UNIVERSIDAD DE CÓRDOBA

Evaluación del tratamiento de lesiones focales de cartílago articular en rodilla empleando una matriz de factores de crecimiento y fragmentos de cartílago autólogos en un modelo experimental ovino

Evaluation of knee focal articular cartilage lesions treatment using an autologous growth factors and particulated cartilage matrix in an experimental model in sheep

Memoria presentada para optar al grado de doctor por la Universidad de Córdoba con mención internacional

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TITULO: *Evaluación del tratamiento de lesiones focales de cartílago articular en rodilla empleando una matriz de factores de crecimiento y fragmentos de cartílago autólogos en un modelo experimental ovino*

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DOCTORANDA/O

LOURDES ALCAIDE RUGGIERO

TÍTULO DE LA TESIS:

Evaluación del tratamiento de lesiones focales de cartílago articular de rodilla empleando una matriz de factores de crecimiento y fragmentos de cartílago autólogos en un modelo experimental ovino

INFORME RAZONADO DE LAS/LOS DIRECTORAS/ES DE LA TESIS
(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma)

La tesis doctoral plantea como objeto de la investigación un tema original y novedoso. Se trata de un estudio de importante relevancia que se encuadra perfectamente dentro de las líneas de trabajo del grupo de investigación que lo desarrolla. Aborda como problema una cuestión importante desde el punto de vista científico y clinico, el tratamiento de lesiones de cartílago articular, vinculado con la realidad práctica susceptible de transformación dada la alta incidencia de esta patología en pacientes humanos y veterinarios, y además estudiando una nueva terapia totalmente autóloga encuadrada dentro de la medicina regenerativa basada en el empleo de factores de crecimiento de origen plasmático y plaquetario para estimular la condrogéneis de reemplazo del tejido cartilaginoso dañado por un nuevo cartílago hialino de reparación equiparable al cartílago sano.

Tras acometer una exhaustiva y actualizada revisión bibliográfica sobre el estado de la cuestión tratada, que enlaza con los conocimientos disponibles hasta el momento, tanto la hipótesis como los objetivos se formulan de manera precisa y clara, cuyo planteamiento esta bien sustentado sobre en un tema de investigacion que esta dentro de los debates disciplinares y cientificos mas actuales. El diseño metodológico del estudio y la ejecución de la investigación son coherentes con la propuesta de método establecida, y es adecuada con los objetivos planteados.

La metodología está bien descrita y con rigor científico correcto. Existe un importante enriquecimiento en la obtención de datos, al tener una muestra amplia y significativa. Tanto la estructuración de la investigación como el método elegido, la especificación de todos los pasos de forma correcta, los métodos y técnicas de análisis, demuestran conocimiento del doctorando sobre cómo investigar.

Los resultados aportados por el desarrollo de la investigación son comprensibles, significativos, novedosos y útiles en la resolución de lesiones focales de cartílago articular. Los resultados de la tesis se presentan de forma adecuada, justificados a partir de los datos obtenidos. El doctorando ha realizado un ingente trabajo en el análisis de los datos generados en la investigación. Se presentan resultados altamente novedosos, como corresponde al objeto de la investigación, siendo muy significativos para el progreso del conocimiento. Estos resultados son discutidos de forma clara, concisa y razonada, contando con la bibliografía más relevante y reciente, lo que le permite aportar trascendentes datos inéditos.

Como director, considero que esta tesis hace una aportación significativa al conocimiento a una tema tan relevante por su alta incidencia epidemiológica como es el tratamiento de las lesiones focales que afectan al cartilago hialino articular, aportando interesantes resultados de regeneración de tejido cartilaginoso como alternativa terapéutica encuadrada dentro de la medicina regenerativa, resultados que son transferibles a la práctica clínica veterinaria y humana.

Significativa también es la difusión de los resultados realizada hasta ahora, con presentaciones de 8 comunicaciones en congresos y 4 publicaciones en revistas de reconocido prestigio que avalan la calidad del trabajo.

A continuación se describen las comunicaciones a congresos generadas por este trabajo:

1. Comunicación oral en el XI Congreso de la Fundación García Cugat en Investigación Biomédica. “Nuevo tratamiento para lesiones de cartílago articular basado en el empleo de una matriz de factores de

crecimiento y cartílago autólogos en ovejas " 13 octubre 2020, Fundación García Cugat.

2. Póster en el IX Congreso Científico de Investigadores en Formación. "Nuevo tratamiento de cartílago articular basado en el empleo de una matriz de factores de crecimiento y cartílago autólogos en ovejas: Resultados preliminares" 2021, Universidad de Córdoba.
3. Póster en el III Congreso de Veterinaria y Ciencia y Tecnología de los Alimentos. "Nueva terapia para lesiones condrales, basada en una matriz de factores de crecimiento y cartílago autólogos. Modelo experimental ovino" 19-21 abril 2021, Universidad de Córdoba.
4. Comunicación oral en el XII Congreso de la Fundación García Cugat en Investigación Biomédica. "Resultados inmunohistoquímicos del cartílago regenerado con CN-Biomatrix. Modelo ovino" Octubre 2021, Fundación García Cugat.
5. Comunicación oral en el 33º Congreso Nacional Sociedad Española de Fertilidad. 8º Congreso. "Determinación de metales no esenciales en semen. Relación con factores de ambientales y potencial en el espermiograma" 6 mayo 2022, Sociedad Española de Fertilidad.
6. Comunicación oral en el X Congreso Científico de Investigadores en Formación. "CN-Biomatrix como tratamiento de lesiones condrales basado en una matriz autóloga de factores de crecimiento y cartílago hialino en modelo ovino. Análisis macroscópico e histopatológico" 3-6 mayo 2023, Universidad de Córdoba.
7. Póster en el XXVII Congreso internacional Sociedad Española de Cirugía Veterinaria. "Reparación de defectos condrales de rodilla en ovejas tras el tratamiento con cartílago autólogo particulado y plasma rico en plaquetas" 17-19 febrero 2023. SECIVE, Alicante.
8. Poster en el 17th World Congress of the International Cartilage Regeneration and Joint Preservation Society. Immunohistochemical analysis of chondral defects repair after autologous particulated cartilage and platelet-rich plasma treatment, 9 -12 septiembre de 2023, Sitges.

A continuación se describen las publicaciones generadas por este trabajo:

1. Main and minor types of collagens in the articular cartilage: The role of collagens in repair tissue evaluation in chondral defects. Este estudio ha sido publicado en la revista International Journal of Molecular Sciences (doi: 10.3390/ijms222413329).
2. Proteoglycans in articular cartilage: Role in chondral injury and repair. Este estudio ha sido publicado en la revista International Journal of Molecular Sciences (doi: 10.3390/ijms241310824).
3. Particulate cartilage and platelet-rich plasma treatment for knee chondral defects in sheep. Este estudio ha sido publicado en la revista Knee surgery, Sports Traumatology, Arthroscopy (doi: 10.1007/s00167-022-07295-7).
4. Immunohistochemical analysis of knee chondral defects repair after autologous particulated cartilage and platelet-rich plasma treatment in sheep. Este estudio ha sido publicado en la revista International Journal of Molecular Sciences (doi: 10.3390/ijms242015157).

Con todo ello se llega a un conjunto de válidas conclusiones que, en gran medida, aportan nuevos e interesantes datos. Además, se reconoce que la tesis no es el punto final de la investigación, pues se abren preguntas, perspectivas, que indican avances en el conocimiento.

Considero que la tesis reúne condiciones adecuadas y suficientes para que el doctorando opte a la aprobación del trabajo de tesis doctoral para su lectura y defensa.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, a 5 de diciembre de 2023

Las/los directoras/es

DOMINGUEZ PEREZ JUAN MANUEL - 31678425L

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Fdo.: Juan Manuel Domíngues Pérez

A mis padres, por su amor y constante motivación, por creer en mí en cada paso del camino.

A mi Yeya, porque a pesar de las sombras has iluminado mi vida.

Este trabajo fue financiado por la Fundación García Cugat para la investigación biomédica.
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GLOSARIO DE TÉRMINOS – GLOSSARY OF TERMS

ACI	Autologous chondrocyte implantation Implante autólogo de condrocitos
ADTT	Autologous-dual-tissue transplantation Trasplante autólogo de doble tejido
AMIC	Autologous-matrix-induced chondrogenesis Condrogénesis inducida por matriz autóloga
CSPG	Chondroitin sulfate proteoglycan Proteoglicano de condroitín sulfato
DMMB	1,9-dimethylmethylene blue 1.9-dimetileno azul
DSPG	Dermatan sulfate proteoglycan Proteoglicano de dermatán sulfato
ECM	Extracellular matrix Matríz extracelular
ELISA	Enzyme-linked immunosorbent assay Ensayo por inmunoadsorción ligado a enzimas
FACITs	Fibril-associated collagens with interrupted tripled hélices Colágenos asociados a fibrillas con hélices triplicadas interrumpidas
FCD	Full-thickness chondral defect Defecto condral de espesor completo
GAG	Glycosaminoglycan Glicosaminoglicano
GPI	Glycosyl-phosphatidyl-inositol Glicosil-fosfatidil-inositol
HA	Hyaluronic acid Ácido hialurónico
HC	Healthy cartilage Cartílago sano
Hep	Heparan Heparán
HSPG	Heparan sulfate proteoglycan Proteoglicano de heparán sulfato
ICRS	International Cartilage Regeneration and Joint Preservation Society Sociedad Internacional de Regeneración del Cartílago y Conservación de las Articulaciones

IHQ	Immunohistochemical staining Tinción Inmunohistoquímica
KSPG	Keratan sulfate proteoglycan Proteoglicano de keratan sulfato
LhCG	Living hyaline cartilaginous graft Injerto cartilaginoso hialino vivo
MACI	Matrix-induced autologous chondrocytes implantation Implantación de condrocitos autólogos inducidos por matriz
MACITs	Membrane-associated collagens with interrupted tripled hélices Colágenos asociados a la membrana con helices triplicadas interrumpidas
MCI	Minced cartilage implantation Implantación de cartílago troceado
MFx	Microfracture Microfractura
MMP13	Matrix metalloproteinase 13 Metaloproteinasa de la matriz 13
n/r	Not reported No comunicado
OA	Osteoarthritis Osteoartritis
OP1-SCS	Osteogenic protein 1 – salmon derived collagen sponge disc Proteína osteogénica 1 – disco de esponja de colágenos derivado de salmón
PACI	Particulated autograft cartilage implantation Implantación de cartílago fragmentado de autoinjerto
PACI+PRP	Autologous matrix composed of healthy hyaline cartilage chips included and mixed in a PRP clot and intraarticular infiltration of PRP Matriz autóloga compuesta por fragmentos de cartílago hialino sano incluidos y mezclados en un coágulo de PRP, y una infiltración intraarticular de PRP
PBS	Phosphate-buffered saline Solución salina tamponada con fosfato
PCD	Partial-thickness chondral defect Defecto condral de espesor parcial
PCM	Pericellular matrix Matriz pericellular
PCR	Polymerase chain reaction Reacción en cadena de la polimerasa

PG	Proteoglycan Proteoglicano
PJAC	Particulated juvenile articular allograft Aloinjerto articular juvenil fragmentado
PRP	Platelet-rich plasma Plasma rico en plaquetas
REAC	Radioelectric asymmetric conveyor Transportador asimétrico radioeléctrico
RER	Rough endoplasmic reticulum Retículo endoplásmico rugoso
RC	Repair cartilage Cartílago reparado
RLS	Ringer's lactate solution Solución de Ringer lactato
RT	Room temperatura Temperatura ambiente
SLRP	Small leucine-rich proteoglycan Pequeño proteoglicano rico en leucina
SMD	Spondylometaphyseal displasia Displasia espondilometafisaria

ÍNDICE

I. RESUMEN – SUMMARY

El cartílago hialino es fundamental para la movilidad, gracias a su baja fricción y alta resistencia a las fuerzas de compresión. Su deterioro limita la calidad de vida, ya que va a provocar una serie de limitaciones que afectan de forma significativa a la movilidad. El cartílago hialino maduro presenta una capacidad de regeneración sumamente restringida, debido a la ausencia de vasos sanguíneos, vasos linfáticos y nervios y a la limitada capacidad de replicación de los condrocitos. Las estrategias terapéuticas actuales para tratar lesiones condrales se han centrado en tratamientos no quirúrgicos y técnicas para estimular la reparación. Sin embargo, estas aproximaciones se han enfrentado a una serie de limitaciones como la formación de fibrocartílago, largos períodos de recuperación, integración insatisfactoria, necesidad de procedimientos en dos etapas, incapacidad para abordar defectos extensos, costes elevados y resultados impredecibles.

El plasma rico en plaquetas (PRP) se usa cada vez más en medicina musculoesquelética debido a su potencial terapéutico basado en la elevada concentración de factores de crecimiento y citoquinas. Estos componentes estimulan la regeneración y reparación en tejidos con limitada capacidad de cicatrización, como es el caso del cartílago articular. El PRP, frecuentemente, se combina con otras técnicas para abordar la reparación de las lesiones condrales. Un enfoque alternativo, normalmente combinado con PRP, implica la implantación de fragmentos de cartílago autólogos (PACI). Estos tratamientos han mostrado resultados alentadores debido a la migración de los condrocitos hacia el biomaterial y la subsiguiente deposición de matriz extracelular (ECM) por parte de estas células.

Dada la prevalencia y las graves consecuencias discapacitantes de los problemas condrales, y teniendo en cuenta la falta de estrategias terapéuticas que posibiliten la regeneración completa del cartílago hialino, surge una necesidad urgente de encontrar tratamientos eficaces. En este contexto, la presente tesis doctoral lleva a cabo un análisis a nivel macroscópico, histológico e inmunohistoquímico de una terapia totalmente autóloga que consiste en la creación de un andamiaje tridimensional compuesto por cartílago hialino sano fraccionado integrado en la estructura de un coágulo de PRP más una infiltración intraarticular de PRP (PACI+PRP).

Los dos primeros capítulos de esta tesis doctoral consistieron en dos revisiones bibliográficas que tienen como objetivo realizar una descripción detallada de las moléculas más relevantes presentes en el cartílago articular, centrándose especialmente en los diferentes tipos de colágenos y los proteoglicanos. Estos capítulos también profundizan en la compresión del papel funcional que desempeñan estas moléculas, tanto en el contexto de un cartílago articular sano como en el proceso de reparación condral, con el fin de establecer sistemas de evaluación más precisos

destinados a la determinación de la calidad del tejido condral. En este sentido, los diversos tipos de colágenos y proteoglicanos juegan un papel fundamental como biomarcadores de interés que posibilitan llevar a cabo una evaluación histológica precisa, tanto del avance como de las características estructurales del cartílago hialino regenerado en el proceso de reparación posterior a una lesión condral.

El objetivo del tercer capítulo consistió en evaluar las propiedades regenerativas condrogénicas a nivel macroscópico e histopatológico tras la aplicación del tratamiento PACI+PRP en defectos condrales, utilizando un modelo experimental en ovejas con el fin de valorar la madurez y morfología de los condrocitos, así como la calidad del tejido reparado.

El cuarto capítulo de esta tesis doctoral se enfocó en realizar un análisis inmunohistoquímico exhaustivo y detallado de los diferentes tipos de colágenos menores (colágenos tipo III, V, VI y X), mayores (colágenos tipo II, IX y XI) y marcadores de fibrocartílago (colágeno tipo I), así como del agrecano, con el objetivo de evaluar la calidad, durabilidad y estructura de la ECM del cartílago reparado mediante la aplicación del tratamiento PACI+PRP.

Para llevar a cabo el tercer y cuarto capítulo, se realizó el mismo diseño experimental y procedimiento quirúrgico. En este sentido, se utilizaron ovejas merinas esqueléticamente maduras, en las que se llevó a cabo una miniartrotomía de 3 a 4 cm en ambas rodillas para crear quirúrgicamente un defecto de espesor completo de 8 mm de diámetro en el área de carga del cóndilo femoral medial. Posteriormente, todas las ovejas recibieron el tratamiento PACI+PRP en las rodillas derechas. Para el desarrollo de esta terapia, la muestra de cartílago obtenido tras la creación del defecto en cada una de las ovejas, se cortó en pequeños fragmentos de 1 a 2 mm^3 y se mezcló con PRP activado, obteniendo un coágulo que sirvió como soporte para los fragmentos de cartílago. A continuación, este coágulo con los fragmentos de cartílago se colocó para rellenar el defecto previamente creado. La incisión se cerró, y se administró PRP activado intrarticularmente. Se siguió el mismo procedimiento quirúrgico para generar los defectos condrales en las rodillas izquierdas de las ovejas, dividiéndose posteriormente de manera aleatoria en dos grupos. La mitad de las rodillas izquierdas fueron sometidas a una inyección intraarticular de solución de Ringer Lactato (RLS) como control negativo, mientras que la otra mitad recibió un tratamiento con ácido hialurónico (HA) como control positivo. Todas las ovejas se dividieron aleatoriamente en dos grupos de estudio: el grupo RLS/PACI+PRP(RLS), que incluía ovejas tratadas con PACI+PRP en las rodillas derechas y RLS en las rodillas izquierdas, y el grupo HA/PACI+PRP(HA) que incluía ovejas tratadas con PACI+PRP en la rodillas derechas y HA en las rodillas izquierdas. A los 9 y 18 meses, se sacrificó de manera aleatoria la mitad de las ovejas en cada grupo para llevar

a cabo las evaluaciones correspondientes. Para evaluar el efecto del tratamiento con PACI+PRP en el capítulo 3, se realizó una evaluación macroscópica utilizando tres sistemas de puntuación diferentes, junto con un análisis histopatológico mediante un sistema de valoración modificado basado en diferentes sistemas de puntuación. En el capítulo 4, se realizó un análisis inmunohistoquímico para evaluar los tipos de colágeno (I, II, III, V, VI, IX, X, XI) y el agrecano, utilizando un sistema de puntuación semicuantitativo basado en diferentes sistemas de puntuación. El análisis histopatológico e inmunohistoquímico, se realizó comparando el tejido reparado con PACI+PRP, RLS o HA, con el cartílago sano adyacente al defecto.

En el tercer capítulo, se observó que los grupos tratados con PACI+PRP mostraron diferencias estadísticamente significativas en cuanto al porcentaje de reparación del defecto y la morfología celular en el tejido cartilaginoso regenerado, comparando los resultados a los 9 y 18 meses de periodo de recuperación. De esta manera, el tejido cartilaginoso regenerado mediante este tratamiento presentó un aspecto macroscópico, una estructura histológica y una apariencia de los condrocitos equiparable a la del cartílago sano adyacente después de 9 y 18 meses de proceso regenerativo.

En el cuarto capítulo, se identificaron diferencias significativas en los colágenos asociados con la madurez de la matriz extracelular (colágenos tipo II y V), la degradación (colágeno tipo IX), la estructura y la mecánica (colágeno tipo VI) y la hipertrofia (colágeno tipo X) entre el cartílago sano y el cartílago reparado con RLS o HA. La utilización de esta terapia, PACI+PRP, para la reparación de lesiones condrales favoreció el avance del proceso de regeneración desde el punto de vista de la evaluación inmunohistoquímica, hacia la formación de cartílago hialino maduro, en relación con los colágenos de tipo II y V. Esta mejora se reflejó en las características estructurales y mecánicas, vinculadas al colágeno de tipo VI, generando un tejido cartilaginoso más consistente, menos propenso a la degradación, la cual está vinculada al colágeno de tipo IX. Además, se observó la ausencia de formaciones hipertróficas incluso después de un periodo de recuperación de 18 meses, relacionadas con el colágeno de tipo X.

Este enfoque integral permitió evaluar de manera exhaustiva las propiedades regenerativas asociadas al tratamiento PACI+PRP, demostrando mejoras en las características estructurales y la calidad del tejido hialino reparado. En este contexto, el tratamiento con PACI+PRP se presenta como una terapia completamente autóloga, de aplicación sencilla y bajo coste, que puede llevarse a cabo en un solo paso quirúrgico. Por lo tanto, se posiciona como una opción terapéutica preferente para la reparación de lesiones condrales en la rodilla, tanto en el ámbito clínico veterinario como en el humano.

I. RESUMEN – SUMMARY

Hyaline articular cartilage is a highly specialized connective tissue, whose main function is to provide a smooth and lubricated surface for the joint and to facilitate the transmission of loads with a low coefficient of friction. The integrity of this tissue is crucial for quality of life; however, injury to this tissue would impose significant limitations on mobility. Mature hyaline cartilage lacks blood vessels, lymphatic vessels, and nerves. Moreover, the chondrocytes present a reduced metabolism and limited replication capacity.

Nowadays, therapeutic approaches for chondral lesions have focused on non-surgical treatments and techniques to stimulate repair. Nevertheless, these strategies have faced several limitations, including the formation of fibrocartilage, long recovery periods, suboptimal integration, the necessity for two-stage procedures, incapacity to address extensive defects, elevated costs, and unpredictable outcomes.

The use of platelet-rich plasma (PRP) has been on the rise in the field of musculoskeletal medicine due to its therapeutic potential derived from the elevated concentration of growth factors and cytokines. These elements have the capacity to stimulate regeneration and repair in tissues with limited healing capabilities, such as articular cartilage. The PRP is often combined with other techniques to address the repair of chondral lesions. An additional approach, frequently combined with PRP, involves the implantation of autologous cartilage fragments (PACI). These therapeutic treatments have shown promising results due to the migration of chondrocytes into a biomaterial and subsequent deposition of extracellular matrix (ECM) by these cells.

Taking into consideration the prevalence and debilitating impacts of chondral issues in the musculoskeletal system, together with the scarceness of therapeutic approaches facilitating the full regeneration of normal hyaline cartilage, there is an urgent need to find effective treatments. In this context, the current PhD thesis performs a macroscopic, histological, and immunohistochemical analysis of a completely autologous therapy. This treatment entails the development of a three-dimensional scaffold comprising healthy fragmented hyaline cartilage integrated into a PRP clot, together with an intra-articular PRP infiltration (PACI+PRP).

The first two chapters of this PhD thesis comprised of two literature reviews providing a detailed description of the most relevant molecules present in articular cartilage, with a particular focus on different types of collagens and proteoglycans. These chapters also delve into understanding the functional roles played by these molecules, both in the context of healthy articular cartilage and the chondral repair process, aiming to establish more precise evaluation

systems for determining the quality of chondral tissue. In this context, various types of collagens and proteoglycans play a crucial role as potential biomarkers, facilitating a meticulous histological evaluation of the progression and structural characteristics of regenerated hyaline cartilage during the post-chondral injury repair process.

The objective of the third chapter was to assess the chondrogenic regenerative properties, maturity, and morphology of chondrocytes, along with the quality of the repaired tissue at macroscopic and histopathological levels. This evaluation was conducted following the performance the PACI+PRP treatment in full-thickness chondral defects using an ovine animal model.

The fourth chapter focused on conducting a comprehensive and detailed immunohistochemical analysis of various types of minor collagens (collagens type III, V, VI y X) and major collagens (collagens type II, IX y XI), as well as aggrecan. The goal was to assess the quality, durability, and structure of the extracellular matrix of repaired cartilage through the application of the PACI+PRP treatment.

In order to carry out the third and fourth chapters, we applied identical experimental designs and surgical procedures. Skeletally mature Merino sheep were employed, undergoing a 3 to 4 cm mini-arthrotomy on both knees, followed by a surgical procedure to create an 8 mm diameter full-thickness defect in the load-bearing area of the medial femoral condyle. Afterward, all sheep underwent the PACI+PRP treatment in their right knees. To develop this therapy, cartilage samples obtained after creating the defect in each sheep were cut into small fragments of 1 to 2 mm3 and mixed with activated PRP, forming a clot that served as a support for the cartilage fragments. This clot, along with the cartilage fragments, was then placed to fill the previously created defect. The incision was closed, and activated PRP was administered intra-articularly. The same surgical procedure was employed to induce chondral defects in the left knees of the sheep, subsequently randomly dividing them into two groups. Half of the left knees underwent an intra-articular injection of Lactated Ringer's solution (LRS) as a negative control, while the other half received hyaluronic acid (HA) treatment as a positive control. The sheep were randomly divided into two study groups: the RLS/PACI+PRP(RLS) group, which included sheep treated with PACI+PRP in the right knees and RLS in the left knees, and the HA/PACI+PRP(HA) group, which included sheep treated with PACI+PRP in the right knees and HA in the left knees. At 9 and 18 months, half of the sheep in each group were randomly euthanized to perform the different evaluations. To evaluate the effect of PACI+PRP treatment in Chapter 3, a macroscopic assessment was conducted using three different scoring systems,

along with a histopathological analysis utilizing a modified scoring system. In Chapter 4, an immunohistochemical analysis was performed to assess several collagen types (I, II, III, V, VI, IX, X, XI) and aggrecan, using a semiquantitative scoring system. The histopathological and immunohistochemical analysis compared the repaired tissue with PACI+PRP, RLS, or HA with the healthy cartilage adjacent to the defect.

In the third chapter, the groups treated with PACI+PRP exhibited statistically significant differences in the percentage of defect repair and cellular morphology within the regenerated cartilaginous tissue when comparing the results at 9 and 18 months into the recovery period. Consequently, the cartilaginous tissue regenerated with this treatment displayed a macroscopic appearance, histological structure, and chondrocyte morphology comparable to that of the healthy adjacent cartilage after 9 and 18 months of the regenerative process.

In the fourth chapter, significant differences were observed in collagens associated with the maturity of the extracellular matrix (specifically, collagens type II and V), degradation (collagen type IX), structure and mechanics (collagen type VI), and hypertrophy (collagen type X) between healthy cartilage and cartilage repaired with RLS or HA. The application of PACI+PRP therapy for chondral lesion repair facilitated the progression of the regeneration process, particularly evident in the development of mature hyaline cartilage, as indicated by collagens type II and V. This improvement extended to the structural and mechanical characteristics associated with collagen type VI, resulting in a more robust and consistent cartilaginous tissue, less susceptible to degradation linked to collagen type IX. Moreover, even after an 18-month recovery period, no hypertrophic formations were observed, indicating the resilience of the repaired tissue in relation to collagen type X.

This in-depth approach performed a comprehensive evaluation of the regenerative properties associated to the PACI+PRP treatment, demonstrating enhancements in structural characteristics and the quality of the repaired hyaline tissue. In this context, PACI + PRP is suggested as a completely autologous, easily applicable, and cost-effective treatment that can be performed in a single surgical step. Therefore, it emerges as the primary therapeutic choice for repairing chondral lesions in the knee, suitable for both veterinary and human clinical procedures.

II. INTRODUCCIÓN

Las articulaciones constituyen unidades funcionales complejas y esenciales en la cadena anatómico funcional que permite el movimiento (1). Estas estructuras incluyen la membrana sinovial, el cartílago articular de tipo hialino y el hueso subcondral. El cartílago hialino desempeña un papel esencial como un tejido de interfase altamente especializado que proporciona una superficie lisa y lubricada facilitando la trasmisión de cargas. Esto se debe a su bajo coeficiente de fricción, lo que le confiere una elevada resistencia a las fuerzas de compresión (2). De su integridad depende, en gran medida, la calidad de vida de las personas, ya que su deterioro o lesión conlleva una grave limitación de la movilidad (3). El cartílago hialino adulto es un tejido carente de vasos sanguíneos, vasos linfáticos y nervios. Su único elemento celular es el condrocito, el cual presenta un bajo metabolismo y una capacidad limitada de replicación conllevando a una capacidad de regeneración del cartílago sumamente restringida (4–6).

Hasta el momento, las estrategias terapéuticas que se han llevado a cabo para tratar las lesiones condrales se han centrado principalmente en tratamientos no quirúrgicos destinados a controlar los síntomas, así como en técnicas diseñadas para estimular el proceso de reparación intrínseca que conducen a la formación de fibrocartílago, o incluso en la opción de recurrir al reemplazo protésico (7–9). Actualmente, se están explorando enfoques novedosos centrados en la regeneración del cartílago (9). Sin embargo, es importante destacar que estas técnicas presentan algunas limitaciones como la formación de fibrocartílago, un prolongado tiempo de recuperación, una integración insuficiente con el cartílago sano, la necesidad de procedimientos en dos etapas, la incapacidad para restaurar defectos grandes, tratamientos costosos o resultados impredecibles, particularmente en atletas de alto rendimiento (10,11).

El plasma rico en plaquetas (PRP) se ha convertido en una opción terapéutica que se está utilizando cada vez con mayor frecuencia en el campo de la medicina musculoesquelética. Su potencial terapéutico se basa en el aporte de factores de crecimiento y citoquinas de origen plaquetario y plasmático en cantidades superiores a las fisiológicas. Estos elementos son esenciales para estimular la regeneración y promover la reparación en tejidos con un limitado potencial de cicatrización (12,13). Además, el PRP se suele utilizar en combinación con otras técnicas para el tratamiento de lesiones condrales (13–15). Otro posible tratamiento, que con frecuencia se combina con el PRP, es la implantación de fragmentos de cartílago autólogos (PACI, del inglés *Particulated Autograft Cartilage Implantation*). Los resultados prometedores de este

tratamiento se deben a la migración de condrocitos hacia un biomaterial y la posterior deposición de matriz extracelular (ECM) por parte de estas células (16).

Debido a que los problemas condrales son muy comunes en el aparato locomotor y representan una de las principales causas de discapacidad, y considerando la dificultad para encontrar una estrategia terapéutica que permita la regeneración completa del cartílago hialino normal, existe una necesidad apremiante de hallar un tratamiento eficaz para la regeneración del cartílago articular hialino. Por esta razón, nuestro grupo de investigación en colaboración con la Fundación García Cugat para la Investigación Biomédica, han implementado y estudiado una nueva estrategia para abordar las lesiones en el cartílago (8,15,17). Este tratamiento consiste en el uso de una matriz autóloga compuesta por fragmentos de cartílago hialino sano en un coágulo de PRP, junto con una infiltración intraarticular de PRP.

1. Cartílago articular

Las superficies articulares están revestidas por un tipo especializado de cartílago hialino conocido como cartílago articular. El cartílago articular es un tejido conectivo cuya función principal es proporcionar una superficie lisa y lubricada para las articulaciones, lo que facilita la transmisión de cargas con un bajo coeficiente de fricción (2,18,19). Este tipo de cartílago se compone de tejido hialino con un grosor que oscila entre 2 y 4 mm (20). Un aspecto que lo diferencia del resto de tejidos es que carece de vasos sanguíneos, vasos linfáticos y nervios. Además, sus células tienen un metabolismo y una capacidad limitada de replicación restringiendo en gran medida su capacidad de reparación intrínseca (4–6,21). Es importante mencionar que las lesiones locales en el cartílago son generalmente irreversibles y pueden progresar gradualmente hacia lesiones más extensas con el paso del tiempo, lo que puede dar lugar a la osteoartritis (OA). Tanto la lesiones locales en el cartílago como la OA pueden provocar rigidez y discapacidad en las articulaciones, lo que perjudica gravemente la calidad de vida del paciente (22).

1.1. Composición del cartílago articular

El cartílago articular cuenta con una ECM que está compuesta principalmente por agua, colágeno y proteoglicanos (PGs), así como por pequeñas cantidades de proteínas y glicoproteínas no colágenas. La combinación de todos estos componentes dan como resultado un material viscoso optimizado para resistir cargas (2,23). En el cartílago, los condrocitos son las únicas células altamente especializadas presentes y constituyen aproximadamente entre el 1% y el 5% del volumen del cartílago (Figura 1). Estos condrocitos, junto con la ultraestructura de

las fibras de colágeno y el resto de los componentes de la ECM, juegan un papel esencial en la secreción, organización y mantenimiento de la ECM. Además, estas células desempeñan una función fundamental proporcionando soporte mecánico (24,25).

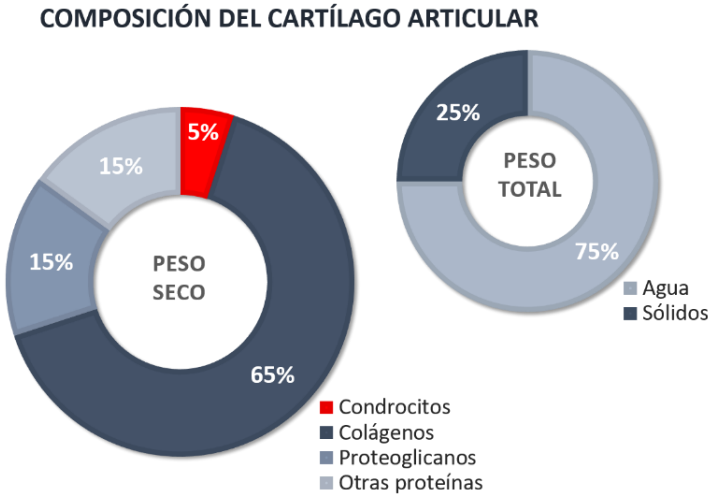

Figura 1. Composición del cartílago articular. Porcentaje de peso seco y peso total *(Fuente propia).*

1.1.1. Condrocitos

Los condrocitos son las células específicas del cartílago articular, y tienen un papel único en el desarrollo, mantenimiento y reparación de la ECM. Éstas células son metabólicamente activas y altamente especializadas, derivando de células madre mesenquimales y constituyendo alrededor del 2% del volumen total del cartílago articular (26). Los condrocitos suelen tener una forma predominantemente redondeada y están organizados en lagunas con una densidad celular relativamente baja, aunque su forma, número y tamaño pueden variar según la región anatómica en la que se encuentre el cartílago articular.

En el ambiente hipóxico del cartílago articular, los condrocitos son principalmente anaeróbicos. Estas células mantienen un equilibrio entre síntesis y descomposición de la matriz, lo que es fundamental para facilitar el metabolismo tisular normal (27). Cada condrocito crea un microambiente especializado y es responsable del recambio de la ECM de su zona inmediata. Esto significa que los condrocitos quedan atrapados dentro de su propia matriz y así evitan cualquier migración a áreas adyacentes del cartílago. Esta baja movilidad y limitada distribución de los condrocitos hace que las interacciones de célula a célula sean poco comunes para la transmisión directa de señales y la comunicación entre células (28). Sin embargo, los condrocitos responden a una gran variedad de estímulos, incluyendo los factores de crecimiento, las cargas mecánicas, las fuerzas piezoeléctricas y las presiones hidrostáticas (2). Además, éstos, poseen receptores de superficie celular para factores de crecimiento y citoquinas, y regulan sus

actividades anabólicas y catabólicas en respuesta a los estímulos mencionados anteriormente (27). Desafortunadamente, los condrocitos tienen un potencial limitado de replicación, un factor que contribuye a la limitada capacidad de reparación del cartílago en respuesta a una lesión. La supervivencia de los condrocitos depende de un entorno químico y mecánico óptimo.

1.1.2. Colágenos

El colágeno constituye aproximadamente el 60-65 % del peso seco del cartílago hialino, siendo la molécula principal en la composición de la ECM del cartílago (29,30). Estas moléculas se ensamblan por sí mismas en forma de fibrillas, brindando una estructura de soporte para el crecimiento celular y siendo las responsables principales de la resistencia mecánica del cartílago articular (31). En el cartílago articular se han identificado numerosos subtipos de colágenos. Los principales y más abundantes son los colágenos tipo II, IX y XI (24,32). Otros tipos de colágeno, como los tipos III, IV, V, VI y X son menos abundantes en el cartílago, pero su presencia es fiable y tienen funciones imprescindibles (24,32,33). Algunos investigadores han descrito que los colágenos tipo XII, XIV, XVI, XXII y XXVII también pueden formar parte del cartílago articular (33), aunque la información que se da sobre ellos es limitada. Además de todos los colágenos mencionados anteriormente, el colágeno tipo I a veces se encuentra en el cartílago articular, lo cual no debería ocurrir en el cartílago articular sano, ya que indicaría la presencia de tejido conectivo fibrótico (34).

El Capítulo 1, sección V, de esta tesis doctoral, titulado "*Main and minor types of collagens in the articular cartilage; the role of collagens in repair tissue evaluation in condral defects*", se centra en una revisión bibliográfica exhaustiva sobre los diversos tipos de colágeno presentes en el cartílago articular y explora cómo esta información puede utilizarse para evaluar la calidad de la reparación en lesiones focales de cartílago articular.

1.1.3. Proteoglicanos

El cartílago hialino se caracteriza, entre otros aspectos, por su alto contenido de PGs, constituyendo el segundo grupo más grande de macromoléculas en la ECM, y representa entre el 10% y el 15% de su peso. Los PGs son monómeros proteicos altamente glicosilados, compuestos por un núcleo proteico que lleva una o más cadenas lineales de glicosaminoglicanos (GAG) unidas de manera covalente. Estas cadenas pueden estar compuestas por más de 100 monosacáridos y se extienden desde el núcleo de la proteína, manteniéndose separadas unas de otras debido a la repulsión de carga (2,18).

El cartílago articular contiene una variedad de PGs esenciales para su funcionamiento normal, entre los que se incluyen el agrecano, decorina, biglicano, versicano y perlecano. De estos PGs, el agrecano es el de mayor tamaño y el más abundante en términos de peso. El agrecano contiene numerosas cadenas de sulfato de condroitina y sulfato de queratina que interactúan con el hialuronano para formar grandes agregados de PGs mediante proteínas enlace (2,35). El agrecano ocupa el espacio interfibrilar de la ECM del cartílago y es responsable de proporcionar al cartílago sus propiedades osmóticas, que son fundamentales para su capacidad de resistir cargas de compresión (2).

El Capítulo 2, sección VI, de esta tesis doctoral, titulado "*Proteoglycans in articular cartilage: role in condral injury and repair*", se centra en una revisión general de los PGs en el cartílago articular y el papel que desempeñan tanto en el cartílago sano como en el dañado.

1.1.4. Agua

El agua constituye el componente más abundante del cartílago articular, llegando a representar hasta el 80% de su peso húmedo. Aproximadamente el 30% de esta agua se encuentra asociada al espacio interfibrilar dentro del colágeno, mientras que un pequeño porcentaje está contenido en el espacio intracelular. El resto está contenida en el espacio poroso de la matriz (36,37). La concentración relativa de agua disminuye desde aproximadamente un 80% en la zona superficial hasta un 65% en la zona profunda (2).

El flujo de agua a través del cartílago y la superficie articular, cumple un papel fundamental en el transporte y distribución de los nutrientes hacia los condrocitos, además de proporcionar lubricación. Gran parte del agua interfibrilar parece existir en forma de gel, y la mayoría puede desplazarse a través de la ECM mediante un gradiente de presión a través del tejido. La resistencia a la fricción contra este flujo a través de la matriz es muy alta, lo que se traduce en una permeabilidad muy baja del tejido (38). En este sentido, la combinación de esta resistencia debida a la fricción en el flujo y la presurización del agua dentro de la matriz constituye los dos mecanismos fundamentales mediante los cuales el cartílago articular adquiere su capacidad para resistir grandes cargas (2).

2. Características mecánicas del cartílago articular

El comportamiento biomecánico del cartílago articular se comprende mejor cuando se considera el tejido como un medio bifásico, compuesto por dos fases: una fase fluida y una fase sólida. La fase fluida está compuesta principalmente por agua, aunque también contiene iones inorgánicos como el sodio, calcio, cloruro y potasio. Por otro lado, la fase sólida se caracteriza por la porosidad y la permeabilidad de la ECM (2).

La mecánica del cartílago articular exhibe diversas características y comportamientos complejos (39). Presenta viscoelasticidad dependiente del flujo, que resulta de las interacciones de fricción entre el líquido intersticial y la matriz sólida y representa un mecanismo de disipación de energía. Además, presenta viscoelasticidad independiente del flujo, que se relaciona con los mecanismos de disipación de energía intrínsecos a los diversos constituyentes de la matriz sólida. La matriz sólida se forma a través de la creación y rotura de enlaces temporales entre moléculas (40).

Debido a la naturaleza fibrilar de la matriz de colágeno y a la capacidad de las fibrillas para resistir mejor las cargas de tracción que las de compresión, el cartílago exhibe una mayor rigidez en tensión que en compresión, un fenómeno conocido como no linealidad de tensión-compresión (41). Además, es un tejido anisotrópico, lo que significa que su rigidez a la tracción varia con la dirección de las cargas (42). El cartílago no es homogéneo y realiza funciones mecánicas divergentes en tensión y compresión, a través de su espesor (43). Por lo tanto, debido a que el cartílago articular es un biomaterial heterogéneo, anisotrópico y multifásico, sus propiedades mecánicas dependen de sus diferentes regiones (44).

Los PGs contienen grandes cantidades de GAG con cargas eléctricas negativas en solución. Debido a la presencia de estas cargas, se producen varios fenómenos fisicoquímicos adicionales que caracterizan el comportamiento del cartílago articular. Con el objetivo de mantener la electroneutralidad, los cationes superan a los aniones en el tejido creando un desequilibrio neto en la concentración de electrolitos entre el líquido intersticial del cartílago y el tejido circundante. Este desequilibrio genera una diferencia de presión osmótica, haciendo que el líquido intersticial exhiba una presión más alta que la solución externa, lo que resulta en la hinchazón de la matriz sólida del tejido (40,45).

3. Lesiones del cartílago articular

El cartílago articular sufre lesiones con frecuencia, ya sea debido a traumas, al proceso de envejecimiento o consecuencia de diversas enfermedades (23). Las lesiones condrales suelen manifestarse con síntomas característicos que incluyen inflamación, dolor local, bloqueo y/o atrapamiento articular, pérdidas de fuerza o debilidad, chasquidos o crujidos al mover la articulación, así como dificultad para soportar el peso. La figura 2 resumen las causas y síntomas más frecuentes de las lesiones condrales (24). Una vez que se produce la lesión condral, el cartílago pierde muchas de sus propiedades de soporte de carga, lo que aumenta la vulnerabilidad del cartílago adyacente al desgaste (46). Además, la lesión puede desencadenar una respuesta inflamatoria que afecte a toda la articulación, aumentando los niveles de citoquinas sinoviales que provocan una mayor degradación y daño del tejido. En estos casos, las lesiones a menudo progresan y pueden dar lugar a una OA que afecte a toda la articulación (47).

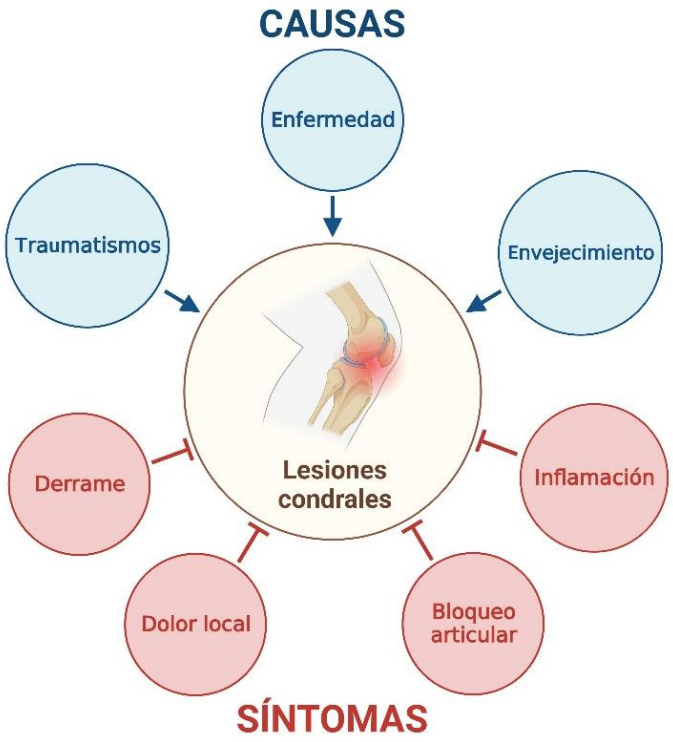

Figura 2. Causas y síntomas asociados a las lesiones condrales *(Fuente propia)*

3.1. Características y clasificación de las lesiones condrales

Las lesiones condrales se pueden clasificar en dos grandes categorías (24):

- Traumatismos mecánicos directos a la matriz sin dañar las células. En este caso, si la pérdida de componentes de la matriz no supera la capacidad de los condrocitos para sintetizar nuevas moléculas, el cartílago puede regenerarse.

- Destrucción mecánica de las células y la matriz por trauma cerrado o penetrante. Esta es la situación más común en la práctica clínica y la reparación es más compleja.

El riesgo de que una lesión condral progrese a artritis degenerativa es multifactorial. Dependerá de las características de la lesión condral y de los factores que la hayan provocado, dando lugar a diferentes respuestas de reparación.

Profundidad del defecto

La profundidad de la lesión es un factor importante en esta clasificación. Dependiendo de la profundidad, se pueden distinguir tres tipos de defectos que afectan al cartílago (48,49):

- Defecto condral de espesor parcial: se limita solo a la zona del cartílago hialino articular sin afectar al cartílago calcificado.
- Defecto condral de espesor completo: abarca el daño en la capa de cartílago calcificado.
- Defecto osteocondral: expone completamente el hueso subcondral y destruye la estructura del tejido osteocondral. Este tipo de defecto cruza la marca de marea, permitiendo el paso de las células madre mesenquimales de la médula ósea migren hacia la lesión (24).

Por lo tanto, la profundidad del defecto es un factor crucial que determinará la estrategia terapéutica en el abordaje de la reparación condral.

Tamaño del defecto

El tamaño del defecto es un factor importante en la respuesta de reparación. Se ha demostrado que los defectos de menor tamaño pueden experimentar una reparación completa, mientras que los defectos más grandes tienden a no repararse por completo (referencia). La respuesta de la reparación del cartílago articular está directamente relacionada con la extensión de la lesión, que se puede medir en función del volumen y el área superficial del defecto. En general, Es menos probable que los defectos con un diámetro inferior a $1cm^2$ afecten significativamente a la distribución de la tensión en el hueso subcondral y, por lo tanto, sería probable que no progresen (24).

Edad

La edad representa un factor de riesgo significativo para el desarrollo de la OA. Así, el proceso de envejecimiento provoca una reducción en la hidratación del carílago, asi como una disminución en la población de condrocitos. Además, se oberva una reducción en la actividad mitótica y sintética de los condrocitos asociada al envejecimiento. Diferentes estudios realizados

en animales han demostrado que la repación de defectos condrales de 2 mm es más efectiva en animales jóvenes en compración con animales mayores (50).

En terminos de lesiones osteocondrales, es importante destacar que los niños y adolescentes suelen desarrollar estas lesiones, mientras que los adultos suelen presentar lesiones condrales puras. Esto podría deberse a que en los adultos, la zona calcificada del cartílago está bien desarrollada y madura (51). En el caso de lesiones osteocondrales en niños con huesos en crecimiento, la repación suele producirse de manera más efectiva y sin complicaciones, sin embargo, en adultos la repación de estas lesiones es poco frecuente (52).

