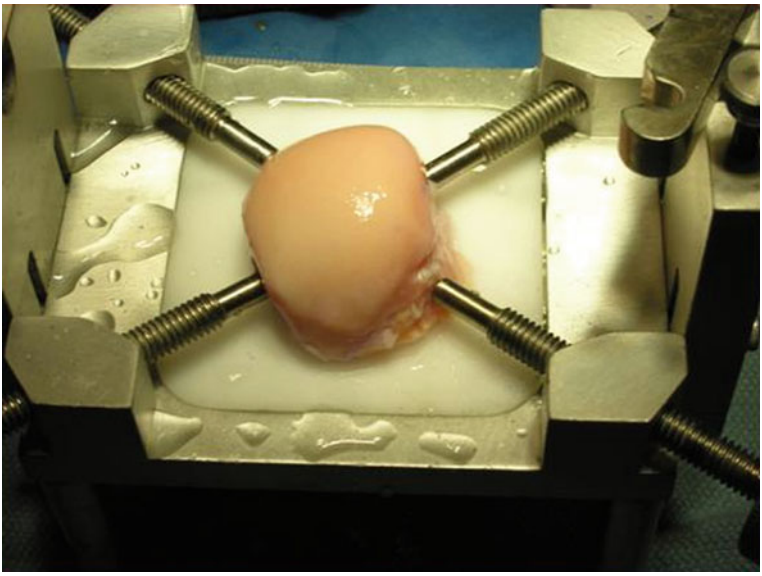

Fig. 9.4 Fresh allograft hemicondyle secured in graft holder

is cut with an oscillating saw, trimmed with a rasp to the appropriate thickness in all four quadrants, and the deep edges of the bone plug can be chamfered with a rongeur and bone rasp. Often this must be done multiple times to ensure precise thickness, preferably refashioning the graft rather than the recipient site and optimally keeping

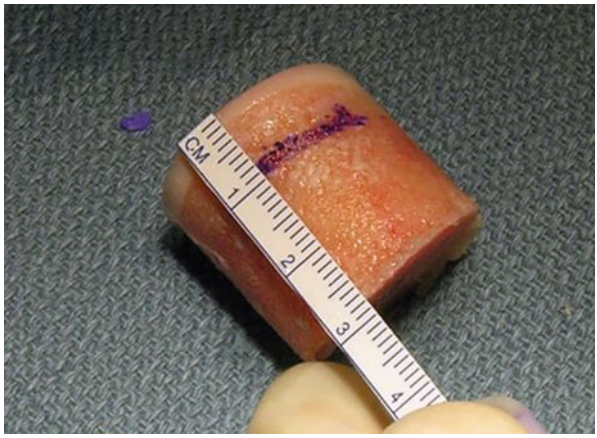

Fig. 9.5 Osteochondral allograft core with *ink mark* correlating to depth of recipient graft bed

Fig. 9.6 The allograft dowel prior to final implantation. Note the *ink marks* for orientation, and the reduced osseous component of the graft

the allograft and host cartilage moist throughout the procedure. Usually, a dowel plug will only comprise several millimeters of subchondral bone (Fig. 9.6). The aim is to transplant as much bone as is necessary to reconstruct an osseous defect, and as little as possible to minimize the bioburden to the host, as well as to optimize the rate limiting step of creeping substitution by minimizing the amount of allogeneic bone to be reconstituted. Prior to final implantation, the graft is irrigated copiously with pulsatile lavage to remove marrow elements and debris, and the recipient site can be dilated using a slightly oversized tamp in order to ease the insertion of the graft to prevent excessive impact loading of the articular surface when the graft is inserted. At this point, any remaining osseous defects are bone grafted. The allograft is then inserted by hand in the appropriate rotation. In case of a line to line fit it is often possible to seat the graft with gentle manual pressure or by using the

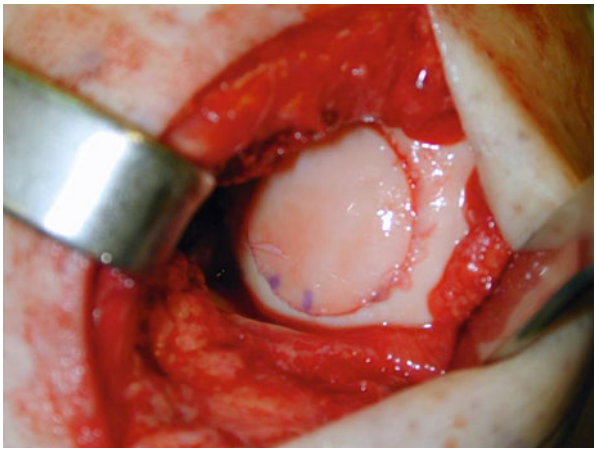

Fig. 9.7 Intraoperative appearance of the final construct at time of implantation. Note the flush alignment of the graft in relation to the surrounding nativearticular surface, and the stable press fit without supplemental fixation

appositional joint surface as a fulcrum while gently cycling the knee through a range of motion. Alternatively, a cupped mallet can be used to gently tamp the graft into place until it is flush, again minimizing mechanical insult to the articular surface of both the native and graft tissue.

Once the graft is seated, a determination is made whether additional fixation is required. Circumferentially contained dowels often provide an inherentlystable press fit that requires no additional fixation (Fig. 9.7). If necessary, bioabsorbable pins are utilized, particularly if the graft is large or borders the intercondylar notch. Sometimes the graft needs to be trimmed in the notch region, to prevent impingement. The knee is then brought through a complete range of motion, in order to confirm that the graft is stable and there is no catching or soft-tissue obstruction noted. At this point, the wound is irrigated copiously, and, if no further adjunct procedures are planned, routine closure is performed.

9.5.3 Shell Allograft

Shell allografts are employed for lesions that cannot be addressed by single or multiple plugs, either due to size, shape, or location, and depend on a free hand technique. The defect is accessed, identified, and assessed through the previously described arthrotomy. The circumference of the lesion is marked with a surgical pen. An attempt is made to create a geometric shape that is amenable to hand crafting a shell graft while minimizing the sacrifice of normal cartilage. A #15 scalpel blade is used to demarcate the lesion, and all tissue inside this mark is removed with ring curettes or other suitable instrumentation. Using motorized burrs and sharp curettes, the defect is then debrided down to a subchondral depth of 4–5 mm. Deeper cystic defects, again, are curetted by hand and later bone grafted. The allograft is fashioned in a freehand fashion, initially slightly over sizing the graft and carefully

removing excess bone and cartilage as necessary through multiple trial fittings. If there is deeper bone loss in the defect, more bone can be left on the graft and the defect can be grafted with cancellous bone prior to graft insertion. The graft is placed flush with the articular surface. The need for fixation is based on the degree of inherent stability. Bioabsorbable pins are typically used when fixation is required but compression screws may be used as an alternative. Wound irrigation and routine closure are performed as previously described.

9.6 Postoperative Regimen

Patients are allowed full range of motion post-operatively, unless there are other additional reconstructive procedures that would dictate alternative rehabilitation. While range of motion exercises and quadriceps strengthening generally are introduced early, patients are usually maintained in a toe-touch-only weight-bearing status for a period of at least 8 weeks, ultimately depending on radiographic evidence of incorporation. At 4 weeks, patients are allowed closed-chain exercises such as cycling. Progressive weight bearing as tolerated usually is allowed at 3 months, and the patient is allowed to return to recreation and sports when functional rehabilitation is complete, usually at 6 months. Typically, braces are not utilized, unless the grafting involves the patellofemoral joint, where flexion is limited to <45° for the first 4–6 weeks, or in cases where bipolar tibial femoral grafts are used, an unloader or range of motion brace can be employed to prevent excessive stress on the grafted surfaces.

9.7 Avoiding Pitfalls and Complications

9.7.1 Graft Selection

In current practice, small-fragment fresh osteochondral allografts are not HLA type or blood group matched between donor and recipient, and no immunosuppression is used. Rather, the allografts are matched to recipients on size alone. Preoperatively, the patient's knee is sized using an anterioposterior radiograph with a standardized magnification marker. A measurement of the medial-lateral dimension of the tibia is then made, just below and parallel to the joint surface. The measurement is accurately adjusted for magnification, and the tissue bank compares this to direct measurements on the donor tibial plateau. A match is considered acceptable within a tolerance of ±2 mm; however, it should be noted that there is a significant variability in anatomy. In particular, in treating osteochondritis dissecans, the pathologic condyle typically is larger, wider, and flatter; therefore, a larger donor should generally be used. In general, it is technically less challenging to fit a larger donor to a smaller recipient condyle than vice versa, due to radius of curvature. The surgeon is ultimately responsible

to inspect the tissue intended for transplantation, optimally before beginning the actual procedure. This should include affirming site, size, and integrity of the tissue including packaging, and adequacy of storage and refrigeration.

9.7.2 Allograft Failure [15]

Failure of the allograft procedure can occur due to nonunion or late fragmentation and graft collapse. While healing of the graft-host interface reliably occurs, particularly with smaller grafts, the degree of revascularization appears to be variable. Fragmentation and collapse typically occurs in areas of unvascularized allograft bone. Since it merely serves as an osteoconductive scaffold for healing to the host by creeping substitution, which is a rate limited process, the portion of transplanted bone should be minimized wherever possible, without compromising stability of the graft as warranted by the clinical situation. This will also minimize the potential antigenic burden of marrow elements possibly remaining in the transplanted cancellous bone. Patients with graft collapse typically present with new onset pain or mechanical symptoms. Radiographs may show joint space narrowing, cysts, or sclerotic regions. Magnetic resonance imaging can help rule out contributory concomitant joint pathology in the differential diagnosis of post-operative symptoms. Depending on the status of the knee joint and patient factors, the treatment options include observation, removal of the fragmented portion of the graft, repeat allografting, or conversion to arthroplasty.

9.8 Conclusion

Fresh osteochondral allografts have a role in the treatment of a wide spectrum of osteoarticular pathology, particularly in combined lesions presenting with an osseous and a chondral component. The operative procedure for the treatment of femoral condylar lesions is straightforward but demands precision to achieve reproducible results and to minimize early graft failures related to surgical technique. While many clinical and basic scientific studies support the theoretical foundation and efficacy of the use of small fragment allografts, more scientific validation of empirical clinical practice is still needed. The indications for the use of fresh osteochondral allografts continue to evolve, including use in other diathrodial joints.

References

1. Görtz S, Bugbee WD. Fresh osteochondral allografts: graft processing and clinical applications. J Knee Surg. 2006;19(3):231–40.
2. Emmerson BC, Görtz S, Jamali AA, Chung C, Amiel D, Bugbee WD. Fresh osteochondral allografting in the treatment of osteochondritis dissecans of the femoral condyle. Am J Sports Med. 2007;35:907–14.

3. Gortz S, De Young AJ, Bugbee WD. Fresh osteochondral allografting for steroid-associated osteonecrosis of the femoral condyles. Clin Orthop Relat Res. 2010;468(5):1269–78.
4. Gross AE, Shasha N, Aubin P. Long-term followup of the use of fresh osteochondral allografts for posttraumatic knee defects. Clin Orthop Relat Res. 2005;435:79–87.
5. Chu CR, Convery FR, Akeson WH, Meyers M, Amiel D. Articular cartilage transplantation. Clinical results in the knee. Clin Orthop Relat Res. 1999;360:159–68.
6. Krych AJ, Robertson CM, Williams 3rd RJ. Return to athletic activity after osteochondral allograft transplantation in the knee. Am J Sports Med. 2012;40(5):1053–9.
7. McDermott AG, Langer F, Pritzker KP, Gross AE. Fresh small-fragment osteochondral allografts. Long-term follow-up study on first 100 cases. Clin Orthop Relat Res. 1985;197:96–102.
8. Ghazavi MT, Pritzker KP, Davis AM, Gross AE. Fresh osteochondral allografts for post-traumatic osteochondral defects of the knee. J Bone Joint Surg Br. 1997;79(6):1008–13.
9. Beaver RJ, Mahomed M, Backstein D, Davis A, Zukor DJ, Gross AE. Fresh osteochondral allografts for post-traumatic defects in the knee. A survivorship analysis. J Bone Joint Surg Br. 1992;74(1):105–10.
10. McCulloch PC, Kang RW, Sobhy MH, Hayden JK, Cole BJ. Prospective evaluation of prolonged fresh osteochondral allograft transplantation of the femoral condyle: minimum 2-year follow-up. Am J Sports Med. 2007;35(3):411–20.
11. Williams 3rd RJ, Ranawat AS, Potter HG, Carter T, Warren RF. Fresh stored allografts for the treatment of osteochondral defects of the knee. J Bone Joint Surg Am. 2007;89(4):718–26.
12. LaPrade RF, Botker J, Herzog M, Agel J. Refrigerated osteoarticular allografts to treat articular cartilage defects of the femoral condyles. A prospective outcomes study. J Bone Joint Surg Am. 2009;91(4):805–11.
13. Garrett JC. Osteochondral allografts for reconstruction of articular defects of the knee. Instr Course Lect. 1998;47:517–22.
14. Jamali AA, Hatcher SL, You Z. Donor cell survival in a fresh osteochondral allograft at twenty-nine years. A case report. J Bone Joint Surg Am. 2007;89(1):166–9.
15. Williams SK, Amiel D, Ball ST, et al. Analysis of cartilage tissue on a cellular level in fresh osteochondral allograft retrievals. Am J Sports Med. 2007;35(12):2022–32.
16. Gortz S, Bugbee WD. Allografts in articular cartilage repair. J Bone Joint Surg Am. 2006;88(6):1374–84.

Chapter 10
The Genesis of Autologous Chondrocyte Transplantation/Implantation: From a Hypothesis via an Animal Model to a Clinical Reality

Lars Peterson

Abstract For more then 2000 years articular cartilage lesions have been recognized as a clinical problem and with no intrinsic healing or optimal treatment. In 1987 the first autologous cell transplantation was performed using isolated and cultured chondrocytes which were injected into an articular cartilage lesion covered with autologous periosteum. This first transplantation was based on solid information from *in vitro* chondrocyte research with animal and human cell culture techniques as well as extensive animal model studies. These animal studies included the first results using a semisynthesized collagen type I sponge injected with chondrocytes and implanted in defects. This research was the beginning for future use of artificial resorbable scaffolds and arthroscopic surgical technique. Since 1994 the results of ACT/ACI have been repeatedly reported. The result of 10–20 years follow up was reported in 2010 showing durable outcomes. The subjective results are supported by objective evaluations of histology of biopsies, immunohistochemistry, mechanical stiffness tests, gadolinium enhanced MRI after 8–20 years showing functional hyaline –like tissue. Even with wider indications towards early posttraumatic osteoarthritis such as large, uncontained, unshouldered lesions, multiple and bipolar lesions, the results have been acceptable. However, this would not have been possible without addressing and correcting concomitant background factors creating an optimal environment for the repair tissue to survive over time. Further improvement and simplifications in the treatment could be expected with optimal cell sources and development of biodegradable scaffolds/membranes and gels allowing arthroscopic technique, early and safe weightbearing but complex cases still may need extensive open surgery for success.

Keywords Autologous • Chondrocyte • Transplantation • Long term results • Background factors

L. Peterson, MD, PhD
Department of Orthopedics, University of Gothenburg, Gothenburg, Sweden
e-mail: peterson.lars@telia.com

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_10, © Springer-Verlag London 2014

Key Points

- ACT/ACI can be successful when choosing the right indication, using adequate surgical techniques, addressing background factors, and understanding the biologic healing process.
- Large, uncontained, unshouldered lesions, multiple lesions, and bipolar lesions can be tried with acceptable results. Concomitant procedures such as stabilizing and unloading procedures must be considered and performed carefully, as well as meniscus deficiencies, bony defects, or other bone pathology which should be corrected.
- The rehabilitation program has to be adjusted to the individual situation; small well contained lesions full WB can be reached at 6–8 weeks, more complex cases usually need a longer period of progressively increased weight bearing, for bipolar and multiple lesions full WB at 16 weeks- is recommended.
- Return to impact sports and heavy labor depends on the maturation time of the repair tissue and the individual injuries and their severity. Concomitant procedures may also play a role and return to football takes between 12 and 18 months.
- The lack of objective noninvasive evaluation methods is a problem, but in our experience gadolinium enhanced MRI is a promising technique but still to expensive for routine evaluations. It takes between 9 and 18 months after cartilage repair before glycosaminoglycan concentrations have returned to 80 % or normal.

10.1 Introduction

In the coming decades we will be seeing an intensive research work to prevent tissue degeneration and promote regeneration of the human body for preserving the health and wellbeing of the aging population. In this research regenerative medicine and preventive medicine will be two important disciplines to overcome and prevent degenerative and other diseases as well as sequelae after trauma. In the regeneration of complete organs e.g. liver, heart, kidney, lungs, as well as regeneration of specific tissues (e.g. in the musculoskeletal system) the fundamental key to success is the choice of the potential stemcell, from embryonic or mesenchymal origin or from tissue specific progenitor cell sources etc. The understanding of the normal and pathological function of the different cell types on a molecular level must be the platform for progress in regeneration as well as repair of organs and specific tissues. The collaboration of multidisciplinary researchers in cell biology at micro- and macromolecular levels, biomaterials, biomechanics and clinical specialities is a necessity for a successful outcome. The great possibility for the future treatment of many diseases and injuries lies in regenerative medicine with multidisciplinary approach. In this work the potential in gene therapy will be of outmost importance.

In the musculoskeletal system it seems that the regeneration of a limb with all tissues involved like bone, muscle, tendon, ligament, periosteum, cartilage, synovium, meniscus, vessels and nerves, is complicated and far away. The separate tissues, however, offer possibilities for local regeneration or repair in case of injury. In that aspect the nerve and cartilage have a minimal capacity of intrinsic healing and in the case of cartilage, an injury may, over time, progress into posttraumatic osteoarthritis by the combined effect of enzymatic autodigestion and mechanical wear accelerated by high activity levels e.g. in sports [1].

More than two thousand four hundred (2400) years ago Hippocrates (460-377b. Chr.), the leading physician at that time, was the first to recognize and treat articular cartilage injuries and in 1743 Hunter stated: "From Hippocrates to the present age, it is universally allowed that ulcerated cartilage is a troublesome thing and that once destroyed it is not repaired" [2]. This insight has over time created a nihilistic approach to the treatment of cartilage injuries by most physicians even up till the present time. In spite of the enormous progress and great development in medicine and related technology in the last centuries, the improvement in treating cartilage injuries has been very slow and not so successful. In osteoarthritis the introduction and improvement of total joint replacement techniques has made a great solution and difference for this patient group. For the traumatic articular cartilage injuries in the young and middle aged patients no optimal treatments have been present. However, during the last decades new treatment techniques for articular cartilage injuries have evolved and opened up for better short and long term results and hope for the future such as autologous chondrocyte transplantation/implantation, microfracture, and osteochondral grafting [3–5]. An exciting development is the fast growing number of centers for cartilage research and repair being established in many universities around the world with multidisciplinary teams ready to work for better understanding and treatment options of articular cartilage injuries and diseases in the future.

10.2 Background

In 1970, after 5 years of general surgery, I was offered a residency at the Department of Orthopaedic Surgery, Sahlgrenska University Hospital, Gothenburg University under the directorship of the late professor Bertil Stener and got involved in an intense period of sports medicine-traumatology, knee surgery, and the introduction of arthroscopy. Professor Stener, my esteemed chief and teacher, encouraged and allowed me to establish a section for reconstructive surgery and arthroscopy with focus on athletic injuries. In the following years we gradually turned from open to arthroscopic surgery and during this period of intense surgical work I noticed a high number of articular cartilage injuries when treating acute and chronic knee and other joint injuries. As the results were improving on meniscus and cruciate ligament surgery, there was no really good treatment for cartilage injuries at the time. This is how I got interested in cartilage injuries.

Going through the literature of previous and actual treatments of cartilage injuries such as debridement (Magnuson), spongialization (Ficat), multiple drilling (Pridie), high tibial osteotomy (Coventry) there were no durable results and no durable repair tissue [6–9].

In other attempts to repair cartilage lesions, perichondrium or periosteum were sutured to the debrided defects [10–13] with initially good short term results but deteriorating with longer follow-up as the repair tissue was fibrous in character and did not resist the wear and tear over time. For some years I tested all these procedures including single bone-cartilage autografts with mostly disappointing results.

In the late 60's Salter and O'Driscoll showed in a rabbit model with periosteum sutured to an osteochondral defect and treated with continuous passive motion a chondrogenic repair potential from the cells in the cambium layer [14]. In 1968 Chesterman and Smith performed homotransplantations of isolated chondrocytes to a tibial defect in the rabbit knee for which they showed that there was no repair of the defect [15].

10.3 Articular Cartilage Structure and Function

Articular cartilage is composed of chondrocytes and matrix built up by collagen type II, proteoglycans and water, is organized in four zones with different appearances and functions from the subchondral bone plate to the superficial layer of artricular cartilage allowing extremely low friction of the surface and diffusion of synovial fluid in and out of the matrix (Fig. 10.1). Articular cartilage is a unique tissue compared to other tissues in the musculoskeletal system by lacking vascular, nerve and lymph supply. This means that there is no inflammatory reparative response to injury and no pain elicited from the cartilage itself. However cartilage degradation products (e.g. after trauma) may cause an inflammatory response of the synovial membrane of the joint. The nutrition of the chondrocytes, which are less than 10 % of the total tissue volume, is maintained via diffusion of synovial fluid passing through the lamina splendens with inflow during non weight-bearing and outflow during weight-bearing. The oxygen tension is low and the metabolism almost anaerobic. The chondrocyte is synthesizing the matrix and maintaining the matrix by a slow turnover of mainly collagen type II, which takes up 10–20 % of the wet weight and proteoglycans (aggrecans), 4–7 % of the wet weight. The collagen type II fibers are anchored in the subchondral plate running up to the surface forming the Benninghoff's arcades and are reinforcing the matrix and adding tensile and compressional strength to the matrix. Together the chondrocytes, collagen type II, the proteoglycans and their content of water, the subchondral bone plate, and the trabecular bone form the osteochondral functional unit. This unit stands for most of the mechanical function such as shockabsorbtion (Fig. 10.1). The water content adds to the shockabsorbtion capacity and stands for between 65 and 80 % of the total cartilage volume and is maintained by the hydrophilic negatively charged proteoglycans [16].

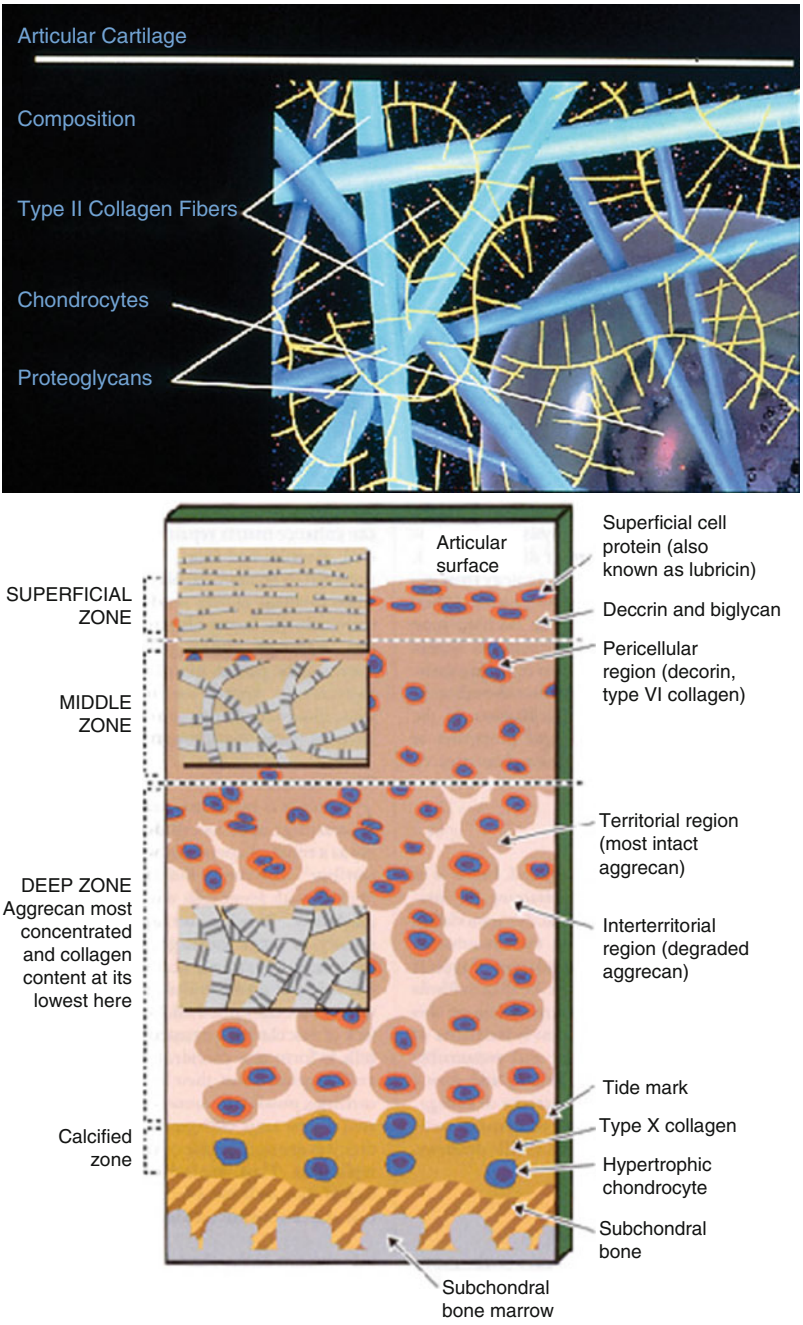

Fig. 10.1 Schematic drawings of articular cartilage structure. *Upper figure*: Close up of main components with the chondrocyte, the collagen type II and the proteoglycans. *Lower figure*: Cellular and matrix structure in different zones, from the lamina splendens to the trabecular bone; the ostechondral functional unit

10.4 Articular Cartilage Healing Capacity

It is claimed that articular cartilage has limited or no intrinsic repair capacity. Injury to the tissue will not cause a reparative inflammatory response to the cartilage itself due to the lack of vascularization, unless the subchondral bone is involved as in an osteochondral fracture [16]. It seems that there is an initial cell mitotic activity immediately after trauma in the area of the injured cartilage, but for unknown reasons this activity ends at about 14 days and leaves no repair. This may be caused by the matrix and cell damage with apoptosis that occurs when the damaged cell membrane starts to leak collagenases and proteases which degrade the newly produced collagens and proteoglycans. Healthy cells which may migrate out from the borders, where about 20 % of the cells are apoptotic, will meet a barrier of necrotic, degrading tissue and released enzymes from damaged or apoptotic cells and will not survive. The lack of sufficient inflammatory response to trauma will not start adequate macrophage or phagocyte activity to remove the necrotic tissue, so the barrier remains and the enzymatic and mechanic breakdown may progress into posttraumatic osteoarthritis over time, as there is no intrinsic cartilage repair or regenerative healing capacity [1] (Fig. 10.3). Cartilage debris and fragments –loose bodies as well as leakage of enzymes through the damaged cell membrane are causing an inflammatory response from the synovial membrane but has no healing effect on the cartilage but will disturb the joint homeostasis.

10.5 How to Address the Situation and Create Healing Conditions?

All damaged, necrotic tissue in the area must be removed down to the subchondral bone and excised 2 mm into healthy surrounding cartilage to minimize the number of apoptotic cells in the excised side (ref. Lindahl A, 2009). The defect has to be repopulated with cells with the capacity to regenerate hyaline cartilage, i.e. articular chondrocyte progenitor cells in increased numbers committed to produce hyaline cartilage. We therefore need to find adequate cell sources from a biopsy harvested from minor weight-bearing areas with minimal donor site morbidity, and develop a safe and optimal cell culture technique to achieve mitotic active and viable cells for implantation and repopulation of defects. We must find adequate autologous tissue or create biocompatible, degradable materials for keeping the cells in the defect. Then develop an animal model to study and evaluate the hypothesis: "*It is possible to heal a full thickness* (*down to the subchondral bone*) *articular defect using enzymatically isolated autologous chondrocytes grown in culture and implanted under an autologous tissue membrane or synthetic degradable biomaterials sutured or fixed to the defect*."

10.6 Cell Culture Technique and Design of a Rabbit Experimental Model

In 1982 I was invited for a year as a visiting professor at the Hospital for Joint Diseases, Orthopaedic Institute, New York City University, in Manhattan, New York City. The director was professor Victor Frankel and he allowed me to use all facilities of the hospital including research laboratories and animal operating resources with the aim to design an animal experimental model in the rabbit to test the hypothesis. In the laboratory there was a small section for cell biology with some experience of growing cells in culture. Dr David Menche and the chief of the Sports Medicine Department, dr Mark Pitman together with a young Ph.D. student Daniel Grande were running a research project and immediately we joined in a team to work on the chondrocyte cell culture and the rabbit model.

Step 1 was to establish a safe, sterile and efficient cell culture technique. Daniel Grande was the key person involved in this first step. Rabbits were operated on both knees and biopsies of articular cartilage were taken from a small area on the upper medial trochlea and from a 3 mm diameter punch defect down to the subchondral bone plate of the central medial femoral condyle or the central patella. The biopsies were brought to the laboratory, prepared and minced in small pieces, and then undergoing enzymatic digestion of the matrix according to the technique described by Audie Smith using collagenase [15]. The cells were then separated from matrix and isolated and grown for 3 weeks in standardized culture media and fetal calf serum added. After some failures with cell death, too small numbers of cells, infections etc. the whole procedure was optimized using a strict and controlled culture technique in which we were able to repeatedly grow sufficient cellnumbers for implantation [17].

Step 2 was to design the optimal experimental model in the rabbit knee. During this initial period we worked on selection of the optimal defect area, the optimal autologous cover to keep the cells in the defects and autologous versus allogenic chondrocytes. After several pre-studies we found that the cartilage thickness for suturing a cover was best on the patella, that the periosteal membrane according to Salter had a chondroid potential in the cambium layer cells and in our tests was superior to synovial membrane, tendon sheath, muscle fascia. We also found the periosteal membrane to be optimal when facing the cambium layer into the defect [18]. For safety reasons we chose the autologous chondrocytes for possible future use of autologous cells in humans. They also seemed superior to allogenic cells from the pre-studies. During this time we also built two continuous passive motion machines for rabbits according to Salter to use in the postoperative care. They were however not used later because the two first transplanted rabbits which were put into the machines, fell out of the machines during the first night and stressed themselves to death. We later found out that Salter did not use the machines during nights.

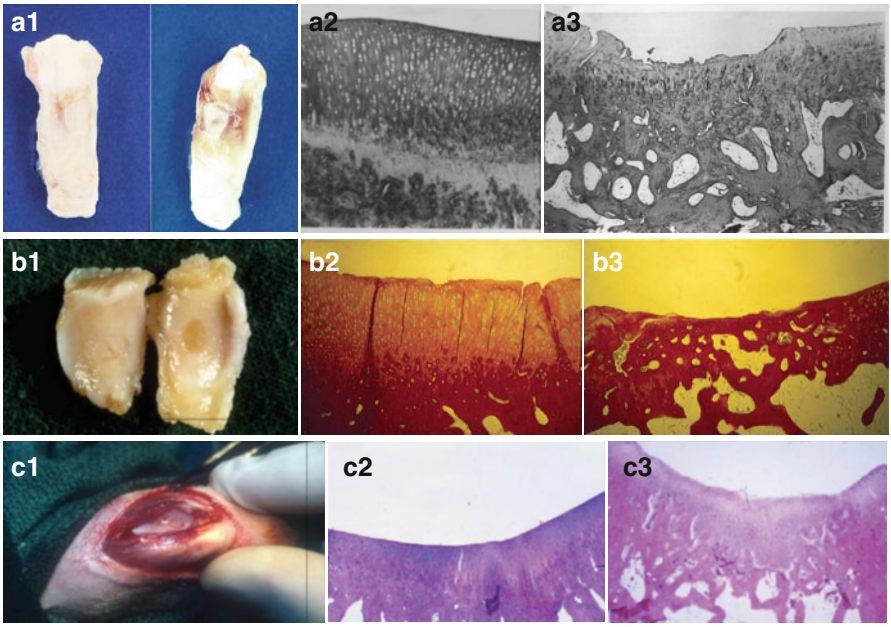

Fig. 10.2 Series of experimental work using a rabbit knee model. A 3 mm diameter articular cartilage defect down to subchondral bone in the rabbit patella. *Upper row* (**a**) showing macroscopic and microscopic results 12 weeks after autologous chondrocyte transplantation; picture (*A1*) showing filling of experimental side to the *left* and control side to the *right*. (*A2*) Histology experimental side showing hyaline cartilage, *A3* the control side showing limited filling of the defect. *Middle row* (**b**) same model with 1 year result. (*B1*) Experimental side showing good filling to the *left* and control side showing no filling to the *right*. (*B2*) Histology experimental side showing hyaline cartilage. (*B3*) The control side showing no filling of the defect. *Lower row* (**c**) same model showing results after a 12 week follow-up in 3 mm defect, (*C1*) surgical picture: the chondrocytes injected into a semi-synthesised collagen type I resorbable sponge from Indian rat tail, implanted with press-fit technique into the defect. (*C2*) Experimental side showing good filling with hyaline appearance. (*C3*) Control side showing no filling of the defect

The first results of the rabbit model were presented at the Orthopaedic Research Society meeting in Atlanta, 1984, showing an 80 % fill in the defects operated with chondrocytes and periosteal cover compared to 20 % fill in the defects without chondrocytes but with periost alone after 12 weeks [17, 19]. Microscopy showed the same staining characteristics as normal cartilage in the experimental group (Fig. 10.2).

In September 1983 I left New York to work in a chief position at the Department of Orthopaedic Surgery at the East Hospital, University of Gothenburg. In 1984 I met Anders Lindahl, who was an MD working on his thesis on epiphyseal cartilage at the Department of Physiology and has been my most important coworker to transfer ACT/ACI into human clinical practice and to continue basic and clinical

research in cartilage regeneration-repair. Together with Anders Nilsson another MD and Ph.D. student we continued the animal experiments and repeated the study from New York with a 1 year follow up. The results at 1 year after transplantation were presented at the yearly meeting of the Swedish Society of Physicians in Stockholm 1986, showing excellent filling and microscopy in the experimental group versus no filling in the control group [20] (Fig. 10.2).

At the same time I continued the collaboration with the New York group. During the early literature review I came over a paper by Shwapil, who was the first to semisynthesize collagen type I resorbable sponges from Indian rat tails. This fitted well into my idea to use scaffolds as a vehicle to support the cells in the early postoperative period but also make it possible to use arthroscopic surgical technique for implantation. Dr. Shwapil allowed us to use the sponches and we injected them with autologous chondrocytes. Using the rabbit model we compared a group with autologous chondrocytes injected into the sponge with a group with the sponge only, fixed by press fit technique into the cartilage defects. The results were presented at the ESSKA meeting in Salzburg, Austria, 1985 and showed excellent filling and histology in the experimental group compared to minimal filling in the control group (Fig. 10.2) This was the first experimental study using a degradable semisynthesized scaffold as a carrier of cells opening up to autologous chondrocyte implantation, second generation and arthroscopic technique.

10.7 Transfer of the Animal Model into Human Clinical Practice

My strategy was to transfer this animal treatment technique as far as possible into human surgical treatment and provide the highest safety for the patients by avoiding any problems like rejections, immunologic reactions, contamination with serious infections etc. The first condition was to use autologous cells, tissues, serum to minimize serious complications.

The transfer of the rabbit articular chondrocyte culture technique into human articular chondrocyte culture technique started in 1984 by Anders Lindahl and myself. Articular cartilage from anterior cruciate ligament reconstructions were harvested from the intended drillhole in the tibia and from the notch plasties under sterile conditions and were, together with the patient's own serum, transported to the laboratory. The standardized culture medium containing Ham's F 12 medium with supplements and 15 % of the patient's own (autologous) serum was added instead of fetal bovine serum [3]. The culture medium was tested for bacteria and fungi, before the cultured chondrocytes were released to use, as well as check of the cell number, character, viability etc. After about 3 years of optimizing and standardizing a safe and optimal cartilage harvesting technique arthroscopically, the work in

the laboratory, studying consequences of cell transportations, freezing of the cells for cell viability or contaminations with repeated tests, we had achieved a safe and reproducible technique for biopsies and optimal cell culture of human chondrocytes in the laboratory using autologous human serum. In 1987 we got the approval by the Ethical Committee of the Medical Faculty of the University of Gothenburg to use autologous chondrocytes cultured in laboratory for the treatment of chondral injuries in the human knee. The first patient was transplanted with autologous chondrocytes in October 1987 at the Department of Orthopaedic Surgery, East Hospital, University of Gothenburg, Sweden. Mats Brittberg at that time a young resident and my Ph.D student assisted me at this "historic surgery" and later defended his thesis on cartilage repair in 1996.

10.8 Indications, Surgical Technique, Classification, and Rehabilitation

10.8.1 Indications

ACT/ACI is indicated in symptomatic full thickness cartilage lesions or osteochondral lesions according to ICRS or Outerbridge classifications III-IV. Age of the patient should be between 15 and 55 years but there is no definite limit. Size of the defect is between 2 and 16 cm^2. Gradually with increased experience the indications have widened and larger (over 16 cm^2) uncontained, multiple defects or bone to bone compartmental lesions could be tried as a relative indication (salvage procedure) in young and active middle age patients (Fig. 10.3).

Contraindications are generalized osteoarthritis, rheumatoid arthritis and other systemic diseases. Background factors such as instability, varus-valgus deformities, patella malalignment or instability, meniscus deficiency, bone pathology or defects must be addressed [21].

10.8.2 Surgical Technique

ACT/ACI is a 2 step procedure. The preoperative arthroscopic evaluation is to decide the indication, to plan the surgical approach and if concomitant procedures like ACL-reconstruction, varus or valgus osteotomies, patellar realignment procedures, meniscus allograft transplantation, bone grafts are needed etc. One can decide if the procedures should be staged or done in a single operation. Then a biopsy is

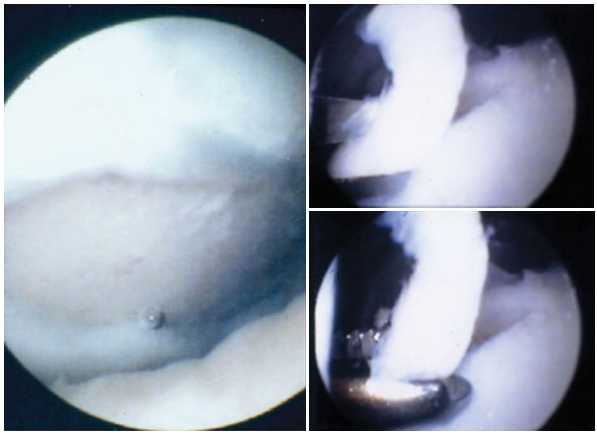

Fig. 10.3 Arthroscopic preoperative assessment; *left image* showing a contained lesion down to subchondral bone. *Right images* showing biopsies taken from upper, medial trochlea

harvested from one of following locations; (1) the upper medial trochlea, (2) the upper lateral trochlea, and (3) the lateral intercondylar notch. Consider possible meniscus surgery at this time (Fig. 10.3).

For implantation of the chondrocytes, adjust the arthrotomy to the location, size and numbers of defect. Radical excision of the defect to healthy cartilage. Debride carefully down to subchondral bone plate. Do not leave any damaged cartilage. Make a template of the prepared defect. A small incision is made medial proximal tibia below the pes anserinus insertion, dissect carefully down to the periosteum, remove fat, fibrous tissue and passing vessels, incise the periosteum around the template. Then dissect the flap from the cortical bone using an elevator (raspartorium), keep the flap moist and go directly to the defect and use 6:o vicryl to suture the flap to the vertical edges of the defect. Seal the intervals between the sutures with fibrin glue (Tisseal), check for tightness by gentle injection of saline, if ok aspirate the fluid and inject the cells, close the injection site and close the incision. If a resorbable membrane is used suture and fix as above [21] (Fig. 10.4). Postoperatively prophylactic antibiotics for 24 h and antithrombotic treatment. CPM 8 h after surgery (cell adhesion time) for 6–8 h/24 h.

10.8.3 Classification of ACT/ACI Cartilage Repair Techniques

The introduction of new materials and techniques has been followed by new classifications regarding the differences between them. The following classification has been proposed:

First generation ACT/ACI: ACT/ACI as first described in 1994 with the use of autologous chondrocytes grown in culture and injected in suspension under a periosteal cover [3].

Second generation ACI: Autologous chondrocytes grown in culture and injected under or into and delivered with tissue engineered matrix support (TEMS) of animal tissue origin (bovine, porcine origin or others) or chemically synthesized matrix support (polyglycolic-polylactic acids), or others. (MACI, Chondrogide) [22, 23].

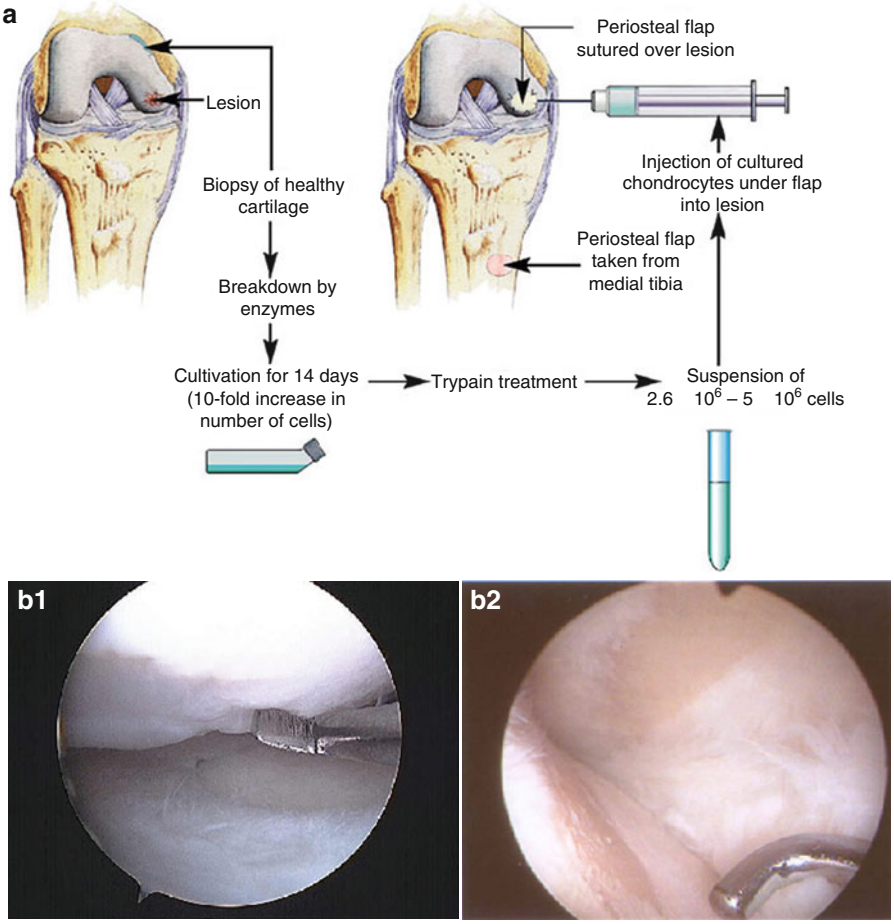

Fig. 10.4 Schematic diagram of ACT/ACI *upper row* (*A*). Case 1, *left row* (*B1-D1*). (*B1*) Isolated lesion treated with microfracture 1 year before, still symptomatic. Arthroscopic assessment. (*C1*) ACT/ACI treatment lateral femoral condyle defect using artrotomy for chondrocyte implantation. (*D1*) Second look arthroscopy at 12 months with excellent healing. Returned to professional football (*soccer*) at 15 months. Case 2, *right row* (*B2-D2*) (*B2*) Preoperative arthroscopy showing bipolar medial femoral and tibial condyle down to bone lesions in 37 year old soccerplayer after total medial meniscectomy at age 16. (*C2*) Bipolar ACT/ACI of large uncontained lesions. Compartment unloaded with a concomitant closing wedge proximal tibial osteotomy. (*D2*) Second look arthroscopy at 4 years showing complete healing. Still asymptomatic 12 years after surgery

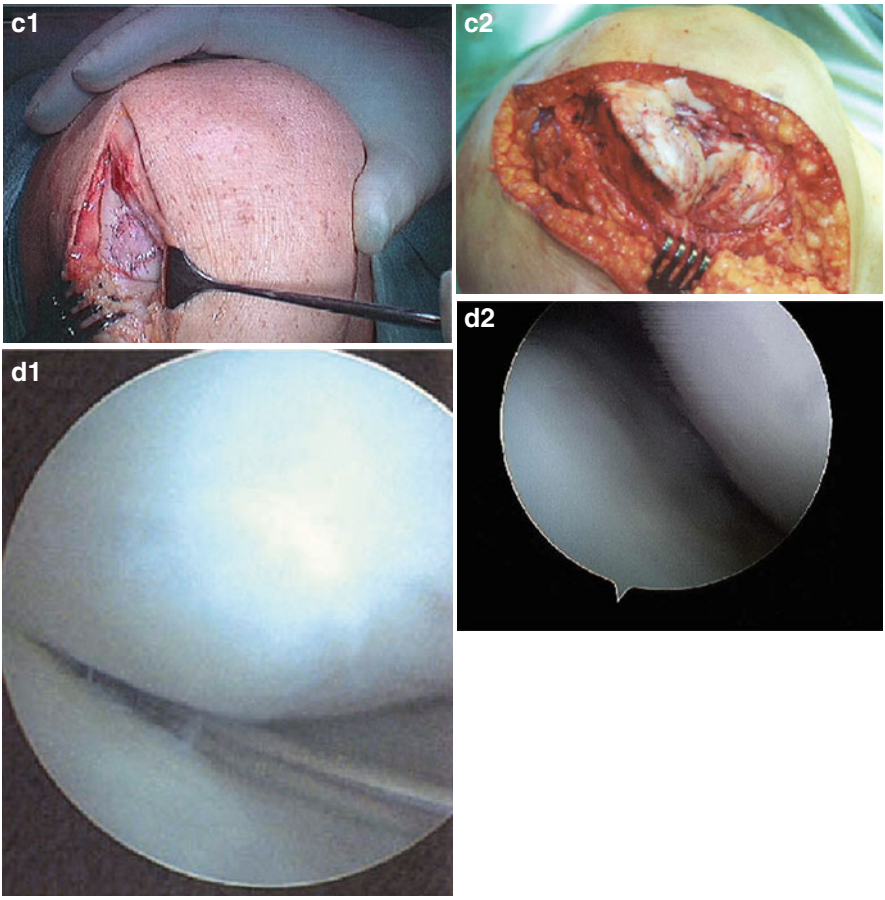

Fig. 10.4 (continued)

Third generation ACI: ACI with three- dimensional TEMS of animal or chemical origin used as scaffolds for growing and delivering the chondrocytes to the joint. (Hyalograft C) [24, 25].

10.8.4 Rehabilitation

The great challenge in the rehabilitation after ACI is to regain full weight-bearing (WB) as early as possible without jeopardizing the new delicate tissue formed by the implanted chondrocytes. Progressive increase of WB is essential to stimulate the matrix production, the remodeling and the maturation of the cartilage. In general the WB could increase after the initial 3 weeks to full WB with the limitation of pain and swelling in contained, sized 4–6 cm^2, isolated lesions. More complex

cases as uncontained, unshouldered, bipolar or multiple lesions are recommended 6–8 weeks of 20–40 kg WB and full WB to be reached at 12–16 weeks. Cases with concomitant procedures will affect the rehabilitation and adjusted accordingly (Fig. 10.6).

The immediate postoperative training includes continuous passive motion, (after 6–8 h to allow the cells to adhere to the subchondral bone and surrounding edges of normal cartilage), during 6–8 h/24 h. Activation of the quadriceps muscle and assisted active flexion from the first postoperative day and mobilization with crutches and partial WB. The early partial loading and unloading is essential for the exchange of fluid for the nutrition of the cartilage as well as a stimulation for the implanted chondrocytes to produce adequate matrix. Non WB is not recommended for this reason.

It is helpful to the patient, physiotherapist and physician to divide the rehabilitation after ACI in four different phases and to understand the healing process, and adjust the training to ensure a short and long term success of the treatment. Each phase has one key word for the actual healing process and repair tissue and one key word for the main focus during this time period. For detailed training activities and instructions [21, 26].

10.8.4.1 Phase I: Proliferation and Protection (1–6 Weeks)

During the first hours the cells are still in suspension and active in mitosis. Within 6–8 h the cells will adhere to the subchondral bone and the edges of surrounding cartilage and start matrix production in the early proliferation phase (Lindahl A, Peterson L, 1996). During this phase the proliferation activity and the newly formed repair tissue are vulnerable to overload, and still the chondrocytes need mechanical stimuli for optimal matrix proliferation by-loading – unloading and by motion for water exchange and nutrition. During this phase the tissue is soft and like a gel under the periosteal flap and has to be protected from overload, too high compression and shear forces. The main goal during phase I is the protection of the fragile tissue proliferation and this needs a progressive increase in partial weightbearing (WB) from 20 to 40 kg for this phase. For patellar and trochlear lesions we allow full WB after 3 weeks but not in up and downstairs climbing not until 10–12 weeks. when loaded full WB in kneeflexion is allowed.

10.8.4.2 Phase II: Transition and Progression (7–12 Weeks)

During this period the repair tissue increases filling the defect and getting more resistant to WB with a transition from partial to full WB and will allow a progressive increase in therapeutic and functional exercises. The main goal is a safe progress to full WB, ROM (full extension and almost full flexion) and increasing quadriceps and hamstring strength preparing for next phase.

10.8.4.3 Phase III: Remodeling and Function (3–6 Months)

During this period there is an ongoing matrix production leading to a continuous remodeling and functional adaptation into a more organized structure. The formation of cells arranged in columns, collagen type II building up the Benninghoff's arcades anchored to the subchondral bone, and filling the interspace with proteoglycans and water, creates a functional, firm, structured tissue with increasing biomechanical properties over time. The training is focused on optimizing in muscle strength, endurance, flexibility and neuromuscular function and gradually increasing functional activities become more important, preparing for going back to low-impact sports.

10.8.4.4 Phase IV: Maturation and Optimizing (7–12–18 Months)

The maturation of the repair tissue is an ongoing process starting in Phase I- III and continues into the normal cartilage tissue turnover which is the ultimate goal and there is no defined endpoint in time. The goal is to gradually return to full preinjury activity level including low-impact sports and hard labour as individually tolerated. The training in high- impact loading sports are gradually started for the definite maturation and tissue healing allowing return to sport specific training and competition which may be possible at an average of 15 months after surgery.

10.9 Results of Autologous Transplantation/Implantation

The results of the first 23 patients operated with autologous chondrocyte transplantation/ implantation were published in The New England Journal of Medicine in October, 1994 [3]. At an average follow up of 36 months, 14 of 16 patients had a good/excellent results on femoral condyle lesions but only 2 out of 7 patients with patellar lesions reported good/excellent results. Biopsies showed hyaline appearance in 11 of 15 patients on the femoral condyle (Fig. 10.5).

