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Cartilage Disorders

Recent Findings and Treatment

Edited by Karl Almqvist, Ahmed Ebrahim El Hamaky and Taiceer Abdulwahab

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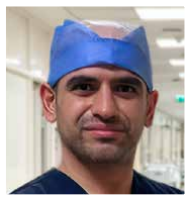
Meet the editors

Prof. Dr. Karl Fredrik Almqvist trained at the University of Ghent, Belgium, and worked at the Ghent University Hospital where he was head of the clinic, Department of Physical Medicine and Orthopaedic Surgery before moving to Dubai in 2014. He has published extensively and written several book chapters. He is a regular speaker at international meetings and is an editorial board and reviewer for the major sports orthopaedics journals. He obtained his Ph.D. in 2001 with his study, "Human differentiated articular cartilage cells in biodegradable matrices – Preparative studies for their use in the repair of cartilage defects." Dr. Almqvist is active in several orthopaedic organizations and is the past chairman of the Sports Committee of the International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine as well as the past chairman of the Cartilage Committee of the European Society of Sports Traumatology, Knee Surgery and Arthroscopy, and ISAKOS Board Director member. His main area of interest is knee surgery. His expertise covers the whole range of knee conditions from sports injuries to knee replacement.



Assistant Prof. Dr. Taiceer Abdulwahab is an orthopaedic surgeon at Mediclinic City Hospital, Dubai, UAE, and Assistant Clinical Professor of Orthopaedic Surgery at the Mohammed Bin Rashid University of Medicine and Health Sciences (MBRU), Dubai, UAE. He obtained a ChM in Trauma and Orthopaedic Surgery from the University of Edinburgh School of Medicine in partnership with the Royal College of Surgeons of Edinburgh, UK. He received an MSc in Trauma and Orthopaedic Surgery from Warwick School of Medicine, University of Warwick, UK, where he also obtained a postgraduate award (PGA) in Understanding Research and Critical Appraisal. Dr. Abdulwahab obtained his MBChB from the Bristol School of Medicine, University of Bristol, UK. He is a member of the Royal College of Surgeons of England (MRCS). He has presented at various international conferences including the International Congress for Joint Arthroplasty (ICJR) and the European College of Sports and Exercise Physicians (ECOSEP) with FIFA update. He has published papers in peer-reviewed journals and edited one book. He is also an investigator in several randomized controlled trials. He is the trauma doctor for the annual Emirates Rugby 7s. He has a special interest in sports surgery (arthroscopic shoulder and knee surgery). Outside of his work, Dr. Abdulwahab enjoys reading and is an active cyclist, competing in an annual 100-km race.

Dr. Ahmed Elhamaky is a qualified orthopedic specialist in trauma and sports medicine, currently practicing at Mediclinic City Hospital, Dubai, UAE. With a deep passion for his field, Dr. Elhamaky has dedicated his career to providing exceptional patient care and advancing the field of orthopedics. Dr. Elhamaky's journey began with his pursuit of higher education at Cairo University, Egypt, where he obtained his master's degree in Orthopedic Trauma and Disease. During his time at Cairo University, he

received comprehensive training in various aspects of orthopedics, honing his skills and knowledge under the guidance of experienced professionals. Driven by a thirst for knowledge and a commitment to excellence, Dr. Elhamaky further expanded his expertise through specialized training at prestigious institutions. He completed training programs at Cairo University's Orthopedic Department, Elhelal Hospital, and the Military Air Force Hospital in Egypt. These experiences allowed him to gain extensive experience in orthopedic trauma and equip him with the necessary skills to handle complex cases. In his pursuit of excellence, Dr. Elhamaky focused on advancing his expertise in hip and knee joint arthroplasty, as well as knee arthroscopic sports injuries. This specialized training enabled him to offer advanced surgical interventions and provide optimal care for patients with joint-related issues and sports-related injuries. Additionally, Dr. Elhamaky holds the esteemed position of adjunct clinical assistant professor at the Mohammed Bin Rashid University of Medicine and Health Sciences (MBRU), Dubai, UAE. This role allows him to contribute to the education of future doctors.

Contents

Preface

This book presents the latest research and treatment options in cartilage disorders. It provides an in-depth understanding of the cutting-edge research, technologies, and treatment options currently available to orthopedic surgeons.

Over the past few decades, there have been significant advances in our understanding of the pathophysiology of cartilage disorders as well as in the development of new treatment options for these conditions. Cartilage disorders are complex and multifactorial, and they can cause significant pain and disability in affected individuals. It is therefore essential to have a thorough understanding of the latest research and treatment options to provide the best possible care to patients.

Written by leading experts in cartilage research, the chapters in this textbook provide an in-depth analysis of the latest research findings and treatment options. The book is divided into two sections. Section I provides an overview of the basic science of cartilage biology, including the structure and function of cartilage, as well as the molecular mechanisms that underlie cartilage disorders. Section II focuses on the clinical aspects of cartilage disorders, including the regenerative techniques and treatment of these conditions.

The chapters in Section I cover topics such as the structure and composition of cartilage, the molecular mechanisms of cartilage degeneration, and the role of inflammation in cartilage disorders. The authors also discuss the latest advances in imaging and biomarker technologies, which have greatly enhanced our ability to diagnose and monitor cartilage disorders.

Section II of the book focuses on the clinical aspects of cartilage disorders, including the evaluation and management of patients with cartilage injuries. The authors provide detailed descriptions of the various techniques used to repair and regenerate damaged cartilage, as well as the latest advances in cell-based therapies and tissue engineering approaches. The association of cartilage lesions and ACL injuries is also highlighted in depth.

The editors of this textbook have taken great care to ensure that the content is both informative and accessible to specialized orthopedic surgeons. The chapters are written in a clear and concise style, and they are accompanied by numerous illustrations and diagrams to aid in understanding. In addition, each chapter includes a comprehensive reference list to facilitate further reading and research.

In conclusion, this textbook represents a comprehensive and up-to-date resource for specialists who are interested in the latest research and treatment options in cartilage disorders. We hope that readers will find this book to be a valuable addition to their professional library and that it will enhance their ability to provide the best possible care to their patients.

We would like to thank our families for their support. Ahmed dedicates this book to his parents, loving wife, daughter, and son. Taiceer dedicates this textbook to his loving parents, brother Omer, sister Noor, and nieces Yasmine and Noora.

And of course, our utmost dedication to our dear friend, role model, the true professor, Prof. Karl Fredrik Almqvist. He has trained us, guided us, and supported us in the operating theatre, the clinic, and life.

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Section 1

Cartilage Degeneration and Biology

Chapter 1

Introductory Chapter: Cartilage Disorders

Taiceer Abdulwahab

1. Introduction

This book demonstrates the pathological changes of cartilage degeneration at the molecular level and during the early stages of osteoarthritis, biochemical changes that precede the later structural changes.

Hyaline Articular Cartilage is critical for the proper functioning of the joint as it provides a smooth and low-friction surface for bone movement. However, hyaline cartilage has a limited ability to repair itself, making it vulnerable to damage and degeneration. In recent years, researchers have developed various techniques for the regeneration of hyaline cartilage, including Confocal Raman Microscopy for identifying tissue damage, tissue engineering for cartilage regeneration and autologous chondral implantations.

2. Confocal Raman microscopy

Confocal Raman Microscopy (CRM) is a promising tool for the characterisation of the collagen and proteoglycan content, as well as for monitoring the tissue damage and repair [1]. CRM can detect subtle changes in the Extra-Cellular Matrix (ECM) of articular cartilage, such as variations in collagen cross-linking, proteoglycan content and mineralisation. Thus, CRM has the potential to be used as a non-invasive diagnostic tool for early detection and monitoring of cartilage degeneration and repair.

3. Tissue engineering for cartilage regeneration

Tissue engineering approaches for cartilage regeneration aim to overcome the limitations of current treatments by providing functional and long-lasting repair tissue [2]. The most commonly used biomaterials for cartilage tissue engineering are hydrogels, which are highly hydrated and have a similar composition to the native ECM [3]. Hydrogels can provide mechanical support and act as a template for cell growth, differentiation and matrix deposition. They can also be modified with bioactive molecules, such as growth factors and cytokines, to promote cell proliferation and matrix synthesis [4].

4. Autologous chondral implantation

Autologous Chondral Implantation (ACI) is a cell-based therapy that involves harvesting healthy chondrocytes from a non-weight-bearing area of the patient's joint

and growing them in vitro. The expanded chondrocytes are then implanted into the damaged area of the joint using a periosteal or collagen membrane. ACI has shown promising results in the treatment of osteochondral defects, especially in the knee joint, with a reported success rate of 75–90% [5].

5. Conclusion

In conclusion, cartilage is a highly specialised tissue that is critical for joint function, but its limited self-repair capacity and the prevalence of joint disorders have created a critical need for advanced cartilage repair therapies. Confocal Raman microscopy, tissue engineering and autologous chondral implantation are among the latest scientific methods that show promise in advancing the field of cartilage regeneration. Although these approaches have shown significant progress, further research is needed to optimise their efficacy and long-term outcomes [6].

We endeavour that this book will update the current Orthopaedic Sports Surgeon on these advances in technique and technology, with explorations of future research and technique modalities.

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References

[1] Marcolongo M, Ateshian GA. Confocal Raman microscopy for cartilage characterization and monitoring. Current Opinion in Biomedical Engineering. 2018;**6**:43-50

[2] Liu Y, Wu X, Liao Y, Zhou N, Wu F, Cui L, et al. Advances and challenges in biomaterials for articular cartilage tissue engineering. Acta Biomaterials. 2020;**112**:1-26

[3] Ng KW, Wang CC, Mauck RL, Kelly TA, Chahine NO, Costa KD, et al. A layered agarose approach to fabricate depth-dependent inhomogeneity in chondrocyte-laden hydrogel constructs. Biomaterials. 2011;**32**(29):7419-7426

[4] Toh WS, Lim TC, Kurisawa M, Spector M. Modulation of mesenchymal stem cell chondrogenesis in a tunable hyaluronic acid hydrogel microenvironment. Biomaterials. 2012;**33**(2):383-391

[5] Giannini S, Buda R, Vannini F, Di Caprio F, Grigolo B. Autologous chondrocyte transplantation in osteochondral lesions of the ankle joint. Foot and Ankle Clinics. 2001;**22**(6):513-517

[6] Hunziker EB. Articular cartilage repair: Are the intrinsic biological constraints undermining this process insuperable? Osteoarthritis and Cartilage. 1999;**7**(1):15-28

Chapter 2

Characterization of Degraded Cartilage Using Confocal Raman Microscopy

N'Dre Jean, Hamideh Salehi, Marie Maumus, Danièle Noël, Yolande Koffi-Gnagne and Frédéric Cuisinier

Abstract

Osteoarthritis is a degenerative disease with pathological changes at the molecular level. Moreover, the damage to articular cartilage is irreversible. Early detection and the ability to follow the progression of osteoarthritis are essential to anticipate management. To characterize degraded human articular cartilage and to identify cellular changes that are precursors of phenotypic matrix changes in osteoarthritis, normal and degraded articular cartilage explants were harvested from the same patient's knee after informed consent. The blocks were washed several times (four times) with phosphate-buffered saline (often abbreviated to PBS) and then fixed on CaF2 slides using Cell-Tak® (an adhesive glue), and the whole set was placed in different Petri dishes containing PBS for Raman measurements. The analysis of the spectroscopic data allowed to differentiate degraded cartilage from normal cartilage by applying intensity ratios of some Raman bands and/or spectral regions. In addition, peaks at 864, 929, 945, 1107, 1386, and 2887 cm^{-1} were identified as characteristic Raman markers of degraded cartilage. The use of confocal Raman microscopy (CRM) has proven to be relevant in providing biochemical information necessary to characterize OA cartilage. CRM appears to be a powerful tool for the diagnosis and therapeutic evaluation of osteoarthritis in both early and late stages.

Keywords: articular cartilage, osteoarthritis, confocal Raman microscopy, degraded cartilage, Raman spectral data

1. Introduction

Osteoarthritis (OA) is the most common degenerative joint disease and one of the main causes of morbidity and economic burden for health resources. It is a slowly progressive disease that alters all tissues of the affected joint, with a long asymptomatic period [1]. According to OARSI (International Association for the Study of Osteoarthritis), OA is a serious disease defined as a disorder involving mobile joints, characterized by adhesive stress and degradation of the extracellular matrix, resulting in macro- and micro-damage that activates abnormal adaptive restorative responses, including pro-inflammatory pathways of the immune system [2]. Emerging evidence

in recent years defines OA as a heterogeneous, multifaceted disease with multiple molecular and clinical phenotypes [3, 4]. Loss of articular cartilage structure and function is one of the main features of OA [5–7].

The current diagnosis of OA is based primarily on radiographic criteria (e.g., joint space width, osteophyte formation, subchondral sclerosis) and clinical symptoms (e.g., pain, stiffness, and loss of function). Radiography is the most accessible tool for assessing OA: It can show lesions and other changes related to OA to confirm its severity according to different classification systems, such as Kellgren's Lawrence classification system [8]. MRI, which does not use radiation, is more expensive than X-rays but can provide better images of cartilage and other structures to detect early abnormalities in OA [9]. Another imaging technique is optical coherence tomography (OCT) imaging, which has the ability to generate cross-sectional images of articular cartilage and can provide quantitative information about the condition of articular cartilage, particularly for OA caused by changes in collagen structure [10]. Although these different medical tests are more sensitive than plain radiography, they cannot be routinely applied to many patients due to its cost, and if so, they are often time-consuming and even destructive. The other disadvantage is that these techniques are only valid and feasible in the advanced stages of osteoarthritis.

Currently, the lack of validated biomarkers and early diagnostic tools is one of the major obstacles to improved diagnosis and therapeutic evaluation of OA [11]. Recently, Raman spectroscopic techniques have been shown to not only provide non-invasive and nondestructive structural information in damaged cartilage, but also to allow spatial resolution at the biomolecular level that can be useful in detailed structural analyses of cartilage diseases. Indeed, these techniques can identify functional groups and chemical bonds present in biological tissues and/or cells. As a result, it is possible not only to assess the structure of proteins, lipids, carbohydrates, and nucleic acids present in a biological molecule [12–15], but also the changes in their chemical structure due to the disease process [3, 11], thus allowing monitoring of the progression of the disease process and prediction of the chemical pathway of the progression. Vibrational spectroscopy thus appears to be a proven analytical tool for understanding chemical changes associated with pathological conditions in tissues. The objective of our study was to characterize degraded human articular cartilage using confocal Raman microscopy (CRM) and to identify early cellular changes that are precursors to the phenotypic change of the matrix in OA.

2. Materials and methods

2.1 Source and preparation of samples

Our samples consisted of human articular cartilage explants taken from the knee of one patient (**Figure 1**). The biopsies were taken from the same knee but at different locations. For example, degraded cartilage (test sample) at the lesion site and normal cartilage (control sample) away from the lesion site. Human tissue was obtained for research purposes with donor consent. The study was also approved for the recovery of OA samples by the Ministry of Research and Innovation and the Comité d'Éthique de la Protection des Données Personnelles (CPP) of Languedoc-Roussillon (approval DC-2010-1185). The cartilage samples were collected from the same patient. The collected sample blocks were washed several times (four times) with phosphate-buffered

Figure 1.
Cartilage samples taken from a patient's knee.

saline (often abbreviated to PBS) and then fixed on CaF2 slides using Cell-Tak® (adhesive glue). The whole set was placed in different Petri dishes containing PBS for Raman measurements. During the whole measurement period, the samples were kept in a refrigerator at 4°C.

2.2 Raman measurements of the samples

All measurements were performed using a Witec α 300R confocal Raman microscope (Witec, Ulm, Germany) (**Figure 2a**). The system was equipped with a dual-frequency Nd: YAG laser (Newport, Evry, France) with a wavelength of 532 nm and a NIKON × 20 aerial lens with a numerical NA of 0.46 (Nikon, Tokyo, Japan). The output laser power was 50 mW. The spatial resolution was 300 nm, and the depth resolution was approximately 1 μm. The microscope was equipped with a piezo-driven scanning stage with a positioning accuracy of 2–3 nm horizontally and 10 nm vertically, respectively. The acquisition time for a single spectrum was set to 0.5 s; 150 × 150 points per image were recorded, resulting in a total of 22,500 spectra for one image. Data acquisition was performed using Image Plus 2.08 software from Witec. Using an edge filter, the backscattered Raman radiation was

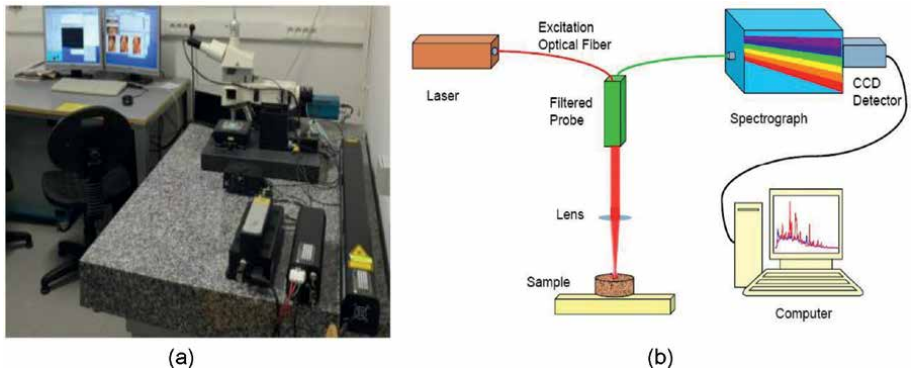

Figure 2.
Measurement of articular cartilage using confocal Raman microscopy. (a) Confocal Raman microscope at Laboratoire Bioingénierie et nanoscience UM_104 Montpellier. (b) Schematic representation of the system. The laser light source irradiates the articular cartilage, and the scattering light is generated by the scattering of the samples. The Raman scattering light is obtained by the filter, and the Raman spectrum is presented after its detection and processing by a CCD detector. The data of the spectra are analyzed using multivariate or chemometric methods.

separated from the scattered Rayleigh light. The Raman photons were transferred to the EMCCD camera (DU 970 N-BV353, Andor, Hartford, USA). The EMCCD chip size is 1600 × 200 pixels, the camera controller is a 16-bit A/D converter operating at 2.5 MHz, and the camera is cooled by a Pelletier system. The UHTS 300 spectroscopy system with 70% transmission and a 600 lines per mm grating (operating at − 60° C) provides a spectral resolution of 3–5 cm^{-1}. This microscope was used for the analysis of articular cartilage structures.

2.3 Analysis of the Raman spectral data

All collected spectra were preprocessed in order to obtain spectra that not only have the same scale, but are comparable to each other. The spectral analysis was performed in the fingerprint range (600–1800 cm^{-1}) due to its higher molecular specificity. Prior to the multivariate statistical analysis, the Raman spectra were preprocessed using well-established techniques. The preprocessing process of the spectra consisted, first, of baseline subtraction following the eighth-order polynomial law. Subsequently, we proceeded to the elimination of the autofluorescence of the tissues and the smoothing of the spectra using the "Savitzky Galay" filter, following a polynomial order = 4 with a number of points (or interval = 13). In order to compare the spectra and allow consistent comparisons where intensity variations may be relative to the intensity of each spectrum, the data were normalized to the area of the region between 600 and 1800 cm^{-1} [15]. These steps of preprocessing the spectra were necessary before subjecting them to statistical methods. All preprocessing, normalization, and determination of the different intensities of the peaks or bands were performed with the non-commercial Spectragryph® software version 1.2.12 developed and kindly offered by Dr. Friedrich Menges.

In addition, multivariate analyses such as principal component analysis (PCA) were applied to the raw dataset collected from the different samples. PCA is a well-established multivariate data analysis method that is well suited to distinguish small recurrent spectral variations from large datasets containing uncorrelated variations. It is a completely unsupervised analysis method for establishing whether or not sample spectra are grouped into classes based on sample type among other factors. This method greatly reduces the size of the dataset into a defined number of principal components (PCs), as all spectra are expressed in terms of a few basic functions (usually <10) and "a score vector" of about p entries. Thus, if clustering is observed, there are quantifiable and significant variations in the spectra that can be used to build discriminative algorithms to distinguish between different samples. PCA describes large global changes in composition without any prior knowledge.

3. Statistical analysis

Given that the distribution of our data does not follow a normal distribution, all our statistical analyses were performed using a non-parametric test, namely the Kruskal-Wallis one-way analysis of Variance on Ranks statistical test with $p < 0.001$ for significance of our results. For comparisons between different areas, the Student-Newman-Keuls statistical test with p-value <0.05 was applied. All our data were processed with SigmaPlot for Windows software version 11.0 Build 11.0.0.77.

4. Results

4.1 Characterization of articular cartilage degradation

From the collected cartilage blocks, a total of 50 Raman spectra were collected at different locations on each normal and degraded cartilage sample. The degraded state of the cartilage was assessed by determining the content of PGs and type II collagen, the main components of the extracellular matrix of cartilage, as reported in previous studies [16]. In **Figure 3**, we can easily see that the content of PGs significantly decreased ($p < 0.001$) in degraded cartilage, with a random coil content, characterized by applying the ratios of the integrated areas of the carbohydrate region ranging from 985 to 1185 cm^{-1} to the amide I region (1601–1776 cm^{-1}) [16], the intensity ratio of the two amide III peaks (1241/1269 cm^{-1}), with the peak 1241 cm^{-1} corresponding to the random coil content = NH2 bending: Random coil Amide III and 1269 cm^{-1} corresponding to the alpha-helix content = NH2 bending: alpha-helix Amide III) [17, 18], respectively.

4.2 Multivariate analysis and discrimination of Raman bands involved in articular cartilage degradation

The application of PCA on the raw spectra of the different samples allowed us to discriminate degraded cartilage from normal cartilage. **Figure 4** shows us the average and differential Raman spectra with the characteristic differential Raman bands. Also, analyzing the PCA score plot using CP1 and CP2, as well as that using CP2 and CP3, we find that only CP2 separates the degraded/normal samples (**Figure 5**). The CP2 principal component shows the intense positive charges for the Raman shifts in the degraded cartilage, characterized by the localized peaks, respectively at: 788 cm^{-1} (⁻ O-P-O ⁻); at 821 cm^{-1} (⁻ O-P-O ⁻); 867 cm^{-1} (RNA); 886 cm^{-1} (collagen I); 926 cm^{-1} (ν (C-C)); 945 cm^{-1} (ν (C-C) backbone); 972 cm^{-1} (ν (C-C) backbone in RNA); 1107 cm^{-1} (ν (C-O)); 1274 cm^{-1} (Amide III); 1386 cm^{-1} (GAG); 1432 cm^{-1} (CH_2 scissoring); 1480 cm^{-1} (CH deformation); 2887 cm^{-1} (CH_2 stretch); and 2947 cm^{-1} (υas CH_2, lipids, fatty acids). These peaks are the expression of vibrations of nucleic

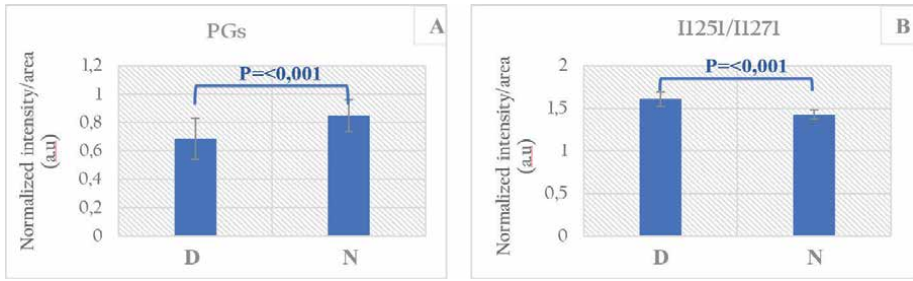

Figure 3.
Characterization of degraded cartilage: (A) determination of PGs content by the ratio of the area of the carbohydrate region [985–1185]/amid region I [1601–1760]. The histogram shows a higher intensity ratio for normal cartilage (CA). This reflects a significant ($p < 0.001$) decrease in PGs in degraded cartilage. (B) The ratio of disordered collagen (random coil) to ordered collagen (α-helix), represented by the intensity ratio I1251/I1271, is significantly greater ($p < 0.001$) and in favor of the random coil in degraded cartilage.
Observations: D: Degraded cartilage; N: Normal cartilage.

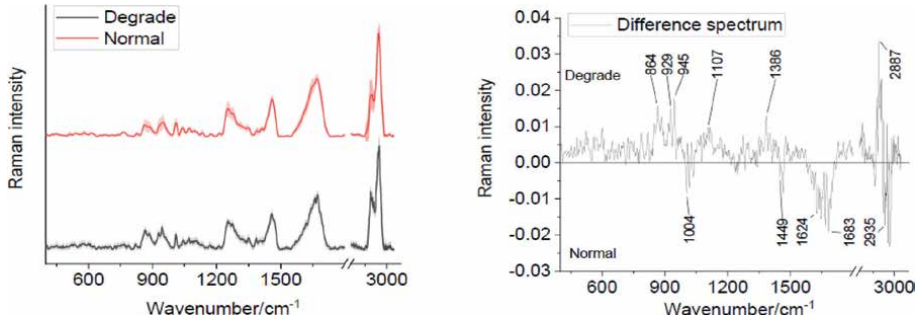

Figure 4.
Average and differential Raman spectra (n = 50 spectra).

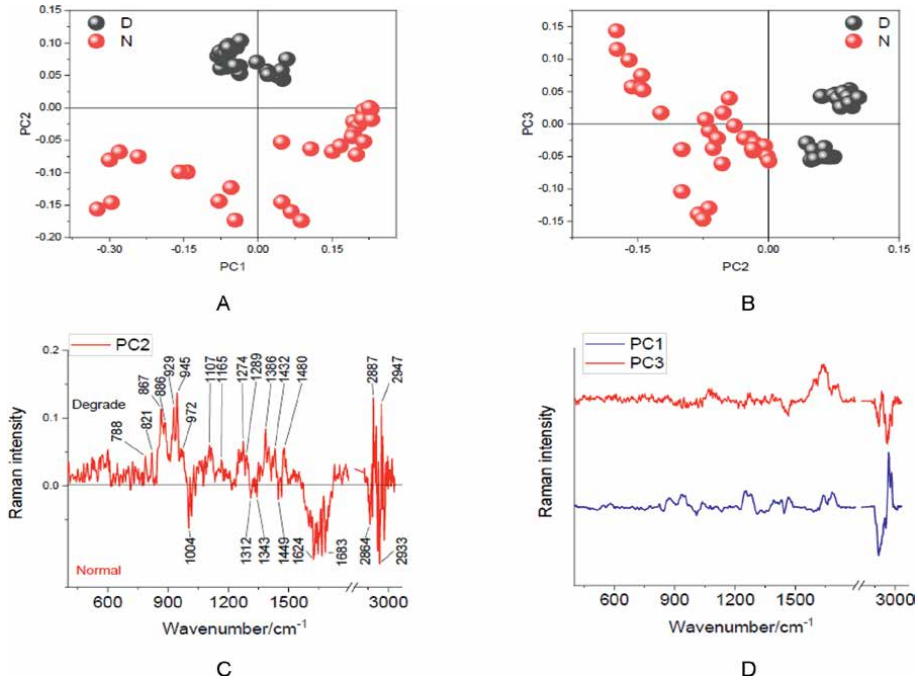

Figure 5.
Principal component analysis. (A) PCA score plot using PC1 and PC2. (B) PCA score plot using PC2 and PC3. Only PC2 separates degraded/normal samples. (C) PC2 loading is responsible for separating degraded and normal cartilage samples. (D) Remaining PC1 and PC3 loadings.

acid bases, collagen, and GAGs. In contrast, the negative charge peaks are the characteristic Raman shifts in normal cartilage, reflected in particular by peaks at 1004 cm^{-1} (Phe), 1624 cm^{-1} (Trp), 1683 cm^{-1} (ν (C=O)), and 2933 cm^{-1} (CH_2 asymmetric stretch). The assignments of these different peaks are recorded in **Table 1**.