Traumatismos

El impacto repentino sobre la superficie de la articulación o la repetida carga sobre el cartílago articular pueden causar daños en los condrocitos provocando la degeneración celular y muerte celular. Esta situación conlleva a la alteración del colágeno de la ECM, lo que a su vez conduce a un aumento en la hidratación, la formación de fisuras en el cartílago y el engrosamiento del hueso subcondral (53). Asimismo, el traumatismo provoca una disminución de la producción de PGs por parte de los condrocitos. Aunque la superficie externa del cartílago puede parecer intacta, su textura tiende a volverse más suave y fibrilada (24).

Desalineación mecánica de la articulación

La carga anormal en una articulación genera tensiones focales excesivas que, a su vez, resultan en la degeneración temprana del cartílago (46). Esta situación sienta las bases para la realización de una osteotomía correctiva alrededor de la articulación de la rodilla. El compartimiento del cartílago varía en respuesta a la carga. La inmovilización o la reducción de la carga conducen a una disminución en la agregación y síntesis de GAG, lo que puede ser reversibles hasta cierto límite. La inmovilización también conduce a una reducción de las moléculas más pequeñas de PGs y a la ruptura irreversible de las fibras de colágeno (24).

La ubicación del defecto influye en la respuesta de reparación del cartílago. Se ha observado que bajo una compresión axial, las tensiones de contacto y los gradientes de tensión aumentan en las superficies del cartílago adyacentes al borde de un defecto (54), provocando una mayor deformación y tensión en este tejido circundante (55). Las tensiones elevadas pueden dar lugar a la muerte celular y daño en la matriz, lo que resultaría en una degeneración progresiva en los tejidos adyacentes al defecto (56).

4. Técnicas y estrategias terapéuticas para tratar defectos condrales. Descripción y limitaciones

A lo lardo de los últimos siglos, médicos y científicos han explorado diversas estrategias para reparar o regenerar la superficie articular tras sufrir daños traumáticos o degeneración del cartílago. La reparación se enfoca en restaurar una superficie articular dañada mediante la creación de neocartílago, que se asemeja al cartílago nativo, pero no necesariamente replica su estructura, composición y función. Por otro lado, la regeneración implica la formación de tejido que es indistinguible del cartílago articular nativo (57).

Una respuesta tisular típica a la lesión abarca una secuencia de eventos que incluyen la activación de la cascada de necrosis, inflamación, reparación y remodelación de la cicatriz. La fase vascular en esta secuencia es determinante en el proceso de curación. El cartílago hialino, al ser una estructura avascular, carece de la capacidad para desencadenar esta respuesta. Por lo tanto, la capacidad intrínseca del cartílago para repararse tras una lesión es muy limitada (4–6). La curación de un defecto condral implica la restauración de la integridad estructural y funcional del tejido dañado. Las estrategias terapéuticas empleadas para tratar las lesiones condrales se han centrado principalmente en tratamientos no quirúrgicos que intentan controlar los síntomas, técnicas para estimular el proceso de reparación intrínseca, pero que a menudo resultan en la formación de fibrocartílago, o finalmente, la utilización de reemplazos protésicos (7–9). A lo largo de los años, se han empleado enfoques clásicos para la reparación y restauración de cartílago, y recientemente, se han introducido numerosas estrategias y productos innovadores para tratar los defectos condrales. Sin embargo, a pesar de los avances realizados en la tecnología e ingeniería de los tejidos, aún es un desafío encontrar un tratamiento eficaz para la reparación del cartílago articular (58). A continuación, se describen algunas de las técnicas más utilizadas hasta el momento.

4.1. Técnicas de estimulación ósea

La penetración en el hueso subcondral es uno de los métodos más antiguos y ampliamente utilizados para estimular la regeneración del neocartílago. Este enfoque es adecuado para defectos condrales de espesor completo con el hueso subcondral expuesto. La penetración en la placa ósea subcondral implica la rotura de vasos sanguíneos subcondrales dando lugar a la formación de uno o varios coágulos de fibrina en la superficie del defecto condral. De esta forma, las células madre mesenquimales de la médula ósea primitiva migran hacia el coágulo, donde proliferan y se diferencian en células que se asemejan morfológicamente a los condrocitos (24,59).

Dentro de este tipo de técnicas encontramos diferentes enfoques:

Perforaciones

Pridie en 1959 (60), fue el primero en introducir este tipo de técnica. Las perforaciones de Pridie implican, mediante cirugía abierta, la creación de perforaciones en el hueso del cóndilo femoral con una aguja de Kirschner o un punzón. Esto tiene como objetivo promover la formación de un fibrocartílago de reparación.

Desbridamiento

El desbridamiento se considera como la primera parte de cualquier técnica de estimulación de la medula ósea (Figura 3). Se trata de un procedimiento quirúrgico variable que incluye lavado articular, meniscectomía parcial, extracción de cuerpos libres, resección de fragmentos condrales inestables y sinovectomías limitadas (61). Sin embargo, los resultados a largo plazo han determinado un deterioro gradual. Además, los estudios de desbridamiento en la OA han arrojado conclusiones contradictorias (62,63).

Microfractura

La microfractura es el método de estimulación de hueso subcondral más utilizado (64). En este procedimiento, se utiliza un conjunto de punzones de diferentes angulaciones para crear manualmente múltiples orificios en el hueso subcondral expuesto (Figura 3). A diferencia de la perforación con una aguja de Kirschner, este enfoque no genera calor, lo que ayuda a reducir el daño térmico en el lecho óseo (65).

A pesar de ser un método técnicamente sencillo y poco invasivo, se ha demostrado que el neocartílago resultante es biomecánicamente inferior y menos duradero en comparación con el cartílago hialino nativo (66). Los productos derivados de la médula ósea que llenan el defecto se remodelan en tejido de fibrocartílago, el cual es histológicamente diferente y biomecánicamente inferior al cartílago hialino nativo. Los resultados a corto plazo han sido alentadores, pero se han observado resultados menos exitosos en seguimientos más prolongados y en pacientes atléticos y de alta demanda (59,67).

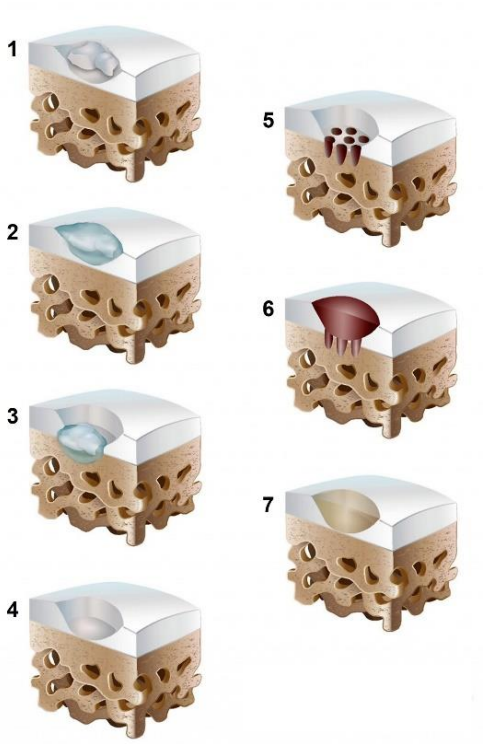

Figura 3. Desbridamiento y microfractura. (1–4) Desbridamiento del cartílago dañado; (5) Microfractura; (6) Formación del coágulo; (7) Formación de fibrocartílago. *(Figura obtenida de ICRS)*

Artroplastia por abrasión

La artroplastia por abrasión se basa en la eliminación del hueso necrótico, exponiendo los vasos subcondrales y permitiendo que se forme un coágulo sanguíneo en su superficie (68). Esta técnica puede dar lugar a la formación de fibrocartílago de reparación, aunque su duración es difícil de determinar. Sin embargo, es un procedimiento que exige un manejo postoperatorio estricto y no es adecuado para todos los pacientes, especialmente aquellos con sobrepeso y edad avanzada. Por lo tanto, la artroplastia por abrasión podría estar contraindicada en pacientes con procesos inflamatorios o en rodillas que presenten rigidez, deformidad o inestabilidad significativa. Además, debe evitarse en pacientes incapaces de mantener un periodo postoperatorio de 2 meses de descarga (61).

4.2. Mosaicoplastia

Esta técnica implica la extracción de tapones osteocondrales cilíndricos de áreas que soportan una menor carga dentro de la articulación de la rodilla. Estos tapones se utilizan para rellenar el defecto condral creando un patrón de “mosaico”. Se utilizan tapones de diferentes tamaños para obtener el máximo relleno del defecto. Sin embargo, los espacios entre los tapones se llenan con fibrocartílago, lo que resulta en una menor estabilidad (69). Además, existen numerosas desventajas asociadas a este procedimiento, como la dificultad de la técnica, la necesidad de un equipo especializado, la incapacidad para restaurar superficies congruentes,

así como la diferencia en las alturas entre el cartílago del defecto y el cartílago nativo circundante (70).

Los trasplantes osteocondrales son las técnicas de mosaicoplastia más utilizadas. El trasplante de autoinjerto osteocondral (OAT) es una opción quirúrgica bien estudiada para el tratamiento de lesiones osteocondrales o condrales de espesor completo pequeñas (69). El trasplante de aloinjerto osteocondral (OCA) es una opción viable para defectos condrales grandes de espesor completo (71). A pesar de la evidencia que respalda a los trasplantes osteocondrales, tanto OAT como OCA se caracterizan por una integración ósea inadecuada o delaminación de la superficie condral. Además, el trasplante de OCA está limitado por un suministro restringido de injertos, y las tecnologías para mejorar la viabilidad de los injertos durante el almacenamiento y facilitar la compatibilidad entre el injerto y el huésped para reducir la delaminación de la superficie articular continúan evolucionando (72).

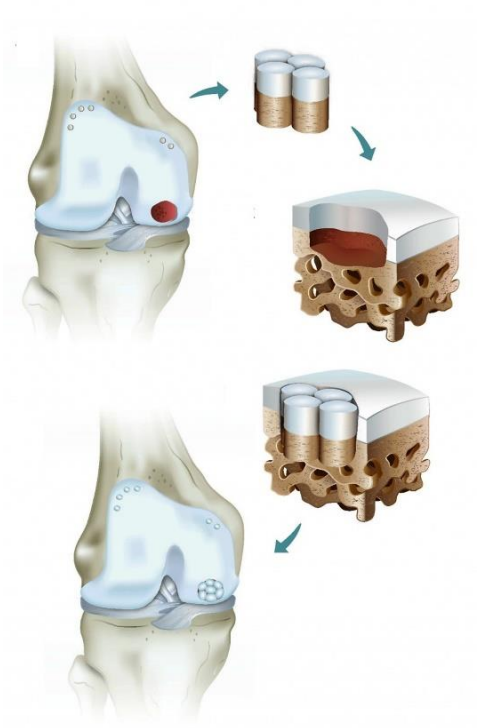

Figura 4. Mosaicoplastia. *(Figura obtenida de ICRS)*

4.3. Implantación de condrocitos autólogos (ACI)

Las técnicas basadas en células se utilizan desde 1994 y son estrategias ampliamente utilizadas para tratar los defectos condrales (73). Sin embargo, estas técnicas presentan algunos obstáculos. En la implantación de condrocitos autólogos (ACI), los condrocitos se extraen del cartílago articular sano en un primera intervención quirúrgica y posteriormente se reimplantan en una segunda cirugía tras su expansión en cultivo celular (73,74). Por lo tanto, ACI requiere

de dos intervenciones quirúrgicas, además de cultivos celulares y los posibles inconvenientes asociados como senescencia celular programada o desdiferenciación (75). El proceso es similar para la ACI inducida en una matriz (MACI) (76). Además, diferentes estudios histológicos han descrito que el tejido de reparación producido con estas técnicas no era morfológica ni histoquímicamente similar al cartílago hialino normal, con la presencia de fibrocartílago (77).

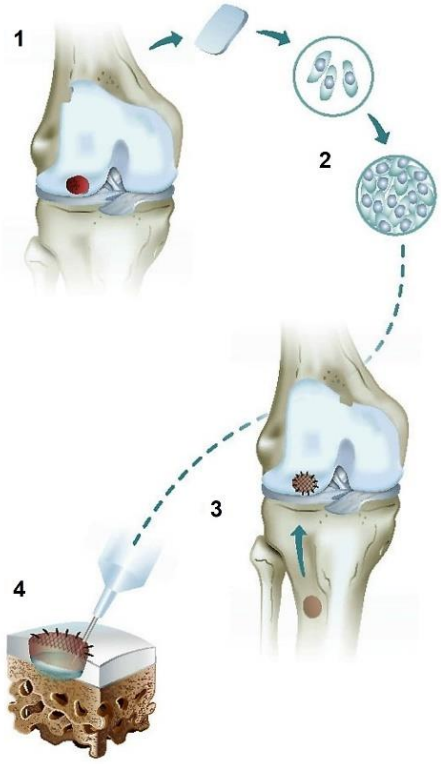

Figura 5. Implantación de condrocitos autólogos (ACI). (1) Biopsia de cartílago; (2) Cultivo celular de condrocitos autólogos; (3) Recolección y fijación del colgajo perióstico; (4) Inyección de la suspensión de condrocitos bajo el colgajo perióstico. *(Figura obtenida de ICRS)*

4.4. Condrogénesis inducida por una matriz autóloga (AMIC)

La condrogénesis inducida por una matriz autóloga (AMIC) implica la estimulación de la médula ósea en combinación con una membrana a base de colágeno o hialuronano porcino de tipo I/III. Este parche se coloca sobre el defecto y actúa como andamio para retener los elementos de la médula ósea y permitirles madurar, facilitando su diferenciación en células condrogénicas (78). Aunque los resultados de AMIC han sido prometedores, la evidencia científica actual es insuficiente para recomendar el tamaño específico del defecto que pueda tratarse con AMIC (79).

5. Matriz autóloga compuesta por cartílago fragmentado incluido en un coágulo de PRP y una posterior infiltración intraarticular de PRP (PACI+PRP) para el tratamiento de defectos condrales

Existe una creciente evidencia de que la calidad del tejido de reparación está relacionada con los resultados clínicos. Sin embargo, a pesar de tantos métodos de tratamientos descritos hasta el momento, no se ha encontrado ninguno que sea capaz de regenerar tejido de reparación con una calidad igual a la de un cartílago articular nativo y sin limitaciones subyacentes. Un posible tratamiento eficaz y que suple las limitaciones de los tratamientos tratados con anterioridad, sería la implantación de una matriz autóloga compuesta por cartílago fraccionado en combinación con plasma rico en plaquetas (PACI+PRP).

5.1. Plasma rico en plaquetas (PRP)

El PRP es un producto biológico derivado de la sangre, que se obtiene mediante un proceso de centrifugación para lograr una concentración plaquetaria superior a la que se encuentra en la sangre circulante (80). El término PRP se utilizó originalmente en 1954 en medicina transfusional (81) para identificar concentrados de trombocitos para el tratamiento de pacientes con trombocitopenia grave. Los estudios de las técnicas para obtener productos derivados de la sangre con el objetivo de mejorar la cicatrización de los tejidos comenzaron en la década de 1970 (82). En los años posteriores, se confirmó el papel que juegan las plaquetas en la cicatrización de los tejidos, y esto se demostró clínicamente mediante el uso de un producto derivado de la sangre conocido como “factores de cicatrización de heridas derivados de plaquetas (PDWHF)” (83). Sin embargo, el término PRP en medicina regenerativa, asociado a factores de crecimiento plaquetarios para promover la cicatrización de los tejidos, fue verdaderamente introducido por Marx et al. en 1998 (84) en un estudio que describió el efecto del PRP en la cicatrización ósea en cirugía maxilofacial.

En los últimos años, los productos derivados de sangre autóloga se han investigado como una herramienta terapéutica útil para el tratamiento de afecciones musculoesqueléticas (85,86). Los concentrados de plaquetas, como el PRP, se incluyen en este tipo de estrategia terapéutica autóloga ya que aportan componentes bioactivos derivados del plasma y las plaquetas como citoquinas, quimiocinas, factores de crecimiento y enzimas (87). De esta forma, las plaquetas activadas se liberan en el sitio de la lesión en el tejido objetivo, contribuyendo así de manera efectiva a la modulación del proceso inflamatorio, la angiogénesis y la respuesta inmune, además de promover la curación y reparación de los tejidos dañados (88). Además, se

ha descrito que los productos biológicos derivados de la sangre tienen efectos antimicrobianos, como la capacidad de inhibir y/o inactivar diferentes cepas bacterianas (89,90).

La aplicación clínica de estos productos biológicos en la medicina musculoesquelética se basa en su capacidad de modular el entorno articular y su papel beneficioso en la reducción de la inflamación local y la promoción del anabolismo del cartílago y la membrana sinovial (88,91,92). Algunos estudios han demostrado que el PRP puede estimular la regeneración del cartílago, mejorar la biosíntesis de las proteínas de la matriz del cartílago y potenciar la proliferación y el metabolismo de los condrocitos (93,94). Esta estrategia terapéutica brinda ventajas en las aplicaciones clínicas debido a su origen autólogo, su perfil de seguridad, la facilidad de obtención y el procedimiento de aplicación mínimamente invasivo (94). Además, el uso de PRP es una estrategia que suele utilizarse en combinación con otras técnicas que buscan la reparación del cartílago (8,17,95,96).

5.1.1. Función plaquetaria en la cicatrización tisular

El proceso de reparación tisular implica una compleja cascada de fenómenos biológicos, regulada por numerosas citoquinas y factores de crecimiento que se liberan de forma cronológica y gradual en el lugar de la lesión (97). Cuando se produce un daño en el tejido, las plaquetas desempeñan un papel inicial en la prevención de la pérdida de sangre, dado el daño vascular inherente a la lesión. Para ello las plaquetas se adhieren y se agregan en el sitio de la lesión, creando una superficie procoagulante que estimula a la generación de trombina y la formación de fibrina (98,99). Una vez se produce la activación plaquetaria, liberan las moléculas biológicamente activas que se encuentran contenidas en sus gránulos citoplasmáticos, promoviendo el reclutamiento, la multiplicación y la morfogénesis de diversas líneas celulares. Estas sustancias se liberan o se presentan en la superficie de las plaquetas tras su activación, así como pueden unirse a una malla o red de fibrina formada durante la cascada de coagulación. En consecuencia, todo el conjunto de citoquinas y factores de crecimiento crean un gradiente quimiotáctico en el lugar de la lesión (100).

Existen varios factores de crecimiento fundamentales para el proceso de reparación tisular, tales como el Factor de crecimiento derivado de plaquetas (PDGF), el Factor de crecimiento transformante β-1 (TGF-β1), el Factor de crecimiento derivado del endotelio vascular (VEGF), el Factor de crecimiento hepatocítico (HGF), el Factor de crecimiento epidérmico (EGF), el Factor de crecimiento insulínico 1 (IGF-1) y el Factor de crecimiento fibroblástico básico (bFGF) (101,102). La mayoría de estos factores de crecimiento se encuentran almacenados en los gránulos α citoplasmáticos de las plaquetas. Cuando las plaquetas se activan

mediadas por un incremento en el calcio intracelular, se forman vesículas secretoras que por exóstosis liberarán al exterior el contenido de estos gránulos (101).

A continuación, se describe brevemente la función que desempeña cada uno de ellos en la cicatrización de los tejidos:

Factor de crecimiento derivado de plaquetas (PDGF)

Es una proteína que juega un papel crucial en la regulación de la proliferación celular y la cicatrización de tejidos. Esta proteína es liberada principalmente por las plaquetas, aunque también puede ser secretada por otras células, como los macrófagos, las células endoteliales y los fibroblastos(103). Se caracteriza por su capacidad de unirse a diversas proteínas plasmáticas y componentes de la ECM, lo que contribuye a su elevada concentración local en el tejido dañado y su detección temprana en el foco de lesión (104,105). Una vez se une a su receptor específico, el PDGF muestra un potente poder quimiotáctico y promueve la proliferación celular, así como la síntesis de componentes de la ECM como colágeno, hialuronato y proteoglicanos (104). Además, el PDGF regula procesos fundamentales relacionados con la remodelación tisular, como la endocitosis o la migración celular (103).

Factor de crecimiento transformante-β1 (TGF-β1)

Se trata de una proteína que desempeña un papel clave en la regulación de diversas funciones biológicas, como la proliferación, la migración y el metabolismo celular. La forma en la que este péptido actúa puede variar según su concentración, el tipo de células a las que está expuesto, y del entorno tisular en el que se encuentra. Puede tanto estimular como inhibir la diferenciación y proliferación celular (103), y se considera un inductor de la síntesis de las proteínas que componen la ECM. Además, promueve la proliferación de fibroblastos (103) y se ha descrito como un potente inductor de fibrogénesis en diversos tejidos, como el riñón, el pulmón, la piel y el músculo (106).

Por otra lado, TGF-β1 también desempeña un papel importante en la modulación de la respuesta inflamatoria en el tejido muscular tras producirse una lesión (107). Además, regula las acciones de otros factores de crecimiento presentes en los gránulos α de las plaquetas, interactuando de forma sinérgica o antagonista con ellos (103).

Factor de crecimiento derivado del endotelio vascular (VEGF)

El VEGF es una proteína que, tras su unión a los receptores específicos, induce la síntesis de enzimas como colagenasas y gelatinasas. Estas enzimas contribuyen a la degradación de la

membrana basal vascular, un proceso fundamental para el inicio de la angiogénesis (104,108). El VEGF estimula la migración y la mitosis de las células endoteliales que componen los vasos sanguíneos en formación.

Además, el VEGF regula la creación del lumen vascular y la formación de las fenestraciones que se encuentran en la red vascular de forma fisiológica. Se ha observado que el VEGF ejerce un efecto quimiotáctico sobre los macrófagos y los granulocitos, así como provoca vasodilatación mediante la liberación de óxido nítrico, lo que aumenta la permeabilidad vascular (105,109). Aunque el VEGF desempeña su función principal durante las fases tempranas de migración y proliferación celular, su efecto angiogénico es fundamental para el proceso de cicatrización de los tejidos musculoesqueléticos durante sus fases más tardías (110).

Factor de crecimiento hepatocítico (HGF)

El HGF es uno de los factores de crecimiento más abundantes liberados tras la activación plaquetaria. Su nombre proviene de su función central en la regulación de la regeneración y proliferación de los hepatocitos, así como en la proliferación de fibroblastos (111). Este péptido desempeña un papel multifacético, ya que, por un lado, tiene un marcado efecto angiogénico, principalmente asociado a la síntesis de VEGF. Por otro lado, promueve la multiplicación de células endoteliales y actúa como agente quimiotáctico para promover la migración celular (112). Estudios experimentales han puesto de manifiesto que el HGF modula la respuesta inflamatoria en las etapas iniciales tras producirse el daño tisular (113). Asimismo, reduce la producción de prostaglandina E2 mediante la inhibición de la enzima ciclooxigenasa (COX) (113) y ejerce un efecto antinflamatorio al inhibir el factor de transcripción NF-κB (114).

Factor de crecimiento epidérmico (EGF)

El EGF es una proteína cuyo efecto se caracteriza por su capacidad para estimular la división celular, dando lugar a un incremento en la producción de proteínas necesarias para la regeneración tisular. A nivel celular, promueve la multiplicación de fibroblastos y células endoteliales, que formarán nuevos vasos sanguíneos. Además, promueve la migración celular hacia la zona de lesión y estimula el crecimiento y la diferenciación de los queratinocitos (115). El EGF también desempeña un papel relevante durante la fase inflamatoria de la reparación de tejidos musculoesquelético tras una lesión, ya que se expresa en las células inflamatorias con el objetivo de producir los efectos beneficiosos mencionados anteriormente (116).

Factor de crecimiento insulínico-1 (IGF-1)

El IGF-1 es una proteína con funciones clave en la promoción de la replicación celular, la síntesis de glucógeno, proteínas y glicosaminoglicanos, así como el transporte de glucosa y aminoácidos a través de la membrana celular (103). En el sistema musculoesquelético, se centra en estimular el crecimiento esquelético al aumentar la formación de hueso y cartílago y reducir la degradación de la ECM (117,118). El IGF-1 también exhibe un fuerte efecto quimiotáctico en las células endoteliales de los vasos sanguíneos estimulando la neoangiogénesis en el área de reparación del tejido (119). Se ha descrito un efecto anti-inflamatorio del IGF-1 en estudios experimentales de lesiones en tendones de Aquiles de ratas, lo que permitió acelerar la recuperación funcional (120).

Factor de crecimiento fibroblástico básico (bFGF)

El bFGF es una proteína con una potente actividad mitogénica, quimiotáctica y angiogénica en diversas estirpes celulares (103). Sus propiedades angiogénicas lo hacen de vital importancia durante los procesos de neovascularización en las diferentes etapas de la cicatrización tisular (115). Además, el bFGF está involucrado en la síntesis de colágeno de la ECM (104,106).

5.2. Implantación de fragmentos de cartílago autólogo (PACI)

El uso clínico de partículas de cartílago para defectos condrales fue descrito por primera vez en 1983 por Albrect et al. (121), y desde entonces, numerosos estudios han respaldado el potencial de cartílago fragmentado como tratamiento para estas lesiones condrales (122,123). Estudios en animales han demostrado que el uso de cartílago fragmentado mejora la calidad de la ECM del cartílago reparado (121–127). Además, los casos y ensayos clínicos en humanos han revelado que el uso de este tipo de tratamiento parece ser una opción reconstructiva efectiva para las lesiones condrales (122,128–130). Se postula que el mecanismo de reparación implicado se basa en la migración de condrocitos desde los fragmentos de cartílago hacia un biomaterial, seguida de la deposición de ECM por estas células (16,124).

El aloinjerto de cartílago articular juvenil fragmentado (PJAC), comercializado con el nombre de DeNovo (DeNovo NT, Zimmer, Warsaw, IN), surgió como una opción de tratamiento en la cual el aloinjerto de cartílago se obtiene de los cóndilos femorales de donantes fallecidos de entre 0 y 13 años de edad (16,130,131). Las ventajas propuestas por este tratamiento incluyen la disponibilidad de material de injerto ilimitado para lesiones de gran tamaño y una actividad más robusta de los condrocitos juveniles en comparación con los condrocitos adultos

(16,130). Aunque el uso de PJAC se ha generalizado con resultados clínicos prometedores, aún existen desafíos en el tratamiento, como los riesgos asociados con el uso de aloinjertos, el coste y el desperdicio de tejido debido a la imposibilidad de volver a utilizarlo (132–134).

Como alternativa, se introdujo la implantación de cartílago fragmentado (MCI) o la implantación de partículas de cartílago autólogo (PACI). Esta técnica implica la obtención de pequeños trozos de cartílago hialino sano y viable que se cortan durante el mismo proceso quirúrgico y se implantan en la lesión condral u osteocondral (Figura 6) (79). En este caso, el cartílago se extrae de los bordes del defecto, lo que elimina el riesgo de morbilidad y se reducen significativamente los costes al ser un material autólogo. Los fragmentos de cartílago autólogo utilizados en PACI funcionan como una matriz bioactiva. Además, esta técnica se puede combinar con otras para obtener mejores resultados (96,135).

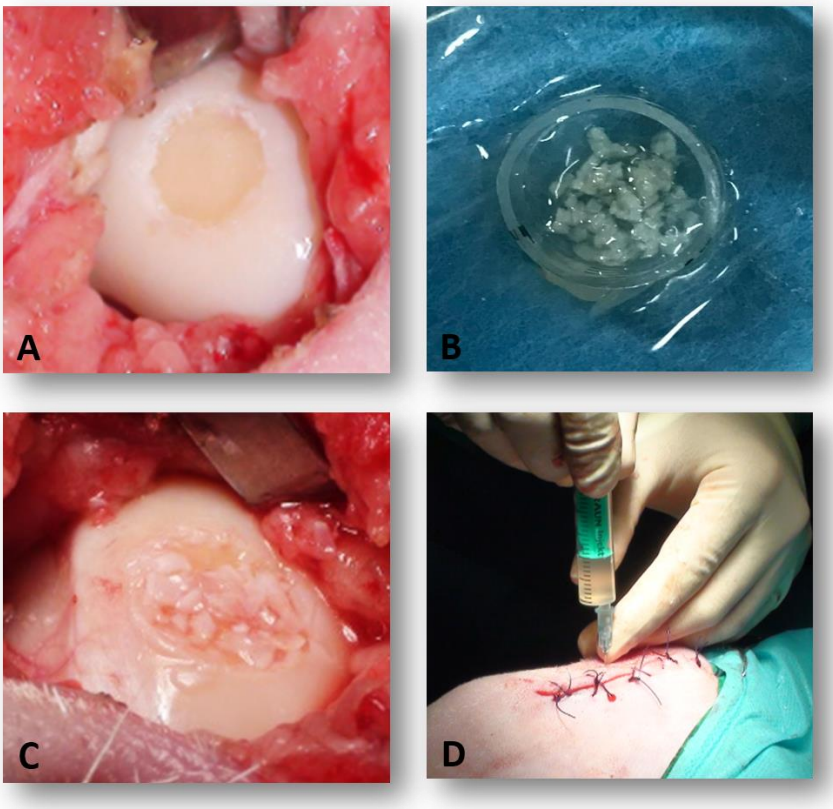

Figura 6. Defecto del cartílago y preparación del tratamiento PACI+PRP. (A) Defecto del cartílago; (B) Cartílago fragmentado con PRP; (C) Defecto cubierto con scaffold de PACI y PRP; (D) Infiltración intraarticular de PRP. *(Fuente propia)*

En estudios realizados en humanos, Cugat et al. (96) y Delman et al. (132) demostraron que PACI es un procedimiento seguro y eficaz con resultados prometedores. Cugat et al. (15) publicaron dos casos de futbolistas profesionales con lesiones condrales de espesor completo en la rodilla que fueron tratados con PACI+PRP. Este estudio describió unos resultados excelentes, incluyendo la recuperación de la función de rodilla, alivio del dolor y regreso al fútbol de alto nivel. Además, Cugat et al. (17), confirmaron estos buenos resultados clínicos y funcionales en un primer estudio clínico que incluyó a 15 pacientes tratados con PACI+PRP. Por otro lado, Domínguez et al. (8) llevaron a cabo un estudio histopatológico e inmunohistoquímico sobre el tratamiento PACI+PRP en defectos condrales de espesor completo en un modelo ovino. Sus hallazgos mostraron una reparación del defecto condral que se asemejaba a un cartílago hialino sano en los análisis macroscópicos e histopatológicos realizados a los 6 meses de la cirugía.

6. Modelos animales usados para el estudio de la reparación de cartílago

Los modelos animales son elementos fundamentales en la investigación científica con fines traslacionales (136). Es imperativo que estos modelos reflejen adecuadamente la diversidad de apariencias y etiologías de los defectos del cartílago, que pueden derivar de la OA, traumatismos, osteocondritis disecante y osteonecrosis. Estos modelos pueden emplearse para investigar la reparación espontánea del cartílago o para perfeccionar las opciones reconstructivas mediante cirugía (137).

Los animales utilizados con mayor frecuencia para el análisis de la regeneración del cartílago son ratones, ratas y conejos, como modelos animales de pequeño tamaño, o perros, ovejas, cabras, mini cerdos y caballos, como modelos de animales de gran tamaño. Se considera que estos últimos reflejan de una manera más precisa las condiciones anatómicas y clínicas humanas y, por esta razón, la Agencia Europea de Medicamentos recomienda su uso en estudios preclínicos (138,139). Los modelos de animales de gran tamaño son una herramienta muy valiosa para desarrollar y evaluar nuevos procedimientos quirúrgicos. La elección adecuada de un modelo animal grande para un procedimiento quirúrgico determinado o el desarrollo de un nuevo tratamiento es esencial para garantizar la correcta interpretación de los resultados y su aplicabilidad en modelos humanos (140).

En la investigación ortopédica traslacional relacionada con la cirugía de rodilla en modelos animales de gran tamaño, no existe un modelo estándar que reproduzca de manera precisa la articulación de la rodilla humana, ya que estos modelos presentan diferencias significativas en términos de estructura y función en comparación con los humanos. A pesar de ello, existen varios criterios generales que se deberían tener en cuenta (141):

1) El modelo debe ser análogo a la especie humana
2) Debe haber disponibles datos comparativos
3) Los resultados deben ser transferibles
4) La cirugía debe ser técnicamente factible
5) Debe haber disponible un centro especializado para animales grandes
6) Los animales deben ser asequibles y accesibles
7) La cirugía debe cumplir con estándares éticos
8) La anestesia y cirugía deben ser toleradas por el animal

Además, al seleccionar modelos para defectos del cartílago articular focal, también se deben tener en cuenta una serie de consideraciones adicionales (141): el grosor del cartílago, el grosor de la placa ósea subcondral, la edad de los animales, el tamaño del defecto, la profundidad del defecto, la anatomía del defecto, la ubicación del defecto, la posición de reposo de la rodilla y los patrones de marcha.

6.1. Modelo ovino

Aunque no existe un solo modelo animal de gran tamaño que replique con precisión la articulación de la rodilla humana, la articulación de la rodilla ovina presenta grandes similitudes con la articulación de la rodilla humana (141).

Las ovejas se utilizan con frecuencia en modelos de reparación de cartílago debido al tamaño adecuado de sus articulaciones de la rodilla, que permiten la creación de lesiones de un tamaño comparable al tratado en pacientes humanos (142). El mayor tamaño de los defectos y el cartílago más grueso permiten realizar ensayos bioquímicos del tejido en reparación y estudios histológicos más detallados (143).

En términos de fuerza femorotibial máxima, se ha estimado que en humanos oscila entre 2,1 y 2,7 veces el peso corporal al caminar y 4,2 veces al trotar (144). En ovejas, se ha estimado que era de 2,3 veces el peso corporal durante la marcha, lo cual es comparable al de los humanos (145). Además, en ambas especies, la mayor parte de la fuerza se trasmite a través del compartimento medial (146). A pesar de ser cuadrúpedas y presentar ligeras diferencias en el

movimiento articular, las rodillas de las ovejas proporcionan un modelo proporcionalmente reducido de la rodilla humana con fuerzas articulares comparables (147).

Estos animales se encuentran disponibles a través de proveedores comerciales y agrícolas como animales de 2 años o más, considerándose esqueléticamente maduros (148).

Además de las consideraciones anatómicas y de composición, existen otras variables que respaldan la elección del modelo ovino (Figura 7). Los equipos, agentes y protocolos de anestesia son en su mayoría similares entre los humanos y muchos de los modelos animales de gran tamaño. Si bien se han realizado investigaciones iniciales en modelos caninos debido a su facilidad de manejo, esto se ha vuelto cada vez más inusual debido a preocupaciones éticas en relación con la investigación terminal en animales de compañía. Aunque las ovejas (50 a 75 kg) son más grandes que los caninos (15 a 30 kg), siguen siendo relativamente fáciles de manejar y es posible el acceso intravenoso a través de la vena yugular o braquial sin premedicación.

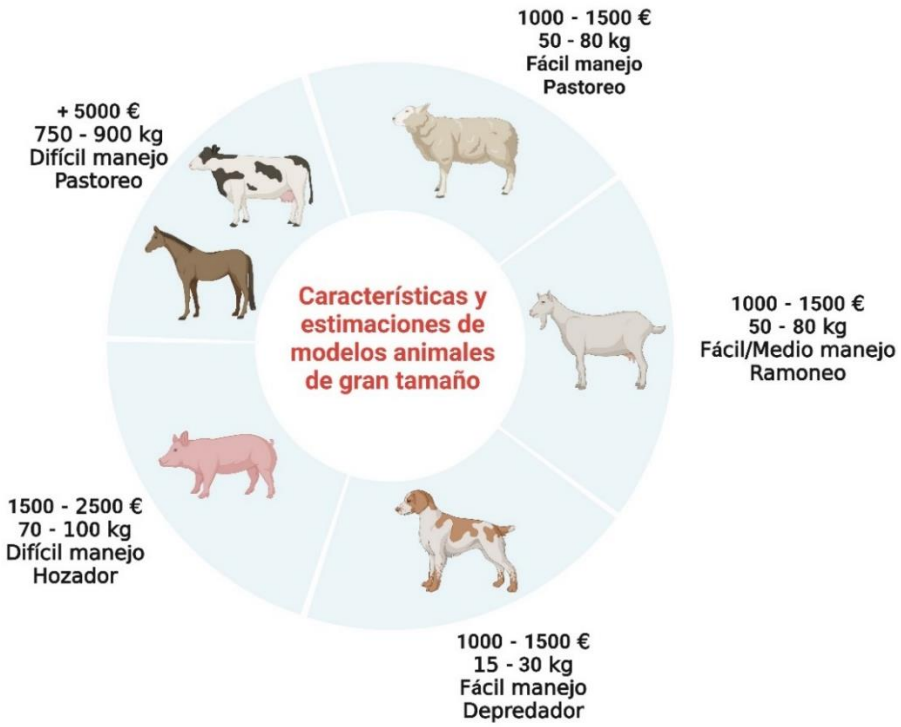

Figura 7. Características y estimaciones consideradas a la hora de elegir un modelo animal de gran tamaño para estudios de reparación de cartílago. *(Fuente propia)*

Por otro lado, tanto vacas como caballos adultos presentan un tamaño drásticamente superior (750 a 900 kg) y requieren de una inversión importante en instalaciones y equipos. Las razas porcinas domésticas son relativamente económicas y de fácil acceso, pero crecen a un ritmo extremadamente rápido y alcanzan pesos de 300-500 kg en la madurez esquelética. Las razas de cerdos minipig solo están disponibles a través de proveedores especializados y los precios son mucho más altos. Además, los cerdos son relativamente difíciles de manejar y

sujetar, por lo que complica su cuidado postoperatorio. Por último, aunque el modelo caprino representa una alternativa asequible y anatómicamente precisa al modelo ovino, limitaciones en su comportamiento restringen su utilidad en cuanto a rehabilitación. Las ovejas generalmente permanecen en cuatro patas, mientras que las cabras a menudo se paran sobre sus patas traseras. Por lo tanto, es mucho menos probable que las ovejas carguen todo su peso sobre las rodillas recién operadas, lo que reduce significativamente el riesgo de daño temprano (147,149).

7. Técnicas histológicas e histoquímicas para el estudio del cartílago articular

La aplicación de técnicas microscópicas junto con técnicas histoquímicas constituye una estrategia idónea para investigar la distribución de los componentes celulares y de la matriz. La evaluación histológica y la caracterización del tejido cartilaginoso en condiciones normales y patológicas se llevan a cabo habitualmente mediante el empleo de microscopía óptica (150). Gracias a los procedimientos de tinción de rutina y a una histoquímica más especializada, es posible obtener información específica sobre el cartílago articular tanto fisiológico como patológico (151).

Existen dos enfoques principales en el estudio histológico, la histología descriptiva y la histomorfometría. Depeniendo de la situación particular, se puede utilizar cualquiera de los dos métodos. La histología descriptiva se utiliza para proporcionar una evaluación integral del tejido de interés, abordando aspectos como la morfología, estructura y disposición de las células, matriz, el implante o la interfaz tejido-implante (152). Se dispone de diversos métodos de tinción, seleccionados en función de los objetivos de estudio. Para tinciones histológicas más convencionales, se pueden realizar procedimientos de descalcificación antes de la inclusión en parafina. Sin embargo, en casos en los que la formación y mineralización ósea sean esenciales, se recomienda el procesamiento no descalcificado con secciones realizadas mediante micrótomos y cuchillas especializadas (153). La tinción clásica con hematoxilina y eosina sigue siendo el método fundamental y ampliamente utilizado, aplicable tanto a muestras descalcificadas como no descalcificadas (154).

Adicionalmente, se llevan a cabo procedimientos de tinción e histoquímica más especializados para obtener información específica sobre hueso, cartílago y matriz extracelular en condiciones normales y patológicas. La inmunohistoquímica ha sido empleada para examinar la composición bioquímica de cartílagos, ligamentos, tendones y otros tejidos, logrando la localización exitosa de macromoléculas comunes de la matriz del cartílago, como colágenos y proteoglicanos (150).

III. HIPÓTESIS / OBJETIVOS – HYPOTHESIS / OBJECTIVES

HIPÓTESIS

En la presente tesis doctoral se evalúa una innovadora terapia regenerativa, dirigida al tratamiento de lesiones focales de cartílago articular, que puede representar una alternativa terapéutica eficaz, segura y económica. Esta nueva terapia totalmente autóloga consiste en la creación de un andamiaje tridimensional compuesto por cartílago hialino sano fraccionado integrado en la estructura de un coágulo de PRP más una infiltración intraarticular de PRP, estudiada para inducir la reparación condrogénica de un defecto condral de rodilla en un modelo ovino. Se plantea la hipótesis de que este tratamiento, favorecerá la regeneración de cartílago articular hialino que rellene la lesión, creando continuidad con el cartílago adyacente, restaurando un tejido cartilaginoso que a nivel celular y de la matriz extracelular tenga organización, arquitectura y calidad equivalente a la del cartílago sano. Este potencial efecto de mejoría de las características estructurales conseguidas con la terapia, implicará una mejora de la funcionalidad y durabilidad del cartílago reparado, abriendo así nuevas perspectivas para su transferencia y aplicabilidad en la práctica clínica veterinaria y humana.

OBJETIVOS

El objetivo general de esta tesis doctoral consistió en evaluar la eficacia de una terapia totalmente autóloga que consiste en la creación de un andamiaje tridimensional compuesto por cartílago hialino sano fraccionado integrado en la estructura de un coágulo de PRP más una infiltración intraarticular de PRP (PACI+PRP), para el tratamiento de lesiones condrales focales de espesor completo en la zona de carga del cóndilo medial del fémur en rodillas de ovejas, tras dos tiempos de recuperación de 9 y 18 meses. Con el fin de establecer comparaciones significativas, se incluyeron grupos control tratados con ácido hialurónico o solución de Ringer Lactato, además de comparar con el cartílago sano adyacente. Para alcanzar este objetivo general, se platearon los siguientes objetivos específicos:

Objetivo I: Realizar una descripción detallada de las moléculas más relevantes presentes en la matriz extracelular del cartílago articular, centrándose especialmente en diferentes tipos de colágenos mayores y menores, y proteoglicanos. Además, profundizar en el papel funcional que desempeñan estas moléculas tanto en el contexto de un cartílago articular sano como en el proceso de reparación tras una lesión condral, con el fin de ampliar este conocimiento y usarlo para establecer un sistema de evaluación más preciso destinado a la determinación de la calidad del tejido cartilaginoso reparado.

Capítulo 1: “Main and minor types of collagens in the articular cartilage: The role of collagens in repair tissue evaluation in chondral defects”. Este estudio ha sido publicado en la revista *International Journal of Molecular Sciences* (doi: 10.3390/ijms222413329) y se presenta como indicio de calidad para la lectura y defensa de esta tesis doctoral.

Capítulo 2: “Proteoglycans in articular cartilage: Role in chondral injury and repair”. Este estudio ha sido publicado en la revista *International Journal of Molecular Sciences* (doi: 10.3390/ijms241310824) y se presenta como indicio de calidad para la lectura y defensa de esta tesis doctoral.

Objetivo II: Evaluar las propiedades regenerativas condrogénicas a nivel macroscópico, utilizando tres sistemas de puntuación, e histopatológico, mediante tinción de hematoxilina-eosina, con el fin de valorar la madurez y morfología de los condrocitos y la calidad del tejido reparado tras la aplicación del tratamiento PACI+PRP en defectos condrales en nuestro modelo experimental.

Capítulo 3: “Particulate cartilage and platelet-rich plasma treatment for knee chondral defects in sheep”. Este estudio ha sido publicado en la revista *Knee surgery, Sports Traumatology, Arthroscopy* (doi: 10.1007/s00167-022-07295-7) y se presenta como indicio de calidad para la lectura y defensa de esta tesis doctoral.

Objetivo III: Realizar un análisis inmunohistoquímico exhaustivo y detallado de los distintos tipos de colágenos mayores y menores del cartílago articular, así como del agrecano, para evaluar la calidad, estructura y durabilidad de la matriz extracelular del cartílago reparado mediante el uso del tratamiento PACI+PRP aplicado a defectos condrales en el modelo experimental evaluado.

Capítulo 4: “Immunohistochemical analysis of knee chondral defects repair after autologous particulated cartilage and platelet-rich plasma treatment in sheep”. Este estudio ha sido publicado en la revista *International Journal of Molecular Sciences* (doi: 10.3390/ijms242015157) y se presenta como indicio de calidad para la lectura y defensa de esta tesis doctoral.

III. HIPÓTESIS / OBJETIVOS – HYPOTHESIS / OBJECTIVES

HYPOTHESIS

The present doctoral thesis evaluates an innovative regenerative therapy designed for treating chondral lesions in the articular cartilage. This approach introduces a cost-effective, swift, easily implementable, and safe therapeutic alternative. This autologous approach involves constructing a three-dimensional scaffold that combines healthy hyaline cartilage integrated into the structure of a platelet-rich plasma treatment clot. This is followed by intra-articular PRP infiltration to induce chondrogenic repair in a knee chondral defect, using an ovine animal model. It is hypothesized that this treatment will establish a favourable cellular environment to achieve effective chondrogenic repair, restoring a durable and high-quality hyaline cartilage exhibiting an organized extracellular matrix analogous to healthy cartilage. This therapy has the potential to improve the structural and functional characteristics of the repaired cartilage, thereby introducing new perspectives for its application in both veterinary and human clinical practice.

OBJECTIVES

The general objective of this doctoral thesis was to evaluate the efficacy of an autologous treatment. This approach involved constructing a three-dimensional scaffold that combines healthy hyaline cartilage integrated into the structure of a platelet-rich plasma treatment clot, followed by intra-articular PRP infiltration (PACI+PRP), for the treatment of focal, full-thickness chondral lesions in the weight-bearing area of the medial condyle of the femur in sheep knees. The evaluation was conducted after recovery periods of 9 and 18 months. In order to establish significant comparisons, control group treated with hyaluronic acid or Ringer´s Lactate solution were included and compared with adjacent healthy cartilage. To achieve this main goal, the following specific objectives were proposed:

Objective I: To provide a detailed description of the key molecules present in articular cartilage, with a specific emphasis on major and minor collagens and proteoglycans. Explore the functional roles of these molecules in both healthy articular cartilage and the chondral repair process after a chondral lesion, with the goal of developing refined evaluation systems to assess the quality of repaired chondral tissue.

Chapter 1: “Main and minor types of collagens in the articular cartilage: The role of collagens in repair tissue evaluation in chondral defects”. This study has been published in the

International Journal of Molecular Sciences (doi: 10.3390/ijms222413329) and it is presented as a quality parameter for the doctoral thesis defence.

Chapter 2: "Proteoglycans in articular cartilage: Role in chondral injury and repair". This study has been published in the *International Journal of Molecular Sciences* (doi: 10.3390/ijms241310824) and it is presented as a quality parameter for the doctoral thesis defence.