Continuously we have published our results. In 2000 we reported 2–9 years outcome after ACT/ACI in Clinical Orthopaedics and Related Research showing an average of 85 % Good/Excellent results in isolated femoral condyle lesions, in multiple (2 or more) lesions, in osteochondritis dissecans, in patella and in isolated femoral condyle lesions combined with ACL reconstructions with a double bundle vascularized graft [27]. The results were supported by objective evaluations such as arthroscopic, macroscopic assessments of repair tissue, showing good filling, good integration to surrounding borders and acceptable surface tissue and biopsies of 2 mm diameter from the center of the repaired defects showed histology with hyaline appearance in over 80 % [27].

In 2002 clinical results, biomechanics of repair tissue were published in the American Journal of Sports Medicine [28]. A 5–11 years outcome of 61 patients showed at 2 years good to excellent (G/E) result in 50 patients, and the same results

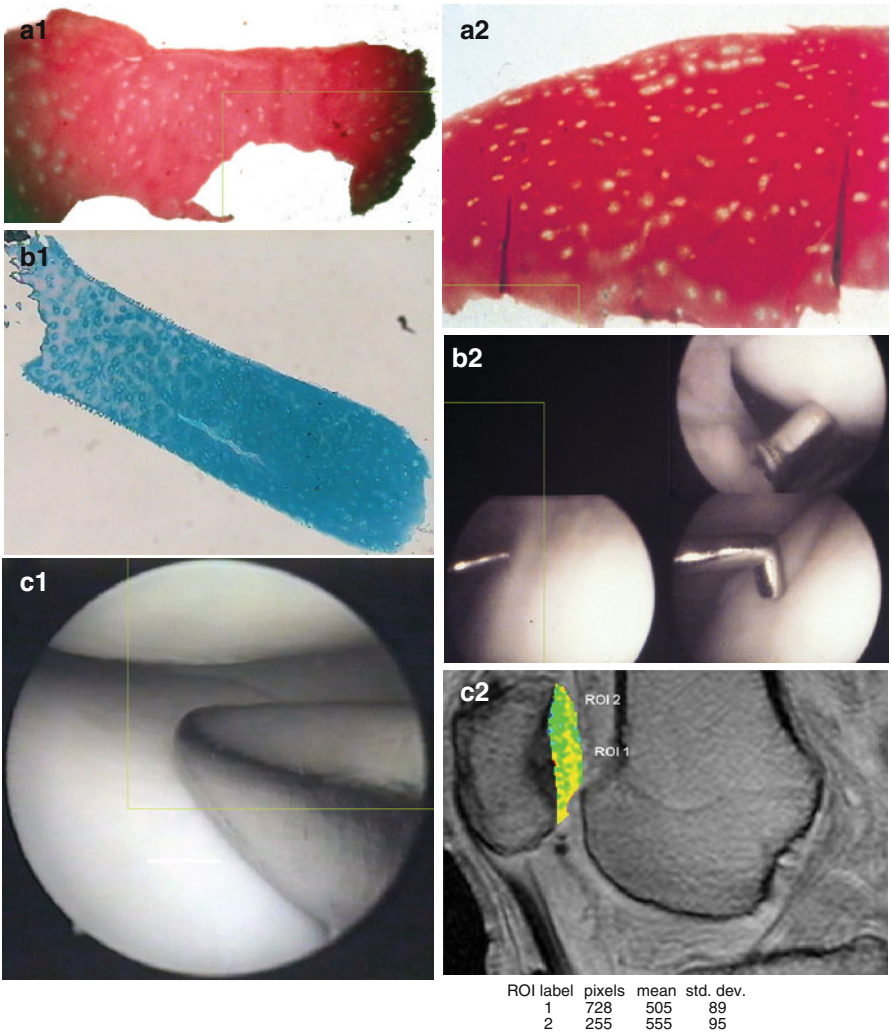

Fig. 10.5 Objective evaluations. (*A1*), (*A2*) and (*B1*): Biopsies 2 mm diameter taken 2–4 years after surgery. Histology showing articular cartilage. (*B2*) Showing artroscopic assessment and probing. (*C1*) Showing arthroscopic indentation test of stiffness of repair tissue. (*C2*) Showing normal glycosaminoglycan concentration in the patella 11 years after surgery

after 5–11 years. The results were supported by biopsies in 12 patients showing hyaline appearance and homogenous structure in polarized light microscopy. Arthroscopic indentation tests of the stiffness of repair tissue with hyaline appearance in biopsies, were equal to the stiffness of normal cartilage tissue. Those with fibrohyaline biopsies had a significant lower stiffness [28] (Fig. 10.5).

In 2003 the long time follow up of osteochondritis dissecans of the knee treated with ACT/ACI was published in Journal of Bone and Joint Surgery, supplement, showing G/E results in 91 % of the patients between 2 and 10 years [29] (Fig. 10.6).

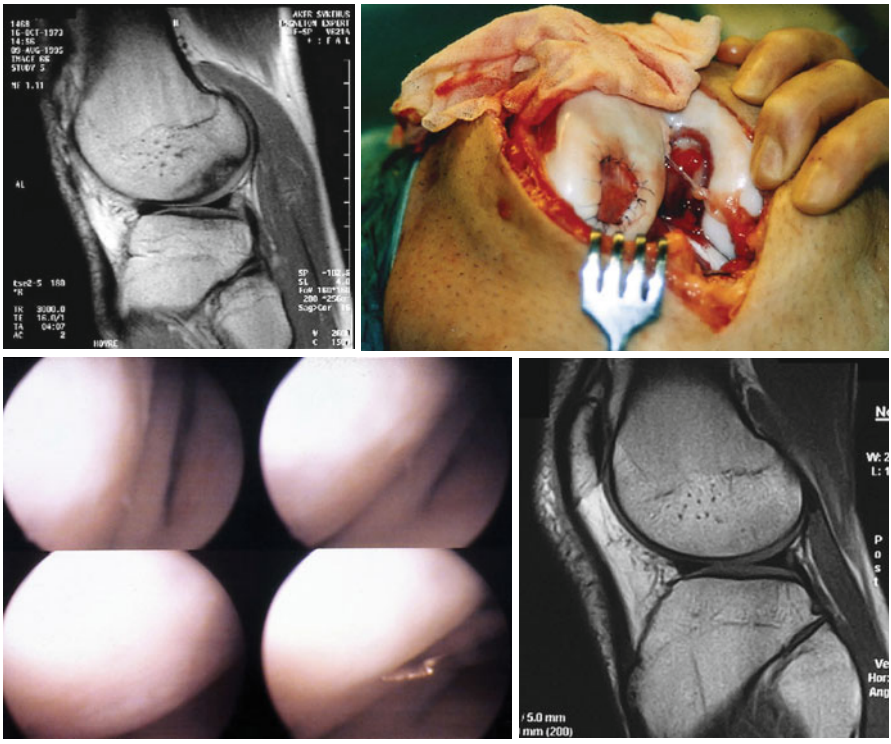

Fig. 10.6 Osteochondritis dissecans treated with ACT/ACI is one of the best treatments reported. *Upper row, left*: preoperative MRI showing large and deep OCD of lateral femoral condyle in 24 year old soccerplayer. *Upper row right*: showing the defect at surgery. *Lower row, left*: second look arthroscopy at 1 year, returned to professional football (*soccer*) at 15 months and are still playing at age 38. *Lower row, right*: MRI 9 years after surgery showing complete healing of both bone and cartilage

The latest and longest follow up reported on 10–20 years in American Journal of Sports Medicine (2010) showed in comparison to previous follow ups no significant differences in results. Ninety-two percent of the patients would have the surgery again [30]. In the same journal May, 2010 we reported on the result of delayed gadolinium enhanced magnetic resonance imaging dGEMRIC in 35 knees in 31 patients treated with ACI 9–18 years ago. In 28 knees the proteoglycan concentration was the same in the operated area as in the normal cartilage and in 7 knees the concentration was somewhat lower both in the operated area as well as in the surrounding cartilage. It shows that the implanted chondrocytes have the capacity to reach and maintain homeostasis in the proteoglycan metabolism in relation to the surrounding cartilage either it is normal or somewhat lowered concentrations [31] (Fig. 10.5).

The initial results of ACI in the patellofemoral joint were not promising. In the patella lesions only 28 % had G/E results in the first publication. Later ACI in trochlear lesions reached over 80 % G/E results and even the patella results improved to 68 %. The improvement was due to concomitant realignment procedures. In a separate follow up of cartilage injuries in the patellofemoral joint with 10–20

years the improvement varied from 44.4 % in kissing lesions, in isolated patellar lesions 79.5–100 % in the isolated trochlear lesions. However, 92 % of the patients would have the operation again [3, 31].

10.9.1 Summary of Objective Evaluations to Support the Clinical Outcome

Arthroscopic macroscopic assessment of repair area according to ICRS showed out of maximal 12 points in isolated femoral condyles in average 10.3 points, in isolated lesions with ACL reconstruction 10.9 points, and in OCD 10.5 points meaning a good filling, a good integration and a good surface. In over 120 biopsies with microscopic, histologic assessments from repair area compared to normal cartilage in the same joint 80 % showing hyaline like appearance. Immunohistochemical analysis of the biopsies showing collagen type II, cartilage oligomeric matrix protein (COMP) and aggrecan similar to normal articular cartilage.

Arthroscopic indentation tests of stiffness in the repair area with hyaline like appearance compared to normal articular cartilage showed no significant difference in stiffness.

With dGEMRIC technique the glycosaminoglycans uptakes were normal in 28 knees and at the same concentration as in the surrounding cartilage in 7 knees in patients 9–18 years after ACI [3, 27, 28, 31] (Fig. 10.5).

10.9.2 What Have We Learned in the Last 25–30 Years with ACT/ACI?

The hypothesis was proven right: It is possible to use isolated and cultured autologous chondrocytes to repair articular cartilage injuries in the human knee. Long term follow up outcome studies show subjectively good results in about 85 % of all diagnoses. The indications have widened from small isolated, contained lesions to large, uncontained, multiple lesions (2 or more) in the same knee, bipolar –kissing lesions in any compartment (medial and lateral tibiofemoral or patellofemoral) or posttraumatic osteoarthritis.

It is necessary for the short and long term success of ACT/ACI that background factors like instability, varus or valgus malalignment, functional meniscus deficiency after subtotal or total meniscectomies as well as bone defects or pathology are addressed adequately [21].

Common concomitant procedures in the tibiofemoral joints are varus and valgus osteotomies to unload the affected compartment. X-rays in standing position with hip – knee – ankle included are valuable to assess the degree of correction-unloading needed. Anterior, posterior, or collateral ligament instability should be reconstructed. Meniscus allograft transplantation to restore the joint mechanics should be performed at the same

time as ACI or 7–8 months later depending on access to the graft and experience. Bony defects after OCD or bone cysts or other bone pathology should be bone grafted with spongious autologous bone from the iliac crest, tibial or femoral condyles depending of the amount of bone needed. Preoperative MRI could help to plan the surgery.

In the patellofemoral joint the background factors have been shown to play an important role for the short and long term results. Patella alta and malalignment, increased q-angle, patellotrochlear dysplasia, patellar instability including patellar lateral tracking, tilt, subluxation and dislocation are important findings to recognize and address properly. That may need tibial tuberosity transfer to correct the q-angle, by medialization, unloading by ventralization and distalization when patella alta is present. The instability also need medial soft tissue stabilization and reinforcement including reconstruction of the medial patellofemoral ligament (medial transverse retinaculum) and vastus medialis obliquus shortening. To achieve this, a lateral release is necessary. If trochlear dysplasia is a part of the instability a proximal trochlea plasty should be done [32, 33]. The unloading by ventralization is important in large uncontained patella or trochlear lesions and in kissing lesions. For a successful ACI all the background factors should be treated. Adequate physiotherapy, mainly with closed chain technique the first 3–4 months and early motion is an important part of the initial treatment. Computerized tomography including quadriceps relaxation and contraction with the knee in extension is a good technique to diagnose instability and trochlear dysplasia (Fig. 10.7a).

10.10 Future of Cartilage Repair/Regeneration

10.10.1 Optimal Cell Sources

The search for other cell sources than autologous articular cartilage has been ongoing for a long time allowing a one step procedure with cells as an on the shelf product. Among new cells explored are cells of allogenic or xenogenic origin, from fetal, juvenile and adult donors. Direct isolation in the operating room by mincing cartilage biopsies, seeding it on resorbable membranes and implant them arthroscopically, as well as directly aspirated and concentrated autologous bone marrow mesenchymal stem cells are under investigational studies.

10.10.2 Tissue Engineered Matrix Support (TEMS)

TEMS include membranes, gels, scaffolds in ACT/ACI and other cartilage repair or regeneration techniques. The ideal TEMS has to be safe for the patient, be compatible, noncarcinogenic, not causing inflammatory or immune reactions, not cytotoxic for the implanted cells or surrounding tissues. However still the mechanical properties and the resorbtion time have to be evaluated and adapted to specific situations

and demands. The development and use of degradable chemically synthesized membranes-gels-scaffolds, such as hyaluronic acid (Hyalograft) [24] or polyglycolic-polylactic acids as well as semisynthesized materials of porcine, bovine or other animal origin such as MACI (Verigen) [22] and Chondro-Gide (Geistlich) [23] have reported medium-term results and are undergoing intense research and clinical trials [25, 34]. It is, however, of outmost importance that the intended functions of different types of TEMS are defined and established, that the resorbtion time is studied and decided, that the mechanical properties are specified, regulated, and tested regarding surface friction coefficient, mechanical stiffness in relation to resorbtion

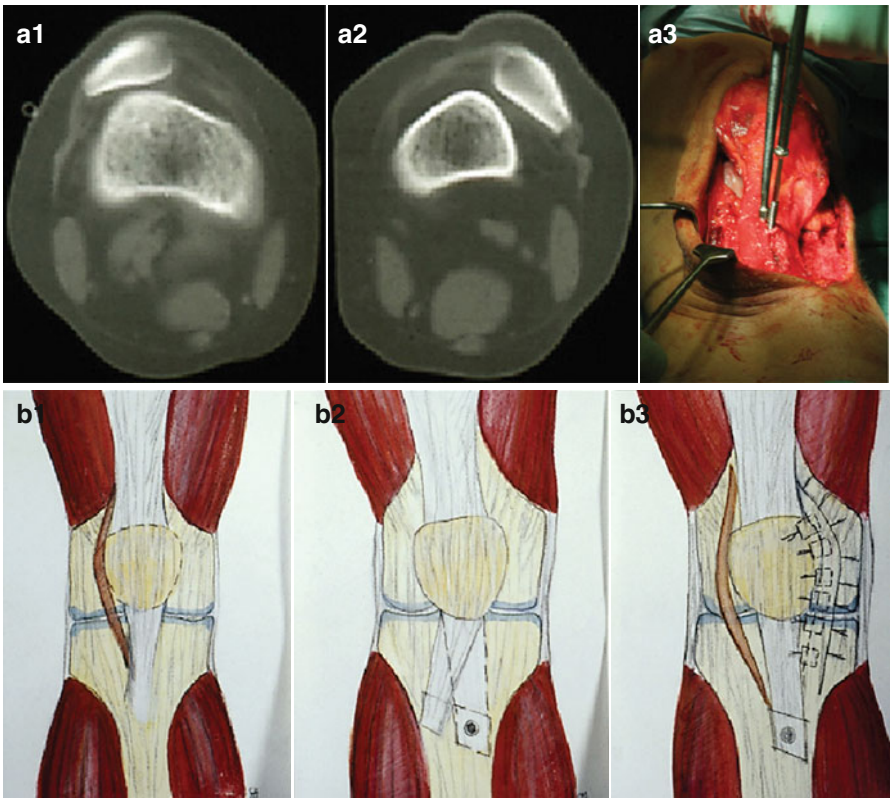

Fig. 10.7 Surgical corrections of background factors to patellofemoral articular cartilage lesions. (*A1-2*) Computerized tomography with the knee in extension and quadriceps contraction. Both patellas dislocated laterally. Note the trochlear dysplasia! (*B1-2-3-A3*) Distal and proximal realignment procedures to correct increased Q-angle as a part of instability or lateral tracking. (*B1*) Lateral release to allow (*B2*) tibial tuberosity transfer (medial or anterior, or distal directions). (*B3*) Medial soft tissue shortening and reinforcement of the medial patellofemoral ligament (medial transverse patellar retinculum) and vastus medialis obliquus. (*A3*) Antero-medial-distalisation of tibial tuberosity and screw fixation. (*C-D-1-2-3*) Schematic and surgical steps in proximal trochleaplasty for correction of trochlear dysplasia. (*C-D1*) Showing trochlear dysplasia and release of the synovial lining from the cartilage. (*C-D2*) Using a curved osteotome or a burr and make 10 × 30 groove in the cartilage and bone. (*C-D3*) suturing the synovial membrane back to the cartilage edge. (*E*) Large kissing lesions need unloading by anteriorisation (ventralisation) to protect the repair

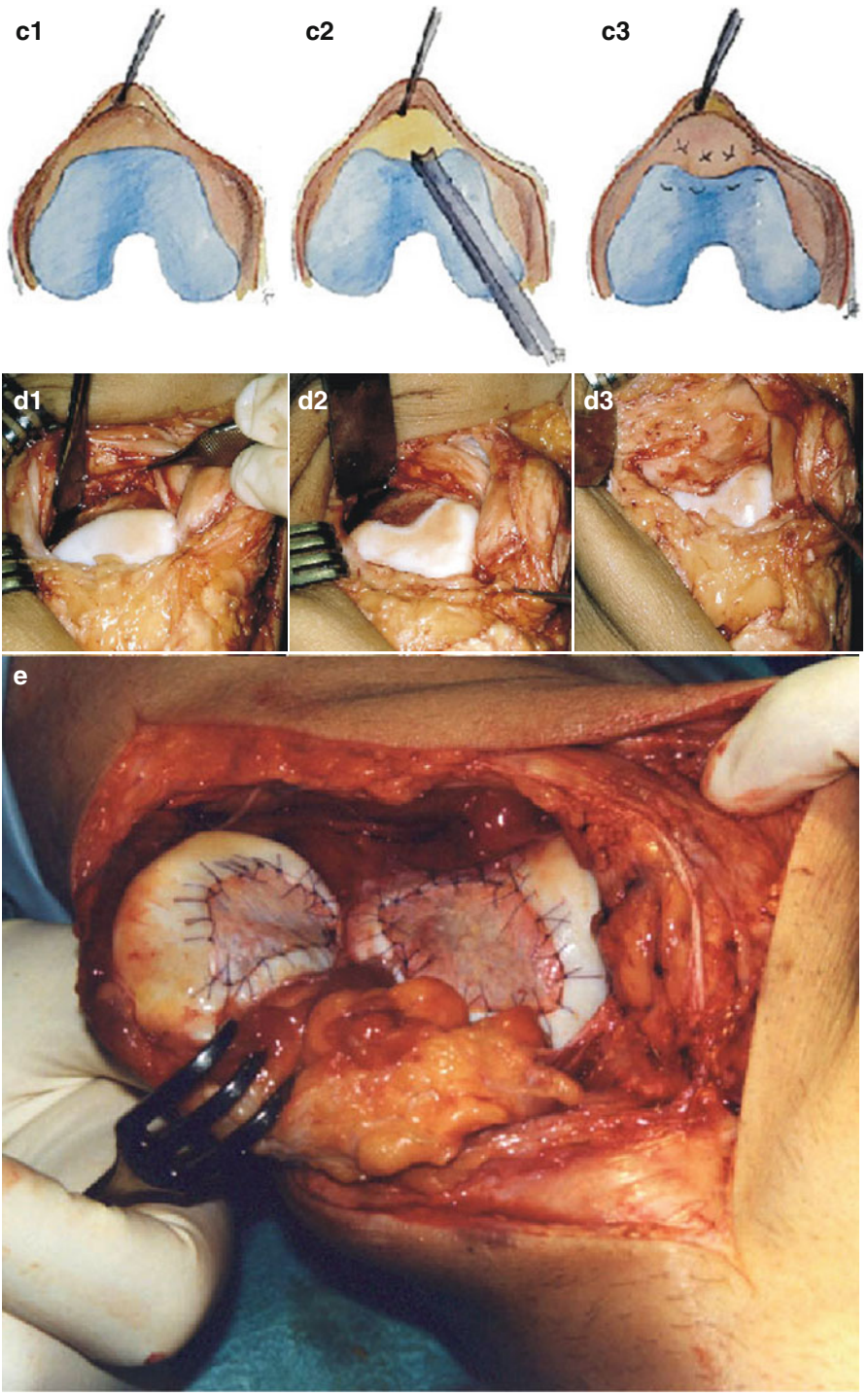

Fig. 10.7 (continued)

time, have the mechanical strength to allow adequate fixation for early and safe weight-bearing etc. Membranes could be used to replace the periosteal membrane, or used as a carrier to be injected with cells and implanted, or to grow the cells in for direct implantation. Should the time to complete resorbtion be short, 6–12 weeks, or medium 3–6 months or long 7 to 12–15 months, to gradually be replaced by regenerated tissue and give mechanical support during part of or whole the maturation process of the regenerating cartilage?

Membranes are now used as cover of defects prepared by microfracturing to host invading mesenchymal stemcells as well as fibroblasts from the subchondral bone and sometimes filled with aspirated and concentrated mesenchymal stemcells. Only short term result have been presented.

Resorbable gels mainly from animal collagen has been used to grow the cells in and used as a carrier to deliver the cells into the defects. Comparable results to ACI has been reported from Japan [35].

Scaffolds of three dimension such as Hyalograft from esterified hyaluronic acid with a resorbtion time of about 4 months, has been used to grow the chondrocytes in for 3 weeks and then used as a carrier to be implanted in small, contained defects using arthroscopic technique or miniarthrotomy [25, 34].

10.11 Summary

It all started with a great interest for sports medicine, traumatology, reconstructive and arthroscopic surgery in a very sports friendly environment under the leadership of professor Bertil Stener, director at the Department of Orthopaedic Surgery II, Sahlgrenska Hospital, University of Gothenburg. During an intense period of acute and reconstructive surgery and the introduction of arthroscopy in the department we established a sportsmedicine section handling most of the athletes in the region. We could diagnose and treat most of the athletic injuries of traumatic or overuse type in an acceptable way but when it came to chondral injuries there was no spontaneous healing or acceptable treatment to use. I tried debridement, periosteum transplantation, spongialization, multiple drilling, osteochondral grafting, most of them with just short relief of symptoms. The review of actual literature was very disappointing.

Injury to articular cartilage does not heal with inflammation as there is no vascular supply and no phagocytic cell activity to remove the necrotic tissue in the injured area. The chondrocytes are not capable of repopulating the area and regenerate new cartilage.

Furthermore no actual treatments at that time showed good long term results. In England Audie Smith was able to isolate rabbit chondrocyte by collagenase degradation. Injections of isolated chondrocytes to an experimental defect in the tibial articular surface of a rabbit knee did not show any healing. Salter showed a chondrogenic repair potential after transplantation of periost to an osteochondral defect in the rabbit knee. Human studies did not show any good long term results.

With the hypothesis: "*It is possible to heal a full thickness articular cartilage defect using enzymatically isolated autologous chondrocytes grown in culture and implanted under an autologous tissue membrane sutured to the defect*", the work started. The hypothesis was proven after 4 years using an experimental rabbit model. Two studies were performed and the first study showed at 3 months follow-up results with over 80 % filling of the defects and hyaline cartilage appearance on microscopy. The second study with 1 year follow up showed over 80 % filling and hyaline cartilage on microscopy. No filling in the control.

From 1984 Anders Lindahl and myself worked on transferring the cell culture technique from the rabbit to the human chondrocyte using autologous serum instead of fetal calf serum. It took us 3 years to reach a safe, efficient and sterile technique and create criteria for an approved cell culture, suitable for autologous transplantation in humans. In 1987 the Ethical Committee of the Medical Faculty of the Gothenburg University approved the technique for clinical use in the human knee. In the autumn, 1987 the first patient was operated with her own isolated and cultured cells implanted in a cartilage defect and still after 25 years she is happy with her knee function.

Since then more than 2,000 defects have been operated with autologous chondrocyte transplantation in Gothenburg and worldwide over 35,000 patients treated.

The results have been reported from medium to long term follow ups showing over 90 % good to excellent results in isolated femoral and trochlear lesions and in osteochondritis dissecans of the knee. The overall results on isolated lesions was 84 % G/E at the 10–20 years latest follow-up and 92 % of the patients would have the surgery again.

The ACT/ACI has the longest follow up, has the most objective data to support the good long-term clinical results, such as arthroscopic macroscopic assessment, indentation test of mechanical stiffness, biopsies showing hyaline cartilage appearance, immunohistochemistry showing collagen type II, aggrecan and COMP concentrations close to normal, and gadolinium enhanced MRI (dGEMRIC) showing normal concentrations of proteoglycans in the repair area 9–18 years after surgery.

For the first time in orthopaedics autologous cells have been isolated, grown in culture and reimplanted into articular cartilage lesions with regeneration and healing with hyaline cartilage appearance and long durable results.

The new emerging techniques using different cell sources and degradable scaffolds, membranes and gels etc., will make cell treatment easier, improve the results, widen the indications, shorten the rehabilitation time and (at least) make some lesions possible to treat with arthroscopic technique and reduce the surgical trauma and the morbidity. However long term randomized studies need to be carried out and the great interest from the young generation of orthopaedic surgeons gives great promises for the future.

The development of cell transplantation is opening up for cell therapy in other tissues in the musculoskeletal system and maybe in the future also for organ regeneration for transplantations.

References

1. Mankin HJ. Current concepts review. The response of articular cartilage to mechanical injury. J Bone Joint Surg. 1982;64A:460–6.
2. Hunter W. On the structure and diseases of articulating cartilage. 1743. Philos Trans R Soc Lond. 42b:514–21.
3. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331:889–95.
4. Steadman JR, Rodkey WG, Singelton SB, et al. Microfracture technique for full- thickness chondral defects: technique and clinical results. Oper Tech Orthop. 1997;7:300–4.
5. Hangody L, Kish G, Karpati Z, et al. Mosaikplasty for the treatment of articular cartilage defects: application in clinical practice. Orthopedics. 1998;21:751–6.
6. Magnuson PB. Technique for debridement of the knee joint for arthritis. Surg Clin North Am. 1946;26:249.
7. Ficat RP, Ficat C, Gedeon P, Toussaint JB. Spongialization: a new treatment for diseased patella. Clin Orthop. 1979;144:74–83.
8. Pridie KH. A method of resurfacing osteoarthritic knee joints. J Bone Joint Surg Br. 1959;41:618.
9. Coventry MB. Osteotomy about the knee degenerative and rheumatoid arthritis: indications, operative technique, and results. J Bone Joint Surg Br. 1973;55A:23.
10. Rubak JM. Reconstruction of articular cartilage defects with free periosteal grafts. Acta Orthop Scand. 1982;53:175–9.
11. Homminga G, van der Linden E, Terwindt-Rouwenhorst W, Drukker J. Repair of articular defects by perichondral grafts. Acta Orthop Scand. 1989;60:326–9.
12. Ritsila VA, Santavirta S, Alhopuro S, et al. Periosteal and perichondral grafting in reconstructive surgery. Clin Orthop. 1994;302:259–65.
13. Lorentzon R, Alfredson H, Hildingsson C. Treatment of deep cartilage defects of the patella with periosteal transplantation. Knee Surg Sports Traumatol Arthrosc. 1998;6:202–8.
14. O'Driscoll SW, Salter RB. The repair of major osteochondral defects in joint surfaces by neochondrogenesis with autogenousosteoperiosteal grafts stimulated by continuous passive motion. An experimental investigation in the rabbit. Clin Orthop. 1986;208:131–40.
15. Chesterman PJ, Smith AU. Homotransplantation of articular cartilage and isolated chondrocytes. J Bone Joint Surg Br. 1968;50B:184–97.
16. Mankin HJ, van Mow C, Buckwalter JA, Iannotti JP, Rathcliffe A. Chapter 1. Form and function of articular cartilage. In: Sheldon S, AAOS, editors. Orthop and basic science. Rosemont: American Academy of Orthopaedic Surgeons; 1994. p. 1–44.
17. Peterson L, Menche D, Grande D, et al. Chondrocyte transplantation-an experimental model in the rabbit. In: Transactions from the 30th annual orthopedic research society, Atlanta, Febr 7–9. Atlanta: Orthopedic Research Society; 1984. p. 218. abstract.
18. Salter RB, Simmonds DF, Malcolm BW, Rumble EJ, MacMichael D, Clements ND. The biological effect of continuous passive motion on the healing of full thickness defects in articular cartilage. An experimental investigation in the rabbit. J Bone Joint Surg Br. 1980; 62A:1232–51.
19. Grande D, Pitman M, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res. 1989;7:208–18.
20. Peterson L, Lindahl A, Nilsson A, et al. Autologous chondrocyte transplantation in a rabbit model- 1 – year result. In: Swedish society of physicians yearly meeting. Stockholm; 1986. Abstract.
21. Peterson L. Chapter 35. Autologous chondrocyte implantation. In: Noyes FR, editor. Noyes: knee disorders, surgery, rehabilitation, clinical outcomes. Saunders-Elsevier: Philadelphia; 2010. p. 931–47.

22. Bartlett W, Skinner JA, Gooding CR, Carrington RW, Flanagan AW, Briggs TW, Bentley G. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomized study. J Bone Joint Surg Br. 2005;87(5):640–5.
23. Niemeyer P, Steinwachs M. Chondrocyte-seeded collagen type I/II membrane (ACT-CS) for autologous chondrocyte transplantation: prospective 2-year results in patients with cartilage defects of the knee joint. Arthroscopy. 2010;11:1539–50.
24. Marcacci M, Berutto M, Brochetta D, et al. Articular cartilage engineering with HyalograftC: 3-years clinical results. Clin Orthop Relat Res. 2005;435:96–105.
25. Peterson L, Vasiliadis H. Chapter 22. International experience with autologous chondrocyte implantation with periosteum (Autologous Chondrocyte Implantation), including scaffold guided techniques and tissue engineered matrix support. In: Scott WN, editor. Insall & Scott, Surgery of the knee. 5th ed. Elsevier-Churchill/Livingston: Philadelphia; 2012. p. 163–77.
26. Wilk K, Reynold M. Chapter 37. Rehabilitation after articular cartilage procedures. In: Noyes FR, editor. Noyes: knee disorders, surgery, rehabilitation and clinical outcomes. Saunders-Elsevier: Philadelphia; 2010. p. 961–80.
27. Peterson L, Minas T, Brittberg M, et al. Two to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res. 2000;374:212–34.
28. Peterson L, Brittberg M, Kiviranta I, et al. Autologous chondrocyte transplantation. Biomechanics and long term durability. Am J Sports Med. 2002;30:2–12.
29. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritisdissecans of the knee with autologous chondrocyte implantation. J Bone Joint Surg Am. 2003;85-A Suppl 2: 17–24.
30. Peterson L, Vasiliadis H, et al. Autologous chondrocyte implantation. A long term follow up. Am J Sports Med. 2010;38:1117–24.
31. Vasiliadis H, Danielsson B, Ljungberg M, McKeon B, Lindahl A, Peterson L. Autologous chondrocyte implantation in cartilage lesions of the knee: long-term evaluation with magnetic resonance imaging and delayed gadolinium-enhanced magnetic resonance imaging technique. Am J Sports Med. 2010;38:943–9.
32. Peterson L, Vasiliadis H, et al. Chapter 31. Long-term results after autologous chondrocyte implantation in cartilage lesions of the patellofemoraljoint. In: Zaffagnini S, editor. patello-femoral pain, instability, and arthritis. Berlin: Springer/ESSKA; 2010. p. 245–54.
33. Peterson L, Vasiliadis H, et al. Chapter 27. Proximal open trochleoplasty (Grooveplasty). In: Zaffagnini S, editor. Patellofemoral pain, instability, and arthritis. Springer Verlag: Berlin-Heidelberg; 2010. p. 217–24.
34. Minas T. Chapter 14. Emerging technologies. In: Minas T, editor. A primer in repair and joint preservation of the knee. Elsevier-Saunders: Philadelphia; 2011. p. 219–49.
35. Ochi M, Uchio Y, Kawasaki T, Wakitani S, Iwasa J. Transplantation of cartilage like tissue made by tissue engineering in the treatment of cartilage defects in the knee. J Bone Joint Surg Br. 2002;84:571–8.

Chapter 11
Characterized Chondrocyte Implantation Challenges Current Paradigms for the Treatment of Symptomatic Joint Surface Lesions

J. Vanlauwe, M. Jelic, M.J. Limbourg, and F.P. Luyten

Abstract The use of autologous cells derived from an articular cartilage biopsy to treat joint surface lesions was introduced in 1994 by Brittberg and Peterson. In a 2-step procedure, chondrocytes were harvested from a minor weight-bearing area of the knee joint during arthroscopy, expanded *ex vivo* and implanted during an arthrotomy 2–3 weeks later. However, throughout the *in vitro* expansion process, articular chondrocytes progressively lose their phenotypic traits and capacity to form stable cartilage tissue, thereby jeopardizing proper *in vivo* repair.

Data on dedifferentiation revealed that *in vivo* tissue formation of stable cartilage is governed by the interaction between environmental factors and inherent phenotypical characteristics. Characterized chondrocytes are an expanded population of cartilage cells that express a marker profile predictive for the formation of ectopic hyaline-like cartilage *in vivo* in a consistent and reproducible manner. A controlled and consistent manufacturing process was developed to maintain this phenotype stability. This involved optimisation of the biopsy procedures and mostly the culture process parameters. Characterized viable autologous cartilage cells expanded *ex vivo* expressing specific marker proteins were introduced in clinical practice in 2004.

JVL and MJ equally contributed to the manuscript

J. Vanlauwe
Department of Orthopedics and Traumatology,
University Hospital Brussels, Brussels, Belgium

M. Jelic
Department of Orthopaedic Surgery, Clinical Hospital Center Zagreb,
School of Medicine, University of Zagreb Salata 6, Zagreb, 10000, Croatia

M.J. Limbourg
Department of Medical and Scientific Affairs, TiGenix NV,
Romeinse Straat 12/2, Leuven 3001, Belgium

F.P. Luyten (✉)
Division of Rheumatology, University Hospitals KU Leuven,
Herestraat 49, Gasthuisberg, Leuven B 3000, Belgium
e-mail: frank.luyten@uzleuven.be

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_11,

A prospective randomized multicenter controlled trial compared characterized chondrocyte implantation (CCI) to microfracture in the treatment of symptomatic cartilage defects of the femoral condyles. The primary endpoint was successfully reached at 1 year, with CCI showing superior tissue regeneration. Clinical outcome at 12–18 months measured by the overall Knee injury and Osteoarthritis Outcome Score (KOOS) was comparable for both treatments. An extension at 3 and 5 years confirmed that a good clinical outcome was maintained over time for both treatments in the overall patient population. Strikingly, sub analysis of the long-term follow-up data revealed that early treatment by CCI resulted in statistically significant and most importantly clinically relevant better results when compared to microfracture, supporting a critical window of opportunity for genuine tissue regeneration. In addition, data from a large compassionate use program, whereby lesions were treated at diverse locations in the knee joint, corroborated the benefit of CCI found in the RCT. These data sets allow now to better define the treatment algorithms for symptomatic joint surface lesions of the knee in clinical practice.

Keywords Cartilage repair • Characterized chondrocyte implantation • Randomized controlled trial • Long term • Treatment algorithm

Key Points

- In order to minimize dedifferentiation during in vitro expansion, a standardized culture procedure for expansion of human articular chondrocytes was optimized resulting in a well characterized product,
- The development of a robust production process and the consistent results in structural benefit and patient outcomes together with an excellent safety profile have led to the approval of ChondroCelect as the first ATMP by EMA in 2009.
- Characterized Chondrocyte Implantation showed superior structural cartilage repair at 1 year as compared to microfracture.
- ChondroCelect treatment results in a significant and clinically relevant benefit compared to microfracture in patients with a symptom onset since less than 3 years.
- The development of cell based approaches in the field of cartilage repair has contributed substantially to regenerative medicine approaches in general.

11.1 Introduction

Cartilage lesions are common disorders of the knee joint and a frequent cause of knee pain and functional disturbances. Hjelle et al. found single, International Cartilage Repair Society (ICRS) grade III or IV defects of at least 1 square centimetre (cm^2), in 7.1 % of arthroscopies in patients under fifty [1]. Next to the negative impact on patients' activities and quality of daily life, functional limitations in this professionally active group cause a considerable societal burden [2].

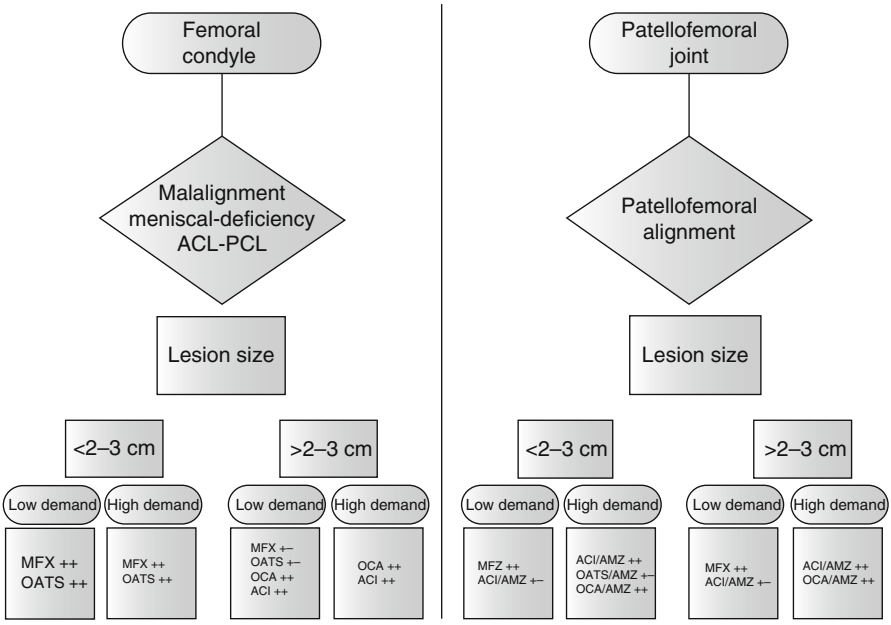

Fig. 11.1 Historical treatment paradigm: lesion size as first criterion in treatment selection. *MFX* microfracture, *OATS* osteoarticular transfer system, *OCA* osteochondral allograft, *ACI* autologous chondrocyte transplantation, *AMZ* anteromedialization (Reproduced with permission from Cole et al. [5])

The natural healing potential of articular cartilage is known to be limited and can predispose to the development of early and established osteoarthritis [3]. Therefore, it is clinically relevant and important to treat cartilage lesions adequately in an early stage, in particular for individuals at risk.

In general, surgical options for knee joint cartilage repair aim at restoring normal, pain-free motion. The procedures can be grouped into three categories: first symptomatic treatments, including arthroscopic debridement and lavage, next repair techniques such as bone marrow stimulation leading to clinical improvement and third restorative and regenerative procedures, including osteochondral grafting and autologous chondrocyte implantation (ACI) aiming to regenerate the joint surface with structural characteristics close to its original integrity [4]. (Fig. 11.1: treatment algorithm). Patient specific variables such as co-morbidities and underlying pathologies play a critical role in the choice and the scope of treatment options. Besides, location and size of the lesion, previous treatment must be taken into account when selecting the most suitable treatment for an individual. In the past, lesion size was applied as a major determinant for defining the best type of surgical technique, being it reparative or restorative, early on in the decision tree [5].

The aim of any surgical treatment is to restore normal, pain-free motion and ultimately postpone or prohibit the onset of osteoarthritis [6]. In the 80's, successful

repair of focal patellar defects were reported by transplantation of cultured autologous cells in the rabbit [7, 8]. In 1994 Brittberg and Peterson published a report on 23 patients in whom deep cartilage defects in the knee were treated by autologous cell implantation (ACI). During an arthroscopic procedure, a biopsy was taken from a minor weight-bearing area. The chondrocytes were cultured *ex vivo* and subsequently, after 14–21 days implanted under a periostal flap during an arthrotomy [9]. Despite overall favourable results, several variables related to the chondrocyte expansion have been identified which may interfere with the *in vivo* formation of stable cartilage. For instance, *in vitro* expansion of articular cartilage derived cells has been known to progressively dedifferentiate and lose their chondrogenic capacity [10, 11]. The loss of phenotypic traits has been shown to result in the formation of disorganized fibrocartilage possibly affecting clinical outcomes [12].

Dell'Accio et al. conducted basic research on mesenchymal cell populations' ability to form stable cartilage *in vivo* in the nude, i.e. immune suppressed, mouse, allowing to study human cell populations and their behaviour *in vivo*. The Ectopic Cartilage Formation Assay (ECFA) was developed, allowing to test and monitor potency and capacity to form a cartilage implant, resistant to vascular invasion and mineralisation, or replacement by bone or fibrous tissue *in vivo*. Furthermore, a set of molecular markers were identified, allowing to predict the outcome of the *in vivo* assay, irrespective of the donor age [13]. The experiments conducted by Dell'Accio et al. not only resulted in the identification of cell populations that retain their cartilage-forming capacity and phenotype *in vivo* but were also at the basis of the development of a standardized and reproducible culturing process aiming at preserving the chondrocyte phenotype capable of producing stable hyaline cartilage.

Based on this research, characterized chondrocyte implantation (CCI) has been developed with the goal to improve the clinical outcome of ACI [6, 14–16]. By definition, characterized chondrocytes are an *in vitro* expanded population of chondrocytes which express a marker profile predictive of the capacity to form hyaline-like cartilage *in vivo* through a standardized, consistent and reproducible process. The expansion procedure, originally optimized by means of the marker profile, was designed as such in order to preserve phenotypic traits and biological activity. As a result, CCI leads to improved potency of individual cell batches and homogeneity in the chondrogenic capacity (Tigenix, data on file). The medicinal product resulting from this manufacturing process, ChondroCelect®, has been granted market authorization by the European regulatory bodies in 2009 as the first centrally approved Advanced Therapy Medicinal Product (ATMP) [17].

A phase III, prospective, multicenter randomized controlled trial was conducted to compare efficacy and safety of CCI versus microfracture (MF) in the repair of single symptomatic cartilage lesions of the femoral condyle [15]. Patients aged between 18 and 50 years, with a single symptomatic cartilage lesion between 1 and 5 cm^2 of the femoral condyles were included. In the CCI arm, 51 patients were treated whereas 61 patients underwent microfracture. The primary endpoint of the trial was the demonstration of structural superiority at 12 months post treatment, both by histological and histomorphometrical assessment. The secondary endpoints related to the clinical outcome, assessed by the overall Knee injury and Osteoarthritis

Outcome Score (KOOS), for which the assumption was made that clinical outcome at 12 and 18 months after CCI should at least be as good as MF.

Histological examination of a biopsy of the repair tissue at 12 months showed superior structural repair in the CCI arm compared to the MF arm [15]. MRI data support the better quality of repair after CCI at 3 years [16]. There was consistent improvement up to 36 months in the clinical outcome as measured by the KOOS in both treatment arms. The estimated benefit at 36 months was larger in the CCI group. The finding that patients with less than 3 years since onset of symptoms (N = 27 in the CC arm and N = 32 in the MF arm) benefited most from CCI allowed to better identify suitable patient populations based on their medical history only [16]. Five year follow-up data on the study patients confirmed the outcomes: patients treated with CCI within 3 years of symptom onset presented with a statistically significantly better clinical outcome, and more importantly a clinically relevant difference versus microfracture [6]. Efficacy results were further corrobated in the compassionate use program in a larger and more varied patient population, presenting with lesions over 5 cm^2, patellar and multiple defects [18].

The safety profile of CCI does not show major differences from that of microfracture. The most commonly found adverse reactions are arthralgia, joint swelling, effusion and crepitation. The majority of observed safety signals in the CCI group relate to the use of the arthrotomy procedure and are present in the early postoperative period. At 60 months, most of the Adverse Events (AEs) had resolved [6].

The good safety profile, and the clinically relevant benefit of CCI over MF provide arguments in favour of revisiting current treatment paradigm, taking into account that time of symptom onset appears to be a crucial determinant of treatment selection. Moreover, basic research reveals more insights into the configuration and functioning of the subchondral region. It becomes clear that knee joint homeostasis as previously suggested [19], and consequently the durable clinical outcome of cartilage repair surgery, is largely defined by proper functioning of the tidemark, the transition zone between cartilage and bone [20].

11.2 From Autologous Chondrocyte Implantation (ACI) to Characterized Chondrocyte Implantation (CCI)

11.2.1 ACI Historically

In the original paper by Brittberg et al., autologous chondrocytes were expanded *in vitro* during a 2 to 3 week culturing process. The chondrogenic phenotype was assessed by microscopical evaluation of clonal growth and metachromatic staining in a small fraction of the isolated cells [9].

However, the loss of the articular cartilage phenotype during *in vitro* expansion culture has been recognized as a major hurdle for ACI [21]. For a long time, monitoring of phenotypic stability throughout the culturing process was based on two

surrogate markers. The expression of type II collagen, a key component of cartilage' extracellular matrix (ECM), basically reflects the differentiation state of the chondrocytes at the time the test is being done. The capacity to form colonies in anchorage-independent conditions moreover seems to be a feature of chondrogenic cells [10] to a large extent but is not a trustworthy variable to predict cartilage forming capacity *in vivo* [13].

11.2.2 Cell Characterization and Technology Development

Dell'Accio et al. [13] developed a nude mouse model resulting in a standardized and validated screening assay which allows to measure the potential of human chondrocytes to form stable cartilage *in vivo*, the Ectopic Cartilage Formation Assay (ECFA). The assay consists of the following steps: in a first step the freshly isolated chondrocytes are obtained from human donors, within 12 h post-mortem. After expansion of the cells in monolayer, four to five million viable cells are re-suspended in 50 μl of phosphate buffered saline and are injected intramuscular (IM) into the thigh of a nude mouse. Thereafter, the cells are allowed to grow in this "in vivo bioreactor" for a period of 3 weeks. Subsequently, the tissue generated at the injection site, is harvested for histological examination.

In all study animals, a distinct cartilage implant was retrieved a week after injection. Safranin O staining, reflecting the presence of sulphated proteoglycans, was nearly comparable to what is seen in normal articular cartilage, however the implants were hypercellular and did not show the typical cartilage architecture. Further histological and immune-staining did not reveal any vascular invasion, bone formation nor the presence of collagen bundles as in fibrocartilage. The properties of serially passaged chondrocytes were assessed, which showed that the cells lose their cartilage-forming potential after 2–3 passages *in vitro*. In an attempt to identify molecular markers with might be predictive of the *in vivo* cartilage forming capacity, the expression of molecules involved in formation and maintenance of chondrocytes' phenotype were monitored throughout the culturing process. The development of consistent and donor age independent parameters, predictive of the *in vivo* cartilage formation potential, have not only been used to identify cell populations but also to design and optimize a reproducible cell culturing process (Fig. 11.2: the mouse model).

The insights that lead to the development of the ECFA allowed to design an *in vitro* assay where the same cell populations tested in the ECFA were analyzed in a comparative micro-array analysis. Cellular expression patterns of genes relevant for cartilage and chondrocyte biology were studied, in an attempt to identify both positive and negative markers predictive of *in vivo* cartilage formation capacity. As such, 150 positive markers, genes that are highly expressed in the cells that produced a cartilage implant in the ECFA and are not or very weak in the cells without

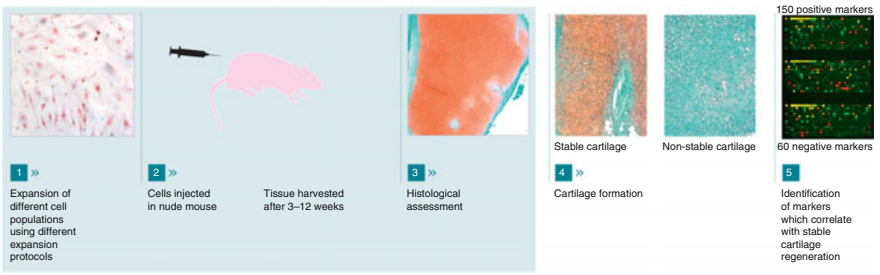

Fig. 11.2 Nude mouse model: comparison of cell populations that pass and fail proprietary in vivo assay on gene expression profiles (Reprinted with permission from Dell'Accio et al. [13])

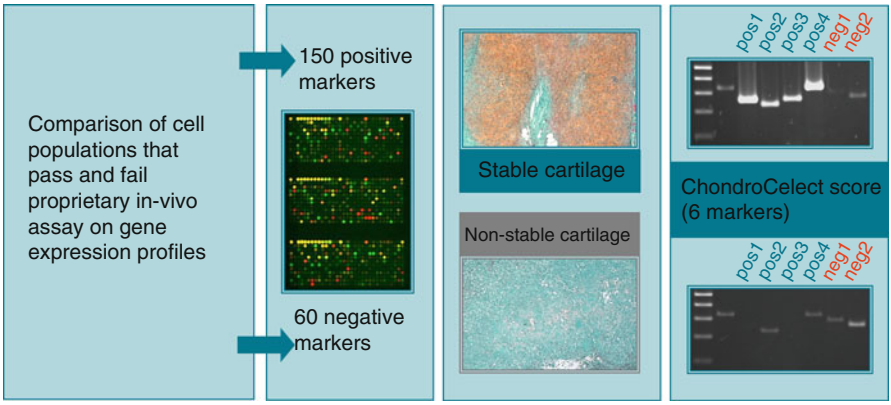

Fig. 11.3 Identification of molecular markers, resulting in ChondroCelect score

chondrogenic potential, and 60 negative markers, genes highly expressed in cells without cartilage formation potential in the ECFA, were identified.

Of these 210 markers, 4 positive and 2 negative markers were selected, based on their capacity to generate cartilage tissue in the ECFA. Each of the individual markers can be scored based on their overall expression level in the assay, adding up to the ChondroCelect score. The score, ranging from −6 to +6, is considered a potency assay for the cartilage forming capacity. A major advantage of the score is that it is compatible with a routine manufacturing setting, by means of Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) methodology (Fig. 11.3: ChondroCelect score).

A set of molecular markers reflecting *in vivo* cartilage formation also enabled to optimize the expansion process. Variables that were investigated include but are not limited to culture media, serum batches, enzymatic treatments, passaging methods, culture vessels and population doublings. These investigations formed the basis for the development of a more robust cell product consisting of a cell suspension of autologous articular cartilage derived cells capable of forming ectopically stable hyaline and not transient cartilage.