5. Discussion

Pathological changes in cartilage begin at the molecular level (at the nanoscale) from where they propagate to higher levels of the hierarchical cartilage architecture

	Degraded cartilage
Raman shift [cm^{-1}]	Assignments
788	– O–P–O – in DNA
821	– O–P–O – in ARN
867	Collagen, ν (C-C) (Pro), Ribose vibration, one of the distinct modes of RNA (with 915 and 974 cm^{-1})
886	Proteins, including collagen I
926	ν (C-C), stretching - probably in amino acids
945	ν (C-C) backbone
972	ν (C-C) backbone in RNA
1107	ν (C-O), GAG
1165	C, G; Tyrosine (type I collagen)
1274	T, A, Amide III; = CH bending
1289	Phosphodiester group in the nucleic acid
1386	GAG, CH_3 band
1432	CH_2 shear
1480	G, A; deformation CH (DNA)
2887	Fermi resonance; CH_2 stretching
2947	υas CH_2, lipids, fatty acids

Table 1.
Table of major Raman peak or band assignments involved in the characterization of degraded versus non-degraded (normal) articular cartilage [12, 17, 19–21].

and cause increasingly irreversible structural and functional damage. Although the etiology of osteoarthritis is largely unknown, its onset is characterized by an imbalance between catabolic and anabolic processes, promoting the degradation of the cartilage matrix. The underlying cause of these macroscopic and microscopic structural features has been linked to the change in biochemical compositions such as alteration and decrease in proteoglycan content but also by the disorganization of type II collagen fibers [18, 22]. The biochemical changes are triggered by the expression of enzymes that are responsible for matrix degradation. The main components of articular cartilage are GAGs (15–30% of dry weight) and collagen (50–60% of dry weight) [23, 24], and their changes can be used as indicators for the early diagnosis of OA. Therefore, any technique to diagnose the onset of OA must detect early changes in cartilage PG gel before significant changes in its collagen matrix occur.

Raman spectroscopy has been shown to provide information on protein structure. Indeed, subtle molecular changes often cause detectable vibrational changes that can be detected by Raman analysis. In recent studies, Raman has been used either to more accurately quantify the distribution of cartilage subcomponents over its entire surface [25] or in some situations to establish the difference between different regions of the tissue over its entire depth the tissue surface [26].

Thus, Raman spectroscopy can be useful in differentiating normal from degraded cartilage. It is now known that the intensity ratio of the two peaks (I1251/I1271 cm^{-1}) provides information about the protein structure [11, 17]. Still called the ratio of

disordered collagen (random coil) to ordered collagen (α-helix), represented by the intensity ratio 1241/1269 cm^{-1}, provides an appreciation of articular cartilage damage [27]. This intensity ratio increases with the progression of osteoarticular diseases following the degree of cartilage damage (ICRS). In the present case, **Figure 3** shows a higher intensity ratio (1251/1271 cm^{-1}) and thus in favor of disordered collagen, characterizing a disordered structure of the protein. This result indicates an increase in defective collagen content. This observation was also made by Kumar et al. [18], who, when analyzing different grades of osteoarthritis, showed that this intensity ratio increased the progression of the cartilage disorder. This alteration of the random coil is associated with a decrease in PGs marking a clear difference between normal and degraded cartilage.

Other more subtle changes were highlighted by the application of PCA, which is a common multivariate statistical method very often used in bioanalytical Raman spectroscopy to reduce dimensionality and identify combinations of the most important spectral markers that maximize data variance and optimize group separation [18]. Indeed, although univariate analysis of Raman intensities in normalized spectra was necessary to differentiate degraded cartilage from normal cartilage by determining proteoglycan and type II collagen contents, multivariate analysis, particularly PCA, has been shown to be very effective in discriminating the two samples and identifying characteristic Raman bands or peaks. This ability of PCA has been widely demonstrated in numerous tissue and cell Raman spectroscopy studies [18, 25].

Figure 5C shows the loadings of the Raman bands responsible for the separation of the degraded and normal cartilage samples. Focusing on the peaks of the degraded cartilage, we find a high nucleotide activity (expressed by the presence of bands at 788, 821, and 1480 cm^{-1}), which could be attributed to the increased internucleosomal DNA cleavage manifested in the advanced stages of OA as revealed also by Verrier et al. [28]. During the progression of ECM degradation, chondrocytes are strongly solicited to renew the matrix proteins subjected to degradation phenomena. This high level of activity can lead to the death of chondrocyte cells by exhaustion, as demonstrated in the work of Zamli et al. [29] after evaluating the role of chondrocyte apoptosis in spontaneous animal models of osteoarthritis. They further demonstrated that chondrocyte death correlated with CA fibrillation ($r = 0.3$), cellularity< ($r = 0.4$), proteoglycan depletion, and overall OA microscopic scores ($r = 0.4$). This supports our results with the depletion of PGs and alteration of type II collagen structure observed in degraded cartilage and characterized by the presence of the high Raman signal intensity peaks at 867, 886, 926, 945 cm^{-1}, and 1274 cm^{-1} (collagen attribution) and peaks at 1107, 1342, and 1386 cm^{-1} (GAGs attribution). Focusing on the 1274 cm^{-1} peak, amide III attribution, Shaikh et al. [30] recently showed significant variations related to the intensity of this band and δCH_2 stretching in a group of cartilage with impact injuries. They therefore inferred that these changes in the intensity of the amide III peak were potentially due to conformational and configurational changes in collagen macromolecules as well as proteoglycan depletion. A first study by Lim et al. [31] had already shown, after an impact on porcine cartilage, a red shift of the 1264–1274 cm^{-1} peak. They correlated this shift of amide III with the compression of the C-N vibration in the collagen fibers. In the case of our study, the presence of the 1274 cm^{-1} peak could therefore reflect an alteration of the random coil associated with the decrease of the PGs content observed above. In addition, the presence of the peak at 1386 cm^{-1}, corresponding to N-acetyl-glucosamine (attribution of hyaluronic acid (HA)), helps to corroborate our observation. It is known that HA constitutes the main skeleton of GAGs. This glycoprotein binds to other aggrecanes to

form superaggregates responsible for tissue hydration, allowing cartilage to respond to mechanical stress. Thus, it helps to organize and stabilize the relationship between PGs and the collagen meshwork. Any changes in this glycoprotein could necessarily lead to disruptions in the structure of the cartilage matrix. As an important component of synovial fluid, HA can increase joint flexibility, thereby reducing cartilage wear. In a study by Buckwalter et al. [32], it could be shown that one of the first changes associated with osteoarthritis and joint immobilization was related to changes in hyaluronic acid glycoprotein. Also, Safiri et al. [33] recently demonstrated that it was associated with the radiological progression of osteoarthritis.

One factor that has also been associated with OA is the appearance of lipid deposits especially in the advanced stages of OA [34]. More recently, Mansfield and Winlove [12] studied the distribution of lipids, identifying two related regions: a band at 1441 cm^{-1} and shifts from 2845 to 2930 cm^{-1}. Generally, the CH vibrational region between 2800 and 3000 cm^{-1} is of particular interest for spectroscopic studies because it provides information about the chemical compositions of the samples due to differences in the C-H bond environment [12, 31]. Thus, it can provide information about the relative total concentration of biomolecules but also about changes in chemical bonds. In a recent study, Gaifulina et al. [35] were able to correlate the alterations observed in cartilage with the loss of tissue constituents and the observed increase in water content. They therefore correlated the observed increase in cartilage hydration with the increase in the intensity of the O-H stretch band. In this study, the Raman shifts of the bands at 2887 and 2947 cm^{-1} (lipid and/or fatty acid vibration) could reflect the strong presence of lipids also in degraded cartilage and explain the possible changes within the cytoplasmic skeleton by lipid production in the face of OA progression. This is justified by the response of chondrocytes to cartilage degradation in order to cope with the physiochemical changes in the cartilage matrix. It should be remembered that lipids are important nutrients in the metabolism of chondrocytes and are available to these cells by *de novo* synthesis, but also by diffusion from the surrounding tissues. Amanda et al. [34], by analyzing the status of cartilage, were able to link the development of osteoarthritis with the availability of lipids. Other more recent studies have reported well-established links with lipid accumulation and the development of OA [36, 37], particularly in its early stages before histological changes occur.

In this study, CRM appears to be very useful in differentiating degraded from healthy articular cartilage tissue. Indeed, during the early stages of osteoarthritis, biochemical changes precede the later structural changes and therefore play a fundamental role in contrast to mechanical factors, which are no longer significant in the advanced stages of disease progression. This makes CRM a privileged tool for early detection of ECM degradation. It is now recognized that CRM can be used for quantitative analysis on thicker, unfixed, hydrated, or even submerged samples, as long as water does not interfere with the Raman signals [13]. Moreover, since depth analysis does not require cutting of the sample, it can be applied to healthy tissue, such as articular cartilage.

In any case, this technique seems to be useful in circumstances where tissue biopsies are recorded or even with Raman-compatible arthroscopic probes [38]. As such, numerous Raman spectroscopy devices in the form of fiber optic probes have been developed and tested for some on human knee joint tissue [39, 40], a common anatomical site for arthroscopic surgery, and allowed detection of cartilage with contributions from subchondral bone [41], others on the colon for real-time *in vivo* assessment of adenomatous polyps [39], or either to nondestructively monitor the

growth of *in vitro* tissue engineering constructs in real time for regenerative medicine applications toward controlled clinical translation [38, 40]. As pointed out by Karen et al. [41], fiber optic probes are a convenient format to couple optical spectroscopies to an arthroscopic probe because they are compatible with the small dimensions of the arthroscope and provide the same chemical information available in a microscopy instrument. In this sense, we agree with Gao et al. [42] that CRM has the potential to be incorporated into a fiber-optic probe device to build a fiber-optic-based confocal Raman microscopy detection unit for an arthroscope for clinical use. As a result, it has the capabilities during arthroscopic procedures to remove the operator from damage to healthy tissue that is usually, in addition to degraded cartilage or sclerotic bone, sometimes included at the microfracture site [41]. Therefore, the coupling of confocal Raman microscopy to arthroscopy could facilitate not only to specifically identify degraded or damaged tissues, but also to better guide surgical interventions by avoiding surrounding healthy tissues. Obviously, such a technology practiced on a daily basis in the clinical setting would strongly contribute to prevent irreversible cartilage damage in the more advanced stages and would favor the institution of adequate management either by stem cell injection to induce regeneration of damaged sites or by other therapeutics without impacting surrounding healthy tissue.

6. Conclusion

Osteoarthritis is a degenerative disease that primarily affects articular cartilage and related joint tissues and imposes an increasing social burden due to overall activity limitation, especially in the elderly. With degradation, the major components of the cartilage ECM, particularly collagen and proteoglycans, are progressively degraded by the released inflammatory factors. Currently, the diagnosis of OA relies on radiographic methods based on the Kellgren-Lawrence scores, in which joint space narrowing is considered the main diagnostic indicator of advanced disease. One of the major challenges in the management of OA is the ability to make an early diagnosis. To date, there are no biomarkers available for early diagnosis of the disease and no effective therapy other than symptomatic treatment and joint replacement surgery.

The results of our study demonstrate the ability of CRM to differentiate damaged from healthy articular cartilage tissue and thus introduce RCM as a future diagnostic tool for the efficient management of OA. The application of CRM has proven to be relevant in providing biochemical information needed to characterize OA cartilage. Combined with multivariate analysis, CRM is able to identify biomarkers to characterize biochemical and structural changes in articular cartilage ECM. The peaks at 864, 929, 945, 1107, 1271, 1386, and 2887 cm^{-1} identified in this work can be considered as major indicators to monitor the physio-pathogenesis of articular cartilage. Since *in vivo* Raman spectra have been reported to be collected from human skin, lung, and bone, this technique can therefore advance the diagnostics of AO at an early stage.

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Authors Contributions

N'DRE NJ was responsible for the realization of the experiments and the preparation of the manuscript. H. SALEHI supervised the Raman experiments and data analysis. M. MAUMUS prepared the samples for the Raman measurements, and ANURADHA R helped us with the analysis and interpretation of the PCA data. D. NOËL supervised the sample preparation and data analysis. KOFFI-GNAGNE and F. CUISINIER edited the manuscript. All authors contributed to the manuscript with comments and suggestions before publication.

Conflicts of interest

No conflicts of interest are reported.

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References

[1] Kraus VB, Blanco FJ, Englund M, Karsdal MA, Lohmander LS. Call for standardized definitions of osteoarthritis and risk stratification for clinical trials and clinical use. Osteoarthritis Cartilage. 2015;**23**(8):1233-1241

[2] Hunter DJ et al. OARSI Clinical Trials Recommendations: Knee imaging in clinical trials in osteoarthritis. Osteoarthritis Cartilage. 2015;**23**(5):698-715

[3] Mobasheri A, Saarakkala S, Finnilä M, Karsdal MA, Bay-Jensen A-C, van Spil WE. Recent advances in understanding the phenotypes of osteoarthritis. F1000Res. 2019;**8**:268

[4] Henrotin Y. Osteoarthritis in year 2021: Biochemical markers. Osteoarthritis Cartilage. 2022;**30**(2):237-248

[5] Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: A disease of the joint as an organ. Arthritis and Rheumatitis. 2012;**64**(6):1697-1707

[6] Tonge DP, Pearson MJ, Jones SW. The hallmarks of osteoarthritis and the potential to develop personalised disease-modifying pharmacological therapeutics. Osteoarthritis and Cartilage. 2014;**22**(5):609-621

[7] Cucchiarini M et al. Basic science of osteoarthritis. Journal of Experimental Otology. 2016;**3**(1):22

[8] Yong CW et al. Knee osteoarthritis severity classification with ordinal regression module. Multimedicine Tools Applied. 2021. DOI: 10.1007/s11042-021-10557-0

[9] Chaudhari AS, Kogan F, Pedoia V, Majumdar S, Gold GE, Hargreaves BA. Rapid knee MRI acquisition and analysis techniques for imaging osteoarthritis. Journal of Magnetic Resonance Imaging. 2020;**52**(5):1321-1339. DOI: 10.1002/jmri.26991

[10] Goodwin M, Workman J, Thambyah A, Vanholsbeeck F. Impact-induced cartilage damage assessed using polarisation-sensitive optical coherence tomography. Journal of the Mechanical Behavior of Biomedical Materials. 2021;**117**:104326

[11] Casal-Beiroa P, González P, Blanco FJ, Magalhães J. Molecular analysis of the destruction of articular joint tissues by Raman spectroscopy. Expert Review of Molecular Diagnostics. 2020;**20**(8):789-802

[12] Mansfield JC, Winlove CP. Lipid distribution, composition and uptake in bovine articular cartilage studied using Raman micro-spectrometry and confocal microscopy. Journal of Anatomy. 2017;**231**(1):156-166

[13] Albro MB et al. Raman spectroscopic imaging for quantification of depth-dependent and local heterogeneities in native and engineered cartilage. NPJ Regeneration Medicine. 2018;**3**(1):1-11

[14] Bergholt MS, Serio A, Albro MB. Raman spectroscopy: Guiding light for the extracellular matrix. Frontier in Bioengineering Biotechnology. 2019;**7**:303

[15] Power L, Wixmerten A, Wendt D, Barbero A, Martin I. Raman spectroscopy quality controls for GMP compliant manufacturing of tissue engineered cartilage. In: Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XVII. San Francisco, United States; 2019. p. 14

[16] Rieppo L, Saarakkala S, Närhi T, Helminen HJ, Jurvelin JS, Rieppo J. Application of second derivative spectroscopy for increasing molecular specificity of Fourier transform infrared spectroscopic imaging of articular cartilage. Osteoarthritis and Cartilage. 2012;**20**(5):451-459

[17] Takahashi Y. Raman spectroscopy investigation of load-assisted microstructural alterations in human knee cartilage_ Preliminary study into diagnostic potential for osteoarthritis. Journal of the Mechanical Behavior of Biomedical Materials. 2014;**3**:77-85

[18] Kumar R et al. Optical investigation of osteoarthritic human cartilage (ICRS grade) by confocal Raman spectroscopy: A pilot study. Analytical Bioanalytical Chemistry. 2015;**407**(26):8067-8077

[19] Czamara K, Majzner K, Pacia MZ, Kochan K, Kaczor A, Baranska M. Raman spectroscopy of lipids: A review: Raman spectroscopy of lipids. Journal of Raman Spectroscopy. 2015;**46**(1):4-20

[20] Mandair GS, Morris MD. Contributions of Raman spectroscopy to the understanding of bone strength. Bonekey Reports. Jan 2015;**4**:620. DOI: 10.1038/bonekey.2014.115. PMID: 25628882; PMCID: PMC4296861

[21] Bergholt MS et al. Raman Spectroscopy reveals new insights into the zonal organization of native and tissue-engineered articular cartilage. ACS Central Science. 2015;**2**(12):885-895

[22] An R et al. Diagnosis of knee osteoarthritis by OCT and surface-enhanced Raman spectroscopy. Journal of Innovative Optical Health Science. 2022;**2022**:2250027

[23] Athanasiou KA, Darling EM, DuRaine GD, Hu JC, Reddi AH. Articular Cartilage. Second ed. Boca Raton London New York: CRC Press, Taylor & Francis Group; 2017

[24] Das Gupta S et al. Raman microspectroscopic analysis of the tissue-specific composition of the human osteochondral junction in osteoarthritis: A pilot study. Acta Biomaterialia. 2020;**106**:145-155

[25] Mason D, Murugkar S, Speirs AD. Measurement of cartilage sub-component distributions through the surface by Raman spectroscopy-based multivariate analysis. Journal of Biophotonics. 2021;**14**(1):e202000289. DOI: 10.1002/jbio.202000289

[26] Jensen M et al. Multiplexed polarized hypodermic Raman needle probe for biostructural analysis of articular cartilage. Optical Letters. 2020;**45**(10):2890-2893

[27] Pavlou E, Zhang X, Wang J, Kourkoumelis N. Raman spectroscopy for the assessment of osteoarthritis. Annotation Joint. 2018;**3**(83):1-10

[28] Verrier S, Notingher I, Polak JM, Hench LL. In situ monitoring of cell death using Raman microspectroscopy. Biopolymers. 2004;**74**:157. DOI: 10.1002/bip.20063

[29] Zamli Z, Adams MA, Tarlton JF, Sharif M. Increased chondrocyte apoptosis is associated with progression of osteoarthritis in spontaneous Guinea pig models of the disease. International Journal of Molecular Sciences. 2013;**14**(9):9. DOI: 10.3390/ijms140917729

[30] Shaikh R et al. Raman spectroscopy is sensitive to biochemical changes related to various cartilage injuries. Journal of Raman Spectroscopy.

2021;**52**(4):796-804. DOI: 10.1002/jrs.6062

[31] Lim NSJ, Hamed Z, Yeow CH, Chan C, Huang Z. Early detection of biomolecular changes in disrupted porcine cartilage using polarized Raman spectroscopy. Journal of Biomedical Optics. 2011;**16**(1):017003. DOI: 10.1117/1.3528006

[32] Buckwalter JA, Lane NE. Athletics and osteoarthritis. American Journal of Sports Medicine. 1997;**25**(6):873

[33] Safiri S et al. Global, regional and national burden of osteoarthritis 1990-2017: A systematic analysis of the Global Burden of Disease Study 2017. Annals of the Rheumatic Diseases. 2020;**79**(6):819

[34] Villalvilla A, Gómez R, Largo R, Herrero-Beaumont G. Lipid transport and metabolism in healthy and osteoarthritic cartilage. IJMS. 2013;**14**(10):20793

[35] Gaifulina R et al. Intra-operative Raman spectroscopy and ex vivo Raman mapping for assessment of cartilage degradation. Clinical Spectroscopy. 2021;**3**:100012

[36] Papathanasiou I, Anastasopoulou L, Tsezou A. Cholesterol metabolism related genes in osteoarthritis. Bone. 2021;**152**:116076

[37] Meng H, Jiang L, Song Z, Wang F. Causal associations of circulating lipids with osteoarthritis: A bidirectional mendelian randomization study. Nutrients. 2022;**14**(7):7. DOI: 10.3390/nu14071327

[38] Bergholt MS, Albro MB, Stevens MM. Online quantitative monitoring of live cell engineered cartilage growth using diffuse fiber-optic Raman spectroscopy. Biomaterials. 2017;**140**:128-137

[39] Bergholt MS et al. Simultaneous fingerprint and high-wavenumber fiber-optic Raman spectroscopy enhances real-time in vivo diagnosis of adenomatous polyps during colonoscopy. Journal of Biophotonics. 2016;**9**(4):333-342. DOI: 10.1002/jbio.201400141

[40] Kandel S, Querido W, Falcon JM, Reiners DJ, Pleshko N. Approaches for in situ monitoring of matrix development in hydrogel-based engineered cartilage. Tissue Engineering Part C: Methods. 2020;**26**(4):225-238

[41] Esmonde-White KA, Esmonde-White FWL, Morris MD, Roessler BJ. Fiber-optic Raman spectroscopy of joint tissues. Analyst. 2011;**136**(8):1675

[42] Gao T, Boys AJ, Zhao C, Chan K, Estroff LA, Bonassar LJ. Non-destructive spatial mapping of glycosaminoglycan loss in native and degraded articular cartilage using confocal raman microspectroscopy. Frontiers in Bioengineering Biotechnology. 2021;**9**:744197

Section 2

Cartilage Regeneration and Biologics

Chapter 3

Evolution of Cartilage Repair Technology

Chao Zhou, Qi Heng Chen, Zhanzhen Li, Xin Wang, Jiang Peng and Changlong Yu

Abstract

Articular cartilage plays an important role in daily joint activities. With the aging of the social population, the degenerative cartilage injury and the sports injury caused by inappropriate exercise of young patients, etc., the incidence rate of articular cartilage injury is constantly rising, and the injured patients tend to be younger. Although articular cartilage has its corresponding metabolic activities, it is difficult to recover and regenerate itself once it is damaged due to lack of nerve, blood vessel, and lymphatic tissue Common articular cartilage injuries can be divided into three types according to the degree of injury: partial cartilage injury, full-thickness cartilage injury, and osteochondral defect. If partial cartilage damage and full-thickness cartilage damage are not found and treated in time in the early stage, further deterioration will lead to serious osteochondral defects. After the corresponding subchondral bone injury, the upward invasion of the upper cartilage layer will also cause the overall osteochondral injury. Therefore, whether the osteochondral injury caused by the top-down or the osteochondral injury caused by the bottom-up, it seriously affects the normal activities of human joints. It not only brings great inconvenience to the daily life of patients, but also causes huge economic and psychological burden to patients. At the same time, it also consumes a large number of social public medical resources. Therefore, seeking an effective osteochondral repair strategy is not only the urgent need and hope of the society, but also one of the clinical scientific problems that clinicians and scientists urgently need to solve.

Keywords: articular cartilage, osteochondral defect, scaffold, autologous chondrocyte transplantation technology, cartilage repair

1. Introduction

The microfracture technique creates a channel between the cartilage defect and the underlying bone marrow by opening the subchondral bone [1]. At present, it is generally believed in clinical research that pluripotent bone marrow mesenchymal stem cells are released and recruited to the defect site through these channels to repair articular cartilage. This technology is often used in clinic because of its simplicity, rapidity, and low cost. However, this method is only effective for small defects,

and cannot form hyaline articular cartilage after repair. It can only provide relative functional improvement after operation, and its clinical effect and significance are relatively limited.

2. Osteochondral transplantation

Autogenous osteochondral transplantation is also used in clinical practice. It is to take out cylindrical osteochondral tissue from the cartilage surface of the non-load bearing area of the joint and implant it into the cartilage defect of the load-bearing area. Although it has been reported in the literature that good clinical results can be obtained by autologous bone and cartilage transplantation, the results vary greatly depending on age, sex, and lesion size. At the same time, the injury and discomfort of the donor site and the limited availability of local donor tissue make the autologous bone and cartilage transplantation only suitable for some small- and medium-sized cartilage defects. At the same time, there are problems in the repair and healing of cartilage between the transplanted bone and cartilage, and the healing of the transplanted cartilage and the surrounding cartilage of the recipient area. In addition, the adverse effects of the wound opening caused by the injury of the donor area and the release of bleeding inflammatory factors on the microenvironment and homeostasis of the joint make its application limited and gradually reduced. Although allogeneic osteochondral transplantation does not have the problems of donor damage and insufficient graft size, there are problems such as the preservation of allografts, the availability of tissues, the immune response of recipients, and the shortage of donor sources and quality that are also practical problems in clinical application.

3. Scaffold

In recent years, with the emergence and continuous development of tissue engineering regenerative medicine, it has brought new hope for the repair and regeneration of bone and cartilage after injury. Tissue engineering regenerative medicine mainly includes three factors: biological scaffolds, seed cells, and growth factors [2]. The scaffold of cartilage tissue engineering technology is equivalent to the extracellular matrix. It should be non-toxic, not causing inflammation, and has high porosity. It can provide a good microenvironment for cell growth and can still maintain its shape after implantation [3]. The earliest scaffold materials used in cartilage tissue engineering are PGA, PLA, PLGA, etc. [4]. When PRP gel is used as a scaffold alone, it is easy to squeeze and deform after subcutaneous implantation, and specific cartilage tissue cannot be formed [5, 6]. 3D printing technology can be used to prepare high-precision scaffolds [7, 8], Plga/dacecm tissue-engineered cartilage scaffolds were prepared by low-temperature deposition 3D printing technology [9, 10], and the results showed that the scaffolds were not cytotoxic and has excellent performance [11]. Lin's [12] study also confirmed that this scaffold can better promote the proliferation of bone marrow mesenchymal stem cells and promote the formation of new cartilage. By integrating the preparation process, it will be a new research direction to construct tissue engineering cartilage scaffolds that cannot only recruit endogenous stem cells but also facilitate the maturation of new tissues.

The first generation of autologous chondrocyte transplantation technology is to take articular cartilage under arthroscopy, isolate chondrocytes, culture and

expand them *in vitro*, inject cell suspension into the defect, and finally cover with autologous periosteum for suture. This method can repair cartilage damage with a depth of more than 6–8 mm [13]. However, there are also many disadvantages, such as the leakage of cell suspension and the proliferation of periosteum, which require arthroscopic surgical resection [14].

The second generation of autologous chondrocyte transplantation technology is to use some biofilms, such as collagen membrane, to suture with surrounding tissues instead of periosteum. Although the cost is higher than that of autologous periosteum, it does not require secondary surgery, so it is more economical [15].

The third-generation matrix-induced autologous chondrocyte transplantation is to expand the chondrocytes cultured *in vitro*, implant them into the rough surface of the I/III double-layer collagen membrane, and then replant them. Finally, the more biocompatible fibrin glue is used for adhesion, eliminating the need for suturing, making the operation easier and reducing the risk of cell leakage [16].

The fourth-generation cartilage repair technology With the continuous development of cartilage repair technology, after the iterative upgrading of the previous three generations of cartilage repair technology, cartilage repair technology has reached a new height. Although the third-generation cartilage repair technology overcomes the shortcomings of the previous two generations of repair technology, such as poor survival of cells in the defect area, poor long-term repair effect, uneven articular surface, and so on, some problems in the past have not been solved. For example, articular cartilage is composed of hyaline cartilage, which is still replaced by fibrocartilage after repair, resulting in unsatisfactory mechanical properties and biological properties.

How can we repair cartilage damage and solve these problems at the same time? With the further in-depth study of the cell microenvironment, it is found that the principle of microfracture technology, for example, is to introduce stem cells in bone marrow into the cartilage defect area, so that the stem cells can grow, and differentiate and repair in the microenvironment of the cartilage itself, playing a good repair effect. It is the so-called "one side of the soil nourishes one side of the people." The scaffold we made not only carries the cell load, but also wants it to grow and differentiate well on the scaffold, which has been imitating the internal environment of the machine. So why not use the microenvironment of the cell itself to make this cell carrier? Therefore, the concept of extracellular matrix (ECM) came into being. It can not only provide a good microenvironment for cell growth and differentiation, but can also solve the problems of cell metabolism and biomechanical properties.