Objective II: Evaluate the quality of the repaired tissue at the macroscopic level using three distinct scoring systems. In addition, evaluate the maturity and morphology of chondrocytes and the chondrogenic regenerative properties of the PACI+PRP treatment in chondral defects at the histopathological level through hematoxylin and eosin staining in our ovine experimental model.

Chapter 3: "Particulate cartilage and platelet-rich plasma treatment for knee chondral defects in sheep". This study has been published in the journal *Knee surgery, Sports Traumatology, Arthroscopy* (doi: 10.1007/s00167-022-07295-7) and it is presented as a quality parameter for the doctoral thesis defence.

Objective III: To conduct a comprehensive and detailed immunohistochemical analysis of several types of collagens, including both major and minor forms, as well as aggrecan. The aim was to evaluate the quality, durability, and structure of the extracellular matrix in the repaired cartilage following the application of PACI+PRP treatment in chondral defects within our ovine experimental model.

Chapter 4: "Immunohistochemical analysis of knee chondral defects repair after autologous particulated cartilage and platelet-rich plasma treatment". This study has been published in the *International Journal of Molecular Sciences* (doi: 10.3390/ijms242015157) and it is presented as a quality parameter for the doctoral thesis defence.

IV. CAPÍTULO I

Objetivo I / Objective I

Main and minor types of collagens in the articular cartilage: the role of collagens in repair tissue evaluation in chondral defects

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Main and minor types of collagens in the articular cartilage: the role of collagens in repair tissue evaluation in chondral defects

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Abstract

Several collagen subtypes have been identified in hyaline articular cartilage. The main and most abundant collagens are type II, IX and XI collagens. The minor and less abundant collagens are type III, IV, V, VI, X, XII, XIV, XVI, XXII, and XXVII collagens. All these collagens have been found to play a key role in healthy cartilage, regardless of whether they are more or less abundant. Additionally, an exhaustive evaluation of collagen fibrils in a repaired cartilage tissue after a chondral lesion is necessary to determine the quality of the repaired tissue and even whether or not this repaired tissue is considered hyaline cartilage. Therefore, this review aims to describe in depth all the collagen types found in the normal articular cartilage structure, and based on this, establish the parameters that allow one to consider a repaired cartilage tissue as a hyaline cartilage.

Keywords: Hyaline articular cartilage. Main collagens. Minor collagens. Chondral defect repair.

1. Introduction

Collagens are the most abundant proteins in mammals accounting for approximately 30% of total protein mass [1]. More than two-thirds of the dry weight of adult articular cartilage, over three-fourths of the dry weight of human skin, over 90% of human tendon and corneal tissues, and almost 80% of the organic matter in bones are composed of collagen, a major component of connective tissues [2,3]. Collagen is approximately 60% of the dry weight of hyaline cartilage, being the main protein in the cartilage extracellular matrix (ECM) composition [4,5]. Since the discovery of the first collagen [6], more than 26 new collagens have been revealed [1,7,8,9,10].

In articular cartilage, characterized by having a hyaline structure, numerous subtypes of collagens have been identified. The main, most abundant collagens, which are mostly studied by researchers are type II, IX, and XI collagens [11,12]. Type III, IV, V, VI, and X collagens are less abundant in cartilage, which is why some authors highlight them and others do not [11,12,13]. Even so, the presence of these minor collagens in healthy articular cartilage is reliable. However, the quantity of some of these minor collagens in healthy cartilage is still controversial. In addition, some researchers mentioned that type XII, XIV, XVI, XXII, and XXVII collagens are also part of articular cartilage [13], although the information given about them is very brief. As well as all the collagens mentioned above, type I collagen can sometimes be found in articular cartilage. However, this should not occur in healthy articular cartilage as it indicates the presence of fibrotic connective tissue [14].

When a chondral defect is treated, an attempt is made to repair the tissue by making the characteristics of the new cartilage as similar as possible to normal hyaline cartilage. To assess the effectiveness of the treatment that has been used, it is necessary to assess the quality of the repaired tissue, and for this purpose we need to establish a threshold that allows us to identify the repaired cartilage as hyaline cartilage. Moreover, it is essential to conduct a study of the collagens present in the repaired cartilage tissue to conduct a good assessment of the quality of the chondral repair.

Therefore, the purpose of this review is to describe in detail all of the collagens that can be found in articular cartilage, as well as describe collagen fibrils assessment considerations in order to identify repaired hyaline cartilage in chondral defects.

2. Articular Cartilage

Hyaline articular cartilage is a highly specialized connective tissue, whose main function is to provide a smooth and lubricated surface for the joint and to facilitate the transmission of loads with a low coefficient of friction [15,16,17]. Articular cartilage is composed of hyaline tissue including a dense ECM with a low distribution of chondrocytes [18]. Unlike most tissues, it lacks blood vessels, lymphatics and nerves, and in addition, its cells have a low replication potential, so the cartilage repair capacity is limited [19,20,21].

ECM consists of interlocking mesh of water, collagens, and proteoglycans (PG), as well as smaller amounts of non-collagen proteins. All components combined generate a single viscous material, optimized to support loads [15,22]. Chondrocytes are the only highly specialized cells found in cartilage that constitute between 1–5% of cartilage volume. These cells play a pivotal role providing mechanical support [11,23].

2.1. Cartilage Zones

Adult cartilage has an architecture divided into zones (Figure 1A), which vary depending on the biochemical composition of ECM, cell density and morphology, and cellular and ECM metabolism [24].

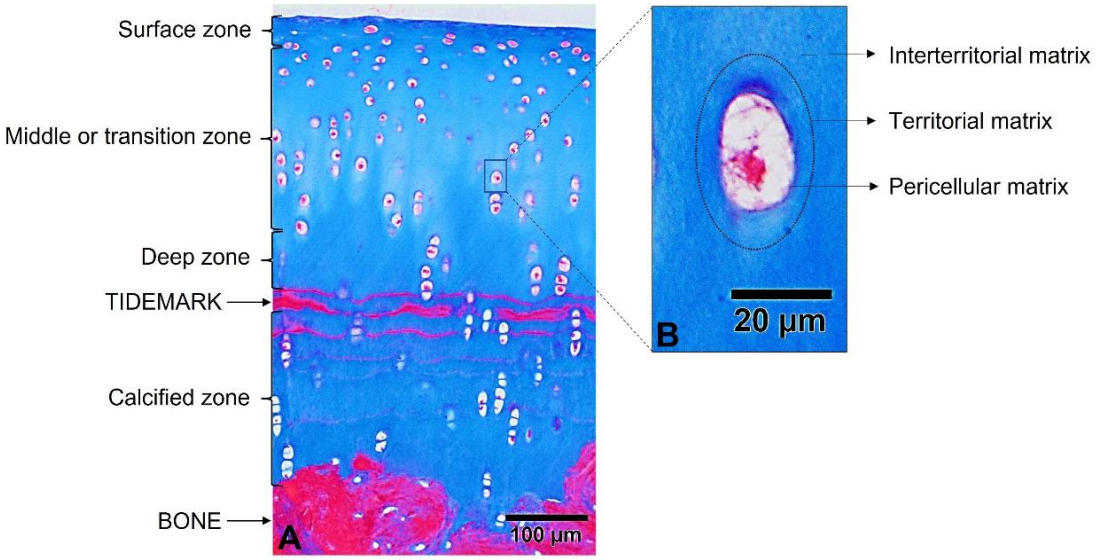

Figure 1. Masson's trichrome staining of a representative section of healthy hyaline cartilage. (A) Cartilage zones. (B) Regions of the ECM.

The surface zone is the outermost and thinnest of all layers and constitutes approximately 10% to 20% of the thickness of the joint cartilage. It is composed of a relatively high number of oval shaped chondrocytes parallel to the surface of the joint. Chondrocytes in

this area synthesize a high concentration of collagen and a low concentration of proteoglycans. This zone has the highest water content. The collagen fibers in this zone are heavily packaged and are also aligned parallel to the joint surface, to provide the greatest resistance to traction and shear. The superficial zone is in contact with synovial fluid and is responsible for most of the traction properties of cartilage [11,15,19,25,26].

Immediately underneath the surface zone is located the middle or transition zone, which provides an anatomical and functional bridge between the superficial and deep zones. The middle zone represents 40% to 60% of the total volume of cartilage and contains thicker PG and collagen fibrils. In this zone, collagen is organized obliquely, and chondrocytes are spherical and have low density. Fundamentally, the middle zone is the first line of resistance to compression forces [11,15,19,25,26].

Then, the deep zone involves the connection between the cartilage tissue and subchondral bone. The deep zone is responsible for providing the greatest resistance to compression forces since the collagen fibrils are arranged perpendicular to the joint surface. This zone contains the largest diameter collagen fibrils in radial arrangement, the highest content of PG and the lowest concentration of water. Chondrocytes are larger and are usually available in a columnar orientation, parallel to the collagen fibers and perpendicular to the surface. The deep zone represents approximately 30% of the joint cartilage volume. Between the deep zone and the calcified cartilage, we find the tidemark that distinguishes both areas [11,15,19,25,26].

The calcified cartilage zone plays an integral role in the fixation of cartilage to the bone by anchoring collagen fibrils from the deep zone to the subchondral bone. In this zone, the cell population is scarce, and chondrocytes are hypertrophic, which makes metabolic activity very low [11,15,19,25,26].

2.2. Regions of the Extracellular Cartilage Matrix

In addition to the zoned architecture, the matrix consists of several regions. The ECM can be divided into three regions, depending on the proximity to chondrocytes, the composition, the diameter, and organization of collagen fibrils: pericellular, territorial and interterritorial (Figure 1B) [15]. The pericellular matrix (PCM) is a thin layer adjacent to the cell membrane that completely surrounds the chondrocyte, playing an important role in the transduction of signals and other aspects of cartilage [24]. The territorial matrix surrounds the PCM, this being thicker and protecting the cartilage cells against mechanical stress. In addition, it contributes to the elasticity of the cartilage structure and its ability to withstand loads. The interterritorial matrix is the biggest, contributing more to biomechanical properties of articular cartilage [11,15,23].

3. Collagens

Collagen is the major ECM molecule that self assembles into cross striated fibrils, provides support for cell growth and is responsible for the mechanical resilience of connective tissues. To date, between 28 and 29 types of collagens have been described [27,28,29].

Collagen belongs to the family of glycoproteins that are characterized by signature features. Each polypeptide chain has a repeating sequence of amino acids [Gly-X-Y]n, with and without interruptions (those with interruptions contain numerous residues). Furthermore, the X and Y positions are frequently occupied by proline and its hydroxylated form, 4-hydroxyproline, respectively. Finally, the right-handed tripe helix is formed from three left-handed polyproline α-chains of identical length, which gives collagen a unique quaternary structure [8,9,27,30].

3.1. Biosynthesis of Collagens Fibers

Collagen fibrils biosynthesis begins with the genetic transcription of genes (Figure 2-1) within the nucleus to the aggregation of collagen heterotrimers into large fibrils [31].

Inside the rough endoplasmic reticulum (RER), the assembly of mainly three amino acids (glycine, proline or its derivative hydroxyproline, and lysine) gives rise to the formation of polypeptide chains (α chains) (Figure 2-2). In the Golgi, three of these α chains are assembled one around the other, along a central axis, to generate a procollagen molecule in the form of right-handed triple-helix (Figure 2-3) [32,33].

Subsequently, procollagen is secreted into the extracellular space, giving rise to tropocollagens units (Figure 2-4). Once in the extracellular space, molecular processing is different depending on the type of collagen in question and the supramolecular structure that it must form in a tissue. In general, in the extracellular space, several tropocollagen molecules associate to form fibrils and fibers (Figure 2-5) [31,32,34].

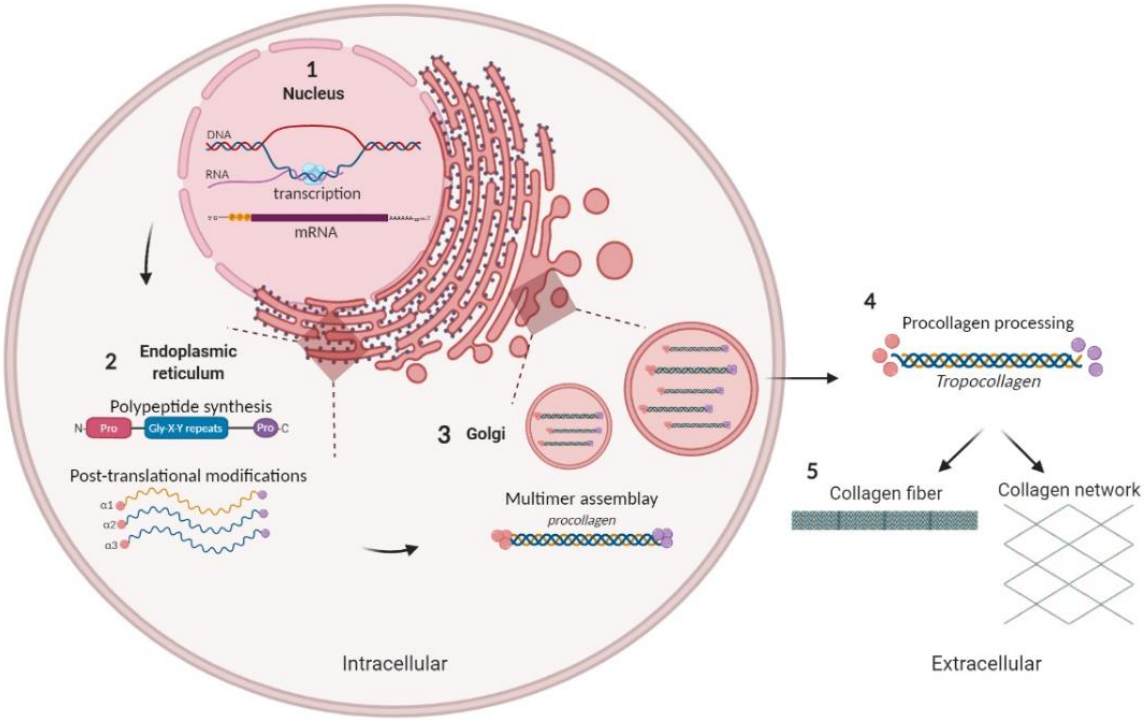

Figure 2. Schematic representation of collagen biosynthesis. (1) Gene transcription. (2) Formation of α-chains. (3) Formation of triple helix procollagen and secretion into extracellular space. (4) Procollagen processing and formation of tropocollagen. (5) Association of tropocollagen molecules to form collagen structures.

3.2. Classification of Collagen Types

Collagens can be grouped based on their structure, function, and tissue distribution. They are designated by Roman numerals according to the order of their discovery and Greek letters to identify the chains, bands, and higher molecular weight components. There are homotrimers, formed by three identical chains, or heterotrimers, formed by two/three different chains [27,32,35].

The different types of collagens and their structure are crucial to provide mechanical stability, elasticity, and strength to tissues and organs. Following a classification based on collagen function and composition, several groups are distinguished (Figure 3): (1) Fibril-forming collagens; (2) Fibril-associated collagens with interrupted tripled helices; (3) Collagens forming networks; (4) Transmembrane collagens; (5) Multiplexins; (6) Anchor fibers; and (7) Beaded filament-forming collagens [9,32].

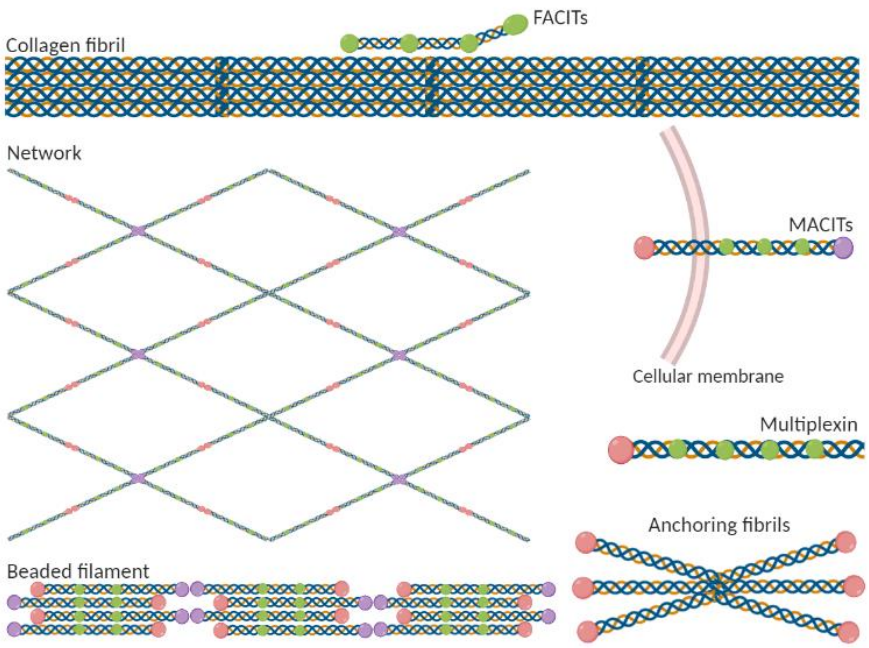

Figure 3. Classification of collagen types based on their structural and organisation.

(1) The classical fibril-forming collagens include types I, II, III, V and XI collagens. They are characterized by appearing as periodic fibrils with an indeterminate in length, depending on the tissue and developmental stage, and range in diameter from 12 nm to 500 nm [36]. All fibril-forming collagens are composed of a large continuous triple helix bordered by the N- and C-propeptide referred as the NC1 domain. The N-propeptide is divided into sub-domains: a short sequence (NC2) that links the major triplex helix to the minor one, and a globular N-terminal end (NC3) [8]. This collagen is the most abundant collagen in vertebrates, and it plays a structural role by contributing to the molecular architecture, shape and mechanical properties of tissues [37].

(2) The types IX, XII, XIV, XVI, XIX and XX collagens belong to the fibril-associated collagens with interrupted tripled helices (FACITs). They are relatively short collagens, with interruptions in the triple helical domain and can be found at the surface of collagen fibrils. These molecules are mostly heterotrimers and carry a glycosaminoglycan side chain [8,31]. These collagens are involved in the integrity and stability of the ECM, modulating the formation and size of the collagen fibrils and controlling cellular organization in the ECM [38].

(3) Collagens forming networks are longer than classical fibril-collagens and can give rise to different kinds of networks depending on the collagen type [10]. These collagens include collagen types IV, VI, VIII and X. They are non-fibrillar collagens that aggregate linearly or laterally to form open networks. The collagen networks act as supporting structures for cells and tissues, serve as selective molecular filters and barriers and function as anchor for neighboring cells [39].

(4) The group of transmembrane collagens (MACITs) is comprised of type XIII, XVII, XXIII and XV collagens. These collagens are homotrimers of an α-chain which contains an N-terminal intracellular domain, a hydrophobic transmembrane stretch, and a large extracellular C-terminus. All members of this group are also shed from the cell surface, generating soluble forms. They are found in numerous cell types and stand out for its cell adhesive properties [10,40].

(5) Type XV and XVIII collagens are multiplexins that are non-fibrillar collagens and have multiple interruptions within its collagenous domain enabling more structural flexibility [41]. These collagens occur in the epithelial and endothelial basement membrane zones of a wide variety of tissues. Their biological roles are essentially separate, that of collagen XV in the muscle and that of collagen XVIII in the eye [8].

(6) Type VII collagen is the main constituent of the anchoring fibrils, structures that mediate the adhesion of the epidermis onto the dermis. It consists of a central collagenous triple-helical domain flanked by NC1 and NC2 domains [42,43].

(7) Type VI collagen is the archetypal beaded filament-forming collagen. It is widely expressed and holds up tissue integrity. Collagen VI monomers are made up of short triple helical domains, which aggregate linearly to form beaded filaments or laterally through their globular domains, thus creating 3D networks [44,45].

4. Types of Collagens in Articular Cartilage

There are numerous subtypes of collagens in the articular cartilage (Table 1). In healthy joint hyaline cartilage, there are main collagens (type II, IX, and XI collagen) and minor collagens (type III, IV, V, VI, X, XII, XIV, XVI, XXII, and XXVII collagens). In articular cartilage with any damage or pathology type I collagen could be found.

Table 1. Types of collagens that may be present in articular cartilage.

Collagen	Chains	Genes	Clasification	%*	Distribution in articular cartilage	Tissue distribution
Type I	$[\alpha1(I)]_2\alpha2(I)$	*COL1A1* *COL1A2*	Fibrill-forming collagen	0 %	Fibrocartilage	Bone, skin, cornea, and many interstitial connective tissues with the exception of hyaline cartilage, brain and vitreous body.
Type II	$[\alpha1(II)]_3$	*COL2A1*	Fibrill-forming collagen	90-95 %	ECM of all zones	Cartilage, vitreous, and intervertebral disc.
Type III	$[\alpha1(III)]_3$	*COL3A1*	Fibrill-forming collagen	n/a	n/a	Bloods vessels, uterus, bowel, skin, tendon, ligament, cartilage, periodontal ligament, and synovial membranes.
Type IV	$[\alpha1(IV)]_2\alpha2(IV)$ $\alpha3(IV)\alpha4(IV)\alpha5(IV)$ $[\alpha5(IV)]_2\alpha6(IV)$	*COL4A1* *COL4A2* *COL4A3* *COL4A4* *COL4A5* *COL4A6*	Network-forming collagen	n/a	PCM	Skin, basement membranes, lung, kidney, cochlea eye, smooth muscle, oesophagus, and cartilage.
Type V	$\alpha1(V)_2\alpha2(V)$	*COL5A1* *COL5A2*	Fibrill-forming collagen	n/a	PCM	Adipose tissue, skeletal muscle, cartilage, pancreatic islets, skin, placenta, and lung.

Type VI	α1(VI)α2(V)α3(V) α1(VI)α2(V)α4(V) α1(VI)α2(V)α5(V) α1(VI)α2(V)α6(V)	*COL6A1* *COL6A2* *COL6A3* *COL6A4* *COL6A5* *COL6A6*	Beaded filament collagen	1-2 %	PCM	Skin, cornea, blood vessels, heart, lung, adipose tissue, nervous, pancreas, bone, cartilage, and muscle.
Type IX	α1(IX)α2(IX)α3(IX)	*COL9A1* *COL9A2* *COL9A3*	FACIT	1-5 %	ECM of all zones and growth plate in adults	Cartilages, eye vitreum, avian cornea, ear, and intervertebral disc.
Type X	$[\alpha1(X)]_3$	*COL10A1*	Network-forming collagen	1 %	Calcified zone and hypertrophic cartilage	Hypertrophic cartilage and the calcified zone.
Type XI	α1(XI)α2(XI)α3(XI)	*COL11A1* *COL11A2* *COL2A1*	Fibrill-forming collagen	1-5 %	ECM of all zones and PCM	Cartilage, tendons, trabecular bone, testis, trachea, skeletal muscle, placenta, ovarian, lung, and brain.

*: percentage of collagen in healthy articular cartilage; ECM: extracellular matrix; PCM: pericellular matrix; FACIT: fibril-associated collagens with interrupted triple helices. n/a: no studies have been found to support it.

4.1. Main Collagens of Healthy Articular Cartilage

4.1.1. Type II Collagen

Type II collagen is a homotrimeric molecule of three α1(II) chains, encoded by the COL2A1 gene, and mainly synthesized by chondrocytes and nucleus pulposus cells [46,47]. It is expressed, synthesized, and secreted into the ECM as two isoforms (IIA and IIB). These isoforms are generated in a developmentally regulated manner by alternative splicing of exon 2. Chondroprogenitor cells synthesize predominantly IIA isoforms (containing exon 2), while differentiated chondrocytes produce mainly IIB transcripts (devoid of exon 2) [46,48].

Type II collagen is a fibrillar collagen that is restricted to cartilages, vitreous and intervertebral discs [49]. The mature articular cartilage comprises more than 90–95% of cartilage collagen. Type II and type XI collagen co-polymerize with type IX collagen to form a heteropolymeric fibrillar framework that gives cartilage its tensile strength [3,50,51].

Type II collagen, together with other proteins and PG, can form complex extracellular scaffolds to bear mechanical forces, maintain physiological homeostasis, and provide anchoring sites for chondrocytes, ECM molecules, and growth factors. Degradation and reduction of type II collagen are frequently observed in osteoarthritic cartilage. The type II collagen decrease in osteoarthritic cartilage is thought to be caused by chondrocyte hypertrophy. In addition to its structural function, type II collagen is an important extracellular signaling molecule that can regulate chondrocytes proliferation, metabolism, and differentiation, similar to soluble signals [52].

4.1.2. Type IX Collagen

Type IX collagen is a heterotrimer composed of α1(IX), α2(IX) and α3(IX) chains encoded by genes COL9A1, COL9A2 and COL9A3, respectively, and belong to the group of FACITs collagens [53,54].

This collagen is found mostly in cartilages, but it also occurs in the eyes, ear, and intervertebral discs, always in co-existence with type II collagen [55].

In regard to articular cartilage, type IX collagen constitutes 1% to 5% of total collagen in adult humans and 10% of that in fetus. It is proposed to stabilize the fibrillar and proteoglycan networks via lateral association with type II and type XI collagen [3,13,56].

Several studies showed that type IX collagen may play important roles in the pathogenesis of arthritis diseases, the formation of a stable collagen network and in the

maintenance of cartilage organization and integrity [57,58]. Loss of type IX collagen in aging articular cartilage may results in a weaker matrix that is more susceptible to degradation [59]. In addition, type IX collagen mutations in human have been linked with the autosomal dominant diseases, multiple epiphyseal dysplasia, characterized by short stature and severe joint pain caused by early onset of osteoarthritis (OA) [59]. Therefore, type IX collagen is crucial for the maintenance of cartilage matrix and formation of collagen meshwork [3,13,53]. The reduced level of type IX collagen may contribute to the pathogenesis of osteoarthritis [13].

4.1.3. Type XI Collagen

Type XI collagen is composed of three α-chains, α1(XI), α2(XI), and α3(XI), which are encoded by COL11A1, COL11A2, and COL2A1, respectively [60]. The α3(XI) chain of type XI procollagen and the α1(II) chain of type II procollagen are encoded by the same gene, being α3(XI) chain, an over-hydroxylated version of α1(II) [46,61].

This collagen is a cartilage-specific ECM protein important for cartilage collagen fibril formation and for ECM organization, but it is also broadly distributed in tendons, trabecular bone, testis, trachea, skeletal muscle, placenta, ovarian, lung and the neoepithelium of the brain [60,62,63].

In fetal cartilage, type XI collagen represents around 10% of total collagen, while in adult human cartilage its presence decreases to 3% [12]. In fetal cartilage, type XI collagen consists of molecules containing three genetically distinct chains α1(XI), α2(XI), and α3(XI) in a 1:1:1 ratio. However, from mature articular cartilage, purified type XI collagen also includes about equal amounts of α1(V) and α1(XI) chains, suggesting the existence of type V/XI hybrid molecules in the matrix [64].

In cartilage, type XI collagen is a minor component of collagen fibrils, but it is essential for the interaction between PG aggregates and collagens [60]. It is polymerized to form the core of type II collagen fibrillogenesis and regulates type II fibril diameters in the cartilage [46]. Type XI collagen molecules are found in thin cartilage fibrils composed of four micro-fibrils, two of which are type II collagen and two of which are type XI collagen, surrounded by ten type II micro-fibrils [65].

Type XI collagen is the first cartilage collagen deposited by mesenchymal stem cells undergoing chondrogenic differentiation [66], suggesting its involvement in the regulation of cartilage formation [67]. It is preferentially retained at the chondrocyte surface and involved in the organization of the PCM via interaction with cartilage PG [13].

Mutations in type XI collagen cause various types of chondrodysplasias which are known as “type XI collagenopathies.” Some of these chondrodysplasias in human include Stickler syndrome type II, Marshall syndrome, and oto-spondylo-megaepiphyseal dysplasia [13,64,67]. Furthermore, it has been shown that a type XI collagen mutation results in increased degradation of type II collagen in articular cartilage [13].

4.2. Minor Collagens of Healthy Articular Cartilage

4.2.1. Type III Collagen

Type III collagen is a homotrimer of three α1(III) chains, which are encoded by COL3A1 gene [49].

This collagen is the second most abundant collagen type in human body, classified as one of the major fibrillar collagens. It constitutes about 5–20% of the entire collagen content in the human body [68,69]. Type III collagen is found as a major structural component in hollow organs such as large bloods vessels, uterus and bowel, tissues that must withstand stretching. It is also found as a copolymer with type I collagen in many tissues, including skin, tendon, ligament, vascular walls, periodontal ligament, and synovial membranes [70].

Type III collagen provides tensile strength and integrity for many organs, but also other different functions has been reported for this collagen. One of the earliest studies on type III collagen, carried out by Balleisen et al. [71], showed that it influences the aggregation of human platelets. Subsequent studies showed that the platelets interact with type III collagen through specific glycoproteins [72,73]. Type III collagen also functions in cell adhesion, migration, proliferation, and differentiation through its interaction with integrins [70].

Regarding the role that type III collagen plays in cartilage, there are some controversies, because we can find it both in healthy cartilage and in aged or osteoarthritic cartilage. Thus, the content of type III collagen could vary markedly at different stages of development and disease. Some studies confirmed that a small but significant amount of type III collagen becomes deposited in articular cartilage of mature joints, concentrated in the matrix surrounding chondrocytes throughout the depth of the tissue and particularly prominent in human osteoarthritic joints [74,75]. According to Hosseininia et al. [76], type III collagen in the human articular cartilage increases in the territorial matrix of aging individuals, although it remains unclear whether this increase represents a protective response to cartilage degeneration or a contributor to the pathological process. Moreover, in human OA, type III collagen is significantly up-regulated in cartilage [76]. Another study revealed that type III collagen is found in the adult

human cartilage, and it has been suggested that its role is to act as a modifier of the fibril network composed of type II collagen together with other minor collagens during tissue healing [49,70]. Wang et al. showed that type III collagen is a crucial matrix constituent for the establishment of normal cartilage ECM [68].

Mutations in the COL3A1 gene cause the vascular type of Ehlers-Danlos syndrome, which is a rare, life-threatening genetic disease. Other disease phenotypes associated with COL3A1 include a brain abnormality characterized by frontoparietal polymicrogyria, and many fibrotic diseases [70].

4.2.2. Type IV Collagen

Type IV collagen has a triple-helical structure composed of 3 of 6 different α- chains (α1, α2, α3, α4, α5, and α6) encoded by COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, and COL4A6. These helical polypeptide α-chains form triple-helical isoforms, which only 3 have been identified: α1(IV)α1(IV)α2(IV) or (α112), α3(IV)α4(IV)α5(IV) or (α345), and α5(IV)α5(IV)α6(IV) or (α556). They are assembled into three major networks (α112:α112, α112:α556, α345:α345), interconnected by NC1 domain [29,77,78,79].

This collagen is identified primarily in the skin, is the most important structural component of basement membranes [31,80]. α1 and α2 chains are expressed ubiquitously in basement membranes, although type IV collagen α3, α4, α5, and α6 chains have a tissue specific distribution [80]. The α3α4α5 network has mainly been identified in lung alveoli, kidney, testis, cochlea, and eye, whereas the α5α5α6 network has been located in skin, smooth muscle cells, oesophagus, and Bowman's capsule of the kidney [29,81].

Regarding cartilage, type IV collagen is observed in the PCM of healthy cartilage tissues but generally absent in degenerated and fibrotic cartilage tissues [79], although there is evidence that it can be found in PCM of degenerative hyaline cartilage [82]. It was observed that type IV collagen was in both in vitro engineered cartilaginous constructs and in vivo cartilage repair samples, in addition they shown that chondrocytes were capable of synthesizing type IV collagen [82].

The specific isoform of type IV collagen α112(IV) was identified as the unique type in articular cartilage. This isoform contains arrestenand canstatin that are anti-angiogenic protein fragments. Since articular cartilage is an avascular structure, type IV collagen isoform α112(IV) with their unique anti-angiogenic properties might be involved in the temporal control of vascularization during cartilage repair and in cartilage homeostasis [79].

Mutations in type IV collagen α3 to α6 chains causes Alport's syndrome associated with glomerulonephritis, sensorineural deafness and eye abnormalities [80,81].

4.2.3. Type V Collagen

The collagen type V triple helix is formed as a heterotrimer by one α1(V) chain and two α2(V) chain, which are encoded by COL5A1 and COL5A2 genes. It is widely distributed in tissues as α1(V)2α2(V) that integrate into fibrils of the abundant type I collagen and regulate the geometry of resulting col(I)/col(V) heterotypic fibrils [83,84]. There is a third col(V) chain, α3(V), which can be found in α1(V)α2(V)α3(V) heterotrimers and has more limited tissue distribution than α1(V)2α2(V) heterotrimers. Tissues in which the α3(V) chain have been detected in white adipose tissue, skeletal muscle, and pancreatic islets, where these chains are important for proper functioning of the adipocytes, myofibers, and pancreatic β cells, respectively [84,85].

This collagen is classified as a minor fibrillar collagen that under normal physiologic conditions assembles into heterotypic fibrils with the major fibrillar collagen type I [86], although it can also be found assembled as type V/XI collagen [87].

Despite being a minor collagen, type V collagen is an abundant protein in skin, placenta and lung, and essential for tissue elasticity and compliance [88]. Type V collagen plays a basic function in the formation of fibrillar collagen mesh and has an important role in fibrogenesis control or fiber size regulation. Furthermore, the type V collagens contribute to the linking between stromal collagen and basement membrane, being important for cellular adhesion and matrix-repairing process [89].

As regards to articular cartilage, D.R. Eyre and J.J. Wu. [50], isolated mature articular cartilage, where type XI collagens included a significate pool of α1(V) chains which implied the presence of V/XI hybrid molecules. Later, a study carried out by J.J. Wu et al. [87], proved the presence of V/XI hybrid molecules in articular cartilage showing an accumulation of collagen α1(V) chains as articular cartilage matures. Although it has been shown that the type V collagen is present in articular cartilage [90], no studies have been found that discuss its role in articular cartilage.

One of the main diseases associated with a defect in type V collagen is classic Ehlers-Danlos syndrome. It is a rare autosomal dominant connective tissue disorder that is primarily characterized by skin hyperextensibility, abnormal wound healing/atrophic scars, and joint hypermobility [91].

4.2.4. Type VI Collagen

Type VI collagen is a heterotrimer that has a characteristic beaded filamentous structure of tetrameric units that consists of three different α-chains, α1(VI), α2(VI), and α3(VI), which are encoded by the genes COL6A1, COL6A2, and COL6A3. Recently, 3 novel subunits of type VI collagen are revealed, α4(VI), α5(VI), and α6(VI) chains encoded by the COL6A4, COL6A5, and COL6A6 genes, which are highly homologous to the α3(VI) chain [92,93,94].

This collagen is found in almost all tissue and distributed among tissues such as skin, cornea, blood vessels, heart, lungs, adipose tissue, nervous tissues, pancreas, bones, cartilage, and muscle [45,92,93].

There is a high affinity between collagen VI and numerous ECM components, as biglycan, decorin, hyaluronan, fibronectin, perlecan and heparin, as well as with the cell membrane. Thus, type VI collagen has been hypothesized to play an essential role in mediating cell-matrix interactions as well as intermolecular interactions in various tissues [95]. In addition, this collagen participates in the maintenance of tissue integrity creating cell-matrix and matrix-matrix interactions [96].

In some studies, total type VI collagen, a putative marker of mesenchymal activation, has been suggested to be an indicator of early architectural remodeling in liver fibrosis [97,98].

In skeletal muscle, type VI collagen is present in the ECM where it functions to anchor the basement membrane to underlying interstitial tissues [99]. Mutations in the COL6A1, COL6A2, and COL6A3 genes lead to a continuous spectrum of disorders characterized by muscle weakness and connective tissue abnormalities [100]. These mutations have been identified as causative in Ullrich congenital muscular dystrophy, Bethlem myopathy and myosclerosis myopathy [45,101].

In articular cartilage, type VI collagen is present in small amounts (1–2%) forming a network that anchors the chondrocytes to the PCM through its interaction with a wide variety of ECM proteins, including type II collagen, type XIV collagen, cartilage matrix protein or matrilin-1, hyaluronan, decorin, and fibronectin; which implies the attachment and integrity of chondrocytes [13,95,102].

In normal adult human articular cartilage, the PCM is typically defined by the exclusive presence and localization of type VI collagen around the chondrocytes [24]. Some studies confirm that type VI collagen could contribute to PCM structural integrity and mechanical

properties. Furthermore, it may serve as a filter or transducer for biochemical and/or biomechanical signals from the cartilage ECM [13,95].

Studies conducted in mice showed that those with type VI collagen deficiency exhibited accelerated development of hip OA, a delayed secondary ossification process, increased chondrocyte swelling and a loss of the stiffness of the articular cartilage PCM. These finding suggest that type VI collagen plays an essential role in transmitting mechanical and osmotic stresses from the ECM to the chondrocytes [95,103].

4.2.5. Type X Collagen

Type X collagen is a homotrimeric collagen which consists of three identical α1(X) chains, encoded by the COL10A1 [104,105].

This collagen is a specific collagen in cartilage. It constitutes about 1% of total collagen in adult human articular cartilage [13]. It is synthesized by hypertrophic chondrocytes during enchondral bone formation and is found exclusively in the hypertrophic cartilage and the calcified zone of articular cartilage [106]. Thus, the 45% of the total collagens produced by mature hypertrophic chondrocytes are type X [104]. As the most widely used marker for chondrocyte hypertrophy, type X collagen in normally expressed in human OA cartilage especially in the vicinity of lesions, but not in human healthy articular cartilage [107,108]. Hypertrophic chondrocytes express a variety of proteins and enzymes as type X collagen, matrix metalloproteinase 13 (MMP13), alkaline phosphatase, which do not seem to exit in normal proliferating chondrocytes [109]. These chondrocytes increase their volume and secrete a specialized ECM rich in type X collagen. This matrix attracts blood vessels and bone precursor cells leading to bone development, but also the process of endochondral ossification has been reported in cartilaginous tumors [110].

The biological function of type X collagen in thought to maintain tissue stiffness, regulate chondrocytes metabolism and interact with hypertrophic chondrocytes [106,111]. It also facilitates the process of calcification, the normal distribution of matrix vesicles and PG within the growth plate [13,111].

Considering that ECM surrounding chondrocytes mineralizes to be replaced by bone marrow and bone, it was suggested that collagen X may be associated with the mineralization process [39]. Its expression at sites of chondrocyte hypertrophy and calcification suggest that type X collagen support endochondral bone growth and development during the degradation of ECM in cartilage [13,109].

Mutations of the COL10A1 gene are causative for the disease Schmid type metaphyseal chondrodysplasia (MCDS; MIM 156500) impeding endochondral ossification in the metaphyseal growth plate. This leads to growth deficiency and skeletal deformities with short limbs [31]. These mutations have been also considered key in spondylometaphyseal dysplasia (SMD) which is a group of genetic skeletal disorders that show abnormal development of spine and metaphysis of long tubular bones [112].

4.2.6. Type XII, XIV, XVI, XXII, and XXVII Collagens

Type XII belong to the group of FACITs collagens. Immunohistochemistry, staining and fibrillogenesis studies showed that type XII collagen can be incorporated into type I collagen fibrils in dense connective tissues and bone [113]. Type XII collagen is associated with articular cartilage and growth plate region during rat forelimb development and may be necessary for microenvironment that support hyaline cartilage formation [13,113]. Type XII collagen was also described in the secretome of human passaged chondrocytes [114]. Type XIV collagen is a large, non-fibrillar ECM protein which also belong to the group of FACITs. Immunofluorescence localization showed that type XIV collagen was prominent at the ligament-bone junction, and in bovine cartilage. Type XIV collagen localizes relatively uniformly throughout the articular cartilage but is absent from growth plate regions [13]. Both, type XII and XIV collagens, are often found in areas of high mechanical stress, and have roles in fibrillogenesis and maintaining the integrity and mechanical properties of the tissue [115].

Type XVI and XXII collagens belong to the group of FACITs collagens. Type XVI collagen has been identified in the territorial matrix of the chondrocytes, associating with thin weakly banded collagen fibrils containing type II and XI collagen [116]. It may be incorporated into structurally and functionally discrete matrix aggregates in cartilage [13]. Type XXII collagen is expressed at the junction between synovial fluid and surface of articular cartilage and associated with the extrafibrillar matrix in cartilage [117].

Type XXVII collagen is a fibril-forming collagen. It is mainly localized at sites of transition from cartilage to bone, and in the matrix surrounding proliferative chondrocytes in the epiphyseal growth plate [118,119]. It is believed to play a key structural role in the ECM of the growth plate and is required for the organization of the proliferative zone [119].

4.3. Articular Collagen Types Synthetized in Pathological Processes

Type I Collagen

The collagen type I triple helix is formed as a heterotrimer by two α1(I) chains and one α2(I) chain [α1(I)2α2(I)], which are encoded by COL1A1 and COL1A2 genes [49,120].

Type I collagen is the most abundant collagen in the body. It forms more than 90% of the organic mass of bone and is the major collagen of tendons, skin, ligaments, cornea, and many interstitial connective tissues with the exception of very few tissues such as hyaline cartilage, brain and vitreous body [31,36]. It is synthesized in large quantities by fibroblasts, osteoblasts, and to a lesser extent by nearly all other tissue cells [36,121,122].

This collagen is always incorporated into heterofibrils containing either type III collagen in skin and reticular fibers, type V collagen in bone, tendon, cornea, and other tissues, or in heterofibrils containing both collagens [36,123,124]. Type I/III collagen heterofibrils are a constituent of reticular fibers of most parenchymal tissues such as lung, kidney, liver, muscle, or spleen, with the exception of hyaline cartilage, brain and vitreous humor [125].

In most organs and notably in tendons and fascia, type I collagen provides tensile stiffness and in bone, it defines considerable biomechanical properties concerning load bearing, tensile strength, and torsional stiffness, ensuring the stability and integrity of the tissues [31]. In addition to its biomechanical properties, type I collagen is important as adhesive substrate for many cells and plays a major role in organ and tissue development, in cell migration, proliferation and differentiation, and in wound healing, tissue remodeling and hemostasis [121].

Type I collagen is generally used as a marker for fibrous connective tissue, bone, and dentin [126,127]. Fibrocartilage is different from articular hyaline cartilage due to the presence of type I collagen and the lower content of glycosaminoglycans [128]. Pathological and disorganized regeneration of tissue in a variety of organs after injury often results in deposition of excessive fibrotic tissue with inferior biomechanical properties, as occurs with articular cartilage, where fibrocartilage is created as repair tissue [128,129]. Full-thickness disruption of articular cartilage by trauma to synovial joints is one example in which highly specialized hyaline cartilage is replaced by biomechanically inferior, disorganized fibrotic tissue enriched in collagen type I [130].

As mentioned above, hyaline articular cartilage contains type II collagen, fibrocartilage contains a mixture of type I and II collagens, and fibrous tissue contains type I collagen [127,128,129,131,132]. In this way, type II collagen cannot be used solely to determine whether

a cartilage is hyaline type, since it can be also found in fibrocartilage, so it would be necessary to determine no type I collagen absence [133].

Mutations in the genes COL1A1 and COL1A2 cause of most cases of osteogenesis imperfecta. It is a heterogeneous hereditary disorder of bone matrix formation and remodeling that causes bone fragility and deformity, blue sclera, short structure, dentinogenesis imperfecta, and hearing loss [134,135].

4.4. Collagens in Pathological Situations

OA is a disease that is often associated with age, female gender, obesity, muscle weakness, and joint injuries [136]. This disease transforms the main collagen of the articular cartilage, type II collagen, into a mixture of type I, II and III collagens. It can induce a 100-fold upregulation of type I collagen and 6-fold of type III collagen, and a 5-fold downregulation of type II collagen [128]. Additionally, it was reported that a higher amount of type VI collagen is found in osteoarthritic cartilage that could be a consequence of a protective effect for chondrocytes [137]. Other authors also described an increase in type III and VI collagen in osteoarthritic articular cartilage, however they observed that a greater amount of type II collagen is also deposited [137,138].

The wound-healing role of major and minor collagens likely to play during the articular regeneration has not been completely deciphered, thus, more efforts should be made to elucidate this potential role.

5. Role of Collagen Fibrils in the Quality of Repaired Tissue in Chondral Defects

As mentioned above, the healthy articular cartilage is characterized by presenting a hyaline structure. Several authors have reported that using different current therapies for chondral lesions they have been able to obtain a hyaline-like repair tissue [139,140,141,142]. Thus, in this context, the basic question that is often asked regarding the cartilage regeneration after damage is about the role of all collagen types in the repair assessment, and the threshold to consider a regenerated cartilage as hyaline-like cartilage including ECM evaluation.

When evaluating the quality of a repaired cartilage, must be assessed the morphological and structural characteristics of the repaired regenerated tissue must be, and the presence and arrangement of collagens in the ECM.

5.1. Methods of Cartilage Tissue Reparation

There are several therapeutic modalities that aim to reduce pain and restoring cartilage function. These strategies can be medical treatments such as pharmacological therapy, or surgical treatments such as palliative therapies (e.g., chondroplasty and debridement), repair techniques (e.g., drilling and microfracture), restorative techniques (e.g., tissue engineering) or prosthetic replacement [90,142,143,144,145,146,147].

Debridement is used to reduce pain; however, there is no obvious physiological or pathological evidence to show that it is beneficial for cartilage repair [148]. Perforation and microfracture repair techniques are considered first-line treatments given their minimally invasive nature, technical ease, and low cost [142,143]. They can be used alone or in combination with other tissue engineering techniques [149]. Tissue engineering seeks to repair damaged cartilage by introducing an optimized combination of cells, scaffold, and bioactive factors that can be transplanted into a patient [150]. Biomaterial scaffolds or hydrogel are being made with collagens given their ability to promote cartilage formation, the most common being type I and II collagen hydrogels [151,152]. Some of the techniques most frequently used in tissue engineering to repair chondral defects are autologous chondrocyte implantation, osteochondral autograft transfer and osteochondral allograft [143,145].

Despite the large number of methods that exist to treat chondral defects, they cannot restore a normal cartilage, and one of the main limitations in fibrocartilage formation without lasting improvements [19,145]. Much information is unknown about the intrinsic repair processes of damaged cartilage, and consequently the search for a treatment that would restore normal articular cartilage. For this reason, it is so important to evaluate in depth the repair quality of the repaired cartilage, including an exhaustive assessment of collagens.

5.2. Methods for Evaluating Morphological and Structural Characteristics of a Repaired Cartilage

The assessment of the morphological and structural characteristics is carried out with histopathological techniques such us HE staining, toluidine blue and Safranin O/fast green staining.