Fig. 11.4 Standardization and optimization starts at the time of biopsy: the ChondroCelect Harvester

11.2.3 ChondroCelect, Standardized Medicinal Product Based on Autologous Cells

The starting material for the production of ChondroCelect consists of 200–300 mg of healthy cartilage, arthroscopically harvested from a minor weight bearing area of the medial or lateral part of the trochlea or the intercondylar notch. To standardize the biopsy process, a specific device has been designed: the ChondroCelect Harvester (Fig. 11.4). According to the estimated size of the debrided lesion, one to three slivers with a length of 8 mm are required for the manufacturing process. Biopsy procurement boxes with sterile biopsy vials are stored in the orthopaedic units under temperature controlled conditions. Once the biopsies are harvested they are immediately sent to the cell expansion facilities (CEF) without interruption of the cold chain. Upon arrival at the CEF, only tissue from donors whose blood samples are negative for HIV type 1 and 2, hepatitis (HBV, HCV) and syphilis will be further processed. This measure of precaution is more stringent than existing regulation, but is primarily introduced to ensure absolute protection of the plant sterility zones.

The manufacturing process starts with the biopsy digestion: cartilage fragments are isolated and chondrocytes are released from the extracellular matrix, washed, counted and seeded in culture medium. Upon confluence of the cell cultures, they are detached from culture flasks and seeded onto new flasks, a process called a 'passage'. This step is being repeated until a sufficient number of cells are obtained, the optimum being one million cells to cover a 1 cm^2 defect, mostly based on the cell number found in corresponding mature articular cartilage . However, as has been demonstrated that cells lose their chondrogenic capacity after four to six passages, the maximum number of passages allowed in the ChondroCelect production process is three [13]. The expansion process is further optimized by comparing the molecular signature of cells with a preserved capacity to form hyaline cartilage to cells which develop inferior cartilage tissue prone to vascular invasion, calcification and bone formation (also in part described in reference [13]), In parallel, culture conditions are set in such a way as not to enrich for other cell lineage populations, e.g. fibroblasts. When the appropriate number of cells is reached, the cells are harvested from the wells, washed, counted and cell viability is checked. The culturing process is variable in time span, partly due to inherent cell characteristics and partly

because the cell yield relates to the defect size. Therefore, and also to offer flexibility for selection of the CCI timing, a cryopreservation step was introduced. The final product can be reconstituted for implantation between 9 and 13 weeks after the biopsy. The dosage required for implantation, 0.8–1 million cells per cm^2, is delivered in vials containing four million cells per 0.4 ml excipiens.

Historically, the technique for performing ACI, consisted of suturing of a periosteal flap (or later on a bio-membrane) over the debrided lesion followed by the injection of the cultured cells beneath the water-sealed membrane. In a recent publication by Steinwachs, a variation is presented: the chondrocyte suspension is applied onto the bio-membrane and after approximately 10 min time needed for the cells to adhere to the membrane, it is sutured into the debrided lesion [22]. No direct comparisons have been made between both techniques, in terms of ease of use or outcomes.

As illustrated above, CCI implies process design, from biopsy to implantation, optimized and standardized to maximally preserve the phenotypic traits and biology, reducing the variability of the final product, despite its autologous origin: for each of these steps the process has been optimized, specific devices have been developed and are being used, and all stakeholders have been trained to comply with preset quality criteria. Thus, a selection at the end of the culturing process itself has become obsolete due to the optimised and robust culturing process which enriches for the superior cartilage forming cells. . In order to investigate its clinical significance, a well designed prospective multicenter trial was initiated.

11.3 Bringing Research to the Bedside: The CCI Randomized Controlled Trial

In 2002, an international prospective randomized multicenter controlled trial was set up involving 13 orthopaedic centres. In consensus, microfracture was chosen as the control arm, because it was considered the existing treatment standard of femoral cartilage lesions, although quite controversial at that time [15].

11.3.1 Microfracture Technique

Microfracture is a surgical technique developed by Steadman to enhance chondral repair by making multiple microfractures in the subchondral bone plate. The mesenchymal stem cells, growth factors and other substances released from the marrow form a ‘super clot’ providing a suitable environment in which the stem cells are believed to differentiate into cartilaginous like tissue within the lesion [23]. The repair tissue consists predominantly of collagen type I and resembles fibrocartilage, thereby less resistant to shear and compression loads as compared to hyaline cartilage [24].

Microfracture is widely used, mainly because it is a one-step procedure and because it has a good potential for symptomatic improvement [25, 26]. In the first randomized controlled trial, published well after the start of the CCI trial, Knutsen compared ACI with MF and found no statistically significant difference with regard to structural outcome at 2 years post surgery [27] and clinical outcome up to 5 years [28]. It is of note that this study confirmed independently the international consensus on the proper clinically relevant comparator for cell based repair at that time being microfracture.

11.3.2 Study Population and Baseline Characteristics [15]

Eligible patients were aged between 18 and 50 years, and had a single symptomatic cartilage lesion (International Cartilage Repair Score III or IV) between 1 and 5 cm^2 of the femoral condyle. Patients with the presence of a clinically relevant patello-femoral cartilage lesion, osteochondritis dissecans (OCD), a lesion over 0.5 cm depth and microfracture performed less than a year before baseline were excluded. Randomized patients were treated with CCI, using periosteum to cover the defect, or MF. For each of the trial arms the same standardized surgical technique and rehabilitation protocol was enforced. Patients who entered the 12 month study were evaluated with 3-month intervals by an independent investigator not involved in the surgery, and were invited to participate in the extension program up 'till 5 years post surgery.

The sample size was determined based on the definition of a treatment success as the presence of hyaline cartilage characteristics of the repair tissue, and in contrast fibro-cartilage or non-cartilage as a failure. It was assumed that 30 % of patients would report with a successful result after MF, and that an improvement in success rate to 60 % with CCI would be a clinically relevant improvement.

A total of 118 patients were randomized to treatment, 57 to CCI and 61 to MF. Of the CCI patients, six subjects could not be treated because they fell out of specs for the CC score criterium which was enforced: they are included for analysis in the safety population but not in the efficacy analysis. The randomisation to CCI and MF was successful for age (mean age 33.9 years and 33.9 years, respectively), gender (61 and 67 % males), and weight (mean 78.1 and 80.6 kg). There was a slightly higher proportion of patients in the MF group with an acute onset of symptoms compared to the ChondroCelect arm. The median duration of time since onset of knee injury was slightly longer in the ChondroCelect group than in the MF group (2.0 years versus 1.6 years). The presence of concomitant cartilage lesions was comparable in both groups (30 % versus 25 %). Proportionally more patients in the CCI group had undergone previous knee surgery (88 % versus 77 %). The size of the lesion post-debridement was similar in both treatment groups (mean 2.64 and 2.44 respectively) and reflects what is typically encountered in the orthopaedic practice. This lesion size was expected to respond well to both techniques in order to avoid bias in favour of cell transplantation.

As for any of the cartilage restoration procedures, it was imperative that concomitant pathology such as mal-alignment or meniscus lesions were corrected prior to or at the time of index surgery.

11.3.3 Histology and Histomorphometry Outcomes After 12 Months [15]

The original primary objective of the study was to show an advantage of ChondroCelect over MF by demonstrating superiority on the structural repair as assessed by histology and histomorphometry. At 12 months post treatment, biopsy specimens were obtained arthroscopically from the centre of the repair tissue.

Staining with safranin O (a measure of proteoglycan) and anticollagen II antibody, reflective of good quality cartilage tissue, was performed and the staining was expressed as a ratio of the total surface by blinded pathologists. From the CCI and the MF group, 50 and 43 biopsy specimens were analysed respectively. The adjusted mean sum of ratios was significantly higher ($P = 0.003$) for the CCI group than for the patients treated with MF (Fig. 11.5a: Collagen type 2 staining above and SafraninO staining for the best samples of both groups, showing a clear morphological superiority of CCI over microfracture in homogeneity and collagen fibre organization).

Histopathologists scored the quality of cartilage repair by means of the Mean Overall Histology Assessment Score (ICRS II score) [29], assessing components related to chondrocyte phenotype, tissue structure and other possible negative characteristics of the repair tissue such as vascularisation or calcification. Each of the items was rated on a visual analogue scale. The adjusted mean overall histology assessment score was significantly higher ($P = 0.012$) for the CCI group. The adjusted mean scores for components of structural repair relating to chondrocyte phenotype and some components relating to tissue structure were also significantly higher in the CCI group (Fig. 11.5b: subscores reflecting chondrocyte phenotype and some scores reflecting tissue structure were significantly better for the CCI group).

Superiority of ChondroCelect over MF could be demonstrated for both efficacy measures for structural repair: the histomorphometric and the histological endpoint. This suggests that after CCI the regenerated tissue is indeed more hyaline-like and richer in chondrocytes and proteoglycan content of the ECM, which is a prerequisite for resistance to compressive strength.

11.3.4 Clinical Outcome as Measured by KOOS at 12 Months [15]

The second primary objective of the study was to demonstrate non-inferiority on the clinical endpoint, measured as change from baseline in Knee injury and Osteoarthritis Outcome Score (KOOS) for the average of the 12- to 18-months follow-up data. The KOOS questionnaire is patient-rated and consists of 42 items divided over 5 subscales: pain (9 items), other symptoms such as swelling (7 items), activities of daily life (17 items), function in sport and recreation (5 items) and knee-related Quality of Life (QoL) (4 items) [30]. Each of the items has to be scored taking into

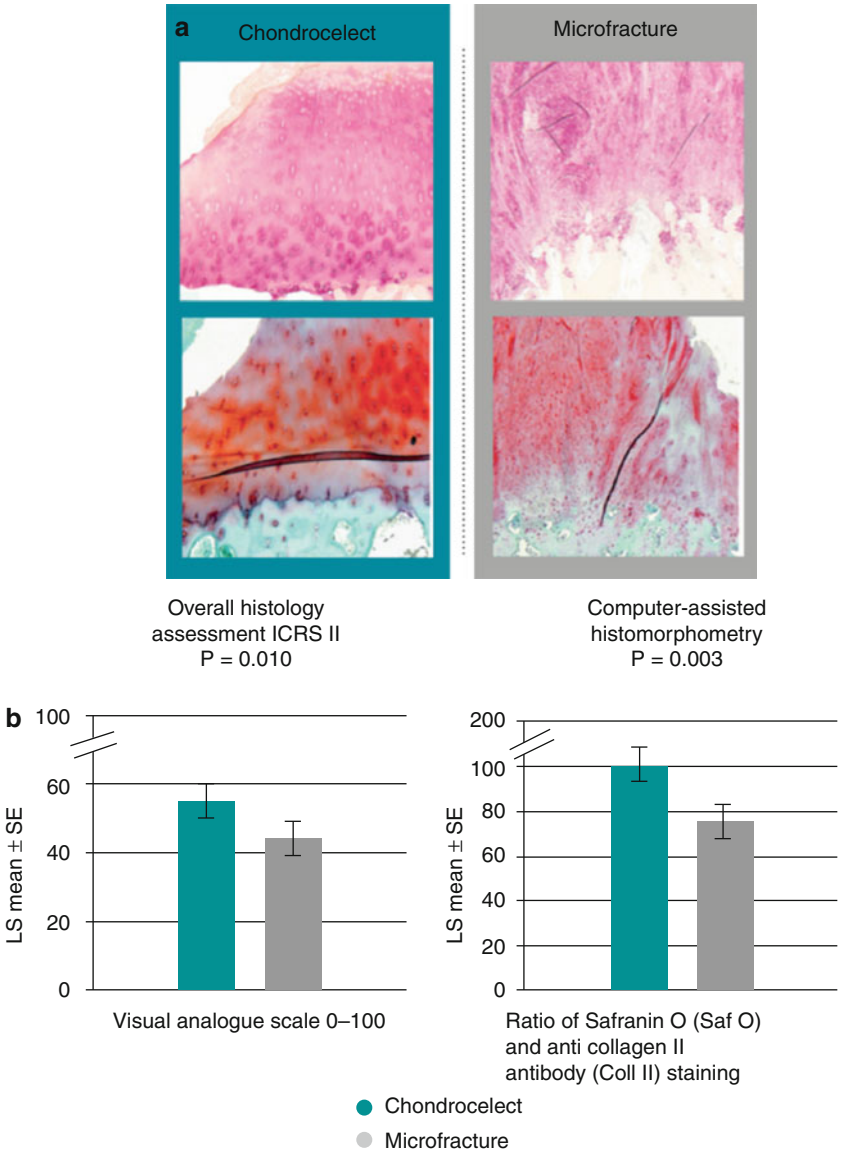

Fig. 11.5 (**a**, **b**) Structural outcome 12 months after CCI, as assessed by histology and histomorphometry

account the previous week. Standardized answer options are given in five Likert boxes leading to a score between 0 and 4. A normalized score whereby 100 meaning no symptoms and 0 indicates extreme symptoms, is calculated for each subscale. The result can be plotted as an outcome profile. KOOS has since been used in a large number of trials and is now considered one of the most meaningful clinical endpoints for knee pathologies to date.

At 18 months, 46 out of the 51 patients treated with CCI were still in the study, and 52 out of the 61 subjects in the MF arm.

The adjusted means for the change from baseline to the mean of 12–18 months in overall KOOS and the subdomains of pain, symptoms/stiffness, ADL and QoL were similar for both study arms. The results fulfill the predefined criteria for non-inferiority in this now co-primary clinical endpoint (as discussed and agreed upon with the regulatory bodies) and both changes are clinically relevant (≥10 points on a scale of 0–100) [31]. Although CCI requires an arthrotomy, which might enforce a slower recovery, this does not appear to affect 1 year outcomes as measured by the KOOS because the clinical improvement versus baseline is comparable in both treatment groups.

11.3.5 Maintenance of Effect in the Long Term [16, 18]

Both treatment groups experienced statistically significant improvements in overall KOOS. Scores continued to improve for 24 months in the CCI group, whereas the maximum for MF was reached approximately 12 months post treatment. In general, the improvement at 2 years was maintained throughout the follow-up period. The clinical benefit versus baseline at 60 months showed a positive trend for CCI versus MF, but no statistically significant differences were found in the overall population (P = 0.116) (Fig. 11.6: the clinical benefit of CCI and MF were maintained at 5 years).

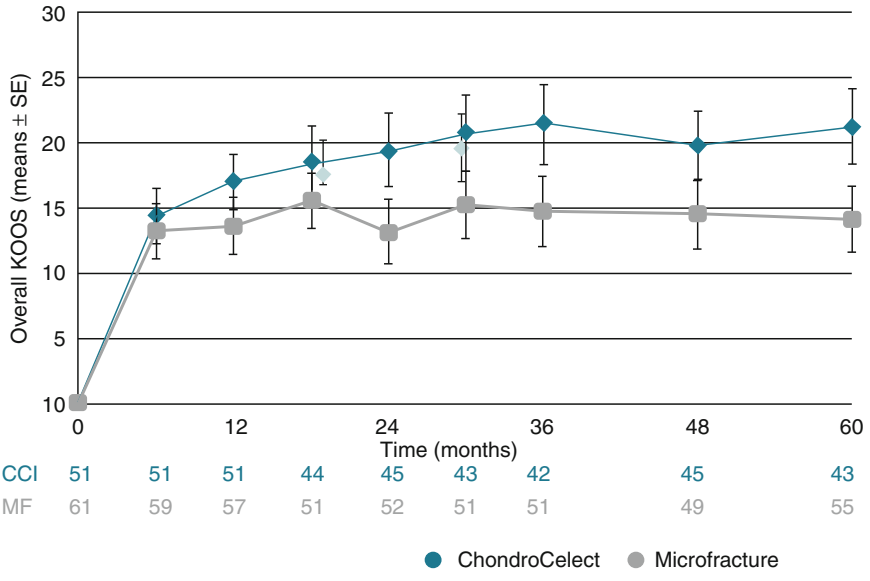

Fig. 11.6 Evolution of overall KOOS (average of all KOOS domains except Sports) throughout 60 month follow-up. Full Analysis Set, including all available data (long-term follow-up) and Last Observation Carried Forward for failures (Vanlauwe et al. [6])

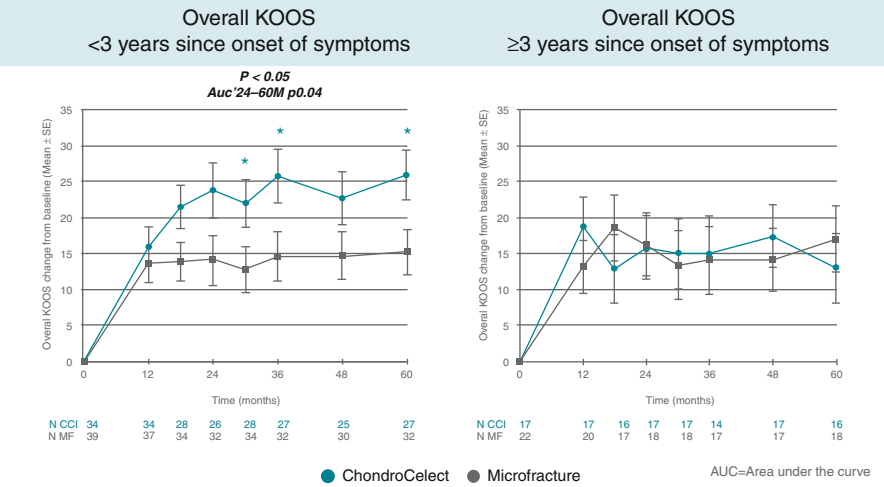

Fig. 11.7 Overall KOOS evolution in patient population with onset of symptoms below and above 3 years. Full Analysis Set and Last Observation Carried Forward for failures (Vanlauwe et al. [6])

Looking for parameters that might predict a favourable outcome, commonly defined as identification of responders to treatment, it was noted that patients with a symptom onset less than 3 years, not only showed a statistically significant difference in overall KOOS improvement compared to MF, but more important had a clinically relevant greater improvement (P = 0.026). Significant differences were also observed in the 'pain' and 'QoL' subscales (Fig. 11.7: Significant difference in overall KOOS in favour of CCI in the <3 years onset group, compared to the >3 years onset group).

Survival analysis did not show statistically significant differences between both treatment arms (Fig. 11.8: Kaplan-Meier survival curve for both treatment arms). However, treatment failures, defined as a re-intervention affecting more than 20 % of the index lesion, seem to occur mostly in the first 3 years post treatment for microfracture treated patients, earlier than in the CCI group. Defining failure has its limitations, in this case it typically relied on clinical symptoms and signs associated with an MRI and/or arthroscopic evaluation to assess whether the cause of failure is due to deterioration of the index lesions.

ACI often is performed in patients who failed traditional first-line treatments such as debridement, MF or osteochondral autograft techniques. However, recent evidence suggests that marrow stimulation techniques have a strong negative effect on subsequent cartilage repair and should be used judiciously in cartilage defects that are amenable to cell based regeneration. In a review of 329 patients, defects that had prior treatment affecting the subchondral bone (microfracture, abrasion chondroplasty and drilling) failed at a rate three times higher than that of non-treated defects [32]. Outcomes were classified as complete failure if more than 20 % of a graft had to be removed in later procedures due to persistent symptoms.

The results from this trial are somewhat different with the outcomes published by Knutsen et al., where no statistically significant differences in clinical outcome, as

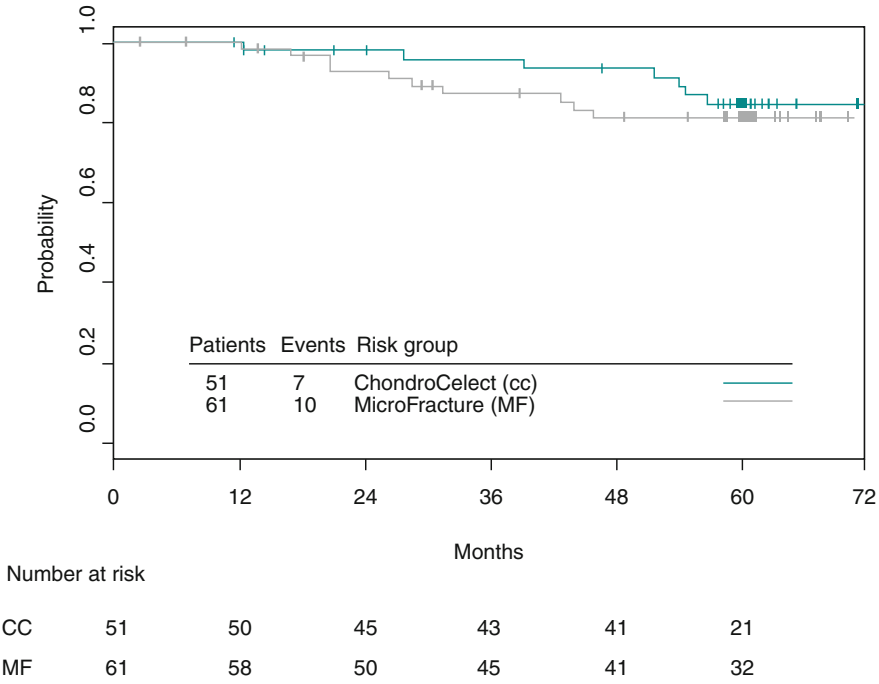

Fig. 11.8 Time to treatment failure (Kaplan Meier graph): Full Analysis Set population at 60 months post surgery (Vanlauwe et al. [6])

measured by the Tegner and International Knee Documentation Committee (IKDC) scores, between MF and ACI at 2 and 5 year follow-up were found [28]. Although osteoarthritis patients were excluded from participation, over a third of the study population in the Knutsen trial displayed radiographic signs of osteoarthritis after 5 years. In contrast, in the RCT comparing CCI to MF, less than 5 % of patients were found to display osteophytes after 5 years [33]. This, together with a higher proportion of chronic lesions in the Knutsen trial (median duration of symptoms 36 months), is reinforcing the hypothesis that cartilage lesions must be treated early on before impairment of the joint biology and loss of homeostasis have led to a 'point of no return'. Many other factors may be of relevance to the different outcomes of the trials including the improved product profile of the cells, a more rigorously controlled trial with respect to inclusion and exclusion criteria, tight control (with audit) of the data and better training of the surgeons.

11.3.6 Imaging Outcomes

Magnetic Resonance Imaging (MRI) scans were performed at baseline, 12, 24 and 36 months. The characteristics of the repair tissue were assessed by means of the Magnetic resonance Observation of Cartilage Repair Tissue (MOCART)

score and nine additional items. Filling of the defect, surface of the repair tissue, subchondral lamina and subchondral bone reaction were identified as the most important determinants of repair tissue quality. At 36 months after surgery, no statistically significant differences for these were observed between the groups, except for subchondral bone reaction which was more prominent in the MF group (P = 0.056). Progressive elevation of the subchondral bone plate was also more pronounced in the MF group than amongst CCI patients (12.1 % versus 8.3 % at baseline; 51.5 % versus 25.0 % at 36 months). In 49 patients, radiographic data were available at baseline and 60 months. No difference in radiographic changes between both treatment arms was observed [6]. In the failure analysis at 5 years in the CCI versus MF trial, the relation with item 5 from the ICRS2 score (subchondral bone changes) was nearly significant (.056) adding to the increasing importance given in literature to the function and the restoration of the subchondral plate as a hallmark in successful cartilage repair, but also in the resistance to development of osteoarthritis.

11.4 Safety Profile of Innovative Treatments: The CCI Experience

11.4.1 Patient Exposure in Clinical Trial and Real Life Setting

A total of 421 patients have been exposed to ChondroCelect in a clinical trial setting.

In the RCT, 61 subjects underwent MF treatment and 57 patients were randomized to CCI, of whom 51 actually underwent CCI. For the safety analysis, all AEs experienced by the patient from the time of the screening visit until the completion of the initial 12-month and extension studies were captured in the case report form (CRF). The retention rate throughout the extension program was high: Forty-three CCI patients (84 %) and 45 MF patients (74 %) provided data for the 5-year follow-up [6].

In the compassionate use program, safety data were available from 334 patients (90.3 %) at database lock. The average exposure, defined as the time between CCI and data capture, was 811 days, ranging between 160 and 1,512 days [18].

In both, the clinical trial and the compassionate use program (CUP), the absolute dose of ChondroCelect received was determined by the size of the lesion(s) treated: ranging between one and five million cells in the RCT, whereas in the CUP lesions up to 20 cm^2 were treated. Despite the relatively short existence of the therapy, patient exposure has thus been quite large both in terms of number of patients, heterogeneity of treated population and follow-up time.

11.4.2 Early AE Profile of CCI [15]

Direct comparison of AE types and frequencies should be evaluated bearing in mind that CCI is a two step procedure with a biopsy arthroscopically harvested, and subsequent implantation of the medicinal product during an arthrotomy. In the RCT, the implanted cells were sealed with a periostal membrane, which necessitated an infrapatellar incision. Thus, 'relatedness' of an AE in the CCI group refers to both, the surgical procedure and the medicinal product itself. In contrast, MF is a single step arthroscopic procedure conducted under local or general anaesthesia [23]. In the first 12-months of the RCT, similar proportions of patients reported treatment-emergent AEs, the majority having a mild or moderate intensity, in the CCI group (50/57, 88 %) versus the MF arm (50/61, 82 %). This was equally true for severe AEs, reported in 12 and 13 % of patients respectively. Relatedness of AEs to the study procedure was confirmed in 67 % of cases in the CCI group and 59 % of cases in the MF arm.

Arthralgia, was the most commonly reported AE in both treatment groups, present in 61 % of CCI subjects and 57 % of MF patients [15].

As CCI requires an arthrotomy, it is not surprising that joint swelling was more frequent in the CCI group versus MF arm. The reported frequency of joint swelling is higher after ChondroCelect (19 %) than after MF (13 %). Joint swelling, a sign of extravasation of fluid in and/or around the knee, is a known symptom after arthrotomy as a result of the inflammatory synovial reaction due to incision [34]. The majority of cases, 7 out of 11, in the CCI group occur in the first 4 weeks after surgery, compared to none in the MF group ($P = 0.003$). One month after surgery, there are no significant differences between both treatments. Apart from the temporary aspect, no cases of postoperative joint swelling were considered severe or serious.

Related AEs of joint crepitation were significantly more common in the CCI arm (12 %) versus MF (1.6 %). Joint crepitation is perceived as being of limited clinical significance and is common even in the normal population [35].

The use of a periosteal flap, conform the initial publication of the technique by Brittberg and Peterson, is known to trigger hypertrophy of the repair tissue, which may cause physical impairment and consequently necessitate arthroscopic shaving. The incidence of hypertrophy at 12 months was 25 % for the CCI group versus 13 % of MF patients ($P = 0.156$). All reported AEs of hypertrophy were mild or moderate in severity in both treatment groups. None was recorded as severe and none was reported as serious.

Overall, no patients were discontinued from the study due to AEs.

11.4.3 Safety and Tolerability in the Long Term [6, 18]

Throughout the whole follow-up period of the RCT, 98 % of patients in the CCI arm and 84 % of MF patients reported at least 1 treatment-emergent AE.

However, all AEs had resolved at 60 months, except for effusion in 3 and 1 cases of after CC and MF respectively, and joint crepitation present in 1 subject of each group.

The most common AE in the early phase, arthralgia, was at 36 months still present in 14 % of CCI cases versus 4 % in the MF group. Joint swelling was not reported in the CCI group beyond 36 months. Joint crepitation, was more frequent in the CCI group compared to MF, but markedly resolves over time: 12 % versus 2 % in the short term, 11 % versus 0 % between 18 and 36 months and 2 % for each treatment group at 60 months [6].

In the compassionate use program, frequencies of AEs are consistently lower as compared to the RCT. Relative underreporting of AEs is indeed one of the methodological limitations of this type of studies. However, with respect to the relative frequency of the AEs, a similar safety profile was observed despite the more heterogenous patient population [18].

In 62.0 % of cases, the reported AE was considered to be related to the surgical procedure. The most commonly reported AEs were knee pain (23.8 %), joint effusion (8.5 %), joint swelling (8.2 %), joint crepitation (6.1 %), muscle atrophy (6.1 %) and decreased joint range of motion (ROM) (5.7 %). The majority of cases (77.6 %) were rated mild to moderate in intensity and 74.4 % were considered unlikely related or unrelated to the medicinal product ChondroCelect.

From 334 patients, 24 serious AEs were received, of which 3 were judged to be possibly related to the product and surgery: 1 in which the ROM was decreased, and 2 cases in which it was judged that the therapeutic product was ineffective.

In contrast with the findings from the RCT (25 % for CCI and 13 % for MF), cartilage hypertrophy was reported overall in 6 of 334 patients (2.1 %). This is most likely explained by the use of a biological membrane, Chondro-Gide™, in the CUP. It is known from the literature that hypertrophy rates are lower in case a biological membrane is used as compared to periostal grafts [36]. Based on these insights, and in order to minimize morbidity, the use of a biomembrane to seal of the implanted chondrocytes has anno 2012 become the standard of CCI . In vitro biocompatibility data for the ChondroGide membrane in combination with ChondroCelect has been generated and approved by EMA.

Interestingly, safety data were collected from 84 patients treated for a patellar lesion. Thus, in the overall safety data, this particular subpopulation contributes for 25.1 %. The observations suggest that patients treated by CCI for a patellar lesion are more prone to developing arthrofibrosis (five patients out of the total of seven patients who developed arthrofibrosis), decreased ROM (8 patellar cases out of the 16 cases which developed decreased ROM) and joint crepitations (9 patellar cases out of the 18 cases which reported crepitations. The rehabilitation program after patellar treatment is indeed clearly different from the femoral protocol in order to prevent early shear and loosening of the graft. This might largely explain these findings.

Patients with lesions larger than 5 cm^2 (range 0.25–20.0 cm^2; median 3.0 cm^2, mean 3.5 cm^2) have been treated under compassionate use only. The safety data obtained in these patients do not indicate a particular safety concern.

In an early phase of the compassionate use program, 16 minors have been treated with ChondroCelect. No specific safety signal was detected in these patients. However, if a surgeon believes that the benefit/risk ratio justifies use of CCI in a particular patient (Marketing Authorisation of ChondroCelect is only granted for patients over 18) complete closure of the growth plate must be documented.

There were no patient deaths recorded during the study. No patients are recorded as being discontinued from the study due to SAEs.

11.5 Discussion and Conclusions

Regenerative Medicine (REGMED) approaches are widely investigated in many fields of Medicine including in musculoskeletal applications. Despite significant advances in the understanding of the processes of tissue repair, the clinical impact of REGMED approaches is still limited. However, some applications have made great progress including skin and cartilage repair. REGMED approaches aim at restoring tissue integrity thereby not leaving any trace behind of the repair process. Fracture healing is a nice example of perfect regeneration in the postnatal mammalian species. Joint surface healing and in particular healing of articular cartilage has been much more challenging as nature is not capable of achieving this. It is an ambition of REGMED to break these boundaries by trying to obtain improved healing of what nature may not always achieve. In view of this, we believe that from the biological perspective comparing microfracture techniques with cell implantation is comparing apples with oranges. Indeed, microfracture induces local fracture healing, ultimately not destined to regenerate an articular surface. The microenvironment may contribute to the maintenance of a cartilage intermediate (a callus type of repair tissue) in the endochondral bone healing process, but there is ample evidence that this is not leading to hyaline articular cartilage. Thus bone marrow stimulation techniques violate the tidemark and subchondral bone plate,and the repair tissue originates from other cellular compartments and go through bone fracture repair pathways [20].

In contrast, articular chondrocyte implantation attempts to preserve the cartilage-bone interface and the resulting regenerate appears to mimick more closely the original and surrounding tissue. Indeed, as soon as cells are implanted a communication is established between the grafted cells and the neighbouring tissue, a phenomenon deemed crucial for the success of the regeneration process. If the implanted or recruited cells are foreign to the articular tissue, this communication might be jeopardized. This is why the data from this CCI trial and other trials, including the failure analyses that have been done, should trigger the orthopaedic community to revisit the treatment algorithm of cartilage lesions. After correction of all surrounding variables such as alignment, ligaments and menisci, more specifically in a patient group which only developed their symptoms recently, the use of autologous chondrocytes should be regarded as a first line regenerative treatment for cartilage in any lesion larger than 2–3 cm^2.

The challenge to prove that REGMED approaches are also of clinical relevance is certainly quite difficult, since several variables are affecting clinical outcomes. They include not only the proper characterization and optimization of the cellular product towards optimal performance, but also factors as microenvironment are of crucial importance. Indeed, proper communication with the surrounding tissues is a key goal for successful regeneration, and we need to translate that into appropriate in- and exclusion criteria, optimized surgical procedures and rehabilitation strategies. Prevention of treatment-emergent side-effects is crucial for any ACI or other cartilage regeneration procedures. In the development of CCI, semi-customized rehabilitation schemes have been specified, which are available to the treating physiotherapist [37]. In addition, we may have to adapt and improve clinical outcome measurements to make them more sensitive to detect and discriminate the distinct mechanisms of repair.

The prospective, multicenter controlled RCT designed to evaluate the efficacy of CCI versus microfracture was the first of its kind. Despite the still somewhat limited number of patients treated when compared to clinical trials in other medical disciplines, there was a lack of evidence for cell based regenerative approaches from controlled trials [38]. This was particularly the case for cartilage repair techniques, including ACI.

It might be considered a shortcoming that CCI was here compared to MF instead of other cell-based technologies, whereas anno 2012 different cell products are available. Differences in efficacy amongst cell products have not been demonstrated in clinical trials. So far, ChondroCelect is the only regenerative cartilage therapy approved as ATMP, which means that efficacy, safety and pharmaceutical quality have strictly been monitored and investigated. For each of these determinants, ample information is available in the public field, which is not necessarily the case for traditional ACIs. In the systematic review by Harris et al., seven trial reports were mentioned in which CCI or ACI was compared to MF, no direct comparisons between ACIs or ACI and CCI do exist [39]. Van Wilder tries to overcome this lack by computing indirect treatment comparisons (ITCs) based on the individual study results of the ACI/CCI versus MF [41]. This methodology was established and validated by the Canadian Agency for Drugs and Technologies. He argues that two out of the seven trials identified by Harris refer to the same study population which was the subject of another publication. Furthermore, he excludes the study by Basad et al. because the large lesions present in this population were in favour of ACI rather than MF treatment [40]. From the four remaining trials, six ITCs were calculated of which four yielded a significant difference, by definition representing ‘large’ treatment effects [41]. Although in ITCs a number of assumptions are being made and one might argue about the validity of the methodology, it is interesting to see that cartilage repair cellular therapies do have different outcomes. It is indeed well know from the literature that minor deviations or variations in culturing conditions can have a huge impact of differentiation of stem cells and adult progenitor cells [42]. Besides divergence at the level of the product used for the treatment, patients presenting with cartilage lesions are a heterogeneous group which is not necessarily adequately addressed when defining inclusion criteria [33].

The lack of correlation between structural improvement from an early stage on and clinical benefit remains a topic for further research. Remodelling and maturation of the cartilage repair tissue after autologous-chondrocyte implantation is progressive and is believed to go on beyond 18 months [43]. It might well be that the advantageous effects resulting from superior quality tissue regeneration require follow-up beyond the time horizons of this trial [44].

The overall safety profile shows that the main difference in treatment related adverse events compared to microfracture is related to the arthrotomy. Many investigational products which can be applied by means of minimally invasive techniques are currently being tested. However, from a patients' perspective, long-term clinical outcomes should be the main driver for any further development. Ease of use and shortening of operation time are features which increase the short term comfort level for surgeon and patient but may not have any inherent long term value.

In conclusion, we believe that the use of cell based approaches in the field of cartilage repair has contributed substantially to REGMED approaches in general, and we and others are capitalizing on this experience to further achieve benefits for our patients. In addition, the lessons learnt from this impressive body of work has triggered new and improved approaches for the prevention and treatment of osteoarthritis, considered as the "holy grail" in the field of musculoskeletal disorders. We hope that young investigators see the ample opportunities to contribute to these major developments and that the field of musculoskeletal disorders and diseases will continue to attract the brightest minds out there!.

References

1. Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. Arthroscopy. 2002;18:730–4.
2. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. Bull World Health Organ. 2003;81:646–56.
3. Trattnig S, Domayer S, Welsch GW, Mosher T, Eckstein F. MR imaging of cartilage and its repair in the knee–a review. Eur Radiol. 2009;19:1582–94.
4. Cole BJ, Pascual-Garrido C, Grumet RC. Surgical management of articular cartilage defects in the knee. J Bone Joint Surg Am. 2009;91:1778–90.
5. Cole BJ, Pascual-Garrido C, Grumet RC. Surgical management of articular cartilage defects in the knee. Instr Course Lect. 2010;59:181–204.
6. Vanlauwe JJE, Saris DB, Victor J, Almqvist KF, Bellemans J, Luyten FP. Five year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. Am J Sports Med. 2011;39(12):2566–74.
7. Peterson L, Menche D, Grande D, et al. Chondrocyte transplantation - an experimental model in the rabbit. In: Transactions from the 30th Annual Orthopedic Research Society, Atlanta. 1984. Palantine: Orthopedic Research Society; 1984. p. 284.
8. Grande DA, Pitman MI, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res. 1989;7:208–18.
9. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331:889–95.

10. Benya PD, Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell. 1982;30:215–24.
11. Giovannini S, Diaz-Romero J, Aigner T, Mainil-Varlet P, Nesic D. Population doublings and percentage of S100-positive cells as predictors of in vitro chondrogenicity of expanded human articular chondrocytes. J Cell Physiol. 2010;222:411–20.
12. Jones DG, Peterson L. Autologous chondrocyte implantation. J Bone Joint Surg Am. 2006;88:2502–20.
13. Dell'accio F, De Bari C, Luyten FP. Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. Arthritis Rheum. 2001;44:1608–19.
14. Vanlauwe J, Almqvist F, Bellemans J, Huskin JP, Verdonk R, Victor J. Repair of symptomatic cartilage lesions of the knee: the place of autologous chondrocyte implantation. Acta Orthop Belg. 2007;73:145–58.
15. Saris DB, Vanlauwe J, Victor J, Haspl M, Bohnsack M, Fortems Y, et al. Characterized chondrocyte implantation results in better structural repair when treating symptomatic cartilage defects of the knee in a randomized controlled trial versus microfracture. Am J Sports Med. 2008;36:235–46.
16. Saris DB, Vanlauwe J, Victor J, Almqvist KF, Verdonk R, Bellemans J, et al. Treatment of symptomatic cartilage defects of the knee: characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. Am J Sports Med. 2009;37 Suppl 1:10S–9.
17. European Medicines Agency, EMEA/724428/2009. 2009. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/000878/WC500026031.pdf.
18. Vanlauwe J, Huylebroek J, Van der Bauwhede J. Clinical outcomes of characterized chondrocyte implantation. Cartilage. 2012;3(2):173–80.
19. Saris DB, Dhert WJ, Verbout AJ. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair. J Bone Joint Surg Br. 2003;85:1067–76.
20. Madry H, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18:419–33.
21. Hoshiba T, Yamada T, Lu H, Kawazoe N, Chen G. Maintenance of cartilaginous gene expression on extracellular matrix derived from serially passaged chondrocytes during in vitro chondrocyte expansion. J Biomed Mater Res A. 2012;100:694–702.
22. Steinwachs M, Peterson L, Bobic V, Verdonk P, Niemeyer P. A consensus statement on surgical technique: cell-seeded collagen matrix-supported autologous chondrocyte transplantation (ACT-CS). Cartilage. 2012;3(1):5–12.
23. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res. 2001;(391 Suppl):S362–9.
24. Bedi A, Feeley BT, Williams III RJ. Management of articular cartilage defects of the knee. J Bone Joint Surg Am. 2010;92:994–1009.
25. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy. 2003;19:477–84.
26. Gobbi A, Nunag P, Malinowski K. Treatment of full thickness chondral lesions of the knee with microfracture in a group of athletes. Knee Surg Sports Traumatol Arthrosc. 2005;13:213–21.
27. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg Am. 2004;86-A:455–64.
28. Knutsen G, Drogset JO, Engebretsen L, Grontvedt T, Isaksen V, Ludvigsen TC, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. J Bone Joint Surg Am. 2007;89:2105–12.
29. Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R, Roberts S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. Am J Sports Med. 2010;38:880–90.

30. Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)–development of a self-administered outcome measure. J Orthop Sports Phys Ther. 1998;28:88–96.
31. Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes. 2003;1:64.
32. Minas T, Gomoll AH, Rosenberger R, Royce RO, Bryant T. Increased failure rate of autologous chondrocyte implantation after previous treatment with marrow stimulation techniques. Am J Sports Med. 2009;37:902–8.
33. Luyten FP, Denti M, Filardo G, Kon E, Engebretsen L. Definition and classification of early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc. 2012;20:401–6.
34. Muckle DS. Open meniscectomy: enhanced recovery after synovial prostaglandin inhibition. J Bone Joint Surg Br. 1984;66:193–5.
35. Jiang CC, Liu YJ, Yip KM, Wu E. Physiological patellofemoral crepitus in knee joint disorders. Bull Hosp Jt Dis. 1993;53:22–6.
36. Gooding CR, Bartlett W, Bentley G, Skinner JA, Carrington R, Flanagan A. A prospective, randomised study comparing two techniques of autologous chondrocyte implantation for osteochondral defects in the knee: Periosteum covered versus type I/III collagen covered. Knee. 2006;13:203–10.
37. Van Assche D, Van Caspel D, Vanlauwe J, Bellemans J, Saris DB, Luyten FP, et al. Physical activity levels after characterized chondrocyte implantation versus microfracture in the knee and the relationship to objective functional outcome with 2-year follow-up. Am J Sports Med. 2009;37 Suppl 1:42S–9.
38. Hanzlik S, Mahabir RC, Baynosa RC, Khiabani KT. Levels of evidence in research published in The Journal of Bone and Joint Surgery (American Volume) over the last thirty years. J Bone Joint Surg Am. 2009;91:425–8.
39. Harris JD, Siston RA, Pan X, Flanigan DC. Autologous chondrocyte implantation: a systematic review. J Bone Joint Surg Am. 2010;92:2220–33.
40. Basad E, Ishaque B, Bachmann G, Sturz H, Steinmeyer J. Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study. Knee Surg Sports Traumatol Arthrosc. 2010;18:519–27.
41. Van Wilder P. Advanced therapy medicinal products and exemptions to the regulation 1394/2007: How confident can we be? An exploratory analysis. Front Pharmacol. 2012;3:12.
42. Roobrouck VD, Clavel C, Jacobs SA, Ulloa-Montoya F, Crippa S, Sohni A, et al. Differentiation potential of human postnatal mesenchymal stem cells, mesoangioblasts, and multipotent adult progenitor cells reflected in their transcriptome and partially influenced by the culture conditions. Stem Cells. 2011;29:871–82.
43. Roberts S, Hollander AP, Caterson B, Menage J, Richardson JB. Matrix turnover in human cartilage repair tissue in autologous chondrocyte implantation. Arthritis Rheum. 2001;44:2586–98.
44. Henderson I, Lavigne P, Valenzuela H, Oakes B. Autologous chondrocyte implantation: superior biologic properties of hyaline cartilage repairs. Clin Orthop Relat Res. 2007;455:253–61.

Part V
Other Aspects of Cartilage Repair

Chapter 12
Autologous Chondrocyte Implantation After Previous Treatment with Marrow Stimulation Techniques

Marco K. Demange, Tom Minas, and Andreas H. Gomoll

Abstract Full-thickness defects of articular cartilage have limited to no spontaneous repair potential and can compromise patients through symptoms such as activity-related pain and swelling. Various techniques have been developed toaddress these defects, including palliative procedures such as debridement and reparative procedures such as marrow stimulation techniques (MST). Marrow stimulation techniques result in changes to the subchondral bone, including osseous overgrowth and intralesional osteophytes. Defects that had prior treatment affecting the subchondral bone have a three to seven times higher failure rate after ACI procedure when compared with non-treated defects.

In this chapter we are going to discuss the role of previous bone marrow stimulation on subsequent cartilage repair and discuss possible surgical techniques to address the altered subchondral bone in order to restore the osteochondral functional unit.

Keywords Cartilage • Marrow stimulation • Autologous chondrocyte implantation

M.K. Demange
Hospital das Clínicas, University of São Paulo,
Rua Ouvidio Pires de Campos, 333,
São Paulo, SP, Brazil

Department of Orthopedic Surgery - Cartilage Repair Center,
Brigham and Women's Hospital, Harvard Medical School,
75 Francis Street, Boston, MA, USA

T. Minas • A.H. Gomoll (✉)
Department of Orthopedic Surgery - Cartilage Repair Center,
Brigham and Women's Hospital, Harvard Medical School,
75 Francis Street, Boston, MA, USA
e-mail: agomoll@yahoo.com

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_12, © Springer-Verlag London 2014

Key Points

- Bone marrow stimulating procedures as drilling or microfracture may affect subchondral bone cause intralesional osteophytes formation.
- Intraleasional osteophytes and alterations in the subchondral bone unit increases autologous chondrocyte implantation failure rate.
- Intralesional osteophytes should be addressed during ACI surgery. High speed burr is effective to remove subchondral bone thickening and intralesional osteophytes.
- Sandwich ACI technique may be performed in the presence of bone cysts or after subchondral bone removal due to sclerotic aspect.
- Better understanding of the osteochondral unit, the subchondral bone itself, and the interface and interaction between cartilageandsubchondral-bone may help us improve surgical procedures after failed marrow stimulation procedures.

12.1 Introduction

Full thickness defects of articular cartilage have limited to no spontaneous repair potential [1] and can compromise patients through symptoms such as activity-related pain and swelling. Cartilage repair should restore joint function, ideally with a near-normal and durable tissue regenerate. Marrow stimulation techniques such as drilling, abrasion arthroplasty, or microfracture are frequently considered first-line treatment options for symptomatic cartilage defects [2, 3]. These techniques attempt to affect filling of a chondral defect with reparative tissue resulting from stimulation of the subchondral bone at the bottom of the defect [4]. Blood and mesenchymal cells from the underlying marrow cavity form a clot in the defect that gradually differentiates into a fibrocartilaginous repair tissue [5]. These techniques have the low morbidity of an all-arthroscopic procedure, with a comparatively quick recovery and low complication rate. Better results are obtained in younger patients, with lesions size smaller than 2–4 cm^2, and without previous surgeries [6]. Durability of the repair tissue, and hence the clinical outcome, is lower in defects that are larger than 2–4 cm^2 and/or located in areas other than the femoral condyles [7, 8]. Autologous chondrocyte implantation (ACI) may be performed as a second-line treatment after failed bone marrow stimulation, as well as first-line treatment in larger lesions [9]. Over the long-term, primary ACI is believed to demonstrate better outcomes, as microfracture-treated patients frequently seem to have recurrence of symptoms 2–5 years after surgery [10]. The ratio of patients maintaining sports activities after 5 years is higher in ACI treated patients compared to microfracture [11, 12].

Whenever a marrow stimulation procedure is chosen as the primary treatment, it is important to evaluate whether the results of a potentially subsequent procedure are not negatively influenced; essentially whether it can truly be considered a "non-bridge-burning" procedure. Recent studies have demonstrated subchondral

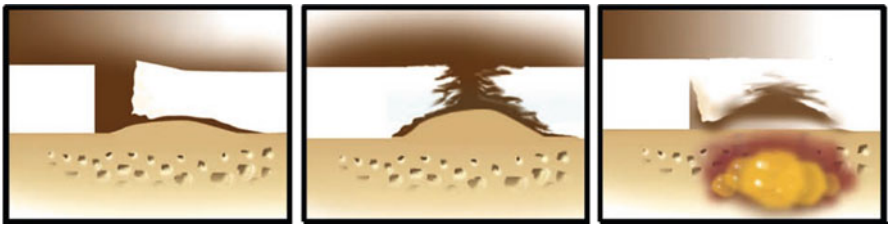

Fig. 12.1 Modes of failure after marrow stimulation. *Left* delamination, *center* intralesional osteophyte, *right* subchondral cyst

changes in up to half of patients treated with microfracture, such as thickening of the subchondral bone, osseous overgrowth and formation of subchondral cysts [8, 13, 14]. Therefore, the interaction of subchondral bone changes with ACI warrant further investigation [15]. This prompted us to review the results of all patients treated at our institution with ACI by the senior author to determine whether defects previously treated with marrow stimulation techniques failed at rates higher than defects that were treated previously with debridement alone.

12.2 Failure Rates of ACI Depending on Previous Treatment with MST Procedures

This cohort study utilizing prospectively collected data was conducted to assess potential differences in failure rates of ACI depending on previous treatment with MST procedures affecting the subchondral bone, such as drilling, abrasion chondroplasty and microfracture.

Hypothesis: Cartilage defects pre-treated with marrow stimulation technique demonstrate an increased failure rate (Fig. 12.1).

Methods: This study reviewed prospectively collected data for 332 patients treated by the senior author between March 1995 and December 2004. Indications for treatment of cartilage defects with ACI were full-thickness chondral defect(s) of the knee with consistent history, physical examination, imaging and arthroscopy; no inflammatory joint disease, no unresolved septic arthritis, no deficient soft tissue coverage, no metabolic or crystal disorders; no or correctable ligamentous instability, malalignment or meniscal deficiency; not more than 50 % loss of joint space on weight-bearing radiographs. All patients had completed more than 2 years of follow-up by the time of data analysis for this study. Eleven patients with potential confounders such as revision ACI, previous bone grafting or osteochondral allograft transplantation were excluded, leaving 321 patients (325 knees) for analysis.

Patients were assigned to one of two groups based on whether they had previously undergone MST for the treatment of cartilage defects or not.

Patients received *ex-vivo* cultured autologous chondrocytes (Genzyme Bio Surgery, Cambridge, MA, USA) injected underneath a periosteal patch, which had

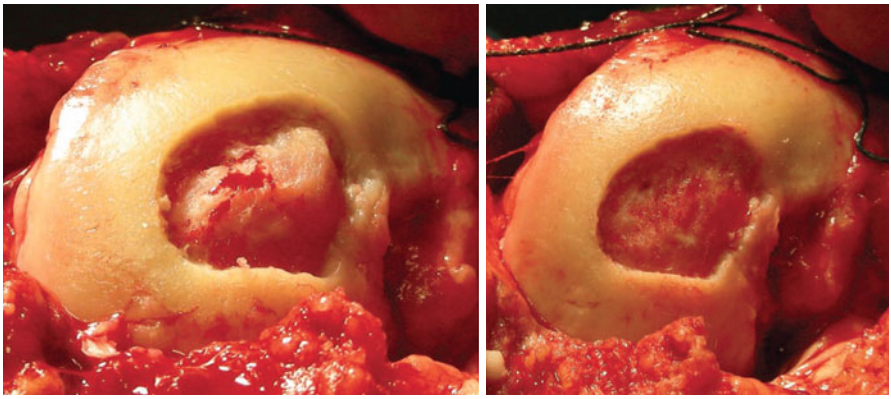

Fig. 12.2 Intralesional Osteophytebefore (*left*) and after debridement with a bur (*right*)

been secured with resorbable sutures and fibrin glue (Tissue Seal, Baxter Biosurgery, Deerfield, IL) sealant [16]. We routinely delayed ACI for 9–12 months after previous MST to allow the subchondral bone to reconstitute and the subchondral edema to resolve. Defect sizes were measured intra-operatively, and concomitant procedures were recorded. Patients with defects of the weightbearing femoral condyles in the setting of 2° or more of malalignment from the neutral mechanical axis were treated with a concurrent valgus- or varus-producing corrective osteotomy. Patients with patellofemoral defects had a concurrent anteromedializationtibial tubercle osteotomy, lateral release and vastus medialis oblique muscle advancement if there was evidence of patellar subluxation and tilt as noted by physical examination, radiographs, and/or CT scan assessment.