3.1 Extracellular matrix (ECM)

Extracellular matrix (ECM) is a matrix structure that is synthesized and secreted by cells and distributed on the cell surface or between cells. It is a complex network composed of proteins and proteoglycans, which can provide support for tissues and regulate cell functions. It is the dynamic microenvironment of the stem cell niche. Therefore, it has attracted the continuous attention of tissue engineering researchers [17, 18]. In addition, cartilage extracellular matrix can activate intracellular signal transduction pathways through various growth factors and cytokines [19]. Due to the complex composition of extracellular matrix, it is almost impossible to fully bionize the cartilage extracellular matrix in terms of composition, morphology, and function in the current progress of cartilage tissue engineering. At present, the bionic biological scaffold materials used in cartilage tissue engineering include extracellular

matrix-derived materials and non-extracellular matrix-derived materials. The extracellular matrix-derived materials include simple-component materials extracted from the extracellular matrix, mixed-component materials, and tissue acellular extracellular matrix materials. The extracellular matrix-derived material is closer to the cartilage extracellular matrix than other materials in composition and is a very excellent biomimetic scaffold material.

1. The composition of extracellular matrix determines the function of extracellular matrix, such as providing support for cells, regulating the dynamic behavior of cells and intercellular communication [20, 21]. The composition of extracellular matrix is different in different tissues [22]. The main components of cartilage extracellular matrix are collagen, proteoglycan and other non-collagen and glycoproteins, as well as a various growth factors, cytokines, and proteases. Collagen is a very important and most abundant macromolecular component of the extracellular matrix of cartilage. In articular cartilage, type II collagen accounts for 90%–95% of the total collagen. The main purpose of collagen is to provide tension and shear force for tissues and to fix proteoglycans in the matrix [23]. Proteoglycan is a macromolecule in the extracellular matrix of cartilage, which is second only to collagen. It is a covalent conjugate composed of glycosaminoglycans and core proteins [24]. Glycosaminoglycans are composed of long-chain unbranched-repeating disaccharide units. Chondroitin sulfate, keratin sulfate, and dermatan sulfate are glycosaminoglycans that covalently bind with core proteins to form proteoglycans in cartilage, of which chondroitin sulfate accounts for 55–90%. 80–90% of proteoglycans in articular cartilage form large polymers, which are called polyproteoglycans. Polyproteoglycan and hyaluronic acid (the only glycosamine polysaccharide that does not undergo sulfation) can bind with connexin in a non-covalent bond to form a stable polyproteoglycan hyaluronic acid connexin complex. The non-covalent binding force between these complexes is very strong, and only proteolytic enzymes can degrade it. In the matrix, polymerization stabilizes polyproteoglycans. Other ingredients include elastin, which forms a network of elastic fibers and gives the tissue elasticity; fibronectin can connect cells to the extracellular matrix; cartilage oligomeric protein, which only appears in cartilage, has the ability to connect chondrocytes and a small amount of lipids. Cartilage extracellular matrix also stores many growth factors and cytokines, bone morphogenetic protein, insulin-like growth factor, basic fibroblast growth factor, platelet-derived growth factor, and chondromodulin, and forms a good storage pool to store them. Changing the conditions can activate the activities of special enzymes, cause the release of factors in the storage pool, and achieve rapid and stable regulation of cell functions [25–27].

2. Extracellular matrix-derived scaffolds in cartilage tissue engineering should simulate the extracellular matrix of cartilage in structure and composition, and provide an ideal microenvironment for the proliferation and differentiation of seed cells. Natural polymer scaffold materials are derived from the organism itself and have the advantages of good biocompatibility, low cytotoxicity, and easy degradation, and the degradation products are easily absorbed by the human body without inflammatory reaction [28, 29]. Extracellular matrix-derived scaffolds are scaffolds made of one or more components of extracellular matrix, which are derived from extracellular matrix and are closer to cartilage tissue than other biomaterials. The commonly used extracellular matrix source scaffold

materials include collagen, glycosaminoglycan, hyaluronic acid, gelatin, chondroitin sulfate, tissue acellular extracellular matrix, etc. These materials can be used alone to make scaffolds, but it is often difficult to imitate all components of the cartilage when applied alone. Therefore, some researchers have also combined two or more of these materials, or combined with other natural materials or artificial materials [30, 31]. Extracellular matrix-derived scaffolds can be divided into four categories according to their composition: monomeric natural polymer materials, multiple natural polymer mixed materials, new biomaterials constructed by combining natural polymer materials with synthetic polymer materials, and tissue acellular extracellular matrix materials.

3. Preparation of tissue acellular extracellular matrix scaffold: Cartilage tissue needs to go through two very important steps, tissue acellularization and scaffold preparation, before it can be prepared as a scaffold derived from extracellular matrix. The purpose of tissue decellularization is to remove the substances that cause immune reaction, such as cell membrane materials, soluble proteins, nucleic acids, while retaining the extracellular matrix components of cartilage as much as possible and maintaining its biological activity. At present, there are two kinds of commonly used decellularization methods: physical method and chemical method. The physical methods include freezing and thawing method, mechanical oscillation method, differential centrifugal method, etc. Chemical methods include enzymatic digestion, high or low osmotic solution decellularization, acid-base decellularization, etc. Usually, one method or a combination of methods is used in the process of decellularization. When preparing acellular extracellular matrix materials, more attention should be paid to whether acellularization is complete and the loss of components. When the extracellular matrix material is prepared, it needs to be made into a scaffold. At present, many methods have been developed to prepare cartilage tissue engineering scaffolds. Different preparation processes can have obvious effects on the performance of scaffolds. In practical application, different preparation processes can be selected according to the conditions [29]. Freeze drying method: The extracellular matrix slurry is poured into the mold, freeze-dried at low temperature, then cross-linked, and finally sterilized to form a 3D scaffold. Immunogenicity and toxicity are removed. Cartilage acellular extracellular matrix scaffold material has many advantages. It is completely derived from biological tissue. After acellular treatment, the immunogenicity is removed to the maximum extent, and many effective components of natural cartilage extracellular matrix are retained. It can provide a better microenvironment for seed cell growth than pure component materials and artificial mixed materials. The extracellular matrix contains natural cytokines, which can promote the proliferation and differentiation of seed cells without adding exogenous cytokines. The PLA General Hospital adopted its own unique preparation process to form a biphasic-oriented stent. The cells implanted in the scaffolds were induced to differentiate into chondrocytes and grow in the direction induced by the scaffolds. Finally, the regeneration and growth of the repaired cartilage become similar to the natural cartilage.

The prepared scaffold had three-dimensional porous sponge-like longitudinally oriented structure. There were cartilage fibers around the scaffold pores. After hematoxylin-eosin staining, no nucleus was observed. Both safranin O and Sirius red staining confirmed that cartilage tissue engineering scaffold contained collagen and

cartilage matrix. The porosity and water absorption of the scaffold was (91.8 ± 2.9)% and (93.5 ± 1.4)%, respectively. MTT results showed that the leaching liquor of human cartilage-derived extracellular matrix was non-toxic to chondrocytes. After co-culture, human chondrocytes adhered, proliferated, and evenly distributed on the peripheral wall of the scaffold pores. The results showed that human articular cartilage-derived extracellular matrix had similar composition to natural cartilage, provided the structure suitable for cell adhesion and proliferation, and exhibited good histocompatibility. Therefore, human articular cartilage-derived extracellular matrix can be used as a scaffold material for repairing cartilage defects by tissue engineering technique.

1. Clinical application and effect of ECM stent. The fourth-generation cartilage scaffold developed by the Orthopaedic Research Institute of the General Hospital of the Chinese people's Liberation Army combined with autologous chondrocytes to repair the local cartilage defect of the femoral condyle has achieved very good results after more than 10 years of clinical verification. It has been popularized in clinic.

2. For summary in recent years, with the rapid development of cartilage tissue engineering, the preparation and selection of scaffold materials have become a hot topic for domestic and foreign scholars. From the current situation, great progress has been made in the research of cartilage tissue engineering scaffold materials. At present, it is recognized that the ideal biomimetic scaffold for cartilage tissue engineering should have the following characteristics: It simulates natural

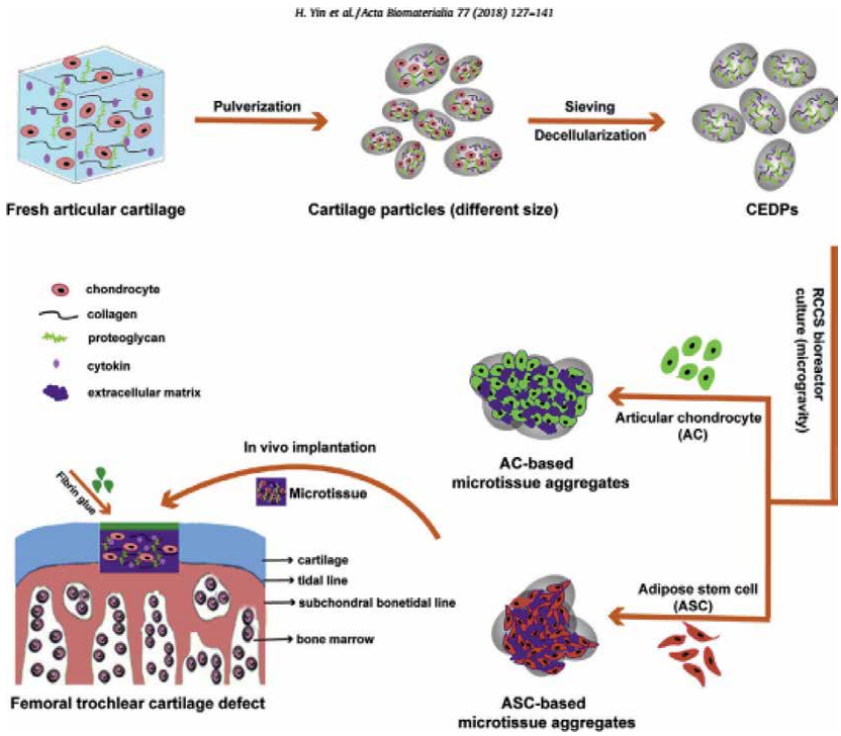

Figure 1.
ECM scaffold preparation, composite cells, cartilage defect model, and treatment plan [33].

cartilage tissue components, has good proliferation and differentiation promoting effects on seed cells, has biomechanical characteristics close to cartilage, and can be degraded *in vivo* and will not cause adverse reactions. Extracellular matrix-derived scaffolds have been used in cartilage tissue engineering and clinical practice because of some characteristics of biomimetic scaffolds, and have been proved to be good biomimetic scaffolds for cartilage tissue engineering. However, the shortcoming is that the scaffold materials derived from extracellular matrix have poor mechanical properties and are not easy to be processed. Extracellular matrix as a scaffold material also has its disadvantages. Because there is almost no way to maintain the original physical properties of cartilage tissue during the fabrication process, the fabricated extracellular matrix scaffold cannot reach the level of natural cartilage in terms of biomechanical properties, and atrophy occurs after seed cells are planted. However, these problems can be greatly improved by using cross-linking method during the fabrication of scaffold. Rowland et al. [32] analyzed the effects of heat crosslinking treatment, ultraviolet irradiation, and chemical crosslinking agent carbodiimide on the contraction of scaffolds, combined with adult bone marrow-derived stem cells for chondrocyte differentiation culture, and proved that crosslinking (non-crosslinking as control) can prevent cell-mediated contraction of extracellular matrix scaffolds (**Figures 1–4**).

Therefore, the future research direction must focus on making full use of the existing materials, continuously improving the preparation process, combining synthetic materials with extracellular matrix-derived scaffold materials to prepare

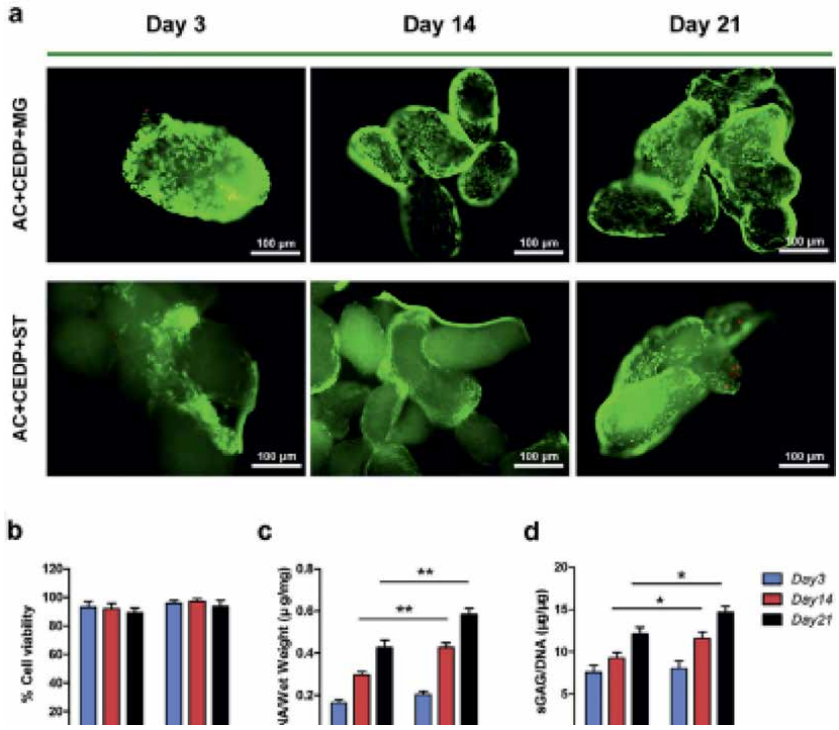

Figure 2.
ECM scaffolds after decellularization of cartilage tissue, and then recombined with autologous chondrocytes or adipose stem cells [33].

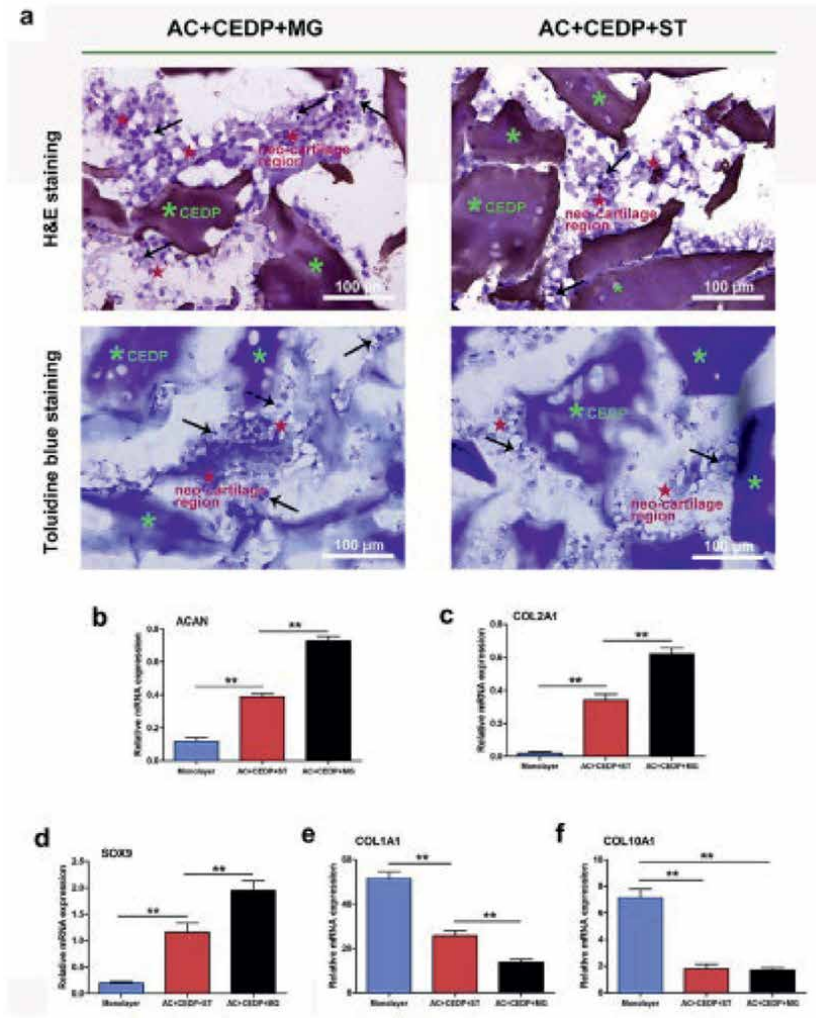

Figure 3.
The ECM scaffolds of cartilage tissue after decellularization treatment were then compounded with autologous chondrocytes or adipose stem cells ECM scaffolds. After the cells were compounded, cell viability and toxicity tests showed that the cells survived well and had strong activity on the scaffolds [33].

cartilage tissue engineering scaffolds, and further exploring methods to change the properties of various materials. That is, the fifth-generation scaffold micro-tissue composite biomimetic scaffold mentioned later (**Figures 5**–7).

Regeneration of articular cartilage is one of the most serious problems facing joint surgeons. In recent years, microcarrier applications have made great progress in cartilage tissue engineering. One advance is the cost-effective expansion of seed cells that provide the necessary microenvironment for cells. Furthermore, microcarriers can also carry proteins, factors that are beneficial for cartilage repair and drugs for cartilage regeneration. Some microcarriers have the advantages on injection. The use of microcarriers having these features avoids the disadvantages of conventional methods and provides unique advantages. For clinical transformation potential, microcarriers have many advantages, such as supplying plenty useful cells, factors, drugs,

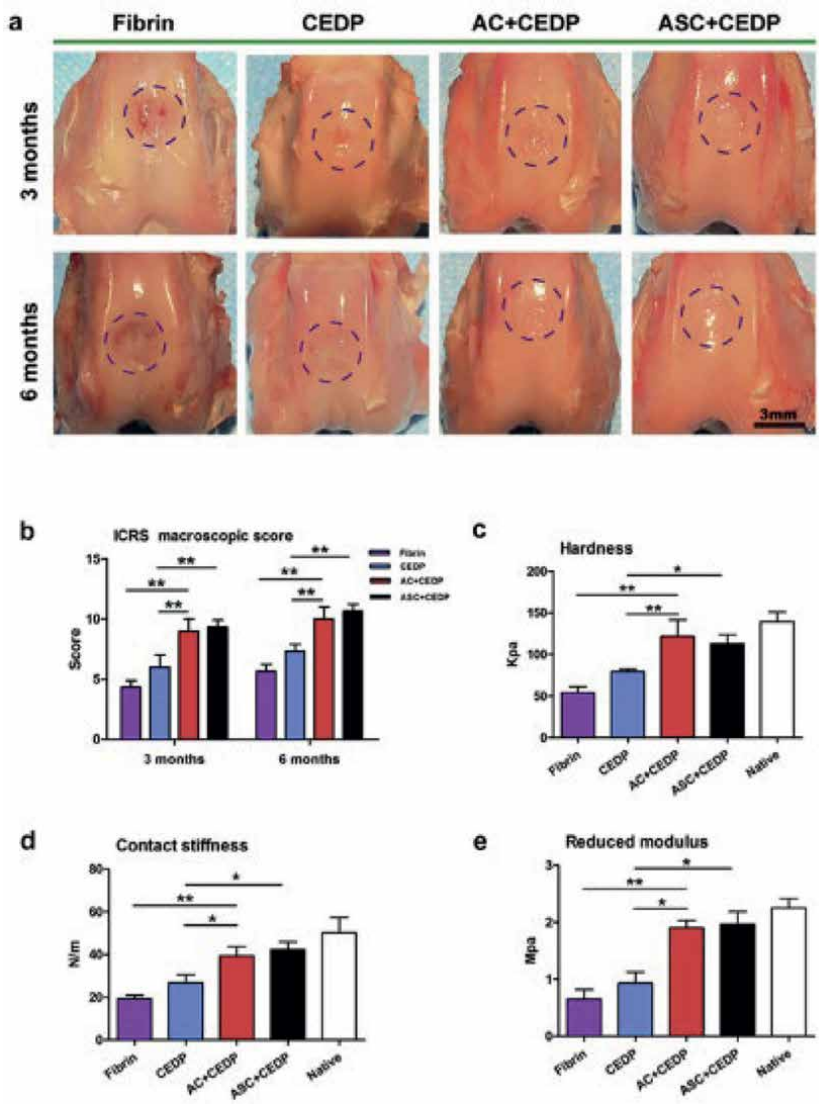

Figure 4.
The ECM scaffolds after decellularization of cartilage tissue, and then composite with autologous chondrocytes or adipose stem cell ECM scaffolds and composite cells to repair the cartilage defect of femoral condyle in rats [33].

microenvironment. Microcarriers also have many application features. First, they can be injected directly into the corresponding site to weaken invasiveness. Second, they can be implanted after organoid formation to enhance repair efficiency. Finally, combining with scaffolds can meliorate the mechanical deficiencies of microcarriers. Thence, the application of microcarriers has great potentiality for clinical translation. A brand new application of microcarriers on tissue engineering is to place them inside hydrogels to make scaffolds or bioinks. Tissue engineering may revolutionize the status quo of cartilage regeneration. However, achieving clinical translation still requires a lot of research support.

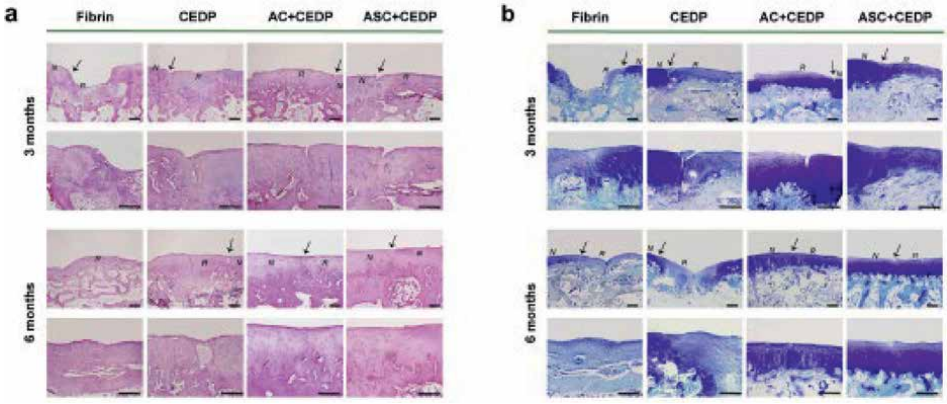

Figure 5.
Pathological section of cartilage defect repaired by ECM scaffolds after decellularization of cartilage tissue and then compounded with autologous chondrocytes or adipose stem cell ECM scaffolds [33].

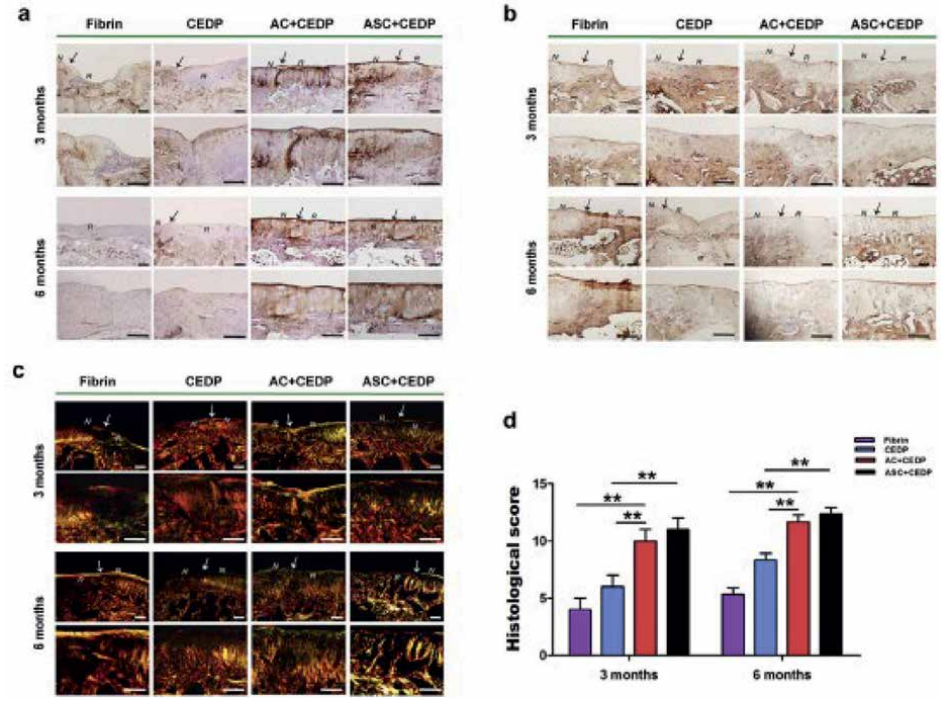

Figure 6.
Pathological section of cartilage defect repaired by ECM scaffolds after decellularization of cartilage tissue and then compounded with autologous chondrocytes or adipose stem cell ECM scaffolds [33].

The advantages of microcarriers are discussed by comparing the characteristics of the microcarrier with other traditional methods. We also discuss the utilization potentiality of the microcarrier and the prospect of future development.

Articular cartilage is very important in the human body. Due to the avascular nature of articular cartilage, it is basically unable to achieve self-healing. Therefore, the treatment of articular cartilage damage is a serious problem for orthopedic

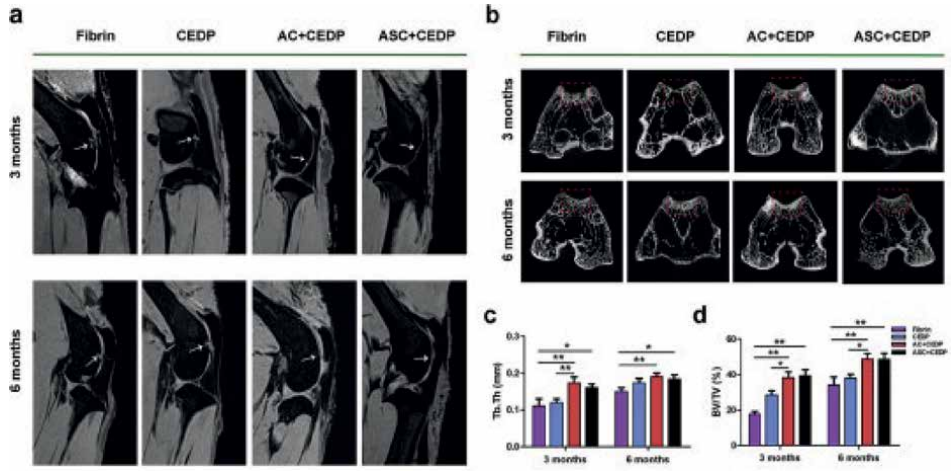

Figure 7.
MRI image of cartilage defect repaired by ECM scaffolds after decellularization of cartilage tissue and then compounded with autologous chondrocytes or adipose stem cell ECM scaffolds [33].

surgeons. If not treated properly, articular cartilage defects can easily lead to osteoarthritis or accelerate the progression of osteoarthritis (OA). OA is one of the most common degenerative joint diseases, and its most notable features are pain and limited joint mobility. The most commonly used methods for clinical treatment of cartilage injury today include microfracture and surgical lavage. Traditional cartilage repair techniques in clinical treatment include bone marrow stimulation techniques (BMS), synthetic, etc. However, all these traditional techniques have shortcomings, such as the inability to repair large-scale cartilage damages, the high rate of secondary operations, and the unsatisfactory prognosis. Tissue engineering (TE) technology has developed speedily in recent decades. Accordingly, a new method for cartilage defect repair is provided. However, traditional tissue engineering techniques have three drawbacks in cartilage regeneration. First, chondrocyte loses certain phenotype in *in vitro* expansion. Second, traditional three-dimensional porous scaffold has hollow phenomenon. Third, tissue-engineered cartilage takes a long period of time to repair the cartilage defect in *in vivo* models. In the field of tissue engineering for cartilage regeneration, many progress have been made in the study of cartilage repair using microcarriers. A microcarrier is a microparticle that can carry cells, factors, or drugs, with a diameter of about 100–300 microns [34]. Microcarriers provide some new ideas for clinical treatment of cartilage injury-induced OA.