There are several histopathological evaluation methods that help us determine how similar repaired cartilage is compared to healthy cartilage [131,133,153,154]. These are detail studies that allow us to obtain standardized assessment methods. In summary, all of them and most authors agree that therapies aimed at developing successful hyaline cartilage regeneration

should obtain a smooth surface predominantly rounded, lagoon-organized chondrocytes of low cell density a repaired tissue capable of integrating with living native cartilage, and good condition of subchondral bone [11,15,17,24,25,155].

5.3. Methods Allowing Evaluating Collagen of a Repaired Cartilage

Collagens have been visualized using a variety of techniques, but the most common are histopathologic techniques because of they are less expensive and sample preparation is less complicated [131]. Toluidine blue and picrosirius red staining are the most widely used. It is important to observe these sections stained with polarized light to view the orientation of the collagen fibers [131,156,157]. In addition, it is very common to use immunohistochemical techniques that allow us to visualize the presence and location of a certain collagen in the cartilage. Immunohistochemically marked tissues are observed under an optical microscope [90,133]. Despite involving a more complicated preparation and being more expensive, some authors use transmission electron microscopy, atomic force microscopy or structural illuminating microscopy, obtaining a higher resolution visualization of collagen fibers [142,157,158].

Regarding assessment methods, there is no standardized evaluation that determine the intrinsic quality of all collagen types in the repaired cartilage. Some of the previously mentioned methods [131,133,153,154], make a brief reference to the main collagens (type II, IX, and XI collagens), but there is a lack of information in considering the rest of the collagens contained in the articular cartilage to evaluate the quality of the repair and whether this repaired tissue can be considered as hyaline cartilage.

Therefore, based on the information reported in several studies [3,12,13,14,24,50,51,56,74,75,76,79,82,90,95,102,106,107,108,113,114,115,116,117,118,119, 127,128,130,131,132,133], the composition of main and minor collagen types in ECM to consider the quality of a repaired cartilage as hyaline-type cartilage should be:

- Main collagens:
  - 90–95% type II collagen distributed throughout all areas of the ECM.
  - 1–5% type IX collagen distributed throughout all areas of the ECM.
  - 1–5% type XI collagen distributed throughout all areas of the cartilage and around the chondrocytes.
- Minor collagens:
  - 1–2% type VI collagen distributed around the chondrocytes.
  - 1% type X collagen present only in the calcified area.

- Presence of type IV and V collagen around the chondrocytes.
- Possible presence of type III, XII, XIV, XVI, XXII, and XXVII collagens.

- Absence of type I collagen.

6. Conclusions

There are many types of collagens present in the articular cartilage, both main and minor collagens, all of which are crucial for maintaining cartilage health. To accurately determine the repair tissue quality after a chondral lesion, the complete structure of the repaired cartilage tissue, including cells and their environment and main and minor collagen types in ECM, should be included in the histopathological evaluation methods.

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References

1. Ricard-Blum S. The Collagen Family. Cold Spring Harb. Perspect. Biol. 2011;3:a004978. doi: 10.1101/cshperspect.a004978.
2. Baumann S., Hennet T. Collagen accumulation in osteosarcoma cells lacking GLT25D1 collagen galactosyltransferase. J. Biol. Chem. 2016;291:18514–18524. doi: 10.1074/jbc.M116.723379.
3. Tanaka N., Tashiro T., Katsuragawa Y., Sawabe M., Furukawa H., Fukui N. Expression of minor cartilage collagens and small leucine rich proteoglycans may be relatively reduced in osteoarthritic cartilage. BMC Musculoskelet. Disord. 2019;20:232. doi: 10.1186/s12891-019-2596-y.
4. Cohen N.P., Foster R.J., Mow V.C. Composition and dynamics of articular cartilage: Structure, function, and maintaining healthy state. J. Orthop. Sports Phys. Ther. 1998;28:203–215. doi: 10.2519/jospt.1998.28.4.203.
5. Cumming M.H., Hall B., Hofman K. Isolation and characterisation of major and minor collagens from hyaline cartilage of hoki (Macruronus novaezelandiae) Mar. Drugs. 2019;17:223. doi: 10.3390/md17040223.
6. Miller E.J., Matukas V.J. Chick cartilage collagen: A new type of alpha 1 chain not present in bone or skin of the species. Proc. Natl. Acad. Sci. USA. 1969;64:1264–1268. doi: 10.1073/pnas.64.4.1264.
7. Miller E.J., Gay S. Collagen: An Overview. Methods Enzymol. 1982;82:3–32.
8. Ricard-Blum S., Ruggiero F. The collagen superfamily: From the extracellular matrix to the cell membrane. Pathol. Biol. 2005;53:430–442. doi: 10.1016/j.patbio.2004.12.024.

9. Kadler K.E., Baldock C., Bella J., Boot-Handford R.P. Collagens at a glance. J. Cell Sci. 2007;120:1955–1958. doi: 10.1242/jcs.03453.

10. Gordon M.K., Hahn R.A. Collagens. Cell Tissue Res. 2010;339:247–257. doi: 10.1007/s00441-009-0844-4.

11. Bhosale A.M., Richardson J.B. Articular cartilage: Structure, injuries and review of management. Br. Med. Bull. 2008;87:77–95. doi: 10.1093/bmb/ldn025.

12. Eyre D. Collagen of articular cartilage. Arthritis Res. Ther. 2002;4:30–35. doi: 10.1186/ar380.

13. Luo Y., Sinkeviciute D., He Y., Karsdal M., Henrotin Y., Mobasheri A., Önnerfjord P., Bay-Jensen A. The minor collagens in articular cartilage. Protein Cell. 2017;8:560–572. doi: 10.1007/s13238-017-0377-7.

14. Wu L., Petrigliano F.A., Ba K., Lee S., Bogdanov J., McAllister D.R., Adams J.S., Rosenthal A.K., Van Handel B., Crooks G.M., et al. Lysophosphatidic acid mediates fibrosis in injured joints by regulating collagen type I biosynthesis. Osteoarthr. Cartil. 2015;23:308–318. doi: 10.1016/j.joca.2014.11.012.

15. Sophia Fox A.J., Bedi A., Rodeo S.A. The basic science of articular cartilage: Structure, composition, and function. Sports Health. 2009;1:461–468. doi: 10.1177/1941738109350438.

16. Luria A., Chu C.R. Articular Cartilage Changes in Maturing Athletes: New Targets for Joint Rejuvenation. Sports Health. 2014;6:18–30. doi: 10.1177/1941738113514369.

17. Thorp H., Kim K., Kondo M., Maak T., Grainger D.W., Okano T. Trends in Articular Cartilage Tissue Engineering: 3D Mesenchymal Stem Cell Sheets as Candidates for Engineered Hyaline-Like Cartilage. Cells. 2011;10:643. doi: 10.3390/cells10030643.

18. Sun Y., Yan L., Chen S., Pei M. Functionality of decellularized matrix in cartilage regeneration: A comparison of tissue versus cell sources. Acta Biomater. 2018;74:56–73. doi: 10.1016/j.actbio.2018.04.048.

19. Decker R.S. Articular cartilage and joint development from embryogenesis to adulthood. Semin. Cell Dev. Biol. 2017;62:50–56. doi: 10.1016/j.semcdb.2016.10.005.

20. Medvedeva E.V., Grebenik E.A., Gornostaeva A.N., Telpuhov V.I., Lychagin A.V., Timashev P.S., Chagin A.S. Repair of damaged articular cartilage: Current approaches and future directions. Int. J. Mol. Sci. 2017;19:2366. doi: 10.3390/ijms19082366.

21. Vinod E., Boopalan P.R.J.V.C., Sathishkumar S. Reserve or Resident Progenitors in Cartilage? Comparative Analysis of Chondrocytes versus Chondroprogenitors and Their Role in Cartilage Repair. Cartilage. 2018;9:171–182. doi: 10.1177/1947603517736108.

22. Patel J.M., Saleh K.S., Burdick J.A., Mauck R.L. Bioactive factors for cartilage repair and regeneration: Improving delivery, retention, and activity. Acta Biomater. 2019;93:222–238. doi: 10.1016/j.actbio.2019.01.061.

23. Phull A.R., Eo S.H., Abbas Q., Ahmed M., Kim S.J. Applications of Chondrocyte-Based Cartilage Engineering: An Overview. BioMed Res. Int. 2016;2016:1879837. doi: 10.1155/2016/1879837.

24. Wilusz R.E., Sanchez-Adams J., Guilak F. The structure and function of the pericellular matrix of articular cartilage. Matrix Biol. 2014;39:25–32. doi: 10.1016/j.matbio.2014.08.009.

25. Tiku M.L., Sabaawy H.E. Cartilage regeneration for treatment of osteoarthritis: A paradigm for nonsurgical intervention. Ther. Adv. Musculoskelet. Dis. 2015;7:76–87. doi: 10.1177/1759720X15576866.

26. Chau M., Lui J.C., Landman E.B.M., Späth S.S., Vortkamp A., Baron J., Nilsson O. Gene expression profiling reveals similarities between the spatial architectures of postnatal articular and growth plate cartilage. PLoS ONE. 2014;9:103061

27. Sorushanova A., Delgado L.M., Wu Z., Shologu N., Kshirsagar A., Raghunath R., Mullen A.M., Bayon Y., Pandit A., Raghunath M., et al. The Collagen Suprafamily: From Biosynthesis to Advanced Biomaterial Development. Adv. Mater. 2019;31:1801651. doi: 10.1002/adma.201801651.

28. Fidler A.L., Boudko S.P., Rokas A., Hudson B.G. The triple helix of collagens—An ancient protein structure that enabled animal multicellularity and tissue evolution. J. Cell Sci. 2018;131:209350. doi: 10.1242/jcs.203950.

29. Regoli M., Tosi G.M., Neri G., Altera A., Orazioli D., Bertelli E. The Peculiar Pattern of Type IV Collagen Deposition in Epiretinal Membranes. J. Histochem. Cytochem. 2020;68:149–162. doi: 10.1369/0022155419897258.

30. Calvo A.C., Moreno L., Moreno L., Toivonen J.M., Manzano R., Molina N., de la Torre M., López T., Miana-Mena F.J., Muñoz M.J., et al. Type XIX collagen: A promising biomarker from the basement membranes. Neural Regen. Res. 2020;15:988–995.

31. Gelse K., Pöschl E., Aigner T. Collagens—Structure, function, and biosynthesis. Adv. Drug Del. Rev. 2003;55:1531–1546. doi: 10.1016/j.addr.2003.08.002.

32. Arseni L., Lombardi A., Orioli D. From structure to phenotype: Impact of collagen alterations on human health. Int. J. Mol. Sci. 2018;19:1407. doi: 10.3390/ijms19051407.

33. Albaugh V.L., Mukherjee K., Barbul A. Proline precursors and collagen synthesis: Biochemical challenges of nutrient supplementation and wound healing. J. Nutr. 2017;147:2011–2017. doi: 10.3945/jn.117.256404.

34. Taye N., Karoulias S.Z., Hubmacher D. The “other” 15–40%: The Role of Non-Collagenous Extracellular Matrix Proteins and Minor Collagens in Tendon. J. Orthop. Res. 2020;38:23–35. doi: 10.1002/jor.24440.

35. Senadheera T.R.L., Dave D., Shahidi F. Sea cucumber derived type I collagen: A comprehensive review. Mar. Drugs. 2020;18:471. doi: 10.3390/md18090471.

36. Bourgot I., Primac I., Louis T., Noël A., Maquoi E. Reciprocal Interplay between Fibrillar Collagens and Collagen-Binding Integrins: Implications in Cancer Progression and Metastasis. Front. Oncol. 2020;10:1488. doi: 10.3389/fonc.2020.01488.

37. León-López A., Morales-Peñaloza A., Martínez-Juárez V.M., Vargas-Torres A., Zeugolis A.I., Aguirre-Álvarez G. Hydrolyzed collagen-sources and applications. Molecules. 2019;24:4031. doi: 10.3390/molecules24224031.

38. Oudart J.B., Monboisse J.C., Maquart F.X., Brassart B., Brassart-Pasco S., Ramont L. Type XIX collagen: A new partner in the interactions between tumor cells and their microenvironment. Matrix Biol. 2017;57–58:169–177. doi: 10.1016/j.matbio.2016.07.010.

39. Knupp C., Squire J.M. Molecular packing in network-forming collagens. Sci. World J. 2003;3:558–577. doi: 10.1100/tsw.2003.40.

40. Nyström A., Kiritsi D. Transmembrane collagens—Unexplored mediators of epidermal-dermal communication and tissue homeostasis. Exp. Dermatol. 2021;30:10–16. doi: 10.1111/exd.14180.

41. Clementz A.G., Harris A. Collagen XV: Exploring its structure and role within the tumor microenvironment. Mol. Cancer Res. 2013;11:1481–1486. doi: 10.1158/1541-7786.MCR-12-0662.

42. Csorba K., Chiriac M.C., Florea F., Ghinia M.G., Licarete E., Rados A., Sas A., Vuta V., Sitaru C. Blister-inducing antibodies target multiple epitopes on collagen VII in mice. J. Cell Mol. Med. 2014;18:1727–1739. doi: 10.1111/jcmm.12338.

43. Wullink B., Pas H.H., Van Der Worp R.J., Kuijer R., Los L.I. Type VII collagen expression in the human vitreoretinal interface, corpora amylacea and inner retinal layers. PLoS ONE. 2015;10:0145502. doi: 10.1371/journal.pone.0145502.

44. Godwin A.R.F., Starborg T., Sherratt M.J., Roseman A.M., Baldock C. Defining the hierarchical organisation of collagen VI microfibrils at nanometre to micrometre length scales. Acta Biomater. 2017;52:21–32. doi: 10.1016/j.actbio.2016.12.023.

45. Izu Y., Ezura Y., Koch M., Birk D.E., Noda M. Collagens VI and XII form complexes mediating osteoblast interactions during osteogenesis. Cell Tissue Res. 2016;364:623–635. doi: 10.1007/s00441-015-2345-y.

46. McAlinden A., Traeger G., Hansen U., Weis M.A., Ravindran S., Wirthlin L., Eyre D.R., Fernandes R.J. Molecular properties and fibril ultrastructure of types II and XI collagens in cartilage of mice expressing exclusively the α1(IIA) collagen isoform. Matrix Biol. 2014;34:105–113. doi: 10.1016/j.matbio.2013.09.006.

47. Lian H., Gao B., Wu Z., Qiu X., Peng Y., Liang A., Xu C., Su P., Huang D. Collagen type II is downregulated in the degenerative nucleus pulposus and contributes to the degeneration and apoptosis of human nucleus pulposus cells. Mol. Med. Rep. 2017;16:4730–4736. doi: 10.3892/mmr.2017.7178.

48. Zhu Y., Oganesian A., Keene D.R., Sandell L.J. Type IIA procollagen containing the cysteine-rich amino propeptide is deposited in the extracellular matrix of prechondrogenic tissue and binds to TGF-β1 and BMP-2. J. Cell Biol. 1999;144:1069–1080. doi: 10.1083/jcb.144.5.1069.

49. Wu J.J., Weis M.A., Kim L.S., Eyre D.R. Type III collagen, a fibril network modifier in articular cartilage. J. Biol. Chem. 2010;285:18537–18544. doi: 10.1074/jbc.M110.112904.

50. Eyre D.R., Wu J.J., Fernandes R.J., Pietka T.A., Weis M.A. Recent developments in cartilage research: Matrix biology of the collagen II/IX/XI heterofibril network. Biochem. Soc. Trans. 2002;30:893–899. doi: 10.1042/BST0300893.

51. Lai C.S., Tu C.W., Kuo H.C., Sun P.P., Tsai M.L. Type II Collagen from Cartilage of Acipenser baerii Promotes Wound Healing in Human Dermal Fibroblasts and in Mouse Skin. Mar. Drugs. 2020;18:511. doi: 10.3390/md18100511.

52. Lian C., Wang X., Qiu X., Gao B., Liu L., Liang G., Zhou H., Yang X., Peng Y., Liang A., et al. Collagen type II suppresses articular chondrocyte hypertrophy and osteoarthritis progression by promoting integrin β1−SMAD1 interaction. Bone Res. 2019;7:1–15. doi: 10.1038/s41413-019-0046-y.

53. Carlsen S., Nandakumar K.S., Holmdahl R. Type IX collagen deficiency enhances the binding of cartilage-specific antibodies and arthritis severity. Arthritis Res. Ther. 2006;8:1–8. doi: 10.1186/ar1989.

54. Bönnemann C.G., Cox G.F., Shapiro F., Wu J.J., Feener C.A., Thompson T.G., Anthony D.C., Eyre D.R., Darras T.B., Kunkel L.M. A mutation in the alpha 3 chain of type IX collagen causes autosomal dominant multiple epiphyseal dysplasia with mild myopathy. Proc. Natl. Acad. Sci. USA. 2000;97:1212–1217. doi: 10.1073/pnas.97.3.1212.

55. Eyre D.R., Pietka T., Weis M.A., Wu J.J. Covalent Cross-linking of the NC1 Domain of Collagen Type IX to Collagen Type II in Cartilage. J. Biol. Chem. 2004;279:2568–2574. doi: 10.1074/jbc.M311653200.

56. Imagawa K., de Andrés M.C., Hashimoto K., Itoi E., Otero M., Roach H.I., Golgring M.B., Oreffo R.O.C. Association of reduced type IX collagen gene expression in human osteoarthritic chondrocytes with epigenetic silencing by DNA hypermethylation. Arthritis Rheumatol. 2014;66:3040–3051. doi: 10.1002/art.38774.

57. Lohiniva J., Paassilta P., Seppänen U., Vierimaa O., Kivirikko S., Ala-Kokko L. Splicing mutations in the COL3 domain of collagen IX cause multiple epiphyseal dysplasia. Am. J. Med. Genet. 2000;90:216–222. doi: 10.1002/(SICI)1096-8628(20000131)90:3<216::AID-AJMG6>3.0.CO;2-1.

58. Czarny-Ratajczak M., Lohiniva J., Rogala P., Kozlowski K., Perälä M., Carter L., Spector T.D., Kolodziej L., Seppänen U., Glazar R., et al. A mutation in COL9A1 causes multiple epiphyseal dysplasia: Further evidence for locus heterogeneity. Am. J. Hum. Genet. 2001;69:969–980. doi: 10.1086/324023.

59. Parsons P., Gilbert S.J., Vaughan-Thomas A., Sorrell D.A., Notman R., Bishop M., Hayes A.J., Mason D.J., Duance V.C. Type IX collagen interacts with fibronectin providing an important molecular bridge in articular cartilage. J. Biol. Chem. 2011;286:34986–34997. doi: 10.1074/jbc.M111.238188.

60. Mio F., Chiba K., Hirose Y., Kawaguchi Y., Mikami Y., Oya T., Mori M., Kamata M., Matsumoto M., Ozaki K., et al. A functional polymorphism in COL11A1, which encodes the α1 chain of type XI collagen, is associated with susceptibility to lumbar disc herniation. Am. J. Hum. Genet. 2007;81:1271–1277. doi: 10.1086/522377.

61. Warner L.R., Blasick C.M., Brown R.J., Oxford J.T. Expression, purification, and refolding of recombinant collagen α1(XI) amino terminal domain splice variants. Protein Expr. Purif. 2007;52:403–409. doi: 10.1016/j.pep.2006.10.016.

62. Luo Y.Y., Karsdal M.A. Biochemistry of Collagens, Laminins and Elastin: Structure, Function and Biomarkers. 1st ed. Elsevier; London, UK: 2016. Type XI Collagen; pp. 77–80.

63. Nallanthighal S., Rada M., Heiserman J.P., Cha J., Sage J., Zhou B., Yang W., Hu Y., Korgaonkar C., Hanos C.T., et al. Inhibition of collagen XI alpha 1-induced fatty acid oxidation triggers apoptotic cell death in cisplatin-resistant ovarian cancer. Cell Death Dis. 2020;11:258. doi: 10.1038/s41419-020-2442-z.

64. Fernandes R.J., Weis M.A., Scott M.A., Seegmiller R.E., Eyre D.R. Collagen XI chain misassembly in cartilage of the chondrodysplasia (cho) mouse. Matrix Biol. 2007;26:597–603. doi: 10.1016/j.matbio.2007.06.007.

65. Tong D., Lönnblom E., Yau A.C.Y., Nandakumar K.S., Liang B., Ge C., Viljanen J., Li L., Bãlan M., Klareskog L., et al. A shared epitope of collagen type XI and type II is recognized by pathogenic

antibodies in mice and humans with arthritis. Front. Immunol. 2018;9:12. doi: 10.3389/fimmu.2018.00451.

66. Xu J., Wang W., Ludeman M., Cheng K., Hayami T., Lotz J.C., Kapila S. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. Tissue Eng. Part A. 2008;14:667–680. doi: 10.1089/tea.2007.0272.

67. Shanmugasundaram S., Logan-Mauney S., Burgos K., Nurminskaya M. Tissue transglutaminase regulates chondrogenesis in mesenchymal stem cells on collagen type XI matrices. Amino Acids. 2012;42:1045–1053. doi: 10.1007/s00726-011-1019-7.

68. Wang C., Brisson B.K., Terajima M., Li Q., Hoxha K., Han B., Goldberg A.M., Liu X.S., Marcolongo M.S., Enomoto-Iwamoto M., et al. Type III collagen is a key regulator of the collagen fibrillar structure and biomechanics of articular cartilage and meniscus. Matrix Biol. 2020;85–86:47–67. doi: 10.1016/j.matbio.2019.10.001.

69. Prockop D.J., Kivirikko K.I. Collagens: Molecular Biology, Diseases, and Potentials for Therapy. Annu. Rev. Biochem. 1995;64:403–434. doi: 10.1146/annurev.bi.64.070195.002155.

70. Kuivaniemi H., Tromp G. Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases. Gene. 2019;707:151–171. doi: 10.1016/j.gene.2019.05.003.

71. Balleisen L., Gay S., Marx R., Kühn K. Comparative investigation on the influence of human bovine collagen types I, II and III on the aggregation of human platelets. Klin. Wochenschr. 1975;53:903–905. doi: 10.1007/BF01468982.

72. Chiang T.M., Seyer J.M., Kang A.H. Collagen-platelet interaction: Separate receptor sites for types I and III collagen. Thromb. Res. 1993;71:443–456. doi: 10.1016/0049-3848(93)90118-8.

73. Monnet E., Fauvel-Lafève F. A new platelet receptor specific to type III collagen. Type III collagen-binding protein. J. Biol. Chem. 2000;275:10912–10917. doi: 10.1074/jbc.275.15.10912.

74. Eyre D.R., Weis M.A., Wu J.J. Articular cartilage collagen: An irreplaceable framework? Eur. Cell Mater. 2006;12:57–63. doi: 10.22203/eCM.v012a07.

75. Young R.D., Lawrence P.A., Duance V.C., Aigner T., Monaghan P. Immunolocalization of collagen Types II and III in single fibrils of human articular cartilage. J. Histochem. Cytochem. 2000;48:423–432. doi: 10.1177/002215540004800312.

76. Hosseininia S., Wei M.A., Rai J., Funk S., Dahlberg L.E., Eyre D.R. Evidence for enhanced collagen type III deposition focally in the territorial matrix of osteoarthritic hip articular cartilage. Osteoarthr. Cartil. 2016;24:1029–1035. doi: 10.1016/j.joca.2016.01.001.

77. Borza D.B., Bondar O., Ninomiya Y., Sado Y., Naito I., Todd P., Hudson B.G. The NC1 Domain of Collagen IV Encodes a Novel Network Composed of the α1, α2, α5, and α6 Chains in Smooth Muscle Basement Membranes. J. Biol. Chem. 2001;276:28532–28540. doi: 10.1074/jbc.M103690200.

78. Maziers N., Dahan K., Pirson Y. From Alport syndrome to benign familial hematuria: Clinical and genetic aspect. Nephrol. Ther. 2005;1:90–100. doi: 10.1016/j.nephro.2005.03.005.

79. Foldager C.B., Toh W.S., Christensen B.B., Lind M., Gomoll A.H., Spector M. Collagen Type IV and Laminin Expressions during Cartilage Repair and in Late Clinically Failed Repair Tissues from Human Subjects. Cartilage. 2016;7:52–56. doi: 10.1177/1947603515604022.

80. Komori T., Ono M., Hara E.S., Ueda J., Nguyen H.T.T., Nguyen H.T., Yonezawa T., Maeba T., Kimura-Ono A., Takarada T., et al. Type IV collagen α6 chain is a regulator of keratin 10 in keratinization of oral mucosal epithelium. Sci. Rep. 2018;8:2612. doi: 10.1038/s41598-018-21000-0.

81. Nakano K., Naito I., Momota R., Sado Y., Hasegawa H., Ninomiya Y., Ohtsuka A. The distribution of type IV collagen α chains in the mouse ovary and its correlation with follicular development. Arch. Histol. Cytol. 2007;70:243–253. doi: 10.1679/aohc.70.243.

82. Jeng L., Hsu H.P., Spector M. Tissue-engineered cartilaginous constructs for the treatment of caprine cartilage defects, including distribution of laminin and type IV collagen. Tissue Eng. Part A. 2013;19:2267–2274. doi: 10.1089/ten.tea.2013.0013.

83. Birk D.E. Type V collagen: Heterotypic type I/V collagen interactions in the regulation of fibril assembly. Micron. 2001;32:223–237. doi: 10.1016/S0968-4328(00)00043-3.

84. Huang G., Ge G., Izzi V., Greenspan D.S. α3 Chains of type v collagen regulate breast tumour growth via glypican-1. Nat. Commun. 2017;8:14351. doi: 10.1038/ncomms14351.

85. Huang G., Ge G., Wang D., Gopalakrishnan B., Butz D.H., Colman R.J., Nagy A., Greenspan D.S. α3(V) Collagen is critical for glucose homeostasis in mice due to effects in pancreatic islets and peripheral tissues. J. Clin. Investig. 2011;121:769–783. doi: 10.1172/JCI45096.

86. Bobadilla J.L., Jankowska-Gan E., Xu Q., Haynes L.D., del Rio A.M., Meyer K., Greenspan D.S., De Oliveira N., Burlingham W.J., Maloney J.D. Reflux-induced collagen type V sensitization: Potential mediator of bronchiolitis obliterans syndrome. Chest. 2010;138:363–370. doi: 10.1378/chest.09-2610.

87. Wu J.J., Weis M.A., Kim L.S., Carter B.G., Eyre D.R. Differences in chain usage and cross-linking specificities of cartilage type V/XI collagen isoforms with age and tissue. J. Biol. Chem. 2009;284:5539–5545. doi: 10.1074/jbc.M806369200.

88. Braun R.K., Molitor-Dart M., Wigfield C., Xiang Z., Fain S.B., Jankowska-Gan E., Seroogy C.M., Burlingham W.J., Wilkes D.S., Brand D.D., et al. Transfer of tolerance to collagen type v suppresses T-helper-cell-17 lymphocyte-mediated acute lung transplant rejection. Transplantation. 2009;88:1341–1348. doi: 10.1097/TP.0b013e3181bcde7b.

89. Parra E.R., Teodoro W.R., Velosa A.P.P., de Oliveira C.C., Yoshinari N.H., Capelozzi V.L. Interstitial and Vascular Type V Collagen Morphologic Disorganization in Usual Interstitial Pneumonia. J. Histochem. Cytochem. 2006;54:1315–1325. doi: 10.1369/jhc.6A6969.2006.

90. Domínguez-Pérez J.M., Fernández-Sarmiento J.A., García D., Granados-Machuca M.M., Morgaz-Rodríguez J., Navarrete-Calvo R., Pérez-Arévalo J., Carrillo-Poveda J.M., Alentorn-Geli E., Laiz-Boada P., et al. Cartilage regeneration using a novel autologous growth factors-based matrix for full-thickness defects in sheep. Knee Surg. Sports Traumatol. Arthrosc. 2019;27:950–961. doi: 10.1007/s00167-018-5107-z.

91. Ritelli M., Dordoni C., Venturini M., Chiarelli N., Quinzani S., Traversa M., Zoppi N., Vascellaro A., Wischmeijer A., Manfredini E., et al. Clinical and molecular characterization of 40 patients with classic Ehlers-Danlos syndrome: Identification of 18 COL5A1 and 2 COL5A2 novel mutations. Orphanet J. Rare Dis. 2013;8:58. doi: 10.1186/1750-1172-8-58.

92. Wang J., Pan W. The biological role of the collagen alpha-3 (Vi) chain and its cleaved c5 domain fragment endotrophin in cancer. OncoTargets Ther. 2020;13:5779–5793. doi: 10.2147/OTT.S256654.

93. Koudouna E., Young R.D., Ueno M., Kinoshita S., Quantock A.J., Knupp C. Three-dimensional architecture of collagen type VI in the human trabecular meshwork. Mol. Vis. 2014;20:638–648.

94. Lettmann S., Bloch W., MaaB T., Niehoff A., Schulz J.N., Eckes B., Eming S.A., Bonaldo P., Paulsson M., Wagener R. Col6a1 null mice as a model to study skin phenotypes in patients with collagen VI related myopathies: Expression of classi cal and novel collagen VI variants during wound healing. PLoS ONE. 2014;9:105686. doi: 10.1371/journal.pone.0105686.

95. Alexopoulos L.G., Youn I., Bonaldo P., Guilak F. Developmental and osteoarthritic changes in Col6a1 -knockout mice: Biomechanics of type VI collagen in the cartilage pericellular matrix. Arthritis Rheum. 2009;60:771–779. doi: 10.1002/art.24293.

96. Veidal S.S., Karsdal M.A., Vassiliadis E., Nawrocki A., Larsen M.R., Nguyen Q.H.T., Hägglund P., Luo Y., Zheng Q., Vainer B., et al. MMP mediated degradation of type VI collagen is highly associated with liver Fibrosis—Identification and validation of a novel biochemical marker assay. PLoS ONE. 2011;6:24753. doi: 10.1371/journal.pone.0024753.

97. Stickel F., Urbaschek R., Schuppan D., Poeschl G., Oesterling C., Conradt C., McCuskey R.S., Simanowski U.A., Seitz H.K. Serum collagen type VI and XIV and hyaluronic acid as early indicators for altered connective tissue turnover in alcoholic liver disease. Dig. Dis. Sci. 2001;46:2025–2032. doi: 10.1023/A:1010616021659.

98. Gerling B., Becker M., Staab D., Schuppan D. Prediction of Liver Fibrosis According to Serum Collagen VI Level in Children with Cystic Fibrosis. N. Engl. J. Med. 1997;336:1611–1612. doi: 10.1056/NEJM199705293362217.

99. Endicott J., Holden P., Fitzgerald J. Authentication of collagen VI antibodies. BMC Res. Notes. 2017;10:358. doi: 10.1186/s13104-017-2674-x.

100. Pan T.C., Zhang R.Z., Arita M., Bogdanovich S., Adams S.M., Gara S.K., Wagener R., Khurana T., Birk D.E., Chu M.L. A mouse model for dominant collagen VI disorders Heterozygous Deletion of Col6a3 EXON 16. J. Biol. Chem. 2014;289:10293–10307. doi: 10.1074/jbc.M114.549311.

101. Allamand V., Briñas L., Richard P., Stojkovic T., Quijano-Roy S., Bonne G. ColVI myopathies: Where do we stand, where do we go? Skelet. Muscle. 2011;1:30. doi: 10.1186/2044-5040-1-30.

102. Jansen I.D.C., Hollander A.P., Buttle D.J., Everts V. Type II and VI collagen in nasal and articular cartilage and the effect of IL-1α on the distribution of these collagens. J. Mol. Histol. 2010;41:9–17. doi: 10.1007/s10735-010-9257-7.

103. Zelenski N.A., Leddy H.A., Sanchez-Adams J., Zhang J., Bonaldo P., Liedtke W., Guilak F. Type VI collagen regulates pericellular matrix properties, chondrocyte swelling, and mechanotransduction in mouse articular cartilage. Arthritis Rheumatol. 2015;67:1286–1294. doi: 10.1002/art.39034.

104. Shen G. The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. Orthod. Craniofacial Res. 2015;8:11–17. doi: 10.1111/j.1601-6343.2004.00308.x.

105. Gu J., Lu Y., Qiao L., Wang Q., Li N., Borgia J.A., Deng Y., Lei G., Zheng Q. Identification and characterization of the novel Col10a1 regulatory mechanism during chondrocyte hypertrophic differentiation. Cell Death Dis. 2014;5:1469. doi: 10.1038/cddis.2014.444.

106. He Y., Siebuhr A.S., Brandt-Hanses N.U., Wang J., Su D., Zheng Q., Simonsen O., Petersen K.K., Arendt-Nielsen L., Eskehave T., et al. Type X collagen levels are elevated in serum from human

osteoarthritis patients and associated with biomarkers of cartilage degradation and inflammation. BMC Musculoskelet. Disord. 2014;15:309. doi: 10.1186/1471-2474-15-309.

107. Brew C.J., Clegg P.D., Boot-Handford R.P., Andrew J.G., Hardingham T. Gene expression in human chondrocytes in late osteoarthritis is changed in both fibrillated and intact cartilage without evidence of generalised chondrocyte hypertrophy. Ann. Rheum. Dis. 2010;69:234–240. doi: 10.1136/ard.2008.097139.

108. Álvarez J., Balbín M., Santos F., Fernández M., Ferrando S., López J.M. Different bone growth rates are associated with changes in the expression pattern of types II and X collagens and collagenase 3 in proximal growth plates of the rat tibia. J. Bone Miner. Res. 2000;15:82–94. doi: 10.1359/jbmr.2000.15.1.82.

109. Van der Kraan P.M., Van den Berg W.B. Chondrocyte hypertrophy and osteoarthritis: Role in initiation and progression of cartilage degeneration? Osteoarthr. Cartil. 2012;20:223–232. doi: 10.1016/j.joca.2011.12.003.

110. De Andrea C.E., Wiweger M.I., Bovée J.V.M.G., Romeo S., Hogendoorn P.C.W. Peripheral chondrosarcoma progression is associated with increased type X collagen and vascularisation. Virchows Arch. 2012;460:95–102. doi: 10.1007/s00428-011-1168-9.

111. Luckman S.P., Rees E., Kwan A.P.L. Partial characterization of cell-type X collagen interactions. Biochem. J. 2003;372:485–493. doi: 10.1042/bj20021572.

112. Zhang X., Liang H., Liu W., Li X., Zhang W., Shang X. A novel sequence variant in COL10A1 causing spondylometaphyseal dysplasia accompanied with coxa valga: A case report. Medicine. 2019;98:16485. doi: 10.1097/MD.0000000000016485.

113. Taylor D.W., Ahmed N., Parreno J., Lunstrum G.P., Gross A.E., Diamandis E.P., Kandel R.A. Collagen type XII and versican are present in the early stages of cartilage tissue formation by both redifferentating passaged and primary chondrocytes. Tissue Eng. Part A. 2015;21:683–693. doi: 10.1089/ten.tea.2014.0103.

114. Polacek M., Bruun J.A., Elvenes J., Figenschau Y., Martinez I. The secretory profiles of cultured human articular chondrocytes and mesenchymal stem cells: Implications for autologous cell transplantation strategies. Cell Transplant. 2011;20:1381–1393. doi: 10.3727/096368910X550215.

115. Hemmavanh C., Koch M., Birk D.E., Espana E.M. Abnormal corneal endothelial maturation in collagen XII and XIV Null mice. Investig. Ophthalmol. Vis. Sci. 2013;54:3297–3308. doi: 10.1167/iovs.12-11456.

116. Kassner A., Hansen U., Miosge N., Reinhardt D.P., Aigner T., Bruckner-Tuderman L., Bruckner P., Grässel S. Discrete integration of collagen XVI into tissue-specific collagen fibrils or beaded microfibrils. Matrix Biol. 2003;22:131–143. doi: 10.1016/S0945-053X(03)00008-8.

117. Koch M., Schulze J., Hansen U., Ashwodt T., Keene D.R., Brunken W.J., Burgeson R.E., Bruckner P., Bruckner-Tuderman L. A novel marker of tissue junctions, collagen XXII. J. Biol. Chem. 2004;279:22514–22521. doi: 10.1074/jbc.M400536200.

118. Boot-Handford R.P., Tuckwell D.S., Plumb D.A., Farrington Rock C., Poulsom R. A novel and highly conserved collagen (proα1(XXVII)) with a unique expression pattern and unusual molecular characteristics establishes a new clade within the vertebrate fibrillar collagen family. J. Biol. Chem. 2003;278:31067–31077. doi: 10.1074/jbc.M212889200.

119. Plumb D.A., Ferrara L., Torbica T., Knowles L., Mironov A., Kadler K.E., Briggs M.D., Boot-Handford R.P. Collagen XXVII organises the pericellular matrix in the growth plate. PLoS ONE. 2011;6:29422. doi: 10.1371/journal.pone.0029422.

120. Pan X., Chen Z., Huang R., Yao Y., Ma G. Transforming Growth Factor β1 Induces the Expression of Collagen Type I by DNA Methylation in Cardiac Fibroblasts. PLoS ONE. 2013;8:60335. doi: 10.1371/journal.pone.0060335.

121. Wilcox W.R. Connective Tissue and Its Heritable Disorders: Molecular, Genetic, and Medical Aspects. Am. J. Hum. Genet. 2003;72:503. doi: 10.1086/345996.

122. Kratochwil K., Ghaffari-Tabrizi N., Holzinger I., Harbers K. Restricted expression of Mov13 mutant α1(I) collagen gene in osteoblasts and its consequences for bone development. Dev. Dyn. 1993;198:273–283. doi: 10.1002/aja.1001980405.

123. Fleischmajer R., Douglas MacDonald E., Perlish J.S., Burgeson R.E., Fisher L.W. Dermal collagen fibrils are hybrids of type I and type III collagen molecules. J. Struct. Biol. 1990;105:162–169. doi: 10.1016/1047-8477(90)90110-X.

124. Birk D.E., Fitch J.M., Babiarz J.P., Linsenmayer T.F. Collagen type I and type V are present in the same fibril in the avian corneal stroma. J. Cell Biol. 1998;106:999–1008. doi: 10.1083/jcb.106.3.999.

125. Von Der Mark K. Localization of collagen types in tissues. Int. Rev. Connect. Tissue Res. 1981;9:265–324.

126. Shibata S., Sakamoto Y., Baba O., Qin C., Murakami G., Cho B.H. An immunohistochemical study of matrix proteins in the craniofacial cartilage in midterm human fetuses. Eur. J. Histochem. 2013;57:262–270. doi: 10.4081/ejh.2013.e39.

127. Xia H., Liang C., Huang J., He J., Wang Z., Cao X., Peng C., Wu S. Pericellular collagen I coating for enhanced homing and chondrogenic differentiation of mesenchymal stem cells in direct intra-articular injection. Stem Cell Res. Ther. 2018;9:174. doi: 10.1186/s13287-018-0916-z.

128. Bielajew B.J., Hu J.C., Athanasiou K.A. Collagen: Quantification, biomechanics, and role of minor subtypes in cartilage. Nat. Rev. Mater. 2020;5:730–747. doi: 10.1038/s41578-020-0213-1.

129. Seo S.S., Kim C.W., Jung D.W. Management of Focal Chondral Lesion in the Knee Joint. Knee Surg Relat Res. 2011;23:185–196. doi: 10.5792/ksrr.2011.23.4.185.

130. Gelber A.C., Hochberg M.C., Mead L.A., Wang N.Y., Wigley F.M., Klag M.J. Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. Ann. Intern. Med. 2000;133:321–328. doi: 10.7326/0003-4819-133-5-200009050-00007.

131. Mainil-Varlet P., Aigner T., Brittberg M., Bullough P., Hollander A., Hunziker E., Kandel R., Nehrer S., Pritzker K., Roberts S., et al. Histological assessment of cartilage repair: A report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS) J. Bone Jt. Surg. 2003;85:45–57. doi: 10.2106/00004623-200300002-00007.

132. Aigner T., Stöve J. Collagens—major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. Adv. Drug Deliv. Rev. 2003;55:1569–1593. doi: 10.1016/j.addr.2003.08.009.

133. Hoemann C., Kandel R., Roberts S., Saris D.B.F., Creemers L., Mainil-Varlet P., Méthot S., Hollander A.P., Buschmann M.D. International cartilage repair society (ICRS) recommended

guidelines for histological endpoints for cartilage repair studies in animal models and clinical trials. Cartilage. 2011;2:153–172. doi: 10.1177/1947603510397535.

134. Lindert U., Gnoli M., Maioli M., Bedeschi M.F., Sangiorgi L., Rohrbach M., Giunta C. Insight into the Pathology of a COL1A1 Signal Peptide Heterozygous Mutation Leading to Severe Osteogenesis Imperfecta. Calcif. Tissue Int. 2018;102:373–379. doi: 10.1007/s00223-017-0359-z.

135. Tabeta K., Du X., Arimatsu K., Yokoji M., Takahashi N., Amizuka N., Hasegawa T., Crozat K., Maekawa T., Miyauchi S., et al. An ENU-induced splice site mutation of mouse Col1a1 causing recessive osteogenesis imperfecta and revealing a novel splicing rescue. Sci. Rep. 2017;7:11717. doi: 10.1038/s41598-017-10343-9.

136. Sen R., Hurley J.A. StatPearls. StatPearls Publishing; Treasure Island, FL, USA: 2021. Osteoarthritis.

137. Hosseininia S., Önnerfjord P., Dahlberg L.E. Targeted proteomics of hip articular cartilage in OA and fracture patients. J. Orthop. Res. 2019;37:131–135. doi: 10.1002/jor.24158.

138. Hosseininia S., Lindberg L.R., Dahlberg L.E. Cartilage collagen damage in hip osteoarthritis similar to that seen in knee osteoarthritis; a case-control study of relationship between collagen, glycosaminoglycan and cartilage swelling. BMC Musculoskelet. Disord. 2013;14:18. doi: 10.1186/1471-2474-14-18.

139. Shi D., Xu X., Ye Y., Song K., Cheng Y., Di J., Hu Q., Li J., Ju H., Jiang Q., et al. Photo-cross-linked scaffold with kartogenin-encapsulated nanoparticles for cartilage regeneration. ACS Nano. 2016;10:1292–1299. doi: 10.1021/acsnano.5b06663.

140. Peck Y., He P., Chilla G.S.V.N., Poh C.L., Wang D.-A. A preclinical evaluation of an autologous living hyaline-like cartilaginous graft for articular cartilage repair: A pilot study. Sci. Rep. 2015;5:16225. doi: 10.1038/srep16225.

141. Mori H., Kondo H., Kawaguchi Y., Kitamura N., Nagai N., Lida H., Yasuda K. Development of a salmon-derived crosslinked atelocollagen sponge disc containing osteogenic protein-1 for articular cartilage regeneration: In vivo evaluations with rabbits. BMC Musculoskelet. Disord. 2013;14:174. doi: 10.1186/1471-2474-14-174.

142. Sobol E., Baum O., Shekhter A., Wachsmann-Hogiu S., Shnirelman A., Alexandrovskaya Y., Sadovskky I., Vinokur V. Laser induced micropore formation and modification of cartilage structure in osteoarthritis healing. J. Biomed. Opt. 2017;22:091515. doi: 10.1117/1.JBO.22.9.091515.

143. Richter D.L., Schenck R.C., Wascher D.C., Treme G. Knee Articular Cartilage Repair and Restoration Techniques: A Review of the Literature. Sports Health. 2016;8:153–160. doi: 10.1177/1941738115611350.

144. Zhang S., Hu B., Liu W., Wang P., Lv X., Chen S., Liu H., Shao Z. Articular cartilage regeneration: The role of endogenous mesenchymal stem/progenitor cell recruitment and migration. Semin. Arthritis Rheum. 2020;50:198–208. doi: 10.1016/j.semarthrit.2019.11.001.

145. Musumeci G., Castrogiovanni P., Leonardi R., Trovato F.M., Szychlinska M.A., Di Giunta A., Loreto C., Castorin S. New perspectives for articular cartilage repair treatment through tissue engineering: A contemporary review. World J. Orthop. 2014;5:80–88. doi: 10.5312/wjo.v5.i2.80.

146. Campbell A.B., Pineda M., Harris J.D., Flanigan D.C. Return to Sport after Articular Cartilage Repair in Athletes' Knees: A Systematic Review. Arthroscopy. 2016;32:651–668. doi: 10.1016/j.arthro.2015.08.028.

147. Ortega-Orozco R., Olague-Franco J.K., Miranda-Ramírez E. Implantación de condrocitos autólogos versus microfracturas para el tratamiento de lesiones del cartílago en la rodilla. Acta Ortopédica Mex. 2018;32:322–328. doi: 10.35366/85425.

148. Jackson R.W., Dieterichs C. The results of arthroscopic lavage and debridement of osteoarthritic knees based on the severity of degeneration: A 4- to 6-year symptomatic follow-up. Arthroscopy. 2003;19:13–20. doi: 10.1053/jars.2003.50022.

149. Stanish W.D., McCormack R., Forriol F., Mohtadi N., Pelet S., Desnoyers J., Restrepo A., Shive M.S. Novel scaffold-based bst-cargel treatment results in superior cartilage repair compared with microfracture in a randomized controlled trial. J. Bone Jt. Surg. 2013;95:1640–1650. doi: 10.2106/JBJS.L.01345.

150. Matsiko A., Levingstone T.J., O'Brien F.J. Advanced Strategies for Articular Cartilage Defect Repair. Materials. 2013;6:637–668. doi: 10.3390/ma6020637.

151. Horbert V., Xin L., Foehr P., Brinkmann O., Bungartz M., Burgkart R.H., Graeve T., Kinne R.W. In Vitro Analysis of Cartilage Regeneration Using a Collagen Type I Hydrogel (CaReS) in the Bovine Cartilage Punch Model. Cartilage. 2019;10:346–363. doi: 10.1177/1947603518756985.

152. Vázquez-PortalatÍn N., Kilmer C.E., Panitch A., Liu J.C. Characterization of Collagen Type i and II Blended Hydrogels for Articular Cartilage Tissue Engineering. Biomacromolecules. 2021;17:145–152. doi: 10.1021/acs.biomac.6b00684.

153. Mainil-Varlet P., Damme B.V., Nesic D., Knutsen G., Kandel R., Roberts S. A New Histology Scoring System for the Assessment of the Quality of Human Cartilage Repair: ICRS II. Am. J. Sports Med. 2010;38:880–890. doi: 10.1177/0363546509359068.

154. Rutgers M., van Pelt M.J.P., Dhert W.J.A., Creemers L.B., Saris D.B.F. Evaluation of histological scoring systems for tissue-engineered, repaired and osteoarthritic cartilage. Osteoarthr. Cartil. 2010;18:12–23. doi: 10.1016/j.joca.2009.08.009.