Intralesional osteophytes were commonly seen after previous MST; initially these were left untreated so as to not create bleeding and admixture of marrow elements with end-differentiated articular chondrocytes. However, when large intra-articular osteophytes presented themselves above the level of the adjacent articular cartilage these were impacted with a bone tamp flush with the adjacent subchondral bone, followed by a standard ACI. In both cases, failures at these sites were seen. The senior author then moved on to removing the osteophytes with a rongeur and noticed no or minimal bleeding easily controlled with epinephrine or fibrin glue. The technique for intralesional osteophytes finally evolved into its current form of microburring to remove the stiffened subchondral bone (Fig. 12.2).

Outcomes were classified as complete failure if more that 25 % of the grafted defect area had to be removed in later procedures due to persistent symptoms and MRI evidence of graft delamination, or surgical removal of more than 25 % of the graft area.

For statistical analysis, the cohort was sub-classified on the basis of size, type and location of the defect into Simple, Complex and Salvage categories. Simple defects were defined as single lesions smaller than 4 cm^2 located on the femoral condyles; the Complex category included both multifocal lesions, as well as single lesions that were either larger than 4 cm^2 or situated on the trochlea, tibia or patella; the Salvage category included all bipolar (kissing) lesions, as well as all defects located in knees with early arthritic changes including osteophyte formation or Ahlback Stage 0–1

Table 12.1 Patient Demographics for Control Group (No MST) and Previously Marrow-Stimulated Group (Prior MST)

	No MST	Prior MST	P value
No. of knees/no. of patients	214/211	111/110	
Average age (years)	35.0 (9.2, 13–60)	35.4 (10.1, 14–55)	0.7
Gender (male/female)	124/87	61/49	0.6
Average follow-up time (months)	54 (27, 24–132)	56 (30, 24–144)	0.4
Average no. of defects per knee	1.7 (0.9, 1–5)	1.7 (0.8, 1–4)	0.9
Average effect size (cm^2)	4.6 (2.7, 0.5–21)	5.2 (3.1, 07–16.8)	0.2
Average transplant area per knee (cm^2)	7.9 (5.0, 1.0–28.3)	8.6 (5.9, 1.5–30.5)	0.3
Worker's compensation patients	28 (13 %)	24 (22 %)	0.1
Patient lost to follow-up after 2 years			
Simple	3 (1 %)	2 (2 %)	>0.5
Complex	16 (8 %)	12 (11 %)	
Salvage	6 (3 %)	4 (4 %)	

Data are given as (*SD* range) or number (%)

changes (<50 % joint space narrowing). Further sub-analyses were performed based on whether the original defect was caused by osteochondritisdissecans (OCD), by type of MST procedure (microfracture, abrasion arthroplasty or drilling) and whether the patient received worker's compensation payments.

Data were collected independent of the surgeon by trained research staff using standardized case report forms or questionnaires, and statistical analysis was conducted by an independent statistician. Statistical analyses were performed using the SAS 8.2 (SAS Institute Inc, Raleigh, N.C.) software package. The Student's *t*-test was used to assess potential differences between the two groups (MST or control) in regards to demographic characteristics, such as average defect size, number and subject age. The chi-square test was utilized to detect differences between the two groups (MST or control), as well as between the three different MST procedures. The level of statistical significance was set at $P < 0.05$.

Results: The patient groups (control and MST) were not significantly different in regard to patient age at implantation ($p = 0.7$), gender ($p = 0.6$), follow-up time ($p = 0.4$), defect size ($p = 0.2$) and number of defects per joint ($p = 0.9$) (Table 12.1). Average follow-up was 55 months: 54 months (range, 24–132) in the control group and 56 months (range, 24–144) in the MST group. In the control group, there were 56 (26 %) varus/valgus producing osteotomies, 55 (26 %) tibial tubercle osteotomies (TTO), and 6 (3 %) ligament reconstructions. This compares with 23 (21 %) varus/valgus osteotomies, 30 (27 %) TTOs and 9 (8 %) ligament reconstructions in the MST group. Average transplant area per knee was 8.2 cm^2 overall: 7.9 cm^2 in the control group and 8.6 cm^2 in the MST group ($p = 0.3$). For non-worker's compensation patients (83 % of patients), the average transplant area per knee was 8.1 cm^2 in the control group and 8.5 cm^2 in the MST group ($p = 0.6$). For worker's compensation (17 % of overall patients), the areas were 6.4 and 8.2 cm^2, respectively ($p = 0.1$).

Approximately half of patients that had failed ACI after having undergone prior marrow stimulation were found to have additional, not pre-treated defects at the time of ACI. In further sub-analysis, the failure rate of these lesions was assessed

Table 12.2 Failure rates for Control (No MST) and Marrow-Stimulated (MST) Groups

	No MST	Prior MST	P Value
Overall	214 (17, 8 %)	111 (29, 26 %)	<0.001
Simple defects	18 (2, 11 %)	9 (1, 11 %)	N/A
Complex defects	97 (9, 9 %)	56 (17, 30 %)	<0.01
Salvage defects	99 (6, 6 %)	46 (11, 24 %)	<0.01
Sub analyses			
Osteochondritisdissecans lesions	23 (2, 9 %)	20 (6, 30 %)	N/A
Worker's comp.	28 (4, 14 %)	24 (9, 38 %)	N/A
Previous microfracture		25 (5, 20 %)	>0.5
Previous abrasion arthroplasty		33 (9, 27 %)	
Previous drilling		53 (15, 28 %)	

separately from the pre-treated defects, acting as an internal control located in the same knee as the latter.

Overall, joints in the control group failed at a rate of 8 % (17 of 214), compared with a failure rate of 26 % (29 of 111) in joints that had been pre-treated with MST (chi-square test, $p<0.001$).

With the exception of defects in the "Simple" category, sub-analysis of the data demonstrated a fairly constant ratio of approximately 3:1 in failure rate between the MST and control groups for "Complex" and "Salvage"-type defects, osteochondritisdissecans lesions and patients receiving worker's compensation (Table 12.2). There were no significant differences in failure rates between the three types of MST (chi-square, $p=0.5$), even though there was a trend towards a lower failure ratio in microfractured defects, which failed at only twice, rather than three times the rate of defects in the control group (Table 12.2).

Within the group of 29 knees that had failed ACI after prior treatment with MST, 14 were implanted for isolated defects and 15 for multiple defects. Among these 15 knees there were a total of 35 implanted defects, some of which had been marrow-stimulated and some of which had not: specifically, 17 had previously been marrow-stimulated (13 knees with 1 defect each and 2 knees with 2 defects each) and 18 lesions had not been treated prior to ACI. Since all knees had at least one marrow-stimulated defect and one untreated defect, we utilized the untreated defect as an internal control. Sixteen of the 17 marrow-stimulated defects failed compared with 2 of the 18 previously untreated lesions.

Conclusion: Defects that had undergone to prior treatment affecting the subchondral bone failed at a rate three times that of nontreated defects (Fig. 12.3).

12.3 Subchondral Bone Unit

The articular cartilage varies throughout its depth from articular surface to subchondral bone. The cartilage can be divided into four zones: superficial, transitional, deep, and calcified cartilage zones. The deepest layer, the zone of calcified cartilage,

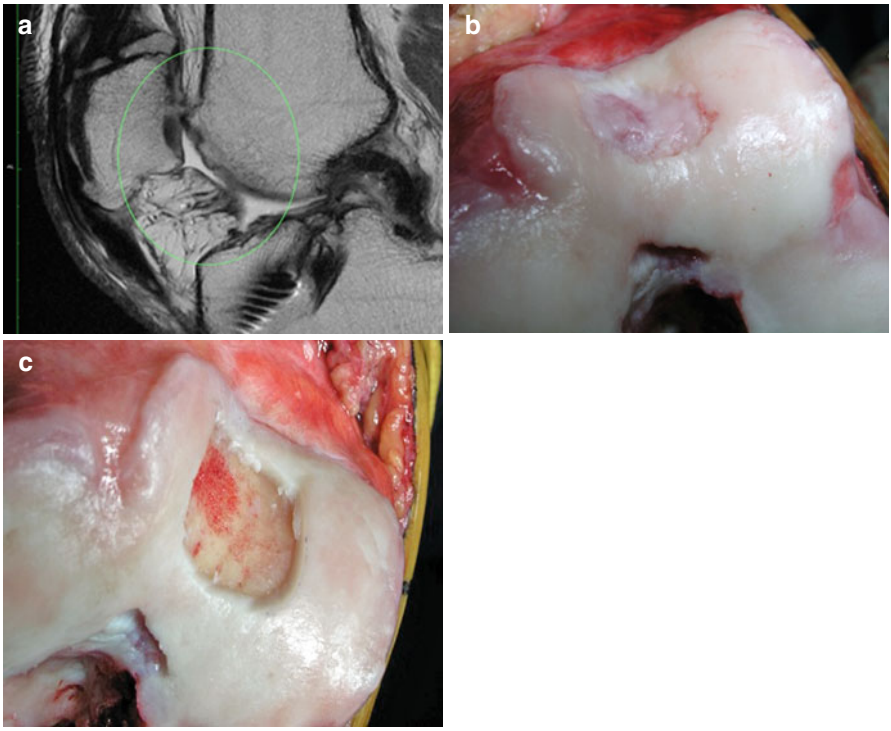

Fig. 12.3 (**a**) Preoperative magnetic resonance imaging (*upper left*), (**b**) intraoperative picture of intralesional osteophyte (*upper right*), (**c**) intraoperative picture of subchondral bone after intralesional osteophyte removal (*bottom*)

separates the hyaline cartilage form the subchondral bone, and it is characterized by small cells distributed in a cartilaginous matrix encrusted with apatitic salts. Histologically, the calcified cartilage zone may be distinguished from the deep zone by the tide-mark, which appears as a bluish line with hematoxylin/eosine staining. Lamellar bone is found throughout the mature skeletal in both trabecular and cortical bone, regardless of whether the bone was formed by intramembranous or endochondral ossification. Bone is a very dynamic and well-organized tissue, and trauma to cortical, trabecular or subchondral bone may activate healing process [17]. One theory suggests microfractures in subchondral bone or calcified cartilage are the potential trigger that provokes reactivation of the secondary center of ossification, with thickening of the subchondral plate and calcified cartilage, and causing the tidemark to advance with corresponding thinning of the overlying cartilage [18]. The activation of secondary centers of ossification in the subchondral plate is considered by some as the initiating event in osteoarthritis [19].

Recently, there has been an increasing interest and awareness of the importance of the subchondral bone and its role in the pathophysiology of osteoarthritis and chondral lesions. Furthermore, studies have demonstrated the necessity to carefully

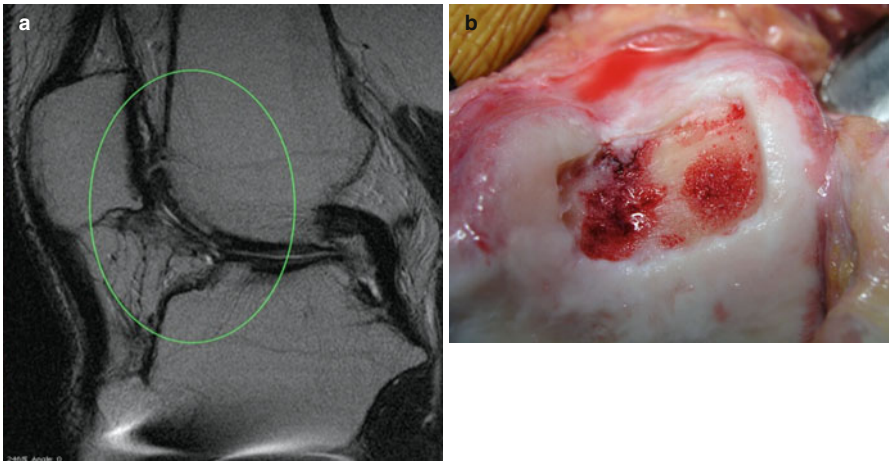

Fig. 12.4 (**a**) MRI scan of an intralesional osteophyte of the trochlea (*left*) and (**b**) intraoperative picture after debridement (*right*). Note the abnormal appearance of the subchondral bone and the holes from the previous microfractures

consider this structure in the treatment of articular surface damage, in the evaluation of the results over time, and in the determination of the patients' prognosis [20].

As our understanding of the underlying pathophysiological changes grow, we realize that cartilage lesions have to be evaluated as an osteochondral unit rather than a disorder limited to the articular cartilage. It is becoming apparent that without support from an intact subchondral bed, any treatment of the surface chondral lesion is likely to fail [20]. Subchondral bone may be affected primarily or secondarily in many diseases of the articular cartilage. Osteochondritis dissecans and spontaneous osteonecrosis of the knee both start in the subchondral bone and progressively affect the articular cartilage. Traumatic osteochondral fractures resulting from impacting may concomitantly affect both, articular cartilage and subchondral bone. Furthermore, several studies have demonstrated a 27–33 % incidence of thickening of the subchondral plate and intralesional osteophytes after microfractures [6, 8, 13]. Animal studies also have demonstrated a high incidence of subchondral bone cysts after microfracture procedures [21]. Finite element analyses suggest that subchondral stiffening and stress concentration causes an elevation in shear stresses in the deep cartilage layers [22, 23]. This thinner layer of viscoelastic cartilage overlies a thickened and stiffened subchondral plate and is therefore more susceptible to damage from shear forces.

In imaging evaluation of the subchondral bone, injury and OA-related changes in bone marrow are manifested by an increase in the signal intensity in bone marrow on fat-saturated T2-weighted images (bone marrow edema, BME). These hyperintense MR imaging abnormalities may be an expression of a number of noncharacteristic histological abnormalities that include bone marrow necrosis, bone marrow fibrosis and trabecular abnormalities [24, 25]. Bone marrow edema has been associated with severity and progression of OA. Evaluationof the subchondral bone after a previous microfracture procedure can be performed with MRI and

should include evaluation of the signal intensity, the appearance of the subchondral-lamina, the presence of intralesional osteophytes, granulation tissue, sclerosis, and cystic formations (Fig. 12.4) [26–28].

Better understanding of technical details to minimize the subchondral bone unit dysfunction after bone marrow stimulation should be pursued. To perform a microfracture technique, all unstable cartilage must be removed, stable perpendicular walls should be obtained at the edges of the lesion in order to contain the blood clot and allow proper edge healing. Currently, complete removal of all calcified cartilage is advised to obtain better filling with repair tissue [29]. Animal studies demonstrated that failure to completely remove the calcified cartilage layer leads to poor healing of the defect. However, Frisbie et al. observed significantly more new bone formation in defects in which the calcified cartilage had been removed completely at the time of surgery (26.5 % against 3.7 %). Subchondral bone cyst prevalence after microfracture was not affected by whether the calcified zone was removed or not [21].

12.4 Surgical Techniques for Autologous Chondrocyte Implantation After Bone Marrow Stimulation Procedures

Initially, a careful clinical history should be obtained, specifically focusing on previous knee surgery. The patient should be asked about any pain-free periods after the previous microfracture procedure to evaluate if they ever experienced pain relief or not. After microfracture, 60–80 % of patients have at least temporary symptomatic improvement, but some are worse even right after surgery.

Arthroscopic pictures of previous procedures help to evaluate the extent of the defect. Radiographic views should include weight-bearing anterior-posterior, 40° flexion weight-bearing posterior-anterior (Rosenberg view), lateral, and axial views. Long-leg weight-bearing views are important for alignment evaluation.

Any surgical intervention should include correction of all articular co-morbidities, such as malalignment, patellofemoralmaltracking, or meniscal and ligament insufficiency. As ACI is a two-stage procedure that requires an arthroscopic cartilage biopsy, we thoroughly evaluate all aspects of the knee during this stage.

During the implantation and after cartilage lesion debridement, the subchondral bone should be assessed for intralesional osteophytes and sclerosis of the subchondral plate. We found the use of a 5-mm bur under continuous irrigation helpful to gently take down any sclerotic cortical bone to the level of native subchondral plate, being mindful not to break into the subchondral bone itself. Bone bleeding may occur and should be addressed with fibrin glue, thrombin, or cauterization if there are distinct vessels. Standard collagen membrane or periosteal suturing is performed afterwards.

In the presence of bone cysts or when the subchondral bone is severely compromised, we elect to perform a sandwich technique. All sclerotic cortical bone and bone cysts are removed down to a healthy bed of subchondral bone, and the resulting defect is filled withautologous bone graft. When a closing wedge high

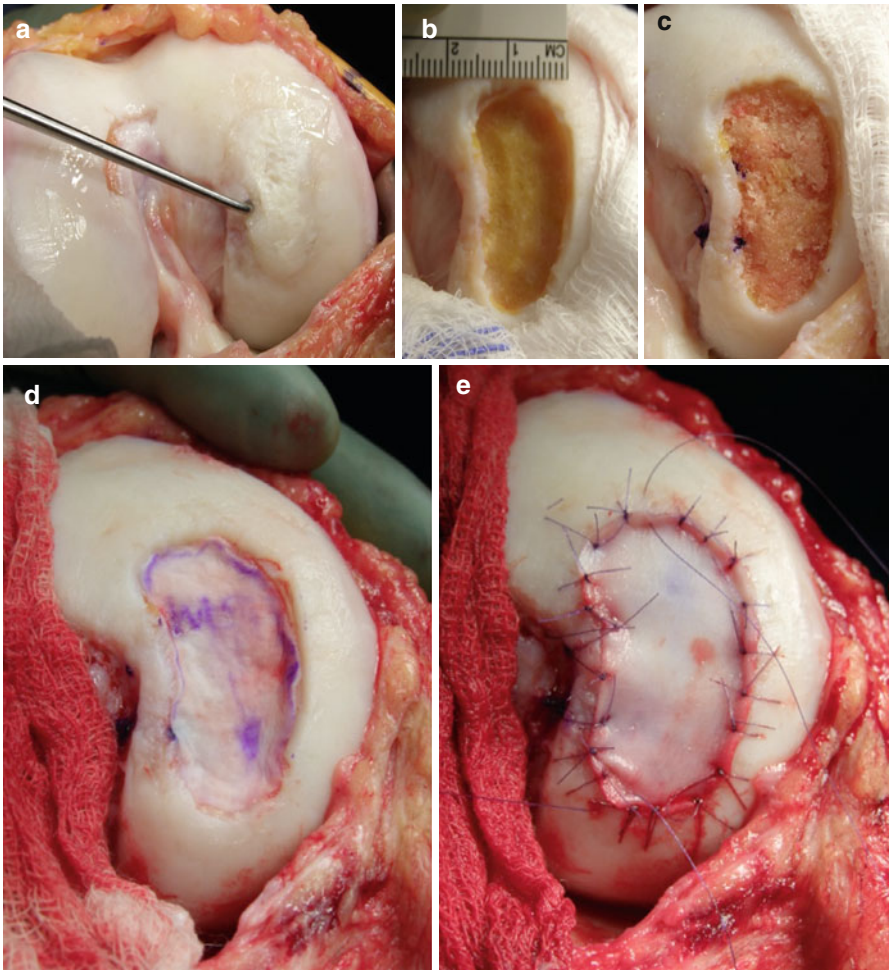

Fig. 12.5 Sandwich technique. Intralesional osteophyte (**a**), after complete debridment (**b**), bone grafting (**c**), membrane covering bone graft (**d**) and final appearance after ACI (**e**)

tibial osteotomy is performed concurrently, we utilize bone from the osteotomy site. Alternatively, bone can be obtained from the medial or lateral femoral or tibialcondyles. A small cortical window of approximately 1 by 1 cm is created with an osteotomeandremoved. Any cancellous bone attached to the cortex can be harvested for graft material. A curette can now be used to harvest as much graft as needed to fill the defect. Alternatively, it has been helpful to utilize a 10-mm harvesting tube from any of the available osteochondralautograft transfer systems by aiming in different directions; at least 3–4 cores of cancellous bone can be obtained. The harvest site can then be filled with allograft chips or putty and the cortical window is replaced. The graft material is now placed into the defect and compacted with a bone tamp. A layer of fibrin glue is placed on top of the bone graft, which is then covered by a size collagen or periosteal membrane. The graft is then compressed with digital

pressure and the tourniquet is released, waiting for the resulting blood clot to solidify and stabilize the graft. Conventional ACI technique is the used from here on. We found second generation ACI techniques simplify the procedure with marked advantages from a biological and surgical point of view (Fig. 12.5) [30, 31].

We are currently reviewing our data on patients with intralesional osteophytes where burring was performed during ACI surgery. We currently reviewed 85 patients that had an osteophyte formation that was removed with high-speed bur or curette prior to ACI. Magnetic resonance imaging at a minimum of 2 years was obtained in 46 patients. Intralesional osteophyte regrowth was observed in ten patients (22 %).

12.5 Conclusion

In cartilage repair, it can be theorized that the altered subchondral plate is responsible for the worse outcomes both in chronic defects, as well as in cartilage defects previously treated with marrow-stimulation techniques [20].

Better understanding of the osteochondral unit, the subchondral bone itself, and the interface and interaction between cartilage and subchondral bone may help us improve surgical procedures after failed marrow stimulation procedures.

Furthermore, future work is also needed to learn how to minimize disruption of the subchondral bone during microfracture, evaluate the subchondral bone before ACI, and treat the subchondral bone unit when necessary during ACI surgery.

References

1. Buckwalter JA. Articular cartilage: injuries and potential for healing. J Orthop Sports Phys Ther. 1998;28:192–202.
2. Sterett WI, Steadman JR, Huang MJ, Matheny LM, Briggs KK. Chondral resurfacing and high tibial osteotomy in the varus knee: survivorship analysis. Am J Sports Med. 2010;38:1420–4.
3. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy. 2003;19:477–84.
4. Frisbie DD, Oxford JT, Southwood L, et al. Early events in cartilage repair after subchondral bone microfracture. Clin Orthop Relat Res. 2003;(407):215–27.
5. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res. 2001;(391 Suppl):S362-9.
6. Mithoefer K, McAdams T, Williams RJ, Kreuz PC, Mandelbaum BR. Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: an evidence-based systematic analysis. Am J Sports Med. 2009;37:2053–63.
7. Gudas R, Kalesinskas RJ, Kimtys V, et al. A prospective randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint in young athletes. Arthroscopy. 2005;21:1066–75.
8. Kreuz PC, Steinwachs MR, Erggelet C, et al. Results after microfracture of full-thickness chondral defects in different compartments in the knee. Osteoarthritis Cartilage. 2006;14:1119–25.

9. Pestka JM, Bode G, Salzmann G, Sudkamp NP, Niemeyer P. Clinical outcome of autologous chondrocyte implantation for failed microfracture treatment of full-thickness cartilage defects of the knee joint. Am J Sports Med. 2012;40:325–31.
10. Vanlauwe J, Saris DB, Victor J, Almqvist KF, Bellemans J, Luyten FP. Five-year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. Am J Sports Med. 2011;39:2566–74.
11. Kon E, Filardo G, Berruto M, et al. Articular cartilage treatment in high-level male soccer players: a prospective comparative study of arthroscopic second-generation autologous chondrocyte implantation versus microfracture. Am J Sports Med. 2011;39:2549–57.
12. Mithoefer K, Hambly K, Della Villa S, Silvers H, Mandelbaum BR. Return to sports participation after articular cartilage repair in the knee: scientific evidence. Am J Sports Med. 2009;37 Suppl 1:167S–76.
13. Mithoefer K, Williams 3rd RJ, Warren RF, et al. The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. J Bone Joint Surg Am. 2005;87:1911–20.
14. Saris DB, Vanlauwe J, Victor J, et al. Treatment of symptomatic cartilage defects of the knee: characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. Am J Sports Med. 2009;37 Suppl 1:10S–9.
15. Kesemenli CC, Memisoglu K, Muezzinoglu US. Bone marrow edema seen in MRI of osteoarthritic knees is a microfracture. Med Hypotheses. 2009;72:754–5.
16. Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. J Bone Joint Surg Am. 2003;85-A Suppl 2:17–24.
17. Miller JD, McCreadie BR, Hankenson KD, Goldstein S. Bone: form and function. In: Einhorn TA, O'Keefe RJ, Buckwalter JA, editors. Orthopaedic basic science foundations of clinical practice. 3rd ed. Doody Enterprises;2007.
18. Goldring MB, Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. Ann N Y Acad Sci. 2010;1192:230–7.
19. Burr DB, Radin EL. Microfractures and microcracks in subchondral bone: are they relevant to osteoarthrosis? Rheum Dis Clin North Am. 2003;29:675–85.
20. Gomoll AH, Madry H, Knutsen G, et al. The subchondral bone in articular cartilage repair: current problems in the surgical management. Knee Surg Sports Traumatol Arthrosc. 2010;18:434–47.
21. Frisbie DD, Morisset S, Ho CP, Rodkey WG, Steadman JR, McIlwraith CW. Effects of calcified cartilage on healing of chondral defects treated with microfracture in horses. Am J Sports Med. 2006;34:1824–31.
22. Wei HW, Sun SS, Jao SH, Yeh CR, Cheng CK. The influence of mechanical properties of subchondral plate, femoral head and neck on dynamic stress distribution of the articular cartilage. Med Eng Phys. 2005;27:295–304.
23. Brown TD, Radin EL, Martin RB, Burr DB. Finite element studies of some juxtarticular stress changes due to localized subchondral stiffening. J Biomech. 1984;17:11–24.
24. Schneider E, Lo GH, Sloane G, et al. Magnetic resonance imaging evaluation of weight-bearing subchondral trabecular bone in the knee. Skeletal Radiol. 2011;40:95–103.
25. Roemer FW, Frobell R, Hunter DJ, et al. MRI-detected subchondral bone marrow signal alterations of the knee joint: terminology, imaging appearance, relevance and radiological differential diagnosis. Osteoarthritis Cartilage. 2009;17:1115–31.
26. Welsch GH, Zak L, Mamisch TC, Resinger C, Marlovits S, Trattnig S. Three-dimensional magnetic resonance observation of cartilage repair tissue (MOCART) score assessed with an isotropic three-dimensional true fast imaging with steady-state precession sequence at 3.0 Tesla. Invest Radiol. 2009;44:603–12.
27. Hayter C, Potter H. Magnetic resonance imaging of cartilage repair techniques. J Knee Surg. 2011;24:225–40.

28. Potter HG, le Chong R, Sneag DB. Magnetic resonance imaging of cartilage repair. Sports Med Arthrosc. 2008;16:236–45.
29. Steadman JR, Rodkey WG, Briggs KK. Microfracture to treat full-thickness chondral defects: surgical technique, rehabilitation, and outcomes. J Knee Surg. 2002;15:170–6.
30. Kon E, Delcogliano M, Filardo G, Montaperto C, Marcacci M. Second generation issues in cartilage repair. Sports Med Arthrosc. 2008;16:221–9.
31. Gomoll AH, Probst C, Sodha S, Minas T. Collagen membrane as cover for autologous chondrocyte implantation. Letter to the editor. Am J Sports Med. 2010;38:NP4.

Chapter 13
Arthroscopic Approaches for Cartilage Repair in the Knee Joint

Enrique Villalobos Jr., Anell Olivos-Meza, Aldo Izaguirre, and Clemente Ibarra

Abstract Articular cartilage injury is a common disorder of the knee and untreated chondral lesions are thus likely to predispose patients to develop osteoarthritis. In the USA more than 500,000 procedures are performed for cartilage-related injuries. Chondral lesions are found in more than 60 % of arthroscopic knee surgeries, according to several authors [1].

Most of the times the diagnosis of the cartilage lesions is performed by the orthopedic surgeon at the time of the arthroscopic evaluation and, in many cases, this is the only opportunity to attempt repairing the damaged cartilage ("the golden moment").

Cartilage repair has developed rather fast in the past 20 years, and so have arthroscopic repair techniques. Despite substantial differences in the complexity and technical application of each method, all are united in the endeavor to restore joint function and prevent joint degeneration.

Surgical techniques to treat cartilage lesions can be grouped in three basic categories: palliative (debridement), restorative (microfracture and retrodrilling) and reparative (mosaicoplasty & MACI).

Debridement smooth fibrillated cartilage, providing relief that may last several years. Microfracture and Retrodrilling techniques stimulate the release of marrow derived cells and growth factors that contribute to fibrocartilage formation with only limited durability. Transplantation techniques as mosaicplasty can result in more durable hyaline cartilage and better integrated weight bearing tissue

E. Villalobos Jr., MD (✉) • A. Olivos-Meza, MD • A. Izaguirre, MD
Orthopedic Sports Medicine and Arthroscopy Division,
National Institute of Rehabilitation, Mexico City 14389, Mexico
e-mail: villalobosenrique@hotmail.com

C. Ibarra, MD
Orthopedic Sports Medicine and Arthroscopy Division and Tissue Engineering Cell Therapy and Regenerative Medicine Unit, National Institute of Rehabilitation, Mexico City, Mexico

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_13, © Springer-Verlag London 2014

improving the joint biomechanical loads. Matrix Autologous Chondrocyte Implantation (MACI) uses biomaterials seeded with chondrocytes as carriers and scaffolds for cell growth that improve arthroscopy delivery providing satisfactory outcomes up to 5 years.

One of the most important issues of cartilage lesions is to perform an appropriate arthroscopic diagnosis of the whole articular surface of the knee, and to enhance the availability of minimally-invasive arthroscopic surgical techniques for the first surgical stage, or as the first or second step of a two-stage arthroscopic chondral repair technique.

This chapter assesses current arthroscopic techniques for chondral repair enhancing the most appropriate indications, advantages and disadvantages of every procedure.

Keywords Cartilage repair • Arthroscopic techniques • Knee joint

Key Points

1. Thorough history and physical examination is mandatory to predict success of cartilage repair.
2. Cartilage lesions and associated injuries should be taken into account completely. Depth and extension of cartilage lesions should be measured with a probe in sagittal and coronal planes.
3. Adequate debridement should be undertaken, and that in grade III-IV lesions special attention to stable borders of healthy cartilage remain.
4. Microfracture techniques can be performed with accessory portals and different angles of awls, and probably scaffold-enhanced techniques will yield better results.
5. Arthroscopic osteochondral transfer is recommended in lesions up to 2 cm^2 and maximum 4–6 plugs.
6. Emergent new cell based approaches with different scaffolds, fixation techniques are in intense research and application and show promising results.

13.1 Introduction

In the present chapter we describe the different arthroscopic approaches that have been emerging as surgical treatment options for cartilage repair. We consider that it is important for the surgeon to know the state of the art of these techniques because sometimes it is not until the arthroscopic procedure that the surgeon becomes aware of the presence of the cartilage lesion [1]. For this reason, it would be a great opportunity to treat it with different arthroscopic techniques as a first line of

treatment, avoiding progression of the damage. If the lesion is treated at that moment, or a cartilage biopsy is obtained for a future second arthroscopic procedure, less pain, morbidity, and probably short-term recovery could be achieved.

We have considered this first diagnostic scope view as the "golden moment" for the repair of a cartilage lesion. Since many procedures to restore articular cartilage are performed arthroscopically, if the surgeon has the knowledge, skills and adequate equipment in the OR, he will be able not only to identify but also to address the cartilage lesions at this unique golden moment, in an appropriate way and contribute to the integral management of the patient with an injured joint.

13.2 Arthroscopic Maneuvers for the Thorough Evaluation of Chondral Lesions

To be able to suspect a chondral lesion in the knee joint, it is important to know the patient clinical history. It is important to identify traumatic events or repetitive micro-traumas, as well as the presence or absence of pain, and the activities that increase or relieve it. During the physical exam we should identify the painful site in the knee, the major symptom of a chondral lesion, asking the patient to point the localization of pain as precisely as possible, and then by means of palpation. Physical examination should also address ligament testing, meniscus testing, patella misalignment, and leg alignment verification. This evaluation should be complemented by weight bearing X-rays to suspect the areas of greater load bearing or hyper pressure, as well as by special views like the axial patellar X-rays. An evaluation with magnetic resonance imaging (MRI) is of great importance in all patients with suspected intra-articular knee lesion. We should observe not only the most evident lesions, such as anterior cruciate ligament or meniscal tears, but also probable chondral lesions, whether partial or total thickness lesions that, in the most severe cases, could be related to areas of subchondral edema. We should start the arthroscopic evaluation with all of this background information. The patients' background will allow us to perform an arthroscopic evaluation of the knee joint being able to find evident and not-so-evident lesions.

We recommend performing three routine portals during the arthroscopic evaluation: superolateral, anterolateral, and anteromedial. Besides the routine arthroscopic evaluation, which includes the three knee compartments, we suggest performing a careful and slow evaluation of patellofemoral tracking, from 0 to 90°, visualizing through the anterolateral portal. This helps us determine whether if there are patellar hyper pressure areas and if they are related to chondral lesions. Lateral patellar hyper pressure, together with a mirror or kissing chondral lesion of the lateral patellofemoral joint, is a common scenario; however, patellar chondral lesions also occur in the medial facet in cases of traumatic patellar dislocation. At the level of the medial and lateral load-bearing compartments, we suggest

assessing them not only with valgus or varus maneuvers, corresponding to the medial or lateral compartments, respectively, but also dynamically performing a screening of the entire articular surface of both condyles, from anterior all the way to the most posterior condylar region. This is achieved by doing simultaneously and slowly a valgus maneuver with maximum flexion-extension, with the arthroscope visualizing the entire articular surface of the medial knee condyle; and a varus maneuver in a 4-shaped position, with slow flexion and extension, and the arthroscope visualizing the entire load-bearing area of the lateral femoral condyle. This dynamic screening evaluation of the articular surface of the knee in the load-bearing area of both compartments allows identifying hidden lesions in the most posterior aspect of either condyles or anterior lesions along the limits of the load-bearing area and the patellofemoral joint.

After identifying the chondral lesion under arthroscopic visualization, the former should be palpated with a probing hook to identify the areas where the lesion reaches the subchondral bone, areas of softened chondral tissue, and unstable chondral flaps. Through palpation the presence of relatively stable healthy cartilage around the identified lesion should be delimited. ICRS grade 3–4 lesions are eligible for chondral repair techniques and thus their debridement is indicated with arthroscopic spoons and curettes to achieve stable lesion borders. Then the lesion should be documented with pictures and a gauge probe that allows estimating the approximate area of the lesion to be treated, measuring it from anterior to posterior and from medial to lateral. Once the lesion has been identified, properly documented and measured (according to ICRS guidelines), the well-known management algorithm may be applied to determine the appropriate choices of chondral repair techniques to treat the total thickness chondral lesion, based on its location, containment, and size [2].

13.3 Arthroscopic Techniques Described for Cartilage Repair

13.3.1 Debridement

It allows removing unstable cartilage fragments or flaps leaving behind stable lesions that can be documented and measured, and it is thus an option for ICRS grade 2 or 3 chondral lesions [3, 4]. In the case of ICRS grade 3–4 chondral lesions, it is a first step towards true chondral repair techniques, like those mentioned below. When performing this technique it is recommended to use the tip of the shaver, placed parallel to the chondral lesion, with the tip opening partially pointing towards the lesion, and use a forward or backward movement with activated suction, instead of an oscillating movement. This will allow properly removing the unstable cartilage fragments, causing the least possible damage to the stable cartilage that is still under the treated area.

13.3.2 Microfracture

Described by Steadman et al. [5], this technique consists of making perforations in the subchondral bone to cause bleeding from the bone marrow that enables a repair consisting of fibrocartilage. This technique is done 100 % arthroscopically, with microfracture awls of different angles, from 30 to 90°, which allow perpendicularly approaching the exposed bone in the ICRS grade 4 chondral lesion. With the appropriate instruments one may access virtually any location in the articular surface that warrants treatment, including posterior chondral lesions in the femoral condyles. The treatment of patellar chondral lesions is also possible with a 90° microfracture awl introduced exerting pressure with the hand instead of using a mallet. We suggest that, to approach these lesions, it may be necessary to introduce the microfracture awl through the superolateral portal, or through the accessory portals at the medial or lateral parapatellar level, allowing a direct and perpendicular approach to the patellar lesions to be treated.

13.3.3 Retrodrilling of the Patella

In case of patellar chondral lesions that cannot be treated with the traditional microfracture technique, either appropriately or perpendicular to the lesion, we suggest to use a subchondral bone perforation technique in a retrograde fashion, what we have called "patellar retrodrilling", which allows perforating the damaged articular surface from outside-in. For this purpose, we suggest using an inverted ACL tibial tunnel guide (DePuyMitek, Inc., Raynham, MA) to locate the outlet where we want to make the perforation, exactly at the level of the lesion, and go in from outside-in, through the upper patellar surface with a 2.0 mm K-wire. The latter should be carefully introduced, under arthroscopic vision, with an extended knee, until it can be seen in the joint. This step may be repeated as often as necessary, depending on the size of the lesion, leaving a 4–5 mm interval between the perforations (Fig. 13.1).

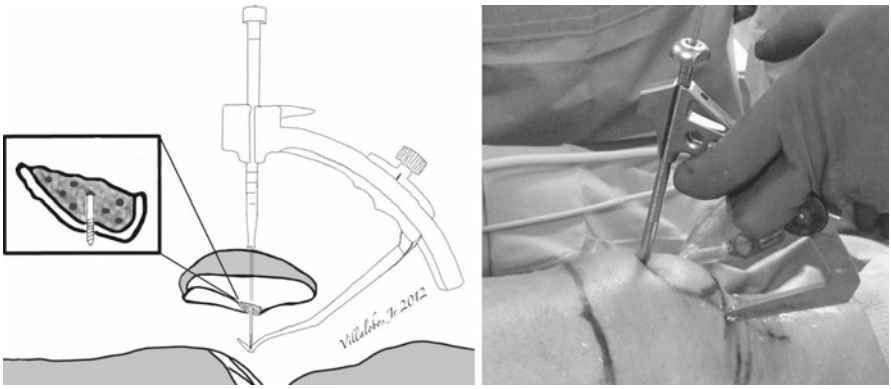

Fig. 13.1 Patellar retrodrilling technique

One should be careful not to perforate the opposite articular surface at the femoral trochlea with the K-wire.

13.3.4 Microfracture with Matrix-based Techniques

Zantop et al. [6] suggested that the use of a matrix to cover a microfractured area for cartilage repair might offer several potential benefits for the marrow-stimulated regeneration. They reported a surgical technique for arthroscopic matrix-covered microfracturing using a tridimensional matrix (Chondrotissue; BioTissue Tech, Freiburg, Germany). They begin by using the microfracture technique and then they cover the chondral defect with the polymer (Chondrotissue; BioTissue Tech), which was previously immersed in 3 ml of autologous serum for 10 min and cut with a scalpel to the size of the defect. It is introduced into the joint through a cannula (Karl Storz) and placed into the defect with an arthroscopic grasp instrument (Karl Storz). Then, through an additional anteromedial portal at an angle perpendicular to the surface of the matrix, a bioresorbable pin (Smart Nail; ConMedLinvatec, Largo, FL) insertion technique is performed to place up to two pins, one anterior and another one posterior, on the polymer, to achieve proper stability [6].

13.3.5 Autologous Matrix-induced Chondrogenesis (AMIC)

Piontek et al. [7] reported the use of autologous matrix-induced chondrogenesis (AMIC) performed as an all-arthroscopic procedure. This technique combines the microfracture technique with matrix-based techniques using a collagen membrane to serve as a scaffold. It consists of the debridement of chondral lesions, measured with a circular sharp punch of the appropriate diameter depending on the lesion. Punches of appropriate sizes may be used according to the lesion size, and then as many circles as necessary to cover the lesion are cut, all of them of the same size, over the chondro-guide collagen membrane (Geistlich Pharma AG, Wolhusen, Switzerland). Later, numerous bores are drilled at 5-mm intervals in the subchondral layer with a 1.1 mm K-wire. Next, Chondro-Guide membrane circles are placed in the cartilage lesion with Pean clamps. If it is a big lesion that needs more than one patch, they can overlap. All used membranes are covered with a tissue glue layer (Tissucol, Baxter, Warsaw, Poland) [7].

13.3.6 Autologous Osteochondral Transplantation

Hangody et al. [8] described the mosaicplasty technique as a solution to bring autologous hyaline cartilage to the chondral lesion sites. Hangody states that mosaicplasty

may be performed either arthroscopically or openly, with a "miniarthrotomy", and that the choice between both depends on the type, size and exact location of the defect, as determined during arthroscopy. Hangody suggests that if the lesion is less than 2 cm in diameter, and no more than four to six grafts are required, the procedure can be done arthroscopically [8]. A key point is that osteochondral plugs should go in totally perpendicular to the lesion. We therefore suggest performing as many portals as necessary in the arthroscopic approach to meet this requirement, using an 18G spinal needle that allows us to plan and visualize the exact site where a new portal is needed. With this technique it is possible to treat lesions located in both femoral condyles, at the central and anterior level, as well as in the medial and lateral trochlea. On the other hand, we should know that the arthroscopic approach with this technique does not allow achieving an appropriate perpendicular access to posterior lesions in both condyles, lesions on the tibial surface, central trochlear lesions, and all patellar lesions, all of which warrant an open approach. Hangody suggests that this procedure involves a learning curve that begins first with open surgery, so that it can later be performed arthroscopically, given that the latter approach increases technical complexity [9].

Marcacci, Kon et al. [10] reported 76.7 % of good to excellent results with a 7-year follow-up in patients treated with arthroscopic autologous osteochondral grafting for cartilage defects of the knee. They used this technique to treat ICRS grade 3-4 articular cartilage lesions less than 2-5 cm^2 in size, located in the weight bearing surface of medial or lateral femoral condyles, with an all-arthroscopic technique. Their technical recommendations include considering that the thickness of the donor's cartilage may be different from the one in the recipient, and thus it is mandatory to achieve a perfect congruence between the grafts and the surrounding articular cartilage surface [10]. For this technique, to harvest arthroscopically donor grafts from the superior and lateral aspects of the intercondylar notch is suggested [11].

13.3.7 Matrix Autologous Chondrocyte Implantation

One of the disadvantages of patients submitted to autologous chondrocyte implantation is the need for a second open surgical procedure. Marcacci, Kon et al. [12] were the first to describe an arthroscopic surgical technique for tissue engineered cartilage grafting. With this technique they reduced the morbidity of classic autologous implantations and avoided open surgery and the use of a periosteal flap, thus reducing also the time and cost of surgery. The technique consists of preparing the chondral defect using an arthroscopic approach, and assessing the lesion size using a delivery device of variable diameter with a sharp edge. A cannula is then inserted and a specifically designed cannulated low profile drill is introduced; it is maintained in the selected position by a Kirschner guide wire (0.9 mm diameter) fixed in the bone. This drill allows creating the predetermined circular area with regular margins for the graft. The inflow is then closed, and the delivery system is beat on

the hyaluronic acid patch containing the autologous chondrocyte culture. The stamp obtained is placed in the chondral defect through the cannula. The procedure is repeated until the entire defect is entirely filled. Finally, implant stability is checked with water flow, and with passive mobilization of the knee under arthroscopic vision [12]. The characteristics and consistency of this hyaluronic acid scaffold (Hyalograft C) make it unnecessary to use any accessory stabilization method. This technique described by Marcacci et al. has been used in several reports to treat chondral lesions in the femoral condyles and trochlea [13–18].

Ergellet et al. [19], designed a new technique for autologous chondrocyte implantation on a resorbable polymer performed by arthroscopy. He described that for a secure fixation the graft have to be armed on the corners with resorbable threads forming loops secured by three-fold knots that tightened pulley slings and served as anchors. On every corner of the defect, a k-wire should be drilled transosseously with an inside-out technique. The pulley slings should be pulled through the femoral bone by the guide wire and the knots guided into the femoral bone, securely anchoring the graft [19].

Abelow, Guillén et al. [20], described an arthroscopic technique of matrix/membrane-induced autologous chondrocyte implantation (MACI) for the treatment of chondral lesions in load-bearing areas of both condyles. To perform this technique it is necessary to use a specially designed arthroscopic cannula. The chondral defect is thoroughly debrided and, using a specially designed caliper and flexible ruler, the size of the lesion is calculated. A template is placed on the defect to test its size. The membrane is then cut to size to match the template. Using a dry scope, two small mini anchors are used with 5-0 Dexon sutures placed at the opposite sides of the periphery of the cartilage lesion. The sutures of the anchors are passed through the MACI membrane and guided down the suture to the cartilage defect. Fibrin glue (Tissucol, Baxter, Spain) is then placed under the membrane. The remnants of the polymer already placed on the defect are remodeled and the sutures are tied over the MACI graft using an arthroscopic knot tying technique. For more accessible lesions, Abelow et al. suggest that the scaffold may be kept in place just with fibrin glue and bioabsorbable pins [20]. Ronga et al. reported the use of arthroscopic autologous chondrocyte implantation for treatment of a chondral defect in the tibial plateau of the knee, in which the site of the lesion could not be reached with an open procedure without sacrificing tendinous or ligamentous structures of the knee [21].

Ibarra, Villalobos et al. presented the clinical results of a pilot study in ten patients with an original all-arthroscopic technique for the implantation of matrix-encapsulated autologous chondrocytes [22] and showed the safety and efficacy of the technique [23]. This technique consists of two surgical stages. The first stage consists of arthroscopically taking a biopsy for culture purposes; the second one is also performed arthroscopically. Initially the chondral lesion is debrided with arthroscopic spoons and curettes trying to form circular lesions 8 mm in diameter, with healthy cartilage borders and without penetrating into the subchondral bone, using the 8 mm COR set (DePuyMitek, Inc., Raynham, MA) (Fig. 13.2, part 1). Then with an 8 mm impactor (COR system, DePuyMitek, Inc., Raynham, MA), the subchondral bone is gently impacted to improve its consistency. Then a

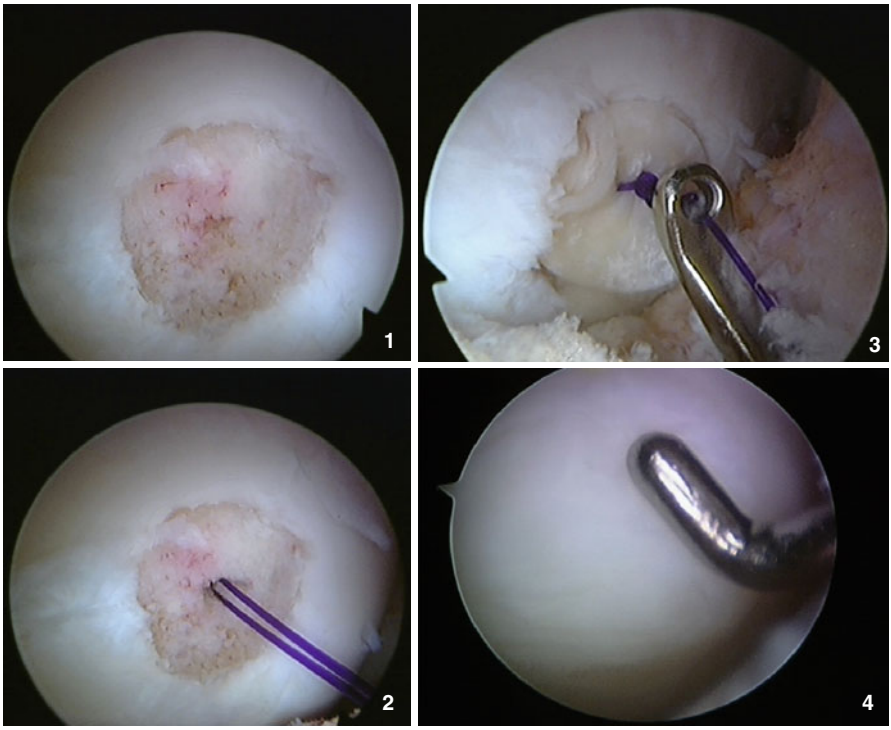

Fig. 13.2 *1* Arthroscopic ICRS grade IV chondral lesion debridment in a circular shape, located at the weightbearing area of medial femoral condyle. *2* Placement of a suture loaded bioaborbable mini anchor at the center of the defect. *3* Fixation of the polymer at the bottom of the defect. *4* Twelve months second look evaluation of the same patient

bioabsorbable mini anchor (DePuyMitek, Inc., Raynham, MA) loaded with a 0 PDS suture is placed at the center of the defect (Fig. 13.2, part 2). Two needles (16G) are passed through a bioabsorbable polymer (Restore, DePuyMitek, Inc., Raynham, MA) cut in the shape of an 8 mm disc, containing the cultured chondrocytes, and the anchor sutures are passed through the needles. A low-profile sliding arthroscopic knot is made with the anchor sutures on the polymer and it is introduced into the joint through a clear 10 mm cannula (Smith & Nephew, Inc., Andover, MA); the arthroscopic water flow is not stopped, it is rather decreased to gravity pressure. The polymer is firmly placed on the defect(Fig. 13.2, part 3), locking the knot placed over it and its stability is checked by means of palpation and dynamically with knee flexion and extension under arthroscopic control. This technique makes it possible to place as many polymers as necessary to completely cover the chondral lesion.