4. Traditional methods for treating cartilage injury and characteristics of microcarrier methods

The function and quality of articular cartilage deteriorates with age. Cartilage damage usually progresses from the surface of the articular cartilage to the subchondral bone, leading to the generation of OA.OA creates a huge economic and social load. Degenerative joint damage is often accompanied by cartilage loss. The study reported that cartilage defects in OA patients with symptoms for more than 2 years were more likely to have accelerated progression.

Hence, the treatment of articular cartilage damages is especially important. The traditional techniques commonly used are as follows: first, BMS technology; second, filling cartilage damages with biological tissue; third, cartilage cell implantation; fourth, use metal or other artificial materials to repair cartilage defects; fifth, cell therapy; sixth, drugs that stimulate cartilage repair.

Microfracture technique is a common technique for repairing cartilage damage in BMS technology. It repairs bone marrow defects by transferring bone marrow mesenchymal stem cells (BMSCs) from the intramedullary cavity to the surface of the cartilage damage through fracture drilling. However, the chondrogenic potential of BMSCs is significantly reduced in the elderly [35]. If the cell source is coupled with the disease state, the repair effect is greatly diminished. Therefore, "vivacious" BMSCs are at the heart of the microfracture technique approach for cartilage repair. The use of autologous cartilage as a biological tissue to fill cartilage defects is also a representative method to repair cartilage damage [35]. Osteochondral transplantation can effectively treat knee joint cartilage friction injury. Symptoms of successful transplant patients improved significantly, but this method does not guarantee a good prognosis and a high success rate. However, not only does the surgery have a low success rate, but autologous cartilage transplantation itself is a method of repairing damaged cartilage by destroying healthy cartilage. Fresh allogeneic osteochondral grafts are primarily used to treat young patients with extremely severe articular cartilage damage. Although allogeneic cartilage transplantation has been reported to relieve symptoms of cartilage damage, the scarcity of donor sources has made it impossible to expand the scope of this technique.

Finding a viable treatment to repair damaged cartilage before osteoarthritis develops has become a worldwide problem. Cartilage TE technology emerges as a new proven treatment that offers hope to those who need to treat articular cartilage injuries. Cartilage TE uses cell differentiation and proliferation factors to culture and expand cartilage seed cells *in vitro*, co-culture high-quality seed cells with bioscaffold materials, fill them into the damaged area of cartilage, and gradually combine with the original cartilage to form new cartilage tissue.

Microcarriers [34] are a type of small functional particles with a diameter of about 100–300 μm. Microcarriers contain a wide variety of materials with good biocompatibility, which can boost seed cells as a suitable support matrix. Compared with the traditional methods, functional TE microcarriers can repair cartilage damage, which can not only play the unique advantages of microcarriers, but also avoid some disadvantages of traditional methods.

Advantages of microcarriers over conventional techniques: First, an important advantage of microcarriers is that they can be loaded with sufficient seed cells, which expand *in vitro*. More BMSCs are carried on microcarriers than BMSCs released from BMS. If microcarriers are fabricated by electrospray method, a large amount of BMSCs can theoretically be carried [36]. Second, the microcarriers made of high-quality biomaterials also take full advantage of the traditional method of filling cartilage defects with biomaterials. Second, microcarriers can also be used to treat cartilage damage by using traditional methods of filling cartilage defects with biomaterials. For example, alginate-based microcarriers resemble extracellular matrix (ECM) and can promote cartilage regeneration even without seed cells. Third, in a sense, microcarriers also belong to an artificial material. High-quality microcarriers with seed cells are cultured to constitute tissue-engineered cartilage microtissues. Microtissue [37] provides cartilage tissue to damaged areas of cartilage in a manner similar to cartilage grafting. The application of microcarriers has

opened up a new way for the treatment of cartilage injury by the method of artificial material implantation. Fourth, microcarriers can target delivery of drugs such as chondroitin sulfate and glucosamine sulfate that are beneficial for the treatment of cartilage damage.

5. Summary of the advantages of microcarriers in the treatment of articular cartilage injury

TE aims to develop biological products to replace damaged tissues or organs with the goal of restoring normal function. Current TE therapies for cartilage regeneration are dominated by the use of synthetic or natural implants. A sufficient amount of high-quality seed cells and a proper supporting matrix are the basic conditions for TE. In the field of TE to repair cartilage damage, microcarriers have advantages in every condition. The advantages of microcarriers from these aspects are summarized below.

5.1 Adequate and "virant" seed cells

Adequate and high-quality seed cells are one of the important components of TE. Microcarriers can carry seed cells and target them to the damaged site for repair. BMSCs have been intensively studied, and many are devoted to their application in cartilage damage repair. BMSCs have been shown to possess immunomodulatory functions, multilineage differentiation potential, and tissue homing properties. Furthermore, it is worth noting that even BMSCs obtained from severe OA patients have chondrogenic capacity and can synthesize cartilage extracellular matrix. Studies [36, 37] pointed out that microcarriers can carry a large number of BMSCs and make them successfully differentiate into chondrocytes, and finally form cartilage TE implants. These studies demonstrate that the microcarriers loaded with BMSCs can be used for cartilage damage repair both *in vitro* and *in vivo*.

Human adipose-derived stem cells (hADSCs) are also able to differentiate into cartilage. A study [38] reported that amplifying ADSCs on microcarriers and then implanting them into defects could well promote cartilage repair. One article [39] demonstrated that rapidly degrading microcarriers promoted cartilage regeneration by promoting the generation of immature bone-like tissue. Another study [40] described the effect of different cell densities and different differentiation states of MSCs in microcarriers on cartilage repair. This research shows that the high density of mesenchymal stem cells is beneficial for cartilage repair, and the well-differentiated state of MSCs also has an important impact on cartilage repair. In another study, the combination of hADSCs and transforming growth factor-beta (TGF-β) 3 microcarriers was much more effective than alone in a rabbit model of OA treatment.

Chondrocytes clearly promote cartilage regeneration [38]. There was no significant difference in yield between chondrocytes carried in dynamic microcarriers and chondrocytes produced in tissue culture plates.

In addition, the seed cells carried by the microcarriers can stay in the cartilage defect for a longer time than the mesenchymal stem cells in the BMS method. One study [37] showed that microcarrier-carried cells or their progeny cells persisted for at least 6 weeks.

5.2 Microenvironment

The microenvironment is also one of the conditions affecting TE. Microcarriers can provide a favorable microenvironment for cartilage defect repair, such as providing an appropriate matrix to help seed cell adhesion and proliferation or carrying cytokines that promote cartilage formation. The microenvironment provided by the microcarriers also has an impact at the protein level, which can guide seed cells into the cellular matrix, which can then be used to restore damaged cartilage.

The results of genetic analysis showed that the chondrocyte phenotype of chondrocytes expanded by dynamic microcarrier culture did not disappear. In a study of chondrocyte expansion methods using dynamic microcarriers, it was found that it could increase the expression levels of hyaline cartilage proteins such as Col2 and Agg compared with the traditional 2D cultures.

Biomaterials such as gelatin can provide a microenvironment conducive to seed cell proliferation and adhesion. According to research [36], microcarriers containing gelatin can be used to repair cartilage damage because they can promote the proliferation of seed cells.

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ECM proteins also play an important role in promoting cartilage regeneration. Microenvironments maintained by high-quality biomaterials, such as alginate hydrogels, also have the potential to repair cartilage damage due to their properties similar to natural ECM. Biomaterial-based approaches, such as the use of pre-formed hydrogel matrices, can provide mechanical stability while also taking advantage of biochemical incentives to maintain cellular propensity for chondrogenic differentiation. Alginate is very suitable as a raw material for the manufacture of microcarriers. A previous study [41] noted that alginate hydrogel microcarriers provided ECM in chondrocyte culture, promoting cell differentiation toward cartilage, compared to conventional 2D cultures with or without TGF-β1 added. The internal structure of these microcarriers is very similar to that of natural ECM, so they can be used as an alternative ECM for implantation in cartilage defect areas (**Figures 8–10**).

The application of appropriate matrix assistance can provide a better microenvironment for cells to differentiate into cartilage. In one study, Ramkumar used microcarriers composed of agarose (AG) and collagen type II (COL-II) to carry bone marrow mesenchymal stem cells. COL-II not only promotes mesenchymal stem cell proliferation but also increases local ECM content [32]; therefore, microcarriers prepared with COL-II and agarose can mimic some of the protein components in ECM to promote cartilage repair. The microspheres prepared by the authors are 80–100 microns in diameter, which does not exactly match the size of the defined microcarriers, but produces basically the same effect. Lineage-specific differentiation of embedded BMSCs did not disappear in culture. More importantly, the microcarriers prepared with COL-II promoted the expression of the chondrogenic phenotype of BMSCs. Furthermore, the microcarriers have excellent physical properties and do not have any negative effects on cell viability. In a study [44], Paulomi Ghosh's group and his team used microcarriers to carry acellular cartilage for related research. The advantage of adding acellular cartilage to microcarriers is that acellular cartilage itself contains a large number of chondrocytes' own proteins, cytokines, GAGs, and TGF,

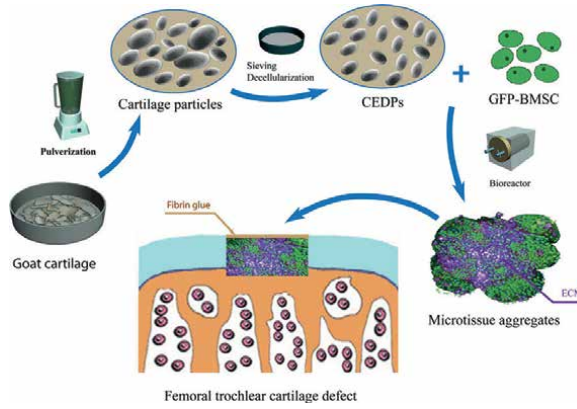


Figure 8.
Flowchart of the experimental design of the fresh goat knee cartilage made into pellets through a series of procedures and co-cultured with BMSCs for repairing rat cartilage damage [42].

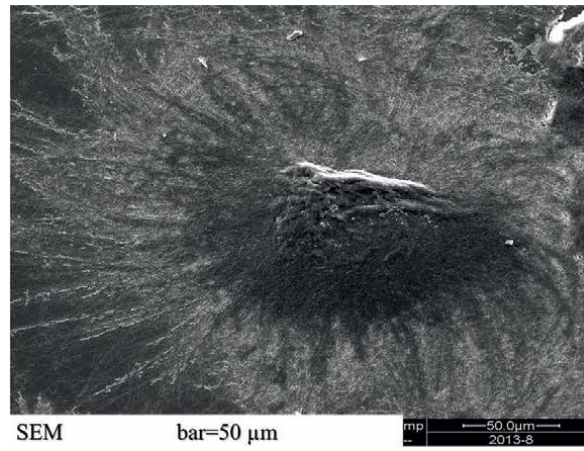


Figure 9.
Scanning electron microscope observation of cartilage-derived microcarriers Villi-like appearance of cartilage particles [43].

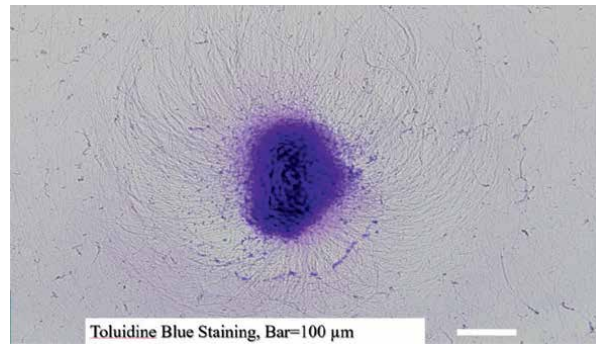


Figure 10.
The cartilage-derived microcarriers stained positively with toluidine blue, and it was strongly positive in the central region near the cartilage particles. The surrounding filament structure is obvious, and it is closely connected with the cartilage particles [43].

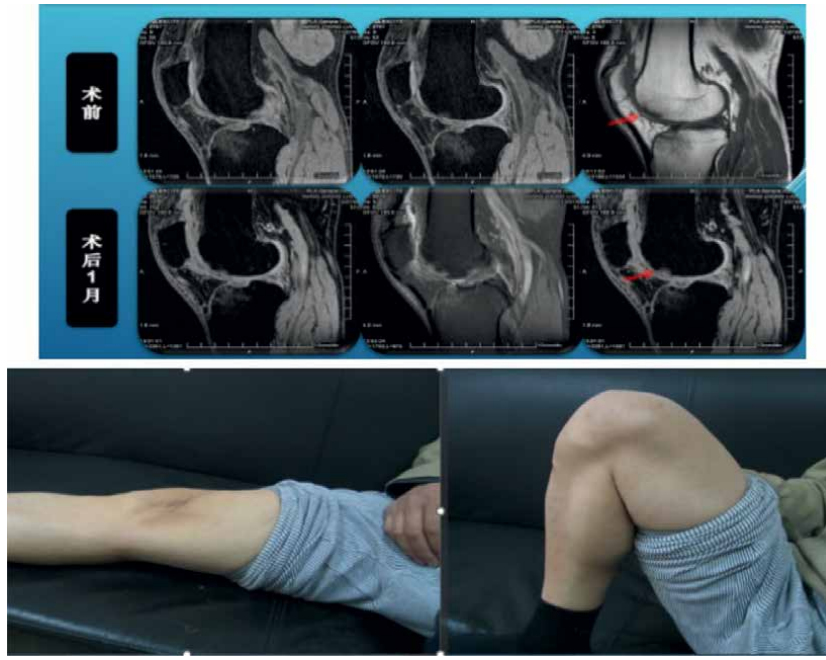


Figure 11.
Source: [45].

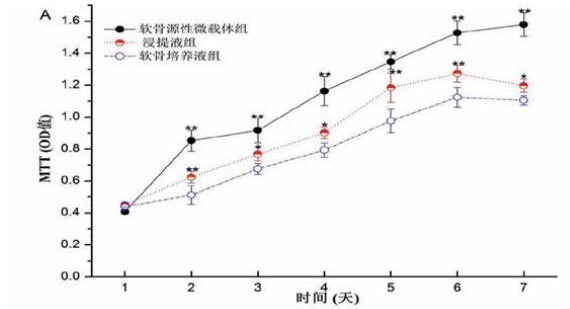
which can provide chondrocytes with a microenvironment that is most in line with the original growth environment of cartilage.

Overall, we can integrate one or more components that are beneficial for the repair of cartilage damage in the microcarrier, provide a microenvironment that is conducive to the growth, proliferation, and differentiation into cartilage of seed cells, and increase the expression of cartilage-related proteins (**Figures 11** and **12**).

6. Suitable supporting matrix

Appropriate supportive matrix is another extremely important reason why cartilage TE can promote cartilage regeneration. We generally divide microcarriers into two categories according to their sources, synthetic microcarriers and natural source microcarriers. The source of synthetic microcarriers is convenient, but their biocompatibility is poor, almost all lack cell-specific recognition sites, and some have some cytotoxicity. Therefore, researchers are now more optimistic about using natural polymers as materials to prepare microcarriers with good biocompatibility, such as gelatin, alginate, chitosan [41].

One study [37] claimed that chitosan microcarriers combined with crocodile dialdehyde bacterial cellulose and DL-allo-hydroxylysine could promote cell proliferation, growth, and migration. Hydroxylysine is a high-quality amino acid with low immunogenicity and good compatibility. Type II collagen contains a large amount of hydroxylysine, and *in vitro* cell culture, hydroxylysine can also promote cell differentiation into cartilage. In addition, oxidized bacterial cellulose can also be used as a raw material for microcarriers, which not only have structural characteristics similar to natural ECM, but also have good physical properties.



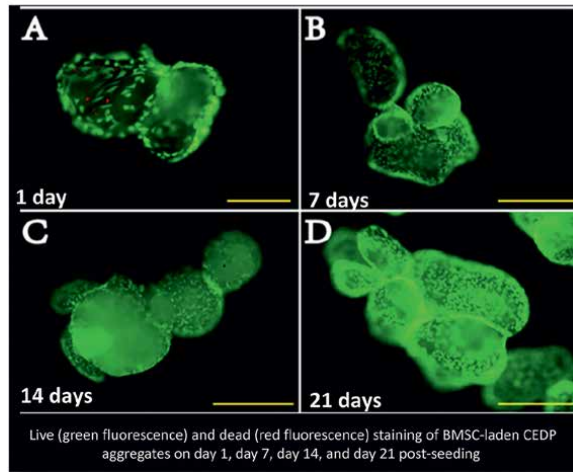


Figure 12.
Source: [42].

In addition, some researchers use both natural and synthetic materials to manufacture the microcarriers. The researchers produced chondrocyte-complexed chitosan polyelectrolyte complex (PEC) microcarriers that significantly outperformed chitosan microcarriers in the ability to generate cartilage matrices. Biomaterials can only be used in the field of cartilage repair if they can promote cell proliferation and differentiation, and have sufficient physical properties. Many scholars have conducted continuous research to make this ideal biological material. Studies by some scholars have shown that porous PLGA microcarriers can promote the formation of cartilage by MSCs. E Filova's team prepared a scaffold of polyε-caprolactone (PCL) porous scaffolds containing chitosan microparticles, which not only possessed good biocompatibility, but also exhibited good mechanical properties. The microcarriers constructed by PCL provide the advantages of sufficiently strong mechanical strength and sufficiently large porosity in cartilage regeneration, and composite chitosan can better promote cartilage repair. And the higher the concentration of chitosan, the better the effect of cartilage repair.

In addition to the above three factors, microcarriers also have many advantages in other aspects (**Figure 13**).

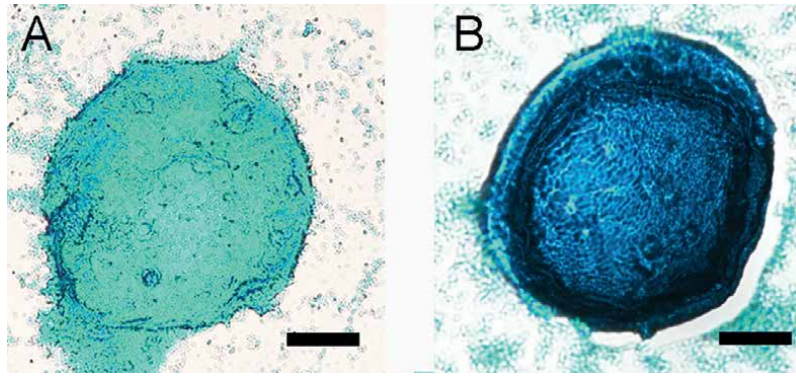


Figure 13.
A is Alcian blue staining of alginate-adipose derived stem cell microspheres after 21 days of chondrogenic induction, blue acid glycosaminoglycan components are visible (scale bar = 100 μm); B is Alcian blue staining of alginate—5 g/L gelatin-adipose derived stem cell microspheres after 21 days of chondrogenic induction, darker acid glycosaminoglycan components were seen (scale bar = 100 μm) [46].

6.1 Microcarriers assist in the formation of microtissues

A study [47] showed that the biocompatibility of the scaffolds was better when the scaffolds were first cultured in a perfusion culture system with exogenous stimulation for a certain period of time and then implanted.

During the construction of tissue-engineered cartilage, the ECM secreted by the cells carried by the microcarriers binds each cartilage microtissue or organoid together, thereby promoting the formation of tissue-engineered cartilage. Seed cell-bound microcarriers were continuously and dynamically cultured in custom-built bioreactors [37]. When the microcarriers begin to secrete ECM, cartilage microtissues can be obtained. In addition, microtissues have other benefits, because functional microtissues can be implanted into the site of cartilage defects, which avoids damage to cell viability and cell numbers caused by the process of digesting cells before the cells are transferred from traditional 2D cultures.

6.2 Microcarriers are injectable

Most of today's tissue engineering treatments for articular cartilage defects require opening the joint cavity to implant bioscaffolds, a process that damages the tissue surrounding the joint. Because of its small diameter, microcarriers can be implanted into the cartilage defect site by injection, thereby reducing the damage to the periarticular tissue caused by the surgical incision [48].

However, in terms of the potential of injectable microcarriers to treat cartilage defects, how to retain or adhere to cartilage defects for a long time after implantation of microcarriers is one of the major issues to be solved. Some studies [37] used bioprotein glue to fix the implanted microtissue in the defect. Although there is nothing wrong with the use of biological protein glue, the microtissue needs to be accurately placed in the cartilage defect, which is difficult to achieve in clinical surgery. Several researchers have developed PLGA microtissues coated with magnetic nanoparticles to enable precise localization of cartilage defects by magnetism. The researchers also prepared alginate-based microtissues that

dispensed MSCs and iron oxide nanoparticles (IONPs) separately, preventing potential damage to cells by IONPs. Another advantage of such microspheres is that they can be loaded with a large number of IONPs, which make them easier to move after magnetization. These magnetic microcarriers play an important role in cartilage repair.

6.3 Incorporating bioscaffolds

Cell-loaded microcarriers can also be used in combination with bioscaffolds for cartilage repair. The combination of PLGA microtissue and collagen/silk fibroin composite scaffold can not only promote cartilage repair, but also promote the fusion of newly formed cartilage with surrounding normal cartilage. It has also been reported that cold atmospheric plasma (CAP)-modified electrospinning scaffolds combined with microtissues can improve the proliferation and differentiation of seeded cells. Another study added hyaluronic acid to the collagen/silk fibroin scaffold, giving it new advantages such as anti-inflammatory and analgesic. At the same time, the researchers added velvet antler polypeptides (PAPs), which can promote cartilage healing, to PLGA microcarriers without seed cells, and found that the scaffolds also promoted the repair of damaged articular cartilage.

6.4 Drug delivery with microcarriers

Microtissues can also be used for drug delivery. Some researchers have repaired pig cartilage by using microtissues to deliver BMP-2 and TGF-3 at the site of injury and release them continuously. One study [49] demonstrated that microtissues containing chondroitin sulfate, a drug that favors cartilage repair, were superior to microfracture techniques for cartilage regeneration. The use of microcarriers as an injectable drug delivery system is not only feasible, but also simple and easy to implement, and effective in the treatment of cartilage damage [50, 51] .The authors [50] infiltrated PLGA microtissues in fluvastatin, which had a significant effect on reducing cartilage degeneration in rabbits. They [51] added tumor necrosis factor-α-stimulated gene 6 in PLGA microtissues and achieved obvious effects in repairing cartilage defects in rats.

In conclusion, microcarriers have multiple and distinct advantages in cartilage repair.

7. Prospects Application prospect of microcarriers in the treatment of cartilage injury

The ability of microcarriers to provide a suitable microenvironment is very important for the repair of cartilage defects. In addition, some studies [37] have shown that microcarriers whose structures are more similar to the cartilage tissue's native ECM perform better in repairing articular cartilage damage. In addition, other studies [48] have shown that there is a higher rate of cell attachment if the cartilage tissue microcarriers contain ECM. Therefore, we reasoned that if the native ECM could be directly incorporated or generated in microcarriers with appropriate physical characteristics, the effect of cartilage repair would be improved.

It is also an unsolved problem at this stage to find raw materials for the production of microcarriers that can perfectly replace the natural cartilage ECM. At present,

there is also a lack of research on whether microcarriers prepared by composite materials can effectively promote the growth, proliferation, and differentiation of seed cells.

Physical properties such as porosity and volume have become key factors affecting the efficacy of cartilage repair. The future development direction of microcarriers should be how to determine the appropriate porosity and volume according to the specific conditions of patients, and establish an efficient way to calculate these physical properties, which will play an important role in future clinical applications.

The source of cells is also an important condition that affects the application effect of microcarriers. The source of chondrocytes is often the patient's own, and obtaining chondrocytes will damage the cartilage. In contrast, ADSCs may be a better choice. Another point to study is how much the patient's age, disease, etc., affect the quality of the obtained seed cells.

Injectable microcarriers allow the treatment of cartilage injuries to be performed minimally invasively, reducing damage to surrounding tissue from the incision. The research and development of magnetic microcarriers has greatly solved the problem that it is difficult to accurately locate cartilage defects by injection methods [37]. If we continue to conduct more in-depth research in this area, or find other methods that can accurately locate under injection treatment conditions, the treatment of cartilage injury with microcarriers is one step closer to clinical application.

Although many *in vivo* and *in vitro* experiments have demonstrated that microcarriers can repair cartilage well, they have not yet reached the clinical trial stage. Orthopedic surgeons who want to pay attention to OA treatment can continue to pay attention to the research progress of microcarriers and confirm their ability to repair human cartilage through clinical studies.