155. Thorp H., Kim K., Kondo M., Grainger D.W., Okano T. Fabrication of hyaline-like cartilage constructs using mesenchymal stem cell sheets. Sci. Rep. 2020;10:20869. doi: 10.1038/s41598-020-77842-0.

156. Figueroa R.O., Dreiman R.W., Montenegro-Rizzardini M.A. Evaluation of Collagen and Elastic Fibers in Human TMJ Tissue. Int. J. Odontostomatol. 2010;4:277–284.

157. Wegner K.A., Keikhosravi A., Eliceiri K.W., Vezina C.M. Fluorescence of Picrosirius Red Multiplexed With Immunohistochemistry for the Quantitative Assessment of Collagen in Tissue Sections. J. Histochem. Cytochem. 2017;65:479–490. doi: 10.1369/0022155417718541.

158. Stolz M., Gottardi R., Raiteri R., Miot S., Martin I., Imer R., Staufer U., Raducanu A., Düggelin M., Baschong W., et al. Early detection of aging cartilage and osteoarthritis in mice and patient samples using atomic force microscopy. Nat. Nanotechnol. 2009;4:186–192. doi: 10.1038/nnano.2008.410.

V. CAPÍTULO II

Objetivo I / Objective I

Proteoglycans in articular cartilage and their contribution to chondral injury and repair mechanisms

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Objective I: To provide a detailed description of the key molecules present in articular cartilage, with a specific emphasis on major and minor collagens and proteoglycans. Explore the functional roles of these molecules in both healthy articular cartilage and the chondral repair process after a chondral lesion, with the goal of developing refined evaluation systems to assess the quality of repaired chondral tissue.

Proteoglycans in articular cartilage and their contribution to chondral injury and repair mechanisms

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Abstract

Proteoglycans are vital components of the extracellular matrix in articular cartilage, providing biomechanical properties crucial for its proper functioning. They are key players in chondral diseases, specifically in the degradation of the extracellular matrix. Evaluating proteoglycan molecules can serve as a biomarker for joint degradation in osteoarthritis patients, as well as assessing the quality of repaired tissue following different treatment strategies for chondral injuries. Despite ongoing research, understanding osteoarthritis and cartilage repair remains unclear, making the identification of key molecules essential for early diagnosis and effective treatment. This review offers an overview of proteoglycans as primary molecules in articular cartilage. It describes the various types of proteoglycans present in both healthy and damaged cartilage, highlighting their roles. Additionally, the review emphasizes the importance of assessing proteoglycans to evaluate the quality of repaired articular tissue. It concludes by providing a visual and narrative description of aggrecan distribution and presence in healthy cartilage. Proteoglycans, such as aggrecan, biglycan, decorin, perlecan, and versican, significantly contribute to maintaining the health of articular cartilage and the cartilage repair process. Therefore, studying these proteoglycans is vital for early diagnosis, evaluating the quality of repaired cartilage, and assessing treatment effectiveness.

Keywords: Proteoglycans. Extracellular matrix. Articular cartilage. Chondral injury. Biomarkers.

1. Introduction

The extracellular matrix (ECM) is widely acknowledged within the scientific community as the most complex structural organization present in an organism [1]. Composed of an intricate and finely organized network of collagens, proteoglycans (PGs), fibronectin, laminins, elastin, glycosaminoglycans (GAGs), and glycoproteins, the ECM serves as a vital support system for cells, tissues, and organs, imparting them with their unique mechanical and chemical properties [2,3]. This network, along with other proteins and growth factors [4], gives each tissue its chemical and mechanical properties [5]. The morphological integrity of articular cartilage ECM mainly determines the proper functioning of the joint [6]. The ECM of articular cartilage can be classified into three regions: The PCM, the territorial matrix, and the interterritorial matrix [7,8]. The PCM is the layer closest to the cell, while the territorial matrix surrounds the cell with a layer of fibrillar collagen. The interterritorial matrix is the largest region and contains most of the material located outside of the cells in cartilage [9]. The unique structure of the PCM allows for chondrocyte–matrix interactions, thereby regulating chondrocyte phenotype and cell survival [10]. The PCM is rich in PGs, collagens, basement membrane proteins, and non-collagenous glycoproteins, which interact and form a mesh-like structure [7,11,12]. Furthermore, the PCM is connected with the neighboring tissue through a meshwork of fine collagen fibrils, PGs, and fibronectin, which together create the territorial matrix [9].

Articular cartilage is a connective tissue that hides the surface of bones where they meet in joints. This cartilage is composed of chondrocytes (between 1% and 5% of the cartilage volume) embedded within an organized ECM of collagen, PGs, and other proteins (approximately 65%, 15%, and 15%, respectively) [13]. It is endowed with a specialized structure that confers upon it the necessary biomechanical characteristics to withstand joint loading. Specifically, this structure imparts compressive strength to the cartilage and ensures maintenance of fluid and electrolyte balance, both of which are critical for the optimal functioning of the joint [14]. This structure has limited reparative and regenerative capabilities due to its lack of vascularity and the fact that mature chondrocytes lose their ability to migrate, proliferate, and synthesize their surrounding matrix [15,16]. Cartilage serves two key functions in the body providing near-frictionless movement between bones and counteracting the compressive forces that are exerted across the joint during movement. These functions are largely attributable to the presence of PGs within the ECM of cartilage [17].

Chondral injuries in patients with healthy cartilage generally have a traumatic origin and can evolve with cartilage degeneration and osteoarthritis (OA) [18]. OA is the primary cause of

disability in adults in the United States [19], with the United Nations projecting that the number of people suffering from OA will reach 130 million by 2050 [20]. Presently, OA affects more than 10% of the elderly population [19]. A defining characteristic of OA is the gradual deterioration of articular cartilage due to the permanent degradation of the cartilage ECM and the remodeling of adjacent joint tissues. This degeneration leads to joint dysfunction, limited mobility, and excruciating pain during routine activities [21,22].

Despite extensive research efforts aimed at elucidating the molecular mechanisms responsible for the onset and progression of OA, as well as intrinsic cartilage repair processes, many aspects of these processes remain unclear [23]. Determining the key molecules involved in these processes would enable early diagnosis and preventive treatment of OA, ideally leading to a 100% effective solution to treat chondral lesions. For these reasons, the present review provides an overview of PGs as one of the main molecules of articular cartilage, describing the PGs present in this particular tissue and the role they play in both healthy and damaged cartilage. Additionally, this review highlights the importance of including PGs assessment to evaluate the quality of repaired articular tissue. Furthermore, a visual and narrative description of the distribution and presence of aggrecan in healthy cartilage is provided.

2. General Characteristics of Proteoglycans

PGs are a class of intricate macromolecules that can be found in various forms within most tissue [24]. These molecules consist of a central protein that has different quantities of GAG side chains linked to it. PGs are categorized into multiple families based on their occurrence in cells and tissues, their interactions with other macromolecular constituents, and the particular structures of their core proteins [25]. In this way, PGs correspond to a wide variety of functions, such as structural functions, regulation of enzymatic activity, and cell surface receptors. During development and tissue repair, PGs play an important role in regulating gradients and the availability of growth factors, cytokines, chemokines, and morphogens [26].

PGs have been a subject of study since the 20th century, but it was not until the late 1960s, when Sajdera and Hascall developed an innovative extraction protocol, that PGs gained recognition [27]. Throughout the 1970s, a considerable amount of research was published on the isolation, purification, and characterization of PGs from ECM in healthy and pathological conditions. During this time, studies primarily focused on tissue with a high PG content, such as cartilage, the aorta, and skin, due to methodological limitations. With the advent of molecular biology techniques, research on PGs has become more accessible, leading to new insights into their structure and function [24].

2.1. Structure

GAGs are covalently attached to the core protein of the PG. They are unbranched and often resemble long polysaccharides with a repeating disaccharide structure [26]. In most PGs, GAGs comprise more than 50% of the total molecular mass and mediate biological functions. The molecular composition of the GAG chains determines the classification of PGs as chondroitin sulfate PGs (CSPGs), heparan sulfate PGs (HSPGs), keratan sulfate PGs (KSPGs), or dermatan sulfate PGs (DSPGs). Some PGs are considered hybrid PGs since they contain multiple types of chains. For instance, aggrecan is the predominant PG in cartilage, consisting of a core protein with three disulfide-linked globular regions (G1, G2, and G3) and an intervening extension region. CSPG is covalently linked from the G3 domain to over half of the protein core, but it also contains KSPG near the G2 domain (Figure 1A) [17,26].

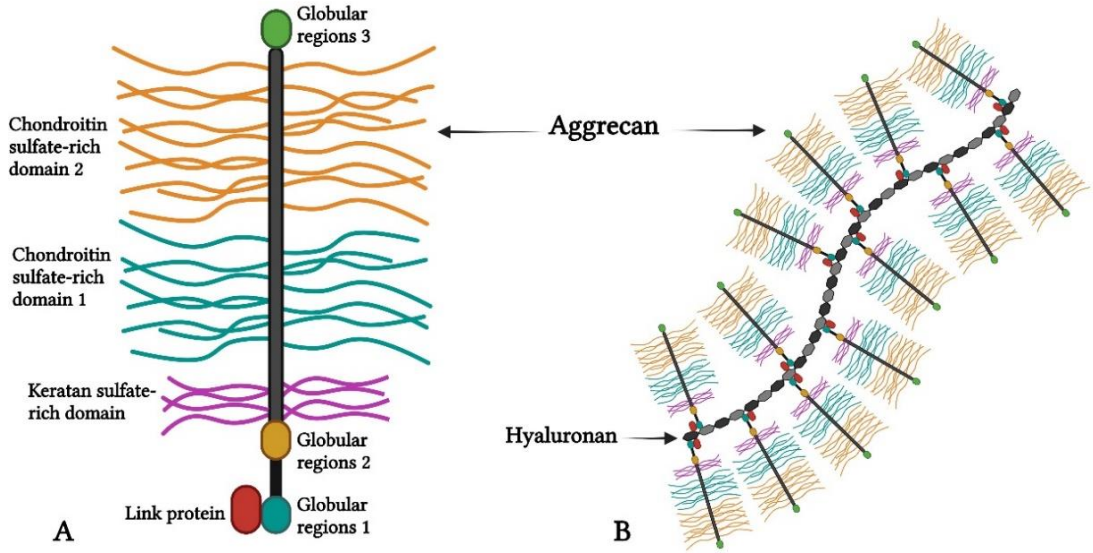

Figure 1. Aggrecan and PG aggregates structure. (A) Aggrecan, a hybrid PG consisting of a protein core with three lobular regions, keratan sulfate, and chondroitin sulfate. (B) PG aggregates composed of a central hyaluronan filament with numerous aggrecan molecules bound together. Modified from Roughley et al. [17].

2.2. Biosynthesis

The protein core of PGs is synthesized by ribosomes and transported to the rough endoplasmic reticulum. Their glycosylation takes place in the Golgi apparatus in multiple enzymatic stages, requiring different glycosyltransferases. A specialized link of tetrasaccharide is attached to the core protein's serine side chain to initiate polysaccharide growth. The PG is subsequently transported to the ECM of the tissue through secretory vesicles. The production of core proteins and carbohydrate chains can be performed independently, owing to the intricate nature of the macromolecules. Glycosylation, a process that necessitates a significant amount of energy, especially in the case of aggrecan, involves the use of several enzymes in

excess. Numerous hydrolases are involved in carbohydrate degradation, either extracellularly or intracellularly in lysosomes [26].

2.3. Classification

The classification of PGs is a complex process. It involves grouping nearly all known PGs of the mammalian genome into four major classes based on factors, such as their cellular localization, similarity in gene/protein structure, and the specific protein units found within their protein cores. PGs can be classified as intracellular PGs, cell surface PGs, pericellular PGs, and extracellular PGs (Table 1) [28].

Serglycin is the only intracellular PG, usually with heparin side chains. Serglycin is a PG present in the granules of mast cells and functions as a binding agent for most of the intracellular proteases that are stored in these granules [29]. This PG is expressed by all inflammatory cells and is stored within intracytoplasmic granules, where it interacts with and regulates the activity of various inflammatory mediators, chemokines, cytokines, and growth factors [30]. Furthermore, serglycin has also been detected in several non-immune cell types, including chondrocytes, endothelial cells, and smooth muscle cells [31].

Cell surface PGs comprise thirteen genes, of which seven are responsible for encoding transmembrane PGs and the other six for glycosyl-phosphatidyl-inositol (GPI)-anchored PGs. All PGs belonging to this group contain heparan sulfate side chains, with the exception of NG2 and phosphacan [28].

Pericellular and basement membrane region PGs consist of four PGs that are closely linked to the surfaces of many types of cells through integrins and other receptors. However, they can also act as components of most basement membranes. Pericellular PGs are mostly HSPGs and include perlecan and agrin, which will be discussed in this review [28].

Extracellular PGs are the largest group with twenty-five different genes, divided into three subgroups. The first subgroup contains four genes that encode hyalectans, including aggrecan, versican, neurocan, and brevican, which are key structural components of cartilage, blood vessels, and nervous systems. The second subgroup includes eighteen small leucine-rich PGs (SLRPs), which perform various functions and signal through different receptors. The third subgroup consists of three testicans, which are calcium-binding HSPGs [28].

Table 1. PGs classification. Modified from Iozzo et al. [22].

Location	Clasification	Eponym	Predominant GAG
INTRACELLULAR	Secretory granules	Serglycin	Hep
CELL SURFACE	Transmembrane	Syndecan, 1-4	HS
		NG2	CS
		Betaglycan	CS/HS
		Phosphacan	CS
	GPI-Anchored	Glypican, 1-6	HS
PERICELLULAR	Basement membrane zone	Perlecan	HS
		Agrin	HS
		Collagen XVIII	HS
		Collagen XV	CS/HS
EXTRACELLULAR	Hyalectan lectican	Aggrecan	CS/KS
		Versican	CS
		Neurocan	CS
		Brevican	CS
	Canonical	Biglycan	CS
		Decorin	DS
		Asporin	
		ECM2	
		ECMX	
		Fibromodulin	KS
		Lumican	KS
		PRELP	
		Keratocan	KS
		Osteoadherin	KS
		Epiphycan	DS/CS
		Opticin	
		Osteoglycin	
	Non-canonical	Chondroadherin	
		Nyctalopin	
		Tsukushi	
		Podocan	
		Podocan-Like 1	
	Spock	Testican, 1-3	HS

GAG: glycosaminoglycan; Hep: heparin; HS: heparan sulfate; CS: chondroitin sulfate; KS: keratan sulfate; DS: dermatan sulfate.

3. Extracellular Matrix Proteoglycans in Articular Cartilage

Aggrecan and versican form large aggregates that are critical for maintaining the pericellular environment around the cell. However, the level of versican decreases with age [7,32]. Apart from large aggregating PGs, non-aggregating PGs, such as biglycan, decorin, versican, and perlecan are also present [7]. Aggrecan is the most abundant PG in terms of weight in articular cartilage, however, in young cartilage, similar amounts of aggrecan, biglycan, and decorin are present on a molecular level [24].

3.1. Aggrecan

Aggrecan is a predominant PG found in typical hyaline cartilage, such as articular cartilage [33], where it exists as PG aggregates [34]. These aggregates consist of a central filament of hyaluronan, to which several aggrecan molecules are attached. The filament of hyaluronan has a protein core of approximately 200 kDa molecular mass, to which chondroitin sulfate, keratan sulfate, and 50 N- and O-linked oligosaccharide chains are attached (Figure 1B) [17,33]. Each PG aggregate can contain over 1000 aggrecan molecules [33]. Aggrecan is responsible for providing the viscoelastic properties of cartilage and plays an important role in cell–ECM interaction, binding, and the release of growth factors and morphogens [35].

When exposed to water, the sulfated GAG chains of aggrecan become hydrated, leading to swelling and the expansion of its molecular domains [17,36]. However, in the ECM, the swelling is mitigated by collagen fibrils that provide the structural support for cartilage. In the presence of an adequate quantity of aggrecan, a state of equilibrium is attained in which the swelling of aggrecan is balanced by the tensile forces generated by stretching the collagen fibrils. To ensure optimal cartilage function, it is necessary to have high concentrations of aggrecan to achieve this balance [17]. This mechanism is the foundation for the hydrodynamic viscoelastic properties of articular cartilage [36].

Although aggrecan is an essential functional element in articular cartilage and plays an important role in OA and chondral repair processes, it is difficult to find accurate information and images of aggrecan's distribution within articular cartilage. For this reason, samples of healthy sheep articular cartilage from a previous study [37] were used to describe the distribution of aggrecan via immunohistochemistry (Figure 2). We observed aggrecan to be present in the cartilage zone, while it was completely absent in bone tissue (Figure 2A). When aggrecan´s presence was analyzed by zones, this PG was highly abundant in the upper zones of the cartilage (superficial, middle, and deep zones), but almost absent in the calcified zone. In the upper zones, aggrecan was found in the territorial and inter-territorial matrix, in the pericellular matrix (PCM), and in chondrocytes. The aggrecan presence decreased when the tidemark was approached. In the calcified zone, this PG was only observed in some chondrocytes and their PCMs (Figure 2B).

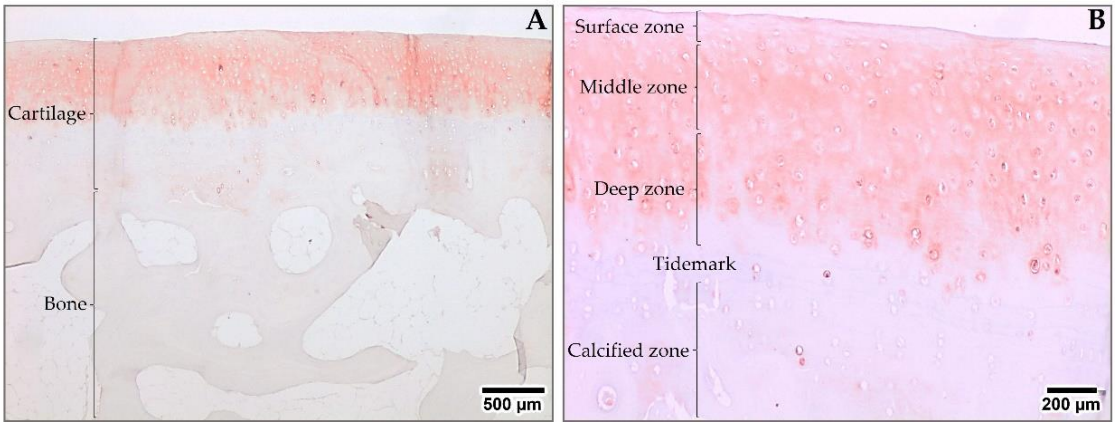

Figure 2. Immunohistochemical staining of aggrecan in healthy sheep articular cartilage [38]. (A) Lower magnification image depicting the cartilage and underlying bone. (B) Higher magnification image revealing intricate details of all cartilage areas. The adivin-biotin-complex method was used for the immunohistochemistry. Enzymatic pre-treatment with hyaluronidase was used. The primary antibody used was anti-aggrecan (ab3778, Abcam, Cambride, UK) at 1/100 dilution in PBS containing 10% normal goat serum.

3.2. Biglycan

Biglycan and decorin belong to SLPRs family of PGs and are characterized by their small size and abundance of leucine repeats. They are composed of a core protein that has a molecular mass of about 40 kDa, to which dermatan sulfate chains are attached [39,40]. In cartilage, biglycan is one of the small PGs present in the ECM, binding to other molecules and helping in stabilizing the matrix [13]. In particular, this PG is located in the PCM [41].

Biglycan can interact with bone morphogenetic protein, which plays an important role in the metabolism of cartilage and bone [24]. During skeletal development, biglycan is present in a rim of chondrocytes close to the articular surface [42]. Furthermore, a study conducted by Han et al. [41] demonstrated that biglycan does not have a significant role in regulating cartilage degradation. However, biglycan has a strong function in the structure of subchondral bone.

Biglycan undergoes proteolytic processing as an individual ages, leading to the removal of the amino-terminal region that carries dermatan sulfate chains. Consequently, biglycan without glycanation tends to accumulate in the cartilage matrix over time [43].

3.3. Decorin

Decorin, along with biglycan, is the most abundant small PG present in the articular cartilage ECM. Decorin is distributed in the pericellular, territorial, and interterritorial matrixes of cartilage [6,41] and has an important role in maintaining the cartilage's structural integrity [41]. It functions as a "physical linker" that regulates the assembly of the aggrecan network in the ECM of the cartilage [6]. Moreover, decorin can also bind to the transforming growth factor

beta and sequester it in the matrix [24]. This PG remains within intact articular cartilage at all ages [43].

3.4. Versican

In the early stages of chondrocyte differentiation, versican is transiently expressed and incorporated into the ECM, but disappears as it is replaced by aggrecan [44]. A previous study showed that mice lacking versican expression achieved endochondral ossification, indicating that versican was not crucial for cartilage development [45].

3.5. Perlecan

The cartilage ECM contains perlecan, whose presence in articular cartilage is unexpected since this PG is typically associated with basement membranes, which are absent in cartilage [43]. Perlecan is a multifunctional PG that promotes the proliferation, differentiation, and matrix synthesis of chondrocytes through its interactions with growth factors, morphogens, and ECM-stabilizing glycoproteins [46,47]. It also contributes to the mechanosensory properties of cartilage through pericellular interactions with fibrillin, type IV, V, VI, and XI collagen, and elastin [48]. These interactions help in stabilizing and enhancing the functional properties of mature cartilaginous ECM [49]. Perlecan plays a role in the maturation of chondroprogenitor stem cells and the development of pluripotent migratory stem cell lineages that contribute to joint formation and early cartilage development [50].

3.6. Proteoglycan Interaction with Other Molecules in the Extracellular Matrix of Articular Cartilage

The PCM, located closest to the chondrocytes, contains type VI collagen, which forms a microfibrillar network anchoring the chondrocyte to the ECM [8]. The PCM binds to type II collagen, aggrecan, and hyaluronan, and interacts with biglycan, decorin, and type IX collagen [11,51]. Type VI collagen is related to the PCM PGs, and biglycan and decorin are essential for the structural integrity of the PCM by connecting it to the territorial/interterritorial matrix. They also serve as functional bridges between type II and VI collagen [11]. Perlecan, along with type VI collagen, contribute to the organization and mechanical stability of the PCM, and affect its modulus [49]. Col6a1 inactivation results in a reduction in genes encoding aggrecan, biglycan, and decorin, which are important during chondrogenesis [52]. When bound to type VI collagen, perlecan has cytoprotective properties [53].

4. Role of Proteoglycans in Chondral Injuries

One pathological feature of chondral repair limitations, such OA, is the depletion of matrix macromolecules from cartilage, particularly PGs [54]. The biosynthesis and degradation of cartilage PGs entail multiple enzymes, and there is evidence suggesting that deficiency or disruption of any of these enzymes can lead to severe cellular or organ dysfunction or damage. The production of deficient PGs can impact their charge density or interactions with other extracellular components, altering the structure and properties of the cartilage. The insufficient degradation of PGs can lead to a limited accumulation of degradation products that can have deleterious effects on organisms [24].

The loss of PGs in the ECM increases hydraulic permeability and decreases solid charge density, thereby reducing the cartilage´s ability to adequately support mechanical loads [55,56].

4.1. Aggrecan

The event that triggers the depletion of aggrecan in cartilage could be caused by trauma, inflammation, or excessive loading of the joint. Once damage has begun, and the endogenous repair capacity of articular cartilage is low, further damage, such as OA, may be an inevitable effect [17].

Aggrecan content and composition appear to be strongly related to tissue status. In aging cartilage, there is a reduction in the overall amount of aggrecan [24]. In the OA joint, catabolic processes destroy both the hyaluronan backbone of the aggregate and the core protein of the aggrecan molecules, thus impairing their function and making articular cartilage susceptible to erosion. Aggrecan degradation is caused by proteinases, hyaluronidases, and free radicals [17]. Some in vivo studies have indicated that levels of aggrecan are initially high in the early stages of OA to prevent cartilage loss. However, over time, the levels of aggrecan decrease due to proteolytic activity, which ultimately leads to the destruction of cartilage [57,58].

Proteolytic cleavage of aggrecan produces two fragments, one of which remains attached to hyaluronan, while the other fragment loses its interaction with hyaluronan and can easily diffuse through the ECM and become lost in the synovial fluid [55,56,57]. The fragments that remain bound to hyaluronan can persist in the tissue for many years, hampering the repair process and occupying space that could be used for binding newly synthesized aggrecan [17]. Aggrecan fragments that appear in the synovial fluid can serve as biomarkers for cartilage degradation, with higher concentrations indicating increased degradation in patients with OA [17,59]. Normal articular cartilage necessitates a high concentration of aggrecan, a high degree

of sulfation, and the capacity to form large aggregates, all of which are compromised in OA joints [17].

4.2. Biglycan and Decorin

In human cartilage, decorin and biglycan are not typically found on the surface of articular cartilage, but their levels increase in the deeper regions of the tissue [60]. In cases of OA, the levels of these PGs are significantly upregulated, which is believed to be a compensatory mechanism by chondrocytes to counteract cartilage degeneration [61]. This is supported by the finding that decorin acts as a "physical linker" to enhance the molecular association of aggrecan, which in turn increases the structural integrity of aggrecan networks in healthy cartilage ECM and reduces the loss of fragmented aggrecan from degenerative cartilage [41].

At present, it is acknowledged that both SLRPs play a crucial role in cartilage function and pathology. Nevertheless, it is still unclear how decorin and biglycan function individually or in conjunction to control the onset and advancement of OA [41]. A recent study reported that decorin insufficiency leads to altered ECM biomechanical characteristic and cartilage stiffness [62].

After tissue injury, biglycan and decorin can be released in a soluble form from the cartilage matrix, which could act as an endogenous warning signal [63,64].

4.3. Perlecan

Perlecan is a molecule that possesses properties that are important for cartilage repair, including chondrogenesis, regulation of cell signaling, matrix architecture, and new tissue formation [47,65]. Therefore, perlecan is a promising candidate molecule to investigate for a better understanding of cartilage repair mechanisms [66]. In adults, perlecan was found to be highly secreted during articular cartilage repair [48]. The role of perlecan in chondrogenesis and cartilage development indicates that it may have a potential function in repairing cartilage by reproducing its developmental roles in damaged or diseased tissues [66,67]. Perlecan plays a critical role in facilitating the maturation of chondroprogenitor stem cells and the creation of pluripotent migratory stem cell lineages, which affect joint formation and early cartilage development [68]. Therefore, heparan sulfate-deficient perlecan may exert inhibitory control over chondrocytes in mature cartilage, resulting in a poor healing response related to cartilage [66]. Curiously, in human knee OA cartilage, perlecan levels are significantly higher in areas close to cartilage defects [69]. Furthermore, collected data indicated that perlecan in the PCM of

cartilaginous tissues is implicated in the regulation of biomechanical properties that characterize PCM´s various matrices, and may thus have unique applications in chondral regeneration [67].

4.4. Molecular Biomarkers

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [70]. These molecules are mainly used to diagnose illness, predict illness, or assess a patient´s physical condition [70,71]. Biomarkers in chondral injuries can be extracted from serum, urine, or synovial fluid samples [72].

Although radiographs and other types of joint imaging are regularly used as diagnostic techniques for chondral injuries, they do not have the capacity to determine dynamic changes in the joint. Therefore, it is important to use molecular biomarkers, which, in addition to complementing biomedical imaging, allow for the monitoring of disease progression and the efficacy of treatment [58,73,74]. In chondral injuries, due to the fragmentation of specific matrix molecules, such as PGs, some of the fragments that are released can be analyzed. Over time, assays have been developed and successfully used to verify that PGs are indeed useful as biomarkers for monitoring the activity of the tissue destruction process [75].

Aggrecan is one of the most commonly studied cartilage proteins for biomarker development [76]. As previously reported, the proteolysis of aggrecan is an early feature of cartilage degradation following chondral injury. Therefore, the elevated presence of aggrecan fragments in synovial fluid is associated with joint injury and/or OA [77]. These fragments are measurable as an increase in the aggrecan released from the cartilage into the synovial fluid [78]. Aggrecan fragments present in synovial fluid can be detected via amino acid sequencing, Western blot, and enzyme-linked immunosorbent assay (ELISA) [77].

The release of soluble forms of biglycan or decorin from the ECM of cartilage into the synovial fluid following tissue injury may act as an internal danger signal [64]. Soluble biglycan and decorin in synovial fluid and serum can be detected using ELISA [63,64,78,79]. Barreto et al. [63] observed high levels of biglycan in advanced OA, concluding that soluble biglycan can serve as a mediator of OA cartilage as well as a potential biomarker. Other studies determined that increased serum decorin levels may indicate changes in ECM and are a risk factor associated with OA [79,80].

5. Analysis of Proteoglycans in Repaired Articular Cartilage

Focal cartilage defects are widespread, with up to 63% of the general population and 36% of athletes affected. In particular, larger defects can be challenging, particularly for individuals leading an active lifestyle [19,81]. These defects can lead to accelerated damage, increased pain, and even the progression of OA [82]. Given the high incidence and associated costs of chondral injuries, it is crucial to identify effective treatments. Finding the molecules involved in chondral repair processes could be key in the search for a treatment.

A decreased PG concentration was shown to predispose cartilage tissue to micro-damage from mechanical loading, thereby weakening and altering the matrix´s structural integrity. Therefore, it is of vital importance to check the concentration and distribution of PGs in repaired articular cartilage [83], bearing in mind that in order to understand the properties of articular cartilage, it is necessary to appreciate the composition of the cartilage according to its layers (superficial, middle, deep, and calcified) and subregions (peri-cellular, territorial, and interterritorial) [84], as shown in Figure 2.

There are several animal and human studies in the literature that investigate the effectiveness of different treatment techniques for chondral and/or osteochondral defects. In human clinical trials and case studies, diagnostic imaging tests are commonly used since they are non-invasive examinations, whereas only a few studies reported the application of histological assessments. In animal experimental studies, histological evaluations have been commonly applied. However, not all these studies included in-depth histological analyses where molecules of great importance in the ECM, such as PGs, were analyzed.

PG analysis has been included in some publications as part of the evaluation processes and the PG content in repaired cartilage has been investigated using different methods. Some techniques, such as Safranin O/fast green staining [84,85,86,87,88,89,90,91,92,93,94], evaluate all PGs as a whole, while other techniques, such as immunohistochemistry [66,85] (Figure 2) and real-time PCR [86] (Table 2 and Table 3), allow for the evaluation of specific PGs.

Table 2. Review of human clinical trials and case report involving PGs analysis in repaired cartilage after different treatments in chondral u osteochondral injuries.

Study	Species	Chondral lesion type	Reparative treatment	Detection technique	PGs analysed	Main results
Garcia et al. 2021 [66]	Human	n/r	Autologous cell therapy	IHQ	Perlecan	Immunostaining for perlecan was significantly greater in autologous cell therapy repair tissues.
Levinson et al. 2019 [90]	Human	n/r	Minced cartilage	Safranin O/fast green	PGs altogether	Staining with Safranin O was positive, however the outgrowth potential, the viability, and the matrix deposition were not different between the mincing techniques.
Hoffman et al. 2015 [91]	Human	FCD	Marrow stimulation with a viable chondral allograft	Safranin O/fast green	PGs altogether	The safranina O staining revealed ample PG content througout the majority of the tissue.

FCD: full-thickness chondral defect; IHQ: immunohistochemical staining; n/r: not reported.

Table 3. Review of animal experimental studies involving PGs analysis in repaired cartilage after different treatments in chondral u osteochondral injuries.

Study	Species	Chondral lesion type	Reparative treatment	Detection technique	PGs analysed	Main results
Yan et al. 2020 [92]	Minipig	FCD	PRP combined with injectable HA hydrogel	Safranin O/fast green	PGs altogether	The HA hedrogel combined with PRP-treated group showed more hyaline-like cartilage with histological staining without formation of hypertrophic cartilage.
Passino et al. 2017 [93]	Ovine	PCD	REAC	Safranin O/fast green	PGs altogether	Histologically, the formation of immature hyaline articular cartilage was reported but with some slight irregularities and deformations on cartilage surface
Pfeifer et al. 2017 [94]	Minipig	FCD and PCD	MFx	Safranin O/fast green	PGs altogether	Quantification of histology showed equal overall assessment for the FCD groups and better overall assessment in juvenile animals treated with microfracture.

FCD: full-thickness chondral defect; HA: hyaluronic acid; MFx: microfracture; n/r: not reported; PCD: partial-thickness chondral defect; PRP: platelet-rich plasma; REAC: radioelectric asymmetric conveyor.

6. Conclusions

Proper articular function depends significantly on the structural integrity and composition of the ECM [15], in which PGs play a major role [18,19]. Aggrecan is the basis of the viscoelastic properties of cartilage and is crucial in cell–ECM interactions. Biglycan and decorin stabilize the ECM by regulating cartilage integrity. Versican is a transient PG present in chondral differentiation and is replaced by aggrecan in the early stages of chondrocyte differentiation.

Perlecan contributes to the mechanosensory and functional properties of cartilage and to the stabilization of the ECM.

Due to the important role of PGs in articular cartilage, it is of vital importance to assess the content and distribution of PGs in terms of chondral injury and/or repair. Additionally, the assessment of PG molecules, such as aggrecan fragments, biglycan, and decorin, could be used as a joint degradation biomarker for OA in patients. Furthermore, in experimental studies, PG analysis is a useful determinant to evaluate the quality of repaired tissue after the application of different treatment strategies for chondral injuries.

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References

1. Piez K.A. History of extracellular matrix: A personal view. Matrix Biol. 1997;16:85–92. doi: 10.1016/S0945-053X(97)90037-8.

2. Theocharis A.D., Skandalis S.S., Gialeli C., Karamanos N.K. Extracellular matrix structure. Adv. Drug Deliv. Rev. 2016;97:4–27. doi: 10.1016/j.addr.2015.11.001.

3. Yue B. Biology of the extracellular matrix: An overview. J. Glaucoma. 2014;23:S20–S23. doi: 10.1097/IJG.0000000000000108.

4. Hastings J.F., Skhinas J.M., Fey D., Croucher D.R., Cox T.R. The extracellular matrix as a key regulator of intracellular signalling networks. Br. J. Pharmacol. 2019;176:82–92. doi: 10.1111/bph.14195.

5. Jurj A., Ionescu C., Berindan-Neagoe I., Braicu C. The extracellular matrix alteration, implication in modulation of drug resistance mechanism: Friends or foes? J. Exp. Clin. Cancer Res. 2022;41:276. doi: 10.1186/s13046-022-02484-1.

6. Han B., Li Q., Wang C., Patel P., Adams S.M., Doyran B., Nia H.T., Oftadeh R., Zhou S., Li C.Y., et al. Decorin regulates the aggrecan network integrity and biomechanical functions of cartilage extracellular matrix. ACS Nano. 2019;13:11320–11333. doi: 10.1021/acsnano.9b04477.

7. Gilbert S.J., Bonnet C.S., Blain E.J. Mechanical Cues: Bidirectional Reciprocity in the Extracellular Matrix Drives Mechano-Signalling in Articular Cartilage. Int. J. Mol. Sci. 2021;22:13595. doi: 10.3390/ijms222413595.

8. Alcaide-Ruggiero L., Molina-Hernández V., Granados M.M., Domínguez J.M. Main and Minor Types of Collagens in the Articular Cartilage: The Role of Collagens in Repair Tissue Evaluation in Chondral Defects. Int. J. Mol. Sci. 2021;22:13329. doi: 10.3390/ijms222413329.

9. Xia Y., Darling E.M., Herzog W. Functional Properties of Chondrocytes and Articular Cartilage using Optical Imaging to Scanning Probe Microscopy. J. Orthop. Res. 2018;36:620–631. doi: 10.1002/jor.23757.

10. Larson C.M., Kelley S.S., Blackwood A.D., Banes A.J., Lee G.M. Retention of the native chondrocyte pericellular matrix results in significantly improved matrix production. Matrix Biol. 2002;21:349–359. doi: 10.1016/S0945-053X(02)00026-4.

11. Wiberg C., Klatt A.R., Wagener R., Paulsson M., Bateman J.F., Heinegård D., Mörgelin M. Complexes of matrilin-1 and biglycan or decorin connect collagen VI microfibrils to both collagen II and aggrecan. J. Biol. Chem. 2003;278:37698–37704. doi: 10.1074/jbc.M304638200.

12. Ulrich-Vinther M., Maloney M.D., Schwarz E.M., Rosier R., O'Keefe R.J. Articular cartilage biology. J. Am. Acad. Orthop. Surg. 2003;11:421–430. doi: 10.5435/00124635-200311000-00006.

13. Bhosale A.M., Richardson J.B. Articular cartilage: Structure, injuries and review of management. Br. Med. Bull. 2008;87:77–95. doi: 10.1093/bmb/ldn025.

14. Simon T.M., Jackson D.W. Articular cartilage: Injury pathways and treatment options. Sport. Med. Arthrosc. Rev. 2006;14:146–154. doi: 10.1097/00132585-200609000-00006.

15. Huey D.J., Hu J.C., Athanasiou K.A. Unlike bone, cartilage regeneration remains elusive. Science. 2012;338:917–921. doi: 10.1126/science.1222454.

16. Roughley P.J., Mort J.S. The role of aggrecan in normal and osteoarthritic cartilage. J. Exp. Orthop. 2014;1:8. doi: 10.1186/s40634-014-0008-7.

17. Prakash D., Learmonth D. Natural progression of osteo-chondral defect in the femoral condyle. Knee. 2002;9:7–10. doi: 10.1016/S0968-0160(01)00133-8.

18. Helmick C.G., Felson D.T., Lawrence R.C., Gabriel S., Hirsch R., Kwoh C.K., Liang M.H., Kremers H.M., Mayes M.D., Merkel P.A., et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis Rheum. 2008;58:15–25. doi: 10.1002/art.23177.

19. Alberton P., Dugonitsch H.C., Hartmann B., Li P., Farkas Z., Saller M.M., Clausen-Schaumann H., Aszodi A. Aggrecan hypomorphism compromises articular cartilage biomechanical properties and is associated with increased incidence of spontaneous osteoarthritis. Int. J. Mol. Sci. 2019;20:1008. doi: 10.3390/ijms20051008.

20. Krishnan Y., Grodzinsky A.J. Cartilage diseases. Matrix Biol. 2018;71–72:51–69. doi: 10.1016/j.matbio.2018.05.005.

21. Martin J.A., Buckwalter J.A. Roles of Articular Cartilage Aging and Chondrocyte Senescence in the Pathogenesis of Osteoarthritis. Iowa Orthop. J. 2001;21:1–7.

22. Masson A.O., Krawetz R.J. Understanding cartilage protection in OA and injury: A spectrum of possibilities. BMC Musculoskelet. Disord. 2020;21:432. doi: 10.1186/s12891-020-03363-6.

23. Vynios D.H. Metabolism of cartilage proteoglycans in health and disease. Biomed. Res. Int. 2014;2014:452315. doi: 10.1155/2014/452315.

24. Lord M.S., Whitelock J.M. Recombinant production of proteoglycans and their bioactive domains. FEBS J. 2013;280:2490–2510. doi: 10.1111/febs.12197.

25. Couchman J.R., Pataki C.A. An introduction to proteoglycans and their localization. J. Histochem. Cytochem. 2012;60:885–897. doi: 10.1369/0022155412464638.

26. Sajdera S.W., Hascall V.C. Proteinpolysaccharide Complex from bovine nasal cartilage: A comparison of low and high shear extraction procedures. J. Biol. Chem. 1969;244:77–87. doi: 10.1016/S0021-9258(19)78194-0.

27. Iozzo R.V., Schaefer L. Proteoglycan form and function: A comprehensive nomenclature of proteoglycans. Matrix Biol. 2015;42:11–55.

28. Douaiher J., Succar J., Lancerotto L., Gurish M.F., Orgill D.P., Hamilton M.J., Krilis S.A., Stevens R.L. Development of mast cells and importance of their tryptase and chymase serine proteases in inflammation and wound healing. Adv. Immunol. 2014;122:211–252.

29. Korpetinou A., Skandalis S.S., Labropoulou V.T., Smirlaki G., Noulas A., Karamanos N.K., Theocharis A.D. Serglycin: At the crossroad of inflammation and malignancy. Front. Oncol. 2014;3:327. doi: 10.3389/fonc.2013.00327.

30. Kolset S.O., Pejler G. Serglycin: A structural and functional chameleon with wide impact on immune cells. J. Immunol. 2011;187:4927–4933. doi: 10.4049/jimmunol.1100806.

31. Matsumoto K., Kamiya N., Suwan K., Atsumi F., Shimizu K., Shinomura T., Yamada Y., Kimata K., Watanabe H. Identification and characterization of versican/PG-M aggregates in cartilage. J Biol. Chem. 2006;281:18257–18263. doi: 10.1074/jbc.M510330200.

32. Aspberg A. The different roles of aggrecan interaction domains. J. Histochem. Cytochem. 2012;60:987–996. doi: 10.1369/0022155412464376.

33. Hascall V.C., Heinegård D. Aggregation of Cartilage Proteoglycans: II. Oligosaccharide competitors of the proteoglycan-hyaluronic acid interaction. J. Biol. Chem. 1974;249:4242–4249. doi: 10.1016/S0021-9258(19)42508-8.

34. Mouw J.K., Ou G., Weaver V.M. Extracellular matrix assembly: A multiscale deconstruction. Nat. Rev. Mol. Cell Biol. 2014;15:771–785. doi: 10.1038/nrm3902.

35. Hayes A.J., Melrose J. Aggrecan, the primary weight-bearing cartilage proteoglycan, has context-dependent, cell-directive properties in embryonic development and neurogenesis: Aggrecan glycan side chain modifications convey interactive biodiversity. Biomolecules. 2020;10:1244. doi: 10.3390/biom10091244.

36. Alcaide-Ruggiero L., Molina-Hernández V., Morgaz J., Fernández-Sarmiento J.A., Granados M.M., Navarrete-Calvo R., Pérez J., Quirós-Carmona S., Carrillo J.M., Cugat R., et al. Particulate cartilage and platelet-rich plasma treatment for knee chondral defects in sheep. Knee Surg. Sport. Traumatol. Arthrosc. 2023;31:2944–2955. doi: 10.1007/s00167-022-07295-7.

37. Alcaide-Ruggiero L., Molina-Hernández V., Morgaz J., Fernández-Sarmiento J.A., Granados M.M., Carrillo J.M., Cugat R., Domínguez J.M. Reparación de defectos condrales de rodilla en ovejas tras

el tratamiento con cartílago autólogo particulado y plasma rico en plaquetas; Proceedings of the XXVII Congreso Internacional de la Sociedad Española de Cirugía Veterinaria; Alicante, Spain. 17–19 February 2023.

38. Neill T., Schaefer L., Iozzo R.V. Decorin: A Guardian from the Matrix. Am. J. Pathol. 2012;181:380–387. doi: 10.1016/j.ajpath.2012.04.029.

39. Nastase M.V., Young M.F., Schaefer L. Biglycan: A Multivalent Proteoglycan Providing Structure and Signals. J. Histochem. Cytochem. 2012;60:963–975. doi: 10.1369/0022155412456380.

40. Han B., Li Q., Wang C., Chandrasekaran P., Zhou Y., Qin L., Liu X.S., Enomoto-Iwamoto M., Kong D., Iozzo R.V., et al. Differentiated activities of decorin and biglycan in the progression of post-traumatic osteoarthritis. Osteoarthr. Cartil. 2021;29:1181–1192. doi: 10.1016/j.joca.2021.03.019.

41. Kram V., Shainer R., Jani P., Meester J.A.N., Loeys B., Young M.F. Biglycan in the skeleton. J. Histochem. Cytochem. 2020;68:747–762. doi: 10.1369/0022155420937371.

42. Roughley P.J. Articular cartilage and changes in arthritis noncollagenous proteins and proteoglycans in the extracellular matrix of cartilage. Arthritis Res. 2001;3:342–347. doi: 10.1186/ar326.

43. Islam S., Watanabe H. Versican: A dynamic regulator of the extracellular matrix. J. Histochem. Cytochem. 2020;68:763–775. doi: 10.1369/0022155420953922.

44. Choocheep K., Hatano S., Takagi H., Watanabe H., Kimata K., Kongtawelert P., Watanabe H. Versican facilitates chondrocyte differentiation and regulates joint morphogenesis. J. Biol. Chem. 2010;285:21114–21125. doi: 10.1074/jbc.M109.096479.

45. Smith S.M., Melrose J. Type XI collagen-perlecan-HS interactions stabilise the pericellular matrix of annulus fibrosus cells and chondrocytes providing matrix stabilisation and homeostasis. J. Mol. Hist. 2019;50:285–294. doi: 10.1007/s10735-019-09823-1.

46. Whitelock J.M., Melrose J., Iozzo R.V. Diverse cell signaling events modulated by perlecan. Biochemistry. 2008;47:11174–11183. doi: 10.1021/bi8013938.

47. Hayes A.J., Farrugia B.L., Biose I.J., Bix G.J., Melrose J. Perlecan, A multi-functional, cell-instructive, matrix-stabilizing proteoglycan with roles in tissue development has relevance to connective tissue repair and regeneration. Front. Cell Dev. Biol. 2022;10:856261. doi: 10.3389/fcell.2022.856261.

48. Guilak F., Hayes A.J., Melrose J. Perlecan in pericellular mechanosensory cell-matrix communication, extracellular matrix stabilisation and mechanoregulation of load-bearing connective tissues. Int. J. Mol. Sci. 2021;22:2716. doi: 10.3390/ijms22052716.

49. Hayes A.J., Hughes C.E., Smith S.M., Caterson B., Little C.B., Melrose J. The CS Sulfation Motifs 4C3, 7D4, 3B3[−]; and perlecan identify stem cell populations and their niches, activated progenitor cells and transitional areas of tissue development in the fetal human elbow. Stem Cells Dev. 2016;25:836–847. doi: 10.1089/scd.2016.0054.

50. Heinegård D., Saxne T. The role of the cartilage matrix in osteoarthritis. Nat. Rev. Rheumatol. 2011;7:50–56. doi: 10.1038/nrrheum.2010.198.

51. Poole C.A., Flint M.H., Beaumont B.W. Chondrons in cartilage: Ultrastructural analysis of the pericellular microenvironment in adult human articular cartilages. J. Orthop. Res. 1987;5:509–522. doi: 10.1002/jor.1100050406.

52. Twomey J.D., Thakore P.I., Hartman D.A., Myers E.G.H., Hsieh A.H. Roles of type VI collagen and decorin in human mesenchymal stem cell biophysics during chondrogenic differentiation. Eur. Cells Mater. 2014;27:237–250. doi: 10.22203/eCM.v027a17.

53. Hayes A.J., Shu C.C., Lord M.S., Little C.B., Whitelock J.M., Melrose J. Pericellular colocalisation and interactive properties of type VI collagen and perlecan in the intervertebral disc. Eur. Cells Mater. 2016;32:40–57. doi: 10.22203/eCM.v032a03.