Recently, our group developed a new technique for the arthroscopic implantation of matrix-encapsulated cultured chondrocytes [22] in chondral patellar lesions. Until now, this had been an inaccessible location for the arthroscopic implantation of matrix-cultured autologous chondrocytes. The first surgical stage includes the identification, debridement, measurement and documentation of an ICRS grade 4 total

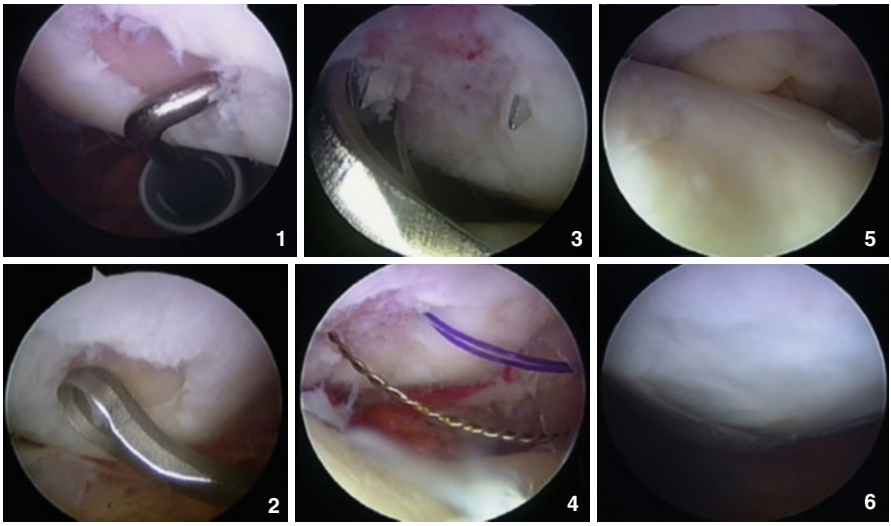

Fig. 13.3 *1* ICRS grade IV chondral injury documentation, located at the patella. *2* Arthroscopic debridment on a circular shape of a chondral lesion located at patella. *3* Arthroscopic retro-drilling of the chondral lesion at patella, using an ACL tibial guide (ACL guide, DePuy Mitek, Inc., Raynham, MA). *4* Passing of the PDS sutures through the chondral lesion using a shuttling device (Chia Percpasser™ - DePuy Mitek, Inc., Raynham, MA). *5* Arthroscopic fixation of a cell-seeded polymer at the bottom of the defect located at patella. *6* Twelve months second look evaluation of an arthroscopic matrix autologous chondrocyte implantation located at patella

thickness chondral lesion located in the patella(Fig. 13.3, part 1). The latter should be contained by healthy or relatively healthy cartilage. Then the cartilage biopsy is taken for culture purposes. It should be taken from the low lateral area of the intercondylar notch to prevent removing cartilage from the patellofemoral joint. During this first surgical stage it is important to understand the cause of the chondral lesion. Preoperative studies should have identified the presence or absence of a patellofemoral misalignment, such as patellar tilt, increased TT-TG (tibial tuberosity-trochlear groove) distance, or patellofemoral instability resulting from medial patellofemoral ligament tear, among others, to perform the necessary procedures to correct such alterations of the patellofemoral joint, without them, any chondral repair procedure would be doomed to failure. The second surgical stage includes the implantation of matrix-encapsulated cultured autologous chondrocytes according to the following steps:

1. Debridement of chondral lesions in the patella, making them circular and approximately 8 mm in diameter, with an arthroscopic angled curette or spoon introduced through any of the three working portals suggested: anteromedial, anterolateral, or superolateral (Fig. 13.3, part 2). In the case of patellar lesions located in the medial facet, it may be necessary to place a fourth portal, located at the superomedial level, to allow a direct approach to the lesion for debridement and implantation.
2. With a tibial tunnel guide for anterior cruciate ligament reconstruction (ACL guide, DePuyMitek, Inc., Raynham, MA), used inversely, with a 55° angulation, the outlet of the guide is placed intra-articularly on one of the sides of the chondral

lesion. The external part of the guide is placed extra-articularly, on the anterior cortical surface of the patella, and a 2 cm-long mini approach is performed on the patella. The patella is drilled with a 2 mm Steinmann pin placed outside-in through the joint, under arthroscopic control, until the outgoing pin is visualized at one of the sides of the chondral lesion, in a similar fashion to the patellar retro-drilling technique(Fig. 13.1). The steps are repeated to perform a second perforation on the opposite side of the lesion (Fig. 13.3, part 3). Two suture shuttling device named Chia Percpasser™ (DePuyMitek, Inc., Raynham, MA) are passed outside-in through each perforation made in the patella; both Chia Percpasser™ (DePuyMitek, Inc., Raynham, MA) are recovered inside the joint, and partially extracted towards one of the portals closest to the lesion(Fig. 13.3, part 4).

3. At the same time, a second assistant mounts the 8 mm circular cell-containing polymer with a PDS suture. Two needles (16G) are passed through the polymer and allow the suture to also pass through it at both of its ends.
4. The next step consists of mounting the ends of the 0 PDS suture bearing the polymer to the loop of each Chia Percpasser™(DePuyMitek, Inc., Raynham, MA). The sutures are recovered by pulling the Chia from the anterior aspect of the patella at the extraarticular level. The polymer bound to the PDS suture is thus introduced inside the joint using a clear 10 mm cannula (Smith & Nephew, Inc., Andover, MA), without stopping the water flow, but only decreasing it to gravity pressure. The polymer is thus firmly placed at the bottom of the chondral defect(Fig. 13.3, part 5).
5. Once the ends of the PDS suture that introduced the polymer inside the joint have been recovered, a knot is made and left at the extra articular level on the anterior cortical surface of the patella, thus definitely fixing the polymer on the chondral defect. This procedure may be done as many times as necessary to cover one or more 8 mm-diameter lesions on the articular surface of the patella. Also different polymer shapes could be done to treat non-circular chondral lesions, using the same principles of this technique.
6. Finally, implant stability is verified by means of palpation and dynamically with knee flexion and extension, under arthroscopic vision, identifying the polymer pressure sites according to the degree of knee flexion. This should be taken into account during the subsequent rehabilitation process.

With these two all-arthroscopic implantation techniques described by Ibarra, Villalobos et al. [22, 23], it is possible to arthroscopically implant matrix autologous chondrocytes in all the areas of the knee, without the need for an open approach, with good to excellent ICRS macroscopic appearance results at 12 months second look evaluation(Figs. 13.2, part 4, and 13.3, part 6).

13.4 Discussion

With the use of these chondral repair arthroscopic techniques, it is possible to restore the articular surface applying several validated open techniques for chondral repair, thus decreasing the morbidity resulting from an open approach, as well as the

costs and the time needed for rehabilitation. Thus chondral repair arthroscopic techniques become more appealing to patients and the big open approaches are increasingly reserved for the time when arthroplasty becomes necessary. However, we should consider what really matters about these new chondral repair arthroscopic techniques. Is it a small incision, an appealing all-arthroscopic approach, or the appropriate restitution of articular congruence that allows for the long term maintenance of the repaired tissue? This type of arthroscopic techniques involves a learning curve in dry and cadaver lab. They are recommended for arthroscopic surgeons interested in chondral repair, and not necessarily for all surgeons doing chondral repair. The basic principles of chondral repair like debridement, shoulder stability, graft stability and articular congruence must be always respected regardless of the selected approach, whether arthroscopic or open technique.

References

1. Villalobos FE, Perez-Jimenez F, Cruz-Lopez F, Almazan A, Izaguirre AF, Ibarra C, et al. Low incidence of chondral injuries candidates for biological repair in 1309 knee arthroscopies. New Orleans: AAOS; 2010. p. 433.
2. Freedman KB, Fox JA, Cole BJ. Kneecartilage: diagnosis and decisionmaking. Textbook of arthroscopy. Philadelphia: Saunders; 2004. p. 555–67.
3. Smith GD, Knutsen G, Richardson JB. A clinical review of cartilage repair techniques. J Bone Joint Surg Br. 2005;87:445–9.
4. Redman SN, Oldfield SF, Archer CW. Current strategies for articular cartilage repair. Eur Cell Mater. 2005;9:23–32.
5. Steadman JR, Rodke WG, Briggs KK. Microfracture chondroplasty: indications, techniques, and outcomes. Sports Med Arthrosc. 2003;11:236–44.
6. Zantop T, Petersen W. Arthroscopic implantation of a matrix to cover large chondral defect during microfracture. Arthroscopy. 2009;25(11):1354–60.
7. Piontek T, Ciemniewska-Gorzela K, Szulc A, Naczk J, Stomczykowski M. All arthroscopic AMIC procedure for repair of cartilage defects of the knee. Knee Surg Sports Traumatol Arthrosc. 2011;30:60–201.
8. Hangody L, Kish G, Kárpáti Z, Szerb I, Udvarhelyi I. Arthroscopic autogenousosteochondral-mosaicplasty for the treatment of femoral condylar articular defects a preliminary report. Knee Surg Sports Traumatol Arthrosc. 1997;5(4):262–7.
9. Hangody L, Duska Z, Karpati Z. Autologous osteochondral mosaicplasty. Knee Surg Sports Traumatol Arthrosc. 2002;1(1):13–22.
10. Marcacci M, Kon E, Delcogliano M, Filardo G, Busacca M, Zaffagnini S. Arthroscopic autologous osteochondral grafting for cartilage defects of the knee. Prospective study results at a minimum 7-year follow-up. Am J Sports Med. 2007;35(12):2014–21.
11. Chow JC, Hantes ME, Houle JB, Zalavras CG. Arthroscopic Autogenous osteochondral transplantation for treating knee cartilage defects: a 2- to 5-year follow-up study. Arthroscopy. 2004;20(7):681–90.
12. Marcacci M, Zaffagnini S, Kon E, Visani A, Lacono F, Loreti I. Arthroscopic autologous chondrocyte transplantation: technical note. Knee Surg Sports Traumatol Arthrosc. 2002;10:154–9.
13. Gobbi A, Kon E, Berruto M, Francisco R, Filardo G, Marcacci M. Patellofemoral full-thickness chondral defects treated with hyalograft: a clinical, arthroscopic, and histologic review. Am J Sports Med. 2006;34:1763–73.

14. Marcacci M, Kon E, Zaffagnini S, Filardo G, Delcogliano M, Neri MP, Iacono F, Hollander A. Arthroscopic second generation autologous chondrocyte implantation. Knee Surg Sports Traumatol Arthrosc. 2007;15:610–9.
15. Kon E, Gobbi A, Filardo G, Delcogliano M, Zaffagnini S, Marcacci M. Arthroscopic second-generation autologous chondrocyte implantation compared with microfracture for chondral lesions of the knee. Prospective nonrandomized study at 5 years. Am J Sports Med. 2009;37(1):33–41.
16. Gobbi A, Kon E, Berruto M, Filardo G, Delcogliano M, Boldrini L, Bathan L, Marcacci M. Patellofemoral full-thickness chondral defects treated with second-generation autologous chondrocyte implantation. Results at 5 years follow-up. Am J Sports Med. 2009;37(6):1083–92.
17. Filardo G, Kon E, Di Martino A, Iacono F, Marcacci M. Arthroscopic second-generation autologous chondrocyte implantation. A prospective 7-year follow-up study. Am J Sports Med. 2011;39:2153–60.
18. Kon E, Gobbi A, Filardo G, Berruto M, Benazzo F, Zanon G, Della-Villa S, Marcacci M. Articular cartilage treatment in Hig-level male soccer players. A prospective study of arthroscopy second-generation autologous chondrocyte implantation versus microfracture. Am J Sports Med. 2011;39(12):2549–57.
19. Erggelet C, Sittinger M, Lahm A. The arthroscopic implantation of autologous chondrocytes for the treatment of full-thickness cartilage defects of the knee joint. Arthroscopy. 2003;19(1):108–10.
20. Abelow SP, Guillen P, Ramos T. Arthroscopic technique for matrix-induced autologous chondrocyte implantation for the treatment of large chondral defects in the knee and ankle. Oper Tech Orthop. 2006;16:257–61.
21. Ronga M, Grassi FA, Bulgheroni P. Arthroscopic autologous chondrocyte implantation for treatment of a chondral defect in the tibial plateau of the knee. Arthroscopy. 2004;20(1):79–84.
22. Masri M, Lombardero G, Velasquillo C, Martinez V, Neri R, Ibarra C, et al. Matrix-encapsulation cell-seeding technique to prevent cell detachment during arthroscopic implantation of matrix-induced autologous chondrocytes. Arthroscopy. 2007;23(8):877–83.
23. Villalobos FE, Velasquillo C, Martinez V, Pérez-Jiménez F, Almazan A, Ibarra C, et al. Hospital-based biotechnology unit facilitates cell therapy strategies for cartilage repair. New Orleans: AAOS; 2010. p. 566.

Chapter 14
Articular Cartilage Repair of the Knee in Athletes

Robert H. Brophy

Abstract Athletes are at an elevated risk for articular cartilage injury of the knee, especially in pivoting sports such as soccer and basketball. Due to the poor intrinsic healing response of articular cartilage, these injuries can be debilitating and even career threatening for high level athletes. Athletes often damage their articular cartilage in conjunction with other pathology such as an anterior cruciate ligament tear or meniscus tear, and these typically need to be addressed at the same time as the articular cartilage. Fortunately, there are an increasing number of surgical treatment options available to restore the articular cartilage of the knee. Outcomes data is still relatively limited, especially for athletes, but there is a growing body of evidence that patients can return to sport after articular cartilage surgery, although the recovery may be lengthy. Microfracture and autologous chondrocyte implantation, and osteochondral autograft to a lesser degree, have demonstrated fair to good rates of return to sport. Appropriate rehabilitation is essential and the quality of the tissue repair appears to be an important factor influencing return to play. Much of the evidence for return to play is in soccer and American football, with less data for other sports. Although promising, more studies are needed to better define and predict return to play and long term outcomes in athletes after articular cartilage surgery in the knee.

Keywords Microfracture • Autologous chondrocyte implantation • Osteochondral autograft • Osteochondral allograft

R.H. Brophy, MD
Department of Orthopaedic Surgery,
Washington University School of Medicine,
14532 South Outer Forty Drive, Chesterfield,
MO 63017, USA
e-mail: brophyr@wudosis.wustl.edu

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_14,

Key Points

- Articular cartilage defects are common in athletes, with studies to date suggesting an estimated overall prevalence of 36 %.
- Good short to medium term results have been reported in athletes after articular cartilage surgery.
- Return to play in athletes after microfracture ranges from 44 to 77 %.
- Return to play in athletes after ACI ranges from 67 to 78 %.
- Return to play after osteochondral autograft has been reported from 86 to 94 %.
- In comparative studies, return to play has been higher after osteochondral autograft and ACI compared to microfracture.
- Younger age, higher levels of competition, shorter duration of symptoms before surgery, and fewer previous surgeries have been associated with a higher rate of return to play for athletes undergoing knee articular cartilage surgery.

14.1 Introduction

Articular cartilage has a poor intrinsic capacity for healing, especially in the knee. Athletes, particularly in pivoting and jumping sports such as soccer (Fig. 14.1) and basketball (Fig. 14.2), put extra demand on the knee joint and are at increased risk for traumatic injuries that can damage the articular cartilage [1–3]. Over time, these demands and injuries can hasten degeneration of the joint and put athletes at risk for early osteoarthritis, often a career ending condition [1, 2]. In the long run, the pain and limitations associated with knee osteoarthritis can limit the activity of former athletes and increase their risk for weight gain and associated health problems.

Fortunately, there are an increasing number of options for restoring articular cartilage. Techniques such as microfracture and osteochondral grafting have been supplemented by new approaches such as MACI and other cell based techniques. As the number of options and their efficacy improve, athletes stand to gain the most both in terms of short term outcomes that can enable return to competition and long term knee and overall health. The purpose of this chapter is to review the prevalence of articular cartilage injury in athletes and outcomes, particularly return to play, from treatment of articular cartilage injury of the knee in this special population.

14.2 Prevalence of Articular Cartilage Injury in Athletes

Athletes, particularly in running, jumping and cutting or pivoting sports, are likely to have a higher incidence of knee articular cartilage injuries than the general population. A number of studies have looked at the incidence of these lesions in athletes

Fig. 14.1 Soccer athletes (Image courtesy of G. Newman Lowrance)

from specific sports, such as American football, basketball and endurance running. A recent systematic review reported an overall prevalence of full thickness cartilage defects in 36 % of athletes [4].

American football athletes are at high risk for articular cartilage injury in the knee for a variety of reasons, including the running, cutting and pivoting nature of the sport, the frequent traumatic contact and higher body mass of many of the athletes (Fig. 14.3). A review of athletes at the National Football League (NFL) combine from 2005 through 2009 reported that articular cartilage abnormalities were seen in 61 % of 704 knees that underwent MR imaging in those years [5]. Full thickness articular cartilage defects were present in 17 % of the knees. Full thickness defects were most common in the lateral compartment (39 % lateral femoral condyle, 19 % lateral tibial plateau), followed by the patellofemoral compartment (14 % patella, 14 % trochlea) and medial compartment (medial femoral condyle 13 %, medial tibial plateau 1 %). A history of previous partial meniscectomy was associated with a higher incidence of full thickness lesions ($p<0.001$) but previous ACL reconstruction was not ($p=0.7$). Full thickness cartilage defects were present in

Fig. 14.2 Basketball athletes (Image courtesy of G. Newman Lowrance)

27 % of knees with a history of previous meniscectomy compared to 12 % of knees with no previous meniscectomy. Meniscal repair appeared to be at least partially protective as the incidence of full thickness cartilage lesions was only 16 % in athletes with a previous meniscal repair, which was not significantly different from knees without previous meniscal surgery. The incidence of full thickness cartilage lesions dropped to 12 % in knees with a successful meniscal repair as defined by no subsequent meniscal surgery. The impact of previous partial meniscectomy was largest in the lateral compartment, where a history of previous partial lateral meniscectomy increased the incidence of full thickness defects from 5 to 25 %. In the medial compartment, a history of previous partial medial meniscectomy increased the incidence of full thickness defects from 2.3 to 7.1 %. The incidence of full thickness cartilage defects was not associated with player BMI or position in this study.

Another study from the NFL combine reported that 38.2 % of athletes at the NFL combine from 2005 through 2007 had full thickness chondral injury [6]. Player position (higher in linebackers, lower in defensive backs), weight and BMI were associated with chondral injury in this study.

Fig. 14.3 American football athletes (Image courtesy of G. Newman Lowrance)

Basketball is another sport that puts tremendous stress on the articular cartilage of the knee, especially in the patellofemoral joint. A study looking at MRIs from 34 knees in asymptomatic college basketball players reported a 41 % incidence of chondral lesions with six full thickness lesions (18 %) [7]. The majority of the lesions were in the patellofemoral joint. A study looking at MRIs from 20 asymptomatic National Basketball Association (NBA) players (40 knees) from 1996 through 1999, reported that 48 % of knees had articular cartilage lesions in the knee, with 77 % in the patellofemoral joint [8]. Only two knees (5 %) had full thickness loss, one on the trochlea and one on the lateral femoral condyle. Another study looking at 28 knees in NBA players reported at least some chondral change in 50 % of knees, with 70 % of the changes occurring in the patellofemoral joint [9]. Only 7.1 % of the knees had a focal chondral defect. The trochlea had chondral change in one fourth of the knees from both studies on NBA players, reinforcing the fact that the patellofemoral joint is at particular risk for breakdown in these athletes.

Running also puts repetitive stress on the knee cartilage. A number of studies have reported the incidence of chondral changes in distance runners, ranging from 18 to 63 % [10, 11]. However, there is relatively limited data on the location of or risk factors for articular cartilage lesions in the knees of runners.

There is relatively limited data on the prevalence of chondral injury in other sports. Despite this lack of evidence, other sports, such as soccer, rugby, volleyball, handball, and skiing, are likely to have similar levels of chondral injury. Further study is needed to better understand the true prevalence of and the risk factors for full thickness chondral defects in the athletic population.

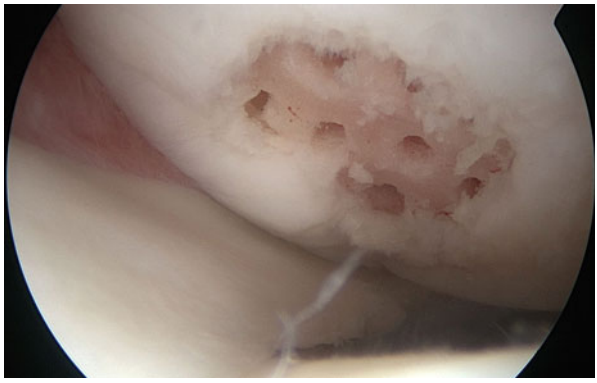

Fig. 14.4 Intraoperative image of microfracture

14.3 Treatment Outcomes of Articular Cartilage Injury in Athletes

Considering their increased risk for articular cartilage injury, athletes are likely to be candidates for articular cartilage surgery. The same factors that put them at risk for articular cartilage injury in the first place could be expected to make their recovery more challenging and their outcomes potentially less optimal. Despite the fact that athletes are a unique population when it comes to articular cartilage injury, there is limited data on the efficacy of articular cartilage surgery in this cohort. Two recent reviews have shown that there is evidence for good short and medium term outcomes in athletes after articular cartilage surgery but that more study is needed [12, 13].

14.3.1 Microfracture

Results have been reported for various techniques of articular cartilage surgery. The majority of evidence to date on outcome from surgical treatment of articular cartilage in athletes is for the microfracture technique [12] (Fig. 14.4) as described by Steadman [14]. In a recent systematic review [12], 8 of 11 studies reporting results of articular cartilage surgery in athletes included patients treated with microfracture and the overall return to play after microfracture was 59 % (range 25–100 %). In another review which pooled the results from reviewed publications, 787 of 1,410 athletes who had undergone articular cartilage surgery in the knee had been treated with microfracture, with a return to play of 66±6 % (range, 44–100 %) in those athletes [13].

A couple of level III studies have reported reasonable results with microfracture in basketball players, with 67–79 % return to play by 25–30 weeks [15, 16]. Athletes were 8.2 times less likely to get back to the NBA than controls and those who did return to play had significantly worse performance than prior to their surgery [16]. Only 58 % were able to play at least one more season, and 76 % of those were on injured reserve at least once during that season [15]. In professional American football, a retrospective series reported that 19 of 25 athletes (76 %) treated with

microfracture returned to football [14]. Those who returned to football managed to play an additional 4.6 seasons and 56 games on average. Other series have reported similar results in mixed cohorts including athletes from several sports, with return to play ranging from 44 to 77 %, with 57–71 % of those returning to sport getting back to a same or better level of competition [17, 18].

14.3.2 ACI

A number of studies have reported outcomes for athletes treated with autologous chondrocyte implantation (ACI). Systematic reviews reported return to sport of 78 % (range 27–100 %) [12] and 67 ± 17 % (range, 33–96 %) [13] for ACI. A prospective cohort of 118 athletes with a mean age of 35 years treated with ACI using a periosteal patch reported a 95 % return to sport by 18 months and 100 % by 3 years [19]. A case series of 45 soccer athletes treated with ACI reported an overall return to play of 33 %, despite 72 % good to excellent clinical outcomes [20]. The return to play varied significantly by level, with 83 % of competitive players getting back to soccer compared to 16 % of recreational level athletes. Another case series of 20 adolescent athletes from a variety of sports including soccer, basketball and American football treated with ACI reported 96 % return to play, with 60 % getting back to the same level or better compared to pre-injury [21]. Duration of preoperative symptoms and the number of previous surgeries impacted return to play in this group.

14.3.3 Osteochondral Graft

14.3.3.1 Autograft

Good rates of return to sport have been reported after osteochondral autograft transplantation (Fig. 14.5). A systematic review reported 93 % return to sport after OAT [12]. Another review pooled results from previously published studies and reported return to sport of 91 ± 2 % (range, 86–94 %) after OAT in 261 patients [13]. These findings are in contrast to the results from a mixed cohort of athletic and non-athletic patients in which 61 % returned to full activity after osteochondral autograft transplantation at an average follow up of 26 months [22]. A long term follow up (mean 9.6 years) of osteochondral autograft transplantation in the knee (303 cases), ankle (39 cases) and elbow (12 cases), reported a 63 % return to previous level of sport and 28 % return to lower level of sport, with 9 % of patients unable to return to any sport [23]. These results were not broken down by joint. Good to excellent clinical outcomes were reported for 91 % of cases on the femur, 86 % on the tibia and 74 % in the patellofemoral joint. Preoperatively, 27 % of the knees were noted to have Fairbank grade I and II changes with no grade III changes. At final radiographic follow-up, only 17 % of knees were noted to have worsening, with 36 % of knees showing grade I or II changes and only five knees (1.6 %) demonstrating grade III changes.

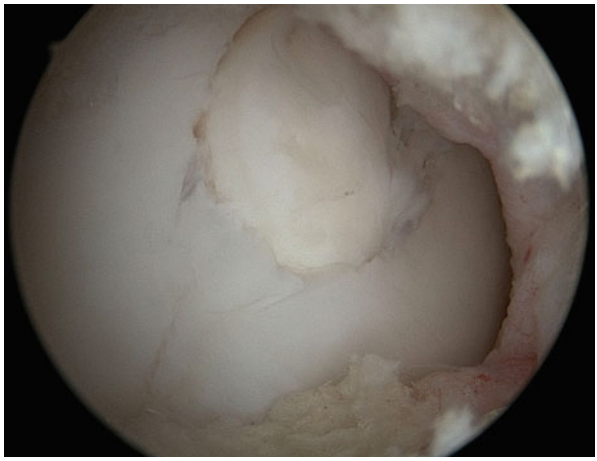

Fig. 14.5 Intraoperative image of osteochondral autograft

14.3.3.2 Allograft

One study reported on outcomes of fresh-stored osteochondral allograft transplantation in 43 athletes [24]. At an average follow up of 2.5 years, 88 % of these athletes had gotten back to at least some activity and 79 % had returned to pre-injury level of activity. In these athletes, average return to sport occurred at 9.6 ± 3.0 months.

14.3.4 Comparative Studies

One Level I prospective, randomized trial compared osteochondral autograft transplantation to microfracture in 57 athletes [25]. Results were better with OAT, including higher return to preinjury level of play (93 % OAT v. 52 % microfracture) and clinical outcomes based on modified Hospital for Special Surgery and ICRS scores at 1 and 2 year follow-up. Worse clinical outcomes were noted in large defects (>2 $cm^{2)}$ treated with microfracture compared to smaller defects treated with microfracture. The same relationship was not present for patients treated with OAT. The samples were small and sports were predominantly soccer and basketball.

A level II cohort study compared the results of microfracture to arthroscopic second-generation ACI (Hyalagraft C) in 41 professional and semi-professional soccer players [26]. There was similar return to play between the two groups with 80 % of athletes returning to high level play after microfracture compared to 86 % following second generation ACI. Return to play was quicker after microfracture (median 8 months compared to 12.5 months). Clinical results were similar based on IKDC scores at 2 years but there was a significant decline in the scores of those treated with microfracture at mean final follow up of 7.5 years. Those treated with second generation ACI did not have the same decline and had significantly better clinical outcomes at final follow-up compared to microfracture.

Table 14.1 Return to sport after knee articular cartilage surgery in athletes

Technique	Evidence	Timing of return to play (months)	Rate of return to play
Microfracture	More than ten studies (~800 athletes)	~8	62.5 % (44–100 %)
ACI	Seven studies (over 350 athletes)	~18	72.5 % (27–100 %)
Osteochondral graft			
Auto	Five studies (over 250 athletes)	~7	92 % (86–94 %)
Allo	One study (43 athletes)	9.6 ± 3.0	79 %

Rate of return to play and timing of return to play are summarized by technique in Table 14.1.

14.3.5 Influencing Factors

The factors affecting the outcome from cartilage repair of the knee in athletes have been similar across various studies and surgical techniques. Patient age has been a consistent factor, with older athletes not doing as well as younger athletes [12, 13]. Studies have used different cut-offs, typically between 25 and 40 years of age [18, 20, 22, 24, 25], to distinguish between younger athletes who have better outcomes and older athletes who have less optimal outcomes. For example, 65–71 % of younger patients have been shown to return to sport after microfracture, compared to 20–29 % of older patients [18, 20]. Following OAT, 90 % of patients under 30 returned to pre-injury participation compared to 23 % of those over 30 (although 70 % got back to some participation) [22]. Age was also a significant factor predicting return to play after osteochondral allograft, with patients under 25 more likely to return [24].

Athletes with a longer duration from onset of symptoms or diagnosis to treatment were also found to have worse return to play after microfracture [18] and ACI [20]. With symptoms less than 12 months prior to surgery, 66 % of athletes returned to play after microfracture [18] while 67 % returned to play after ACI [20]. If treatment was received more than 12 months after the onset of symptoms, return to play dropped to 14 % after microfracture [18] and 15 % after ACI [20]. In a cohort of adolescent athletes treated with ACI, all of the patients treated within 12 months of symptoms returned to play while only a third of those treated after a longer interval returned to competition [21]. Athletes treated with osteochondral allograft transplantation within 12 months of symptoms were also more likely to get back to sports with an odds ratio of 37 (range 3.4–298) compared to those treated after a longer duration of symptoms [24].

The number of previous knee surgeries has also been shown to impact return to play. Fewer previous surgeries have been associated with higher return to play after microfracture [18] and ACI [21]. Higher level athletes have also been shown to get

Table 14.2 Factors influencing return to sport after knee articular cartilage surgery in athletes

Technique	Age	Duration of symptoms	Number of previous surgeries	Level of athlete
Microfracture	↑ in younger athletes	↑ with shorter duration of symptoms	↑ if fewer previous surgeries	↑ in higher level athletes
ACI	↑ in younger athletes	↑ with shorter duration of symptoms	↑ if fewer previous surgeries	↑ in higher level athletes
Osteochondral graft				
Auto	↑ in younger athletes	↑ with shorter duration of symptoms		
Allo	↑ in younger athletes	↑ with shorter duration of symptoms		

back to sport at a higher rate after ACI [21] and microfracture [17]. The evidence is mixed on how the size and location of the cartilage lesion, as well as concomitant procedures, impact return to play, with results varying by study and surgical technique.

Factors influencing return to play are summarized in Table 14.2.

14.4 Conclusion

In conclusion, articular cartilage injury is common in the knee of athletes although the true incidence and prevalence of these lesions remains to be fully elucidated. Treatment of these lesions is likely to be more difficult in this population due to the unique demands that make the injury more likely to occur. Evidence to date suggests athletes can have good outcomes from articular cartilage surgery in the knee. Rate of return and performance upon return may be better after ACI and OAT compared to microfracture. Young age and early treatment are associated with greater return to play. More and higher level studies are needed to better understand optimal treatment techniques and outcomes in this population.

References

1. Drawer S, Fuller CW. Propensity for osteoarthritis and lower limb joint pain in retired professional soccer players. Br J Sports Med. 2001;35(6):402–8.
2. Kujala UM, Kettunen J, Paananen H, Aalto T, Battie MC, Impivaara O, et al. Knee osteoarthritis in former runners, soccer players, weight lifters, and shooters. Arthritis Rheum. 1995; 38(4):539–46.

3. Mandelbaum BR, Browne JE, Fu F, Micheli L, Mosely Jr JB, Erggelet C, et al. Articular cartilage lesions of the knee. Am J Sports Med. 1998;26(6):853–61.
4. Flanigan DC, Harris JD, Trinh TQ, Siston RA, Brophy RH. Prevalence of chondral defects in athletes' knees: a systematic review. Med Sci Sports Exerc. 2010;42(10):1795–801.
5. Nepple JJ, Wright RW, Matava MJ, Brophy RH. Full-thickness knee articular cartilage defects in national football league combine athletes undergoing magnetic resonance imaging: prevalence, location, and association with previous surgery. Arthroscopy. 2012;28(6):798–806.
6. Hirshorn KC, Cates T, Gillogly S. Magnetic resonance imaging-documented chondral injuries about the knee in college football players: 3-year National Football League Combine data. Arthroscopy. 2010;26(9):1237–40.
7. Major NM, Helms CA. MR imaging of the knee: findings in asymptomatic collegiate basketball players. AJR Am J Roentgenol. 2002;179(3):641–4.
8. Kaplan LD, Schurhoff MR, Selesnick H, Thorpe M, Uribe JW. Magnetic resonance imaging of the knee in asymptomatic professional basketball players. Arthroscopy. 2005; 21(5):557–61.
9. Walczak BE, McCulloch PC, Kang RW, Zelazny A, Tedeschi F, Cole BJ. Abnormal findings on knee magnetic resonance imaging in asymptomatic NBA players. J Knee Surg. 2008;21(1):27–33.
10. Schueller-Weidekamm C, Schueller G, Uffmann M, Bader TR. Does marathon running cause acute lesions of the knee? Evaluation with magnetic resonance imaging. Eur Radiol. 2006;16(10):2179–85.
11. Krampla W, Mayrhofer R, Malcher J, Kristen KH, Urban M, Hruby W. MR imaging of the knee in marathon runners before and after competition. Skeletal Radiol. 2001; 30(2):72–6.
12. Harris JD, Brophy RH, Siston RA, Flanigan DC. Treatment of chondral defects in the athlete's knee. Arthroscopy. 2010;26(6):841–52.
13. Mithoefer K, Hambly K, Della Villa S, Silvers H, Mandelbaum BR. Return to sports participation after articular cartilage repair in the knee: scientific evidence. Am J Sports Med. 2009;37 Suppl 1:167S–76.
14. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res. 2001;(391 Suppl):S362–9.
15. Cerynik DL, Lewullis GE, Joves BC, Palmer MP, Tom JA. Outcomes of microfracture in professional basketball players. Knee Surg Sports Traumatol Arthrosc. 2009;17(9): 1135–9.
16. Namdari S, Baldwin K, Anakwenze O, Park MJ, Huffman GR, Sennett BJ. Results and performance after microfracture in National Basketball Association athletes. Am J Sports Med. 2009;37(5):943–8.
17. Blevins FT, Steadman JR, Rodrigo JJ, Silliman J. Treatment of articular cartilage defects in athletes: an analysis of functional outcome and lesion appearance. Orthopedics. 1998;21(7):761–7; discussion 7–8.
18. Mithoefer K, Williams 3rd RJ, Warren RF, Wickiewicz TL, Marx RG. High-impact athletics after knee articular cartilage repair: a prospective evaluation of the microfracture technique. Am J Sports Med. 2006;34(9):1413–8.
19. Kreuz PC, Steinwachs M, Erggelet C, Lahm A, Krause S, Ossendorf C, et al. Importance of sports in cartilage regeneration after autologous chondrocyte implantation: a prospective study with a 3-year follow-up. Am J Sports Med. 2007;35(8):1261–8.
20. Mithofer K, Peterson L, Mandelbaum BR, Minas T. Articular cartilage repair in soccer players with autologous chondrocyte transplantation: functional outcome and return to competition. Am J Sports Med. 2005;33(11):1639–46.
21. Mithofer K, Minas T, Peterson L, Yeon H, Micheli LJ. Functional outcome of knee articular cartilage repair in adolescent athletes. Am J Sports Med. 2005;33(8):1147–53.
22. Kish G, Modis L, Hangody L. Osteochondral mosaicplasty for the treatment of focal chondral and osteochondral lesions of the knee and talus in the athlete. Rationale, indications, techniques, and results. Clin Sports Med. 1999;18(1):45–66, Vi.

23. Hangody L, Dobos J, Balo E, Panics G, Hangody LR, Berkes I. Clinical experiences with autologous osteochondral mosaicplasty in an athletic population: a 17-year prospective multicenter study. Am J Sports Med. 2010;38(6):1125–33.
24. Krych AJ, Robertson CM, Williams 3rd RJ. Return to athletic activity after osteochondral allograft transplantation in the knee. Am J Sports Med. 2012;40(5):1053–9.
25. Gudas R, Stankevicius E, Monastyreckiene E, Pranys D, Kalesinskas RJ. Osteochondral autologous transplantation versus microfracture for the treatment of articular cartilage defects in the knee joint in athletes. Knee Surg Sports Traumatol Arthrosc. 2006;14(9):834–42.
26. Kon E, Filardo G, Berruto M, Benazzo F, Zanon G, Della Villa S, et al. Articular cartilage treatment in high-level male soccer players: a prospective comparative study of arthroscopic second-generation autologous chondrocyte implantation versus microfracture. Am J Sports Med. 2011;39(12):2549–57.

Chapter 15
Meniscus Substitution: Scaffolds, Allografts and Prosthetic Implants

Peter C.M. Verdonk, Aad A.M. Dhollander, Thomas Tampere, and René Verdonk

Abstract The impact of meniscectomy on the articular cartilage is very significant. At 10 years, 30–60 % of patients have some kind of medial cartilage degeneration and 12–40 % have lateral cartilage degeneration on X-ray. Moreover, if the ACL is also torn almost all patients will end up with osteoarthritis (OA) in the long run.

Replace the meniscus rather than resecting it

Meniscus lesions are tremendously common: one million patients in the USA and 400,000 in Europe have operations for meniscus lesions every year. Even though partial meniscectomy is still the gold standard, orthopaedic surgeons should try to save or repair it because of its effect on the knee in the long term. If these options are not possible, they should consider replacing it.

The polyurethane scaffold

A polyurthane scaffold is intended for partial defects and has the following concept: once it is implanted into a partial defect, cells from the synovium and probably pluripotent cells in the articulation and the joint will grow in. A meniscus-like tissue will be formed after implantation.

The results on the cartilage are interesting: at 1 and 2 years regrowth of both meniscus tissue and cartilage can be observed, indicating that the protection of the meniscus leads to protection of the cartilage. A significant improvement in pain and function is observed at 12 and 24 months. Continuing follow-up is needed.

P.C.M. Verdonk (✉)
Orthopaedic Center Antwerp, Monica Hospitals Antwerp,
Harmoniestraat 68, 2018 Antwerp, Belgium

Faculty of Medicine, Department of Physiotherapy and Orthopaedic Surgery,
Ghent University, De Pintelaan 185 9000, Ghent, Belgium
e-mail: pverdonk@yahoo.com

A.A.M. Dhollander • T. Tampere • R. Verdonk
Department of Orthopaedic Surgery and Traumatology, Ghent University Hospital,
Ghent, Belgium

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_15, © Springer-Verlag London 2014

Meniscus allograft transplantation (MAT)

This kind of intervention is indicated for large meniscus lesions. The papers published on this topic show very satisfactory results in the long term, both clinically and radiographically. The evidence is still inconclusive in relation to chondroprotection with these allograft transplantations.

Total meniscus prosthetic implant

This is a new and upcoming technique which is still under clinical investigation. In-vitro studies have shown that it made it possible to reduce the major stresses on the cartilage. Apparently patients are functioning quite well. This seems to be a very attractive potential solution for a specific population of patients.

Keywords Meniscus • Scaffold • Allograft • Prosthesis

Key Points

- When possible, the meniscus should be preserved or repaired.
- Meniscus scaffolds are indicated for partial meniscus defects (ideally less than 5 cm) while meniscus allografts are indicated in larger defects.
- Prosthetic meniscus implants are a promising new tool for meniscus deficiency but are currently still under investigation.
- Meniscus substitution is a treatment option for non-arthritic, well-aligned and stable knees.
- Significant osteophytes or flattening of the femoral condyle, often observed in chronic meniscectomy cases, eliminates the possibility for meniscus substitution.

15.1 General Introduction

Treatment of meniscal lesions is the most common surgical intervention performed by orthopedic surgeons today, with over one million surgical interventions involving the meniscus performed annually in the United States (US) and approximately 400,000 in Europe. Metcalf and Barett prospectively reviewed 1,370 patients with 1,485 tears to determine the overall incidence of meniscal tears. Fewer females (31 %) than males (69 %) reported meniscal tears. The mean age of a meniscal tear was 46 years. More medial tears (73 %) than lateral tears (19 %) were reported and 8 % of all patients had both medial and lateral [1].The menisci are semilunar, fibrocartilaginous structures and provide several elements to knee function, including load transmission, shock absorption, joint lubrication, joint nutrition and stability.

For many years, the function of the meniscus was not completely known and thus treated as an unnecessary accessory that could be sacrificed if needed. However, over the last few decades, the understanding of meniscal functions has continued to evolve with an increasing commitment among physicians to preserve the meniscus

consequently. It is now accepted that loss of all or part of the meniscus leads to long term degenerative changes due to higher peak stresses on the articular cartilage in the meniscectomized compartment as a result of the decreased contact area. Thus, it seems logical to repair or preserve the meniscus, especially in young patients. If this is not possible one should aim to regenerate or substitute lost meniscus tissue with a scaffold, allograft or implant in order to restore the function of the knee joint and to possibly prevent further joint degeneration.

In this chapter, the different options for meniscus substitution will be discussed and elaborated. Specific attention will be paid to the important differences in indication and surgical technique.

15.2 Meniscus Scaffolds for the Treatment of *Partial* Meniscus Defects

15.2.1 Introduction

Meniscal regeneration appears to require the physical presence of a scaffold to encourage successful migration and colonization with precursor cells and vessels eventually leading to the formation of organized meniscal tissue [2, 3].

Currently, two scaffolds for the treatment of partial meniscus defects are available in Europe. The authors have acquired extensive experience with a novel, biodegradable, synthetic, a cellular scaffold composed of aliphatic polyurethane (Actifit™, Orteq Ltd, London, UK) which was designed to fulfill an unmet clinical need in the treatment of patients with irreparable partial meniscal tissue lesions. The treatment objective of the scaffold is to provide pain relief and restore lost meniscus function.

The scaffold has been shown to support generation of new meniscus-like tissue. When attached to the vascularized portion of the meniscus, the scaffold acts as a template for proliferation and organization of cells with extracellular matrix formation within the interconnected, highly porous scaffold. Importantly, the scaffold is not designed to provide mechanical support to the knee joint; rather, it is anticipated that such a function will be provided by the new tissue generated after scaffold implantation. The scaffold slowly degrades over an anticipated period of 5 years and is replaced by regenerated tissue with meniscus-like characteristics [3].

15.2.2 Indications

The key inclusion criteria are (1) irreparable medial or lateral meniscal tear or partial meniscus loss, with intact rim. This synthetic meniscus substitute is not intended for the treatment of total or subtotal meniscus defects. Ideally, the defect length should be limited to 5–6 cm; (2) skeletally mature male or female patients; (3) age 16–50 years; (4) stable knee joint or knee joint stabilization procedure within

12 weeks of index procedure; (5) International Cartilage Repair Society (ICRS) classification ≤3.

The key exclusion criteria are (1) total meniscus loss or unstable segmental rim defect; (2) multiple areas of partial meniscus loss that could not be treated by a single scaffold; (3) any significant malalignment (varus or valgus); (4) ICRS classification >3; and (5) body mass index ≥35.

15.2.3 Surgical Technique

The Actifit® meniscal scaffold is placed in the subject's knee using a standard arthroscopic surgery procedure and standard equipment. Verification of cartilage status and integrity of the meniscal rim and both the anterior and posterior horns should be performed. In the case of a tight medial compartment, distending the medial collateral ligament using the outside-in puncture method (several passes with a spinal needle from outside-in) or the inside-out piecrusting release technique described by Steadman allows the surgeon to adequately visualize both the femoral and the tibial cartilage status and to create an adequate working space for meniscus reconstructive surgery.

To allow for easy insertion of these scaffold, an enlargement of the portal used for insertion of the device may be required (the size of the little finger is typically sufficient).

Preparation of the damaged meniscus includes surgical debridement and removal of all pathological tissue and ensuring that the resulting defect site extends into the vascularized red-on-red or red-on-white zone of the damaged portion of the meniscus. Lesions situated further away from the synovial border are known to have only very limited healing potential and therefore should be excluded from this type of meniscoplasty. To enhance healing, the meniscal rim may be punctured in order to create vascular access channels. Gentle rasping of the synovial lining may further stimulate meniscal intergration and tissue ingrowth.

The meniscal defect should be measured along the curvature of its inner edge using the accompanying specially designed meniscal ruler and meniscal ruler guide.

Actifit® is then measured, and using a scalpel, cut to fit in such a place and manner as to ensure that sterility is maintained at all times. To allow for shrinkage caused by suturing of the sponge like material and to ensure a snug optimal fit into the prepared defect, oversizing of the length by 10 % is advised (3 mm for defects <3 cm and 5 mm for defects ≥3 cm). In order to achieve a perfect fit of the scaffold with the native meniscus at the anterior junction, the anterior side should be cut at an oblique angle of 30–45° (Fig. 15.1).

Fixation of Actifit® is achieved by suturing the scaffold to the native meniscus tissue.

Fixation of the device should begin with a horizontal all-inside suture from the posterior edge of the scaffold to the native meniscus. Suturing should be secure; however, attention must be paid not to over tighten sutures, as this may alter and indent the surface of the scaffold. In line with wellknown meniscal suturing

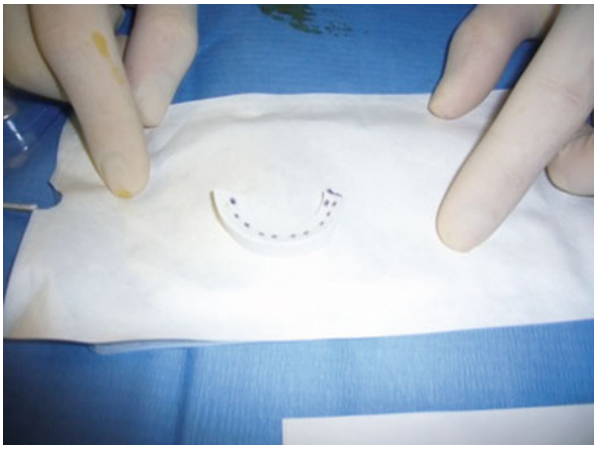

Fig. 15.1 The Actifit® meniscal scaffold is tailored on surgical field using a scalpel for a perfect fit to the meniscus defect

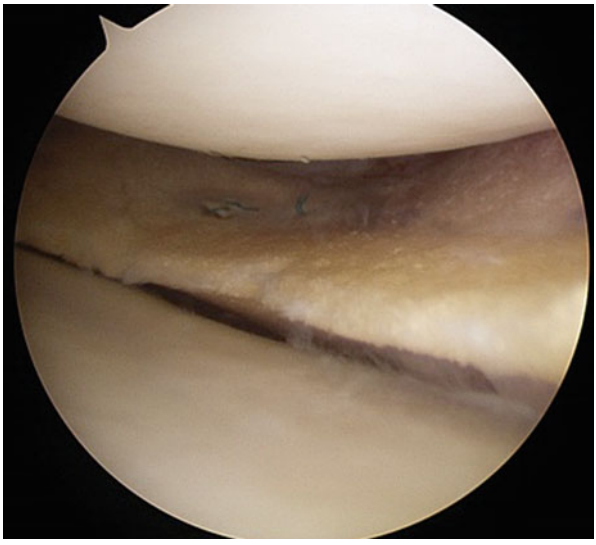

Fig. 15.2 Macroscopic aspect of the scaffold 1 year after implantation showing full incorporation and intergration to the rim and horns

techniques, the distances between the sutures should be kept to approximately 0.5 cm. Each suture should be placed at one-third to one-half of the scaffold's height, as determined from the lower surface of the scaffold.

Following suturing, if required, the scaffold may be further trimmed and fine-tuned intraarticularly using a basket punch. Once the scaffold is securely fixed, stability of the fixation is tested using the probe and moving the knee through a range of motion (0–90°).

To enhance the healing response, a bone marrow aspirate can be performed from the notch area and directly applied on the dry scaffold after implantation. Figure 15.2 depicts a scaffold 1 year after implantation showing full peripheral integration to the meniscus rim.

15.2.4 Rehabilitation Protocol

In order to ensure protection of the newly formed fragile tissue and to provide optimum conditions for healing, all patients were required to undergo a conservative rehabilitation program similar to that for a meniscal allograft. The rehabilitation protocol was followed for 16–24 weeks, with the patient non-weight bearing for the first 3 weeks. Partial weight bearing was permitted from Week 4 onwards, with a gradual increase in loading up to 100 % load at 9 weeks post-implantation. The progressive weight bearing is initiated in stages, increasing by 10 kg/week for patients weighing ≤60 kg and by 15 kg/week for patients weighing >60 to ≤90 kg. Full weight bearing with an unloader brace is allowed from Week 9 onwards, and without the use of the unloader brace from Week 14 onwards. Range of motion exercises are gradually initiated but limited to 90° of flexion the first 6 weeks. Gradual resumption of sports was generally commenced as of 6 months at the discretion of the responsible orthopaedic surgeon; however, contact sports were to recommence only after 9 months.

15.2.5 Conclusion

Meniscus scaffolds are a safe and viable option for the treatment of painful partial meniscus defects. Current literature has provided short-term 2 year evidence for a continued significant improvement in pain and function [4]. In addition, a recent histological study provides insights into the regeneration of an immature meniscus-like tissue at 1 year after implantation [5].

15.3 Meniscus Allografts for the Treatment of *Large* Meniscus Defects

15.3.1 Introduction

In this update we will discuss our experience with arthroscopic meniscus allograft transplantation using a soft tissue fixation technique. While scaffolds are mainly used to substitute for partial loss, meniscus allografts are generally used in total or subtotal meniscectomized patients. Meniscal allograft transplantation can be considered as safe and reliable for the treatment of refractory postmeniscectomy symptoms in selected patients [5]. Due to the specific biomechanical characteristics of the lateral compartment, most patients develop early postoperative pain in the lateral compartment after a large meniscectomy and hence the majority of allograft transplants will be performed in the lateral compartment [6, 7].

15.3.2 Indications

A meniscal allograft transplantation is indicated in the young or middle-aged (<50 years) patient, who has undergone a previous *total* meniscectomy. The patient should complain of moderate to severe pain due to excessive joint loading secondary to meniscal deficiency. Professional incapacity is commonly present. Joint space narrowing should be limited to grade 0 (no narrowing) or 1 (<50 % narrowing) as measured on plain postero-anterior weight bearing radiographs according to the International Knee Documentation Committee system. At this point, we do not recommend prophylactic viable meniscus allograft transplantation in the meniscectomized but asymptomatic patient. Ideally, degenerative cartilage changes should be limited (grade III is considered borderline) and/or focal. If necessary, focal cartilage defects can be treated concomitantly. Because of the usually mild degenerative cartilage disease, the relative young age of the patients and their desire to lead an active lifestyle, these patients are not candidates for a unicompartimental or total knee arthroplasty. The lower limb axial alignment should be normal and the knee joint should be stable. Otherwise, an associated corrective osteotomy or stabilization procedure is indicated.

15.3.3 Contraindications

Meniscus allograft transplantation is contraindicated in:

Generalized/Grade IV degenerative compartmental cartilage changes
Marked radiographic changes such as femoral condyle flattening and osteophyte formation
Axial malalignment exceeding 2°
Ligamentous instability
Inflammatory joint disease
A history of infection in the knee

15.3.4 Surgical Technique

15.3.4.1 Allograft Preparation

Non-resorbable high-strength (Fibre wire, Arthrex, Naples, USA) sutures are placed in the anterior and posterior horn of the allograft. Generally, three whipstitches are placed on the inner and outer rim of the horn of the allograft (Fig. 15.3). An additional vertical non-resorbable suture (Ethibond 2, Somerville, NJ, USA) is placed at the posteromedial or posterolateral corner of the medial or lateral allograft,

Fig. 15.3 Prepared lateral meniscal allograft for arthroscopic meniscal transplantation. Whipstiches on inner an outer rim of anterior and posterior horn. A vertical non-resorbable suture is placed on the posterolateral corner in front of the popliteus hiatus

respectively. For the lateral allograft, the posterolateral suture is positioned just anteriorly to the popliteus tendon hiatus as this will serve as a landmark during arthroscopy.

15.3.4.2 Meniscus Allograft Transplantation

The classic anteromedial and anterolateral portals are made. Using shaver and punch the remnant meniscus is debrided to the level of the meniscal rim.

A modified ACL aiming device, with a low profile tip, is inserted through the portal and positioned at the anatomical posterior horn of the meniscus (Fig. 15.4). A guide pin is drilled first and subsequently overdrilled by a 4.5 mm cannulated drill. A double loop metal wire is introduced through the tunnel from outside-in and picked up intra-articularly with an arthroscopical grasper and pulled out through the portal. Subsequently, a suture passer (Acupass, Smith and Nephew, Memphis, Tennessee, USA) is introduced twice from outside-in in the posteromedial or posterolateral corner: one just below and the second above the native meniscal rim. The looped wires are picked up and pulled out again through the portal. Next, the posterior horn pull suture and the posteromedial/lateral pull suture are pulled through using the double looped metal wire and the double looped suture pass wire. The prepared allograft is subsequently introduced into the compartment throughout an enlarged portal by pulling progressively on the pull suture and the posterior horn pull suture. Care should be taken that the graft does not flip upon introduction and that pull wires do not intertwine. Risk for intertwining wires is greatly reduced by using a double loop metal wire for the posterior horn.