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References

[1] Madry H, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. Knee Surgery, Sports Traumatology, Arthroscopy. 2010;**18**(4):419-433

[2] Vinatier C, Bouffi C, Merceron C, et al. Cartilage tissue engineering: Towards a biomaterial-assisted mesenchymal stem cell therapy. Current Stem Cell Research & Therapy. 2009;**4**(4):318-329

[3] Madeira C, Santhagunam A, Salgueiro JB, et al. Advanced cell therapies for articular cartilage regeneration. Trends Bio-technol. 2015;**33**(1):35-42

[4] Langer R, Vacanti JE. Tissue engineering. Science. 1993;**260**(5110):920-926

[5] Wu W, Chen F, Liu Y, et al. Autologous injectable tissue engineered cartilage by using platelet – Rich plasma: Experimental study in a rabbit model. Oral and Maxillofacial Surgery. 2007;**65**(10):1951-1957

[6] Pujol N, Salle De Chou E, Boisrenoult P, Beaufils P. Platelet-rich plasma for open meniscal repair in young patients: Any benefit? Knee Surgery, Sports Traumatology, Arthroscopy. 2015;**23**(1):51-58

[7] Algul D, Gokce A, Onal A, et al. In vitro release and In vivo biocompatibility studies of biomimetic multilayered alginate-chitosan/β-TCP scaffold for osteochondral tissue. Journal of Biomaterials Science. Polymer Edition. 2016;**27**(5):431-440

[8] Kaifeng L, Shu H, Yue S, et al. Low-temperature additive manufacturing of biomimic three-dimensional hydroxyapatite/collagen scaffolds for bone regeneration. ACS Applied Materials & Interfaces. 2016;**8**(11):6905-6916

[9] Nowicki MA, Castro NJ, Plesniak MW, et al. 3D printing of novel osteochondral scaffolds with graded microstructure. Nanotechnology. 2016;**27**(41):414001

[10] Gauvin R, Chen YC, Lee JW, et al. Microfabrication of complex porous tissue engineering scaffolds using 3D projection stereolithography. Biomaterials. 2012;**33**(15):3824-3834

[11] Dutta RC, Dey M, Dutta AK, et al. Competent processing techniques for scaffolds in tissue engineering. Biotechnology Advances. 2017;**35**(2):240-250

[12] Kaifeng L, Shu H, Yue S, et al. Low - temperature additive manufacturing of biomimic three-dimensional hydroxyapatite/collagen scaffolds for bone regeneration. ACS Applied Materials & Interfaces. 2016;**8**(11):6905-6916

[13] Renkawitz T, Benditz A, Rüsseler M, et al. Teaching in orthopaedic and trauma surgery. Der Orthopäde. 2017;**46**(11):969-978

[14] Peterson L, Minas T, Brittberg M, et al. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clinical Orthopaedics and Related Research. 2000;**374**:212-234

[15] Samuelson EM, Brown DE. Cost - effectiveness analysis of autologous chondrocyte implantation a comparison of periosteal patch versus type I/III collagen membrane. The American Journal of Sports Medicine. 2012;**40**(6):1252-1258

[16] Edwards PK, Ackland T, Ebert JR. Clinical rehabilitation guidelines for matrix – Induced autologous chondrocyte implantation on the tibiofemoral joint. Journal of Orthopaedic and Sports Physical Therapy. 2014;**44**(2):102-119

[17] Manabe R, Tsutsui K, Yamada T, et al. Transcriptome-based systematic identification of extracellular matrix proteins. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(35):12849-12854

[18] Badylak SF, Freytes DO, Gilbert TW. Reprint of: Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomaterialia. 2015;**23**(Suppl):S17-S26

[19] Hynes RO. The extracellular matrix: Not just pretty fibrils. Science. 2009;**326**(5957):1216-1219

[20] Michel G, Tonon T, Scornet D, et al. The cell wall polysaccharide metabolism of the brown alga *Ectocarpus siliculosus*. Insights into the evolution of extracellular matrix polysaccharides in Eukaryotes. The New Phytologist. 2010;**188**(1):82-97

[21] Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials. 2007;**28**(25):3587-3593

[22] Abedin M, King N. Diverse evolutionary paths to cell adhesion. Trends in Cell Biology. 2010;**20**(12):734-742

[23] Ohno M, Motojima K, Okano T, et al. Maturation of the extracellular matrix and cell adhesion molecules in layered co-cultures of HepG2 and endothelial cells. Journal of Biochemistry. 2009;**145**(5):591-597

[24] Fraser JR, Laurent TC, Laurent UB. Hyaluronan: Its nature, distribution, functions and turnover. Journal of Internal Medicine. 1997;**242**(1):27-33

[25] Takahashi I, Onodera K, Bae JW, et al. Age-related changes in the expression of gelatinase and tissue inhibitor of metalloproteinase genes in mandibular condylar, growth plate, and articular cartilage in rats. Journal of Molecular Histology. 2005;**36**(5):355-366

[26] Wu CH, Ko CS, Huang JW, et al. Effects of exogenous glycosaminoglycans on human chondrocytes cultivated on type II collagen scaffolds. Journal of Materials Science. Materials in Medicine. 2010;**21**(2):725-729

[27] Honore S, Pichard V, Penel C, et al. Outside-in regulation of integrin clustering processes by ECM components per se and their involvement in actin cytoskeleton organization in a colon adenocarcinoma cell line. Histochemistry and Cell Biology. 2000;**114**(4):323-335

[28] Loeser RF. Integrins and cell signaling in chondrocytes. Biorheology. 2002;**39**(1-2):119-124

[29] Ab-Rahim S, Selvaratnam L, Kamarul T. The effect of TGF-beta1 and beta-estradiol on glycosaminoglycan and type II collagen distribution in articular chondrocyte cultures. Cell Biology International. 2008;**32**(7):841-847

[30] Fischer BA, Mundle S, Cole AA. Tumor necrosis factor-alpha induced DNA cleavage in human articular chondrocytes may involve multiple endonucleolytic activities during apoptosis. Microscopy Research and Technique. 2000;**50**(3):236-242

[31] Sajedeh K, Akbar K. A review on gradient hydrogel/fiber scaffolds for osteochondral regeneration. Journal of Tissue Engineering and Regenerative Medicine. 2018;**12**(4):e1974-e1990

[32] Rowland CR, Lennon DP, Caplan AI, et al. The effects of crosslinking of scaffolds engineered from cartilage ECM on the chondrogenic differentiation of MSCs. Biomaterials. 2013;**34**(23): 5802-5812

[33] Yin H, Wang Y, Sun X, Cui G, Sun Z, Chen P, et al. Functional tissue-engineered microtissue derived from cartilage extracellular matrix for articular cartilage regeneration. Acta Biomaterialia. 2018;**77**:127-141. DOI: 10.1016/j.actbio.2018.07.031 Epub 2018 Jul 18

[34] van Wezel AL. Growth of cell-strains and primary cells on micro-carriers in homogeneous culture. Nature. 1967;**216**(5110):64-65

[35] Smeriglio P, Lai JH, Dhulipala L, Behn AW, Goodman SB, Smith RL, et al. Comparative potential of juvenile and adult human articular chondrocyte for cartilage tissue formation in three-dimensional biomimetic hydrogels. Tissue Engineering. 2015;**21**:147-155

[36] Xu Y, Peng J, Richards G, Lu S. Optimization of electrospray fabrication of stem cell-embedded alginate-gelatin microspheres and their assembly in 3D-printed poly(ε-caprolactone) scaffold for cartilage tissue engineering. Journal of Orthopaedic Translation. 2019;**18**:128-141

[37] Wang Y, Yuan X, Yu K, Meng H, Zheng Y, Peng J, et al. Fabrication of nanofibrous microcarriers mimicking extracellular matrix for functional microtissue formation and cartilage regeneration. Biomaterials. 2018;**171**:118-132

[38] Musumeci G, Mobasheri A, Kalamegam G, Batt ME. Chondrocyte and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopaedic conditions. Maturitas. 2014;**78**(3):188-198

[39] Sedlaczek J, Leslie SK, Cohen DJ, Hyzy SL, Dosier CR, Nicolini A, et al. Microencapsulated rabbit adipose stem cells initiate tissue regeneration in a rabbit ear defect model. Journal of Tissue Engineering and Regenerative Medicine. 2018;**12**(7):1742-1753

[40] Chan D, Li YY, Cheng HW, Cheung KM, Chan BP. Mesenchymal stem cell-collagen microspheres for articular cartilage repair: Cell density and differentiation status. Acta Biomaterialia. 2014;**10**(5):1919-1929

[41] Ab-Rahim S, Selvaratnam L, Raghavendran HR, Kamarul T. Chondrocyte-alginate constructs with or without TGF-β1 produces superior extracellular matrix expression than monolayer cultures. Molecular and Cellular Biochemistry. 2013;**376**:11-20

[42] Yin H, Wang Y, Sun Z, Sun X, Xu Y, Li P, et al. Induction of mesenchymal stem cell chondrogenic differentiation and functional cartilage microtissue formation for in vivo cartilage regeneration by cartilage extracellular matrix-derived particles. Acta Biomaterialia. 2016;**33**:96-109. DOI: 10.1016/j.actbio.2016.01.024 Epub 2016 Jan 21

[43] Lu Qiang Z, Li PJ, et al. Micromechanism and biocompatibility of cartilage derived microcarrier for injectable tissue engineering cartilage. Orthopedic Journal of China. 2009;**17**(09):688-691

[44] Ghosh P, Gruber SMS, Lin CY, Whitlock PW. Microspheres containing decellularized cartilage induce chondrogenesis in vitro and remain functional after incorporation within a

poly(caprolactone) flament useful for fabricating a 3D scaffold. Biofabrication. 2018;**10**(2):025007

[45] Li B, Peng J, Wang X, Wang Y, Gou W, Yuan X, et al. Biocompatibility and chondrogenesis of articular cartilage-derived microcarrier. Chinese Journal of Joint Surgery. 2013;**7**(06):815-820

[46] Liao S, Meng H, Li J, Xu Y, Li H, Tian X, et al. Preparation of alginate-gelatin-adipose-derived stem cells microspheres by electrospray and feasibility on repairing articular cartilage injury. Chinese Journal of Tissue Engineering Research. 2021;**25**(28):4473-4479

[47] Zhou Y, Chen M, Zhou M, Ye Z, Tan WS. Ectopic osteogenesis of macroscopic tissue constructs assembled from human mesenchymal stem cell-laden microcarriers through in vitro perfusion culture. PLoS One. 2014;**9**(10):e109214

[48] Sivandzade F, Mashayekhan S. Design and fabrication of injectable microcarriers composed of acellular cartilage matrix and chitosan. Journal of Biomaterials Science. Polymer Edition. 2018;**29**(6):683-700

[49] Mohan N, Gupta V, Sridharan BP, Mellott AJ, Easley JT, Palmer RH, et al. Microsphere-based gradient implants for osteochondral regeneration: A long-term study in sheep. Regenerative Medicine. 2015;**10**(6):709-728

[50] Goto N, Okazaki K, Akasaki Y, Ishihara K, Murakami K, Koyano K, et al. Single intra-articular injection of fluvastatin-PLGA microspheres reduces cartilage degradation in rabbits with experimental osteoarthritis. Journal of Orthopaedic Research Society. 2017;**35**(11):2465-2475

[51] Tellier LE, Treviño EA, Brimeyer AL, Reece DS, Willett NJ, Guldberg RE, et al. Intra-articular TSG-6 delivery from heparin-based microparticles reduces cartilage damage in a rat model of osteoarthritis. Biomaterials Science. 2018;**6**(5):1159-1167

Chapter 4

Bio-Orthopedics: A New Approach to Osteoarthritis and Joint Disorders

Alberto Gobbi, Katarzyna Herman and Dawid Szwedowski

Abstract

Osteoarthritis is a major cause of functional limitation and a raising burden in aging population. Lately more research is directed into finding biological enhancement of healing processes in joint dysfunctions. Biological cell-based therapies for cartilage restoration treatment were created to address the need for the long-term viability of repaired tissues. Additionally, the use of biologic therapies is also considered in common disorders affecting ligaments and cartilage. However, if inevitable arthritic changes commence biological therapies offer options to delay the need for arthroplasty. This chapter provides insights into these regenerative, joint preservation techniques for cartilage treatment, osteoarthritis, and other joint disorders.

Keywords: cartilage, osteoarthritis, bio-orthopedics, PRP, BMAC, knee articular preservation

1. Introduction

Osteoarthritis (OA) is one of the most common joint diseases; characterized mainly by joint pain and functional impairment, due to cartilage degeneration, subchondral bone remodeling, and synovial inflammation [1]. OA affects 7% of the global population [2], making it an important problem to solve for the orthopedic surgeons.

Tissue healing is limited not only by the biochemical environment but also by other coexisting factors such as diabetes, smoking, hypercholesterolemia or local factors, which can further weaken healing [3]. Biological response has been studied for many years in order to optimize the healing processes. These have resulted in cell-based, cytokine-based, and scaffold-based therapies [4]. In this chapter, we analyze current concepts in the use of biological treatments. This book chapter intends to provide a review of the current status of biological therapies for OA and other joint disorders in orthopedics.

2. Biological options

2.1 PRP

Lately, research in OA has moved the attention toward biochemical pathways that can be aimed therapeutically through biological intervention. In the past decade, a great interest has been focused on platelet-rich plasma (PRP). It is one of the "hot topics" of regenerative medicine due to its potential to help in different conditions. PRP has surfaced as a biological therapy for the treatment of cartilage injuries and for intra-articular application to address knee pain. PRP contains cytokines, growth factors, and inflammatory mediators, all capable of stimulating cartilage, subchondral bone, and soft tissue healing [5]. PRP contains a significant number of growth factors and proteins in the alpha granules of platelets, that were observed to have regenerative and analgesic effect [6, 7]. What is more, studies demonstrated that platelet-rich plasma (PRP) has an anti-inflammatory influence and counteracts catabolic processes within the joint [8–10]. Additionally, it has been shown to promote chondrocyte proliferation and increase the synthesis of collagen and proteoglycans and for this reason, the application of PRP for osteochondral pathologies has increased [5, 11]. PRP can be categorized as leucocyte-rich PRP (L-PRP), leucocyte-poor PRP (LP-PRP), or platelet-poor plasma (PPP) depending on the preparation technique. The ingredients of PRP categories vary, depending on which system is used to prepare the autologous blood, so care must be taken when choosing the method. There is no one gold standard, so injections of PRP can be done once or in cycles of 3 or more injections. PRP is acquired from patients' peripheral blood. The venous blood is centrifuged in a special probe, according to instructions provided by the manufacturer. When using intra-articularly the PRP is injected into the joint after careful skin disinfection at the needle entry point. The highest beneficial effect is seen at 6 months after cycle of injections, however it may last up to two years [12]. Authors of a meta-analysis of randomized controlled trials using PRP in treatment of knee OA concluded that a statistically significant beneficial effect over placebo was observed at 6 and 12 months [13]. It was also shown that PRP is more effective both in short- or long-term pain and functional recovery when compared to HA [14].

2.2 Hyaluronic acid

HA is a non-sulphated glycosaminoglycan that consists of D-glucuronic acid and N-acetylglucosamine units. It ensures tissue hydration due to its hydrophilic properties and high solubility in an aqueous environment [15]. In a healthy joint it is produced by the synovium, exactly the type B synoviocytes and fibroblast. Its role is to preserve the viscoelastic and functional characteristics of the articular cartilage [16] in the same time promoting chondrocytes proliferation and differentiation [17]. Additionally, it provides joint lubrication and shock absorption. Interestingly in a study HA was shown to inhibit tissue nociceptors [16]. HA also inhibits IL-1β-induced oxidative stress and the inflammatory mediators, such as metalloproteases (MMP-13), nitric oxide (NO), and prostaglandin (PGE2) [18]. Various HA compounds are available that differ in molecular weight (MW), composition, and dosage regimens. High MW HA consists of molecules ranging from 3000 kDa to 6000 kDa; medium MW from 1500 to 3000 kDa; low MW has been described as ≤1500 kDa. High molecular weight was proven to increase the fluid retention into the joint and to have greater anti-inflammatory effect than low MW HA [19]. Clearance rate of HA is another important factor influencing its effectiveness. That is why stabilizers have been

introduced to slow down the clearance rate. One of the used options is trehalose, a disaccharide that acts as a protector of HA. In an in vitro model hyaluronic acid combined with trehalose had improved resistance to hyaluronidase enzyme compared to standard therapy [20]. Additionally in a randomized controlled trial comparing 1% trehalose hyaluronic acid (T-HA) versus non-trehalose hyaluronic acid (NT-HA) for patients with OA KOOS, VAS and IKDC improved for T-HA group at 6 months post-injection while for NT-HA patients it decreased to baseline. The difference was reported statistically significant ($P < 0.05$) [21]. At our institution we are currently conducting two clinical trials on the use of new formulations of HA for the treatment of knee OA. Particularly, one consists of a high chain (1800–2600 KDalton) combined with Niacinamide, an excipient that protects the acid from hyaluronidase degradation activity, promoting longer HA stability. Our preliminary results confirm a more durable effect of HA with Niacinamide compared to standard HA in patients affected with knee OA, at 6-month follow-up. We are also evaluating the efficacy of a second product of HA combined with Collagen type I which reinforces the cartilaginous matrix deteriorated by the ongoing pathological processes of osteoarthritis. We are testing it in young athletic patients to evaluate if they can have more benefits when compared with other therapies.

2.3 Adipose stem cells

An Autologous Microfragmented Adipose Tissue (MFAT) and Stromal Vascular Fraction (SVF) in contrast to peripheral blood has 25,000 times more reparative cells [22]. MFAT is acquired from adipose tissue from abdominal or supragluteal area with a special lipoaspirate cannula. Lipoaspirate is transferred to the special low-pressure cylindrical system and combined with saline solution and then mixed. A final product is a concentration of pericytes and MScs. This is then applied into the joint through an injection or during arthroscopy. Promising results have been shown in literature. In multi-centric, international, open-label study evaluating the outcomes of MFAT injections in patients with knee OA at 2-year follow-up significant functional and quality of life improvement was seen in 88% of patients [23]. In a prospective randomized control trial comparing leukocyte-poor PRP combined with HA and MFAT no significant superiority was seen for either group, both procedures were found to be safe with no major complications showing good results at mid-term follow-up [24]. Additionally, a new technique using SVF and platelet-rich plasma to treat knee osteoarthritis (OA) has been recently described. First, the adipose tissue is harvested from abdominal area with the harvester cannula connected to syringe (**Figure 1**). Meanwhile, leucocyte-poor platelet-rich plasma (LP-PRP) preparation is performed. The harvested fat is transferred to an ACP double syringe for first-round centrifugation with an end product of a middle fraction that is the condensed fatty tissue containing the stromal vascular fraction (SVF) (**Figure 2**). After fragmentation the lipoaspirate is transferred back to the ACP double syringe for second-round centrifugation. After centrifugation the SVF fraction is retrieved. Lastly the SVF is combined with LP-PRP injected into the patient's knee joint (**Figure 3**) [25].

2.4 Bone marrow aspirate

MSCs have multi-differentiation ability, which is why they have been identified as an attractive cell source to regenerate tissue. MSC represents only 0.0001 to 0.01%

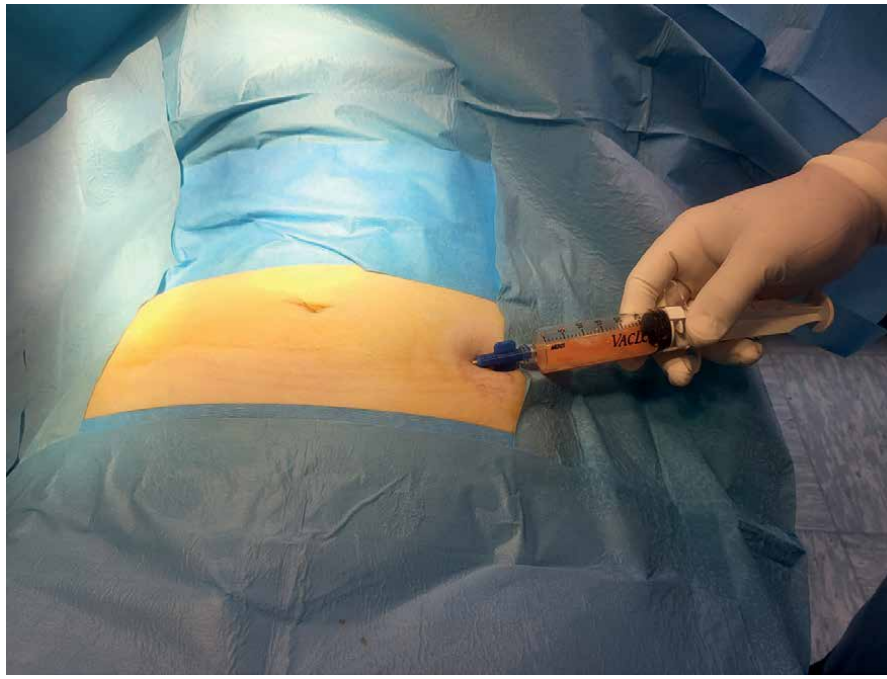

Figure 1.
Lipoaspiration with a 2.1 mm × 15 cm harvester cannula connected to syringe with Johnnie snap locking system through 5 mm skin cut after infiltrating the fatty tissue with a tumescent solution.

mononuclear cells in bone marrow aspirates [26]. Bone marrow aspirate concentrate (BMAC) consists of variety of growth factors such as the platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and bone morphogenetic proteins (BMP)-2 and BMP-7, known to have anabolic and anti-inflammatory effects [27]. Hypothetically MSCs, rather than contributing to tissue formation, act as site-regulated "drugs stores" by releasing immunomodulatory factors activated by local injury [28].

BMAC with a Hyaluronic scaffold (Hyaff) has been used to treat chondral lesions of the knee with good clinical outcomes at long-term follow-up using [29–31]. This treatment was not only successful for single lesions but also in cases of multiple compartment injury, extensive lesions, or in older patients [30, 32, 33]. The composition of BMAC and what could be its mechanism of action has been a point of interest lately. Preliminary data seem to indicate there was no correlation between the clinical outcome and the number of Colony Forming Units (CFU; indirect estimation of the number of MSCs) found in bone marrow aspirate [29]. Interestingly, bone marrow aspirate harvested with Marrow Cellution system was shown to contain a relatively high CFU-fs/mL and CD34+/mL and therefore not requiring centrifugation. The level of CFU-fs/mL was significantly higher in comparison to BMAC in side-by-side evaluation from the same patient [34].

Although the use of BMAC shows good effects in cartilage repair surgery, its use as an injectable therapy is not so common. As a recent RCT has shown, there was no superiority of BMAC over PRP in knee OA therapy [35]. Though BMAC is one of the most appealing sources for cartilage defect repair, several aspects such as safety, amount of aspirate, and scaffold requirement require further investigation.

Figure 2.
Lipoaspirate transferred into ACP double syringe.

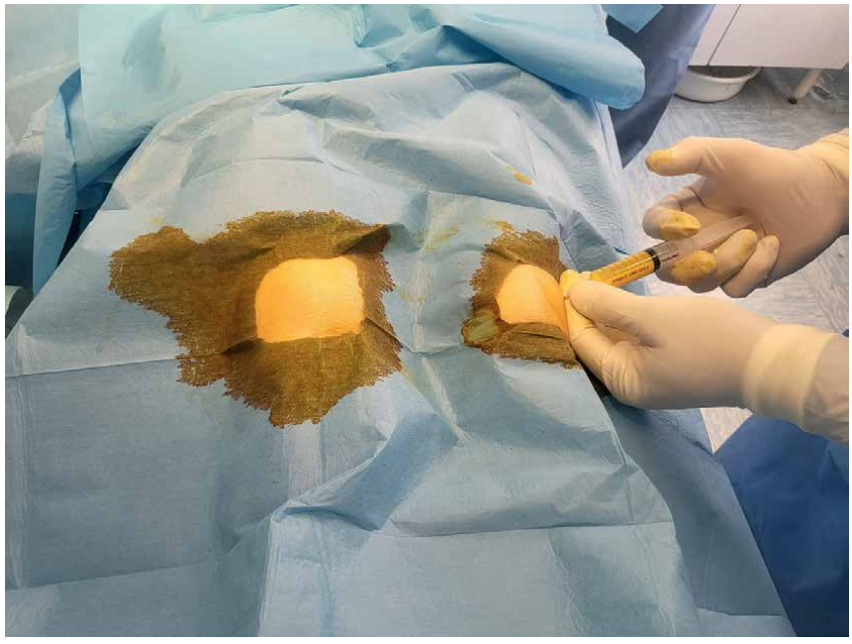

Figure 3.
Injection of the final product, 5 mL of leucocyte-poor platelet-rich plasma (LP-PRP) and 3 mL of SVF in a 10-mL syringe.

3. Applications

3.1 Ligament repair

After a ligament is injured, a cascade of processes begins, it is characterized by cellular proliferation, migration, and collagen production. However, the process of platelet fibrin clot formation is significantly deficient in the cases of intra-articular injury [36]. Synovial fluid has been shown to reduce ACL fibroblast proliferation and migration, thus delaying tissue healing [37]. What is more, the presence of circulating plasmin in the joint space has been suggested to be the cause of suboptimal clot formation [38]. Without clot, the torn ligamentous fibers remain separated after injury and subsequent tissue repair is impaired.

PRP contains growth factors that enhance the processes related to cellular activity and their arrival at the area of the lesion. Platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) stimulate cell population's activation, migration, and proliferation. Fibroblast growth factor (FGF) is vital in collagen synthesis stimulation and fibroblast proliferation, both important elements in the tendon structure [39]. Due to these biological properties, PRP has been suggested to enhance the healing processes in ligaments.

For example, one study showed that the addition of PRP to the sutured ACL repairs did not improve AP knee laxity, maximum tensile load, or linear stiffness of the ACL after 14 weeks in vivo, compared to ACL repair without the addition of PRP [40]. However, when used with a collagen-PRP scaffold, the same author reported a significant improvement in load at yield, maximum load, and linear stiffness at four weeks. The PRP effect alone in ACL repair remains debatable, with significant graft maturation enhancement over time but no clear benefits on clinical outcomes [39].

Mixed results were also found with PRP administration in MCL lesions. LaPrade et al. described that one dose of either PPP or increasing twice the amount of PRP injected at the time of injury did not enhance ligament healing [41]. Furthermore, authors found that a four-time fold increase in the administered dose of PRP showed a significant adverse outcome on ligament strength six weeks after injury. In contrast, in a biomechanical analysis study by Yoshioka et al., there was a significant increase in the structural properties of MCLs in rabbits given leukocyte-poor PRP relative to controls [42].

Other cell therapies reported for ligament healing enhancement included dermal fibroblasts, which were shown to have promising properties in an in vivo models [43]. Adult MSCs represent a known cellular therapy used for tendon engineering; these cells' self-renewal capacity and multi-lineage differentiation potential have become common treatment. Bone marrow stromal cells embedded on polylactide or glycolide sutures have shown higher collagen production and DNA content than sutures seeded with anterior cruciate ligament fibroblasts and skin fibroblasts [41].

Combinations of cellular isolates and scaffolding have a promising role in treating tendon and ligament injury. In a prospective case series involving 50 patients (mean age 28.3 years), it was shown that ACL primary repair combined with bone marrow stimulation and BMAC-Hyaluronic acid bioabsorbable scaffold is an efficient method to restore knee stability and function in young athletes with acute partial ACL tears, after 10-year of follow-up [44, 45].

3.2 Meniscal repair

Even though techniques of repair have changed, many new instruments have been developed, still in the literature the failure rate of meniscus repair is reported to be around 20–25% [46]. Biological healing enhancement for the meniscal lesions treatment may be one of possible options to alter the outcomes [47].

Possible options for biological enhancement of menisci healing include rasping, needling, using a fibrin clot, platelet-rich plasma, bone marrow aspirate, and a scaffold with bone marrow addition [48].

In the 2019 ESSKA consensus on traumatic meniscus tears rasping, needling, and fibrin clot weak evidence was reported [48]. Although similar conclusion was reached in case of PRP use, a study that was not included in the consensus shown promising results. A prospective, randomized, double-blind, placebo-controlled trial with 37 patients with unstable complete vertical longitudinal tears, half treated with repair and placebo and half with repair and PRP. Same suturing all-inside technique was used. At 18-month follow-up the healing rate of tears was superior in the PRP-treated group (85% versus 47%) [49].

In another study on 550 patients treated for meniscal tears, authors found that the use of PRP resulted in improved survival of isolated meniscal repairs, but had no effect on survival of meniscal repairs with coexisting ACL reconstruction [50].

Bone marrow represents a great potential in healing enhancement. A prospective, randomized, double-blind, parallel-group, placebo-controlled study analyzing complete vertical meniscus tears in 40 patients randomized into two groups both using same suturing technique but one with addition of bone marrow venting procedure. Interestingly, the authors found a significant improvement of healing rate (rated by Second-Look Arthroscopy) in the bone marrow venting group compared to repair alone [51]. Another interesting study by Piontek et al. involved the use of meniscus suture with a collagen membrane wrapping together with bone marrow aspirate to treat combined and complex meniscal tears. They found a statistically significant improvement in subjective scores between the preoperative, 2-year follow-up, and 5-year follow-up [52, 53].

3.3 Healing the cartilage

Bone marrow stimulation techniques refer to methods using bleeding from subchondral marrow space and further formation of fibrin clot, which functions as scaffold for subchondral stem cell migration and consequent formation of fibrocartilage. In general, full-thickness cartilage lesion of a surface area < 1 cm^2 is considered an indication for a bone marrow stimulation technique as an isolated procedure [54]. Although, one should be aware that these recommendations should be carefully considered for every patient individually.

Microfracture is most commonly known and used procedure due to availability, simplicity, and small cost [55–57]. The lesion should be prepared, loose cartilage should be removed, and borders made perpendicular to subchondral bone and then holes should be made with an arthroscopic awl. As the saline pressure is lowered, the release of fat droplets and the bleeding begins which will later form a clot on the defect [56, 58, 59]. Studies have shown that this fibrocartilage matrix consists mainly of type I collagen and other non-collagenous proteins, making this tissue more delicate and less elastic, that is why it is common for the initial satisfactory results to deteriorate over long term [60–62]. Better results may be expected in younger patients with smaller lesions. This technique should be used cautiously as it may damage the

subchondral bone and lead to the formation of microcysts, therefore, compromising the articular surface for future procedures [63].

Autologous Matrix-Induced Chondrogenesis (AMIC) is based on the same idea as microfracture but supported with a porcine collagen scaffold [64]. This technique is indicated for focal chondral or osteochondral defects, Outerbridge classification grade 3–4 with a defect size of 1.0–8.0 $cm^{2,}$ and patient age of 18 to 55 years old [3]. After the defect is treated with microfracture a scaffold is added to cover the lesion and to allow the ingrowth of mesenchymal stem cells (MSCs) from the subchondral bone. The main advantage of the AMIC procedure is no donor site morbidity and the possibility of arthroscopic approach. The procedure is also inexpensive compared to autologous chondrocyte implantation (ACI). Good clinical results of AMIC in mid-term follow-ups have been described [65].