54. Suutre S., Kerna I., Lintrop M., Tamm H., Aunapuu M., Arend A., Tamm A. Evaluation of correlation of articular cartilage staining for DDR2 and proteoglycans with histological tissue damage and the results of radiographic assessment in patients with early stages of knee osteoarthritis. Int. J. Clin. Exp. Pathol. 2015;8:5658–5665.

55. Lu X.L., Mow V.C., Guo X.E. Proteoglycans and mechanical behavior of condylar cartilage. J. Dent. Res. 2009;88:244–248. doi: 10.1177/0022034508330432.

56. Maroudas A., Ziv I., Weisman N., Venn M. Studies of hydration and swelling pressure in normal and osteoarthritic cartilage. Biorheology. 1985;22:159–169. doi: 10.3233/BIR-1985-22206.

57. Sandy J.D., Verscharen C. Analysis of aggrecan in human knee cartilage and synovial fluid indicates that aggrecanase (ADAMTS) activity is responsible for the catabolic turnover and loss of whole aggrecan whereas other protease activity is required for C-terminal processing in vivo. Biochem. J. 2001;358:615–626. doi: 10.1042/bj3580615.

58. Kumavat R., Kumar V., Malhotra R., Pandit H., Jones E., Ponchel F., Biswas S. Biomarkers of Joint Damage in Osteoarthritis: Current Status and Future Directions. Mediat. Inflamm. 2021;2021:5574582. doi: 10.1155/2021/5574582.

59. Larsson S., Englund M., Struglics A., Lohmander L.S. Association between synovial fluid levels of aggrecan ARGS fragments and radiographic progression in knee osteoarthritis. Arthritis Res. Ther. 2010;12:R230. doi: 10.1186/ar3217.

60. Poole A.R., Rosenberg L.C., Reiner A., Ionescu M., Bogoch E., Roughley P.J. Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. J. Orthop. Res. 1996;14:681–689. doi: 10.1002/jor.1100140502.

61. Ni G.X., Li Z., Zhou Y.Z. The role of small leucine-rich proteoglycans in osteoarthritis pathogenesis. Osteoarthr. Cartil. 2014;22:896–903. doi: 10.1016/j.joca.2014.04.026.

62. Gronau T., Krüger K., Prein C., Aszodi A., Gronau I., Iozzo R.V., Mooren F.C., Clausen-Schaumann H., Bertrand J., Pap T., et al. Forced exercise-induced osteoarthritis is attenuated in mice lacking the small leucine-rich proteoglycan decorin. Ann. Rheum. Dis. 2017;76:442–449. doi: 10.1136/annrheumdis-2016-209319.

63. Barreto G., Soininen A., Ylinen P., Sandelin J., Konttinen Y.T., Nordström D.C., Eklund K.K. Soluble biglycan: A potential mediator of cartilage degradation in osteoarthritis. Arthritis Res. Ther. 2015;17:379. doi: 10.1186/s13075-015-0902-0.

64. Anders H.J., Schaefer L. beyond tissue injury—Damage-associated molecular patterns, toll-like receptors, and inflammasomes also drive regeneration and fibrosis. J. Am. Soc. Nephrol. 2014;25:1387–1400. doi: 10.1681/ASN.2014010117.

65. Gomes R.R., Farach-Carson M.C., Carson D.D. Perlecan functions in chondrogenesis: Insights from in vitro and in vivo models. Cells Tissues Organs. 2004;176:79–86. doi: 10.1159/000075029.

66. Garcia J., McCarthy H.S., Kuiper J.H., Melrose J., Roberts S. Perlecan in the natural and cell therapy repair of human adult articular cartilage: Can modifications in this proteoglycan be a novel therapeutic approach? Biomolecules. 2021;11:92. doi: 10.3390/biom11010092.

67. Gao G., Chen S., Pei Y.A., Pei M. Impact of perlecan, a core component of basement membrane, on regeneration of cartilaginous tissues. Acta Biomater. 2021;135:13–26. doi: 10.1016/j.actbio.2021.08.032.

68. Melrose J., Hayes A.J., Whitelock J.M., Little C.B. Perlecan, the "jack of all trades" proteoglycan of cartilaginous weight-bearing connective tissues. BioEssays. 2008;30:457–469. doi: 10.1002/bies.20748.

69. Tesche F., Miosge N. Perlecan in late stages of osteoarthritis of the human knee joint. Osteoarthr. Cartil. 2004;12:852–862. doi: 10.1016/j.joca.2004.07.004.

70. Biomarkers Definitions Working Group Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin. Pharmacol. Ther. 2001;69:89–95. doi: 10.1067/mcp.2001.113989.

71. Mobasheri A., Henrotin Y. Biomarkers of (osteo)arthritis. Biomarkers. 2015;20:513–518. doi: 10.3109/1354750X.2016.1140930.

72. Boffa A., Merli G., Andriolo L., Lattermann C., Salzmann G.M., Filardo G. Synovial Fluid Biomarkers in Knee Osteoarthritis: A Systematic Reviewand Quantitative Evaluation Using BIPEDs Criteria. Cartilage. 2021;13:82S–103S. doi: 10.1177/1947603520942941.

73. Braun H.J., Gold G.E. Diagnosis of Osteoarthritis: Imaging. Bone. 2012;51:278–288. doi: 10.1016/j.bone.2011.11.019.

74. Marouf B.H. Effect of Resveratrol on Serum Levels of Type II Collagen and Aggrecan in Patients with Knee Osteoarthritis: A Pilot Clinical Study. BioMed Res. Int. 2021;2021:3668568. doi: 10.1155/2021/3668568.

75. Heinegård D. Proteoglycans and more-from molecules to biology. Int. J. Exp. Pathol. 2009;90:575–586. doi: 10.1111/j.1365-2613.2009.00695.x.

76. Bay-Jensen A.C., Mobasheri A., Thudium C.S., Kraus V.B., Karsdal M.A. Blood and urine biomarkers in osteoarthritis—An update on cartilage associated type II collagen and aggrecan markers. Curr. Opin. Rheumatol. 2022;34:54–60. doi: 10.1097/BOR.0000000000000845.

77. Larsson S., Lohmander L.S., Struglics A. Synovial fluid level of aggrecan ARGS fragments is a more sensitive marker of joint disease than glycosaminoglycan or aggrecan levels: A cross-sectional study. Arthritis Res. Ther. 2009;11:92. doi: 10.1186/ar2735.

78. Lohmander L.S., Dahlberg L., Ryd L., Heinegård D. Increased levels of proteoglycan fragments in knee joint fluid after injury. Arthritis Rheum. 1989;32:1434–1442. doi: 10.1002/anr.1780321113.

79. Ozler K. Relationship between increased serum & synovial fluid decorin levels & knee osteoarthritis. Indian J. Med. Res. 2021;153:453–458.

80. Anwar R., Habib B., Mustafa Y., Kazi A., Farooq O., Nishat M. Validation of Levels of Decorin as A Reliable Biomarker of Osteoarthritis: Comparison of Serum and Synovial Fluid Levels. Pak. J. Med. Health Sci. 2022;16:11. doi: 10.53350/pjmhs20221611324.

81. Perera J.R., Gikas P.D., Bentley G. The present state of treatments for articular cartilage defects in the knee. Ann. R. Coll. Surg. Engl. 2012;94:381–387. doi: 10.1308/003588412X13171221592573.

82. Everhart J.S., Boggs Z., DiBartola A.C., Wright B., Flanigan D.C. Knee Cartilage Defect Characteristics Vary among SymptomaticRecreational and Competitive Scholastic Athletes Eligible for Cartilage Restoration Surgery. Cartilage. 2021;12:146–154. doi: 10.1177/1947603519833144.

83. Pastrama M.I., Ortiz A.C., Zevenbergen L., Famaey N., Gsell W., Neu C.P., Himmelreich U., Jonkers I. Combined enzymatic degradation of proteoglycans and collagen significantly alters intratissue strains in articular cartilage during cyclic compression. J. Mech. Behav. Biomed. Mater. 2019;98:383–394. doi: 10.1016/j.jmbbm.2019.05.040.

84. Hsueh M.F., Khabut A., Kjellström S., Önnerfjord P., Kraus V.B. Elucidating the Molecular Composition of Cartilage by Proteomics. J. Proteome Res. 2016;15:374–388. doi: 10.1021/acs.jproteome.5b00946.

85. Pretzel D., Linss S., Ahrem H., Endres M., Kaps C., Klemm D., Kinne R.W. A novel in vitro bovine cartilage punch model for assessing the regeneration of focal cartilage defects with biocompatible bacterial nanocellulose. Arthritis Res. Ther. 2013;15:R59. doi: 10.1186/ar4231.

86. Mori H., Kondo E., Kawaguchi Y., Kitamura N., Nagai N., Iida H., Yasuda K. Development of a salmon-derived crosslinked atelocollagen sponge disc containing osteogenic protein-1 for articular cartilage regeneration: In vivo evaluations with rabbits. BMC Musculoskelet. Disord. 2013;14:174. doi: 10.1186/1471-2474-14-174.

87. Yan W., Xu X., Xu Q., Sun Z., Jiang Q., Shi D. Platelet-rich plasma combined with injectable hyaluronic acid hydrogel for porcine cartilage regeneration: A 6-month follow-up. Regen. Biomater. 2019;7:77–90. doi: 10.1093/rb/rbz039.

88. Levinson C., Cavalli E., Sindi D.M., Kessel B., Zenobi-Wong M., Preiss S., Salzmann G., Neidenbach P. Chondrocytes From device-minced articular cartilage show potent outgrowth into fibrin and collagen hydrogels. Orthop. J. Sport. Med. 2019;7:2325967119867618. doi: 10.1177/2325967119867618.

89. Passino E.S., Rocca S., Caggiu S., Columbano N., Castagna A., Fontani V., Rinaldi S. REAC regenerative treatment efficacy in experimental chondral lesions: A pilot study on ovine animal model. Clin. Interv. Aging. 2017;12:1471–1479. doi: 10.2147/CIA.S140976.

90. Pfeifer C.G., Fisher M.B., Saxena V., Kim M., Henning E.A., Steinberg D.A., Dodge G.R., Mauck R.L. Age-dependent subchondral bone remodeling and cartilage repair in a minipig defect model. Tissue Eng. Part C Methods. 2017;23:745–753. doi: 10.1089/ten.tec.2017.0109.

91. Shi D., Xu X., Ye Y., Song K., Cheng Y., Di J., Hu Q., Li J., Ju H., Jiang Q., et al. Photo-cross-linked scaffold with kartogenin-encapsulated nanoparticles for cartilage regeneration. ACS Nano. 2016;10:1292–1299. doi: 10.1021/acsnano.5b06663.

92. Hoffman J.K., Geraghty S., Protzman N.M. Articular Cartilage Repair Using Marrow Stimulation Augmented with a Viable Chondral Allograft: 9-Month Postoperative Histological Evaluation. Case Rep. Orthop. 2015;2015:617365. doi: 10.1155/2015/617365.

93. Christensen B.B., Foldager C.B., Olesen M.L., Vingtoft L., Rölfing J.H.D., Ringgaard S., Lind M. Experimental articular cartilage repair in the Göttingen minipig: The influence of multiple defects per knee. J. Exp. Orthop. 2015;2:13. doi: 10.1186/s40634-015-0031-3.

94. Peck Y., He P., Chilla G.S.V.N., Poh C.L., Wang D.A. A preclinical evaluation of an autologous living hyaline-like cartilaginous graft for articular cartilage repair: A pilot study. Sci. Rep. 2015;5:16225. doi: 10.1038/srep16225.

95. Milano G., Sanna-Passino E., Deriu L., Careddu G., Manunta L., Manunta A., Saccomanno M.F., Fabbriciani C. The effect of platelet rich plasma combined with microfractures on the treatment of chondral defects: An experimental study in a sheep model. Osteoarthr. Cartil. 2010;18:971–980. doi: 10.1016/j.joca.2010.03.013.

VI. CAPÍTULO III

Objetivo II / Objective II

Particulated cartilage and platelet-rich plasma treatment for knee chondral defects in sheep

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Objective II: Evaluate the quality of the repaired tissue at the macroscopic level using three distinct scoring systems. In addition, evaluate the maturity and morphology of chondrocytes and the chondrogenic regenerative properties of the PACI+PRP treatment in chondral defects at the histopathological level through hematoxylin and eosin staining in our ovine experimental model.

Particulated cartilage and platelet-rich plasma treatment for knee chondral defects in sheep

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Abstract

Purpose Articular cartilage is vulnerable to multiple types of damage, and, it has limited reparative and regenerative capacities due to its absence of vascularity. Although a large number of therapeutic strategies exist to treat chondral defects, they have some limitations, such as fibrocartilage formation. Therefore, the goal of the present study was to evaluate the chondrogenic regenerative properties of an autologous-made matrix of particulated cartilage and platelet-rich plasma (PACI+PRP) implantation for the treatment of full-thickness chondral defects in sheep.

Methods A full-thickness 8 mm diameter cartilage defect was created in the weight-bearing area of the medial femoral condyle in both knees of 16 sheep. The right knees of all animals were treated with particulated autograft cartilage implantation and platelet-rich plasma, while the left knees were injected with Ringer's lactate solution or hyaluronic acid. The sheep were sacrificed 9 or 18 months after surgery. Macroscopic evaluations were performed using three different scoring systems, and histopathological evaluations were performed using a modified scoring system based on different scoring systems.

Results The PACI+PRP groups showed statistically significant differences in the percentage of defect repair and chondrocytes in the newly formed cartilage tissue at 18 months compared to 9 months.

Conclusions The results suggest that macroscopic appearance, histological structure, and chondrocyte repair were improved when using PACI+PRP treatment for chondral defects, producing an outcome similar to the surrounding healthy cartilage. PACI+PRP is a totally autologous, easy, and unexpensive treatment that can be performed in one-step procedure and is useful as a therapeutic option for knee chondral defects.

Keywords: Articular cartilage. Chondrogenesis. Particulated cartilage. Platelet-rich plasma. Sheep.

1. Introduction

Articular cartilage is a highly specialized tissue that provides a lubricious, low friction gliding surface for joints [47]. Articular cartilage is vulnerable to trauma, overloading, aging, and inflammation. In fact, these chondral injuries are some of the most common injuries of the musculoskeletal system and may lead to progressive damage and joint disorders such as osteoarthritis (OA) [43]. Cartilage has limited reparative and regenerative capacities due to its lack of vascularity [25]. In addition, mature chondrocytes lose their ability to migrate, proliferate, and synthesize their surrounding matrix [47]. The present therapeutic strategies applied to treat chondral lesions have some limitations, such as fibrocartilage formation without lasting improvements, prolonged recovery time, insufficient integration with healthy cartilage, the need to employ two-stage procedures, an inability to restore large defects, expensive treatments, or unpredictable results in athletes [10,24,37,41,44,57]. Platelet-rich plasma (PRP) is a therapeutic option that is being increasingly used in musculoskeletal medicine. Its therapeutic potential is based on the supraphysiological supply of growth factors and cytokines, which promote the repair of tissues with low healing potential [12,52,54]. In vitro studies have reported that growth factors can stimulate chondrogenic regeneration, improving cartilage matrix protein biosynthesis and enhancing chondrocyte proliferation and metabolism [1]. In addition, PRP has been used in vivo to repair chondral defects in combination with other techniques, improving the quality of the repaired tissue [14,38,54,58]. One of these techniques is particulated autograft cartilage implantation (PACI), which has been shown to lead to a better quality of repair of chondral lesions in animal and human studies [4,8,13,14,17]. However, the studies conducted to date have some relevant limitations, such as small sample size, the absence of comparative groups, short follow-up, and/or the absence of a meticulous study of the quality of the repaired tissue.

Therefore, the goal of the present study was to evaluate the chondrogenic regenerative properties of an autologous-based matrix composed of healthy hyaline cartilage chips with a clot of PRP and an intra-articular infiltration of PRP (PACI+PRP) for full-thickness defects in the weight-bearing area of the medial femoral condyle in sheep, and meticulous macroscopical and histopathological studies were carried out. It was hypothesized that this technique can restore chondral lesions in normal articular cartilage in sheep.

2. Materials and Methods

2.1. Ethical statement

The present study was approved by the Bioethical Committee on Animal Research of the Regional Government of Andalusia (Junta de Andalucía 12/06/2016/109—reference SSA/SIS/MD/jv) and was conducted in accordance with the protection regulations for animals utilized for scientific purposes (Directive 2010/63/UE; Decision 2020/569/UE and RD 1386/2018).

2.2. Animals and surgical procedure

Sixteen skeletally mature Merino sheep (n=16), each weighing between 50 and 60 kg, were used for this study. A veterinary examination guaranteed that the animals were healthy and showed no musculoskeletal clinical signs before the surgical procedure, described as follows:

Both hind limbs of the anesthetized sheep were prepared for knee surgery. After a medial parapatellar approach, a 3–4 cm mini-arthrotomy was performed. The knees were flexed, and an 8 mm diameter punch was used to create a full-thickness cartilage defect in the weight-bearing area of the medial femoral condyle, without opening the subchondral bone. The right knees were treated with PACI+PRP, and the cartilage sample obtained was particulated in small 1–2 mm fragments and mixed with the activated PRP to obtain a clot used as a scaffold for the cartilage chips. A PACI+PRP matrix was placed to fill the cartilage defect. The adhesion of the clot was confirmed by performing flexion–extension of the knee. Then, the arthrotomy was closed, and 2 mL of activated PRP was injected intra-articularly. Moreover, the same surgical procedure for chondral defects was performed in the left knees, and the knees were randomly divided into two groups. Half of the left knees received an intra-articular injection of 2 ml of Ringer's lactate solution (RLS, n=8) as a control, and the other half were treated with 2 ml of hyaluronic acid (Synvisc One, Hylan G-F 20) (HA, n=8). Finally, antibiotic (amoxicillin–clavulanic acid, 10 mg/kg IM) and analgesic (buprenorphine 0.02 mg/kg/8 h IM) treatments were given for 3 and 5 days after surgery, respectively.

Sheep were randomly divided into two study groups: the RLS/PACI+PRP (RLS) group, which included eight sheep treated with RLS in the left knee and PACI+PRP in the right knee (n=8), and the HA/PACI+PRP (HA) group, which included eight sheep treated with HA in the left knee and PACI+PRP in the right knee (n=8). Sacrifice times were established at 9 or 18 months

after surgery. Four randomly chosen sheep from each study group were sacrificed at each time point.

2.3. Platelet-rich plasma

PRP total treatment was prepared using the PRGF-Endoret system (Biotechnology Institute, Vitoria, Spain). Blood was collected in four extraction tubes from the jugular vein of each animal and was centrifuged for 8 min at 630× g according to a published method [4]. Then, the centrifugated plasma volume was divided by 50%, so the upper layer and the deeper layer just over the buffy coat were labeled as fraction 1 and fraction 2, respectively. Fraction 2 was obtained by avoiding the aspiration of white and blood cells. The platelets were activated by adding 50 µL of calcium chloride 10% per 1 mL of plasma just prior to the use of both fractions. The whole treatment was studied, including PACI+PRP application and intra-articular PRP injection. For the PACI+PRP treatment, activated fraction 1 and fraction 2 were combined with the particulated cartilage at a 50/50 ratio and left for 30 minutes to obtain a semisolid scaffold. Additionally, a PRP intra-articular injection of 2 mL of activated fraction 2 was used.

The blood for PRP total treatment preparation was collected just prior to surgery and was processed and provided intraoperatively. The time delay between blood collection and the application of both fractions was less than 1.5 h. No postoperative PRP injection was given.

2.4. Macroscopic evaluation

After sacrifice at 9 or 18 months, digital high-resolution photographs were taken of the medial femoral condyle articular surface.

Macroscopic evaluation of cartilage repair was carried out according to three evaluation systems (Tables S1, S2, and S3). The validated International Cartilage Regeneration and Joint Preservation Society (ICRS) [50] scoring system analyzes the degree of defect repair, the integration of the border zone, and the macroscopic appearance (Table S1). The evaluation described by Jung et al. [26] uses a semiquantitative score, which notes the filling of the defect, surface, integration, and color (Table S2). The Goebel et al. [22] scoring system analyzes the color of the repair tissue, presence of blood vessels, surface, filling of the defect, and degeneration of adjacent articular cartilage (Table S3). In the three evaluation systems, the highest score is achieved for the best possible result. Scoring was performed by three different researchers for all three evaluation systems.

2.5. Histopathological study

For the histopathological analysis, medial femoral condyles were harvested and fixed in 10% neutral buffered formalin for 24 h, decalcified, and then routinely processed and embedded in paraffin wax. Tissue sections (4 µm thick) were stained with hematoxylin and eosin (H&E). Histological images were taken using a photomicroscope (Olympus BX43), and photomicrographs were analyzed with Image J software. To determine the quality of the repaired tissue, chondral defects were histologically evaluated and compared with the surrounding normal hyaline cartilage within the same sheep.

The regenerated tissue was scored using a modified scoring model based on different scoring systems [33,34,45,51,53] (Table 1). This scoring system included a total of 10 parameters. Six parameters were studied for a quantitative evaluation of chondrocytes and chondral repair: defect regeneration (%), cartilage thickness (µm), cell count (cell/mm2), lacunae area (µm2), cell area (µm2), and cell morphology (form factor = [π*area]/perimeter2). Four parameters were assessed for a semiquantitative evaluation of the cartilage structure: cartilage areas, tidemark formation, lateral integration of the defect, and cell distribution. The structure of the cartilage was scored on a scale of 0 to 14 (Table 1), with 14 points indicating a completely normal cartilage structure.

2.6. Statistical analyses

A statistical analysis was performed using GraphPad Prism software 7.0 (Inc., San Diego, CA, USA). After performing the Kolmogorov–Smirnoff normality test, the quantitative and semiquantitative variables were analyzed using nonparametric tests. For the comparisons made in the same sacrifice group, between treatments carried out in the same individual, the Wilcoxon test was performed. However, the Mann–Whitney U test was used to make comparisons between treatments carried out in different sheep. To compare the results of the same treatment group between the two sacrifice periods, the Mann–Whitney U test was performed. The variables in the tables are expressed as means (minimum value – maximum value). The variables were considered statistically significant when the p value < 0.05*, p<0.01**, or p<0.001***.

Significant differences between groups as they pertained to the lacuna area were considered to be detectable, with a statistical power of 0.8 and, a significance level of 0.05. When considering a SD of 50 µm2 and a difference between groups of 100 µm2, 4 animals per group would be required.

Table 1. Histological parameter analyzed and score system used [33,34,45,53,55].

Histological Parameter			Score system
Defect regeneration (%)			Pineda [33] and Wakitani [51]
Cartilage thickness (μm)			n/a
Cell count (cell/mm^2)			n/a
Lacuna area (μm^2)			n/a
Cell area (μm^2)			n/a
Cell morphology (form factor = [π*area]/perimeter^2)			ICRS II [34]
		Points	
Cartilage structure	Cartilage areas		ICRS II [34] and Mankin [53]
	Normal (superficial, middle, deep, and calcified zone)	4	
	Absence of one of the zones	3	
	Absence of two of the zones	2	
	Presence of only one zone	1	
	Total absence of cartilage	0	
	Tidemark formation		ICRS II [34] and Mankin [53]
	Yes, defined	2	
	Yes, blurred	1	
	No	0	
	Lateral integration of the defect		O´Driscoll [45]
	Complete integration of both sides	4	
	Full integration of one side	3	
	Partial integration of both sides	2	
	Partial integration of one side	1	
	No side integration	0	
	Cell distribution		Mankin [53]
	Normal	4	
	Diffuse hypercellularity	3	
	Diffuse hypercellularity and isogenic groups	2	
	Abundant isogenic groups	1	
	Hypocellularity	0	

n/a: not reported in previous studies.

3. Results

All the animals reached the end of the study without incidents or adverse effects.

3.1. Macroscopic appearance and scoring

After sacrificing the animals, no gross changes consistent with inflammation or other pathological changes were observed in the knee joints.

At 9 and 18 months, a clear trend in the scores was observed in the macroscopic repair evaluation of the chondral defects treated with PACI+PRP, with the scores of the PACI+PRP knees being substantially higher according to the three evaluation systems used than those of their respective control knees treated with RLS or HA. Moreover, at 18 months after the administration of PACI+PRP treatment, the scores tended to be considerably higher than those

obtained at 9 months for all of the studied groups, independent of the evaluation system used (Table 2).

Table 2. Score obtained from the macroscopic evaluation.

Evaluation system	Time of sacrifice	RLS	PACI+PRP (RLS)	HA	PACI+PRP (HA)	Healthy cartilage[Ψ]
ICRS	9 months	2.6 (1.0-4.0)	7.6 (5.7-9.0)	4.2 (0.7-10.3)	6.6 (4.7-9.7)	12
	18 months	5.2 (4.0-6.3)	9.3 (6.7-11.7)	6.5 (5.3-8.3)	10.3 (8.0-11.7)	
GOEBEL	9 months	9.3 (5.3-12.0)	14.8 (13.3-16.3)	10.7 (4.7-17.0)	12.6 (9.7-16.0)	20
	18 months	11.2 (10.3-1.0)	16.6 (12.7-19.7)	14.8 (11.7-16.3)	17.8 (15.3-19.0)	
JUNG	9 months	1.8 (1.0-2.7)	4.0 (3.3-5.3)	2.1 (0.0-5.0)	3.0 (1.7-4.7)	6
	18 months	1.6 (1.0-2.3)	4.7 (2.7-6.0)	2.9 (1.7-4.0)	5.2 (3.7-6.0)	

RLS: left knee treated with Ringer's lactate solution; PACI+PRP (RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP (HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee. Data are expressed as mean (minimum – maximum values). [Ψ] Maximum score value.

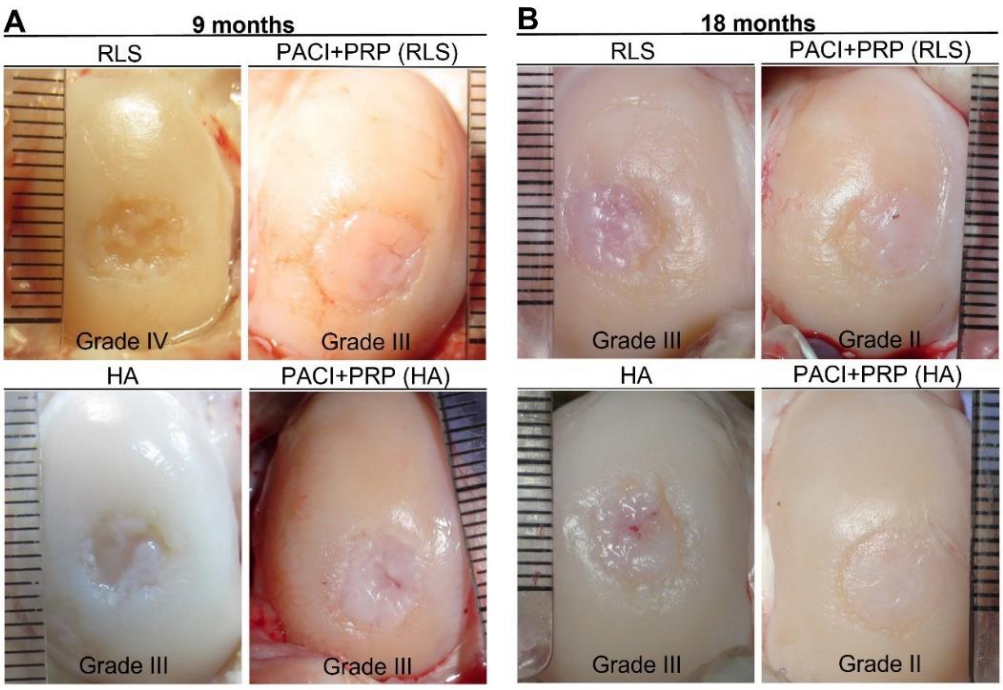

Figure 1. Macroscopic digital photographs of chondral defects after sacrifice at 9 months (A) and 18 months (B) for RLS, PACI+PRP (RLS), HA, and PACI+PRP (HA) groups. Degree of repair according to the ICRS scoring system 19, with Grade II being nearly normal; Grade III being abnormal; Grade IV being severely abnormal. RLS: left knee treated with Ringer's lactate solution; PACI+PRP (RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP (HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee.

Furthermore, the ICRS scoring system allowed for the classification of the degree of chondral repair based on the score obtained (Table S1). The knees treated with RLS had the lowest degree of repair at 9 months (Figure 1A). In addition, the PACI+PRP treated knees showed a nearly normal degree of repair at 18 months (Figure 1B).

3.2. Histopathological analysis

At 9 and 18 months, a percentage of the defect was regenerated, and the thickness of the cartilage regenerated with PACI+PRP tended to be closer to that of the normal hyaline cartilage among the three groups (Tables 3 and 4), showing the highest regeneration percentage at 18 months (90.4%). Likewise, the cell count, area of the lacuna, and cells of the regenerated cartilage did not differ between the treatments. The cell morphology values of the PACI+PRP groups tended to be closer to those of the surrounding normal hyaline cartilage, being equal at 18 months (0.93), and they were higher than those of the RLS and HA groups (Tables 3 and 4).

Table 3. Results of the histopathological analysis at 9 months (quantitative parameters).

	9 months group							
	RLS	**Hyaline cartilage** RLS	**PACI+PRP (RLS)**	**Hyaline cartilage** PACI+PRP(RLS)	**HA**	**Hyaline cartilage** HA	**PACI+PRP (HA)**	**Hyaline cartilage** PACI+PRP(HA)
Defect regeneration (%)	56.1 (37.9-84.8)	100 (100-100)	64.4 (45.5-86.2)	100 (100-100)	44.9 (17.5-88.1)	100 (100-100)	68.52 (62.2-80.0)	100 (100-100)
Cartilage thickness (μm)	300.7 (175.5-368.1)	496.5 (452.5-525.0)	417.9 (365.6-507.2)	575.2 (499.8-713.2)	347.0 (241.7-446.9)	859.5 (385.2-1262.0)	333.5 (243.3-423.9)	461.0 (293.6-718.6)
Cell count (cell/mm²)	244.8 (223.8-262.9)	94.7 (69.9-107.2)	197.5 (163.5-229.1)	74.8 (68.4-85.1)	276.2 (177.9-381.1)	68.5 (54.6-87.7)	231.5 (214.6-256.1)	69.8 (51.3-91.2)
Lacunae area (μm)	102.6 (92.6-121.3)	208.3 (190.5-221.5)	103.2 (81.6-124.0)	193.7 (170.2-227.2)	77.5 (37.0-101.5)	154.5 (54.1-198.5)	97.9 (88.3-114.5)	220.9 (202.5-249.7)
Cell area (μm)	36.3 (32.9-39.7)	20.3 (16.7-25.4)	28.0 (24.7-31.9)	19.6 (18.3-20.2)	38.1 (24.7-45.5)	22.3 (17.1-31.3)	30.2 (26.6-33.5)	17.1 (15.6-20.0)
Cell morphology (form factor)	0.83 (0.78-0.86)	0.92 (0.91-0.93)	0.92 (0.89-0.93)	0.90 (0.88-0.93)	0.84 (0.82-0.85)	0.92 (0.90-0.95)	0.89 (0.86-0.91)	0.91 (0.90-0.92)

RLS: left knee treated with Ringer's lactate solution; PACI+PRP (RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP (HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee.

Table 4. Results of the histopathological analysis at 18 months (quantitative parameters).

	18 months group							
	RLS	**Hyaline cartilage** RLS	**PACI+PRP (RLS)**	**Hyaline cartilage** PACI+PRP(RLS)	**HA**	**Hyaline cartilage** HA	**PACI+PRP (HA)**	**Hyaline cartilage** PACI+PRP(HA)
Defect regeneration (%)	66.0 (50.1-82.0)	100 (100-100)	90.4 (78.4-99.3)	100 (100-100)	73.0 (27.9-90.2)	100 (100-100)	88.3 (74.9-96.7)	100 (100-100)
Cartilage thickness (µm)	287.2 (134.0-425.1)	639.6 (436.0-808.9)	341.5 (271.5-461.8)	534.6 (408.4-701.7)	250.7 (53.5-471.3)	515.9 (229.4-690.2)	248.8 (118.6-339.1)	537.7 (181.2-938.6)
Cell count (cell/mm²)	218.5 (189.4-257.7)	69.3 (56.3-88.7)	154.4 (141.2-170.5)	62.0 (56.6-67.7)	247.5 (130.4-377.8)	60.2 (45.3-72.3)	149.5 (129.6-165.0)	67.8 (57.6-82.8)
Lacunae area (µm)	82.4 (37.7-167.4)	121.2 (46.2-200.7)	92.8 (42.8-146.2)	131.8 (51.4-215.7)	103.2 (40.7-142.7)	204 (189.3-224.1)	114.1 (39.8-162.6)	162.8 (49.8-223.6)
Cell area (µm)	27.0 (21.2-36.5)	20.7 (19.0-24.0)	24.3 (18.2-28.0)	19.9 (16.0-23.1)	30.2 (19.2-46.2)	20.7 (16.7-23.4)	20.0 (15.6-23.5)	21.1 (15.7-27.6)
Cell morphology (form factor)	0.89 (0.81-0.94)	0.93 (0.90-0.95)	0.93 (0.92-0.94)	0.92 (0.90-0.92)	0.89 (0.85-0.92)	0.92 (0.91-0.94)	0.93 (0.92-0.94)	0.93 (0.92-0.93)

RLS: left knee treated with Ringer's lactate solution; PACI+PRP (RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP (HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee.

Regarding the evaluation of the cartilage structure, the PACI+PRP groups showed scores closer to those of the normal hyaline cartilage (Figure 2). Despite not being statistically significant, the PACI+PRP-repaired cartilage was found to have better lateral integration with the surrounding cartilage, and the tidemark formation was more defined (Figure 3). In terms of cellular organization, no major differences were observed between the different groups.

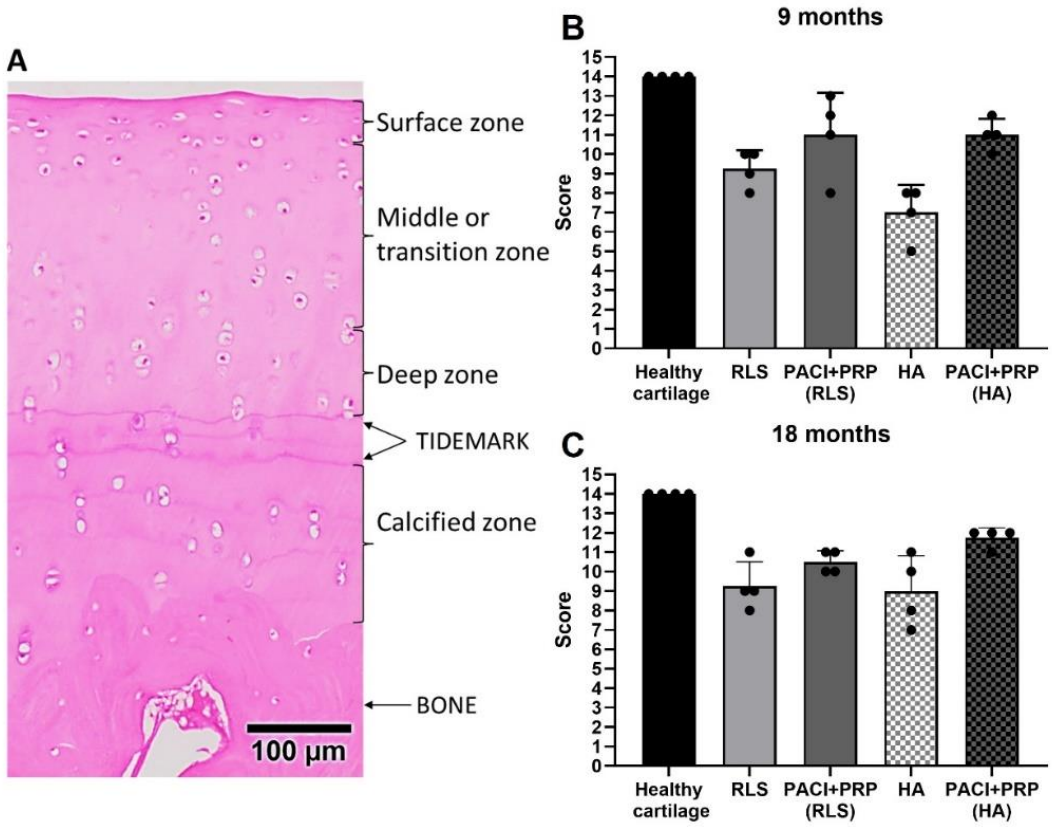

Figure 2. Analysis of the cartilage structure (semiquantitative parameters). (A) Histological image showing the different layers of healthy cartilage structure used to evaluate histopathological parameters (H&E stain). (B and C) Score obtained in each group evaluating the structure of the repaired cartilage at 9 and 18 months, respectively. RLS: left knee treated with Ringer's lactate solution; PACI+PRP (RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP (HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee.

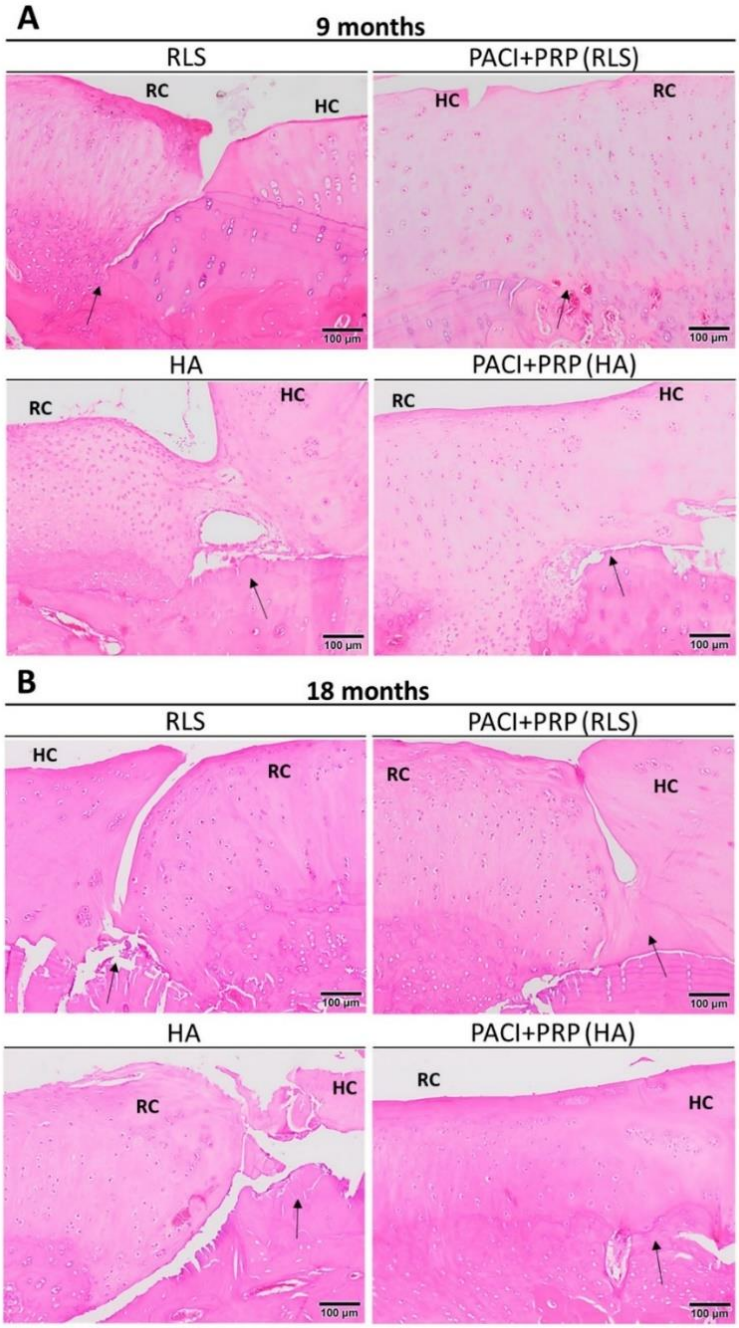

Figure 3. Histological images showing the structure of articular cartilage after administration of the different treatments at 9 and 18 months (H&E stain). The arrows indicate the boundary between healthy cartilage (HC) and repaired cartilage (RC), observing the degree of repair and lateral integration. (A) Histological images at 9 months showing complete lateral integration of the repaired cartilage with the surrounding healthy cartilage in PACI+PRP (RLS) and PACI+PRP (HA) groups, in contrast to the observation for the RLS and HA groups. (B) Histological images at 18 months showing better lateral integration of repaired cartilage with the surrounding healthy cartilage in PACI+PRP (RLS) group than in RLS group. Complete lateral integration of the repaired cartilage with healthy cartilage was observed in the PACI+PRP (HA) group compared with incomplete lateral integration of repaired cartilage in the RLS and HA groups. RLS: left knee treated with Ringer's lactate solution; PACI+PRP (RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP (HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee.

3.3. Chondrocytes and chondral repair: recovery at 9 and 18 months

The sheep treated with PACI+PRP showed statistically significant increases in the percentage of regenerated defect (25.6%; p=0.002) (Figure 4A) and cell morphology (0.02 form factor; p=0.021) (Figure 4D) in repaired cartilage tissue at 18 months compared to 9 months of recovery. Statistically significant decreases were observed in cell count (Figure 4B) (64.7 cell/mm2; p=0.001) and cell area (7.0 µm2; p=0.005) (Figure 4C) in cartilage regenerated with PACI+PRP treatment at 18 months compared to 9 months of recovery.

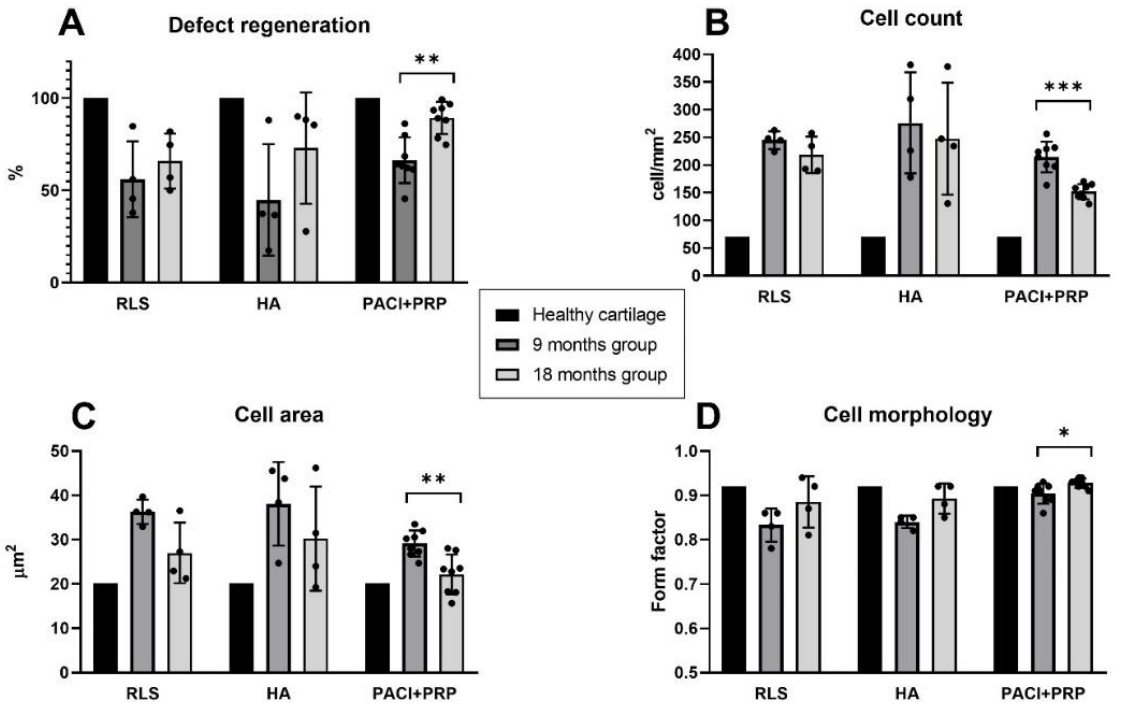

Figure 4. Comparison of the different treatments according to percentage of cartilage defect repair and chondrocyte recovery time studied (9 and 18 months). (A) Defect regeneration (%). (B) Cell count (cell/mm2). (C) Cell area (µm2). (D) Cell morphology (form factor). RLS: left knee treated with Ringer's lactate solution; HA: left knee treated with hyaluronic acid; PACI+PRP: right knee treated with particulated autograft cartilage implantation and platelet-rich plasma. *p<0.05; **p<0.01; ***p<0.001.

4. Discussion

Considering the key role of chondrocytes in the maintenance and repair processes of hyaline cartilage, the most important finding of the present study was the improved quality observed in the cartilage cells in the PACI+PRP groups, which were very similar to those of healthy cartilage.

Despite improvements in technology and tissue engineering, an effective treatment for articular cartilage repair remains elusive [25,39]. Techniques such as autologous chondrocyte implantation (ACI) or matrix-induced ACI (MACI), require two surgical interventions, in addition

to cell cultures, and they have possible drawbacks, such as cell senescence and dedifferentiation [35,37,48]. The autologous matrix-induced chondrogenesis technique (AMIC) needs to be combined with other techniques [40]. Despite reporting substantial improvements, the obstacles surrounding cell-based techniques have led experts to study and use other therapeutic strategies. A possible alternative is the application of small cartilage chips that have been previously particulated or minced (PACI or MCI) [2,31,32,36]. This technique has the advantages of being able to be applied in a single surgical intervention and not requiring cell cultures or scaffolds. A growing number of animal and human studies support the potential of better repaired cartilage quality using minced or particulated cartilage techniques [2,8,11,21,23,32,35,56]. These promising results stem from the migration of chondrocytes into a biomaterial and the subsequent cartilaginous extracellular matrix (ECM) deposition by these cells [19].

In this study, a therapy for chondral lesions based on the combination of PACI and autologous growth factor treatment was presented. In this method, the autologous hyaline cartilage chips serve as a bioactive matrix. Cugat et al. [15] and Delman et al. [16] showed in studies with humans that PACI is a safe and efficient surgical procedure with promising results. Therefore, the goal of this study was to provide in vivo evidence on chondrogenic repair quality following treatment with PACI+PRP by carrying out a detailed study of the quality of repaired tissue in sheep over two prolonged periods of time, 9 and 18 months after surgery, and by comparing the results with those of RLS and HA treatments.