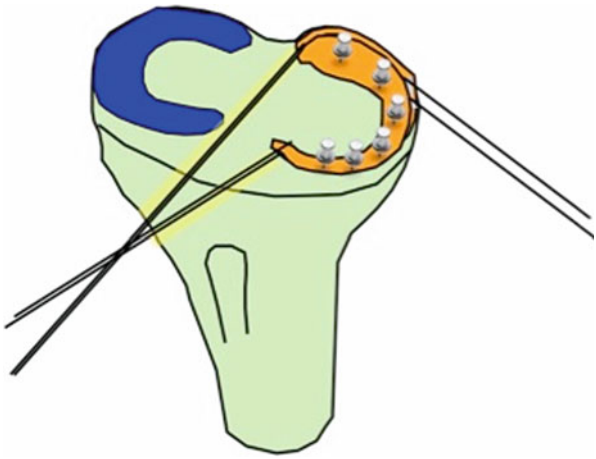

Fig. 15.4 Diagram illustrating the meniscus allograft with two transosseus fixation in combination with all-inside and inside-out sutures. The posteromedial holding suture is also illustrated

The posterior horn is now positioned correctly. Its position can be slightly modified more towards the posteromedial/lateral corner or more towards the posterior horn by pulling more on the posteromedial/lateral or posterior horn traction wire. One or two all-inside meniscal fixation devices (Fastfix, Smith and Nephew, Memphis, Tennessee, USA) are used to fix the allograft to the meniscal rim. Fixation should be started in the posteromedial/lateral corner. Subsequently inside out horizontal Ethibond 2/0 sutures are used for fixing the body of the allograft. The anterior horn is fixed using outside in PDS or Ethibond 2/0 sutures.

Prior to making the sutures knots, the anterior horn is introduced into the knee joint and the anatomical insertion site is identified and prepared is a same manner as for the posterior tunnel. If necessary, its position can be slightly adapted to the graft position. Similar to the procedure of the posterior horn, the anterior tunnel is prepared and the traction suture is pulled through.

First, the meniscal inside out sutures are knotted. Subsequently, the anterior and posterior horn traction sutures are knotted to each other over a bone bridge on the anteromedial side of the tibia. This procedure reduces the possibly stretched capsule and native meniscal rim tied to the meniscal allograft, by pulling on the anterior and posterior horn by a transosseus suture fixation.

15.3.5 Rehabilitation

Rehabilitation is initially focused on providing mobility to the joint without endangering ingrowth and healing of the graft. Therefore, 3 weeks of non-weight-bearing are prescribed followed by 3 weeks of partial weight bearing (50 % of body weight). Progression to full weight bearing is allowed from week 6 on to week 10 postoperatively. The use of a knee unloader knee brace is advised for a period of 3–6 months. For the same reasons, range of motion is limited during the first 2 weeks from 0 to 30, to

increase by 30° each 2 weeks. Isometric muscle tonification and co-contraction exercises are prescribed from day 1 post-surgery on. Straight leg raise however, is prohibited during the first 3 weeks. Proprioception training is started after week 3. Swimming is allowed after week 6, biking after week 12 and running is progressively promoted starting at week 20.

15.4 Results

All mid- and long-term studies have shown that medial and lateral meniscal allograft transplantation significantly reduces pain and improves function of the involved knee joint [5, 8]. Previous studies have shown that risk factors for failure and reduced survival time are lower limb malalignment, ACL deficiency and grade IV cartilage lesions. Moreover, the additional beneficial effect of a corrective osteotomy in case of a varus malalignment, and the importance of a stable knee joint have been clearly demonstrated. The exact position of an associated corrective osteotomy in the valgus knee needs further refinement. More recent studies have not confirmed a significant correlation between the initial cartilage status and clinical failure, challenging the contraindications for osteoarthritis severity.

15.4.1 Conclusion

Since the first human meniscal transplantation in 1984, thousands of patients have received allografts with more than one thousand documented cases in the literature. Ligamentous instability, axial malalignment and/or cartilage lesions should be concomitantly addressed. The consistently high clinical success rates and the acceptable incidence of nonmajor complications make meniscal allograft transplantation a reliable solution for postmeniscectomy symptoms in selected patients. It enables them to resume high levels of activity/productivity and works, at least, as a long-term "bridging" procedure before arthroplasty. Meniscal allograft transplantation is safe, reliable and should no longer be considered experimental.

15.5 Prosthetic Polycarbonate-Urethane Meniscus Implant for the Treatment of *Medial Meniscus Deficiency*

15.5.1 Introduction

While, in recent years meniscal scaffolds have emerged as a treatment option for younger patients, less than 45 years of age, the efficacy of such treatment options

may decline with age and with the progression of symptoms of osteoarthritis. At a later age, e.g. >65, clinicians often choose to practice more invasive treatment arthroplasty options to treat joint pain by performing unicompartmental or total joint replacement. Based on the above, there is a clear treatment gap creating a need to accommodate middle-aged patients with a non-biological treatment option which can delay more aggressive arthroplasty treatments by relieving pain associated with meniscal dysfunction and the associated joint overload.

A prosthetic medial meniscal implant is proposed as a bridge treatment for middle-aged patients suffering from joint pain associated with a dysfunctional meniscus. The concept of a meniscal implant with a reliable biomechanical performance which does not rely on regeneration has thus far not been offered in the clinical practice. Clinical indications for the use of such implant must be determined.

15.5.2 Methods and Materials

A composite, non-fixed, self-centering, discoid-shaped meniscus implant composed of polycarbonate-urethane, reinforced circumferentially with UHMWPE fibers was produced (NUsurface, Active Implants Corp, Memphis, TN). The concept of a non-anchored device was considered since it allows a simple implantation, through a mini-arthrotomy and without damaging the bone, cartilage or ligaments, thus leaving all successive treatment options open. The implants form was based on extensive MRI study that included the geometrical analysis of more than 100 knee scans, and differs from previous inter-positional devices by its distinct curb which runs along the tibial spine and femoral notch to restrict excessive motion and dislocation. Biomechanical optimization of the material properties of the implant was based on *in-vitro* measurements of contact pressure under the implant in cadaver knees and computational finite element (FE) analyses [9]. The last pre-clinical stage was a sheep study in which an extensive quantitative cartilage evaluation was conducted microscopically, post implantation [10]. The material properties of the device were tailored to provide it with an optimal pressure distribution ability, to reduce cartilage loads and thus, relieve pain. Being able to conform moderately under load, without risking its integrity is another important feature of this concept which distinguishes it from other inter-positional devices.

15.5.3 Surgical Procedure

Briefly, in a first stage the remaining meniscus tissue is debrided to a stable meniscus rim. The continuity of the meniscus rim and horns is checked, the stability of the cruciate ligaments is documented and the cartilage degeneration is evaluated. Subsequently, a mini-arthrotomy of approximately 5 cm is made over the medial compartment, the appropriately sized trial implant is introduced into the medial compartment and its

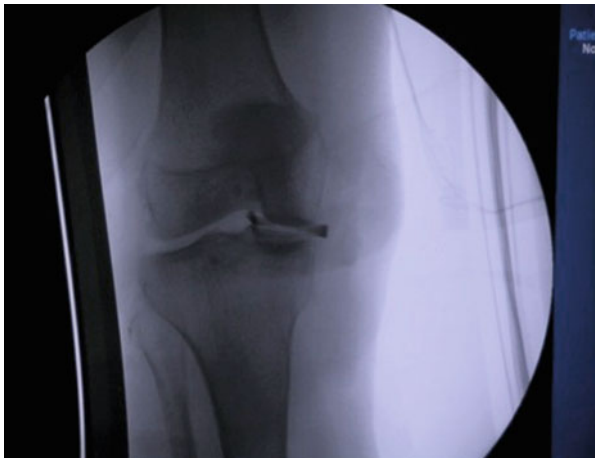

Fig. 15.5 Prosthetic trial meniscus implant in extension

stability and lift-off of are evaluated clinically and using fluoroscopy (Fig. 15.5). If correct sizing and biomechanical behavior is confirmed, the final implant is introduced.

15.5.4 Results

The *in-vitro* evaluation of the final implant in cadaver knees showed that pressure distribution maps under the fiber reinforced PCU were similar to those attained for the natural meniscus. Importantly, the contact area was predominantly in the outer third of the tibial plateau surface and was not concentrated in the central region. It was found that the synthetic meniscus performs equally well in distributing joint loads in a 5 % range around the 'true' size. Additional kinetic evaluation in cadaver knees using fluoroscopy, demonstrated good functionality in terms of maintaining contact with the cartilage and smoothness of motion due to the self-adjustment ability of the implant. A multicenter European trial is currently being conducted. Although the clinical results should still be considered very preliminary, a clear and significant clinical improvement based on KOOS could be observed in all patients.

15.5.5 Conclusions

The proposed implant concept is considered a feasible treatment option for patients suffering from medial pain associated with dysfunctional meniscus due to tear or previous meniscectomy in the middle-aged patient. It was found to reduce

cartilage contact pressures to normal levels, without relying on tissue regeneration. With its simple implantation, and joint sparing use, this implant has good potential to postpone more aggressive treatment options to a later age. Currently, the device is under clinical investigation for safety and efficacy in a multicenter European study.

15.6 General Conclusion

Meniscus scaffolds are indicated for partial meniscus defects (ideally less than 5 cm) while meniscus allografts are indicated in larger defects. Prosthetic meniscus implants are a promising new tools for meniscus deficiency but are currently still under investigation.

References

1. Metcal MH, Barett GR. Prospective evaluation of 1485 meniscal tear patterns in patients with stable knees. Am J Sports Med. 2004;32:675–80.
2. Rodkey WG, Steadman JR, Li ST. A clinical study of collagen meniscus implants to restore the injured meniscus. Clin Orthop Relat Res. 1999;367(Suppl):S281–92.
3. Verdonk R, Verdonk P, Huysse W, Forsyth R, Heinrichs EL. Tissue ingrowth after implantation of a novel, biodegradable polyurethane scaffold for treatment of partial meniscal lesions. Am J Sports Med. 2011;39(4):774–82.
4. Verdonk P, Verdonk R, Huysse W, Heinrichs EL, Laprell H, Siebold R. Successful treatment of painful irreparable partial meniscal defects with a polyurethane scaffold – two year safety and clinical outcomes. Am J Sports Med. 2012;40(4):844–53.
5. Elattar M, Dhollander A, Verdonk R, Almqvist KF, Verdonk P. Twenty-six years of meniscal allograft transplantation: is it still experimental? A meta-analysis of 44 trials. Knee Surg Sports Traumatol Arthrosc. 2011;19(2):147–57.
6. Paletta Jr GA, Manning T, Snell E, Parker R, Bergfeld J. The effect of allograft meniscal replacement on intraarticular contact area and pressures in the human knee. A biomechanical study. Am J Sports Med. 1997;25:692–8.
7. Huang A, Hull ML, Howell SM. The level of compressive load affects conclusions from statistical analyses to determine whether a lateral meniscal autograft restores tibial contact pressure to normal: a study in human cadaveric knees. J Orthop Res. 2003;21:459–64.
8. Cole BJ, Carter TR, Rodeo SA. Allograft meniscal transplantation: background, techniques, and results. Instr Course Lect. 2003;52:383–96.
9. Elsner JJ, Portnoy S, Zur G, Guilak F, Shterling A, Linder-Ganz E. Design of a free-floating polycarbonate-urethane meniscal implant using finite element modeling and experimental validation. J Biomech Eng. 2010;132(9):095001.
10. Zur G, Linder-Ganz E, Elsner JJ, Shani J, Brenner O, Agar G, Hershman EB, Arnoczky SP, Guilak F, Shterling A. Chondroprotective effects of a polycarbonate-urethane meniscal implant: histopathological results in a sheep model. Knee Surg Sports Traumatol Arthrosc. 2011;19(2):255–63.

Part VI
Clinical Studies/Overview Ankle

Chapter 16
Cartilage Repair, Replacement, and Regenerative Strategies for Osteochondral Lesions of the Talus

Samuel B. Adams, Selene G. Parekh, Diego H. Zanolli de Solminihac, Evgeny E. Krynetskiy, Lew C. Schon, and Mark E. Easley

Abstract Osteochondral lesions of the talus present a formidable treatment challenge to the orthopaedic surgeon. Historical cartilage repair strategies often result in the formation of fibrocartilage leading to suboptimal clinical results. With advances in regenerative medicine, modern surgical techniques are diverse and employ autograft, allograft, and tissue-engineered constructs for cartilage repair. Fresh and particulated juvenile allograft transplantation have become popular options in the United States. Wordwide, both cellular and acellular tissue-engineered constructs are utilized. In all cases, there is still debate as to the optimal cell source and scaffold material and only short-term clinical results are available. This chapter will review these current as well as experimental techniques for cartilage repair of osteochondral lesions of the talus.

Keywords Osteochondral lesions of the talus • Particulated juvenile allograft transplantation • Fresh structural allograft transplantation • Tissue-engineered • Autologous chondrocyte implantation • Matrix-induced autologous chondrocyte implantation • Collagen scaffold • Bone marrow aspirate concentrate • Stem cells

S.B. Adams, MD (✉)
Department of Orthopaedic Surgery, Duke University Medical Center,
4709 Creekstone Drive, Suite 200, Durham, NC 27703, USA
e-mail: samuel.adams@duke.edu

S.G. Parekh, MD, MBA • L.C. Schon, MD
Department of Orthopaedic Surgery, Duke University Medical Center,
North Carolina Orthopaedic Clinic, 3609 Southwest Durham Drive, Durham, NC 27707, USA

D.H.Z. de Solminihac, MD
Department of Orthopaedic Surgery, Clinica Alemana, 5951 Vitacura Avenue,
Santiago, Chile

E.E. Krynetskiy, MD
Joint Implant Surgeons of Florida, 7331 College Parkway, Suite 300,
Fort Myers, FL 33907, USA

M.E. Easley, MD
Department of Orthopaedic Surgery, Duke University School of Medicine,
Durham, NC, USA

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_16,

Key Points

- The preferred nomenclature for any pathology of the talar articular cartilage and corresponding subchondral bone is osteochondral lesion of the talus (OLT).
- Many surgical techniques are available for the treatment of OLTs. These techniques involve cartilage repair, replacement, or regeneration. However, there is a lack of generally agreed-upon surgical guidelines and a paucity of comparative studies examining these techniques.
- Talar articular cartilage is distinctly different from other joint cartilage and therefore, results of cartilage repair, regeneration, and replacement from other joints cannot simply be inferred to OLTs.
- In our clinical experience, conservative management of symptomatic OLTs offers little success with regard to pain relief and functional improvement and surgical management is often performed.
- Cartilage debridement and marrow stimulation techniques (i.e. microfracture) are the most commonly employed surgical treatments because of their relatively simple techniques and low cost. The success rate for these procedures as a group ranges from 65 to 90 %.
- In general, microfracture should not be used in lesions with an average (longitudinal and transverse) diameter greater than 15 mm, an area greater than 150 mm^2.
- Autologous chondrocyte implantation (ACI) is a popular treatment option for OLTs deemed inappropriate for microfracture or after failed microfracture. The literature is lacking to draw concrete conclusions about ACI, but it seems from the available studies that ACI can be successfully used on OLTs with cartilage lesions >15 mm average diameter and those with subchondral cystic areas.
- More recently, the ACI technique has been modified to allow chondrocytes to be delivered to a cartilage defect in a biodegradable scaffold. This technique is termed matrix induced autologous chondrocyte implantation (MACI) obviates the need for periosteal harvesting, thereby reducing the operative time and potential postoperative complications. Additionally, the matrix eliminates the potential of the cells from leaking under the periosteal flap, uneven distribution of the cells, or periosteal hypertrophy, all concerns with the ACI technique.
- Osteochondral autografts are a promising single-stage procedure for delivering hyaline cartilage to the OLT. However, there are concerns about donor site morbidity.
- Osteochondral allografts negate the concerns of donor site morbidity but the quality of the cartilage and potential disease transmission remain concerns of these techniques.
- Increasing attention is being paid to tissue engineering and biologic techniques for cartilage regeneration.

16.1 Introduction

The term osteochondral lesion of the talus (OLT) refers to any pathology of the talar articular cartilage and corresponding subchondral bone. A variety of names have been given to these lesions, including osteochondritis dissecans, osteochondral fracture, transchondral fracture, and osteochondral defect, but currently, OLT is the preferred nomenclature. Alternatively, osteochondral lesion (OCL) of the talus is used. Regardless of name, these lesions present a challenging problem for orthopaedic surgeons secondary to the poor intrinsic reparative capacity of cartilage. Historically, debridement and an attempt to stimulate cartilage repair (marrow stimulation) was the mainstay of treatment. With advances in regenerative medicine, modern surgical techniques are diverse; employing autograft, allograft, and alternate cell sources for cartilage repair. However, despite the myriad treatment options, there is a lack of generally agreed-upon surgical guidelines and a paucity of comparative studies examining these techniques. The purpose of this chapter is to introduce the reader to the various techniques for cartilage repair, replacement, or regeneration for OLTs (Table 16.1).

16.2 Etiology, Presentation, and Classification

A plethora of information concerning etiology and classification of OLTs has been presented in multiple formats. This body of knowledge is so vast and is beyond the intent of this chapter. A brief introduction will be included.

Kappis [1] initially described this pathology as osteochondritis dissecans, suggesting spontaneous necrosis of bone as the primary etiology. However, contemporary data supports trauma as the cause of most OLTs, with repetitive micro-trauma, avascular necrosis, and congenital factors as the remaining etiologies [2]. In a review of 582 patients with OLTs, ankle trauma was reported by 76 % of patients [3].

In a cadaveric study, Berndt and Harty [4] proposed the mechanisms by which traumatic OLTs occur. Axial loading of the ankle with the foot inverted and dorsiflexed produced lateral talar dome lesions. Conversely, axial loading of the ankle with the foot inverted and plantarflexed, with external tibial rotation, produced

Table 16.1 Classification of OLT cartilage treatment strategies

Repair	Replacement	Regeneration
Marrow stimulation (microfracture)	Osteochondral autograft transfer (OAT)	Autologous chondrocyte implantation (ACI)
Retrograde drilling	Osteochondral allografting	Matrix induced chondrocyte implantation (MACI)
	Particulated juvenile cartilage allograft transplantation	Bone marrow derived cell transplantation
	Metallic resurfacing	

Table 16.2 Berndt and Hardy classification of OLTs [4]

Stage	Radiographic findings
I	Focal subchondral bone compression
II	Focal subchondral bone compression with partial detachment (partially noncontiguous but not displaced)
III	Focal subchondral bone compression with complete detachment (completely noncontiguous but not displaced)
IV	Focal subchondral bone compression with complete detachment and displaced

medial talar dome lesions. These biomechanical mechanisms can occur with both ankle fracture and ankle sprain. Alexander and Lichtman [5] observed that associated ankle fractures occur with 28 % of OLTs. Van Bueken et al. [6] reported that OLTs occur in 6.5 % of ankle sprains. However, Takao et al. reported OLTs in 38 % of patients with residual ankle disability after ankle sprain [7]. Tibial lesions are rarely seen with traumatic OLTs. This might be secondary to significantly increased stiffness seen in tibial cartilage [8].

Tol et al. [3] reported that 56 % of OLTs were located medially, and 44 % were located laterally. Of the medial lesions, trauma was implicated in only 62 %, whereas trauma was implicated in 94 % of the laterally located lesions. Elias et al. [9] reported similar results regarding location, in an MRI examination of 424 OLTs. The talar dome was divided into nine equal size zones. Sixty-two percent of lesions were located medially, whereas 34 % were located laterally. In the sagittal plane, 80 % of lesions were located centrally. The medial-central zone was the most common location for lesions (53 %). The authors also reported that medial lesions were significantly larger and deeper.

An OLT should be suspected in anyone presenting after acute traumatic injury to the ankle, chronic ankle sprains, or chronic instability. Patients may complain of pain, stiffness, catching, and swelling of the ankle [10]. However, none of these complaints are specific to OLTs.

Often in the acute setting, a detailed examination is limited secondary to pain and swelling. The ankle and foot should be palpated for areas of tenderness. Ankle range-of-motion (ROM) should be recorded and compared to the contralateral extremity. Ankle stability, including the anterior drawer and talar-tilt tests should be performed and compared to the contralateral extremity.

The differential diagnosis of OLTs is wide and includes: occult fracture of the foot or ankle, tarsal coalition, syndesmosis injury, synovitis, degenerative arthrosis, peroneal tendonitis, soft-tissue or bony impingement, ankle instability, or subtalar arthritis.

The radiographic classification most widely used today was introduced in 1959 by Berndt and Harty (Table 16.2) [4]. However, this classification system has been criticized as having poor correlation with arthroscopic findings. Pritsch et al. [11] arthroscopically examined 24 OLTs at an average follow-up of 30 months. Fifty percent of lesions classified as stage IV (displaced) according to the Berndt and Hardy [4] system were found to be intact under arthroscopic visualization. They concluded that there was lack of correlation between radiographic appearance and

the findings at arthroscopy. These authors devised an arthroscopic grading scheme: grade I, intact, firm, shiny cartilage; grade II, intact but soft cartilage; grade III, frayed cartilage. Ferkel et al. [12] expanded on this classification system. However, it is easy to conceive that a purely radiographic classification system does not properly address the damage to the cartilage, and an arthroscopic classification system does not properly address the damage to the subchondral bone. Therefore, with the advent of newer imaging modalities, several authors have proposed CT or MRI classification schemes. However, these systems are not much different from the original Berndt and Hardy system [12–14]. MRI has been demonstrated to be 81–92 % accurate in staging OLTs [15–17]. Currently, it is unclear as to if any classification system can be used as a guide for treatment. However, what can be used to guide treatment are the characteristics of the OLT, including intact versus disrupted articular surface, displaced versus non-displaced, and cystic versus non-cystic [10].

16.3 Talar Cartilage

Talar articular cartilage is distinctly different from other joint cartilage and therefore, results of cartilage repair, regeneration, and replacement from other joints cannot simply be transposed to OLTs. Talar cartilage is thinner than knee and hip cartilage. The mean thickness of talar cartilage is 0.89 mm, whereas the mean thickness of femur, patella, and tibial plateau cartilage thickness is 2.0, 3.33, and 2.92 mm, respectively [18, 19]. Compared to knee cartilage, ankle cartilage has a higher content of proteoglycans, as well as an increased rate of proteoglycan and collagen turnover and synthesis, contributing to increased compressive stiffness and reduced permeability [20–22]. Additionally, ankle cartilage is less responsive to catabolic stimulation and more responsive to anabolic stimulation than knee cartilage [23]. These differences are likely adaptations of ankle cartilage to withstand the increased force per unit area compared to knee and hip cartilage [24]. In fact, Kempson [25] demonstrated that femoral articular cartilage resistance to fissuring decreases 67 % with age (from 7 to 90 years) compared to only 20 % for talar articular cartilage. Additionally, the tensile stiffness of femoral articular cartilage decreases 45 % but only 20 % in the talus.

16.4 Nonoperative Treatment

The initial treatment for a newly diagnosed OLT should be based on the patient's age, symptoms, chronicity, and stage of the lesion. Incidentally found asymptomatic lesions do not need treatment but should be followed with serial radiographs. For symptomatic non-displaced lesions, some authors recommend a trial of conservative management for a period of 3–6 months [26–28]. Nonoperative modalities include protected weightbearing, physical therapy, and NSAIDS. Protected

weightbearing can range from cast immobilization and nonweightbearing status to weightbearing as tolerated in a walking boot.

One study attempted to treat OLTs with intra-articular injection of platelet-rich plasma (PRP) or hyaluronic acid (HA) [29]. Fifteen ankles received three weekly injections of HA and another 15 ankles received a total of three PRP injections (initial, 2 and 4 weeks later). Patients were assessed using a modified ankle-hindfoot scale and a visual analog scale for pain. After a short follow-up of 28 weeks, both groups demonstrated significant improvement in the ankle-hindfoot questionnaire and pain scores. However, the magnitude of improvement was significantly greater in the PRP group. This study demonstrated the potential positive benefit of PRP injections but more literature is needed before widespread usage is agreed upon.

Based on the available literature, no specific recommendations can be given regarding weightbearing status, type of immobilization, or length of therapy. The long-term outcome of conservatively treated OLTs is not well understood. Bauer et al. [26] performed a retrospective study of 30 patients with radiographically diagnosed OLTs. At a mean follow-up of 21 years, the OLTs demonstrated only minimal change in size and only two ankles were found to have radiographic signs of osteoarthritis. However, in our clinical experience, conservative management of symptomatic OLTs offers little success with regard to pain relief and surgical management is often performed.

16.5 Operative Treatment

Chronic lesions, lesions that remain symptomatic despite 3–6 months of nonoperative treatment, or displaced OLTs of any chronicity should be considered for operative treatment. Many operative therapies have been described for OLTs. In order to choose the most appropriate treatment option, several characteristics of the OLT are important for operative planning, including location, size, and quality of the subchondral bone. These characteristics can be elucidated through a series of imaging studies.

16.5.1 Preoperative Assessment

Every patient should have weightbearing anteroposterior (AP), lateral, and mortise radiographic views of the ankle joint. A debate exists as to the choice of MRI or CT following negative plain radiographs in a patient with a suspected OLT. Verhagen et al. [30] reported no significant difference in the sensitivity or specificity of MRI, CT, or arthroscopy in the diagnosis of OLTs. On the contrary, Anderson et al. [13], in a series of 14 OLTs that were not evident on plain radiographs, reported that CT scanning only identified 4 of these lesions, whereas MRI identified all 14. Additionally, an MRI may identify other bony or soft-tissue pathology involved in

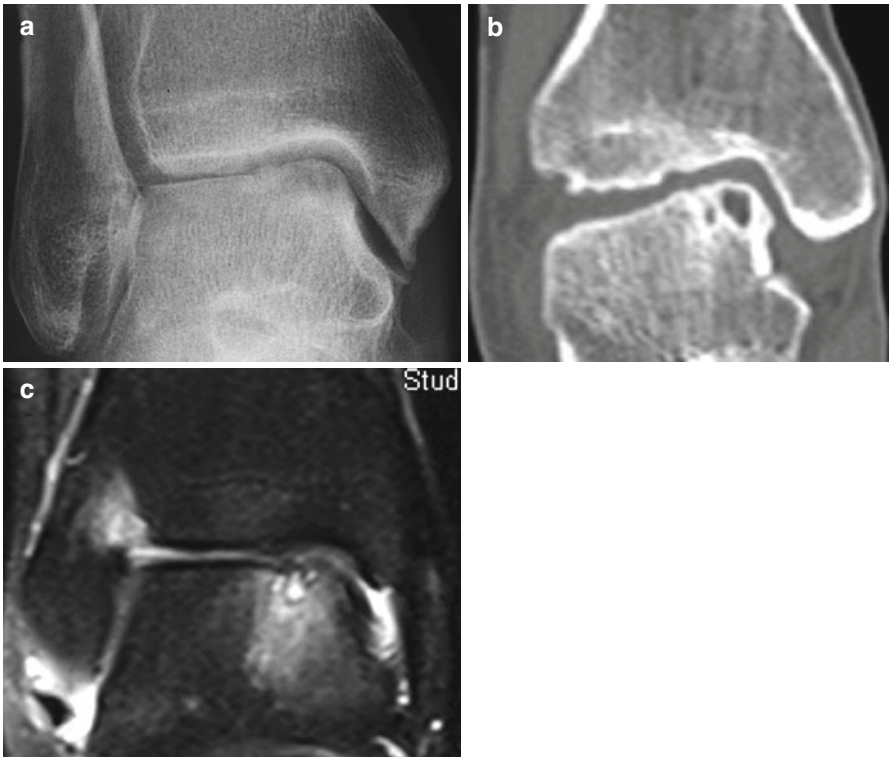

Fig. 16.1 Anteroposterior plain radiograph (**a**), coronal CT scan (**b**), and coronal MRI (**c**) images of a medial OLT depicting the different characteristics of the lesion that can be obtained from the different imaging modalities

a painful ankle, and therefore should be obtained in a patient with persistent ankle pain when an OLT is suspected but the plain radiographs are negative.

In a known OLT, MRI is better at visualizing the articular surface, whereas CT is better at assessing the subchondral bone (Fig. 16.1). Stroud and Marks [31] proposed the following algorithm regarding OLTs diagnosed on plain radiographs. If the OLT is nondisplaced, an MRI is recommended to evaluate the integrity of the articular cartilage and assess the true stability of the lesion. If the lesion appears displaced on plain radiographs, a CT scan is preferred to accurately assess the lesion size and location. Additionally, in some cases where an OLT is diagnosed via MRI, a CT scan can be beneficial for determining the treatment modality, as estimation of the size and stage of the lesion can be obscured by bone-marrow edema on MRI [15]. We routinely obtain both an MRI and a CT scan to aid in treatment decision making.

Recently, the combined imaging modality of single-photon emission computed tomography-computed tomography (SPECT-CT) has been used for the diagnosis of OLTs and the subsequent treatment decision making [32, 33]. The SPECT-CT allows localization of scintigraphic osteoblastic activity around the OLT in

combination with the anatomic resolution of a CT scan, providing both morphologic and biologic information about the OLT. However, with SPECT-CT, the cartilage cannot be directly interpreted and an MRI is warranted.

16.5.2 Cartilage Debridement and Marrow Stimulation Techniques

These arthroscopically assisted techniques include debridement (chondroplasty) with microfracture, abrasion arthroplasty, and antegrade drilling. These techniques are typically used as the initial operative management after failed conservative treatment and are intended to penetrate the subchondral bone, providing a pathway for bone marrow precursor cells and cytokines to populate the lesion (Fig. 16.2). The cartilage formed after marrow stimulation techniques is not hyaline cartilage. Typically, fibrocartilage is formed which consists of both type I and type II collagen, whereas true hyaline cartilage is composed of mostly type II collagen. Although undoubtedly better than exposed subchondral bone, fibrocartilage has been shown to be biomechanically weaker than hyaline cartilage after microfracture in the knee [34].

Success rates for marrow stimulation techniques range from 65 to 90 % [3, 35–38]. Because these techniques are relatively simple and inexpensive, historically, they have been used on a variety of OLTs with different characteristics. However, newer literature is starting to define the most appropriate lesion type for marrow stimulation. One study noted that younger patients with traumatic lesions and a shorter interval between injury and drilling had improved results, while patients with subchondral cysts had poorer outcomes [39].On the contrary, Choi et al. [40] demonstrated that age was not a significant predictor of postoperative outcome, but size and the number of associated intra-articular lesions were predictors of a poor outcome. Becher and Thermann [41] reported on the outcome of microfracture and found no correlation with patient age, grade or location of the defect, only degenerative post-traumatic lesions with arthrosis had less satisfactory results. Kelberine and Frank [35] reported a more favorable outcome for lesions that were treated acutely than for those that had become chronic, 85 and 67 %, respectively.

OLT size is an important characteristic in treatment decision making. Chuckpaiwong et al. [42], reported on a series of 105 osteochondral lesions of the ankle (tibial and talar) treated with ankle arthroscopy, debridement, and microfracture. They found that lesion size was the overwhelming variable influencing success. There were no treatment failures in lesions with an average (longitudinal and transverse) diameter less than 15 mm. However, only one (3 %) patient had a successful outcome with a lesion $\geq$15 mm. Similar work by Choi et al. [43], reports a cut-off cartilage defect area of 150 mm^2, based on MRI imaging, for successful clinical outcome.

Marrow stimulation techniques have been employed in OLTs where the underlying bone has been replaced with cysts. Kumai et al. [39] suggested that poor results are to be expected in the management of OLTs associated with subchondral

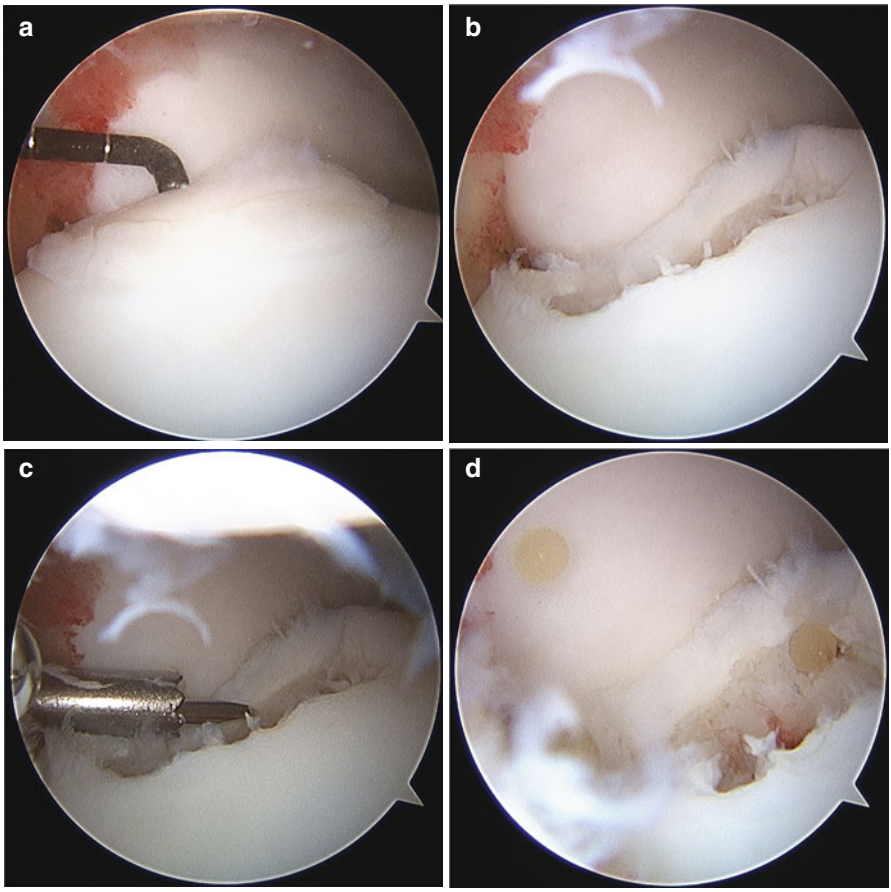

Fig. 16.2 (**a**) An arthroscopic image of a lateral OLT. (**b**) The same lesion has now been debrided. (**c**) Marrow stimulation was performed. (**d**) Marrow elements are seen egressing from marrow stimulation holes

cysts, irrespective of treatment method. Robinson et al. [36] reported a 53 % poor outcome in debridement/curettage and drilling of OLTs with subchondral cysts. However, Han et al. [44] performed arthroscopic microfracture or abrasion arthroplasty on 20 OLTs with subchondral cysts, and 18 OLTs without cysts. At a minimum follow-up of 2 years, there were no differences in AOFAS scores between the two groups. At final post-operative assessment, the cystic area significantly decreased. All of the cystic lesions in this study, as measured by AP radiographs, were less than 1.5 cm^2. Saxena and Eakin [45] found that the time to return to activity was longer for cystic lesions treated with bone grafting than it was for non-cystic lesions treated with microfracture (19 versus 15 weeks), but that functional scores were not different.

Gobbi et al. [46] performed a prospective randomized trial of chondroplasty alone, microfracture, or osteochondral autograft for the treatment of OLTs in 31

patients. At a minimum of 2-year follow-up, there was essentially no difference in outcome scores among the three treatment groups.

The authors routinely perform debridement and marrow stimulation as the initial surgical treatment for lesions less than 15 mm in diameter without cystic changes.

Second-look arthroscopy findings following marrow stimulation have rarely been reported. One study reported on 20 ankles that underwent second-look arthroscopy at 12 months after microfracture. The cartilage was graded according to the Ferkel and Cheng [47] staging system and the International Cartilage Repair Society (ICRS) Score [48].According to the Ferkel and Cheng staging, 35 % of the lesions demonstrated incomplete healing. With regard to the ICRS grades, 60 % of the lesions were grade I or II (normal or near normal) and 40 % were grade III (abnormal).

Although rare, complications with these techniques have been reported and include: superficial and deep infection, deep vein thrombosis, stiffness requiring manipulation, plantar fasciitis, complex regional pain syndrome, and saphenous and superficial peroneal nerve injury secondary to arthroscopic portal placement [10].

Repeat debridement and marrow stimulation after failed initial surgery may provide some benefit. Olgilvie-Harris and Sarrosa [49] reported on eight patients who underwent arthroscopic debridement to bleeding bone after previous open debridement. At a mean of 38 months follow-up, significant improvement of pain was maintained. The authors concluded that the failure of the initial open procedures was due to lack of debridement to bleeding bone. Likewise, Savva et al. reported improvement in AOFAS ankle-hindfoot scores, at a mean of 5.9 years of follow-up, in 12 patients who underwent repeat debridement. Mitchell et al. [50] perform repeat debridement in athletes desiring an early return to sport and in lesions of $<1\ cm^2$.

Typically, we do not perform repeat debridement and marrow stimulation on our own patients. If the patient was initially treated at an outside facility and the extent of debridement is unknown, we do entertain the idea of a repeat debridement and microfracture.

16.5.2.1 Postoperative Supplementation of Marrow Stimulation

Recently, Doral et al. [51]. reported on postoperative injection of hyaluronan after microfracture. Fifty-seven patients with 57 OLTs were prospectively randomized to receive microfracture only (16 patients) or microfracture plus three weekly injections of 12.5 mg/1.25 ml hyaluronan (41 patients). The injections started in the third postoperative week. All patients were available for 2 year follow-up. Both groups demonstrated significant improvement in the Frieburg scoring system and AOFAS ankle-hindfoot questionnaire. However, in both of these scoring systems, the magnitude of improvement was significantly greater in the microfracture plus hyaluronan injection group. The primary author routinely performs bone marrow aspirate concentrate injection into the ankle joint after microfracture. Although no human OLT data is available to support this practice, Fortier et al. [52] demonstrated

superior results with the application of bone marrow aspirate concentrate after microfracture to microfracture alone in an equine model of full-thickness cartilage defects of the knee.

16.5.3 Retrograde Drilling

In unique cases of OLTs where the overlying cartilage is intact, but the subchondral bone is cystic, retrograde drilling without microfracture can be performed with excellent clinical outcomes [39, 53, 54]. This is a rare instance and in fact, there is debate as to whether this situation is truly an OLT. However, when preoperative MRI reveals that the articular surface over the OLT is intact and stable, retrograde drilling and potentially bone grafting can be entertained. We routinely perform diagnostic arthroscopy to confirm the integrity of the cartilage as well as to ensure that healthy cartilage is not penetrated during retrograde drilling. Kono et al. [54] reported on 11 patients whom underwent retrograde drilling for symptomatic OLTs. Second-look arthroscopy, performed at 1 year after surgery, demonstrated that none of the lesions deteriorated. Additionally, the AOFAS ankle-hindfoot score was significantly improved in this patient population at 2 year follow-up. Autologous bone graft, bone marrow aspirate concentrate, or bone graft substitutes can be used to fill large areas of avascular necrosis.

16.5.4 Autologous Chondrocyte Implantation

Autologous chondrocyte implantation (ACI) is a staged technique, typically performed after failed conservative treatment or microfracture [55].The defect should be focal and be well contained by a peripheral rim of intact cartilage. In the first stage, hyaline cartilage is harvested from an appropriate donor site, such as the anterior talus [56] or interchondylar notch or other nonweightbearing portion of the ipsilateral knee [55]. The harvest typically yields 200–300 mg of cartilage. The cells are cultured for a variable amount of time (3–8 weeks) to increase the number of chondrocytes, but can be stored for >1 year [50]. The cells are delivered to the OLT in a second stage procedure. Typically, the filled defect is covered with a periosteal patch, from the tibia, that is sewn into place, or alternatively, the chondrocytes are carried in a matrix [57], obviating the need to apply a periosteal patch. The latter procedure is termed matrix-induced chondrocyte implantation (MACI).

Like marrow stimulation techniques, ACI has generally had favorable results. Nam et al. [55] reported on 11 patients with OLTs. All had failed previous surgery including debridement, drilling, pinning, or abrasion arthroplasty. The mean size of the OLTs was 273 mm^2(range, 80–500 mm^2). Six patients had extensive subchondral cysts that were debrided and bone-grafted at the time of implantation. At a mean follow-up of 38 months, there was significant improvement in the AOFAS

ankle-hindfoot score and the Tegner activity score. Nine (82 %) patients reported good or excellent results, and would have the surgery again. There was no correlation with the size of the OLT, or presence of a cyst and outcome. However, these authors mention that ACI should not be performed on OLTs with a cartilage defect of >4 cm^2. Similarly, Baums et al. [58] reported on 12 OLTs with a mean size of 2.3 cm^2 (range, 1.0–6.25 mm^2). At a mean follow-up of 63 months, there was significant improvement in the AOFAS ankle-hindfoot score. These authors did not mention the number of patients, who failed previous attempts at microfracture or drilling, but these patients were included in the study.

Whittaker et al. [59] reported their results in 10 patients with 4-year follow-up. The lesions had a mean area of 1.95 cm^2. Eight of 10 patients had failed some type of prior arthroscopic debridement. Cartilage was harvested from the ipsilateral knee. Ninety percent of the patients were "pleased" or "extremely pleased" with the outcome. Nine patients underwent second-look arthroscopy at a mean of 13 months. All lesions were macroscopically filled. Biopsies were performed in five patients, with two biopsies containing hyaline cartilage and three biopsies containing fibrocartilage. The Lysholm knee score normalized in three patients and remained reduced by 15 % in seven patients.

Zenerink et al. [60] reported on 11 patients who underwent ACI for a mean lesion size of 13.1 mm by 20.7 mm. All patients had failed prior surgery. The mean follow-up period was 38 months (range, 24–60 months). Ten patients reported improvement. Outcomes were good to excellent in 9/11 patients. The AOFAS ankle-hindfoot score improved from 47.4 preoperatively to 84.3 postoperatively. Second-look arthroscopy was performed in 10 patients at a mean of 14.2 months after surgery. Complete defect coverage was seen in all 10 patients but the cartilage at the repair site was noted to be softer than the surrounding native articular cartilage. The authors subjectively observed a correlation between increased firmness of the graft and time from implantation. Periosteal overgrowth was noted in two patients.

Lee et al. [61] evaluated the factors influencing the results of ACI. They performed second-look arthroscopy on 38 patients who received ACI 1 year earlier. A modification to a magnetic resonance scoring system (MOCART) was used to assess the cartilage through arthroscopy. The authors examined the relationship of age, sex, location, depth, size, preoperative AOFAS score, and additional procedures to outcome. The authors found that lesion size and patient age significantly affected the quality of cartilage repair. The authors chose to classify the lesion size based on whether it was greater or less than 137.6 mm^2 which was the average size of the lesions in this patient population. Interestingly, patients with lesions greater than 137.6 mm^2 had significantly better modified MOCART scores. Patients with age less than 26 years old also had significantly better modified MOCART scores.

The use of the detached cartilage fragment of OLTs as a cell source for ACI has been investigated. Giannini et al. [62] harvested the detached cartilage fragments from 20 patients with chronic traumatic OLTs. The authors found a 99.9 % chondrocyte viability rate. Immunohistochemistry analysis revealed a positive signal for type-II collagen and no signal for type-I collagen in the chondral fragments. Sixteen

of the patients received expanded cells from their detached fragment during the ACI procedure. These patients experienced significant improvement in the AOFAS ankle-hindfoot score at a minimum of 12 months. The magnitude of improvement in this group was not statistically different from a group of patients receiving ACI with chondrocytes from the ipsilateral knee. However, Candrian et al. [63] demonstrated that chondrocytes from OLTs have inferior cartilage-forming capacity when compared to normal ankle cartilage. Specifically, the chondrocytes from OLTs demonstrated significantly lower amounts of DNA, glycosaminoglycan (GAG), and collagen type II. Additionally, they were found to have a statistically greater amount of collagen type I.

The literature is lacking to draw concrete conclusions about ACI, but it seems from the available studies that ACI can be successfully used on OLTs with cartilage lesions >15 mm average diameter and those with subchondral cystic areas. In fact, van Bergen et al. [64] prefer the lesion to be greater than 15 mm in diameter for ACI.

16.5.5 Matrix Induced Chondrocyte Implantation

More recently, the ACI technique has been modified to allow chondrocytes to be delivered to a cartilage defect in a biodegradable scaffold. This technique is termed matrix induced autologous chondrocyte implantation (MACI) or less often, matrix-associated autologous chondrocyte transplantation (MACT) and is considered a second generation of ACI. The use of a matrix obviates the need for periosteal harvesting, thereby reducing the operative time and potential postoperative complications. Additionally, the matrix eliminates the potential of the cells from leaking under the periosteal flap, uneven distribution of the cells, or periosteal hypertrophy, all concerns with the ACI technique [65]. However, MACI remains a two-stage procedure.

Schneider and Karaikudi [66] reported their results on 20 patients who underwent open MACI for full-thickness OLTs at a mean follow-up of 21 months. The cartilage was harvested from the perimeter of the OLT and the matrix was a porcine collagen membrane seeded with 1×10^6 chondrocytes per cm^2. The grafts were secured with fibrin glue. The patients experienced both a significant reduction in pain and an improvement in AOFAS ankle-hindfoot scores. The open technique was performed through malleolar osteotomies which all healed. However, four cases required osteotomy hardware removal. Two of the grafts failed. Six of the patients had second-look arthroscopy and the articular surfaces were determined to have healed.

Giza et al. [67] described their results of ten patients who underwent MACI using either an anteromedial or anterolateral arthrotomy with plafondplasty without a malleolar osteotomy. The cartilage was harvested from the perimeter of the OLT. A type I/III collagen bilayer matrix was used. The authors did not mention the cell-seeding density. Arthroscopic evaluation revealed that the defects, according to the

ICRS grading system, were three grade 3b, four grade 3c, two grade 3d, and one grade 4 lesion. The mean time between cartilage harvest and MACI implantation was 56 days. There was significant improvement in the AOFAS ankle-hindfoot score at 1 year. However, at 2-year postoperative follow-up, there was improvement in the AOFAS score over baseline, but it was no longer significant. However, significant improvement was found in the SF-36 subscores of physical functioning and bodily pain at one and 2 years after surgery. Additionally, subjectively, all patients believed the procedure helped them and there were no failures.

One study performed diffusion-weighted three Tesla magnetic resonance imaging on OLTs treated with MACI or microfracture (10 each) [68]. The patients in each group were matched by age, body mass index, and follow-up for comparison. The treated OLTs were assessed using the magnetic resonance observation of cartilage repair tissue (MOCART) score and diffusion weighted imaging (DWI). There was no difference in postoperative MOCART scores between groups. Additionally, the AOFAS ankle-hindfoot scores significantly improved at final follow-up in both groups but there was not a significant difference in the magnitude of improvement between groups. DWI demonstrated no significant difference between MACI repaired cartilage and healthy control cartilage. However, there was a significant difference in the DWI diffusion quotient between microfracture repair tissue and control cartilage. The authors concluded that although clinically these techniques provided similar results, DWI indicated that quality of cartilage repair may be better with MACI.

Niemeyer et al. [69] performed a meta-analysis of the available data of ACI/MACI for OLTs. The authors systematically reviewed 16 studies that met their inclusion criteria. Six studies used ACI and the rest performed MACI. There were 213 patients included in this analysis with a mean postoperative follow-up of 32 months. The mean defect size was 2.3 cm^2. Various outcomes measures were used with the AOFAS ankle-hindfoot being the most common. The mean clinical success rate was 89.9 % (range, 50–100 %). However, there were no controlled studies. In fact, all studies reviewed in this meta-analysis were classified as case series. This meta-analysis illustrates the lack of controlled clinical trials concerning the treatment of OLTs.

16.5.6 Bone Marrow Derived Cell Transplantation and Platelet-Rich Plasma

Currently, this category of cartilage regeneration encompasses autologous matrix-induced chondrogenesis (AMIC) and other “one-step” techniques that use bone marrow derived cells and/or PRP. These relatively new techniques are varied, but typically combine lesion debridement, microfracture, and then the addition of autologous iliac crest spongiosa bone, bone marrow aspirate concentrate, and/or platelet-rich plasma to the lesion using a collagen matrix carrier and fibrin glue to secure the carrier. The advantages of this technique are the use of autologous tissue, it is a

one-step surgical procedure, it can be performed arthroscopically, and there is minimal to no donor site morbidity (definitely no intra-articular morbidity). Because it is a relatively new technique, there is minimal outcome data.

Giannini et al. [70] reported prospective results on 48 patients at a mean of 29 months follow-up who received a "one-step" arthroscopic transplantation of bone marrow derived cells (BMDCs). In this study, the OLTs were debrided and the subchondral bone was penetrated. The scaffold was either a porcine collagen powder or a hyaluronic acid membrane. Each scaffold was loaded with 2 ml of concentrated bone marrow aspirate and 1 ml of platelet-rich fibrin gel (to provide growth factors). The scaffolds were shaped or cut to the appropriate size and delivered arthroscopically to the lesion. In vitro analysis, although not quantified, demonstrated BMDC viability in each of the scaffolds. The authors also demonstrated chondrogenic and osteogenic differentiation of the BMDCs. There was significant improvement in AOFAS scores for both types of scaffolds at 6, 12, 18, and 24 months. There was no difference in outcomes between scaffolds. Twenty-four month MRIs demonstrated new tissue formation at the lesion site in all patients. Five patients underwent second-look arthroscopy which demonstrated normal appearing cartilage in three patients and hypertrophied cartilage in two patients. Cartilage biopsies demonstrated various stages of tissue remodeling. The authors concluded that this arthroscopic one-step technique provides similar outcomes to other techniques without the disadvantages.

Giannini et al. [71] also compared 56 patients who received ACI (arthroscopic or open-field) to 25 patients who underwent one-step BMDC cell transplantation as previously described. The patients who underwent arthroscopic ACI and the one-step technique demonstrated significant improvement at 36 months. There was not a significant difference of the magnitude of improvement amongst the groups. The only complication in the one-step groups was superficial infection at a portal site. Both MRI and second-look arthroscopy demonstrated moderate cartilage hypertrophy in a small amount of lesions from all groups.

Wiewiorski et al. [72, 73] presented two case reports on patients successfully treated with OLT debridement, microfracture, autologous iliac crest cancellous bone graft and an overlying collagen I/III membrane secured with fibrin glue.

16.5.7 Osteochondral Autograft Transfer

Osteochondral autograft transfer (OAT) techniques are performed to restore hyaline cartilage to the osteochondral defect. One graft, or multiple plugs (mosaicplasty) can be harvested from a non-weightbearing portion of the ipsilateral knee, the anterior talus, or an allograft talus. One theoretical advantage over ACI is the need for only one procedure for harvesting and implantation. However, several complications have been reported with harvesting osteochondral plugs from the ipsilateral knee. They include persistent pain, pain on heavy exertion, patellar instability, giving way, difficulty kneeling or squatting, and the need for additional surgery [10].