In acute lesions, use of autologous cartilage is an optimal alternative to repair a cartilage defect. It is described that covering an acute cartilage defect with minced fragments from a large piece of cartilage achieves good clinical results [66]. In this technique a large chondral fragment is minced into multiple small ones (<1 x 1 x 1 mm) with a scalpel. First, the cartilage lesion is debrided and drilled into the subchondral bone using a 1.4 mm K-wire. Then, minced cartilage fragments are placed into the defect and attached using fibrin glue. This concept is known since 1980s. The procedure using minced cartilage was modified and combined with various materials to become Cartilage Autograft Implantation System (CAIS) [67]. A cartilage paste (smaller cartilage size) was demonstrated to significantly increase extracellular matrix production [68]. Recently 10–23-year long-term results were reported in 74 patients cured with Articular Cartilage Paste Graft, the biopsies of the healed tissue revealed that 14 (48.3%) contained hyaline-like cartilage, 24 (82.8%) fibrocartilage with GAG, 10 (34.5%) fibrocartilage without GAG, and 3 (10.3%)fibrous tissue [69].

Osteochondral Autograft Transfer System (OATS) may be done in a single-stage procedure, arthroscopically or through arthrotomy. Cylindrical plugs are collected from donor sites from non-articulating regions. The plug consists of not only cartilage but also the subchondral bone, that is why it may recreate the osteochondral unit in cartilage lesions with damaged subchondral bone. This method is one of the few that has the advantage of restoring the hyaline cartilage. OATS is usually used for lesions smaller than 2 cm^2. In a 17-year prospective multicentric study performed in 383 found good to excellent results in 91% of femoral mosaicplasty, 86% of tibial, and 74% of patellofemoral mosaicplasty [70]. However, Wu et al. have shown that osteochondral plugs protruding 1 mm caused drastically increased contact pressures within the joint. Additionally, treatment using the OAT technique is restricted by the availability of autologous tissue, as donor site morbidity is a concern if multiple grafts are harvested.

Fresh osteochondral allograft is used mostly in lesions where OATS cannot be performed. The advantages of using allografts include the plasticity of graft sizing and the chance to treat the entire lesion with a one transplanted plug and no donor site morbidity. Some disadvantages include lower chondrocyte viability due to storage and managing and potential immunogenic response concerns.

ACI consists of two steps: first, a piece of healthy cartilage is obtained from a non-weight bearing area and subsequently expanded in vitro. The second step is grafting of the chondrocyte suspension into the defect [57]. Four generations of ACI have been introduced through the years. The first generation [71] is based on infusion of the chondrocyte suspension under periosteal flap, while in second generation the chondrocyte suspension is injected under a collagen membrane. The third generation also known as matrix-induced autologous chondrocyte implantation (MACI)

is a combination of the expanded chondrocytes embedded on a scaffold which is implanted in the cartilage defect. The fourth generation which is a one-step procedure with chondrocyte isolation through biopsies and direct implantation. Long-term results with ACI first-generation method were published with good term results at 20-year follow-up [72]; However, some studies report significantly better functional outcomes in patients who underwent second-generation ACI compared to patients with first-generation ACI [73, 74] ACI in comparison with bone marrow stimulating techniques such as microfracture has shown to be superior to time due to longer-lasting effects. Although the final tissue is still fibrocartilage, it is more "hyaline-like" in contrast to the one after microfracture procedure [32, 75]. ACI has proven a durable solution in treatment of large full-thickness cartilage lesions, but the need for two surgical interventions, the cost of chondrocyte culture, and equal results compared to one-step biological scaffolds stay the ACI technique's major drawbacks.

HA-BMAC developed 30 years ago, allows the treatment of larger cartilage lesions in a one-step surgery. This method provided good long-term results [29, 30, 32, 33] and demonstrated its superiority to microfracture. Additionally, it can be used in multiple compartment and extensive lesions or in older patients [30, 32, 33]. The senior authors' selected method is a one-step cartilage repair with a three-dimensional hyaluronic acid-based scaffold "Hyalofast" (Anika Therapeutics, Bedford, Massachusets, U.S.) combined with activated bone marrow aspirate concentrate (HA-BMAC). Contrasted with two-step MACI, the clinical outcomes have not shown a significant difference and also there was no relationship between the clinical outcome and the number of Colony Forming Units (CFU) found in bone marrow aspirate [29].

Depending on the lesion's extent and location the procedure is done through a small arthrotomy or arthroscopically. Loose cartilage is removed, vertical walls are made around the periphery of the defect with special chondrectomes. Then the calcified cartilage layer must be thoroughly removed without damaging the subchondral plate (**Figure 4A**). The defects are measured with aluminum foil templates which are later used to cut out a matching hyaluronic acid scaffold. Bone marrow is collected from the iliac crest and centrifuged to obtain a concentrated bone marrow which is later mixed with Batroxobin (Plateltex®act-Plateltex S.R.O. Bratislava, SK) to create a clot. The hyaluronic acid-based scaffold and clot are combined to create a biologically active structure for cartilage repair. The HA-BMAC is placed on the lesion and secured with fibrin glue (**Figure 4B**). Afterward, the knee is cycled to check stability [63]. Sadlik et al. demonstrated a variation of this technique using morselized bone graft to fill the lesion and then covered with hyaluronic acid scaffold embedded with BMAC [76].

3.4 Healing the subchondral bone (OCP)

BMAC may as well be used to treat subchondral bone pathologies. Cartilage and subchondral bone work as a unit and over the last few years a debate on the function of subchondral bone has been going on. Bone marrow lesions (BMLs) are the focal defects in the subchondral bone and can be identified by magnetic resonance imaging (MRI). Number of pathologies may be causing such lesions with an ischemic, mechanical or reactive background. When assessing a patient with BML it is vital to evaluate whether the lesion is reversible and irreversible [77]. Both biological and mechanical improvement of osteochondral unit can be achieved with Osteo-Core-Plasty (Marrow Cellution™) a minimally invasive subchondral bone augmentation. This technique may also be used to treat insufficiency fractures, subchondral cysts, and osteonecrosis [78].

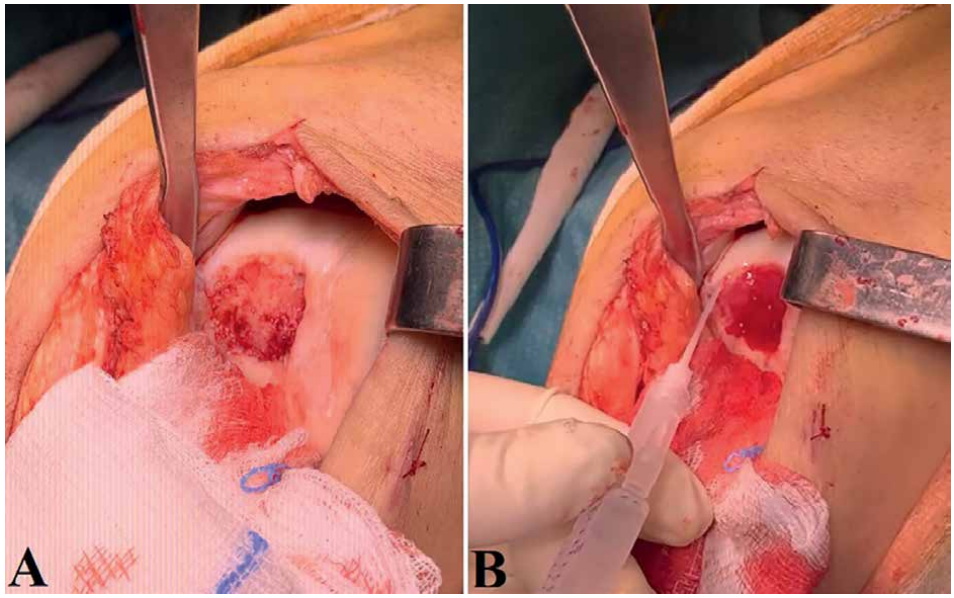

Figure 4.
(A) chondral defect on the patella after removal of loose cartilage and a calcified layer of subchondral bone with perpendicular borders of the lesion prepared for scaffold implantation. (B) The lesion after implantation of the HA-BMAC secured with fibrin glue.

This method is made of two parts, first being the aspiration of bone marrow and second application of the material into the defect. Bone marrow is aspirated from the iliac crest. Application may be done arthroscopically or through an open approach, both need fluoroscopic assistance. Necrotic Tissue Zone is recognized under fluoroscopy on AP and lateral images. In an open technique the lesion is debrided, and necrotic bone underneath is separated and removed. In an arthroscopic technique a K-wire is put to target zone from outside the joint and a cannulated drill bit is inserted over the K-Wire. Then Marrow Cellution Bone Core Graft is delivered to the necrotic zone with Extraction/Delivery Tool in both open and arthroscopic approach. The bone core graft is pressed with a probe to aim point. Finally, aspirated Marrow Cellution™ is injected to the necrotic site or in case of an open procedure the Marrow Cellution™ Saturated Matrix Scaffold Membrane is used [79]. In Osteo-Core-Plasty, there is no need for centrifugation and the surgeon can apply the aspirate to the target zone [80].

4. Conclusion

OA is a raising problem and many opportunities to treat cartilage lesions and early OA have been reported. Cell therapies using chondrocytes, MSCs, and other cell sources have been used to treat joint pathologies. In order to obtain the best cartilage quality, these cartilage preserving/regenerating methods combined with addressing coexisting intraarticular pathologies or limb alignment issued. The biology of the articular cartilage must be fully understood before cartilage repair technologies can advance further. Collecting evidence of experimental studies on novel techniques for biological healing is vital to advance the treatment possibilities for patients. Therefore, use of the most appropriate line of treatment and proper patient selection is key to improving results.

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References

[1] Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: A lancet commission. The Lancet. 2020;**396**(10264):1711-1712

[2] Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) results. 2020. [Internet]. [cited 2021 Aug 10]. Available from: http://ghdx.healthdata.org/gbd-results-tool. [Accessed: August 8, 2020]

[3] Gobbi A, Lane JG, Espregueira-Mendes J, Karahan M. Bio-Orthopaedics: A New Approach. Bio-orthopaedics: A New Approach; 2017. pp. 1-696

[4] Herman K, Gobbi A. Evidence-based approach to Orthobiologics for osteoarthritis and other joint disorders. Physical Medicine and Rehabilitation Clinics of North America. 2023;**34**(1):71-81

[5] Salamanna F, Veronesi F, Maglio M, Della Bella E, Sartori M, Fini M. New and emerging strategies in platelet-rich plasma application in musculoskeletal regenerative procedures: General overview on still open questions and outlook. BioMed Research International. 2015;**2015**:846045. DOI: 10.1155/2015/846045. E

[6] Ulrich-Vinther M, Maloney MD, Schwarz EM, Rosier R, O'Keefe RJ. Articular cartilage biology. Journal of the American Academy of Orthopaedic Surgeons. 2003;**11**(6):421-430

[7] Asfaha S, Cenac N, Houle S, Altier C, Papez MD, Nguyen C, et al. Protease-activated receptor-4: A novel mechanism of inflammatory pain modulation. British Journal of Pharmacology. 2007;**150**(2):176-185

[8] Sánchez M, Anitua E, Delgado D, Sanchez P, Prado R, Goiriena JJ, et al. A new strategy to tackle severe knee osteoarthritis: Combination of intra-articular and intraosseous injections of platelet rich plasma. Expert Opinion on Biological Therapy. 2016;**16**(5):627-643

[9] Cole BJ, Karas V, Hussey K, Pilz K, Fortier LA. Hyaluronic acid versus platelet-rich plasma: A prospective, double-blind randomized controlled trial comparing clinical outcomes and effects on intra-articular biology for the treatment of knee osteoarthritis. The American Journal of Sports Medicine. 2017;**45**(2):339-346

[10] Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clinical Orthopaedics and Related Research. 2011;**469**(10):2706-2715 [Internet] [cited 2023 Feb 25] Available from: https://pubmed.ncbi.nlm.nih.gov/21403984/

[11] 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**(9739):440-448

[12] Gobbi A, Lad D, Karnatzikos G. The effects of repeated intra-articular PRP injections on clinical outcomes of early osteoarthritis of the knee. Knee Surgery, Sports Traumatology, Arthroscopy. 2015;**23**(8):2170-2177 [Internet] [cited 2023 Nov 18] Available from: https://pubmed.ncbi.nlm.nih.gov/24748286/

[13] Filardo G, Previtali D, Napoli F, Candrian C, Zaffagnini S, Grassi A. PRP injections for the treatment of knee osteoarthritis: A meta-analysis of randomized controlled trials. Cartilage. 2021;**13**(1):364S-375S

[14] Tang JZ, Nie MJ, Zhao JZ, Zhang GC, Zhang Q, Wang B. Platelet-rich plasma versus hyaluronic acid in the treatment of knee osteoarthritis: A meta-analysis. Journal of Orthopaedic Surgery and Research. 11 Sep 2020;**15**(1):403. DOI: 10.1186/s13018-020-01919-9

[15] Santilli V, Paoloni M, Mangone M, Alviti F, Bernetti A. Hyaluronic acid in the management of osteoarthritis: Injection therapies innovations. Clinical Cases in Mineral and Bone Metabolism. 2016;**13**(2):131

[16] Waddell DD, Bert JM. The use of Hyaluronan after arthroscopic surgery of the knee. Arthroscopy - Journal of Arthroscopic and Related Surgery. 2010;**26**:105-111

[17] Watterson JR, Esdaile JM. Viscosupplementation: Therapeutic Mechanisms and Clinical Potential in Osteoarthritis of the Knee. Journal of the American Academy of Orthopaedic Surgeons. Sep 2000;**8**(5):277-284

[18] Mongkhon JMM, Thach M, Shi Q, Fernandes JC, Fahmi H, Benderdour M. Sorbitol-modified hyaluronic acid reduces oxidative stress, apoptosis and mediators of inflammation and catabolism in human osteoarthritic chondrocytes. Inflammation Research. 2014;**63**(8):691-701

[19] Bagga H, Burkhardt D, Sambrook P, March L. Longterm effects of Intraarticular Hyaluronan on synovial fluid in osteoarthritis of the knee. The Journal of Rheumatology. 2006;**33**:5

[20] Giardina S. In vitro efficacy study: Evaluation of susceptibility of a hyaluronate- based product to the activity of the hyaluronidase. Report number E.IT.076_2018/2116 B. performed by Complife group. 2018.

[21] Gobbi A, Morales M, Avio G, D'Ambrosi R. Double-blinded prospective randomized clinical trial in knee joint osteoarthritis treatment: Safety assessment and performance of trehalose hyaluronic acid versus standard infiltrative therapy based on medium-weight sodium hyaluronate. Journal of Cartilage & Joint Preservation. 2022;**2**(3):100043. [Internet]. DOI: 10.1016/j.jcjp.2022.100043

[22] Russo A, Screpis D, di Donato SL, Bonetti S, Piovan G, Zorzi C. Autologous micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis: An update at 3 year follow-up. Journal of Experimental Orthopaedics. 19 Dec 2018;**5**(1):52. DOI: 10.1186/s40634-018-0169-x

[23] Gobbi A, Dallo I, Rogers C, Striano RD, Mautner K, Bowers R, et al. Two-year clinical outcomes of autologous microfragmented adipose tissue in elderly patients with knee osteoarthritis: A multi-centric, international study. International Orthopaedics. 2021;**45**(5):1179-1188 [Internet] [cited 2023 Mar 6] Available from: https://pubmed.ncbi.nlm.nih.gov/33649891/

[24] Gobbi A, Dallo I, D'Ambrosi R. Autologous microfragmented adipose tissue and leukocyte-poor platelet-rich plasma combined with hyaluronic acid show comparable clinical outcomes for symptomatic early knee osteoarthritis over a two-year follow-up period: A prospective randomized clinical trial. European Journal of Orthopaedic Surgery and Traumatology. 2022. Available from: https://link.springer.com/article/10.1007/s00590-022-03356-2#article-info

[25] Dallo I, Morales M, Gobbi A. Platelets and adipose stroma combined for the treatment of the arthritic knee. Arthroscopy Techniques. 2021;**10**(11):e2407 [Internet] [cited

2023 Mar 6] Available from: http://pmc/articles/PMC8626615/

[26] Martin DR, Cox NR, Hathcock TL, Niemeyer GP, Baker HJ. Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. Experimental Hematology. 2002;**30**(8):879-886

[27] McCarrel T, Fortier L. Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. Journal of Orthopaedic Research. 2009;**27**(8):1033-1042

[28] Caplan AI. Mesenchymal stem cells: Time to change the name! Stem Cells Translational Medicine. 2017;**6**(6):1445-1451

[29] Gobbi A, Karnatzikos G, Scotti C, Mahajan V, Mazzucco L, Grigolo B. One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions: Results at 2-year follow-up. Cartilage. 2011;**2**(3):286-299 [Internet] Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4300809&tool=pmcentrez&rendertype=abstract

[30] Gobbi A, Scotti C, Karnatzikos G, Mudhigere A, Castro M, Peretti GM. One-step surgery with multipotent stem cells and Hyaluronan-based scaffold for the treatment of full-thickness chondral defects of the knee in patients older than 45 years. Knee Surgery, Sports Traumatology, Arthroscopy. Aug 2017;**25**(8):2494-2501

[31] Gobbi A, Whyte GP. One-stage cartilage repair using a hyaluronic acid-based scaffold with activated bone marrow-derived mesenchymal stem cells compared with microfracture: Five-year follow-up. The American Journal of Sports Medicine. 2016;**44**(11):2846-2854 [Internet] [cited 2022 Oct 15] Available from: https://pubmed.ncbi.nlm.nih.gov/27474386/

[32] Gobbi A, Chaurasia S, Karnatzikos G, Nakamura N. Matrix-induced autologous chondrocyte implantation versus multipotent stem cells for the treatment of large patellofemoral chondral lesions: A nonrandomized prospective trial. Cartilage. 2015;**6**(2):82-97

[33] Gobbi A, Karnatzikos G, Sankineani SR. One-step surgery with multipotent stem cells for the treatment of large full-thickness chondral defects of the knee. American Journal of Sports Medicine. 2014;**42**(3):648-657

[34] Scarpone M, Kuebler D, Chambers A, De Filippo CM, Amatuzio M, Ichim TE, et al. Isolation of clinically relevant concentrations of bone marrow mesenchymal stem cells without centrifugation 11 medical and health sciences 1103 clinical sciences. Journal of Translational Medicine. 2019;**17**(1):1-10

[35] Anz AW, Plummer HA, Cohen A, Everts PA, Andrews JR, Hackel JG. Bone marrow aspirate concentrate is equivalent to platelet-rich plasma for the treatment of knee osteoarthritis at 2 years: A prospective randomized trial. The American Journal of Sports Medicine. 2022;**50**(3):618-629 [Internet] [cited 2022 Oct 16] Available from: https://pubmed.ncbi.nlm.nih.gov/35289231/

[36] Murray MM, Martin SD, Martin TL, Spector M. Histological changes in the human anterior cruciate ligament after rupture. Journal of Bone and Joint Surgery. 2000;**82**(10):1387-1397

[37] Rość D, Powierza W, Zastawna E, Drewniak W, Michalski A, Kotschy M.

Post-traumatic plasminogenesis in intraarticular exudate in the knee joint. Medical Science Monitor. 2002;**8**(5):CR371-8

[38] Andrish J, Holmes R. Effects of synovial fluid on fibroblasts in tissue culture. Clinical Orthopaedics and Related Research. 1979;**138**:279-283

[39] di Matteo B, Andriolo L, Filardo G, Loibl M, Zellner J, Koch M, et al. Biologic agents for anterior cruciate ligament healing: A systematic review. World Journal of Orthopedics. 2016;**7**(9):592-603

[40] Murray MM, Palmer M, Abreu E, Spindler KP, Zurakowski D, Fleming BC. Platelet-rich plasma alone is not sufficient to enhance suture repair of the ACL in skeletally immature animals: An in vivo study. Journal of Orthopaedic Research. 2009;**27**(5):639-645

[41] LaPrade RF, Goodrich LR, Phillips J, Dornan GJ, Turnbull TL, Hawes ML, et al. Use of platelet-rich plasma immediately after an injury did not improve ligament healing, and increasing platelet concentrations was detrimental in an In vivo animal model. American Journal of Sports Medicine. 2018;**46**(3):702-712

[42] Yoshioka T, Kanamori A, Washio T, Aoto K, Uemura K, Sakane M, et al. The effects of plasma rich in growth factors (PRGF-Endoret) on healing of medial collateral ligament of the knee. Knee Surgery, Sports Traumatology, Arthroscopy. 2013;**21**(8):1763-1769

[43] van Eijk F, Saris DBF, Riesle J, Willems WJ, van Blitterswijk CA, Verbout AJ, et al. Tissue engineering of ligaments: A comparison of bone marrow stromal cells, anterior cruciate ligament, and skin fibroblasts as cell source. Tissue Engineering. 2004;**10**(5-6):893-903 [Internet] [cited 2022 Oct 7] Available from: https://www.liebertpub.com/doi/10.1089/1076327041348428

[44] Gobbi A, Whyte GP. Biological Augmentation in Acute ACL Repair. Bio-Orthopaedics: A New Approach. 2017. pp. 325-35. [Internet] [cited 2022 Oct 7] Available from: https://link.springer.com/chapter/10.1007/978-3-662-54181-4_25

[45] Murray MM, Flutie BM, Kalish LA, Ecklund K, Fleming BC, Proffen BL, et al. The bridge-enhanced anterior cruciate ligament repair (BEAR) procedure: An early feasibility cohort study. Orthopaedic Journal of Sports Medicine. 21 Nov 2016;**4**(11):2325967116672176. DOI: 10.1177/2325967116672176

[46] Nepple JJ, Block AM, Eisenberg MT, Palumbo NE, Wright RW. Meniscal repair outcomes at greater than 5 years: A systematic review and meta-analysis. The Journal of Bone and Joint Surgery. American Volume. 2022;**104**(14):1311-1320 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/35856932/

[47] DePhillipo NN, LaPrade RF, Zaffagnini S, Mouton C, Seil R, Beaufils P. The future of meniscus science: International expert consensus. Journal of Experimental Orthopaedics. 31 Mar 2021;**8**(1):24. DOI: 10.1186/s40634-021-00345-y

[48] Kopf S, Beaufils P, Hirschmann MT, Rotigliano N, Ollivier M, Pereira H, et al. Management of traumatic meniscus tears: The 2019 ESSKA meniscus consensus. Knee Surgery, Sports Traumatology, Arthroscopy. 2020;**28**(4):1177-1194 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/32052121/

[49] Kaminski R, Kulinski K, Kozar-Kaminska K, Wielgus M,

Langner M, Wasko MK, et al. A prospective, randomized, double-blind, parallel-group, placebo-controlled study evaluating meniscal healing, clinical outcomes, and safety in patients undergoing meniscal repair of unstable, complete vertical meniscal tears (bucket handle) augmented with platelet-rich plasma. BioMed Research International. 11 Mar 2018;**2018**:9315815. DOI: 10.1155/2018/9315815

[50] Everhart JS, Cavendish PA, Eikenberry A, Magnussen RA, Kaeding CC, Flanigan DC. Platelet-rich plasma reduces failure risk for isolated meniscal repairs but provides No benefit for meniscal repairs with anterior cruciate ligament reconstruction. The American Journal of Sports Medicine. 2019;**47**(8):1789-1796 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/31166699/

[51] Kaminski R, Kulinski K, Kozar-Kaminska K, Wasko MK, Langner M, Pomianowski S. Repair augmentation of unstable, complete vertical meniscal tears with bone marrow venting procedure: A prospective, randomized, double-blind, parallel-group, placebo-controlled study. Arthroscopy. 2019;**35**(5):1500-1508.e1. [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/30902532/

[52] Piontek T, Ciemniewska-Gorzela K, Naczk J, Jakob R, Szulc A, Grygorowicz M, et al. Complex meniscus tears treated with collagen matrix wrapping and bone marrow blood injection: A 2-year clinical follow-up. Cartilage. 2016;**7**(2):123-139 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/27047635/

[53] Ciemniewska-Gorzela K, Bąkowski P, Naczk J, Jakob R, Piontek T. Complex meniscus tears treated with collagen matrix wrapping and bone marrow blood injection: Clinical effectiveness and survivorship after a minimum of 5 years' follow-up. Cartilage. 2021;**13**(1_suppl):228S-238S [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/32476440/

[54] Chahla J, Stone J, Mandelbaum BR. How to manage cartilage injuries? Arthroscopy - Journal of Arthroscopic and Related Surgery. 2019;**35**(10):2771-2773

[55] Gobbi A, Karnatzikos G, Kumar A. Long-term results after microfracture treatment for full-thickness knee chondral lesions in athletes. Knee Surgery, Sports Traumatology, Arthroscopy. 2013;**22**(9):1986-1996

[56] Steadman JR, Rodkey WG, Briggs KK. Microfracture: Its History and Experience of the Developing Surgeon. Cartilage. 2010;**1**(2):78-86. DOI: 10.1177/1947603510365533

[57] Gobbi A, Lane JG, Dallo I. Editorial commentary: Cartilage restoration—What is currently available? Arthroscopy - Journal of Arthroscopic and Related Surgery. 2020;**36**(6):1625-1628

[58] Hoemann CD, Gosselin Y, Chen H, Sun J, Hurtig MB, Carli A, et al. Characterization of initial microfracture defects in human condyles. The Journal of Knee Surgery. 2013;**26**(5):347-355

[59] Gobbi A, Herman K, Grabowski R, Szwedowski D. Primary anterior cruciate ligament repair with hyaluronic scaffold and autogenous bone marrow aspirate augmentation in adolescents with open Physes. Arthroscopy Techniques. 2019;**8**(12):e1561-e1568

[60] Mithoefer K, Mcadams T, Williams RJ, Kreuz PC,

Mandelbaum BR, et al. Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: An evidence-based systematic analysis. American Journal of Sports Medicine. Oct 2009;**37**(10):2053-2063. DOI: 10.1177/0363546508328414

[61] Gobbi A, Whyte GP. One-stage cartilage repair using a hyaluronic acid-based scaffold with activated bone marrow-derived mesenchymal stem cells compared with microfracture. American Journal of Sports Medicine. 2016;**44**(11):2846-2854

[62] Gobbi A, Karnatzikos G, Kumar A. Long-term results after microfracture treatment for full-thickness knee chondral lesions in athletes. Knee Surgery, Sports Traumatology, Arthroscopy. 2014;**22**(9):1986-1996

[63] Frank RM, Cotter EJ. Nassar I. Failure of Bone Marrow Stimulation Techniques: Cole B; 2017

[64] Behery O, Siston RA, Harris JD, Flanigan DC. Treatment of cartilage defects of the knee: Expanding on the existing algorithm. Clinical Journal of Sport Medicine. 2014;**24**:21-30

[65] Gille J, Behrens P, Volpi P, De Girolamo L, Reiss E, Zoch W, et al. Outcome of autologous matrix induced Chondrogenesis (AMIC) in cartilage knee surgery: Data of the AMIC registry. Archives of Orthopaedic and Trauma Surgery. 2013;**133**(1):87-93

[66] Salzmann GM, Baumann GA, Preiss S. Spontaneous minced cartilage procedure for unexpectedly large femoral condyle surface defect. Case Reports in Orthopedics. 2016;**2016**:1-3

[67] Bonasia DE, Marmotti A, Rosso F, Collo G, Rossi R. Use of chondral fragments for one stage cartilage repair: A systematic review. World Journal of Orthopedics. 2015;**6**(11):1006

[68] Bonasia DE, Marmotti A, Mattia S, Cosentino A, Spolaore S, Governale G, et al. The degree of chondral fragmentation affects extracellular matrix production in cartilage autograft implantation: An In vitro study. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 2015;**31**(12):2335-2341

[69] Stone KR, Pelsis JR, Na K, Walgenbach AW, Turek TJ. Articular cartilage paste graft for severe osteochondral lesions of the knee: A 10- to 23-year follow-up study. Knee Surgery, Sports Traumatology, Arthroscopy. 2017;**25**(12):3824-3833

[70] Hangody L, Dobos J, Balo E, Panics G, Rudolf Hangody L, Berkes I. Clinical experiences with autologous osteochondral mosaicplasty in an athletic population: A 17-year prospective multicenter study. The American Journal of Sports Medicine. 2010;**38**(6):1125-1133

[71] Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. The New England Journal of Medicine. 1994;**331**(14):889-895

[72] Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: A long-term follow-up. American Journal of Sports Medicine. 2010;**38**(6):1117-1124

[73] Niemeyer P, Salzmann G, Feucht M, Pestka J, Porichis S, Ogon P, et al. First-generation versus second-generation autologous chondrocyte implantation for treatment of cartilage defects of the knee: A matched-pair analysis on long-term clinical outcome. International Orthopaedics. 2014;**38**(10):2065-2070

[74] McCarthy HS, Roberts S. A histological comparison of the repair tissue formed when using either Chondrogide(®) or periosteum during autologous chondrocyte implantation. Osteoarthritis and Cartilage. 2013;**21**(12):2048-2057

[75] Mistry H, Connock M, Pink J, Shyangdan D, Clar C, Royle P, et al. Autologous chondrocyte implantation in the knee: Systematic review and economic evaluation. Health Technology Assessment. 2017;**21**(6):5-160

[76] Sadlik B, Gobbi A, Puszkarz M, Klon W, Whyte GP. Biologic inlay Osteochondral reconstruction: Arthroscopic one-step Osteochondral lesion repair in the knee using Morselized bone grafting and hyaluronic acid-based scaffold embedded with bone marrow. Aspirate Concentrate. 2017;**6**(2):e383-e389

[77] Gobbi A, Alvarez R, Irlandini E, Dallo I. Current concepts in subchondral bone pathology. In: Gobbi A, Lane JG, Longo UG, Dallo I, editors. Joint Function Preservation. Cham: Springer; 2022. DOI: 10.1007/978-3-030-82958-2_15

[78] Herman K, Whyte GP, Montagna A, Bizzoco L, Shabshin N, Szwedowski D, et al. Osteo-core plasty: Minimally invasive approach for subchondral pathologies. Journal of Cartilage & Joint Preservation. 2023;**3**(1):100101. DOI: 10.1016/J.JCJP.2023.100101

[79] Gobbi A, Dallo I. Osteo-Core-Plasty Technique for the Treatment of a Proximal Tibial Subchondral Cystic Lesion. A Case report of non-published data. Aspire Innovations June 2021. Available from: https://aspire-medical.eu/wp-content/uploads/2021/06/Osteo-Core-Plasty_CaseReport_Gobbi-Dallo_Jun21.pdf

[80] Szwedowski D, Dallo I, Irlandini E, Gobbi A. Osteo-core Plasty: A minimally invasive approach for subchondral bone marrow lesions of the knee. Arthroscopy Techniques. 20 Nov 2020;**9**(11):e1773-e1777. DOI: 10.1016/j.eats.2020.07.023

Chapter 5

Cartilage Restoration and Allogeneic Chondrocyte Implantation: Innovative Technique

Anell Olivos-Meza, Mats Brittberg, Carlos Landa-Solis and Carlos Suárez-Ahedo

Abstract

Articular cartilage lesions are frequent in young people with deleterious results if not treated properly. Various restorative techniques have been developed with the aim to overcome the limitations and short-term results of cartilage repair procedures. Cell therapy and tissue engineering techniques as Autologous Chondrocyte Implantation (ACI) have proved to induce cartilaginous tissue in joint defects with considerable long-term durability, currently being the gold standard in the treatment of medium to large cartilage injuries. Although results are encouraging and overall, the patients are satisfied, this technique is not exempt of limitations. These include the technical complexity and the costs of the two surgical procedures, de-differentiation of chondrocytes during in-vitro expansion and the limited amount of cartilage from a small biopsy. Here, we describe the recent advances in chondrocytes-based therapies for cartilage restoration, with a focus on the latest development in the use of allogeneic chondrocytes as a cell source. In allogeneic chondrocyte implantation, cells are harvested from cadaveric articular cartilage, and implanted in a scaffold into the cartilage defect. The advantages of this procedure are that there is no need for double surgeries, reduced patient morbidity and the availability of a large chondrocyte depot.