The degree of fragmentation in particulated cartilage is a critical parameter for the amount of ECM production, which is hypothetically due to chondrocyte activation upon the mechanical stimulation of the cut [7,45]. Bonasia et al. [7] recommended fragmenting cartilage into cubes of about 2 mm3 or using minced cartilage. Farr et al. [20] carried out a study comparing particulated cartilage and minced cartilage, concluding that both techniques appear to be similar. In this study, the cartilage particles were fragmented into cubes that were between 1 and 2 mm3 in size. Another important consideration is the material used as a scaffold to support the particulated cartilage. No consensus exists on the nature of the most appropriate biomaterial to be used to promote chondrocyte migration. Fibrin glue is the material most often used as a scaffold in chondral lesions treated with particulated cartilage [16,21,23,28,42,46,55]. However, the source and concentration of its components vary widely, affecting both its mechanical strength and adhesive properties [6]. When PRP was used as scaffold, it promoted cell migration but also served as bioactive scaffolds to promote chondrocyte viability, proliferation, and differentiation, acting as reservoirs of growth factors and cytokines [12,27].

Furthermore, growth factors and cytokines released by PRP have been shown to play a key role in chondrogenesis during cartilage repair [4,59]. A 100% autologous bioactive matrix was used in PACI+PRP treatment, which combined hyaline cartilage chips that can generate a chondrogenic environment once mixed with PRP clots, and allowing the construct to reach a semisolid state before implantation. The further intra-articular injection of PRP after scaffold implantation provides an additional source of growth factors to enhance the regenerative joint environment within the defect.

The quality of cartilage formed in a chondral defect treated with PACI+PRP in sheep was investigated in this study, and exhaustive macroscopic and microscopic analyses were carried out over a long period of time after surgery. In this study, an attempt was made to obtain more robust results. For this reason, different validated assessment systems were used. Furthermore, the assessment of some other parameters (cartilage thickness, cell count, lacuna area, and cell area) was considered important, so these were added after the literature review was performed for this study.

The macroscopic evaluation showed that the PACI+PRP groups had a better appearance than the RLS and HA groups, especially after 18 months of recovery. The cartilage obtained in the RLS group showed the worst macroscopic appearance. The histological analysis showed that the cartilage repair tissue structure in the PACI+PRP groups was of a higher quality than that in the RLS and HA groups at both 9 and 18 months of recovery. Furthermore, the chondrocytes showed a statistically significant more advanced repair process in the PACI+PRP groups after 18 months of recovery. It is recognized that chondrocytes play a unique role in the development, maintenance, and repair of ECM [3].

In this study, most of the observed results were not statistically significant despite the promising results of the cartilage being repaired to a nearly normal state after 18 months of treatment with PACI+PRP. This is probably due to the great variability that exists between sheep [30,49], so a large dispersion of the data were found. However, in the present study, some of the limitations of previous studies were able to overcome. Control groups were included to compare the results of the treatments at different times, and a longer follow-up study was also carried out.

5. Conclusion

The macroscopic and histological structure evaluations of cartilage repair showed that the newly formed tissue after the PACI+PRP treatment tended to be more similar to healthy articular cartilage than that after RLS and HA treatments at 9 and 18 months. The percentage of cartilage defect repair and chondrocytes resulted in a statistically significant and more advanced repair process in the PACI+PRP groups after 18 months of recovery.

Supplementary Information: The online version contains supplementary material available at https:// doi. org/ 10. 1007/ s00167- 022- 07295-7.

Table S1. ICRS macroscopic evaluation of repair [50].

Cartilage repair assessment ICRS	**Points**
Degree of defect repair	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4th of graft integrated, 1/4th with a notable border >1mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1 mm	1
From no contact to 1/4th of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several, small or few but large fissures	1
Total degeneration of grafted area	0
Overall repair assessment	
Grade I: normal	12
Grade II: nearly normal	11 – 8
Grade III: abnormal	7 – 4
Grade IV: severely abnormal	3 – 1

Table S2. Adaptation of Jung´s semi-quantitative score [26].

Parameter	Points
Defect filling	
Empty	0
Half	1
Complete	2
Defect surface	
Rough	0
Smooth	1
Defect integration	
Bad	0
Good	1
Defect colour	
Dark	0
Bright	1
Normal	2
Total score	6

Table S3. Adaptation of Goebel macroscopic evaluation of repair [22].

Parameter	Points
Color of the repair tissue	
Hyaline or white	4
Predominantly white (>50%)	3
Predominantly translucent (>50%)	2
Translucent	1
No repair tissue	0
Presence of blood vessels in the repair tissue	
No	4
Less than 25% of the repair tissue	3
25-50% of the repair tissue	2
50-75% of the repair tissue	1
More than 75% of the repair tissue	0
Surface of the repair tissue	
Smooth, homogenous	4
Smooth, heterogeneus	3
Fibrillated	2
Incomplete new repair tissue	1
No repair tissue	0
Filling of the defect	
In level with adjacent cartilage	4
>50% repair of the defect depth or hypertrophy	3
<50% repair of defect depth	2
0% repair of defect depth	1
Subchondral bone damage	0
Degeneration of adjacent articular cartilage	
Normal	0
Cracks and/or fibrillations in integrations zone	1
Diffuse osteoarthritic changes	2
Extension of the defect into the adjacent cartilage	3
Subchondral bone damage	4
Total score	20

Author contributions: Conceptualization: RC, JMD and JMC; Methodology: JMD, RC, LAR, JMC, VMH, MMG, RNC, SQC and JAFS; Formal analysis and investigation: LAR, JMD, VMH, JM, JP and JAFS; Writing- original draft preparation: LAR and JMD; Writing – review and editing: JMD, VMOH, JP, RC and JM; Funding acquisition: RC; Supervision: JMD.

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Data availability: The data presented in this study are available on request from the corresponding author.

Declarations

Conflicts of interest: The authors declare that they have no conflict of interest.

Ethical approval: All animal experimental protocols were approved by Bioethical Committee on Animal Research of the Regional Government of Andalusia (Junta de Andalucía 12/06/2016/109-reference SSA/SIS/MD/jv) and conducted in accordance to the protection of animals utilized for scientific purposes (Directive 2010/63/UE, Decision 2020/569/UE and RD 1386/2018).

References

1. Abrams G.D., Frank R.M., Fortier L.A., Cole B.J. Platelet-rich plasma for articular cartilage repair. Sports Med Arthrosc. 2013;21:213-219. doi:10.1097/JSA.0b013e3182999740.

2. Albrecht, F. Closure of joint cartilage defects using cartilage fragments and fibrin glue. Fortschr Med. 1983;101:1650–1652.

3. Alford J.W., Cole B.J. Cartilage restoration, part 1: basic science, historical perspective, patient evaluation, and treatment options. Am J Sports Med. 2005;33:295-306. doi: 10.1177/0363546504273510.

4. Anitua E., Sanchez M., Nurden A.T., Zalduendo M., de la Fuente M., Orive G., Azofra J., Andia I. Autologous fibrin matrices: A potential source of biological mediators that modulate tendon cell activities. J Biomed Mater Res. 2006;77:285-293. doi:10.1002/jbm.a.30585.

5. Bellis S.L. Advantages of RGD peptides for directing cell association with biomaterials. Biomaterials. 2011;32:4205-4210. doi:10.1016/J.BIOMATERIALS.2011.02.029.

6. Bhagat V., Becker M.L. Degradable Adhesives for Surgery and Tissue Engineering. Biomacromolecules. 2017;18:3009-3039. doi:10.1021/ACS.BIOMAC.7B00969.

7. Bonasia D.E., Marmotti A., Mattia S., Cosentino A., Spolaore S., Governale G., Castoldi F., Rossi R. The Degree of Chondral Fragmentation Affects Extracellular Matrix Production in Cartilage Autograft Implantation: An In Vitro Study. Arthroscopy. 2015;31:2335-2341. doi: 10.1016/j.arthro.2015.06.025.

8. Bonasia D.E, Marmotti A., Rosso F., Collo G., Rossi R. Use of chondral fragments for one stage cartilage repair: A systematic review. World J Orthop. 2015;6:1006–1011. doi:10.5312/WJO.V6.I11.1006.

9. Brittberg M., Lindahl A., Nilsson A., Ohlsson C., Isaksoon O., Peterson L. Treatment of Deep Cartilage Defects in the Knee with Autologous Chondrocyte Transplantation. N Engl J Med. 1994;331:889-895. doi:10.1056/NEJM199410063311401.

10. Campbell A.B., Pineda M., Harris J.D., Flanigan D.C. Return to Sport after Articular Cartilage Repair in Athletes' Knees: A Systematic Review. Arthroscopy. 2016;32:651-668. doi:10.1016/j.arthro.2015.08.028.

11. Christensen B.B., Olesen M.L., Hede K.T.C., Bergholt N.L., Foldager C.B., Lind M. Particulated Cartilage for Chondral and Osteochondral Repair: A Review. Cartilage. 2021;13:1047S-1057S. doi: 10.1177/1947603520904757.

12. Chu J., Duan W., Yu Z., Tao T., Xu J., Ma Q., Zhao L., Guo J.J. Intra-articular injections of platelet-rich plasma decrease pain and improve functional outcomes than sham saline in patients with knee osteoarthritis. Knee Surg Sports Traumatol Arthrosc . 2022;30:4063-4071. doi: 10.1007/s00167-022-06887-7.

13. Cugat R., Alenton-Geli E., Navarro J., Cuscó X., Steinbacher G., Seijas R., Álvarez-Díaz P., Barastegui D., Laiz P., Samitier G., García-Balletbó M. A novel autologous-made matrix using hyaline cartilage chips and platelet-rich growth factors for the treatment of full-thickness cartilage or osteochondral defects: Preliminary results. J Orthop Surg. 2020;28:2309499019887547. doi:10.1177/2309499019887547.

14. Cugat R., Alentorn-Geli E., Steinbacher G., Álvarez-Díaz P., Cuscó X., Seijas R., Barastegui D., Navarro J., Laiz P., García-Balletbó M. Treatment of Knee Osteochondral Lesions Using a Novel Clot of Autologous Plasma Rich in Growth Factors Mixed with Healthy Hyaline Cartilage Chips and Intra-Articular Injection of PRGF. Case Rep Orthop. 2017;2017:8284548. doi:10.1155/2017/8284548.

15. Cugat R., Samitier G., Vinagre G., Sava M., Alentorn-Geli E., García-Balletbó M., Cuscó X., Seijas R., Barastegui D., Navarro J., Laiz P. Particulated Autologous Chondral–Platelet-Rich Plasma Matrix Implantation (PACI) for Treatment of Full-Thickness Cartilage Osteochondral Defects. Arthrosc Tech. 2021;10:e539-e544. doi:10.1016/J.EATS.2020.10.038.

16. Delman C., Wuellner J., Kreulen C., Lundeen G., Giza E. Particulated Autograft Cartilage Implantation for the Treatment of Osteochondral Lesions of the Talus: A Novel Technique. Foot Ankle Spec. 2018;11:365-371. doi:10.1177/1938640018770277.

17. Domínguez-Pérez J.M., Fernández-Sarmiento J.A., Aguilar-García D., Granados-Machuca M.M., Morgaz-Rodríguez J., Navarrete-Calvo R., Pérez-Arévalo J., Carrillo-Poveda J.M., Alentorn-Geli E., Laiz-Boada P., Cugat-Bartomeu R. Cartilage regeneration using a novel autologous growth factors-based matrix for full-thickness defects in sheep. Knee Surg Sports Traumatol Arthrosc. 2019;27:950-961. doi:10.1007/s00167-018-5107-z.

18. Dunkin B.S, Lattermann C. New and Emerging Techniques in Cartilage Repair: MACI. Oper Tech Sports Med. 2013;21:100–107. doi:10.1053/J.OTSM.2013.03.003.

19. Farr J., Cole B.J., Sherman S., Karas V. Particulated articular cartilage: CAIS and DeNovo NT. J Knee Surg. 2012;25:23-29. doi:10.1055/s-0031-1299652.

20. Farr J., Gomoll A. Particulated/Minced Cartilage. Cartil Restor. 2014;161–168. doi:10.1007/978-1-4614-0427-9_15.

21. Farr J., Yao J.Q. Chondral Defect Repair with Particulated Juvenile Cartilage Allograft. Cartilage. 2011;2:346–353. doi:10.1177/1947603511405838.

22. Goebel L., Orth P., Müller A., Zurakowski D., Bücker A., Cucchiarini M., Pape D., Madry H. Experimental scoring systems for macroscopic articular cartilage repair correlate with the MOCART score assessed by a high-field MRI at 9.4 T – comparative evaluation of five macroscopic scoring systems in a large animal cartilage defect model. Osteoarthritis Cartilage. 2012;20:1046-1055. doi:10.1016/J.JOCA.2012.05.010.

23. Grawe B., Burge A., Nguyen J., Strickland S., Warren R., Rodeo S., Stein B.S. Cartilage Regeneration in Full-Thickness Patellar Chondral Defects Treated with Particulated Juvenile Articular Allograft Cartilage: An MRI Analysis. Cartilage. 2017;8:374–383. doi:10.1177/1947603517710308.

24. Hinckel B.B., Thomas D., Vellios E.E., Hancock K.J., Calcei J.G., Sherman S.l., Eliasberg C.D., Fernandes T.L., Farr J., Lattermann C., Gomoll A.H. Algorithm for Treatment of Focal Cartilage Defects of the Knee: Classic and New Procedures. Cartilage. 2021;13:473S-495S. doi: 10.1177/1947603521993219.

25. Huey D.J., Hu J.C., Athanasiou K.A. Unlike bone, cartilage regeneration remains elusive. Science. 2012;338:917-921. doi: 10.1126/science.1222454.

26. Jung M., Tuischer J.S., Sergi C., Gotterbarm T., Pohl J., Richter W., Simank H.G. Local application of a collagen type I/hyaluronate matrix and growth and differentiation factor 5 influences the closure of osteochondral defects in a minipig model by enchondral ossification. Growth Factors. 2006;24:225-232. doi:10.1080/08977190600926969.

27. Kreuz P.C, Krüger J.P, Metzlaff S., Freymann U., Endres M., Pruss A., Petersen W., Kaps C. Platelet-Rich Plasma Preparation Types Show Impact on Chondrogenic Differentiation, Migration, and Proliferation of Human Subchondral Mesenchymal Progenitor Cells. Arthroscopy. 2015;31:1951-1961. doi:10.1016/J.ARTHRO.2015.03.033.

28. Kwon H., Brown W.E., Lee C.A., Wang D., Paschos N., Hu J.C., Athanasiou K.A. Surgical and tissue engineering strategies for articular cartilage and meniscus repair. Nat Rev Rheumatol. 2019;15:550-570. doi:10.1038/S41584-019-0255-1.

29. Levinson C., Cavalli E., Sindi D.M., Kessel B., Zenobi-Wong M., Preiss S., Salzmann G., Neidenbach P. Chondrocytes From Device-Minced Articular Cartilage Show Potent Outgrowth Into Fibrin and Collagen Hydrogels. Orthop J Sports Med. 2019;7:2325967119867618. doi:10.1177/2325967119867618.

30. Levinson C., Cavalli E., von Rechenberg B., Zenobi-Wong M., Darwiche S.E. Combination of a Collagen Scaffold and an Adhesive Hyaluronan-Based Hydrogel for Cartilage Regeneration: A Proof of Concept in an Ovine Model. Cartilage. 2021;13:636S-649S. doi: 10.1177/1947603521989417.

31. Lorenz C.J., Freislederer F., Salzmann G.M., Scheibel M. Minced Cartilage Procedure for One-Stage Arthroscopic Repair of Chondral Defects at the Glenohumeral Joint. Arthrosc Tech. 2021;10:e1677-e1684. doi: 10.1016/j.eats.2021.03.012.

32. Lu Y., Dhanaraj S., Wang Z., Bradley D.M., Bowman S.M., Cole B.J., Binette F.Minced cartilage without cell culture serves as an effective intraoperative cell source for cartilage repair. J Orthop Res. 200624:1261-1270. doi: 10.1002/jor.20135

33. Mainil-Varlet P, Aigner T, Brittberg M, Bullough P, Hollander A, Hunziker E, Kandel R, Nehrer S, Pritzker K, Roberts S, Stauffer E, International Cartilage Repair Society (2003) Histological

assessment of cartilage repair: A report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). J Bone Joint Surg Am 85-A Suppl 2:45-57.

34. Mainil-Varlet P., van Damme B., Nesic D., Knutsen G., Kandel R., Roberts S. A New Histology Scoring System for the Assessment of the Quality of Human Cartilage Repair: ICRS II. Am J Sports Med. 2010;38:880-890. doi: 10.1177/0363546509359068.

35. Marmotti A., Bruzzone M., Bonasia D.E., Castoldi F., Mauthe Von Degerfels M., Bignardi C., Mattia S., Maiello A., Rossi R., Peretti G.M. Autologous cartilage fragments in a composite scaffold for one stage osteochondral repair in a goat model. Eur Cell Mater. 2013;26:15-31. doi:10.22203/ECM.V026A02.

36. Massen F.K., Inauen C.R., Harder L.P., Runer A., Preiss S., Salzmann G.M. One-Step Autologous Minced Cartilage Procedure for the Treatment of Knee Joint Chondral and Osteochondral Lesions: A Series of 27 Patients With 2-Year Follow-up. Orthop J Sports Med. 2019;7:2325967119853773. doi:10.1177/2325967119853773.

37. Mehl J., Feucht M., Achtnich A., Imhoff A.B., Niemeyer P., Angele P., Zinser W., Spahn G., Loer I., Kniffler H., Schauf G., Schmitt A. Autologous chondrocyte implantation combined with anterior cruciate ligament reconstruction: similar short-term results in comparison with isolated cartilage repair in ligament intact joints. Knee Surg Sports Traumatol Arthrosc. 2022;30:3249-3257. doi: 10.1007/s00167-021-06764-9.

38. Milano G., Sanna-Passino E., Deriu L., Careddu G., Manunta L., Manunta A., Saccomanno M.F., Fabbriciani C. The effect of platelet rich plasma combined with microfractures on the treatment of chondral defects: An experimental study in a sheep model. Osteoarthritis Cartilage .12010;8:971-980. doi: 10.1016/j.joca.2010.03.013.

39. Monaco G., El Haj A.J., Alini M., Stoddart M.J. Ex Vivo Systems to Study Chondrogenic Differentiation and Cartilage Integration. J Funct Morphol Kinesiol. 2021;6:6. doi: 10.3390/jfmk6010006.

40. Muschler G.F., Nitto H., Boehm C.A., Easley K.A. Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. J Orthop Res. 2006;1:117-125. doi: 10.1016/S0736-0266(00)00010-3.

41. Musumeci G., Castrogiovanni P., Leonardi R., Trovato F.M., Szychlinska M.A., Di Giunta A., Loreto C., Castorina S. New perspectives for articular cartilage repair treatment through tissue engineering: A contemporary review. World J Orthop. 2014;5:80–88. doi: 10.5312/wjo.v5.i2.80

42. Oshima Y., Iizawa N., Takai S., Majima T. Maturation process of regenerated tissues after single-stage simultaneous autologous particulated cartilage implantation and open wedge high tibial osteotomy for articular cartilage defects with medial osteoarthritis of bilateral knees: a case report. BMC Musculoskelet Disord 22:502. doi:10.1186/S12891-021-04368-5.

43. Palazzo C., Nguyen C., Lefevre-Colau M.M., Rannou F., Poiraudeau S. Risk factors and burden of osteoarthritis. Ann Phys Rehabil Med. 2016;59:134-138. doi:10.1016/j.rehab.2016.01.006.

44. Richter D.L., Schenck R.C., Wascher D.C., Treme G. Knee Articular Cartilage Repair and Restoration Techniques: A Review of the Literature. Sports Health. 2016;8:153-160. doi:10.1177/1941738115611350.

45. Rutgers M, van Pelt M J P, Dhert W J A, Creemers L B, Saris D B F (2010) Evaluation of histological scoring systems for tissue-engineered, repaired and osteoarthritic cartilage. Osteoarthritis Cartilage 18:12-23.

46. Saltzman B.M., Lin J., Lee S. Particulated Juvenile Articular Cartilage Allograft Transplantation for Osteochondral Talar Lesions. Cartilage. 2017;8:61-72. doi:10.1177/1947603516671358.

47. Simon T.M., Jackson D.W. Articular Cartilage: Injury Pathways and Treatment Options. Sports Med Arthrosc Rev. 2006;14:146-154. doi:10.1097/JSA.0000000000000182.

48. Sumida Y., Nakamura K., Feil S., Siebold M., Kirsch J., Siebold R. Good healing potential of patellar chondral defects after all-arthroscopic autologous chondrocyte implantation with spheroids: a second-look arthroscopic assessment. Knee Surg Sports Traumatol Arthrosc. 2022;30:1535-1542. doi: 10.1007/s00167-021-06584-x.

49. Takroni T., Laouar L., Adesida A., Elliott J.A.W., Jomha N.M. Anatomical study: comparing the human, sheep and pig knee meniscus. J Exp Orthop. 2016;3:35. doi: 10.1186/s40634-016-0071-3.

50. Van den Borne M.P.J., Raijmakers N.J.H., Vanlauwe J., Victor J., de Jong S.N., Bellemans J., Saris D.B.F. International Cartilage Repair Society (2007) International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation scores validated for use in Autologous Chondrocyte Implantation (ACI) and microfracture. Osteoarthritis Cartilage. 2007;15:1397-1402. doi:10.1016/j.joca.2007.05.005.

51. Wakitani S., Goto T., Pineda S.J., Young R.G., Mansour J.M., Caplan A.I., Goldberg V.M. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am. 1994;76:579-592. doi:10.2106/00004623-199404000-00013.

52. Wu P.I.K., Diaz R., Borg-Stein J. Platelet-Rich Plasma. Phys Med Rehabil Clin N Am. 2016;27:825-853. doi:10.1016/j.pmr.2016.06.002.

53. Xu L., Flahiff C.M., Waldman B.A., Wu D., Olsen B.R., Setton L.A., Li Y. Osteoarthritis-like changes and decreased mechanical function of articular cartilage in the joints of mice with the chondrodysplasia gene (cho). Arthritis Rheum. 2003;48:2509-2518. doi:10.1002/art.11233.

54. Xue Y., Su X., Jiang M., Yu Z., Yang H., Qin L., Giannoudis P.V., Guo J.J. Pure platelet-rich plasma facilitates the repair of damaged cartilage and synovium in a rabbit hemorragic arthritis knee model. Arthritis Res Ther. 2020;22:68. doi:10.1186/s13075-020-02155-6.

55. Yan W., Maimaitimin M., Zhao F., Fan Y., Yang S., Li Y., Cao C., Shao Z., Liu Z., Hu X., Ao Y., Cheng J. The transplantation of particulated juvenile allograft cartilage and synovium for the repair of meniscal defect in a lapine model. J Orthop Translat. 2022;33:72-89. doi:10.1016/J.JOT.2022.02.004.

56. Zhang C., Zhao X., Ao Y., Cao J., Yang L., Duan X. Proliferation ability of particulated juvenile allograft cartilage. J Orthop Surg Res. 2021;16:56. doi:10.1186/S13018-020-02199-Z.

57. Zhang S., Binwu H., Weijian L., Peng W., Xiao L., Songfeng C., Hongjian L., Zengwu S. Articular cartilage regeneration: The role of endogenous mesenchymal stem/progenitor cell recruitment and migration. Semin Arthritis Rheum. 2020;50:198-208. doi:10.1016/j.semarthrit.2019.11.001.

58. Zhao J., Huang H., Liang G., Zeng L.F., Yang W., Liu J. Effects and safety of the combination of platelet-rich plasma (PRP) and hyaluronic acid (HA) in the treatment of knee osteoarthritis: a systematic review and meta-analysis. BMC Musculoskelet Disord. 2020;21:224. doi:10.1186/s12891-020-03262-w.

59. Zhu Y., Yuan M., Meng H.Y., Wang A.Y., Guo Q.Y., Wang Y., Peng J. Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: a review. Osteoarthritis Cartilage. 2013;21:1627-1637. doi: 10.1016/j.joca.2013.07.017.

VII. CAPÍTULO IV

Objetivo III / Objective III

Immunohistochemical analysis of knee chondral defect repair after autologous particulated cartilage and platelet-rich plasma treatment in sheep

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Objective III: To conduct a comprehensive and detailed immunohistochemical analysis of several types of collagens, including both major and minor forms, as well as aggrecan. The aim was to evaluate the quality, durability, and structure of the extracellular matrix in the repaired cartilage following the application of PACI+PRP treatment in chondral defects within our ovine experimental model.

Immunohistochemical analysis of knee chondral defect repair after autologous particulated cartilage and platelet-rich plasma treatment in sheep

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Abstract

This study performs an analysis that will enable the evaluation of the quality, durability, and structure of repaired cartilaginous extracellular matrix tissue using an autologous-based particulated autograft cartilage and platelet-rich plasma treatment (PACI + PRP). A single-blind controlled experiment was conducted on 28 sheep to evaluate the efficacy of the PACI + PRP treatment for cartilage defects. Full-thickness 8 mm diameter defects were created in the weight-bearing area of both knees. The right knees received PACI + PRP. The left knees were treated with Ringer's lactate solution (RLS) or hyaluronic acid (HA) injections. Sheep were euthanized at 9- or 18-months post-surgery. An extensive immunohistochemical analysis was performed to assess collagen types (I, II, III, V, VI, IX, X, XI) and aggrecan positivity. A semiquantitative scoring system provided a detailed evaluation of immunostaining. Collagens and aggrecan scores in the PACI + PRP groups were similar to healthy cartilage. Significant differences were found in collagens associated with matrix maturity (II and V), degradation (IX), structure and mechanics (VI), and hypertrophy (X) between healthy cartilage and RLS- or HA-repaired cartilage. The PACI + PRP treatment advanced the repair cartilage process in chondral defects with mature hyaline cartilage and enhanced the structural and mechanical qualities with better consistent cartilage, less susceptible to degradation and without hypertrophic formation over time.

Keywords: Chondral defect, Knee, Particulated cartilage, Platelet-rich plasma, Immunohistochemical.

1. Introduction

Articular cartilage is characterized by a hyaline structure composed of water, collagen, proteoglycan, and chondrocytes. Collagen is the main protein in the composition of the extracellular matrix (ECM), comprising approximately 60% of the dry weight of hyaline cartilage. Collagens provide tensile strength, regulate cell adhesion, support chemotaxis and migration, and direct tissue development [1,2].

Injuries in this tissue commonly cause knee pain and dysfunction. Additionally, these injuries may lead to early-onset osteoarthritis and have a huge negative impact on patients' functions and quality of life [3]. Unfortunately, chondral lesions are difficult to treat because of their poor healing and regeneration potential [4,5]. Despite the improvement in technology and tissue engineering, it remains difficult to find an effective treatment for articular repair that is capable of restoring normal hyaline cartilage [4]. Minimal data are available regarding the intrinsic repair processes of damaged cartilage, and consequently, the search for a treatment that can restore normal hyaline cartilage is difficult. For this reason, it is critical to evaluate, in depth, the repair quality of repaired cartilage, including an exhaustive assessment of collagen types [6].

A possible treatment is the application of small, previously particulated or minced cartilage chips, described for the first time by Albrecht et al. [7]. The use of particulated autograft cartilage implantation (PACI) for the treatment of cartilage lesions relies on the migration of chondrocytes into a biomaterial and subsequent cartilaginous ECM deposition by these cells [8]. Platelet-rich plasma (PRP) has been proposed as a therapeutic option in musculoskeletal conditions given the important role of platelets in hemostasis, inflammation, and proliferation for tissue remodeling and healing [9]. Some studies have reported that PRP can stimulate chondrogenic regeneration, enhance the biosynthesis of cartilage matrix proteins, and enhance chondrocyte proliferation and metabolism [10]. In addition, PRP has been used in vivo in combination with other techniques to treat chondral defects, improving the quality of the repaired tissue [11].

In this study, a PACI + PRP therapeutic technique based on an autologous matrix composed of healthy hyaline cartilage chips included and mixed in a PRP clot, and an intraarticular infiltration of PRP was used to treat full-thickness chondral defects in the weight-bearing area of the medial femoral condyle of sheep. Two preliminary case reports provided excellent clinical, functional, and MRI-based outcomes in young active individuals with full-thickness cartilage or osteochondral defects treated with PACI + PRP [3,12]. Moreover, a

preliminary study conducted on sheep treated with PACI + PRP after 1, 3, and 6 months of recovery revealed improvements in chondrogenesis and the regeneration of newly formed cartilage after 6 months of recovery [13]. However, these preliminary studies have certain limitations, such as a small sample size and a lack of a deep evaluation of the quality and organization of the ECM in the repaired cartilage tissue.

The appropriate zonal organization of the components of the ECM is a feature that distinguishes articular hyaline cartilage from other types of cartilage. Additionally, collagen and proteoglycan organization in adult hyaline articular cartilage is important for the maintenance of tissue load-bearing capacity [6,14,15]. Therefore, the goal of the present study is to perform an extensive and detailed immunohistochemical analysis of major and minor collagen types and aggrecan, allowing for the evaluation of the quality, durability, and structure of repaired cartilaginous ECM tissue using an autologous-based PACI + PRP treatment. We hypothesized that this treatment could restore durable and good-quality hyaline cartilage with an organized ECM.

2. Materials and Methods

2.1. Ethical statement

The present study was approved by the Bioethical Committee on Animal Research of the Regional Government Andalusia (Junta de Andalucía 12/06/2016/109—reference SSA/SIS/MD/jv) and conducted in accordance with guidelines for the protection of animals utilized for scientific purposes (Directive 2010/63/UE, Decision 2020/569/UE, and RD 1386/2018).

2.2. Study design and surgical procedure

Twenty-eight skeletally mature and healthy Merino sheep (n = 28), weighing between 50 and 60 kg were used for this study.

A medial mini-arthrotomy of 4 cm was performed on both knees of the sheep. An 8 mm full-thickness cartilage defect was created in the weight-bearing area of the medial femoral condyle, and the cartilage was excised. The right hind limbs of the sheep (28 right knees) were treated with the PACI + PRP technique. The excised cartilage sample was sliced into small, 1–2 mm3 fragments and mixed with activated PRP to obtain a clot used as a scaffold for cartilage chips. The clot and cartilage chips were placed, filling the created cartilage defect. Then, it remained in a stationary position for 5 min to ensure the adherence of the clot to the defect. Following adherence, the surgical site was sutured, and 2 mL of activated PRP was

intraarticularly injected. The left knees of the sheep were randomly divided to use two different control treatments. Subsequently, after performing the same surgical procedure of creating a chondral defect and closing the incision, half of the left knees received an intra-articular injection of 2 mL of Ringer's lactate solution (RLS) (14 left knees), and the other half were treated with 2 mL of hyaluronic acid (HA) (Synvisc One, Hylan G-F 20) (14 left knees). Finally, antibiotic (amoxicillin–clavulanic acid, 10 mg/kg IM) and analgesic (buprenorphine 0.02 mg/kg/8 h IM) treatments were administered for three and five days after surgery, respectively. The animals were allowed to move freely without splints in an indoor stable.

Animals were randomly divided into two study groups: the RLS/PACI + PRP(RLS) group, which included fourteen sheep treated with RLS in the left knee and PACI + PRP in the right knee (n = 14), and the HA/PACI + PRP(HA) group, which included fourteen sheep treated using HA in the left knee and PACI + PRP in the right knee (n = 14). The time of sacrifice was initiated 9 or 18 months after surgery. Seven sheep from each study group were randomly sacrificed at the allotted time.

Throughout the study, three animals were lost: one sheep from the RLS/PACI + PRP(RLS) group at 9 months, one sheep from the HA/PACI + PRP(HA) group at 9 months, and one sheep from the RLS/PACI + PRP(RLS) group at 18 months.

2.3. Preparation and use of autologous PRP

The PRP treatment was prepared according to a previously reported method [16]. Blood was collected from the jugular vein of each animal in 5 mL collection tubes with 0.5 mL of sodium citrate solution (3.8%) as an anticoagulant and centrifuged over 8 min at 630× g. Blood was collected just prior to surgery and was processed intraoperatively. The plasma volume was divided into two (50%). The upper layer of centrifuged plasma was fraction 1, and the deeper layer of the centrifuged plasma just over of the buffy coat was fraction 2. Fraction 2, including a platelet concentration of 2- to 2.5-fold higher than peripheral blood, was obtained by pipetting with precision to avoid the aspiration of white and red blood cells. Platelets were activated by adding 50 µL of calcium chloride (10%) for 1 mL of plasma ratio just prior to use. To obtain the semisolid scaffold, including the cartilage chips, activated fraction 1 and fraction 2 were used in equal parts (50/50). For the intraarticular injection of 2 mL of PRP, only activated fraction 2 was used. The entire treatment included PACI + PRP application and an intraarticular PRP injection. The time delay between blood collection and both fractions' use was less than 1.5 h. The PRP treatment was only applied intraoperatively with no postoperative doses administered.

2.4. Immunohistochemical evaluation

An immunohistochemical study was used to assess collagen types (types I, II, III, V, VI, IX, X, XI) and proteoglycan (aggrecan) positivity in cartilage samples using the avidin–biotin–peroxidase method. After the sacrifice of each sheep, the medial femoral condyles were harvested from both knees and then immediately preserved in 10% buffered formaldehyde for 24 h, decalcified for 72 h (Thermo ScientificTM Shandon TBD-1TM Decalcifier, Cheshire, United Kingdom), and then processed and embedded in paraffin wax in the customary fashion. Sections of 4 µm were obtained for immunohistochemical staining. Each sample was coded to blind the analysis to the researchers. Samples were routinely processed and embedded in paraffin wax. Tissue sections were dewaxed and rehydrated, and endogenous peroxidase activity was exhausted via incubation with 0.3% hydrogen peroxidase (Panreac, Barcelona, Spain) in methanol (Panreac, Barcelona, Spain) at room temperature (RT). Two different antigen retrieval pre-treatments were used, heat-induced antigen retrieval with 0.01 M sodium citrate buffer pH6 (antibody to collagen type IX) and an enzymatic pre-treatment with hyaluronidase (antibodies to collagen types I, II, III, V, VI, X, XI and aggrecan), both using a laboratory oven at 50 °C for 45 min. Sections were washed in phosphate-buffered saline (PBS) at pH 7.2 for 10 min and incubated with 20% normal goat serum (MP Biomedicals, San Francisco, CA, United States) at RT for 30 min. A panel of primary antibodies was diluted in PBS containing 10% normal goat serum (Table 1) and incubated overnight at 4 °C. According to the manufacturer, all primary antibodies show negligible cross-reaction with non-specific collagen types, and all cross-react with several species of mammals, including sheep [16]. Following washing in PBS, the sections were incubated with biotinylated goat anti-rabbit or anti-mouse (Table 1) secondary antibodies (Dako, Agilent, Santa Clara, CA, United States; E0432 and E0433, respectively) diluted to 1:200 and 1:50, respectively, for 30 min at RT. After washing in PBS, the sections were incubated with ABC complex (Vector Laboratories, Burlingame, CA, United States) for 1 h at RT in darkness, washed in Tris-buffered saline pH 7.6, and then incubated in chromogen solution (Vector NovaRed Peroxidase Substrate Kit, Vector Laboratories, Burlingame, CA, United States). Finally, the sections were counterstained with Mayer's hematoxylin and mounted with Eukitt (Freiburg, Germany). Tissue sections in which primary antibodies were substituted with non-immune serum were used as negative controls.

To determine the quality of the repaired tissue, chondral defects treated with RLS, HA, and PACI+PRP were immunohistochemically evaluated and compared with surrounding normal hyaline cartilage.

Table 1. Immunohistochemical scoring system

Parameters		Points	Final score
Immunostaining Pattern	Severely abnormal	0	6
	Abnormal	1	
	Nearly normal	2	
	Normal	3	
Immunostaining Intensity	Severely abnormal	0	
	Abnormal	1	
	Nearly normal	2	
	Normal	3	

2.5. Development of a semiquantitative scoring system

The assessment system used in this study was based on previous publications [6,14,41,42,43]. In this scoring system, a semiquantitative assessment of immunostaining pattern and intensity was performed. Both assessed parameters were evaluated separately using a scale ranging from 0 to 3 (Table 2).

Table 2. Collagen types and aggrecan score results for all studied groups at 9 or 18 months.

Month	Variable	Group				
		HC	RLS	PACI+PRP (RLS)	HA	PACI+PRP (HA)
9	Col I	6 ± 0	6 ± 2.04	6 ± 0.41	6 ± 1.33	6 ± 0
	Col II	6 ± 0[b]	5 ± 1.22	6 ± 1.22	4 ± 1.17[b]	5.50 ± 0.82
	Col III	6 ± 0	4.50 ± 1.86	6 ± 1.67	6 ± 0.82	6 ± 2.04
	Col V	6 ± 0[a]	2.50 ± 0.98[a]	4 ± 1.03	2.50 ± 1.79	4 ± 1.03
	Col VI	6 ± 0[a,b]	3 ± 0.75[a]	4.50 ± 1.05	3.50 ± 0.82[b]	4.50 ± 1.21
	Col IX	6 ± 0	3 ± 1.86	4 ± 1.22	3.50 ± 2.06	6 ± 1.03
	Col X	6 ± 0	4.50 ± 1.37	6 ± 0.82	5 ± 1.17	6 ± 1.03
	Col XI	6 ± 0	4 ± 1.17	4.50 ± 1.05	4.50 ± 1.67	4.50 ± 1.83
	Aggrecan	6 ± 0	3.50 ± 1.87	3 ± 1.76	3.50 ± 1.26	4.50 ± 1.21
18	Col I	6 ± 0	6 ± 2.04	6 ± 0	6 ± 2.27	6 ± 0
	Col II	6 ± 0	5 ± 1.03	6 ± 0.41	6 ± 1.25	6 ± 0.38
	Col III	6 ± 0	3.50 ± 1.67	5.50 ± 1.27	3 ± 2.34	6 ± 1.53
	Col V	6 ± 0	4.50 ± 0.82	6 ± 0.52	4 ± 1.90	6 ± 0.97
	Col VI	6 ± 0 [a,b]	4 ± 0.41[a]	6 ± 1.33	3 ± 1.21 [b]	5 ± 0.75
	Col IX	6 ± 0[b]	2 ± 2.34	4 ± 1.60	4 ± 1.99[b]	6 ± 0.75
	Col X	6 ± 0[a]	3 ± 1.03[a]	4.5 ± 1.21	5 ± 1.60	6 ± 0.53
	Col XI	6 ± 0	4.5 ± 1.05	5± 0.89	5 ± 0.90	5 ± 0.69
	Aggrecan	6 ± 0	4.5 ± 1.47	5 ± 0.75	5 ± 1.38	5 ± 0.82

HC: healthy cartilage; RLS: left knee treated with Ringer´s lactate solution; PACI+PRP(RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP(HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee. [a] Significant difference ($p<0.05$) between healthy cartilage and RLS; [b] Significant difference ($p<0.05$) between healthy cartilage and HA.

For this evaluation, the whole repaired tissue area was divided into four zones considering the physiological zones that comprised the healthy hyaline cartilage (superficial zone, middle or transition zone, deep zone, and calcified zone). Subsequently, to evaluate the

structure of the repaired cartilage, the same zones were compared with repaired and normal cartilage tissue. First, when three of the zones compared yielded differences, the assigned score was 0 (severely abnormal). Second, when two zones differed, the score was 1 (abnormal). Third, when one zone differed, the score was 2 (nearly normal). Fourth, when no zones differed, the score was 3 (normal). Finally, a score from 0 to 6 points was obtained, where 6 points indicated that the repaired cartilage immunostaining was equal to that of the surrounding normal hyaline cartilage. This evaluation was graded blindly.

2.6. Statistical Analyses

Statistical analysis was performed using GraphPad Prism 7.0 (Inc., San Diego, CA, United States). First, a Kolmogorov–Smirnoff normality test was used to determine whether the studied variables were normally distributed. A Wilcoxon test was performed to compare treatments used on the same sheep at the same time of sacrifice, while a Mann–Whitney U test was carried out to compare treatments between different animals. In addition, a Mann–Whitney U test was performed to compare the two sacrifice periods (9 vs. 18 months after surgery) for the same treatment group. The variables in the tables were expressed as mean ± standard deviation. The variables were considered significant when $p < 0.05$ (* $p < 0.05$).

3. Results

As presented in Figure 1 and Figure 2, at 9 and 18 months, respectively, the score of the studied collagens and aggrecan obtained from the PACI + PRP groups (n = 25) were the closest to the surrounding healthy cartilage compared with the other study groups. In contrast, for some of the collagens (types II, V, VI, IX, and X) the RLS or HA groups scored significantly lower than the surrounding healthy tissue (Figure 1 and Figure 2) (Table 3).

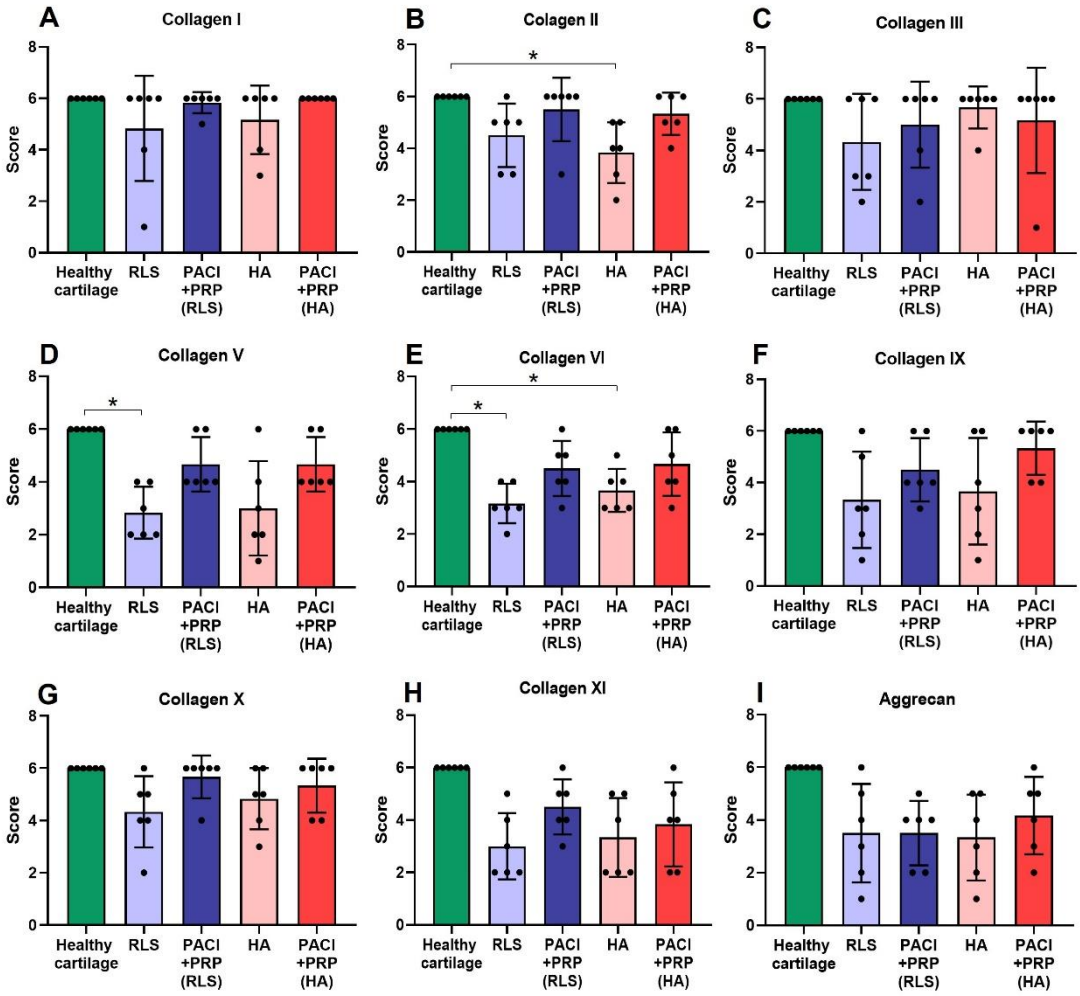

Figure 1. Immunostaining scores of collagen types and aggrecan studied for RLS, PACI + PRP(RLS), HA, and PACI + PRP(HA) at 9 months. (A) Type I collagen; (B) type II collagen; (C) type III collagen; (D) type V collagen; (E) type VI collagen; (F) type IX collagen; (G) type X collagen; (H) type XI collagen; (I) aggrecan. RLS: left knee treated with Ringer's lactate solution; PACI + PRP(RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI + PRP(HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee. * Significant difference between groups, $p < 0.05$.

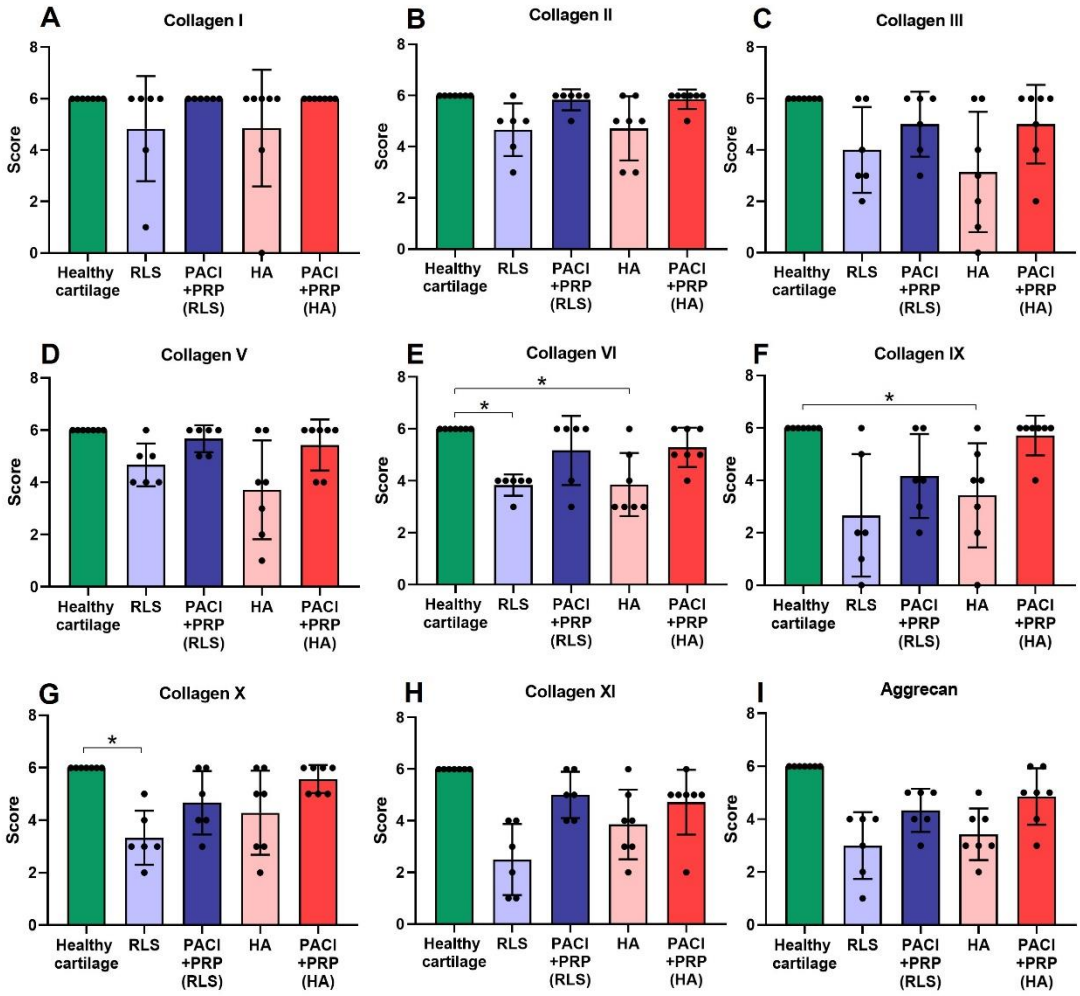

Figure 2. Immunostaining scores of collagen types and aggrecan studied for RLS, PACI + PRP(RLS), HA, and PACI + PRP(HA) at 18 months. (A) type I collagen; (B) type II collagen; (C) type III collagen; (D) type V collagen; (E) type VI collagen; (F) type IX collagen; (G) type X collagen; (H) type XI collagen; (I) aggrecan. RLS: left knee treated with Ringer's lactate solution; PACI + PRP(RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI + PRP(HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee. * Significant difference between groups, $p < 0.05$.