Valderrabano et al. [74] reported on 12 patients who underwent knee-to-ankle mosaicplasty for OLTs. At a mean of 72 months of follow-up, these patients reported significant pain relief and improvement in AOFAS ankle-hindfoot scores. However, sports activity remained significantly decreased and ankle dorsiflexion was significantly reduced. Moreover, six patients reported knee pain and 10 patients developed recurrent ankle lesions and demonstrated some degree of joint degeneration. Gobbi et al. [75] reported on 12 patients who underwent osteochondral allografting via one to three plugs harvested from the lateral femoral condyle or trochlear notch. The mean size of the lesions was 3.7 cm^2 (range, 1.2–5 cm^2). The AOFAS ankle-hindfoot score significantly improved at final follow-up. There were no harvest site complications. At a mean of 36 month follow-up, Scranton et al. [76] reported 90 % satisfaction in 50 patients who underwent osteochondral autograft transplantation for cystic OLTs. Similarly, Hangody and Fules [77] reported 94 % good to excellent results in 36 patients at a mean follow-up of 4.2 years who received mosaicplasty.

Alternatively, the osteochondral plugs can be harvested from the talus. Sammarco et al. [78] reported on 12 patients in which osteochondral plugs were harvested from the medial or lateral talar facet. The largest graft size was 8 mm in diameter. At a mean follow-up of 25 months, there was significant improvement in the AOFAS ankle-hindfoot score. The most common complaint was aching over the anterior aspect of the ankle, although this did not detract from activities of daily living or sports. Kruez et al. [79], in series of 35 patients who underwent ipsilateral talus articular facet osteochondral plug harvesting and implantation through either no osteotomy, a medial malleolar osteotomy, or a tibial wedge osteotomy, reported no complications related to graft harvesting. The largest diameter single graft in this series was 10 mm. Two patients required multiple grafts. At a mean follow-up of 48.9 months, there was significant improvement in AOFAS ankle-hindfoot scores. These authors did find significantly better results in patients that did not need an osteotomy to access the lesion and concluded that lesions accessible through an anterior approach without additional osteotomy have the best prognosis. Although there is insufficient evidence in the literature to conclude size limitations of osteochondral autografting, it seems that lesions requiring an osteochondral plug less than 10 mm in diameter can successfully be treated with local talar autografting, avoiding the complications of ipsilateral knee harvesting.

OAT is a technically demanding procedure and it is imperative to restore the native joint height with the transplanted plug. One biomechanical study on porcine knees found that small elevations in graft height led to a significant increase in joint contact pressure at the graft site [80]. Subsequently, Latt et al. [81] examined the effect of graft height mismatch for osteochondral plugs in the talus. They found that flush graft placement can restore near-normal joint contact pressure whereas elevated graft placement lead to increased joint contact pressure at the graft site and recessed graft placement lead to a transfer of pressure to the opposite facet of the talus.

Giannini et al. [82] demonstrated that osteochondral autografts maintain the presence of type II collagen at their implantation site.

The authors typically perform OAT for lesions greater than 1.5 cm in diameter with a relatively flat two dimensional geometry. Typically, the donor site is the knee. However, to avoid donor site morbidity, we are exploring other treatment options.

16.5.8 Osteochondral Allografting

We reserve osteochondral allografting for large lesions, typically involving the talar shoulder, with significant subchondral cysts or avascular necrosis. The grafts are obtained from deceased individuals by licensed tissue banks. They may be fresh or fresh-frozen, although modern techniques mostly employ fresh grafts. We recommend fresh grafts. The tissue bank delivers the entire talus that has been size-matched based on recipient radiographic parameters. Advantages of using osteochondral allografting include the ability to restore multiple dimensions of cartilage loss, treat large lesions, and eliminate donor site morbidity in the knee or multiple procedures. Disadvantages include disease transmission, failure of the graft to incorporate, and necessity for hardware fixation.

Few studies exist on osteochondral allografting for OLTs. Gross et al. [75] reported on nine patients who underwent fresh osteochondral allograft transplantation. At a mean follow-up of 11 years, six grafts remained in situ. The three failed grafts demonstrated radiographic and intraoperative evidence of fragmentation or resorption, and these patients went on to ankle fusion at 36, 56, and 83 months following allograft surgery. Raikin [83] reported on six patients with bulk allografting of OLTs with a mean size of 4.38 cm^3. At nearly 2-year average follow-up, five grafts continued to remain in situ with satisfactory results. More recently, Raikin [84] published on 15 patients with cystic OLTs with a mean lesion size of just over 6 cm^3 (range, 3–10 cm^3). At a mean follow-up of 54 months, 13 allografts remained in situ with significant improvement in the AOFAS ankle-hindfoot score. Some evidence of collapse, resorption, or joint space narrowing was observed in all patients. The two patients with failed grafts underwent ankle arthrodesis.

Adams et al. [85] reported on the mid-term results of eight talar shoulder lesions treated with this technique. At a mean follow-up of 4 years, patients experienced significant reduction in pain and improvement in functional outcomes scoring. All grafts were still in place. However, 50 % of patients required additional surgical procedures including debridement and revision medial malleolar osteotomy. El-Rashidy et al. [86] retrospectively reported on 38 patients who received transplanted fresh osteochondral allografts to the talus. The authors' indications for fresh allograft were failed prior operative intervention, lesion size of >200 mm^2, lesion depth of >5 mm, or lesions deemed unsuitable for other options. At a mean of 37 months follow-up, AOFAS ankle-hindfoot scores were significantly improved. Seven patients had second-look arthroscopy revealing one graft with 5–6 mm area of denuded cartilage, one graft with diffuse cartilage degeneration, and three loose grafts. Overall, four of the grafts failed and required additional surgery.

Although it is generally thought that chondrocytes are immunoprivileged secondary to their surrounding extracellular matrix [87], a recent report indicated the presence of an immunologic response to cartilage-specific protein in 8 of 14 osteochondral allografts [88]. Additionally, marrow elements are not considered immunoprivilaged [89]. Meehan et al. [90] demonstrated evidence of serum anti-HLA cytotoxic antibodies in 10 of 11 patients who underwent fresh tibiotalar osteochondral allografting. Interestingly, the one patient without cytotoxic antibodies was on

immunosuppressant medications for a kidney transplant. Although a correlation of positive cytotoxic antibodies and graft survival was not made, the authors speculated that immune response may play an important role in graft survival. However, the role of cytotoxic antibodies or HLA matching remains unknown. When using this technique, the authors do not routinely perform these tests. We do copiously irrigate the allograft, prior to implantation, to reduce the antigenic load.

The basis of allograft transplantation is to provide viable chondrocytes that can maintain the cartilage extracellular matrix for long-term survival of the joint. Fresh osteochondral allografts have been shown to contain viable chondrocytes for up to 17 years post transplantation [91, 92]. On the contrary, Enneking et al. [93, 94] demonstrated absence of viable chondrocytes and cartilage breakdown as early as 1 year after transplantation. Therefore, we prefer to use fresh osteochondral allografts over cryopreserved grafts. However, it is important to note that chondrocyte viability decreases with time in fresh allografts. Williams et al. [95] reported a significant decrease in viable chondrocytes of human fresh osteochondral allografts by day 28. However, at day 28, the mean percentage of remaining viable chondrocytes was still 70 % of the starting amount. Every effort should be made to perform the transplantation as soon as the allograft is released, but there are many patient, surgeon and hospital factors that ultimately dictate the timing of the procedure.

As with any transplantation, disease transmission in osteochondral allografting remains a concern. The current estimated risk of HIV transmission in allograft tissue is one in one million [96]. There have been three reported cases of HIV transmission, two cases of hepatitis C virus transmission, and one case of hepatitis B virus transmission in allograft tissue [96].The authors counsel our patients about the risks and benefits of receiving allograft tissue.

16.5.9 Particulated Juvenile Cartilage Allograft Transplantation

Particulated Juvenile Cartilage Allograft Transplantation (PJCAT) is a new technique of transplantation of multiple fresh juvenile cartilage allograft pieces, containing live cells within their native extracellular matrix, with fibrin adhesive securing the tissue pieces firmly inside the lesion. Currently, the only product available for this procedure is DeNovo® NT Natural Tissue Graft (Zimmer, Inc., Warsaw, IN). This product is considered a minimally manipulated tissue by the United States Food and Drug Administration and is only available in the United States. The cartilage pieces of this product are obtained, in compliance with Good Tissue Practice, from donors ranging in age from newborn to age 13. No stillborn or fetal tissue is used. Standard disease screening is performed on each lot (one lot of tissue comes from a single donor). The first clinical implantation of DeNovo® NT Graft was performed in May 2007 for a patella lesion [97]. PJCAT can be performed through an arthrotomy (Fig. 16.3) with or without a plafondplasty, through a medial malleolar osteotomy, or arthroscopically. One of the major advantages of this technique is that

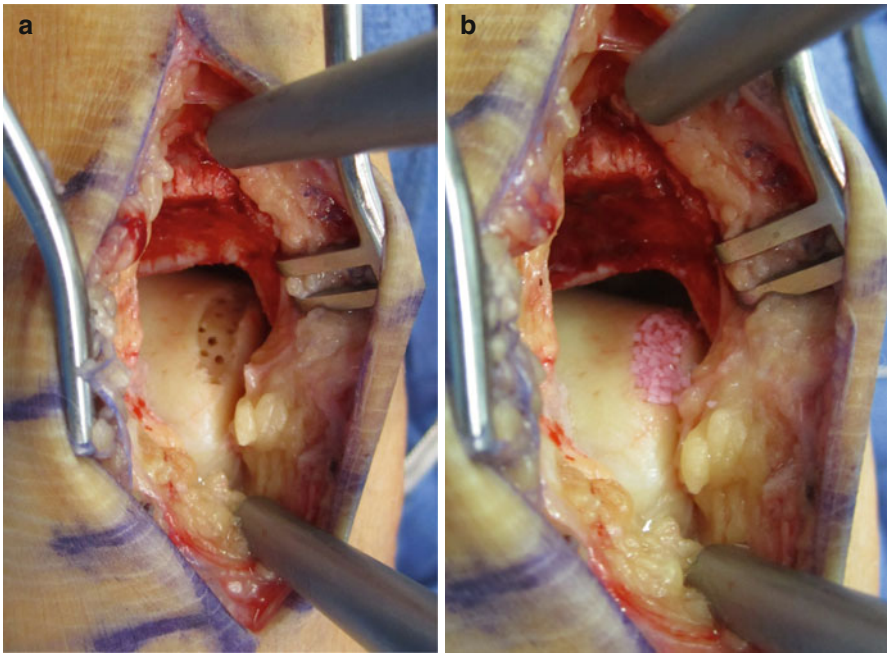

Fig. 16.3 (**a**) Debridement and marrow stimulation of a medial OLT performed through anteromedialarthrotomy and plafondplasty. (**b**) Application of particulated juvenile cartilage

the particulated nature of the graft does not require perpendicular access, thereby obviating the need for malleolar osteotomies and the potential associated complications. Additional advantages of this technique are that it is a technically simple procedure without the need for graft press-fitting/contouring (as needed for osteochondral autograft or allograft transplantation), it is a single-stage procedure, there is no donor site morbidity, and there is a minimal chance for immunological reaction (cartilage is considered immune privileged). The disadvantages of this technique are the fact that it is a relatively new procedure, there is a limited supply of juvenile donor cartilage, and as with any allograft tissue, disease transmission concerns exist.

Currently, there are no published results of PJCAT in any joint other than case reports. Bonner et al. [97] reported 2-year follow-up on a PJCAT to a full-thickness retropatellar lesion. The patient was reported to be pain free with return to preinjury lifestyle. A 12-month MRI demonstrated a full-thickness repair. Kruse et al. [98] presented a case report of arthroscopic PJCAT to an OLT. The patient was a 30-year-old female with a full thickness posteromedial lesion. At 2 years follow surgery, the patient was pain free without activity limitations.

The exact indications for this technique have yet to be elucidated. The authors use PJCAT for lesions that have failed previous debridement and marrow stimulation techniques or primary lesions that are greater than 15 mm in diameter. We are moving toward performing the majority of PJCATs arthroscopically even in when

bone graft is needed. However, if a large amount of bone grafting is required, the authors prefer a structural allograft.

16.5.10 Metallic Resurfacing

Van Bergen et al. [99] first described the use of a metal resurfacing implant for failed curettage of an OLT. The patient returned to preinjury sports activity level at 1 year after surgery. At 2-year follow-up the implant remained in position and there were no signs of osteolysis or degenerative changes in the ankle. A cadaver study from the same group demonstrated decreased joint contact pressures compared to the native joint but this is likely secondary to the component being implanted in a recessed position [100].

16.6 Summary

OLTs remain a treatment challenge. There are many surgical treatment strategies available encompassing cartilage repair, replacement, and regeneration. The myriad treatment strategies are impart due to the widely variable characteristics of OLTs as well as the scientific advances related to surgical techniques and cartilage tissue-engineering. This review indicates that clinical improvement can be found with most of these techniques. However, long-term comparative studies are needed to evaluate the efficacy of these techniques.

References

1. Kappis M. Weitere Beitrage zur traumatisch-mechanischen Entstehung der “spontanen” Knorpelablosungen (sogen. Osteohondrisit dessecans). Dtsch Z Chir. 1922;171:13–20.
2. Cambell C, Ranawat C. Osteochondritis dissecans: the question of etiology. J Trauma. 1966;6:201–21.
3. Tol JL, Struijs PA, Bossuyt PM, Verhagen RA, van Dijk CN. Treatment strategies in osteochondral defects of the talar dome: a systematic review. Foot Ankle Int. 2000;21(2):119–26.
4. Berndt AL, Harty M. Transchondral fractures (osteochondritis dissecans) of the talus. J Bone Joint Surg Am. 1959;41-A:988–1020.
5. Alexander AH, Lichtman DM. Surgical treatment of transchondral talar-dome fractures (osteochondritis dissecans). Long-term follow-up. J Bone Joint Surg Am. 1980;62(4):646–52.
6. Van Buecken K, Barrack RL, Alexander AH, Ertl JP. Arthroscopic treatment of transchondral talar dome fractures. Am J Sports Med. 1989;17(3):350–5; discussion 5–6.
7. Takao M, Uchio Y, Naito K, Fukazawa I, Ochi M. Arthroscopic assessment for intra-articular disorders in residual ankle disability after sprain. Am J Sports Med. 2005;33(5):686–92.
8. Athanasiou KA, Niederauer GG, Schenck Jr RC. Biomechanical topography of human ankle cartilage. Ann Biomed Eng. 1995;23(5):697–704.

9. Elias I, Zoga AC, Morrison WB, Besser MP, Schweitzer ME, Raikin SM. Osteochondral lesions of the talus: localization and morphologic data from 424 patients using a novel anatomical grid scheme. Foot Ankle Int. 2007;28(2):154–61.
10. McGahan PJ, Pinney SJ. Current concept review: osteochondral lesions of the talus. Foot Ankle Int. 2010;31(1):90–101.
11. Pritsch M, Horoshovski H, Farine I. Arthroscopic treatment of osteochondral lesions of the talus. J Bone Joint Surg Am. 1986;68(6):862–5.
12. Ferkel RD, Zanotti RM, Komenda GA, Sgaglione NA, Cheng MS, Applegate GR, et al. Arthroscopic treatment of chronic osteochondral lesions of the talus: long-term results. Am J Sports Med. 2008;36(9):1750–62.
13. Anderson IF, Crichton KJ, Grattan-Smith T, Cooper RA, Brazier D. Osteochondral fractures of the dome of the talus. J Bone Joint Surg Am. 1989;71(8):1143–52.
14. Hepple S, Winson IG, Glew D. Osteochondral lesions of the talus: a revised classification. Foot Ankle Int. 1999;20(12):789–93.
15. Lee KB, Bai LB, Park JG, Yoon TR. A comparison of arthroscopic and MRI findings in staging of osteochondral lesions of the talus. Knee Surg Sports Traumatol Arthrosc. 2008;16(11):1047–51.
16. Dipaola JD, Nelson DW, Colville MR. Characterizing osteochondral lesions by magnetic resonance imaging. Arthroscopy. 1991;7(1):101–4.
17. Mintz DN, Tashjian GS, Connell DA, Deland JT, O'Malley M, Potter HG. Osteochondral lesions of the talus: a new magnetic resonance grading system with arthroscopic correlation. Arthroscopy. 2003;19(4):353–9.
18. Al-Ali D, Graichen H, Faber S, Englmeier KH, Reiser M, Eckstein F. Quantitative cartilage imaging of the human hind foot: precision and inter-subject variability. J Orthop Res. 2002;20(2):249–56.
19. Ateshian GA, Soslowsky LJ, Mow VC. Quantitation of articular surface topography and cartilage thickness in knee joints using stereophotogrammetry. J Biomech. 1991;24(8):761–76.
20. Huch K. Knee and ankle: human joints with different susceptibility to osteoarthritis reveal different cartilage cellularity and matrix synthesis in vitro. Arch Orthop Trauma Surg. 2001;121(6):301–6.
21. Kuettner KE, Cole AA. Cartilage degeneration in different human joints. Osteoarthritis Cartilage. 2005;13(2):93–103.
22. Treppo S, Koepp H, Quan EC, Cole AA, Kuettner KE, Grodzinsky AJ. Comparison of biomechanical and biochemical properties of cartilage from human knee and ankle pairs. J Orthop Res. 2000;18(5):739–48.
23. Eger W, Schumacher BL, Mollenhauer J, Kuettner KE, Cole AA. Human knee and ankle cartilage explants: catabolic differences. J Orthop Res. 2002;20(3):526–34.
24. Stauffer RN, Chao EY, Brewster RC. Force and motion analysis of the normal, diseased, and prosthetic ankle joint. Clin Orthop Relat Res. 1977;127:189–96.
25. Kempson GE. Age-related changes in the tensile properties of human articular cartilage: a comparative study between the femoral head of the hip joint and the talus of the ankle joint. Biochim Biophys Acta. 1991;1075(3):223–30.
26. Bauer M, Jonsson K, Linden B. Osteochondritis dissecans of the ankle. A 20-year follow-up study. J Bone Joint Surg Br. 1987;69(1):93–6.
27. McCullough CJ, Venugopal V. Osteochondritis dissecans of the talus: the natural history. Clin Orthop Relat Res. 1979;144:264–8.
28. Pettine KA, Morrey BF. Osteochondral fractures of the talus. A long-term follow-up. J Bone Joint Surg Br. 1987;69(1):89–92.
29. Mei-Dan O, Carmont MR, Laver L, Mann G, Maffulli N, Nyska M. Platelet-rich plasma or hyaluronate in the management of osteochondral lesions of the talus. Am J Sports Med. 2012;40(3):534–41.
30. Verhagen RA, Maas M, Dijkgraaf MG, Tol JL, Krips R, van Dijk CN. Prospective study on diagnostic strategies in osteochondral lesions of the talus. Is MRI superior to helical CT? J Bone Joint Surg Br. 2005;87(1):41–6.

31. Stroud CC, Marks RM. Imaging of osteochondral lesions of the talus. Foot Ankle Clin. 2000;5(1):119–33.
32. Leumann A, Valderrabano V, Plaass C, Rasch H, Studler U, Hintermann B, et al. A novel imaging method for osteochondral lesions of the talus–comparison of SPECT-CT with MRI. Am J Sports Med. 2011;39(5):1095–101.
33. Meftah M, Katchis SD, Scharf SC, Mintz DN, Klein DA, Weiner LS. SPECT/CT in the management of osteochondral lesions of the talus. Foot Ankle Int. 2011;32(3):233–8.
34. Nehrer S, Spector M, Minas T. Histologic analysis of tissue after failed cartilage repair procedures. Clin Orthop Relat Res. 1999;365:149–62.
35. Kelberine F, Frank A. Arthroscopic treatment of osteochondral lesions of the talar dome: a retrospective study of 48 cases. Arthroscopy. 1999;15(1):77–84.
36. Robinson DE, Winson IG, Harries WJ, Kelly AJ. Arthroscopic treatment of osteochondral lesions of the talus. J Bone Joint Surg Br. 2003;85(7):989–93.
37. Savva N, Jabur M, Davies M, Saxby T. Osteochondral lesions of the talus: results of repeat arthroscopic debridement. Foot Ankle Int. 2007;28(6):669–73.
38. Schuman L, Struijs PA, van Dijk CN. Arthroscopic treatment for osteochondral defects of the talus. Results at follow-up at 2 to 11 years. J Bone Joint Surg Br. 2002;84(3):364–8.
39. Kumai T, Takakura Y, Higashiyama I, Tamai S. Arthroscopic drilling for the treatment of osteochondral lesions of the talus. J Bone Joint Surg Am. 1999;81(9):1229–35.
40. Choi WJ, Kim BS, Lee JW. Osteochondral lesion of the talus: could age be an indication for arthroscopic treatment? Am J Sports Med. 2012;40(2):419–24.
41. Becher C, Thermann H. Results of microfracture in the treatment of articular cartilage defects of the talus. Foot Ankle Int. 2005;26(8):583–9.
42. Chuckpaiwong B, Berkson EM, Theodore GH. Microfracture for osteochondral lesions of the ankle: outcome analysis and outcome predictors of 105 cases. Arthroscopy. 2008;24(1):106–12.
43. Choi WJ, Park KK, Kim BS, Lee JW. Osteochondral lesion of the talus: is there a critical defect size for poor outcome? Am J Sports Med. 2009;37(10):1974–80.
44. Han SH, Lee JW, Lee DY, Kang ES. Radiographic changes and clinical results of osteochondral defects of the talus with and without subchondral cysts. Foot Ankle Int. 2006;27(12):1109–14.
45. Saxena A, Eakin C. Articular talar injuries in athletes: results of microfracture and autogenous bone graft. Am J Sports Med. 2007;35(10):1680–7.
46. Gobbi A, Francisco RA, Lubowitz JH, Allegra F, Canata G. Osteochondral lesions of the talus: randomized controlled trial comparing chondroplasty, microfracture, and osteochondral autograft transplantation. Arthroscopy. 2006;22(10):1085–92.
47. Ferkel RD, Cheng J. Operative treatment of the foot and ankle. New York: Appleton-Croft; 1999.
48. van den Borne MP, Raijmakers NJ, Vanlauwe J, Victor J, de Jong SN, Bellemans J, et al. International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation scores validated for use in Autologous Chondrocyte Implantation (ACI) and microfracture. Osteoarthritis Cartilage. 2007;15(12):1397–402.
49. Ogilvie-Harris DJ, Sarrosa EA. Arthroscopic treatment after previous failed open surgery for osteochondritis dissecans of the talus. Arthroscopy. 1999;15(8):809–12.
50. Mitchell ME, Giza E, Sullivan MR. Cartilage transplantation techniques for talar cartilage lesions. J Am Acad Orthop Surg. 2009;17(7):407–14.
51. Doral MN, Bilge O, Batmaz G, Donmez G, Turhan E, Demirel M, et al. Treatment of osteochondral lesions of the talus with microfracture technique and postoperative hyaluronan injection. Knee Surg Sports Traumatol Arthrosc. 2012;20:1398–403.
52. Fortier LA, Potter HG, Rickey EJ, Schnabel LV, Foo LF, Chong LR, et al. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. J Bone Joint Surg Am. 2010;92(10):1927–37.
53. Geerling J, Zech S, Kendoff D, Citak M, O'Loughlin PF, Hufner T, et al. Initial outcomes of 3-dimensional imaging-based computer-assisted retrograde drilling of talar osteochondral lesions. Am J Sports Med. 2009;37(7):1351–7.

54. Kono M, Takao M, Naito K, Uchio Y, Ochi M. Retrograde drilling for osteochondral lesions of the talar dome. Am J Sports Med. 2006;34(9):1450–6.
55. Nam EK, Ferkel RD, Applegate GR. Autologous chondrocyte implantation of the ankle: a 2- to 5-year follow-up. Am J Sports Med. 2009;37(2):274–84.
56. Baums MH, Heidrich G, Schultz W, Steckel H, Kahl E, Klinger HM. The surgical technique of autologous chondrocyte transplantation of the talus with use of a periosteal graft. Surgical technique. J Bone Joint Surg Am. 2007;89(Suppl 2 Pt.2):170–82.
57. Cherubino P, Grassi FA, Bulgheroni P, Ronga M. Autologous chondrocyte implantation using a bilayer collagen membrane: a preliminary report. J Orthop Surg (Hong Kong). 2003;11(1):10–5.
58. Baums MH, Heidrich G, Schultz W, Steckel H, Kahl E, Klinger HM. Autologous chondrocyte transplantation for treating cartilage defects of the talus. J Bone Joint Surg Am. 2006;88(2):303–8.
59. Whittaker JP, Smith G, Makwana N, Roberts S, Harrison PE, Laing P, et al. Early results of autologous chondrocyte implantation in the talus. J Bone Joint Surg Br. 2005;87(2):179–83.
60. Zengerink M, Szerb I, Hangody L, Dopirak RM, Ferkel RD, van Dijk CN. Current concepts: treatment of osteochondral ankle defects. Foot Ankle Clin. 2006;11(2):331–59, vi.
61. Lee KT, Lee YK, Young KW, Park SY, Kim JS. Factors influencing result of autologous chondrocyte implantation in osteochondral lesion of the talus using second look arthroscopy. Scand J Med Sci Sports. 2012;22:510–5.
62. Giannini S, Buda R, Grigolo B, Vannini F, De Franceschi L, Facchini A. The detached osteochondral fragment as a source of cells for autologous chondrocyte implantation (ACI) in the ankle joint. Osteoarthritis Cartilage. 2005;13(7):601–7.
63. Candrian C, Miot S, Wolf F, Bonacina E, Dickinson S, Wirz D, et al. Are ankle chondrocytes from damaged fragments a suitable cell source for cartilage repair? Osteoarthritis Cartilage. 2010;18(8):1067–76.
64. van Bergen CJ, de Leeuw PA, van Dijk CN. Treatment of osteochondral defects of the talus. Rev Chir Orthop Reparatrice Appar Mot. 2008;94(8 Suppl):398–408.
65. Brittberg M, Peterson L, Sjogren-Jansson E, Tallheden T, Lindahl A. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. J Bone Joint Surg Am. 2003;85-A Suppl 3:109–15.
66. Schneider TE, Karaikudi S. Matrix-Induced Autologous Chondrocyte Implantation (MACI) grafting for osteochondral lesions of the talus. Foot Ankle Int. 2009;30(9):810–4.
67. Giza E, Sullivan M, Ocel D, Lundeen G, Mitchell ME, Veris L, et al. Matrix-induced autologous chondrocyte implantation of talus articular defects. Foot Ankle Int. 2010;31(9):747–53.
68. Apprich S, Trattnig S, Welsch GH, Noebauer-Huhmann IM, Sokolwski M, Hirschfeld C, et al. Assessment of articular cartilage repair tissue after matrix-associated autologous chondrocyte transplantation or the microfracture technique in the ankle joint using diffusion-weighted imaging at 3 Tesla. Osteoarthritis Cartilage. 2012;20:703–11.
69. Niemeyer P, Salzmann G, Schmal H, Mayr H, Sudkamp NP. Autologous chondrocyte implantation for the treatment of chondral and osteochondral defects of the talus: a meta-analysis of available evidence. Knee Surg Sports Traumatol Arthrosc. 2012;20:1696–703.
70. Giannini S, Buda R, Vannini F, Cavallo M, Grigolo B. One-step bone marrow-derived cell transplantation in talar osteochondral lesions. Clin Orthop Relat Res. 2009;467(12):3307–20.
71. Giannini S, Buda R, Cavallo M, Ruffilli A, Cenacchi A, Cavallo C, et al. Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: from open field autologous chondrocyte to bone-marrow-derived cells transplantation. Injury. 2010;41(11):1196–203.
72. Wiewiorski M, Miska M, Nicolas G, Valderrabano V. Revision of failed osteochondral autologous transplantation procedure for chronic talus osteochondral lesion with iliac crest graft and autologous matrix-induced chondrogenesis: a case report. Foot Ankle Spec. 2012;5(2):115–20.
73. Wiewiorski M, Leumann A, Buettner O, Pagenstert G, Horisberger M, Valderrabano V. Autologous matrix-induced chondrogenesis aided reconstruction of a large focal osteochondral lesion of the talus. Arch Orthop Trauma Surg. 2011;131(3):293–6.

74. Valderrabano V, Leumann A, Rasch H, Egelhof T, Hintermann B, Pagenstert G. Knee-to-ankle mosaicplasty for the treatment of osteochondral lesions of the ankle joint. Am J Sports Med. 2009;37 Suppl 1:105S–11.
75. Gross AE, Agnidis Z, Hutchison CR. Osteochondral defects of the talus treated with fresh osteochondral allograft transplantation. Foot Ankle Int. 2001;22(5):385–91.
76. Scranton Jr PE, Frey CC, Feder KS. Outcome of osteochondral autograft transplantation for type-V cystic osteochondral lesions of the talus. J Bone Joint Surg Br. 2006;88(5):614–9.
77. Hangody L, Fules P. Autologous osteochondral mosaicplasty for the treatment of full-thickness defects of weight-bearing joints: ten years of experimental and clinical experience. J Bone Joint Surg Am. 2003;85-A Suppl 2:25–32.
78. Sammarco GJ, Makwana NK. Treatment of talar osteochondral lesions using local osteochondral graft. Foot Ankle Int. 2002;23(8):693–8.
79. Kreuz PC, Steinwachs M, Erggelet C, Lahm A, Henle P, Niemeyer P. Mosaicplasty with autogenous talar autograft for osteochondral lesions of the talus after failed primary arthroscopic management: a prospective study with a 4-year follow-up. Am J Sports Med. 2006;34(1):55–63.
80. Koh JL, Wirsing K, Lautenschlager E, Zhang LO. The effect of graft height mismatch on contact pressure following osteochondral grafting: a biomechanical study. Am J Sports Med. 2004;32(2):317–20.
81. Latt LD, Glisson RR, Montijo HE, Usuelli FG, Easley ME. Effect of graft height mismatch on contact pressures with osteochondral grafting of the talus. Am J Sports Med. 2011;39(12):2662–9.
82. Giannini S, Buda R, Grigolo B, Vannini F. Autologous chondrocyte transplantation in osteochondral lesions of the ankle joint. Foot Ankle Int. 2001;22(6):513–7.
83. Raikin SM. Stage VI: massive osteochondral defects of the talus. Foot Ankle Clin. 2004;9(4):737–44, vi.
84. Raikin SM. Fresh osteochondral allografts for large-volume cystic osteochondral defects of the talus. J Bone Joint Surg Am. 2009;91(12):2818–26.
85. Adams SBJ. Midterm results of osteochondral lesions of the talar shoulder treated with fresh osteochondral allograft transplantation. J Bone Joint Surg Am. 2011;93:648–54.
86. El-Rashidy H, Villacis D, Omar I, Kelikian AS. Fresh osteochondral allograft for the treatment of cartilage defects of the talus: a retrospective review. J Bone Joint Surg Am. 2011;93(17):1634–40.
87. Langer F, Gross AE. Immunogenicity of allograft articular cartilage. J Bone Joint Surg Am. 1974;56(2):297–304.
88. Phipatanakul WP, VandeVord PJ, Teitge RA, Wooley PH. Immune response in patients receiving fresh osteochondral allografts. Am J Orthop (Belle Mead NJ). 2004;33(7):345–8.
89. Langer F, Czitrom A, Pritzker KP, Gross AE. The immunogenicity of fresh and frozen allogeneic bone. J Bone Joint Surg Am. 1975;57(2):216–20.
90. Meehan R, McFarlin S, Bugbee W, Brage M. Fresh ankle osteochondral allograft transplantation for tibiotalar joint arthritis. Foot Ankle Int. 2005;26(10):793–802.
91. Convery FR, Akeson WH, Amiel D, Meyers MH, Monosov A. Long-term survival of chondrocytes in an osteochondral articular cartilage allograft. A case report. J Bone Joint Surg Am. 1996;78(7):1082–8.
92. McGoveran BM, Pritzker KP, Shasha N, Price J, Gross AE. Long-term chondrocyte viability in a fresh osteochondral allograft. J Knee Surg. 2002;15(2):97–100.
93. Enneking WF, Campanacci DA. Retrieved human allografts : a clinicopathological study. J Bone Joint Surg Am. 2001;83-A(7):971–86.
94. Enneking WF, Mindell ER. Observations on massive retrieved human allografts. J Bone Joint Surg Am. 1991;73(8):1123–42.
95. Williams SK, Amiel D, Ball ST, Allen RT, Wong VW, Chen AC, et al. Prolonged storage effects on the articular cartilage of fresh human osteochondral allografts. J Bone Joint Surg Am. 2003;85-A(11):2111–20.

96. Mroz TE, Joyce MJ, Steinmetz MP, Lieberman IH, Wang JC. Musculoskeletal allograft risks and recalls in the United States. J Am Acad Orthop Surg. 2008;16(10):559–65.
97. Bonnor KF, Daner W, Yao JQ. 2-Year postoperative evaluation of a patient with a symptomatic full-thickness patellar cartilage defect repaired with particulated juvenile cartilage tissue. J Knee Surg. 2010;23(2):109–14.
98. Kruse DL, Ng A, Paden M, Stone PA. Arthroscopic De Novo NT(®) juvenile allograft cartilage implantation in the talus: a case presentation. J Foot Ankle Surg. 2012;51(2):218–21.
99. van Bergen CJ, Reilingh ML, van Dijk CN. Tertiary osteochondral defect of the talus treated by a novel contoured metal implant. Knee Surg Sports Traumatol Arthrosc. 2011;19(6):999–1003.
100. van Bergen CJ, Zengerink M, Blankevoort L, van Sterkenburg MN, van Oldenrijk J, van Dijk CN. Novel metallic implantation technique for osteochondral defects of the medial talar dome. A cadaver study. Acta Orthop. 2010;81(4):495–502.

Chapter 17
How to Treat Cartilage Injuries in the Ankle Joint by BMDC's Transplantation

Francesca Vannini, Roberto Emanuele Buda, Marco Cavallo, Maria Chiara Bulzamini, and Sandro Giannini

Abstract Osteochondral lesions of the ankle joint are defects of the cartilaginous surface and underlying subchondral bone, typically affecting the talus, most frequently traumatic in origin.

Osteochondral lesions are often asymptomatic and may be treated conservatively, taking care to follow the patient over time. Bigger lesions, or higher grade lesions, especially in adult patients, are usually painful and hardly respond to a conservative treatment. So((that,)) surgical treatment is frequently indicated.

Thanks to technical advancements, regenerative techniques are quickly moving from traditional periostium based autologous chondrocyte implantation (ACI) to bone marrow derived cell transplantation (BMDCT).

The introduction of a biodegradable scaffold based on the benzylic ester of hyaluronic acid for cell support and proliferation represented a first advancement toward a full arthroscopic procedure and significatively decreased the morbidity of ACI procedure in the ankle joint. Still two surgeries were required.

Recently, BMDCT has been proposed as a technique capable to provide a repair of the lesion by hyaline cartilage in a one step procedure. Mesenchymal stem cells have the ability to differentiate into osteoblasts and chondroblasts. The rationale of the "one-step technique" is to transplant the entire bone-marrow cellular pool instead of isolated and expanded mesenchymal stem cells. This allows cells to be processed directly in the operating room, without the need for a laboratory phase, and BMDCT to be performed in "one step".

With a dedicated kit a total amount of about 60 ml of bone marrow aspirate is harvested from the posterior iliac crest, with the patient in prone decubitus.

F. Vannini, MD, PhD (✉) • R.E. Buda, MD • M. Cavallo, MD • M.C. Bulzamini, MD
S. Giannini, MD
I Clinic of Orthopaedics and Traumatology, Rizzoli Orthopaedic Institute, via Pupilli 1, Bologna 40136, Italy
e-mail: france_vannini@yahoo.it; robertoemanuele.buda@ior.it; cavallo.mrc@libero.it; mariachiara.bulzamini@yahoo.it; sandro.giannini@ior.it

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_17,

A scaffold and the instrumentation previously used for ACI are then used for an entirely arthroscopic implantation. Autologous platelet-rich fibrin (PRF) is added in order to provide a supplement of growth factors.

The results of this procedure have been confirmed by biopsies and T2 mapping MRI and are clinically encouraging at mid-term.

Evolution in surgical technique, new biomaterials and more recently the use of BMDCs permitted a marked reduction in procedure morbidity and costs up to a "one step" technique able to overcome the drawbacks of previous repair techniques. The stability of the results needs to be followed long term.

Keywords Cartilage repair • Ankle joint • Bone marrow derived cell transplantation • Mesenchymal stem cells

Key Points

- Osteochondral lesion of the ankle are common occurrences, typically affecting young people practicing sports activities.
- There are different surgical options, in particular the arthroscopic bone marrow derived cells transplantation demonstrated good results, comparable to the autologous chondrocyte implantation.
- The arthroscopic bone marrow derived cell transplantation is a procedure able to obtain cartilage regeneration with the advantage of only one arthroscopic surgery. This gives the advantages of less patient's discomfort and lower costs.
- The procedure involves the bone marrow harvesting from the posterior iliac crest, where the cell count is higher than different donor sites. The cells are concentrated directly in OR, and arthroscopically implanted onto a reabsorbable three dimensional scaffold.
- Platelet rich fibrin gel, produced from autologous peripheral blood, is added to the biomaterial to give extra growth factors and further improve the stability of the implant. The outcome of this procedure has been validated both by biopsies and T2 mapping MRI, capable to give a qualitative assessment of the repaired cartilage.

17.1 Introduction

Osteochondral lesions of the ankle joint are defects of the cartilaginous surface and underlying subchondral bone, typically affecting the talus, and more rarely, the tibial plafond [1, 2]. Most frequently these lesions have traumatic origin and represent a challenging issue for the orthopedic surgeon, since the ideal treatment is still controversial [3, 4] (Fig. 17.1).

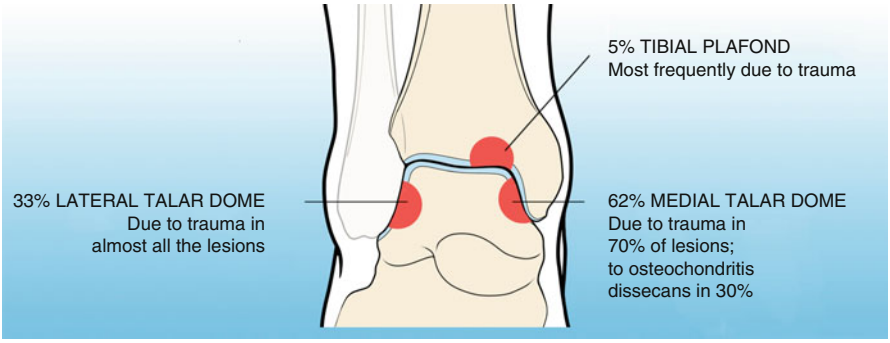

Fig. 17.1 Location and etiology of osteochondral lesions in the ankle joint [1, 2, 5]

The typical patient affected by an osteochondral lesion is a young male (20–30 years) involved in non-competitive sport activities, who reported an inversion trauma of his ankle.

In acute, the osteochondral lesion may be not be recognized, since it does not always appear at the standard radiographical examination. So that, the patient may come to the physician's attention because of persistent ankle pain, usually following a previous traumatic event. More rarely it is not possible to recall any specific etiology.

The chondral tissue shows a poor healing capacity, with the consequence that a primary injury to the talar cartilage may progress, leading to a disability status with pain, recurrent swelling, limited function and finally, to an early osteoarthritis.

17.2 Classification

The first classification system for osteochondral lesions of the ankle joint was introduced in 1959 by Berndt e Hardy. This 4 grades classification system bases on plain radiography [6]. It is to be considered, nevertheless, that 50 % of the osteochondral lesions of the talus are not evident on X-rays and to date the newer diagnostic methods available permitted the development of other classification systems.

In 2000, during an International Cartilage Repair Society Standards Workshop, a classification system was developed, based on the depth of the osteochondral lesion [7–9].

In 2005, Giannini et al. [10] introduced a new classification system specific for the ankle, considering also other labelling factors which were never taken into account before, such as the differentiation between acute and chronic lesions and the lesion size (Table 17.1, Fig. 17.2). The rationale of this MRI based system is to provide an algorithm capable to give indications on the surgical treatment to be associated to each kind of lesion. Nevertheless the age of the patient and also the

Table 17.1 Classification of the osteochondral lesions of the ankle joint

Giannini Classification for Osteochondral lesions of the talus			
Type of the lesion	Articular surface	Lesion size	Surgical treatment
Acute			
I	Damaged	<1 cm^2	Debridement
II	Damaged	>1 cm^2	Fixation
Chronic			
0	Intact	Any	Retrograde drilling
I	Damaged	<1.5 cm^2	Microfracture
II	Damaged	≥1.5 cm^2	Cartilage regeneration
IIA	Damaged	≥1.5 cm^2, ≥5 mm deep	Cartilage regeneration + bone graft
III	Damaged	Massive anatomy disruption	Allograft

According to Giannini et al. [10]

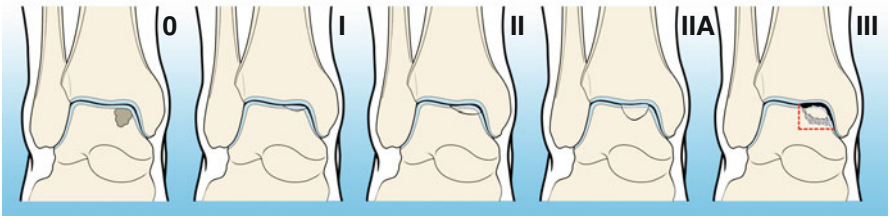

Fig. 17.2 Scheme showing chronic osteochondral lesions (Type 0, I, II, IIA, III) [10]

presence of open or closed epiphysises should be taken into consideration when planning the surgical treatment.

No classifications up to now have taken into account the presence of tibial plafond lesions.

17.3 Treatment

Osteochondral lesions which are not symptomatic may be treated conservatively, with a carefull follow-up of the patient over time. The lowest grades of osteochondritis dissecans, such as lesions with the fragment not yet clearly demarcated, and small osteochondral lesions may have a positive course if treated with boot or plaster cast with weight bearing restriction, in particular in very young patients [11].

Bigger lesions, or higher grade OCD are usually painful and hardly respond to a conservative treatment. So that, surgical treatment is frequently indicated [11].

A variety of different techniques have been reported over time for the treatment of osteochondral lesions in the ankle, ranging from a curettage of the lesion to a series of respective or regenerative procedures. Marrow stimulation techniques such as drilling and microfracture, are simple and still widely used, although the

regenerated tissue obtained is fibro-cartilaginous in nature. Otherwise, osteochondral segments transplants (mosaicplasty, osteochondral autografts transfer system), regenerative techniques such as ACI and bone marrow derived cells transplantation have been developed in order to provide a repair by cartilage as close as possible to hyaline cartilage [12].

In particular, recent acquisitions in the field of regenerative medicine demonstrated that mesenchymal stem cells (MSCs) could replicate and regenerate both bone as well as cartilaginous tissue, therefore without any need for a laboratory treatment [13, 14].

MSCs differentiation into various lineages, such as osteoblasts and chondrocytes, can be influenced by mechanical stimulation and growth factors previously added in the platelet gel. The role of the surrounding microenviroment is also a key factor, since the potential of a multipotent cell is to be considered not only an intrinsic capability of the cell alone but also the interaction between a cell with its physiologic niche, that provides a signaling network [15, 16]. In fact, adhesion molecules, extracellular matrix, growth factors, cytokines and chemokines secreted by the resident cell, along with MSCs, are believed to be necessary for bone and cartilage regeneration [17–20].

The bone marrow derived cells transplantation (BMDCT) in fact allows the implantation of MSC together with all the mononuclear cells and high regenerative potential present in the bone marrow [17–19]. Furthermore, autologous bone marrow contains not only stem cells and precursor cells, but also accessory cells that support angiogenesis by producing several growth factors [17, 19]. This ability is particulary useful in the treatment of osteochondral lesions, where the possibility to regenerate the subchondral bone is strictly dependent on angiogenesis. According to this rationale, it is possible to implant into the defect the entire regenerative potential of the niche, without the need to select and expand the MSC in a lab phase making a one step procedure possible [21].

The autologous platelet gel is used in order to provide directly *in-situ* additional growth factors. The platelet gel is a very effective "accelerator" for healing processes [22]. The secretory granules of platelets, the α granules, contain platelet-derived growth factors AA, BB, and AB; transforming growth factors β1 (TGF-β1) and β2; platelet-derived epidermal growth factor; platelet-derived angiogenesis factor (PDAF); insulin growth factor 1(IFG-1), and platelet factor 4, the latter which influences bone regeneration [23]. Moreover, the Platelet Rich Fibrin (PRF) is richest in fibrin and is able to coagulate faster than PRP, providing, an additional stability of the implant due to its jelly consistence.

17.4 Surgical Technique: BMDC's "One Step" Arthroscopic Transplantation

The surgical technique for the BMDCT consists of several phases, all to be performed during the same surgical session.

17.4.1 Platelet Rich Fibrin gel Production

The PRF is produced with an automatic system the day before the operation or the same day. 120 ml of venous blood is harvested with a needle size 16G connected to a bowl previously prepared with the anticoagulant solution. The bowl is then inserted inside the Vivostat System (Vivolution A/S, 3460 Birkeroed, Denmark) and processed. At the end of the machine cycle a syringe containing 6 ml of PRF is extracted to be stored at −35 °C or either used immediately. In case of storage, the PRF needs to be slowly heated to room temperature 30 min before its use.

17.4.2 Bone Marrow Concentration Aspiration Procedure

The bone marrow is aspirated from the posterior superior iliac crest after preparation of a sterile surgical field with the patient lying prone and already under spinal or general anesthesia. The equipment for the bone marrow harvesting, concentration and implant are part of a dedicated kit for osteochondral regeneration developed by Novagenit (Mezzolombardo, Trento, IT).

By insertion of a needle size 11G to a depth of about 3 cm in the iliac crest a total of 60 ml of bone marrow is harvested as a result of subsequent aspirations. The bone marrow aspirate is placed in a bag preloaded with 500 U of heparin in 10 ml of saline solution. The harvesting is made in little steps on different locations on the crest in order to maximize the collection of stromal cells useful for the regeneration and minimize the diluting effect coming from the aspiration of the peripheral blood (Fig. 17.3). In our cases the cell count highlighted huge differences, with a mean value of nucleated cells in the bone marrow aspirate of 26.6×10^6 per ml. The minimum was 7.1×10^6 per ml and the maximum 68.7×10^6 per ml. A progressive decrease was noticed with the age, and no differences were reported according to gender. The proximal tibia was investigated as source of bone marrow cells, but unfortunately the cell count was 60 % lower than the iliac crest.

17.4.3 Bone Marrow Concentration

The previously extracted bone marrow volume is then reduced by eliminating the plasma and the red cells, thereby increasing its stem cell concentration. This procedure is performed directly at the end of the aspiration phase in the operating room using a cells separator-concentrator (Res-Q, ThermoGenesis, Rancho Cordova, CA) and its related sterile and disposable kit. In order to perform this procedure the kit must be filled with all 60 ml of bone marrow. In 15 min, 6 ml of concentrated cells, containing the mesenchymal stem cells and other cell populations which constitute the nucleated bone marrow microenvironment, are obtained. With this procedure it was possible to obtain a mean of 164.4 nucleated cells $\times 10^6$ per ml. The minimum was 11.5×10^6 per ml and the maximum 440.0×10^6 per ml, with a concentration factor of 6.1 ± 0.3.

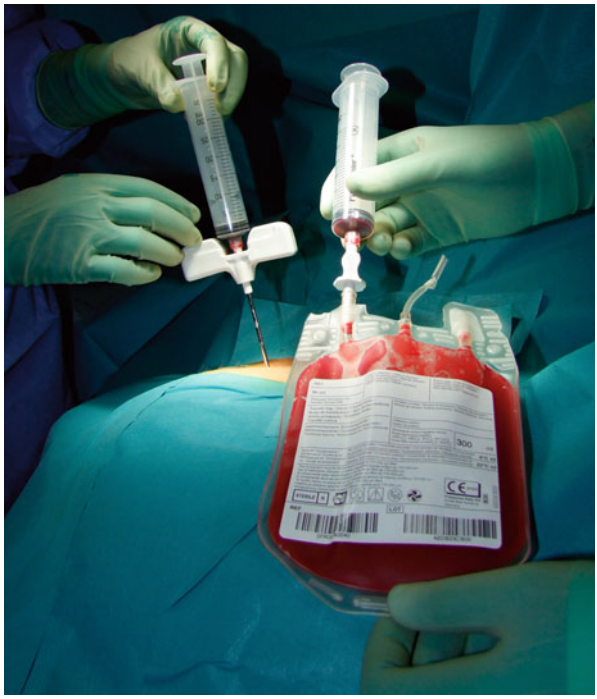

Fig. 17.3 Surgical field showing aspiration of the bone marrow from the posterior iliac crest

17.4.4 Surgical Procedure

The patient is positioned in supine decubitus with a tourniquet at the leg to be operated. A standard ankle arthroscopy is performed through antero-medial and antero-lateral portals.

The lesion is inspected: articular fibrosis, intra-articular loose bodies or osteophytes are to be removed. Theosteochondral lesion is detected and shaved, until healthy sub-chondral bone bed is reached (Fig. 17.4).

The size of the lesion is measured with the aid of a millimeter probe and recorded. The biomaterial to be implanted is then prepared in the same size and shape of the lesion.

A collagen membrane comes with the kit and is used for cell support. Approximately 2 ml of marrow concentrate are loaded on the highly hydrophilic membrane and fastly absorbed, together with 1 ml of PRF using a dedicated spray pen. Following the size and the dimension of the lesion previously measured using the sizers provided by a special instrument set the biomaterial is accurately cut. The cannula is then inserted through the arthroscopic portal closer to the lesion with the help of the trocar, then the trocar is extracted and the joint distension is stopped. The fluid is completely removed from the joint. The final biomaterial is applied in the window cut out of the cannula and guided to the edge of the lesion. At this point, the cannula is removed and the biomaterial is made to adhere perfectly to the lesion through the use of a flat probe (Fig. 17.5).