Keywords: cartilage restoration, cartilage treatment, chondral lesions, chondrocyte implantation, allogeneic chondrocytes

1. Introduction

Injured cartilage and lack of intrinsic tissue healing capacity leave a relatively young and healthy population to the risk of development of degenerative osteoarthritis (OA). Currently, the standard surgical intervention for end-stage degenerative joint pathology is total joint replacement. Early surgical interventions for symptomatic focal cartilage injuries include reparative and restorative techniques. The restorative strategies include cell-based (with or without scaffolds) or whole-tissue transplantation techniques.

The short-term outcomes of reparative techniques prompted the development of Autologous Chondrocyte Implantation (ACI) by Mats Brittberg and Lars Peterson

in Sweden in 1987 [1, 2]. Chondrocyte implantation is a cell-based cartilage repair technique in which transplanted cells are used to allow de novo development of the articular hyaline cartilage. Over time, the original technique evolved with the aim of facilitating implantation, reducing complications and improving results. These modifications of the original ACI technique are popularly known as so-called "ACI generations" [3]. Since more than 30 years ago, there are now four generations described in the literature [4]. In the first and second generations the chondrocytes are injected under a membrane (living periosteal and collagen, respectively) while in the third generation the cells are seeded on a three-dimensional porous scaffold, then this construct is place in the cartilage damage area secured with a layer of fibrine glue. Unlike these three generations are performed in two steps, the fourth one can be done in one step using pieces of cartilage fragments or isolated chondrocytes, either autologous or allogeneic (**Table 1**).

The ACI procedure requires an in-vitro expansion of autologous chondrocytes harvested from a non-weight-bearing area of the articular joint and subsequent implantation into the defect after 4 to 8 weeks [2, 4]. Long-term case series with >10 years follow-up have demonstrated that ACI is an effective and durable treatment for large knee cartilage lesions being superior to other standard treatments in prospective randomized controlled clinical trials [5–7]. Compared to other reconstructive therapy options for cartilage defects, ACI shows the best quality of the induced repair tissue [8].

Although the implantation of mature cultured chondrocytes have shown good to excellent long-term results, there are still unresolved challenges associated with the maintenance of those cells in a stable state. The in vitro expansion of autologous chondrocytes is associated with de-differentiation [4]. Dedifferentiation refers to chondrocytes with a phenotype more reminiscent of fibroblasts with the consequent modification in the expressed proteins and the formation of fibrous like-tissue with inferior biochemical and biomechanical properties [9].

Allogenic transplantation of chondrocytes has been used with some success in animal models involving rabbits [10–12]. In those studies, chondrocytes did not show positive immunomodulatory properties [13]. However, chondrocytes express class I and, in some species, class II major histocompatibility complex (MHC). Successful allogeneic chondrocyte transplants in rabbits and hens could be due to an inbreeding among experimental animals, by the use of chondrocytes cultivated before grafting in artificial scaffolds and thus protected by matrix produced in vitro [14]. It subsequently seems that encasing the allogeneic chondrocytes in a matrix reduce the immunological activity. The advantages of the allogenic approach are a single surgery, high seeding densities with early or non-culture and decreased dedifferentiated cell use.

Generation	Chondrocytes	Membrane	Source	Steps
1st	In suspension	Living Periosteal	Autologous	2
2nd	In suspension	Collagen Flap	Autologous	2
3rd	Grown in scaffold	3D-Scaffold	Autologous	2
4th	Seeded in a scaffold	3D-Scaffold	Autologous / Allogeneic	1

Table 1.
Autologous chondrocyte implantation has evolved with the aim of facilitating cartilage treatment, reducing complications and improving results. The first three generations are performed in two steps with autologous chondrocytes while the fourth one can be done in one step using either autologous or allogeneic cells.

Although the use of allogeneic chondrocytes has been explored as an alternative technique of cartilage repair, the literature is limited regarding human practice [15]. Interesting are the pioneer results from Almqvist and Dhollander [16, 17]. They used in vitro expanded allogeneic chondrocytes seeded in alginate beads. No signs of clinical deterioration or adverse reactions to the alginate beads/allogenic chondrocyte implantation were observed after mean 6.3 years [17]. However, also with in vitro expansion of allogeneic chondrocytes, de-differentiation is a dis-advantage. The ideal is to use allogeneic chondrocytes without in vitro culture for cell expansion. Use of a cadaveric source of allogeneic chondrocytes offers a possibility to obtain a large number of chondrocytes ready to use fast and easy. We present here a model of how to make use of cadaveric chondrocytes.

2. Allogeneic chondrocyte implantation

In order to reduce costs with a multi-step procedure, a one-step technique called ALCI-Graft (Allogeneic Chondrocyte Implantation Graft) has been developed. In this technique chondrocytes are isolated from cartilage obtained from the knee of young cadaveric donors. The isolated cells are seeded in a membrane of hyaluronic acid sealed with a fibrin adhesive and left for a short period of time (4 days) in an incubator with culture media enriched with autologous serum. The ALCI-Graft is subsequently a one-stage cell-based repair therapy for isolated articular cartilage lesions.

This orthobiologic implant consists of 10 million of primary chondrocytes in 20 mm of hyaluronic acid membrane in Tisseel® tissue glue (Baxter B.V, Utrecht, the Netherlands) which will act as a cell carrier for implantation.

The number of allogeneic chondrocytes incorporated into a hyaluronic acid scaffold is determined by the area of the membrane using an injection of 10×10^6 cells in defects with a mean size of 2 cm^2. The calculated seeding density used is generally close to 5×10^6 cells/cm^2. It is important that the surgeon estimates the volume of the defect, in order to implant an amount of 50 million of chondrocytes for every 10 millimeters of cartilage lesion.

We have performed a pilot study with symptomatic patients that have full-thickness cartilage lesions in the hip, knee and ankle diagnosed by clinical examination and MRI. The ALCI-Graft surgery consists of an arthroscopy approach during which the cartilage defect is localized and debrided to create stable borders (**Figure 1**). Allogeneic cryopreserved chondrocytes are thawed 4 days before surgery and seeded in the hyaluronic acid membrane embedded in Tisseel® (**Figure 2**). Ten million of primary cadaveric chondrocytes per 20 millimeters of membrane are implanted into the cartilage defect.

In the 17 cases that we have treated with this technique we have not observed any early or late immunological rejection data, infection or foreign body response after surgery. Special attention was kept on the articular volume, local temperature, and possibility of detachment of the implant, the latter evaluated by MRI after 2 weeks of surgery.

Another great advantage of ALCI-Graft is that it is easy to obtain enough donor cartilage to treat large lesions and no limit of number of cells to carry out the surgical treatment as we can see in a bipolar lesion in trochlea and patella in the **Figure 3**. Furthermore, the graft requires only a short time to become ready for use in the operating room (< 1 week) (**Table 2**).

An ALCI-Graft consists of chondrocytes isolated from articular cartilage (knee and/or patella) from young donors (<20 years), seeded on a three-dimensional

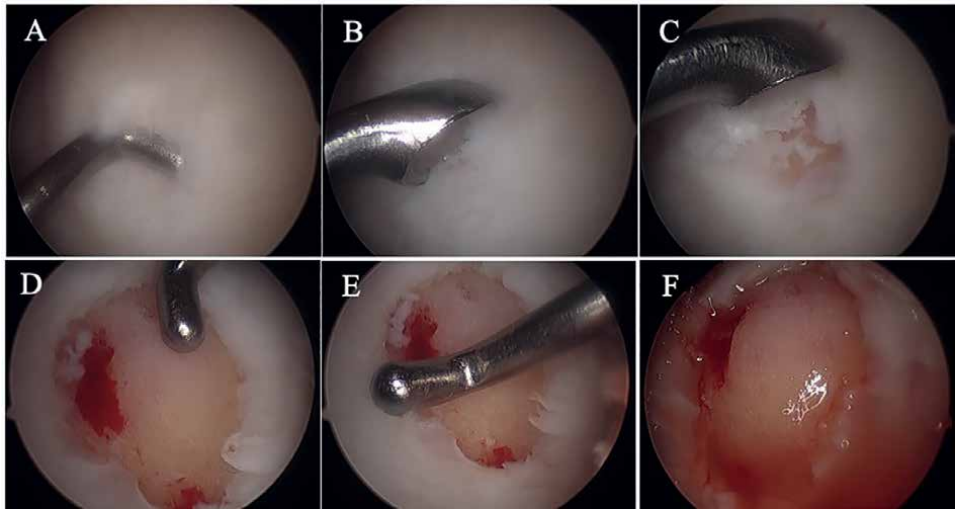

Figure 1.
Cartilage lesion in medial femoral condyle (A). The lesion is stabilized by debridement around the edges and down to the subchondral bone using a curette. Any delaminated cartilage is removed from the defect (B and C). Vertical walls are established to maximize the probability of complete cartilage fill (D and E). The calcified layer of cartilage is removed before implantation (F).

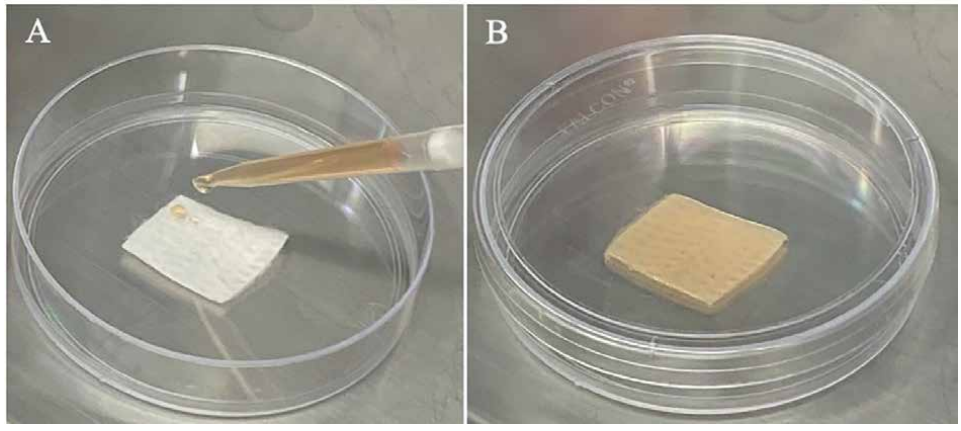

Figure 2.
Allogeneic chondrocytes seeding. The cells diluted in culture medium enriched with autologous patient serum are seeded in the hyaluronic acid membrane and then covered by fibrin glue.

membrane, composed of one of the main chondrogenic-promoting substances (hyaluronic acid, HYALOFAST®, Anika Therapeutics, Inc), in high densities (10×10^6) with autologous serum and sealed with a fibrin adhesive. The structural nature of the hyaluronic acid membrane allowed us to obtain an implant with the desirable flexibility and strength to be manipulated during arthroscopy (**Figure 4**). Likewise, during the development of the present invention and with the described methods, we found that more than 80% of the chondrocytes obtained from cadaver donor cartilage, up to 48 hours after death, maintain their viability and preserve the capacity to form cartilage in in-vitro cultures.

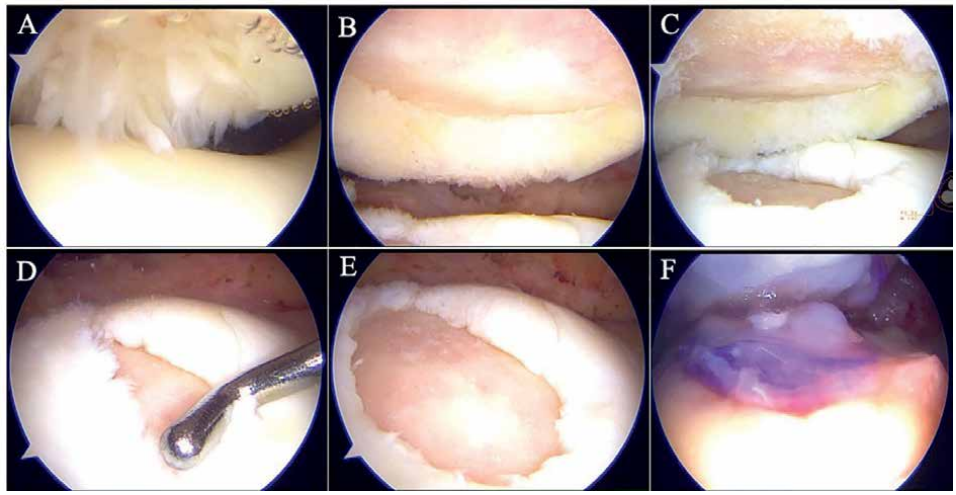

Figure 3.
Treatment of big size bipolar cartilage lesion in the patella (A) and trochlea (D) with ALCI-graft technique. The chondral defects are cleaned with a curette to remove the pathologic cartilage from within the defect (B and E). Once the cartilage lesions have stable borders (C) a final measurement is performed to cut the graft to the appropriate sizes, then the pump water is closed and the construct introduced into the joint and place in the bottom of the lesion until completely covered, finally fibrin glue is applied on the surface to fix the implant (F).

Characteristic	ALCI	ACI
Source of cells	Allogeneic	Autologous
Number of surgeries	One	Two
Cell culturing	No	Yes
Days to prepare the graft	4	42–56
Limit of lesion size	No limit	4 cm^2
Recommended age of patient	No limit	<50 years

Table 2.
Comparative characteristics of allogeneic versus autologous chondrocyte implantation. ALCI-graft does not require cell culture or biopsy of the patient, so it becomes a one-step technique with which it is possible to treat large lesions (> 4 cm^2).

Once a cadaveric donor is selected, the cartilage is harvested in sterile conditions and then sent to a panel of safety tests to rule out the presence of infectious diseases that can be transmitted through the donated tissue. The panel includes antibodies against hepatitis B (Core and Surface), hepatitis C, HTLV, syphilis, HIV and detection of nucleic acids (NAT test) against hepatitis B, C and HIV (Viromed/Labcorp laboratory, in Minnesota, United States). Once the biopsies or the tissue samples show negative serology, they are considered suitable for use in the formation of the implant.

3. Implant formation

Cryopreserved chondrocytes stored in a tissue banking are thawed and seeded 4 days before patient is scheduled for chondrocyte implantation. Every vial of stored

Figure 4.
ALCI-graft in the operating room. The construct is transported in a sterile culture box to the operating room, where it is appropriate to the size and shape of the lesion to be treated.

cells contains $10x10^6$ primary chondrocytes that are seeded in every 20 mm parts of hyaluronic membrane. The seeded cells are then covered by tissue glue and left in the incubator for 15 minutes before to be started in the media culture (Dulbecco's Modified Eagle Medium F12 GIBCO, Grand Island, NY with 20% autologous serum) and finally left in the incubator at 37°C, 5% CO2 and 5% humidity. The autologous serum is obtained from the patient to whom the construct is to be implanted, thus avoiding the use of fetal bovine serum which may cause secondary reactions in the patient.

4. Arthroscopic implantation

The ALCI-Graft is useful for the treatment of cartilage lesions in all kinds of articular joints. The construct is provided as a kit comprising a culture box transported under sterile conditions in a portable incubator that maintain standard parameters (37°C, 5% CO2 and 5% humidity). It is recommended that prior to the use of the implant, the treating physician should evaluate the injury during surgery, identifying the location, size, and shape.

In order to have a good integration of the graft to surrounding native cartilage, it is recommended that, before the implantation of the construct, an adequate debridement of the lesion should be performed (**Figure 3B** and **E**). All damaged tissue should be removed creating stable vertical walls of healthy cartilage. It is important that after debridement the lesion is measured again, as it is very certain that the size of the lesion is now larger than before removal of the injured and unstable edges. If the surgery is open, the measurement can be made with a surgical ruler; if the procedure is arthroscopic, the hook probe, or a flexible ruler, is used to determine the exact size of the lesion in the proximal-distal and medio-lateral planes (**Figure 1D** and **E**). Likewise, it is highly recommended that the lesion be given a square or rectangular shape, to facilitate measuring and matching the size and shape of the graft. It is equally important not to damage the subchondral bone.

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Once the final lesion measurements are obtained, after debridement of the lesion, it is recommended to trim the graft with an excess of at least 2 mm at each edge. It is preferable to have a larger construct, which can be compressed and adapted to the lesion, than to leave a fair or smaller construct, with the consequent risk that one or more of its walls will fail to integrate with the edges of the healthy cartilage.

The graft is fixated at the bottom of the cartilage lesion with fibrin glue. When the construct is placed into the lesion, fluid entry into the arthroscopy is closed to prevent loss of chondrocytes. A cannula is placed in the portal of best access to the lesion, and through this portal the graft is introduced into the joint (**Figure 5A**), placed and spread over the entire area (**Figure 5B**) until the lesion is completely covered and ensuring existing contact of the graft with all edges of the adjacent native cartilage (**Figure 5B**). Finally, fibrin glue is applied to the edges and surface of the graft to ensure fixation to the lesion, (**Figure 5C** and **D**). In the case of the knee, it is recommended to keep it in extension for at least a couple of days to avoid friction and loss of the graft, when a lesion is treated in hip or ankle, we also recommend a couple of days of immobilization.

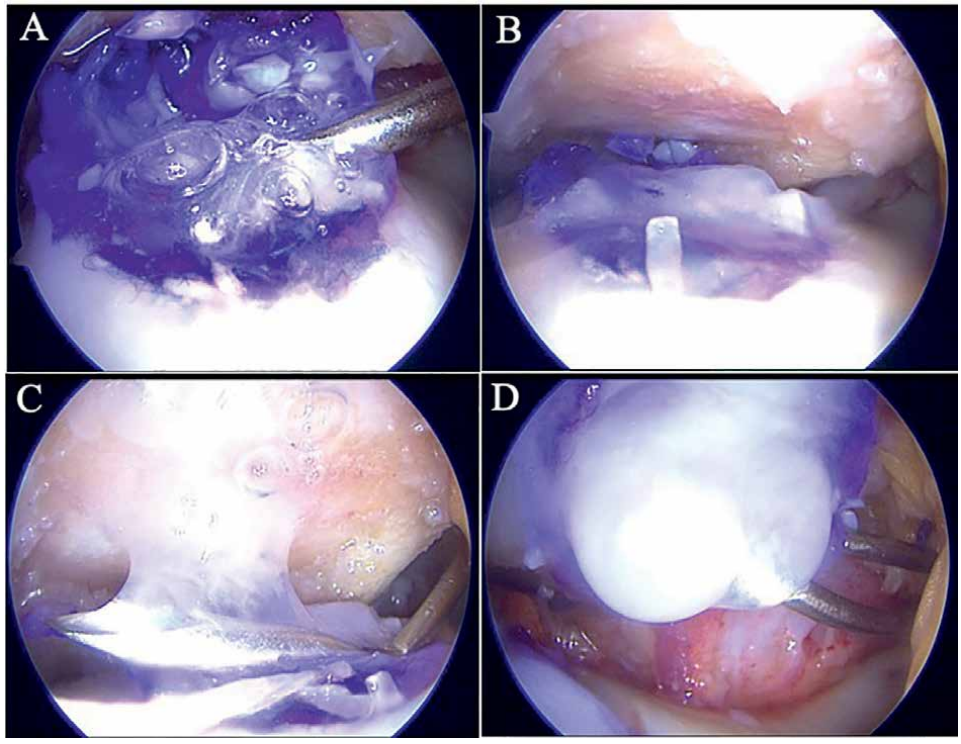

Figure 5.
Allogeneic chondrocyte implantation in a bipolar lesion in the patella and trochlea. A) At the time the construct is placed into the lesion, water entry into the arthroscopy is closed to prevent loss of chondrocytes. B) the graft is introduced into the joint, placed and spread over the entire area until the lesion is completely covered and ensuring contact of the graft with all edges of the adjacent native cartilage. Fibrin glue is applied to the edges and surface of the graft to ensure fixation to the lesion (C and D).

5. Rehabilitation protocol

Post-operatively the rehabilitation starts directly the same day with cryotherapy, after 2 days of immobilization in a brace locked in extension, continuous passive motion is started with gradually movement that increases over the time depending of the location of the graft and its stability during arthroscopy motion. Weight bearing is avoided during 4 weeks and if the repaired lesion is in a loading zone, then partial discharge is permitted at week 5 and 6. Progressive open-chain strengthening is started after the first iso-kinetic evaluation at 3 months after surgery. Patients are allowed to return to sports activities after 12 months and when isokinetic evaluation reported 90% of strength of the contralateral limb.

6. Post-operative follow-up

Different evaluation system can be used for post-operative evaluation. Visual analog scale (VAS) for pain, the IKDC knee scoring, Lysholm knee score, and Tegner activity scale are all used to assess the clinical evolution. With MRI, T2-mapping evaluation is performed after surgery to evaluate the graft integration and maturity compared to a native cartilage adjacent zone.

7. Conclusions

Since the first ACI-autologous chondrocyte implantation was performed in humans in 1987, several variants of ACI have been developed. All those generations of ACI are based on autologous cells. To use allogeneic cells, the availability to treat patients faster, with a smaller number of operations and with more stable repair possibility with differentiated cells is possible. Implantation of allogeneic chondrocytes in focal full cartilage lesions in hip, knee and ankle has been shown to be a safe procedure that can be performed arthroscopically and improve pain, function and quality of life of the patients in studies up to 3 years post-surgery. Use of a cadaver allogeneic chondrocytes gives benefits in terms of a possible one step technique, minor cell culturing, larger treatment sizes and short period of graft preparation compared to traditional Gold ACI Standard technique.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. The New England Journal of Medicine. 1994;**331**:889-895

[2] Makris EA, Gomoll AH, Malizos KN, Hu JC, Athanasiou KA. Repair and tissue engineering techniques for articular cartilage. Nature Reviews Rheumatology. 2015;**11**(1):21-34

[3] Bohacek I, Bojanic I, Gajovic S, Josipovic M, Jelic M. Articular cartilage repair techniques exploiting intrinsic healing capacity – Which one is the best. Periodicum Biologorum. 2015;**117**(1):125-133

[4] Brittberg M. Clinical articular cartilage repair—An up-to-date review. Annals of Joint Journal. 2018;**3**:1-9

[5] Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: A long-term follow-up. The American Journal of Sports Medicine. 2010;**38**:1117-1124

[6] Minas T, Von Keudell A, Bryant T, Gomoll AH. The John Insall award: A minimum 10-year outcome study of autologous chondrocyte implantation. Clinical Orthopaedics and Related Research. 2014;**472**:41-51

[7] Behery OA, Harris JD, Karnes JM, Siston RA, Flanigan DC. Factors influencing the outcome of autologous chondrocyte implantation: A systematic review. The Journal of Knee Surgery. 2013;**26**:203-211

[8] Vavken P, Samartzis D. Effectiveness of autologous chondrocyte implantation in cartilage repair of the knee: A systematic review of controlled trials. Osteoarthritis and Cartilage. 2010;**18**(6):857-863

[9] Medvedeva EV, Grebenik EA, Gornostaeva SN, Telpuhov VI, Lychagin AV. Repair of damaged articular cartilage: Current approaches and future directions. International Journal of Molecular Sciences. 2018;**19**(8):2366

[10] Boopalan PR, Sathishkumar S, Kumar S, Chittaranjan S. Rabbit articular cartilage defects treated by allogenic chondrocyte transplantation. International Orthopaedics. 2006;**30**:357-361

[11] Schreiber RE, Ilten-Kirby BM, Dunkelman NS, Symons KT, Rekettye LM, Willoughby J, et al. Repair of osteochondral defects with allogeneic tissue engineered cartilage implants. Clinical Orthopaedics and Related Research. 1999;**367S**:S382-S395

[12] Wakitani S, Goto T, Young RG, Mansour JM, Goldberg VM, Caplan AI, et al. Repair of large full-thickness articular cartilage defects with allograft articular chondrocytes embedded in a collagen gel. Tissue Engineering. 1998;**4**:429-444

[13] Davies RL, Kuiper N. Regenerative medicine: A review of the evolution of autologous chondrocyte implantation (ACI) therapy. Bioengineering. 2019;**6**(22):2-16

[14] Moskalewski S, Hyc A, Osiecka-Iwan A. Immune response by host after allogeneic chondrocyte transplant to the cartilage. Microscopy Research and Technique. 2002;**58**(1):3-13

[15] Lohan P, Treacy O, Lynch K, Barry F, Murphy M, Griffin MD, et al. Culture

expanded primary chondrocytes have potent immunomodulatory properties and do not induce an allogeneic immune response. Osteoarthritis and Cartilage. 2016;**24**:521-533

[16] Almqvist KF, Dhollander AA, Verdonk PC, Forsyth R, Verdonk R, Verbruggen G. Treatment of cartilage defects in the knee using alginate beads containing human mature allogenic chondrocytes. The American Journal of Sports Medicine. 2009;**37**(10):1920-1929

[17] 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. The American Journal of Sports Medicine. 2012;**40**(1):75-82

Chapter 6

ACL Tear and Cartilage Lesions

Philippe Landreau, Antoine Catteeuw, Karl Almqvist and Prashant Meshram

Abstract

Articular cartilage injuries are not uncommon finding in patients with anterior cruciate ligament (ACL) tear. There are several ways to address the cartilage injuries when encountered during ACL reconstruction. The favorable treatment of cartilage injuries during ACL reconstruction is controversial. Indeed, the treatment of cartilage injuries depends on multiple factors including patient variables and severity of lesion. It is unclear whether cartilage lesions affect the recovery after ACL reconstruction and vice versa. Whether ACL reconstruction has a preventive effect on further progression of cartilage lesions is also unclear. This chapter gives an overview of current literature related to cartilage injuries with ACL tear in terms of epidemiology, clinical presentation, and management.

Keywords: ACL, anterior cruciate ligament, cartilage, chondral, lesions, arthritis, knee, laxity, outcomes

1. Introduction

Articular cartilage injuries are a frequent finding in patients with anterior cruciate ligament (ACL) tear. The cartilage lesions may be pre-existing, especially in athletes, or may occur at the same traumatic episode of the ACL injury. The chondral injuries may be asymptomatic and difficult to identify on preoperative imaging; but discovered only at the time of ACL reconstruction (ACLR). The treatment options for cartilage lesions include nonoperative, chondroplasty, microfracture, mosaicplasty, osteochondral allograft, autologous chondrocyte implantation, and artificial joint replacement. When encountered with ACL tear, the influence of concomitant cartilage injuries on clinical outcomes, the return to sport, and long term progression to osteoarthritis is unclear [1]. The ideal treatment of cartilage injuries during ACLR is also controversial. In general, the treatment of cartilage injuries depend on multiple factors including patient demographics and activity level, severity and location of the lesion, and concomitant pathologies such as knee varus malalignment. The aim of this chapter is to highlight the current literature related to cartilage injuries with ACL tear in terms of epidemiology, clinical presentation, and management.

2. Epidemiology

The prevalence of cartilage injuries with ACL tear is not uncommon. Flanigan et al. in a systematic review of 11 studies involving 931 athletes reported the prevalence of full-thickness focal chondral defects of 36%, of whom, 14% were asymptomatic [2]. Brophy et al. in a systematic review of 5 studies reported that the incidence of severe articular injury identified during ACLR to be between 16% and 46% [3]. Wyatt et al. in a case series of 261 patients reported a prevalence of chondral injuries of 15% in primary and 32% in revision ACLR [4]. Rotterud et al. [5] studied the Norwegian and the Swedish National Knee Ligament Registry between 2005 and 2008 with a total of 8476 ACLRs. They found that 20% of the patients had a grade 1 or 2 International Cartilage Research Society (ICRS) cartilage lesion, and 7% had a grade 3 or 4 ICRS cartilage lesion and approximately half of the lesion had a surface area less than 2 cm^2. The most common location of chondral lesion was the medial femoral condyle (34–51%). Tandogan et al. [6] in their multicentric study of 764 patients who underwent arthroscopy for ACL tear found that at least one chondral injury was present in 19% of patients, of which 60% were observed in weight-bearing area of the medial condyle. One third of the chondral injuries were grades 3 and 4 ICRS. The mean surface was 219 ± 175 mm^2. Thus, current literature suggests that severe cartilage injuries are encountered in about one third of patients undergoing ACLR.

3. Effect of ACL injury on cartilage lesions

The cartilage injury accompanying ACL tears could occur at the time of the initial trauma that tore ACL or as a sequalae due to knee instability and altered tibiofemoral biomechanics. Notably, the literature indicates an increase in incidence of cartilage lesions in chronic ACL tear in comparison with acute cases. Sommerfeldt et al. [7] evaluated the 860 patients who underwent ACLR with a single surgeon and found that increased time duration between injury to surgery associated with higher odds of chondral damage of the medial femoral condyle, early degenerative changes of the medial tibiofemoral compartment, and medial meniscal tears, including irreparable medial meniscal tears. Shelbourne et al. [8] studied 2770 patients who underwent ACLR and found that the incidence of cartilage lesions was twice as common in patients with chronic ACL tear (54%) as compared to those with acute ACL tear (23%).

Several studies have evaluated the influence of duration between injury and ACLR on the incidence of chondral injury. Tandogan et al. in their multicentric study [6] found that the risk of grade 3 and 4 ICRS chondral injury increased with longer duration between ACL injury and arthroscopy and older age. The odds of grade 3 and 4 chondral injury at 2 to 5 years after ACL injury were 2.7 times which increased to 4.7 times after 5 years of ACL injury. Joseph et al. [9] studied the arthroscopic findings of 575 athletes and 800 non-athletes undergoing ACLR and found an increase in chondral injuries in both the groups who had a delay of 1 year for ACLR since injury. Yüksel et al. [10] evaluated the arthroscopic findings of patients with ACL tear who elected not to restrict their daily activities after the initial trauma and reported the characteristics of meniscal and chondral lesions. The prevalence of chondral injuries was significantly higher in patients who had a delay of 1 year (70%) from injury until treatment as compared to those with a delay of 6 weeks to 1 year (26%), and less than 6 weeks (9%). Michalitsis et al. [11] in their case series of 109 consecutive patients

with ACL tear reported increased incidence of high-grade cartilage lesion when reconstruction was performed more than 12 months after injury. Similarly, Anderson et al. [12] studying pediatric patients with ACL tear demonstrated that delayed ACLR increased the risk of secondary meniscal and chondral injuries. Taketomi et al. [13] in their retrospective study involving 226 patients concluded that ACLR should be performed within 6 months after the ACL injury to prevent cartilage and meniscus lesions. Bambrilla et al. [14] found that ACLR within 12 months of injury can significantly reduce the risk of meniscal tears and chondral lesions. In their study, older age and increased BMI were risk factors for the occurrence of at least one associated lesion. Prodromidis et al. [15] in their meta-analysis published in 2022 of 14 studies found that a delay in ACLR by 3 months after injury increased the odds of low-grade chondral injuries by 1.9 times and a delay of 1 year increased the odds of high-grade chondral injuries by 3 times. They recommended performing ACLR, when indicated, within 3 months of ACL injury. Wyatt et al. [4] reported in their case series of 261 patients that the prevalence of cartilage injuries increased from 14.9% at primary ACLR to 31.8% at revision ACLR. Thus, the current literature indicates that it may be critical to perform ACLR as early as 3 months or at least within 1 year of ACL injury and perhaps earlier in obese, older, and failed previous ACLR patients to prevent further damage to the knee cartilage and meniscus.

4. Does ACLR prevent from progression of chondral damage?

Whether ACLR prevents an increase in the identified chondral lesions and progression to osteoarthritis is controversial. MARS and MOON group [16] evaluated 134 patients undergoing revision ACLR for progression of chondral lesions as compared to primary ACLR. Significant progression of articular cartilage damage was defined in each compartment according to progression on the modified Outerbridge scale (increase ≥1 grade) or > 25% enlargement in any area of damage. Partial meniscectomy was found as a risk factor for progression of chondral lesions and older age, higher body mass index, and use of allograft in primary ACLR were associated with osteoarthritis progression. Sugiu et al. [17] in their study of 37 patients that underwent double bundle ACLR found an increase in the cartilage lesions from 11 sites at index ACLR surgery to 54 sites at the second look arthroscopy at a mean of 17 months follow up. The authors concluded that the knee articular cartilage lesions after ACL rupture cannot be completely suppressed, even with anatomical ACLR technique. Nakamae et al. [18] in a study of 174 patients who had second look arthroscopy after double bundle ACLR found that the chondral damage progression was strongly associated with partial meniscectomy. Huang et al. [19] studying the patellofemoral cartilage lesions detected during ACLR at a follow up of mean of 2 years found progression of lesions in 45 out of 129 patients. They identified medial or lateral partial meniscectomy and quadriceps muscle weakness to be the associated with progression of patellofemoral cartilage lesions. Hence, the role of ACLR to prevent progression of chondral lesions is controversial.

5. Effect of cartilage injuries on clinical outcomes after ACLR

Several studies have evaluated the influence of concomitant cartilage lesion on short-, mid-, and long-term clinical outcomes after ACLR. Everhart et al. [20] studied

508 patients undergoing ACLR and found that those with grade 2 or higher chondral damage had quadriceps weakness at 6 months follow up as compared to those without chondral damage. They also found that patients with chondral damage had lesser risk of ipsilateral and contralateral ACL injury, perhaps due to reduced activity level. Rotterud et al. [5] found worse functional patient-reported outcomes at short term follow up of 2 years after ACLR when concomitant cartilage lesion of grades 3 and 4 ICRS were present. Kowalchuk et al. [21] studied 402 patients of ACLR at a midterm follow up of mean 6.3 years and found lower International Knee Documentation Committee (IKDC) score in patients with concomitant chondral injuries. Similarly, Cox et al. [22] in another midterm (mean 6 years follow up) multicentric study in patients with ACLR found that grade 3 and 4 ICRS cartilage lesions and meniscal injury were the predictors of lower IKDC and KOOS scores. Another study by Webster et al. [23] of 180 patients undergoing revision ACLR evaluated at a mean of 4.6 years found that patients with ICRS 3 or 4 chondral pathology had significantly lower IKDC, KOOS-Quality of life, Marx activity, and Single Numerical Assessment scores as well as a lower rate of return to the same level of pre-injury sport. Brophy et al. [24] in a retrospective study of a cohort of 2575 patients who had ACLR found that the risk factors for worse IKDC and KOOS scores at 10 years follow up were chondral lesions in patellofemoral or medial compartments and previous meniscal surgery. In a prospective study of 100 patients undergoing ACLR, Janssen et al. [25] reported radiological signs of osteoarthritis in 53.5% of the cases at a long term follow of 10 years. They reported cartilage lesion and meniscectomy to be the risk factors for osteoarthritis after ACLR.

On the other hand, there are few studies [8, 26–28] that did not find any influence of concomitant cartilage lesions on outcomes of ACLR. Shelbourne et al. [8] evaluated 2770 ACLR patients and found no difference in IKDC scores at midterm follow up of mean 6.3 years between patients with or without concomitant grade 3 and 4 chondral lesions. Widuchowski et al. [28] in a long term of 10 and 15 years follow up study found that the patients with grade 3 and 4 Outerbridge chondral lesions identified during ACLR and left alone without treatment had similar Lysholm, Tegner, and IKDC scores as compared to those without chondral lesions.

Despite the discrepancy in literature on influence of chondral lesion on outcomes of ACLR, there is adequate evidence and rational favoring treatment of grade 3 and 4 chondral lesions when encountered during ACLR. In a systematic review of 37 studies, Filardo et al. [29] concluded that most of the studies in the literature showed a correlation between lesions of the articular surface and a poor outcomes after ACLR. Furthermore, it is well known that isolated grade 3 and 4 cartilage knee injuries can cause pain and effusion and affect the return to sport. Thus, it seems appropriate to treat high-grade focal chondral defects simultaneously with ACLR in order to give the best chance of return to function and sports and reduce chances of rapid progression to osteoarthritis.

6. Results of concomitant cartilage repair and ACLR

The preferred method of cartilage treatment during ACLR such as simple debridement, microfracture, mosaicplasty, osteochondral allograft, and biological cell based treatment like chondrocyte implantation or stem cell therapy is based on multiple factors and controversial. One cannot emphasize enough on the importance of preoperative assessment of imaging for the size and location of chondral lesion to

plan its treatment. Debridement, microfracture procedure, or mosaicplasty are simple techniques that will not require postoperative immobilization of the knee. On the other hand, more sophisticated and staged procedures, such as scaffolds and biological cell based treatment, usually requires a period of postoperative immobilization which could compromise the rehabilitation of the ACL based on early mobilization.

In a nationwide prospective cohort study from Norway and Sweden of 368 patients, comparing simple debridement, microfracture, and nonsurgical treatment of concomitant full-thickness cartilage lesions after ACLR, Ulstein et al. [30] showed no difference on KOOS scores at 5-year follow-up. Nakamura et al. [31] demonstrated that at second-look arthroscopy, after ACLR without any intervention to the articular cartilage, there was a significant recovery of chondral lesions by Outerbridge grading on both the medial and lateral femoral condyles. Differently for chondral lesions of patellofemoral joint or tibial plateau, there was no significant recovery of chondral lesions. They concluded that there was a location-specific difference in the natural healing response of chondral injury favoring those on femoral condyles. One of the reasons of spontaneous healing of chondral lesions with simple debridement in patients undergoing ACLR could be due to biological factors released during the surgery. ACLR involves drilling of bone tunnels, intra-articular enrichment in growth factors and progenitor cells from bone marrow might be involved in the repair processes of injured cartilage. Another study by Imade et al. [32] in a cohort of 40 patients undergoing ACLR with cartilage treatment either by microfracture or autologous osteochondral grafting at 1 year follow up found no differences in IKDC scores.

In our experience, only grade 3 and 4 ICRS lesions are treated at the same time as ACLR and grade 1 and 2 are left alone. For grade 3 and 4 lesions of size less than 3 cm^2 or less, microfractures or mosaicplasty are preferred. Among these, mosaicplasty is preferred over microfracture if the cartilage lesions involve substantial surface of weight bearing surface of the condyle as the literature shows better results of return to sports with mosaicplasty [33]. Above 3 cm^2, if available, osteochondral allograft is a good option because it allows starting an early mobilization after the combined surgery. More literature evidence is needed to ascertain the best method of cartilage treatment during ACLR.

7. Physiotherapy protocol after ACLR with concomitant chondral lesion

The presence of concomitant cartilage lesions, treated or not, in the setting of ACLR represents a peculiar and controversial challenge in terms of postoperative physiotherapy management. Most of the authors recommend caution in the rehabilitation in such cases. There is limited evidence in the literature to recommend a specific physiotherapy protocol. Thrush et al. [34] in their systematic review of 6 studies on physiotherapy after concomitant ACLR and chondral injury found very little uniformity, and no strong recommendations could be concluded regarding the most appropriate rehabilitation. Interestingly, use of aggressive rehabilitation, early weightbearing, no immobilization, and immediate range of motion did not compromise outcomes compared to more conservative protocols. The authors' preferred physiotherapy protocol for cases of microfracture in any area or auto- or allo-graft cartilage transplantation or autologous chondrocyte implantation in nonweight bearing area, is no restriction of range of motion and full weight bearing. In cases of auto- or allo-graft cartilage transplantation in the weight bearing area, we prefer partial weight bearing for 6 weeks without any restriction of range of motion.

As with any surgery, patient expectation is key to optimize satisfaction [35]. It is crucial for surgeons to inform patients undergoing ACLR about the additional treatment and modifications in physiotherapy in case full-thickness cartilage lesion is encountered. This is particularly important in athletic population who may be disappointed if the cartilage lesion leads to residual pain, swelling, and limitation to return to sport.

8. Conclusion

The presence of concomitant grade 3 and 4 cartilage injury leads to worse clinical outcomes after ACLR than those without cartilage lesion or even partial thickness cartilage lesion. Current evidence favors the treatment of the cartilage lesion at the time of ACLR to optimize the clinical outcomes and return to sports. The preferred treatment of cartilage lesion is controversial and depends on the size, location, and experience of the surgeon. Future studies should focus on refining the selection criteria for cartilage lesions that will benefit from treatment during ACLR and also the preferred method of treatment.

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References

[1] Øiestad BE, Engebretsen L, Storheim K, Risberg MA. Knee osteoarthritis after anterior cruciate ligament injury: A systematic review. The American Journal of Sports Medicine. 2009;**37**(7):1434-1443

[2] Flanigan DC, Harris JD, Trinh TQ, Siston RA, Brophy RH. Prevalence of chondral defects in athletes knees: A systematic review. Medicine and Science in Sports and Exercise. 2010;**42**(10):1795-1801

[3] Brophy RH, Zeltser D, Wright RW, Flanigan D. Anterior cruciate ligament reconstruction and concomitant articular cartilage injury: Incidence and treatment. Arthroscopy. 2010;**26**(1):112-120

[4] Wyatt RW, Inacio MC, Liddle KD, Maletis GB. Prevalence and incidence of cartilage injuries and meniscus tears in patients who underwent both primary and revision anterior cruciate ligament reconstructions. The American Journal of Sports Medicine. 2014;**42**(8):1841-1846

[5] Røtterud JH, Sivertsen EA, Forssblad M, Engebretsen L, Arøen A. Effect of meniscal and focal cartilage lesions on patient-reported outcome after anterior cruciate ligament reconstruction: A nationwide cohort study from Norway and Sweden of 8476 patients with 2-year follow-up. The American Journal of Sports Medicine. 2013;**41**(3):535-543

[6] Tandogan RN, Taşer O, Kayaalp A, et al. Analysis of meniscal and chondral lesions accompanying anterior cruciate ligament tears: Relationship with age, time from injury, and level of sport. Knee Surgery, Sports Traumatology, Arthroscopy. 2004;**12**:262-270

[7] Sommerfeldt M, Goodine T, Raheem A, Whittaker J, Otto D. Relationship between time to ACL reconstruction and presence of adverse changes in the knee at the time of reconstruction. Orthopaedic Journal of Sports Medicine. 2018;**6**(12):2325967118813917

[8] Shelbourne KD, Jari S, Gray T. Outcome of untreated traumatic articular cartilage defects of the knee: A natural history study. The Journal of Bone and Joint Surgery. American Volume. 2003;**85-A**(Suppl. 2):8-16

[9] Joseph C, Pathak SS, Aravinda M, Rajan D. Is ACL reconstruction only for athletes? A study of the incidence of meniscal and cartilage injuries in an ACL-deficient athlete and non-athlete population: An Indian experience. International Orthopaedics. 2008;**32**(1):57-61. DOI: 10.1007/s00264-006-0273-x

[10] Yüksel HY, Erkan S, Uzun M. The evaluation of intraarticular lesions accompanying ACL ruptures in military personnel who elected not to restrict their daily activities: The effect of age and time from injury. Knee Surgery, Sports Traumatology, Arthroscopy. 2006;**14**(11):1139-1147

[11] Michalitsis S, Vlychou M, Malizos KN, Thriskos P, Hantes ME. Meniscal and articular cartilage lesions in the anterior cruciate ligament-deficient knee: Correlation between time from injury and knee scores. Knee Surgery, Sports Traumatology, Arthroscopy. 2015;**23**(1):232-239

[12] Anderson AF, Anderson CN. Correlation of meniscal and articular cartilage injuries in children and

adolescents with timing of anterior cruciate ligament reconstruction. The American Journal of Sports Medicine. 2015;**43**(2):275-281

[13] Taketomi S, Inui H, Yamagami R, Kawaguchi K, Nakazato K, Kono K, et al. Surgical timing of anterior cruciate ligament reconstruction to prevent associated meniscal and cartilage lesions. Journal of Orthopaedic Science. 2018;**23**(3):546-551. DOI: 10.1016/j.jos.2018.02.006

[14] Brambilla L, Pulici L, Carimati G, Quaglia A, Prospero E, Bait C, et al. Prevalence of associated lesions in anterior cruciate ligament reconstruction: Correlation with surgical timing and with patient age, sex, and body mass index. The American Journal of Sports Medicine. 2015;**43**(12):2966-2973. DOI: 10.1177/0363546515608483

[15] Prodromidis AD, Drosatou C, Mourikis A, Sutton PM, Charalambous CP. Relationship between timing of anterior cruciate ligament reconstruction and chondral injuries: A systematic review and meta-analysis. The American Journal of Sports Medicine. 2022;**50**(13):3719-3731. DOI: 10.1177/03635465211036141

[16] MARS Group, Magnussen RA, Borchers JR, Pedroza AD, Huston LJ, Haas AK, et al. Risk factors and predictors of significant chondral surface change from primary to revision anterior cruciate ligament reconstruction: A MOON and MARS cohort study. The American Journal of Sports Medicine. 2018;**46**(3):557-564. DOI: 10.1177/0363546517741484

[17] Sugiu K, Furumatsu T, Kodama Y, Kamatsuki Y, Okazaki Y, Okazaki Y, et al. Post-traumatic articular cartilage lesions increase at second-look arthroscopy following primary anterior cruciate ligament reconstruction. Acta Medica Okayama. 2019;**73**(3):223-228. DOI: 10.18926/AMO/56864

[18] Nakamae A, Adachi N, Deie M, Ishikawa M, Nakasa T, Ikuta Y, et al. Risk factors for progression of articular cartilage damage after anatomical anterior cruciate ligament reconstruction: a second-look arthroscopic evaluation. Bone Joint Journal. 2018;**100-B**(3):285-293. DOI: 10.1302/0301-620X.100B3.BJJ-2017-0837.R1

[19] Huang H, Li Z, Luo S, Zheng J, Zhou G, Wang G. Factors influencing the progression of patellofemoral articular cartilage damage after anterior cruciate ligament reconstruction. Orthopaedic Journal of Sports Medicine. 2022;**10**(7):23259671221108362. DOI: 10.1177/23259671221108362

[20] Everhart JS, DiBartola AC, Swank K, Pettit R, Hughes L, Lewis C, et al. Cartilage damage at the time of anterior cruciate ligament reconstruction is associated with weaker quadriceps function and lower risk of future ACL injury. Knee Surgery, Sports Traumatology, Arthroscopy. 2020;**28**(2):576-583. DOI: 10.1007/s00167-019-05739-1

[21] Kowalchuk DA, Harner CD, Fu FH, Irrgang JJ. Prediction of patient-reported outcome after single-bundle anterior cruciate ligament reconstruction. Arthroscopy. 2009;**25**(5):457-463

[22] Cox CL, Huston LJ, Dunn WR, Reinke EK, Nwosu SK, Parker RD, et al. Are articular cartilage lesions and meniscus tears predictive of IKDC, KOOS, and Marx activity level outcomes after anterior cruciate ligament reconstruction? A 6-year multicenter cohort study. The American Journal of Sports Medicine. 2014;**42**(5):1058-1067. DOI: 10.1177/0363546514525910

[23] Webster KE, Feller JA, Kimp A, Devitt BM. Medial meniscal and chondral pathology at the time of revision anterior cruciate ligament reconstruction results in inferior mid-term patient-reported outcomes. Knee Surgery, Sports Traumatology, Arthroscopy. 2018;**26**(4):1059-1064. DOI: 10.1007/s00167-018-4880-z

[24] Brophy RH, Huston LJ, Briskin I, MOON Knee Group, Amendola A, Cox CL, et al. Articular cartilage and meniscus predictors of patient-reported outcomes 10 years after anterior cruciate ligament reconstruction: A multicenter cohort study. The American Journal of Sports Medicine. 2021;**49**(11):2878-2888. DOI: 10.1177/03635465211028247

[25] Janssen RP, du Mée AW, van Valkenburg J, Sala HA, Tseng CM. Anterior cruciate ligament reconstruction with 4-strand hamstring autograft and accelerated rehabilitation: A 10-year prospective study on clinical results, knee osteoarthritis and its predictors. Knee Surgery, Sports Traumatology, Arthroscopy. 2013;**21**(9):1977-1988. DOI: 10.1007/s00167-012-2234-9

[26] Hjermundrud V, Bjune TK, Risberg MA, Engebretsen L, Arøen A. Full-thickness cartilage lesion do not affect knee function in patients with ACL injury. Knee Surgery, Sports Traumatology, Arthroscopy. 2010;**18**(3):298-303. DOI: 10.1007/s00167-009-0894-x

[27] Spindler KP, Warren TA, Callison JC Jr, Secic M, Fleisch SB, Wright RW. Clinical outcome at a minimum of five years after reconstruction of the anterior cruciate ligament. The Journal of Bone and Joint Surgery. 2005;**87**(8):1673-1679. DOI: 10.2106/JBJS.D.01842

[28] Widuchowski W, Widuchowski J, Koczy B, Szyluk K. Untreated asymptomatic deep cartilage lesions associated with anterior cruciate ligament injury: Results at 10- and 15-year follow-up. The American Journal of Sports Medicine. 2009;**37**(4):688-692. DOI: 10.1177/0363546508328104

[29] Filardo G, de Caro F, Andriolo L, Kon E, Zaffagnini S, Marcacci M. Do cartilage lesions affect the clinical outcome of anterior cruciate ligament reconstruction? A systematic review. Knee Surgery, Sports Traumatology, Arthroscopy. 2017;**25**(10):3061-3075. DOI: 10.1007/s00167-016-4097-y

[30] Ulstein S, Årøen A, Engebretsen L, Forssblad M, Lygre SHL, Røtterud JH. A controlled comparison of microfracture, debridement, and no treatment of concomitant full-thickness cartilage lesions in anterior cruciate ligament-reconstructed knees: A nationwide prospective cohort study from Norway and Sweden of 368 Patients With 5-Year Follow-up. Orthopaedic Journal of Sports Medicine. 2018;**6**(8):2325967118787767. DOI: 10.1177/2325967118787767

[31] Nakamura N, Horibe S, Toritsuka Y, Mitsuoka T, Natsu-ume T, Yoneda K, et al. The location-specific healing response of damaged articular cartilage after ACL reconstruction: Short-term follow-up. Knee Surgery, Sports Traumatology, Arthroscopy. 2008;**16**(9):843-848. DOI: 10.1007/s00167-008-0565-3

[32] Imade S, Kumahashi N, Kuwata S, Kadowaki M, Tanaka T, Takuwa H, et al. A comparison of patient-reported outcomes and arthroscopic findings between drilling and autologous osteochondral grafting for the treatment of articular cartilage defects combined with anterior cruciate ligament injury. The Knee. 2013;**20**(5):354-359. DOI: 10.1016/j.knee.2012.07.007

[33] Krych AJ, Harnly HW, Rodeo SA, Williams RJ 3rd. Activity levels are higher after osteochondral autograft transfer mosaicplasty than after microfracture for articular cartilage defects of the knee: A retrospective comparative study. The Journal of Bone and Joint Surgery. 2012;**94**(11):971-978. DOI: 10.2106/JBJS.K.00815

[34] Thrush C, Porter TJ, Devitt BM. No evidence for the most appropriate postoperative rehabilitation protocol following anterior cruciate ligament reconstruction with concomitant articular cartilage lesions: A systematic review. Knee Surgery, Sports Traumatology, Arthroscopy. 2018;**26**(4):1065-1073. DOI: 10.1007/s00167-018-4882-x

[35] Meshram P. CORR Insights®: What are culturally relevant activities of daily living in the Asian-Indian population? A survey of 402 patients with knee pain. Clinical Orthopaedics and Related Research. 2023. DOI: 10.1097/CORR.0000000000002599

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Over the past few decades, there have been significant advances in our understanding of the pathophysiology of cartilage disorders, for example, the characterization of degraded cartilage using Confocal Raman Microscopy as well as the evolution of cartilage repair technologies. Cartilage disorders are complex and multifactorial, and they can cause significant pain and disability in affected joints/individuals. As such, it is essential for orthopedic surgeons to have a thorough understanding of the latest research and treatment options to provide the best possible care to their patients. This book provides a comprehensive overview of the latest research and treatment options in cartilage disorders. It presents cutting-edge research, technologies, and treatment options for orthopedic surgeons. Organized into two sections, the chapters of this book address such topics as the evaluation and management of patients with cartilage injuries, imaging and biomarker technologies, techniques to repair and regenerate damaged cartilage, and more.

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