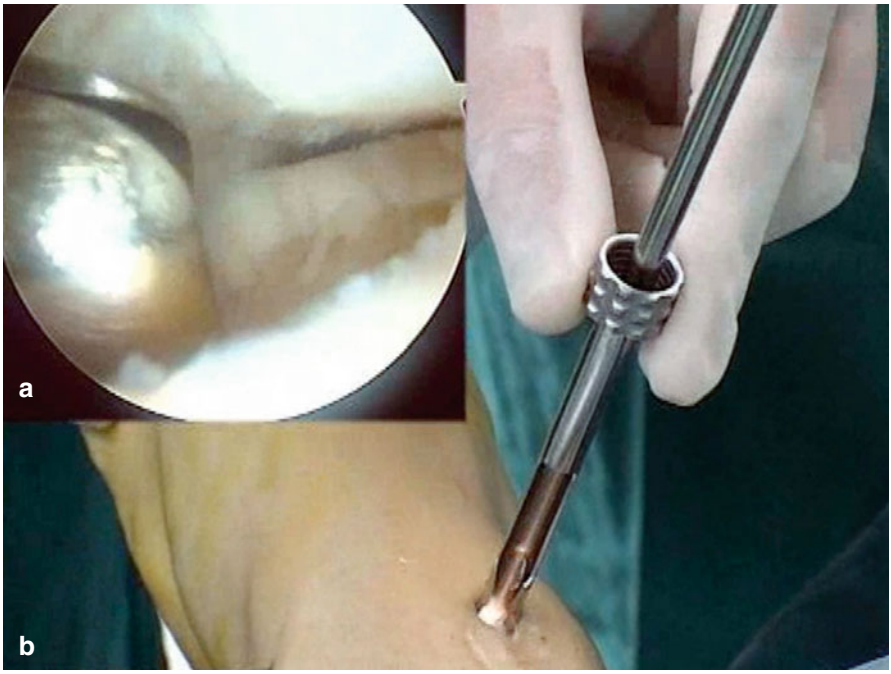

Fig. 17.4 (**a**) Arthroscopic view: the lesion is inspected and shaved until healthy sub-chondral bone bed is reached; (**b**) Intraoperative view: a standard ankle arthroscopy is performed through antero-medial and antero-lateral portals

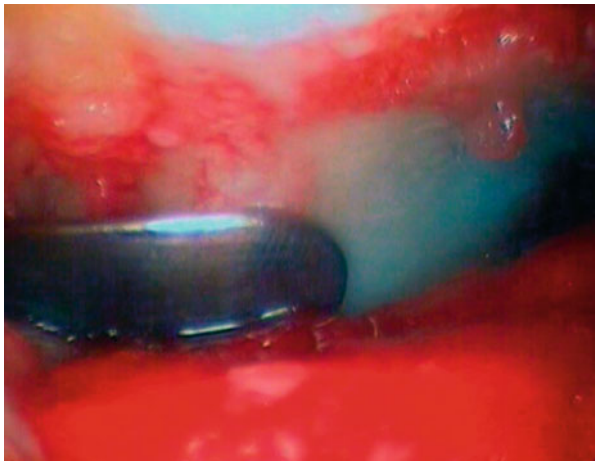

Fig. 17.5 The biomaterial is made to adhere perfectly to the lesion through the use of a flat probe

In order to provide a high concentration of growth factors and to further promote the stability of the implant due to coagulation, PRF is applied to cover the lesion. The implant stability is checked performing ankle flexion/extension movements. The skin accesses are closed with a 3-0 absorbable suture wire covered by a bandage, replaced the following day by a flat dressing.

17.5 Post Operative Treatment and Rehabilitation Protocol

Patients are usually dismissed the day after surgery.

Continuous passive motion is immediately performed the day after surgery and gradually increased as tolerated. Starting from the second day post-op the use of a passive mobilization device is recommended. During the first 6 weeks post-op walking is allowed using two crutches without applying any load on the operated leg. Partial weightbearing is started 6 weeks after the surgery, and total weightbearing is allowed at 8–10 weeks. Low impact sport activity such as swimming and cycling are allowed 4 months after the surgery, while high impact sport activities such as tennis, soccer or running at 10–12 months.

17.6 Conclusions

Osteochondral lesions of the ankle are recognized with increasing frequency. This has resulted in an increase in research and proposed solutions for the treatment of osteochondral lesions of the ankle. There is still controversy concerning the ideal treatment of chronic lesions.

Autologous chondrocyte implantation has been considered a gold standard for repair of osteochondral lesions with hyaline cartilage, however, disadvantages such as the need for two surgical procedures and high costs have led to the search for new methods.

The idea to transplant the entire bone marrow cellular pool permits the cells to be processed directly in the operating room, without the need for a laboratory phase, allowing BMDC's transplantation to be performed in "one step", with reduction of morbidity and costs.

The results obtained to date with the transplantation of BMDCs are confirmed clinically, histologically and by T2 mapping. Bone marrow-derived cell transplantation resulted in AOFAS scores similar to those reported for other widely used techniques [21, 24]. Histologic and immunohistologic results by biopsy samples indicated a good quality of the regenerated tissue still immature with remodeling features at 2 years of follow-up [21, 24]. T2 mapping MRI has proven capable, even in osteochondral defects of the talus, to give a qualitative assessment of the cartilage in terms of percentage of water quantity. The advantage of T2 mapping is to evaluated noninvasively the presence, in articular surface, of hyaline cartilage, fibrocartilage or tissue in remodeling phase [25, 26]. T2 mapping MRI results demonstrate the presence of a hyaline like tissue regeneration and also that the presence of a high percentage of hyaline regenerated correlates with the maintenance of a high clinical score at mid-term of follow-up [25, 27].

Although a long term follow-up is needed, the arthroscopic "one-step" BMDCT represents an improvement in osteochondral lesions repair in the ankle join, achieving high clinical scores with the formation of repair tissue and without the limits of previous techniques.

References

1. Bui-Mansfield L, Chew FS, Rogers LF. Original report osteochondritis dissecans of the tibial plafond: imaging characteristics and a review of the iterature. Society. 2000;175:1305–8.
2. Elias I, Raikin SM, Schweitzer ME, Besser MP, Morrison WB, Zoga AC. Osteochondral lesions of the distal tibial plafond: localization and morphologic characteristics with an anatomical grid. Foot Ankle Int. 2009;30(6):524–9.
3. Pritsch M, Horoshovski H, Farine I. Arthroscopic treatment of osteochondral lesions of the talus. J Bone Joint Surg Am. 1986;68(6):862–5.
4. Flick AB, Gould N. Osteochondritis dissecans of the talus (transchondral fractures of the talus): review of the literature and new surgical approach for medial dome lesions. Foot Ankle. 1985;5(4):165–85.
5. Roden S, Tillegard P, Unanderscharin L. Osteochondritis dissecans and similar lesions of the talus: report of fifty-five cases with special reference to etiology and treatment. Acta Orthop Scand. 1953;23(1):51–66.
6. Berndt AL, Harty M. Transchondral fractures (osteochondritis dissecans) of the talus. J Bone Joint Surg Am. 1959;41-A:988–1020.
7. Brittberg M, Peterson L. Introduction to an articular cartilage classification. ICRS Newsl. 1998;1:5–8.
8. Mainil-Varlet P, Aigner T, Brittberg M, Bullough P, Hollander A, Hunziker E, et al. Histological assessment of cartilage repair: a report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). J Bone Joint Surg Am. 2003;85-A(Suppl):45–57.
9. Brittberg M, Winalski CS. Evaluation of cartilage injuries and repair. J Bone Joint Surg Am. 2003;85-A(Suppl):58–69.
10. Giannini S, Buda R, Faldini C, Vannini F, Bevoni R, Grandi G, et al. Surgical treatment of osteochondral lesions of the talus in young active patients. J Bone Joint Surg Am. 2005;87 Suppl 2:28–41.
11. Navid DO, Myerson MS. Approach alternatives for treatment of osteochondral lesions of the talus. Foot Ankle Clin. 2002;7(3):635–49.
12. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis and cartilage/OARS, Osteoarthritis Research. Society. 2002;10(6):432–63.
13. Galois L, Freyria A-M, Herbage D, Mainard D. Cartilage tissue engineering: state-of-the-art and future approaches. Pathol Biol. 2005;53(10):590–8.
14. Oreffo ROC, Cooper C, Mason C, Clements M. Mesenchymal stem cells: lineage, plasticity, and skeletal therapeutic potential. Stem Cell Rev. 2005;1(2):169–78.
15. Lucarelli E, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, et al. Platelet-derived growth factors enhance proliferation of human stromal stem cells. Biomaterials. 2003;24(18):3095–100.
16. Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. Biotechnol Bioeng. 2006;93(6):1152–63.
17. Dominici M, Pritchard C, Garlits JE, Hofmann TJ, Persons DA, Horwitz EM. Hematopoietic cells and osteoblasts are derived from a common marrow progenitor after bone marrow transplantation. Proc Natl Acad Sci U S A. 2004;101(32):11761–6.
18. Kacena MA, Gundberg CM, Horowitz MC. A reciprocal regulatory interaction between megakaryocytes, bone cells, and hematopoietic stem cells. Bone. 2006;39(5):978–84.
19. Olmsted-Davis EA, Gugala Z, Camargo F, Gannon FH, Jackson K, Kienstra KA, et al. Primitive adult hematopoietic stem cells can function as osteoblast precursors. Proc Natl Acad Sci U S A. 2003;100(26):15877–82.
20. Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood. 2005;105(7):2631–9.

21. Giannini S, Buda R, Cavallo M, Ruffilli A, Cenacchi A, Cavallo C, et al. Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: from open field autologous chondrocyte to bone-marrow-derived cells transplantation. Injury. 2010;41(11):1196–203.
22. Sánchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. Int J Oral Maxillofac Implants. 2012;18(1):93–103.
23. Nair MB, Varma HK, John A. Platelet-rich plasma and fibrin glue-coated bioactive ceramics enhance growth and differentiation of goat bone marrow-derived stem cells. Tissue Eng Part A. 2009;15(7):1619–31.
24. Giannini S, Buda R, Vannini F, Cavallo M, Grigolo B. One-step bone marrow-derived cell transplantation in talar osteochondral lesions. Clin Orthop Relat Res. 2009;467(12):3307–20.
25. Battaglia M, Rimondi E, Monti C, Guaraldi F, Sant'Andrea A, Buda R, et al. Validity of T2 mapping in characterization of the regeneration tissue by bone marrow derived cell transplantation in osteochondral lesions of the ankle. Eur J Radiol. 2011;80(2):e132–9.
26. Giannini S, Battaglia M, Buda R, Cavallo M, Ruffilli A, Vannini F. Surgical treatment of osteochondral lesions of the talus by open-field autologous chondrocyte implantation: a 10-year follow-up clinical and magnetic resonance imaging T2-mapping evaluation. Am J Sports Med. 2009;37 Suppl 1:112S–8.
27. Battaglia M, Vannini F, Buda R, Cavallo M, Ruffilli A, Monti C, et al. Arthroscopic autologous chondrocyte implantation in osteochondral lesions of the talus: mid-term T2-mapping MRI evaluation. Knee Surg Sports Traumatol Arthrosc. 2011;19(8):1376–84.

Part VII
Emerging Techniques

Chapter 18
Pure Cartilage-Based Repair Modalities of Focal Cartilage Lesions

Tamas Bardos

Abstract Focal cartilage lesions in diarthrodial joints have a limited capacity to heal, and repair techniques used at present are still unable to provide a universal solution. Osteochondral auto- and allografts are accepted and successful methods for the treatment of these lesions, but occasionally the osseal incorporation is delayed or insufficient and graft integration might be unsuccessful. Failure at this level generates a large osseos crater and the consequences can prove challenging.

Until just a few years ago, it was a generally accepted dogma that when cartilage is detached from the subchondral bone it would fail to reintegrate to its bed and its surrounding cartilage. Recently, innovative approaches have been established to repair cartilage defects using pure cartilage-based implants, and so far they seem to have had considerable success.

One of the available options is to use autologous minced cartilage in a single-stage procedure. Cartilage tissue is obtained from the less-weight-bearing surface of the affected joint and the sample is processed *in situ* resulting in a cartilage fragment-loaded scaffold that can be applied to the lesion of the weight-bearing area. Another system repairs with cadaveric juvenile articular hyaline cartilage cut into 1 mm^3-cartilage cubes and using fibrin glue as vehicle the tissue particles are evenly distributed on the defected articular surface. Both methods are relatively new and therefore lacking long-term follow up, but the short-term results seem encouraging.

An additional concept for cartilage-based repair is when pure cartilage allograft is peeled from the subchondral bone, and instead of mincing the tissue it is repeatedly incised on its basilar surface rendering the rigid cartilage graft into a rather pliable graft. Since the superficial layer is preserved the graft is similar to any scaffold used in cartilage repair, and it can be secured to the lesion site using sutures and

T. Bardos, MD, PhD
Department of Orthopedics, University of Pecs, Pecs, Hungary

Emersons Green NHS Treatment Centre, Bristol, UK
e-mail: tbardos@hotmail.com

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_18,

fibrin glue. Although this method is in the experimental phase, a short term clinical trial has proved it to be safe.

In this chapter we will briefly describe the physiology of cartilage integration; summarize the basis and the potential pitfalls of these methods; and provide a review of the available data on the clinical outcomes.

Keywords Focal lesion • Pure cartilage • Cartilage allograft • Cartilage fragmentation

Key Points

- Recently novel cartilage repair methods have emerged, which are based on processed pure cartilage tissue placed on the focal chondral lesion.
- Cartilage Autograft Implantation System (CAIS) uses minced autologous hyaline cartilage from lessweight-bearing surfaces and secures it to the lesion site with resorbable mesh and anchors. This method is in the clinical trial phase.
- DeNovo NT system operates with minced juvenile hyaline cartilage allograft that is delivered to the lesion site with fibrin glue and it exploits the high growth capacity of the young cartilage cells. This technique is already in clinical use.
- If a larger pure cartilage piece is peeled off from its osseal base and is multiply incised on one surface, the graft appears to have good potential to repair focal lesions by adhering to the recipient subchondral bone. This approach is only in the pre-clinical phase.
- It was a generally accepted view amongst orthopeadic surgeons that once cartilage tissue is delaminated from the subchondral bone it is destined to degenerate. In view of these new cartilage repair methods this theory might need some adjustments.

18.1 Introduction

Articular cartilage lesion is still considered a difficult problem due to its poor regenerative potential [1, 2]. Many distinct cartilage repair modalities are available but none of them offer a universal solution for the various types of lesions. The most frequently used surgical solutions employ the reparative capacity of the subchondral bone (chondral debridement, marrow stimulation) or provide restoration of the articular surface by transplantation of cartilage either on a cellular level (ACI with or without matrix) or as part of an osteochondral graft (autologous or allogeneic). More recently a new concept has come to light. It is postulated that cartilage tissue alone could be transplanted to the site of the lesion and it would eventually integrate

to the lytic site providing a durable hyaline or hyaline-like coverage for the affected area [3].

The reason why cartilage cannot re-integrate to the osseal bone has been investigated. Both the cartilage matrix and surviving chondrocytes may play a role in the failure to integration to the subchondral bone. Many factors have been held responsible for this phenomenon, such as impaired collagen network cross linking [4, 5] or proteoglycan components (lubricin) in the synovial fluid [6] preventing integration. Today it is still not clear why articular cartilage has such poor vertical and lateral integration, but gap formation between host and graft cartilage tissue is hypothesized to be very significant in the long term survival of any repair tissue [7, 8]. In earlier studies articular cartilage was demonstrated to have limited capacity to heal to the subchondral bone once detached [9, 10], therefore the idea of transplanting pure cartilage onto the subchondral bone would have been considered unorthodox- to say the least. In the last few years this long-standing dogma has been challenged and it appears that the concept of cartilage as an indifferent and passive part of the reintegration process is to be re-evaluated. Many studies showed that cartilage cells can be rather motile and can move through the extracellular matrix [11, 12] once adequate signals are present. Cutting the cartilage tissue has been shown to induce cell death in the adjacent zone of cartilage [13]. On the other hand, cartilage cells show good motility after cutting (mincing) and evade the original cartilage tissue [14]. The result of the evasion is neocartilage formation which varies depending on the zone (superficial vs. deep) and the age of the cartilage (young vs. adult) [14].

While autologous chondrocyte implantation has been a promising repair technique its drawbacks prevent it from a broader acceptance: the need of a 2-stage surgery, significant expenses of laboratory culturing, cumbersome arrangements for adequate quality insurance and transportation. To explore other possible techniques, research groups started to investigate the potential of pure hyaline cartilage-based repair modalities. The concept of mincing tissue laid onto the defect site is not entirely new. In the early 90's Stone et al. implanted a paste of minced autologous osteochondral tissue onto the defect sites following microfracture [15]. In their concept the lesion had to be properly debrided and the mesenchymal stem cells were stimulated by fracturing the subchondral plate; the scaffold for these cells was a "putty" derived from osteochondral autografts from the intercondylar notch (8 mm trephine – 1.5 cm long cylinder) that was crushed into a paste and impacted onto the fractured subchondral boneplate of the defect – not to fill but rather to cover the lesion and the penetrations of the bone [15]. In the 2- to 12-year follow-up of 136 procedures Stone et al. showed a relatively small portion of failures (14.4 %) especially considering the stage and size of the lesions (all grade IV lesions with average size of 28.6 cm^2) [16]. The 65 follow-up biopsies showed mostly fibrocartilage with or without GAG, and only 18 of the 65 biopsies showed areas of hyaline cartilage; yet overall 82 % of patients reported improved pain scores.

The need for a single-stage procedure from the surgeons, patients and insurance companies and the encouraging data of the morselized tissue grafting led to new investigations and resulted in new cartilage repair procedures.

18.2 Cartilage Autograft Implantation System (CAIS™)

Cartilage Autograft Implantation System (CAIS) is one of the new repair procedures for the treatment of cartilage lesions in the knee. It uses autologous cartilage obtained from the less-weight-bearing surface of the affected joint; the sample is processed *in situ* resulting in a cartilage fragment-loaded scaffold that can be applied to the lesion of the weight-bearing area. In contrast with the earlier osteochondral morselized grafting method in this approach the pure cartilage graft is delivered on a non-bleeding chondral defect thus the repair is likely to be driven by the chondrocytes rather than the mesenchymal stem cells from the bone marrow.

A minimum of 200 mg cartilage tissue (approximately two 13 × 5 mm pieces) is harvested from the non weight-bearing surfaces (intercondylar notch or trochlear ridge), similarly to the first step of ACI. Using a disposable device (CAIS Harvester and Dispenser, DePuy Mitek, Raynham, MA) the harvested cartilage sample is minced into 1–2 mm particles and using surgical vacuum and irrigation fluid the minced cartilage is evenly dispersed onto a scaffold. The scaffold is made from an absorbable copolymer foam of 35 %polycaprolactone (PCL) and 65 % polyglycolic acid (PGA), that is augmented with a polydioxanone(PDO) mesh (DePuy Mitek, Raynham, MA). The mesh provides the mechanical strength for the graft to be easily handled during implantation. Fibrin glue is used to keep the pieces on the foam; to ensure that the prepared graft is secured on the defect site firmly, two or more biodegradable staple anchors are used to affix the graft (side with the cartilage particle facing the osseal base) (Fig. 18.1). The staple anchors (DePuy Mitek, Raynham, MA) consist of a PDO strap and a PGA tip, and were shown in a cadaver knee study to hold scaffolds in place even after 10,000 cycles of CPM [18].

Large animal studies using goats and horses proved CAIS feasible and safe [17, 19]. In the equine experiment 15 mm full thickness cartilage defects of the trochlea were treated in 2- to 5-year old horses and various techniques were used for repair: scaffold only, CAIS, modified-ACI or left empty [17]. The gross examination of the repair tissues showed significantly firmer tissue in the CAIS and the ACI groups. The total histology scores were significantly better in modified-ACI- or CAIS-treated defects than in the empty or PDS scaffold-treated groups.

Based on the preclinical results a randomized clinical trial has been commenced to determine the safety and efficacy of CAIS compared to microfracture in reducing pain and improving function at 24 months. The study is designed to enroll a rather large number of patients (over 300). Patients are included with 1–2 focal chondral lesions that are less than 6 mm in depth with the affected area between 1 and 10 cm^2. The major exclusion criteria are bilateral disease, advanced radiological OA, >5° of malalignment and bipolar (kissing) lesions.

Two-year follow-up data of a prospective clinical safety trial has already been published [20]. In this study 29 patients were randomized to the CAIS treatment (20) or to the microfracture active control (9) group. In addition to knee-specific outcome data (IKDC and KOOS) MRI was performed at 3 weeks, 6, 12 and 24 months.

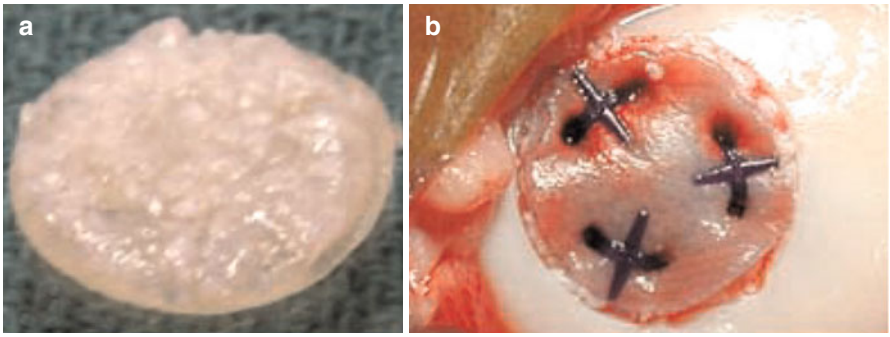

Fig. 18.1 CAIS in equine model. Morselized cartilage on top of the scaffold before implantation (**a**). Graft after implantation to the trochlea using three resorbable staples (with scaffold side up) (**b**) (Picture modified reproduction from Frisbie et al. [17])

The surgical procedure was carried out using a similar method to that described above. The rehabilitation protocol was adjusted to the site of the lesion (condylar or trochlear lesion) using the basic principle of non-weight-bearing for 2 weeks and 50 % weight-bearing until week 6 on the repaired area.

Complete blinding was not possible due to the different nature of the two procedures (microfracture – wholly arthroscopic, CAIS – mini arthrotomy). MRI showed comparable results in both groups with 76–100 % fill of the repair sites in nearly all lesions by 24 months (Fig. 18.2). There was no significant difference in terms of the subchondral cyst formation or the graft integration, but the intralesional osteophytes were significantly more common in the microfractured group at 6 and 12 months. The Short Form 36 (SF-36) score did not show significant differences between the two groups, but the knee-specific outcomes (IKDC and KOOS) showed significantly higher scores for the CAIS-treated group at 24 months. Although the study is limited by the small sample size and the lack of total blinding, the authors conclude that CAIS is well tolerated among the patients, and it is a safe and feasible treatment technique for focal chondral lesion in the knee [20]. Short term follow-up results of larger patient cohorts are expected in the next few years.

18.3 DeNovo® NT Natural Tissue Graft

DeNovo NT (Zimmer, Warsaw, IN / ISTO, St Louis, MO) is a distinct technique to repair focal chondral defects, but it shows some similarities with CAIS. DeNovo NT repairs the focal cartilage lesions with particulated juvenile articular hyaline cartilage allograft. The tissue is cut into 1 mm^3-cartilage cubes and using fibrin glue as a vehicle the tissue particles are evenly distributed on the defected articular surface.

Previous animal studies have showed that mature articular cartilage pieces can remodel and form adequate cartilage repair tissues in rabbits, goats and horses [17,

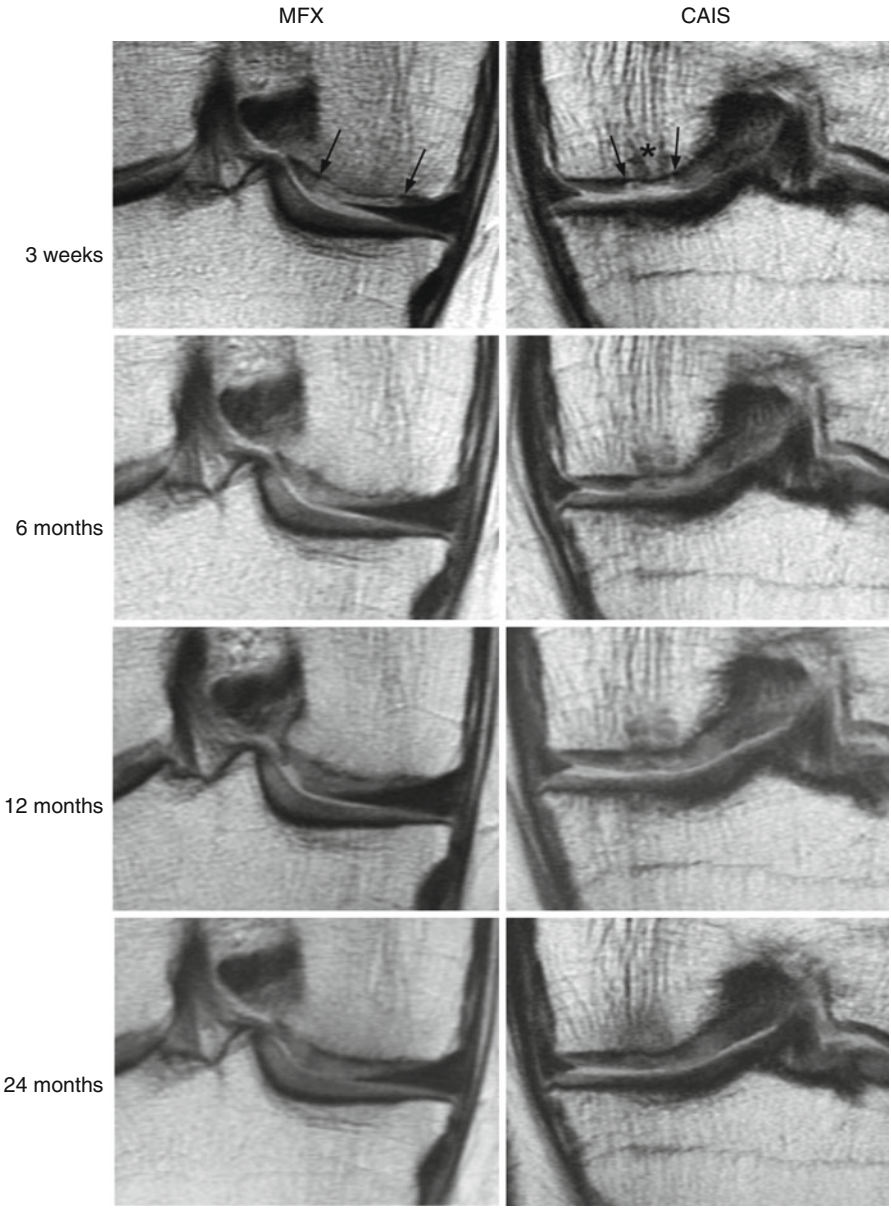

Fig. 18.2 Magnetic resonance images of two representative patients treated with microfracture (*MFX*) or Cartilage Autograft Implantation System (*CAIS*) in coronal view at 3 weeks, 6-, 12- and 24 months. The two low intensity structures (next to *) in CAIS at 3 weeks represent the staples (which gradually absorbed) (Picture modified reproduction from Cole et al. [20])

19, 21]. When juvenile and adult cartilage have been compared in terms of cell proliferation, potential to generate neocartilage, and production of GAG – juvenile cartilage was found to be dramatically better and the results in all aspect were

superior in the groups using juvenile cartilage [14, 22, 23]. Also, injured immature cartilage has a good healing potential compared to that of an adult [2], which is often explained by the higher cell density in the juvenile cartilage [24]. Unpublished data on equine trochlear lesion repaired with human particulated juvenile cartilage showed no necrotic changes in the subchondral bone, with full thickness continuous layer of cartilage repair tissue after 6 months, with better appearance than the control fibrin glue repaired defects (Frisbie et al. 2007, data on file at ISTO Technology, Inc.). Based on these results DeNovo NT was introduced to the market as a novel cartilage repair kit in 2007.

During the production of DeNovo NT graft pure cartilage tissue is harvested from fresh cadaveric juvenile (<13 years old excluding stillbirth and fetal donors) femoral condyles, and the tissue pieces are manually minced under aseptic conditions (Good Tissue Practice, ISTO Tech Inc.) without enzymatic digestion. In accordance with the FDA guidance (21 CFR Part 1271 subpart C and Guidance for Industry), strict viral and bacterial screening is completed using a similar method to other fresh osteochondral allografts. The single donor-derived minced sample is aliquoted and sealed with medium in blister packs and is to be used within 52 days after packaging.

As DeNovo NT graft is "minimally manipulated tissue", the product does not require premarketing approval from the FDA, and so post-launch clinical studies are conducted. The special requirements for donor eligibility have been speculated to limit the supply but following an early period of relative shortage of donor tissues the company now claims to have a good supply of donor tissues and to deliver the product without major interruption. DeNovo NT has been used for cartilage repair in various joints like knee, ankle, elbow, shoulder and hip.

The surgical technique in the knee is straightforward: after confirmatory arthroscopy the defect is approached via limited arthrotomy. The lesion is prepared with ring curette by removing the affected cartilage, forming vertical shoulder around the defect (Fig. 18.3). The congruent replica of the lesion is prepared using thin aluminum foil. Depending on the size of the lesion (each package is used for 2.5 cm^2) the appropriate amount of cartilage particles are evenly distributed on the foil mold and immobilized using fibrin glue. After setting, the construct (fibrin glue patch containing the cartilage particles) is removed from the foil in one piece and secured on the defect site using fibrin glue. The repair construct should be recessed relative to the surrounding cartilage to avoid dislodging (so far reported only in one case- Zimmer Orthobiologics, Inc. internal data). The postoperative rehabilitation protocol is identical to that used in similar repair techniques (e.g., following CAIS, see above).

Notwithstanding the rapidly increasing popularity of this technique, the available DeNovo NT clinical studies are limited. One case study reported an arthroscopic repair of a posteromedial talar osteochondral lesion using DeNovo NT, and found that the procedure is well tolerated, the patient returned to full activity within 6 months and remained free of pain at 24 months [26]. Another case study found this technique to be clinically successful for patellar cartilage defect and the 2 years MRI demonstrated good fill of the defect [27].

An additional case study series of four patients who completed the 24 months follow-up period has also just recently been published [25]. Five focal lesions with an average size of 2.7 cm^2 were treated using DeNovo NT. At 24 months all four

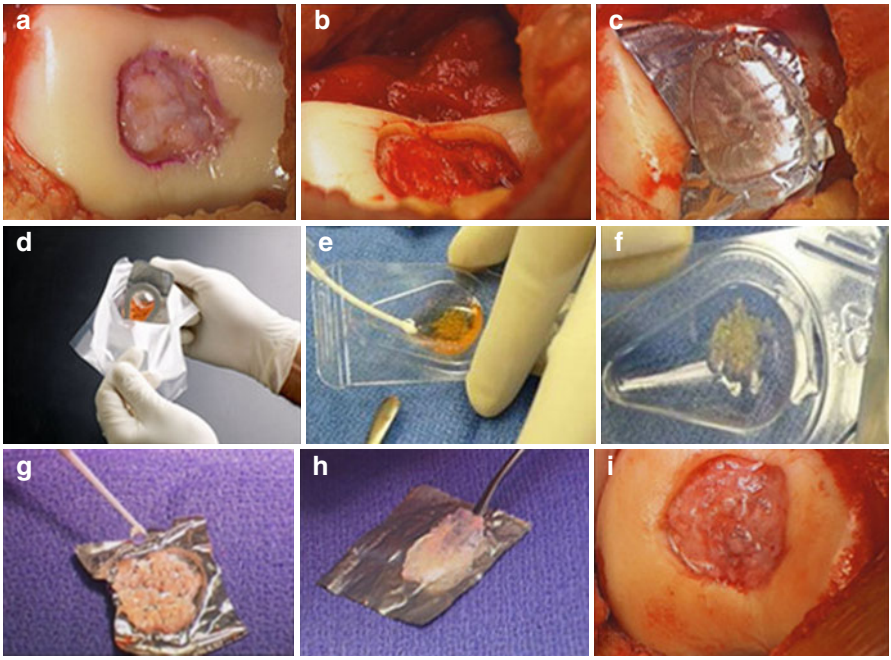

Fig. 18.3 Clinical steps of DeNovo NT technique. The cartilage defect (**a**) is debrided (**b**) and the sterile foil is pressed to form a replica (**c**). The blister pack of DeNovo NT is opened (**d**) and the culture medium is aspirated (**e**). The particulated cartilage (**f**) is distributed evenly in the foil and fibrin glue is allowed to solidify (**g**). The graft is carefully removed from its mold (**h**) and secured to the defect site using fibrin glue (**i**) (Picture reproduced from Farr et al. [25])

patients showed improvements in KOOS and IKDC scores as well as in VAS pain scores. MRI showed good filling of the defects (Fig. 18.4), however, due to the low number of patients the interpretation is tempered.

The DeNovo NT technique is suggested to have several advantages. As it is a single-stage cartilage repair technique, there is no need to compromise the subchondral bone, therefore it doesn't "burn bridges", no autologous tissue needs to be harvested and the juvenile tissue has great proliferative potential. As of today more than 2,500 DeNovo NT implantations were performed, and it is claimed that soon it may be used for cartilage repair more often than ACI. Although the results are very promising, further clinical data will have to be collected and analyzed in the next few years allowing better insight into this technique.

18.4 Processed Chondro-Graft

The central dogma of orthopedics, i.e., once cartilage is separated from its osseal base, it cannot grow back – seems to weaken with the emerging results of CAIS and DeNovo NT. Yet the question, whether pure cartilage peeled off the subchondral

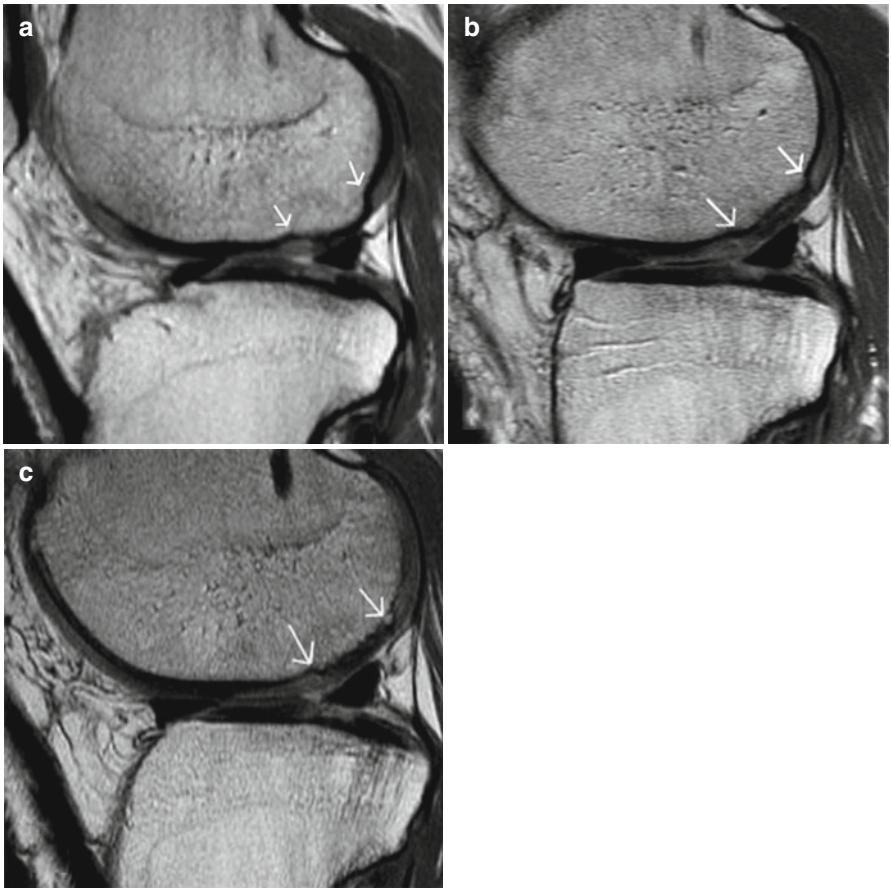

Fig. 18.4 Magnetic resonance images (sagittal plane) of a representative patient with focal chondral lesion of the knee treated with DeNovo NT technique: preoperative (**a**), at 12-months (**b**) and at 24 months (**c**). Arrows mark the margins of the lesion. (Picture reproduced from Farr et al. [25])

bone (the "absolute shell graft") could ever be used for repairing cartilage defects is still unanswered.

Processed chondrograft is a technique that lies somewhere between the large osteochondral shell allograft and the minced cartilage allograft implantation. Occasionally it is also called the "hedgehog graft" after its appearance (when the incised cartilage is turned "inside-out"). The basic steps of this technique are the harvesting of the pure cartilage allograft from an adult cadaveric knee joint (large intact piece peeled from the femoral condyle), the multiple incision of the deep zone of the cartilage graft (Fig. 18.5) to improve handling and healing characteristics, and the implantation via mini arthrotomy.

For graft harvesting the cadaveric knee joints are prepared under aseptic conditions. The condylar surfaces are used as donor sites. Approximately 2.5×4 cm grafts are harvested by peeling the cartilage off of the subchondral bone. Calcified

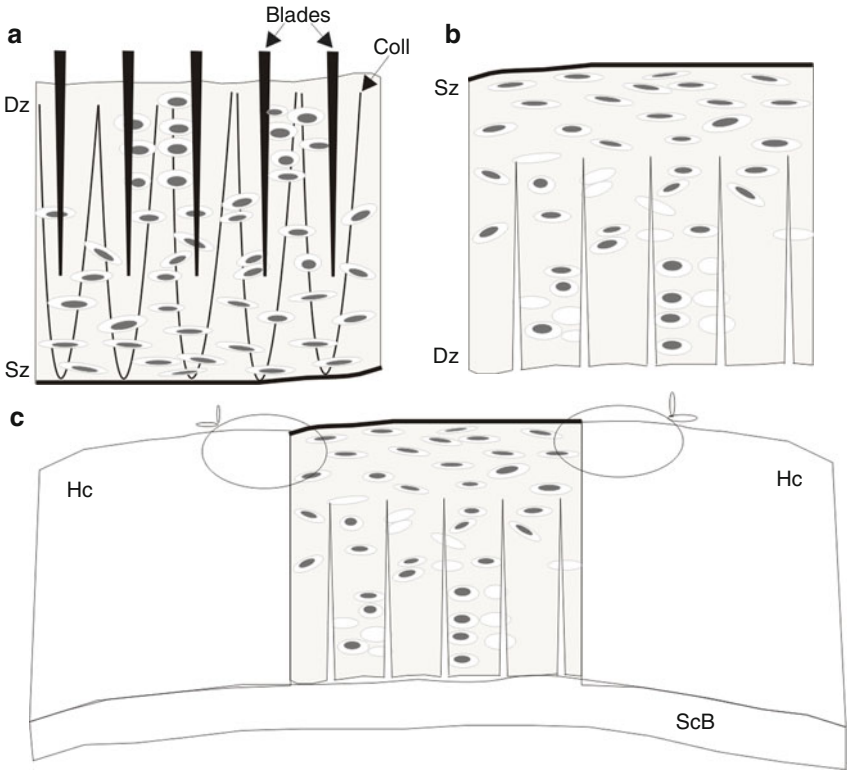

Fig. 18.5 The basic concept of processed cartilage allograft. (**a**) Articular cartilage specimen multiply incised through the deep zone (*Dz*). Note that the collagen fibers (*Coll*) run parallel with the incisions in this zone. Graft before (**b**) and after (**c**) implantation. (*ScB* subchondral bone, *Hc* healthy hyaline cartilage, *Sz* superficial zone) (Picture reproduced from Bardos et al. [3])

cartilage remnants are meticulously removed from the graft after harvesting. The graft is kept moist throughout the whole process (to prevent cell death in the superficial zone of the graft) and stored in medium until used (within 35 days).

During surgery a specific incisor is used to create parallel incisions in the deep zone of the graft limiting the incision depth to 600 μm from the superficial zone, therefore preserving the arcading collagen network in the superficial zone and providing the pliable graft with good tensile strength. The incisions convert the graft into a pliable, well accommodating tissue that can be firmly positioned and fixed to the cartilage site but the incisions also significantly increase the surface area for attachment. The incisions can be performed in two or more different directions, creating larger surface for cell invasion/evasion at the integration site. The graft must be recessed relative to the contiguous host cartilage surface and it is secured to the defect site using 6.0 PDF stitches and Fibrin Glue (Fig. 18.6).

Recently we showed in a porcine model that fresh pure cartilage allograft incised through the deep zone has a good healing potential to the subchondral bone [3]. The

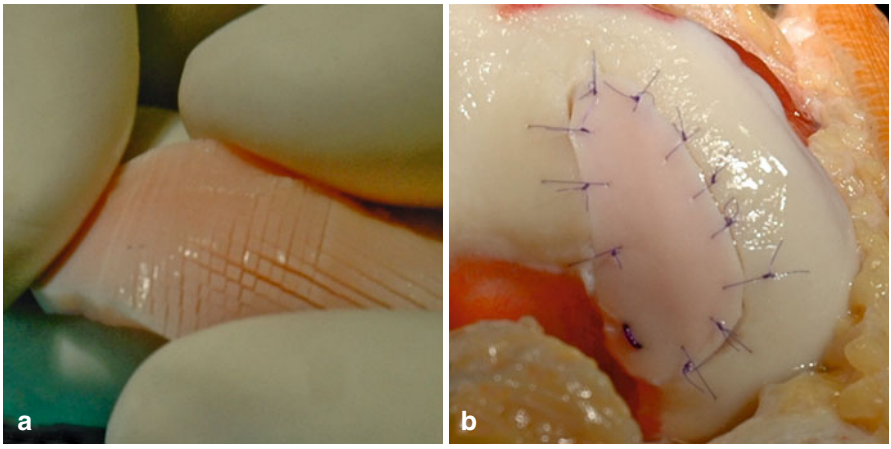

Fig. 18.6 (**a**) Hedgehog appearance of the basal surface of a processed cartilage allograft before implantation. (**b**) Intraoperative picture of the graft after securing with stitches, trans-osseal knot and fibrin glue

study showed that the grafts had good primary stability and the grafts were held in place at 6 weeks even though the securing stitches were reabsorbed by then. The basal incisions were visible with histology at 6 weeks postoperatively without any obvious tissue formation within the incisions (almost only virtual space), nevertheless at 6 months the former incisions were not detectable anymore (unpublished data from the author). Lateral integration was incomplete in most grafts, and the quality of the subchondral bone appeared somewhat irregular (less trabecula in the subchondral bone, with slightly thicker appearance than at the recipient site). However, the microscopic assessment showed good preservation of the graft with hyaline cartilage on the recipient site.

A small clinical trial has been introduced to assess the repair potential of processed chondrograft in human knee joints. Seven patients with eight focal chondral lesions were enrolled into the study. The average size of the chondral defects was 3.9 cm^2 (2.0–5.4 cm^2). The lesions were accessed via a small arthrotomy and prepared for the graft implantation with thorough debridement of the lesion down to the underlying subchondral plate. Fresh human chondral allografts (obtained from knee joints, containing no bony component, stored at 4 °C) were tailored to match the shape of the lesion, incised on the formerly osseous side and secured with stitches and fibrin glue as described above. Postoperative rehabilitation was similar to the previous methods. At an average of 18 months follow up no adverse reaction was reported; patients did not experience any sudden deterioration in the joint functions (e.g., loose body sensation, joint locking, or sharp pain). The patients were satisfied with the outcome and the SF-36 health survey revealed significant increase (the mean combined SF-36 scores increased from 57 ± 19 at baseline to 85 ± 13 at the latest examination, $p < 0.001$). The knee functions improved in all patients (Lysholm's knee score from 62 ± 18 preoperatively to 74 ± 14 postoperatively,

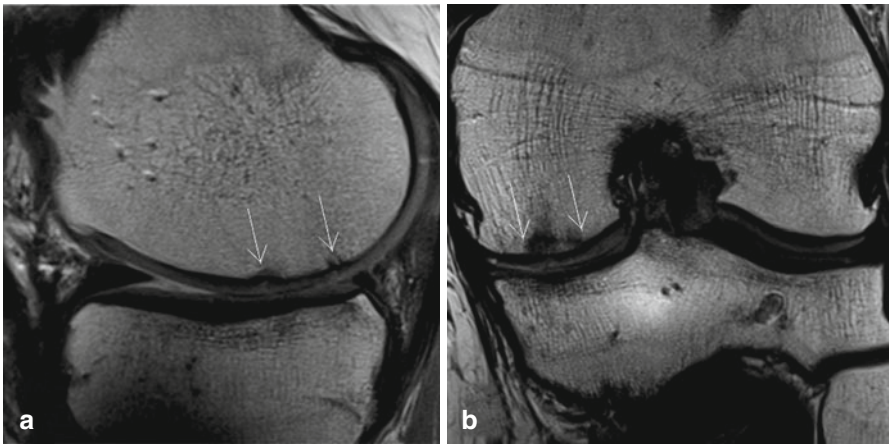

Fig. 18.7 Magnetic resonance images in sagittal (**a**) and coronal plane (**b**) of a representative patient 12 months after focal defect repair of the medial femoral condyle using processed allograft (hedgehog). Please note that the patient does not correspond with the pictures shown in Fig. 18.6

$p < 0.05$). At 12 months MRI suggested possible partial delamination of the chondral graft (fluid signal beneath the graft) in only one patient.

In the other patients MRI proved good osteochondral integration without any sign of degeneration or rejection of the cartilage graft (Fig. 18.7). The grafts demonstrated good congruency with the host condyle, and the cartilage signal was isointense to that of the host area with only mild subchondral edema. It appears that processed chondrograft has capacity to integrate to the subchondral bone following surface augmentation, and the straightforward technique seems to be safe and feasible as well. Additional long term clinical investigations must be carried out to assess the possible role of processed pure cartilage allografts in cartilage repair.

18.5 Conclusion

The new concept in cartilage repair- that pure cartilage tissue following mincing or incisions could be used in a single-stage procedure – is very promising and opens a whole new field for research and investigations. The results of any cartilage repair technique can only be judged years following the initial treatment. Maturation of the cartilage tissue, vascular invasion from the subchondral bone and transformation into fibrous cartilage are still feared possible long term outcomes in all these techniques, but the data available in the literature to date is very encouraging, and the attractiveness of a single stage procedure with off-the-shelf or readily-available repair kits are difficult to resist for any cartilage surgeon when providing similar or better results compared to existing techniques. These techniques may have potential and if the outcome meets the expectations, they may become repair techniques in the future.

Acknowledgement The author gratefully acknowledges the help from Brian J. Cole, Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL; Jack Farr, Orthoindy Cartilage Restoration Center, Greenwood, IN; David D. Frisbie, Orthopaedic Research Center, CSU, Fort Collins, CO and Patrick D. Reischling, Clinical Affairs Biologics, Zimmer.

References

1. Buckwalter JA, Mankin HJ. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr Course Lect. 1998;47:487–504.
2. Namba RS, Meuli M, Sullivan KM, Le AX, Adzick NS. Spontaneous repair of superficial defects in articular cartilage in a fetal lamb model. J Bone Joint Surg Am. 1998;80:4–10.
3. Bardos T, Farkas B, Mezes B, et al. Osteochondral integration of multiply incised pure cartilage allograft: repair method of focal chondral defects in a porcine model. Am J Sports Med. 2009;37 Suppl 1:50S–7.
4. DiMicco MA, Sah RL. Integrative cartilage repair: adhesive strength is correlated with collagen deposition. J Orthop Res. 2001;19:1105–12.
5. DiMicco MA, Waters SN, Akeson WH, Sah RL. Integrative articular cartilage repair: dependence on developmental stage and collagen metabolism. Osteoarthritis Cartilage. 2002;10:218–25.
6. Schaefer DB, Wendt D, Moretti M, et al. Lubricin reduces cartilage–cartilage integration. Biorheology. 2004;41:503–8.
7. Balint L, Park SH, Bellyei A, Luck Jr JV, Sarmiento A, Lovasz G. Repair of steps and gaps in articular fracture models. Clin Orthop Relat Res. 2005;403:208–18.
8. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis Cartilage. 2002;10:432–63.
9. Pap K, Krompecher S. Arthroplasty of the knee : experimental and clinical experiences. J Bone Joint Surg Am. 1961;43:523–37.
10. Bentley G, Greer III RB. Homotransplantation of isolated epiphyseal and articular cartilage chondrocytes into joint surfaces of rabbits. Nature. 1971;230:385–8.
11. Morales TI. Chondrocyte moves: clever strategies? Osteoarthritis Cartilage. 2007;15:861–71.
12. Lyman JR, Chappell JD, Morales TI, Kelley SS, Lee GM. Response of chondrocytes to local mechanical injury in an Ex vivo model. Cartilage. 2012;3:58–69.
13. Huntley JS, Bush PG, McBirnie JM, Simpson AH, Hall AC. Chondrocyte death associated with human femoral osteochondral harvest as performed for mosaicplasty. J Bone Joint Surg Am. 2005;87:351–60.
14. Bos PK, Kops N, Verhaar JA, van Osch GJ. Cellular origin of neocartilage formed at wound edges of articular cartilage in a tissue culture experiment. Osteoarthritis Cartilage. 2008;16:204–11.
15. Stone KR, Walgenbach AW. Surgical technique for articular cartilage transplantation to full thickness cartilage defects in the knee joint. Oper Tech Orthop. 1997;7:305–11.
16. Stone KR, Walgenbach AW, Freyer A, Turek TJ, Speer DP. Articular cartilage paste grafting to full-thickness articular cartilage knee joint lesions: a 2- to 12-year follow-up. Arthroscopy. 2006;22:291–9.
17. Frisbie DD, Lu Y, Kawcak CE, DiCarlo EF, Binette F, McIlwraith CW. In vivo evaluation of autologous cartilage fragment-loaded scaffolds implanted into equine articular defects and compared with autologous chondrocyte implantation. Am J Sports Med. 2009;37 Suppl 1:71S–80.
18. Zheng N, Lu Y, Boock R, Conrad B, Binette F. Retention of scaffold with staple fixation for cartilage repair during cyclic motion of human cadaveric knees. J Musculoskelet Res. 2008;11:151–9.
19. Lu Y, Dhanaraj S, Wang Z, et al. Minced cartilage without cell culture serves as an effective intraoperative cell source for cartilage repair. J Orthop Res. 2006;24:1261–70.

20. Cole BJ, Farr J, Winalski CS, et al. Outcomes after a single-stage procedure for cell-based cartilage repair: a prospective clinical safety trial with 2-year follow-up. Am J Sports Med. 2011;39:1170–9.
21. Albrecht F, Roessner A, Zimmermann E. Closure of osteochondral lesions using chondral fragments and fibrin adhesive. Arch Orthop Trauma Surg. 1983;101:213–7.
22. Adkisson HD, Gillis MP, Davis EC, Maloney W, Hruska KA. In vitro generation of scaffold independent neocartilage. Clin Orthop Relat Res. 2001;391:S280–S94.
23. Adkisson HD, Martin JA, Amendola RL, et al. The potential of human allogeneic juvenile chondrocytes for restoration of articular cartilage. Am J Sports Med. 2010;38:1324–33.
24. Stockwell RA. The interrelationship of cell density and cartilage thickness in mammalian articular cartilage. J Anat. 1971;109:411–21.
25. Farr J, Yao JQ. Chondral defect repair with particulated juvenile cartilage allograft. Cartilage. 2012;4:346–53.
26. Kruse DL, Ng A, Paden M, Stone PA. Arthroscopic De Novo NT((R)) juvenile allograft cartilage implantation in the talus: a case presentation. J Foot Ankle Surg. 2012;51:218–21.
27. Bonner KF, Daner W, Yao JQ. 2-year postoperative evaluation of a patient with a symptomatic full-thickness patellar cartilage defect repaired with particulated juvenile cartilage tissue. J Knee Surg. 2010;23:109–14.

Index

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6, © Springer-Verlag London 2014

N

O