Table 3. Collagen types and aggrecan score results for all studied groups at 9 or 18 months.

Month	Variable	Group				
		HC	RLS	PACI+PRP (RLS)	HA	PACI+PRP (HA)
9	Col I	6 ± 0	6 ± 2.04	6 ± 0.41	6 ± 1.33	6 ± 0
	Col II	6 ± 0[b]	5 ± 1.22	6 ± 1.22	4 ± 1.17[b]	5.50 ± 0.82
	Col III	6 ± 0	4.50 ± 1.86	6 ± 1.67	6 ± 0.82	6 ± 2.04
	Col V	6 ± 0[a]	2.50 ± 0.98[a]	4 ± 1.03	2.50 ± 1.79	4 ± 1.03
	Col VI	6 ± 0[a,b]	3 ± 0.75[a]	4.50 ± 1.05	3.50 ± 0.82[b]	4.50 ± 1.21
	Col IX	6 ± 0	3 ± 1.86	4 ± 1.22	3.50 ± 2.06	6 ± 1.03
	Col X	6 ± 0	4.50 ± 1.37	6 ± 0.82	5 ± 1.17	6 ± 1.03
	Col XI	6 ± 0	4 ± 1.17	4.50 ± 1.05	4.50 ± 1.67	4.50 ± 1.83
	Aggrecan	6 ± 0	3.50 ± 1.87	3 ± 1.76	3.50 ± 1.26	4.50 ± 1.21
18	Col I	6 ± 0	6 ± 2.04	6 ± 0	6 ± 2.27	6 ± 0
	Col II	6 ± 0	5 ± 1.03	6 ± 0.41	6 ± 1.25	6 ± 0.38
	Col III	6 ± 0	3.50 ± 1.67	5.50 ± 1.27	3 ± 2.34	6 ± 1.53
	Col V	6 ± 0	4.50 ± 0.82	6 ± 0.52	4 ± 1.90	6 ± 0.97
	Col VI	6 ± 0 [a,b]	4 ± 0.41[a]	6 ± 1.33	3 ± 1.21 [b]	5 ± 0.75
	Col IX	6 ± 0[b]	2 ± 2.34	4 ± 1.60	4 ± 1.99[b]	6 ± 0.75
	Col X	6 ± 0[a]	3 ± 1.03[a]	4.5 ± 1.21	5 ± 1.60	6 ± 0.53
	Col XI	6 ± 0	4.5 ± 1.05	5± 0.89	5 ± 0.90	5 ± 0.69
	Aggrecan	6 ± 0	4.5 ± 1.47	5 ± 0.75	5 ± 1.38	5 ± 0.82

HC: healthy cartilage; RLS: left knee treated with Ringer´s lactate solution; PACI+PRP(RLS): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with RLS in the left knee; HA: left knee treated with hyaluronic acid; PACI+PRP(HA): right knee treated with particulated autograft cartilage implantation and platelet-rich plasma from the group treated with HA in the left knee. [a] Significant difference ($p<0.05$) between healthy cartilage and RLS; [b] Significant difference ($p<0.05$) between healthy cartilage and HA.

The presence of type I collagen, both in normal hyaline cartilage and in the PACI + PRP groups, is confined to the bone and is completely absent in the cartilage. However, in the RLS and HA groups, some positivity for this collagen in the ECM can be observed at 9 and 18 months (Figure 1A and Figure 2A).

At 9 or 18 months, statistically significant differences were observed between healthy cartilage and the HA group for type II collagen ($p = 0.03$) (Figure 1B) or type IX collagen ($p = 0.03$) (Figure 2F), respectively. The HA group presented a higher percentage of type II (Figure 3A,B) and type IX (Figure 4A,B) collagens than the percentage found in healthy cartilage. In healthy cartilage and the PACI + PRP groups, type II collagen was found throughout the cartilage ECM (Figure 3B,C). In the HA group, type II collagen was observed throughout the ECM, although it was also observed with more intensity in the pericellular matrix of chondrocytes in the superficial, deep, and calcified zones (Figure 3A). Type IX collagen was observed in some chondrocytes of the superficial zone in healthy cartilage and the PACI + PRP groups (Figure 4B,C), while in the HA group, type IX collagen was identified in all chondrocytes (Figure 4A).

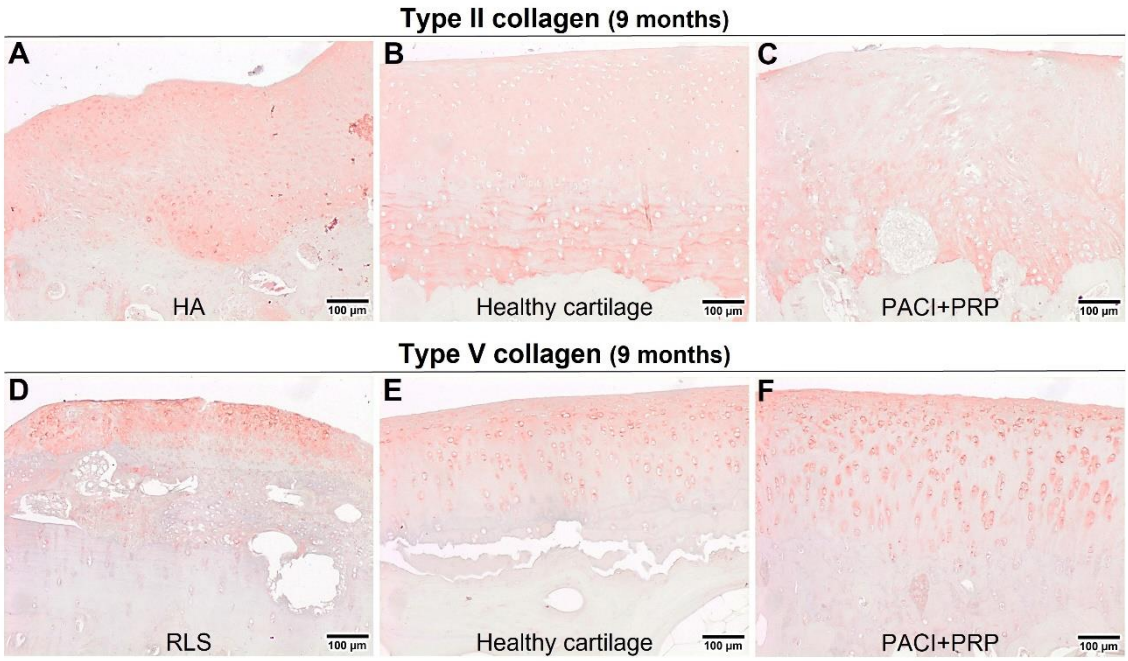

Figure 3. Immunohistochemical evaluation of healthy cartilage and repaired tissue at 9 months after the administered treatments. (A) Evaluation of type II collagen in HA group at 9 months; (B) example of healthy cartilage surrounding the chondral defect immunostained with type II collagen at 9 months; (C) evaluation of type II collagen in the PACI + PRP group at 9 months; (D) evaluation of type V collagen in the RLS group at 9 months; (E) example of healthy cartilage surrounding the chondral defect immunostained with type V collagen at 9 months; (F) evaluation of type V collagen in the PACI + PRP group at 9 months. RLS: left knee treated with Ringer's lactate solution; HA: left knee treated with hyaluronic acid; PACI + PRP: right knee treated with particulated autograft cartilage implantation and platelet-rich plasma.

Both type V collagen at 9 months ($p = 0.03$) (Figure 1D) and type X collagen at 18 months ($p = 0.03$) (Figure 2G) showed statistically significant differences between healthy cartilage and the RLS group. The RLS group presented a higher percentage of type V (Figure 3D,E) and type X (Figure 4D,E) collagen than the healthy hyaline cartilage. Additionally, the pattern and intensity of immunostaining differed between the RLS group and healthy cartilage. In healthy cartilage and the PACI + PRP groups, type V collagen was found mainly in the pericellular matrix of chondrocytes and with decreasing intensity from the upper superficial to the bottom calcified zones (Figure 3E,F), while in the RLS group, type V collagen was found in the pericellular matrix of chondrocytes but also in the rest of the ECM, with much more immunostaining intensity (Figure 3D). Type X collagen was found in bone and in some superficial chondrocyte zones, as it was observed in healthy cartilage (Figure 4E). However, in the RLS group, the presence of type X collagen was observed in most of the chondrocytes in all the cartilage zones (Figure 4D).

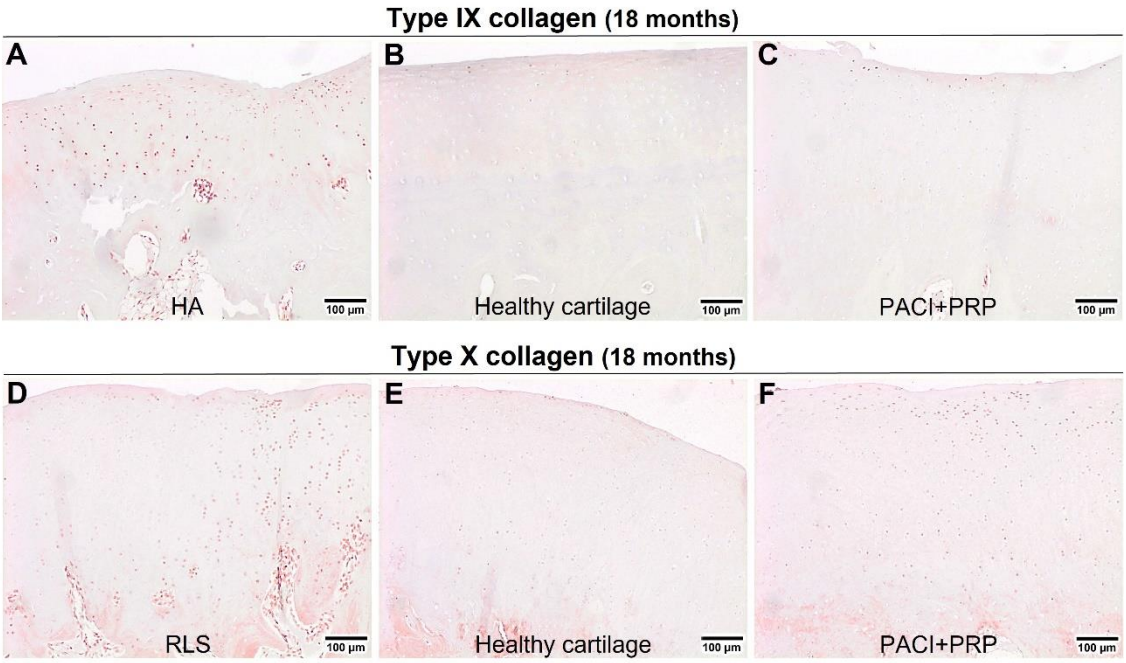

Figure 4. Immunohistochemical evaluation of healthy cartilage and repaired tissue at 18 months after the treatments administered. (A) Evaluation of type IX collagen in the HA group at 18 months; (B) example of healthy cartilage surrounding the chondral defect immunostained with type IX collagen at 18 months; (C) evaluation of type IX collagen in the PACI + PRP group at 18 months; (D) evaluation of type X collagen in the RLS group at 18 months; (E) example of healthy cartilage immunostained surrounding the chondral defect with type X collagen at 18 months; (F) evaluation of type X collagen in the PACI + PRP group at 18 months. RLS: left knee treated with Ringer's lactate solution; HA: left knee treated with hyaluronic acid; PACI + PRP: right knee treated with particulated autograft cartilage implantation and platelet-rich plasma.

Positivity for type VI collagen showed statistically significant differences between healthy cartilage and the RLS group ($p = 0.03$ and $p = 0.03$) and between healthy cartilage and HA-treated cartilage ($p = 0.03$ and $p = 0.03$), both at 9 and 18 months (Figure 1E and Figure 2E). Groups treated with RLS or HA presented a higher percentage of type VI collagen than the percentage found in healthy cartilage (Figure 5). In healthy cartilage, type VI collagen was found with great intensity in the pericellular matrix of all chondrocytes and in the rest of the ECM of the superficial and middle zones, and it was absent in the ECM of the deep and calcified zones (Figure 5B,E,H,K). At 9 months, a decrease in the intensity of immunostaining in the chondrocyte pericellular matrix was observed in the RLS- and HA-treated groups (Figure 5A,G). Additionally, at 9 months, type VI collagen was perceived in the ECM of the deep and calcified zones of the RLS group (Figure 5A). At 18 months, an improvement in the RLS group was detected related to the high intensity of immunostaining in the chondrocyte pericellular matrix, and type VI collagen was not observed in the ECM of the calcified zone.

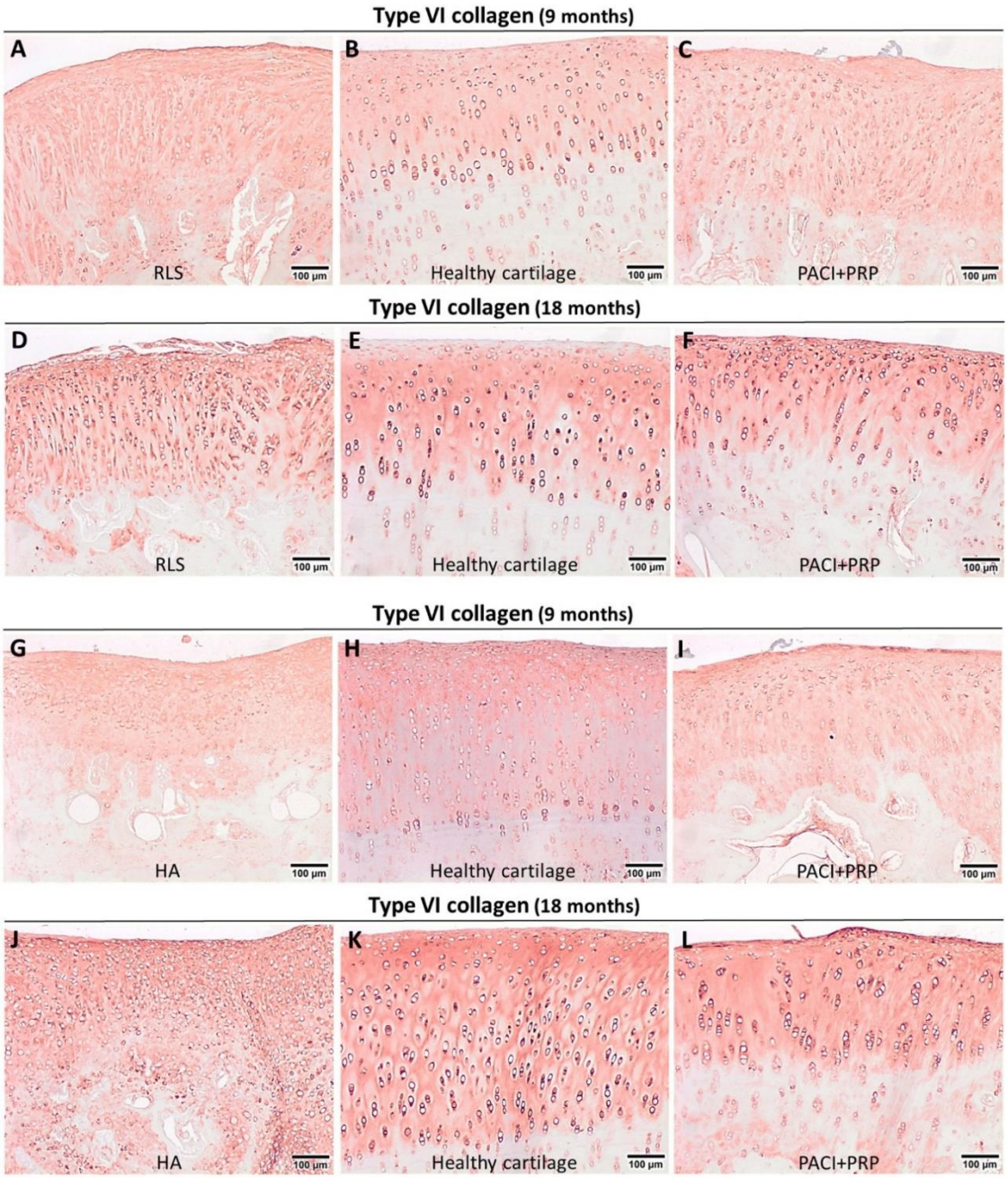

Figure 5. Immunohistochemical evaluation of healthy cartilage and the repaired tissue at 9 and 18 months after the treatments administered. (A) Evaluation of type VI collagen in the RLS group at 9 months; (B) example of healthy cartilage surrounding the chondral defect immunostained with type VI collagen at 9 months; (C) evaluation of type VI collagen in the PACI + PRP group at 9 months; (D) evaluation of type VI collagen in the RLS group at 18 months; (E) example of healthy cartilage surrounding the chondral defect immunostained with type VI collagen at 18 months; (F) evaluation of type VI collagen in the PACI + PRP group at 18 months; (G) evaluation of type VI collagen in the HA group at 9 months; (H) example of healthy cartilage surrounding the chondral defect immunostained with type VI collagen at 9 months; (I) evaluation of type VI collagen in the PACI + PRP group at 9 months; (J) evaluation of type VI collagen in the HA group at 18 months; (K) example of healthy cartilage surrounding the chondral defect immunostained with type VI collagen at 18 months; (L) evaluation of type VI collagen in the PACI + PRP group at 18 months. RLS: left knee treated with Ringer's lactate solution; HA: left knee treated with hyaluronic acid; PACI + PRP: right knee treated with particulated autograft cartilage implantation and platelet-rich plasma.

However, type VI collagen was already distinguished in the ECM of the deep zone (Figure 5D). In the HA group at 18 months, the intensity of the immunostaining in the pericellular matrix was stronger; however, type VI collagen was observed in the ECM of the deep and calcified zones (Figure 5J). The intensity of immunostaining in the pericellular matrix in the PACI + PRP groups was lower than in healthy cartilage at 9 months; however, type VI collagen distribution was the same as in healthy cartilage (Figure 5C,I), whereas, at 18 months, type VI collagen distribution and the intensity of immunostaining in the PACI + PRP groups was the same as healthy cartilage (Figure 5F,L).

4. Discussion

Hyaline articular cartilage is a highly specialized connective tissue, optimized to support and transmit loads with a low coefficient of friction. This, to a large extent, is attributed to the collagens present in the ECM [16]. However, given some of its distinctive features, such as the absence of blood vessels, lymphatic vessels, and nerves, as well as the fact that its cells have low mitotic potential, the repair capacity of cartilage is very limited [17,18]. Despite the large number of existing methods for the treatment of chondral lesions [4,17,19], it has not been possible to restore completely normal hyaline cartilage. An increasing number of studies support the potential of techniques based on particulated cartilage, such as PACI or minced cartilage implantation (MCI) [12,20,21]. The use of MCI or PACI for the treatment of chondral defects relies on the migration of chondrocytes into a biomaterial and subsequent cartilaginous ECM depositions by these cells [22]. Recent in vitro research that studied the repair process mechanisms behind PACI + PRP therapy showed that the cartilage fragments embedded in the three-dimensional PRGF scaffold contain viable chondrocytes that were able to migrate into the fibrin network, proliferate, and synthesize ECM [23]. The better quality of repaired tissue matrix has been shown in animal [13,22,24,25] and human [3,12,22] studies. Previously, a study was published where the chondrogenic regenerative properties of PACI + PRP were analyzed at a macroscopic and histological level. Improved macroscopic appearance, enhanced histological structure, and chondral repair were observed following the application of PACI + PRP as a treatment for chondral defects in sheep [25].

In this study, a therapy for chondral lesions based on a combination of treatment with PACI and PRP was investigated. This treatment was an entirely autologous therapy, and the surgery was performed in an on-step procedure, so the cost can be significantly reduced [20,21]. These cartilage chips and PRP were part of a bioactive matrix that conducted the repair process in the chondral defect. It is essential to take into account their degree of fragmentation since it

correlates positively with the exposed surface area of the biomaterial in which the particles are embedded [26]. Bonasia et al. [27] recommended fragmenting the cartilage into cubes of about 2 mm3. In our study, the excised cartilage was fragmented into cubes of between 1 and 2 mm3. Another important parameter of PACI treatment is the scaffolds used to support the cartilage particles. Protein-based scaffolds such as collagen and fibrin provide binding sites for chondrocytes to adhere to, making them good candidates for scaffolding. Fibrin glue is one of the most commonly used; however, some of its components affect both mechanical strength and adhesive properties [28]. The use of alternative scaffolds has also been described. Marmotti et al. [24] carried out a study on rabbits in which they treated chondral defects using PACI on a scaffold composed of a derivate of HA, fibrin glue, and PRP. Furthermore, in vivo evaluation with PRP scaffolds promoted cell migration but also served as a bioactive scaffold in order to promote chondrocyte viability, proliferation, and differentiation, acting as a reservoir of growth factors and cytokines in [29]. The use of growth factors plays a key role in chondrogenesis during cartilage repair [30,31].

Minimal information is available regarding the repair processes of damaged cartilage, and consequently, there is increased difficulty in finding a treatment that can restore normal articular cartilage. For this reason, it is critical to evaluate, in depth, the repair quality of the repaired cartilage, including an exhaustive evaluation of collagen types. Numerous important collagen subtypes have been identified in healthy articular cartilage, which can be classified as main collagens (types II, IX, and XI) and minor collagens (types III, V, VI, and X) [6]. Additionally, it was determined that type I collagen can be identified in damaged or pathologic articular cartilage [32]. Studies that have evaluated the quality of chondral repair during their treatment have analyzed only some of these collagen types. For instance, Yvonne et al. [33] analyzed type I, II, and VI collagens; Dongquan et al. [34] analyzed type I and II collagens; and Wenqiang et al. [35] analyzed type I, II, and X collagens. However, in this study, we employed a scoring system that allowed us to analyze, in depth, these main and minor collagens, as well as type I and aggrecan, thus obtaining greater evidence on the quality and durability of the repaired tissue.

Type II and V collagens are fibril-forming collagens, characterized by periodic fibrils with an indeterminate length according to the stage of development [36]. In our results, at 18 months, we did not observe significant differences between healthy cartilage and the PACI + PRP, RLS, or HA groups for type II and V collagens. However, at 9 months, significant differences were detected between healthy cartilage and the HA group for type II collagen and between healthy cartilage and the RLS group for type V collagen, which indicated that, at this time, the RLS and HA groups did not reach a fully mature stage of development. These results showed

that the PACI + PRP treatment generated mature hyaline cartilage in a shorter period of time than the RLS or HA groups.

Type IX collagen is a main collagen that stabilizes fibrillar networks by laterally associating with type II and type XI collagens. Type IX collagen reduction in articular cartilage produces a weaker ECM that is predisposed to degradation [37]. Type X collagen is specific to the calcified cartilage zone [38], is synthesized by hypertrophic chondrocytes, and is used as a marker of chondrocyte hypertrophy [39]. In our results, at 9 months, significant differences were not detected between healthy cartilage and the PACI + PRP, RLS, or HA groups for type IX and X collagens. However, at 18 months, significant differences were observed between healthy cartilage and the HA group for type IX collagen and the RLS group for type X collagen. These findings suggested that repaired cartilage tissue deteriorates over time in both the HA and RLS groups, being more susceptible to degradation and hypertrophic cartilage formation, respectively. Therefore, these results showed that the PACI + PRP treatment regenerated consistent cartilage without hypertrophic cartilage formation at the studied times.

Type VI collagen plays a key role in interactions between chondrocytes and ECM, contributing to the ECM's structural integrity and mechanical properties [38]. In our research, significant differences were observed for type VI collagen between healthy cartilage and the RLS and HA groups at 9 and 18 months. In contrast, significant differences were not detected for this collagen in the PACI + PRP groups at either time point. Thus, the PACI + PRP treatment induced better structural and mechanical qualities in the repaired cartilage.

Sheep have been shown to be a viable option as an experimental model for translational investigation in new treatment options for knee joint injuries [40]. However, it is a very demanding animal model because rest is not possible. Postoperative rehabilitation is also impossible. Although in this study the results showed that the PACI + PRP treatment improved the quality of chondral repair, there was a large dispersion in the data obtained, without which, we suspect we could have obtained more significant results. This dispersion is probably due to the postoperative limitations encountered. Furthermore, these results could be even more promising when they are translated to humans, as it would be possible to carry out the necessary rest and subsequently carry out rehabilitation.

5. Conclusion

From an immunohistochemical point of view, the PACI + PRP treatment advanced the repair cartilage process in chondral defects with mature hyaline cartilage (related to type II and V collagens) and enhanced the structural and mechanical qualities (related to type VI collagen) with better consistent cartilage that was less susceptible to degradation (related to type IX collagen) and without hypertrophic formations over time (related to type X collagen).

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Institutional Review Board Statement: All animal experimental protocols were approved by the Bioethical Committee on Animal Research of the Regional Government of Andalusia (Junta de Andalucía 12/06/2016/109—reference SSA/SIS/MD/jv) and conducted in accordance to guidelines for the protection of animals utilized for scientific purposes (Directive 2010/63/UE, Decision 2020/569/UE, and RD 1386/2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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References

1. Cohen N.P., Foster R.J., Mow V.C. Composition and dynamics of articular cartilage: Structure, function, and maintaining healthy state. J. Orthop. Sports Phy. Ther. 1998;28:203–2015. doi: 10.2519/jospt.1998.28.4.203.

2. Rozario T., DeSimone D.W. The extracellular matrix in development and morphogenesis: A dynamic view. Dev. Biol. 2012;34:126–140. doi: 10.1016/j.ydbio.2009.10.026.

3. Cugat R., Alentorn-Geli E., Navarro J., Cuscó X., Steinbacher G., Seijas R., Álvarez-Díaz P., Barastegui D., Laiz P., Samitier G., et al. A novel autologous-made matrix using hyaline cartilage chips and platelet-rich growth factors for the treatment of full-thickness cartilage or osteochondral defects: Preliminary results. J. Orthop. Surg. 2019;28:2309499019887547. doi: 10.1177/2309499019887547.

4. Huey D.J., Hu J.C., Athanasiou K.A. Unlike bone, cartilage regeneration remains elusive. Science. 2012;338:917–921. doi: 10.1126/science.1222454.

5. Simon T.M., Jackson D.W. Articular cartilage: Injury pathways and treatment options. Sports Med. Arthrosc. Rev. 2006;14:146–154. doi: 10.1097/00132585-200609000-00006.

6. Alcaide-Ruggiero L., Molina-Hernández V., Granados M.M., Domínguez J.M. Main and minor types of collagens in the articular cartilage: The role of collagens in repair tissue evaluation in chondral defects. Int. J. Mol. Sci. 2021;22:13129. doi: 10.3390/ijms222413329.

7. Albrecht F., Roessner A., Zimmermann E. Closure of osteochondral lesions using chondral fragments and fibrin adhesive. Arch. Orthop. Trauma. Surg. 1983;101:213–217. doi: 10.1007/BF00436773.

8. Farr J., Cole B.J., Sherman S., Karas V. Particulated articular cartilage: CAIS and DeNovo NT. J. Knee Surg. 2012;25:23–29. doi: 10.1055/s-0031-1299652.

9. Mariani E., Pulsatelli L. Platelet concentrates in musculoskeletal medicine. Int. J. Mol. Sci. 2020;21:1328. doi: 10.3390/ijms21041328.

10. Abrams G.D., Frank R.M., Fortier L.A., Cole B.J. Platelet-rich plasma for articular cartilage repair. Sports Med. Arthrosc. Rev. 2013;21:213–219. doi: 10.1097/JSA.0b013e3182999740.

11. Zhao J., Huang H., Liang G., Zeng L.F., Yang W., Liu J. Effects and safety of the combination of platelet-rich plasma (PRP) and hyaluronic acid (HA) in the treatment of knee osteoarthritis: A systematic review and meta-analysis. BMC Musculoskelet. Disord. 2020;21:224. doi: 10.1186/s12891-020-03262-w.

12. Cugat R., Alentorn-Geli E., Steinbacher G., Álvarez-Díaz P., Cuscó X., Seijas R., Barastegui D., Navarro J., Laiz P., García-Balletbó M. Treatment of knee osteochondral lesions using a novel clot of autologous plasma rich in growth factors mixed with healthy hyaline cartilage chips and intra-articular injection of PRGF. Case Rep. Orthop. 2017;2017:8284548. doi: 10.1155/2017/8284548.

13. Domínguez-Pérez J.M., Fernández-Sarmiento J.A., Aguilar-García D., Granados-Machuca M.D.M., Morgaz-Rodríguez J., Navarrete-Calvo R., Pérez-Arévalo J., Carrillo-Poveda J.M., Alentorn-Geli E., Laiz-Boada P., et al. Cartilage regeneration using a novel autologous growth factors-based matrix for full-thickness defects in sheep. Knee Surg. Sports Traumatol. Arthrosc. 2019;27:950–961. doi: 10.1007/s00167-018-5107-z.

14. Hoemann C., Kandel R., Roberts S., Saris D.B.F., Creemers L., Mainil-Varlet P., Méthot S., Hollander A.P., Buschmann M.D. International cartilage repair society (ICRS) recommended guidelines for histological endpoints for cartilage repair studies in animal models and clinical trials. Cartilage. 2011;2:153–172. doi: 10.1177/1947603510397535.

15. Alcaide-Ruggiero L., Cugat R., Domínguez J.M. Proteoglycans in articular cartilage and their contribution to chondral injury and repair mechanisms. Int. J. Mol. Sci. 2023;24:10824. doi: 10.3390/ijms241310824.

16. Sophia-Fox A.J., Bedi A., Rodeo S.A. The basic science of articular cartilage: Structure, composition, and function. Sports Health. 2009;1:461–468. doi: 10.1177/1941738109350438.

17. Decker R.S. Articular cartilage and joint development from embryogenesis to adulthood. Semin. Cell Dev. Biol. 2017;62:50–56. doi: 10.1016/j.semcdb.2016.10.005.

18. Medvedeva E.V., Grebenik E.A., Gornostaeva S.N., Telpuhov V.I., Lychagin A.V., Timashev P.S., Chagin A.S. Repair of damaged articular cartilage: Current approaches and future directions. Int. J. Mol. Sci. 2018;19:2366. doi: 10.3390/ijms19082366.

19. Musumeci G., Castrogiovanni P., Leonardi R., Trovato F.M., Szychlinska M.A., Di Giunta A., Loreto C., Castorina R. New perspectives for articular cartilage repair treatment through tissue engineering: A contemporary review. World J. Orthop. 2014;5:80–88. doi: 10.5312/wjo.v5.i2.80.

20. Cugat R., Samitier G., Vinagre G., Sava M., Alentorn-Geli E., García-Balletbó M., Cuscó X., Seijas R., Barastegui D., Navarro J., et al. Particulated autologous chondral−platelet-rich plasma matrix implantation (PACI) for treatment of full-thickness cartilage osteochondral defects. Arthrosc. Tech. 2021;10:239–544. doi: 10.1016/j.eats.2020.10.038.

21. Delman C., Wuellner J., Kreulen C., Lundeen G., Giza E. Particulated autograft cartilage implantation for the treatment of osteochondral lesions of the talus: A novel technique. Foot Ankle Spec. 2018;11:365–371. doi: 10.1177/1938640018770277.

22. Bonasia D.E., Marmotti A., Rosso F., Collo G., Rossi R. Use of chondral fragments for one stage cartilage repair: A systematic review. World J. Orthop. 2015;6:1006–1011. doi: 10.5312/wjo.v6.i11.1006.

23. Anitua E., Prado R., Guadilla J., Alkhraisat M.H., Laiz P., Padilla S., García-Balletbó M., Cugat R. The dual-responsive interaction of particulated hyaline cartilage and plasma rich in growth factors (PRGF) in the repair of cartilage defects: An in vitro study. Int. J. Mol. Sci. 2023;24:11581. doi: 10.3390/ijms241411581.

24. Marmotti A., Bruzzone M., Bonasia D.E., Castoldi F., Von-Degerfeld M.M., Bignardi C., Mattia S., Maiello R.R., Peretti G.M. Autologous cartilage fragments in a composite scaffold for one stage osteochondral repair in a goat model. Eur. Cell Mater. 2013;26:15–31. doi: 10.22203/eCM.v026a02.

25. Alcaide-Ruggiero L., Molina-Hernández V., Morgaz J., Fernández-Sarmiento J.A., Granados M.M., Navarrete-Calvo R., Pérez J., Quirós-Carmona S., Carrillo J.M., Cugat R., et al. Particulate cartilage and platelet-rich plasma treatment for knee chondral defects in sheep. Knee Surg. Sports Traumatol. Arthrosc. 2023;31:2944–2955. doi: 10.1007/s00167-022-07295-7.

26. Levinson C., Cavalli E., Sindi D.M., Kessel B., Zenobi-Wong M., Preiss S., Salzmann G., Neidencach P. Chondrocytes from device-minced articular cartilage show potent outgrowth into fibrin and collagen hydrogels. Orthop. J. Sports Med. 2019;7:2325967119867618. doi: 10.1177/2325967119867618.

27. Bonasia D.E., Marmotti A., Mattia S., Cosentino A., Spolaore S., Governale G., Castoldi F., Rossi R. The degree of chondral fragmentation affects extracellular matrix production in cartilage autograft implantation: An in vitro study. Arthroscopy. 2015;31:2335–2341. doi: 10.1016/j.arthro.2015.06.025.

28. Bhagat V., Becker M.L. Degradable adhesives for surgery and tissue engineering. Biomacromolecules. 2017;18:3009–3039. doi: 10.1021/acs.biomac.7b00969.

29. Kreuz P.C., Krüger J.P., Metzlaff S., Freymann U., Endres M., Pruss A., Petersen W., Kaps C. Platelet-rich plasma preparation types show impact on chondrogenic differentiation, migration, and proliferation of human subchondral mesenchymal progenitor cells. Arthroscopy. 2015;31:1951–1961. doi: 10.1016/j.arthro.2015.03.033.

30. Anitua E., Sanchez M., Nurden A.T., Zalduendo M., De La Fuente M., Orive G., Azofra J., Andia I. Autologous fibrin matrices: A potential source of biological mediators that modulate tendon cell activities. J. Biomed. Mater. Res. A. 2006;77:285–293. doi: 10.1002/jbm.a.30585.

31. Zhu Y., Yuan M., Meng H.Y., Wang A.Y., Guo Q.Y., Wang Y., Peng J. Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: A review. Osteoarthr. Cartil. 2013;21:1627–1637. doi: 10.1016/j.joca.2013.07.017.

32. Bielajew B.J., Hu J.C., Athanasiou K.A. Collagen: Quantification, biomechanics, and role of minor subtypes in cartilage. Nat. Rev. Mater. 2020;5:730–747. doi: 10.1038/s41578-020-0213-1.

33. Peck Y., He P., Chilla G.S.V.N., Poh C.L., Wang D.A. A preclinical evaluation of an autologous living hyaline-like cartilaginous graft for articular cartilage repair: A pilot study. Sci. Rep. 2015;5:16225. doi: 10.1038/srep16225.

34. Shi D., Xu X., Ye Y., Song K., Cheng Y., Di J., Hu Q., Li J., Ju H., Jiang Q., et al. Photo-cross-linked scaffold with kartogenin-encapsulated nanoparticles for cartilage regeneration. ACS Nano. 2016;10:1292–1299. doi: 10.1021/acsnano.5b06663.

35. Yan W., Xu X., Xu Q., Sun Z., Jiang Q., Shi D. Platelet-rich plasma combined with injectable hyaluronic acid hydrogel for porcine cartilage regeneration: A 6-month follow-up. Regen. Biomater. 2020;7:77–90. doi: 10.1093/rb/rbz039.

36. Bourgot I., Primac I., Louis T., Noël A., Maquoi E. Reciprocal interplay between fibrillar collagens and collagen-binding integrins: Implications in cancer progression and metastasis. Front. Oncol. 2020;10:1488. doi: 10.3389/fonc.2020.01488.

37. Parsons P., Gilbert S.J., Vaughan-Thomas A., Sorrell D.A., Notman R., Bishop M., Haynes A.J., Mason D.J., Duance V.C. Type IX collagen interacts with fibronectin providing an important molecular bridge in articular cartilage. J. Biol. Chem. 2011;286:34986–34997. doi: 10.1074/jbc.M111.238188.

38. Luo Y., Sinkeviciute D., He Y., Karsdal M., Henrotin Y., Mobasheri A., Önnerfjord P., Bay-Jensen A. The minor collagens in articular cartilage. Protein Cell. 2017;8:560–572. doi: 10.1007/s13238-017-0377-7.

39. He Y., Siebuhr A.S., Brandt-Hansen N.U., Wang J., Su D., Zheng Q., Simonsen O., Petersen K.K., Arendt-Nielsen L., Eskehave T., et al. Type X collagen levels are elevated in serum from human osteoarthritis patients and associated with biomarkers of cartilage degradation and inflammation. BMC Musculoskelet. Disord. 2014;15:309. doi: 10.1186/1471-2474-15-309.

40. Takroni T., Laouar L., Adesida A., Elliott J.A.W., Jomha N.M. Anatomical study: Comparing the human, sheep and pig knee meniscus. J. Exp. Orthop. 2016;3:35. doi: 10.1186/s40634-016-0071-3.

41. Kaul G., Cucchiarini M., Remberger K., Kohn D., Madry H. Failed cartilage repair for early osteoarthritis defects: A biochemical, histological and immunohistochemical analysis of the repair tissue after treatment with marrow-stimulation techniques. Knee Surg. Sports Traumatol. Arthrosc. 2012;20:2315–2324. doi: 10.1007/s00167-011-1853-x.

42. Landmann M., Scheibner D., Graaf A., Gischke M., Koethe S., Fatola O.I., Raddatz B., Mettenleiter T.C., Beer M., Grund C., et al. A semiquantitative scoring system for histopathological and immunohistochemical assessment of lesions and tissue tropism in avian influenza. Viruses. 2021;13:868. doi: 10.3390/v13050868.

43. Walker R.A. Quantification of immunohistochemistry—Issues concerning methods, utility and semiquantitative assessment I. Histopathology. 2006;49:406–410. doi: 10.1111/j.1365-2559.2006.02514.x.

VIII. DISCUSIÓN GENERAL

El cartílago articular, un tejido conjuntivo altamente especializado, desempeña un papel esencial en la transmisión de cargas debido a su bajo potencial de fricción y alta resistencia a las fuerzas de compresión, en gran medida gracias a los colágenos presentes en la ECM (2). Sin embargo, el cartílago hialino maduro presenta características distintivas, como la ausencia de vasos sanguíneos, vasos linfáticos y nervios, siendo los condrocitos la única estirpe celular que podemos encontrar en este tejido. La capacidad de reparación del cartílago es considerablemente limitada debido al metabolismo reducido y al bajo potencial mitótico de los condrocitos (4,5).

En este contexto, a pesar de la gran variedad de estrategias terapéuticas disponibles para el tratamiento de las lesiones condrales y los avances tecnológicos en ingeniería de tejidos en los últimos años (4,10,58), continúa siendo un reto encontrar un tratamiento eficaz capaz de restaurar por completo cartílago hialino maduro (4,5).

En el ámbito de la medicina musculoesquelética, el PRP se ha estado utilizando cada vez con más frecuencia en los últimos años, debido a su destacado potencial terapéutico. Este se deriva de su elevada concentración de factores de crecimiento y citoquinas, las cuales promueven activamente la regeneración y reparación del cartílago articular. La aplicación del PRP combinado con la implantación de PACI, tratamiento que se conoce como PACI+PRP, ha demostrado resultados alentadores tanto en modelos animales (15,17) como en humanos (15,122).

Teniendo en cuenta estos precedentes, en la presente tesis doctoral se llevó a cabo un análisis a nivel macroscópico, histológico e inmunohistoquímico del tratamiento PACI+PRP para la reparación de este tipo de lesiones, utilizando un modelo ovino. Este enfoque terapéutico implica el uso de una matriz autóloga compuesta por fragmentos de cartílago hialino sano en un coágulo de PRP, acompañado de una infiltración intraarticular de PRP.

En nuestro estudio, el grupo tratado con PACI+PRP presentó características macroscópicas superiores en comparación con aquellos recuperados con HA y RLS, especialmente después de 18 meses de recuperación, siendo el cartílago recuperado con RLS el que mostró un aspecto macroscópico más deficiente. Este análisis macroscópico coincidió con los resultados a nivel histológico, así el cartílago reparado con PACI+PRP mostró una mayor calidad en cuanto a sus características histológicas, y los condrocitos mostraron un proceso de reparación mucho más avanzado en comparación con los grupos tratados con HA y RLS, tanto a los 9 como a los 18 meses de recuperación. Esto se debe principalmente a la migración de condrocitos hacia el

biomaterial y la subsiguiente deposición de ECM en la zona de reparación, poniendo de manifiesto el papel que juegan estas células en el desarrollo, mantenimiento y reparación de la ECM (26).

Posteriormente, se llevó a cabo un análisis detallado de los colágenos mayores y menores (colágenos tipo II, III, V, VI, IX, X y XI), así como del agrecano, como biomarcadores de interés para evaluar la calidad de la reparación del cartílago hialino maduro. Se incluyó el colágeno tipo I como marcador de fibrocartílago (31). Además, se desarrolló un sistema de valoración que permitió una evaluación más precisa de la calidad y durabilidad del tejido reparado, respaldando así los resultados obtenidos previamente en los análisis macroscópico e histológico (31,155,156).

Los colágenos tipo II y V se caracterizan por formar fibrillas periódicas con una longitud variable en función de la etapa de desarrollo del cartílago hialino (157). Nuestros resultados indican que, a los 9 meses, el cartílago tratado con PACI+PRP mostraba una similitud significativa en cuanto a los colágenos tipo II y V en comparación con el cartílago sano, a diferencia de los grupos tratados con HA y RLS. A los 18 meses de recuperación, no se observaron diferencias entre los diferentes tratamientos y el cartílago sano en relación con estos colágenos formadores de fibrillas. Estos resultados ponen en evidencia la capacidad del tratamiento con PACI+PRP para regenerar el cartílago hialino maduro en un periodo de recuperación más corto.

El colágeno tipo IX es un componente clave al estabilizar las redes fibrilares mediante su asociación lateral con los colágenos tipo II y XI. La disminución del colágeno tipo IX en el cartílago articular resulta en una ECM más frágil y propensa a la degradación (158). Por otro lado, el colágeno tipo X es específico de la zona de cartílago calcificado y se produce en condrocitos hipertróficos, actuando como marcador de la hipertrofia condrocitaria (33,159). En relación con los colágenos tipo IX y X, a los 9 meses de recuperación, no se observaron diferencias significativas entre el cartílago sano y los grupos tratados con PACI+PRP, HA o RLS. Sin embargo, a los 18 meses, el tratamiento con PACI+PRP demostró regenerar un cartílago más consistente y sin la formación de cartílago hipertrófico, a diferencia de los grupos HA y RLS. Estos últimos presentaron diferencias en cuanto a los colágenos tipo IX y X, indicando que el tejido cartilaginoso reparado es más susceptible a la degradación y a la formación de cartílago hipertrófico, mostrando una tendencia al deterioro con el tiempo.

El colágeno tipo VI es esencial para la interacción entre los condrocitos y la ECM, contribuyendo tanto a la integridad estructural de esta como a sus propiedades mecánicas (33). En este contexto, el tratamiento con PACI+PRP indujo mejores cualidades estructurales y

mecánicas en el cartílago reparado, tanto a los 9 como a los 18 meses de recuperación, a diferencia de los grupos HA y RLS.

En conjunto, en esta tesis doctoral, destacamos el tratamiento PACI+PRP como una terapia completamente autóloga de aplicación relativamente sencilla y bajo coste. Este enfoque no solo favorece la regeneración del cartílago hialino en lesiones condrales, sino que este también exhibe una estructura histológica comparable al cartílago sano circundante. Además, ha demostrado la capacidad de mejorar las características estructurales y mecánicas del cartílago, generando un tejido más consistente y menos propenso a la degradación en un periodo de recuperación más breve en comparación con otros tratamientos evaluados.

IX. CONCLUSIONES - CONCLUSIONS

Conclusión 1 (Objetivo I – Capítulos 1 y 2)

Los diferentes tipos de colágenos, tanto mayores como menores, así como los proteoglicanos desempeñan un papel fundamental como biomarcadores de interés que permitirían realizar una evaluación histológica precisa del cartílago hialino reparado. Estas moléculas son de vital importancia para el diagnóstico precoz, evaluación de la calidad y características del cartílago reparado y valoración de la eficacia del tratamiento tras una lesión condral.

Conclusión 2 (Objetivo II – Capítulo 3)

El cartílago hialino regenerado tras la aplicación del tratamiento PACI+PRP presentó un aspecto macroscópico, una estructura histológica y una apariencia de los condrocitos equiparable a la del cartílago sano adyacente después de 9 y 18 meses de proceso regenerativo. Los grupos PACI + PRP mostraron diferencias estadísticamente significativas en el porcentaje de reparación del defecto, así como en el área celular, la morfología y el número de condrocitos presentes en el tejido cartilaginoso reparado, al comparar los resultados a los 18 meses con los obtenidos a los 9 meses.

Conclusión 3 (Objetivo III – Capítulo 4)

El análisis inmunohistoquímico demostró que el tratamiento PACI+PRP estudiado promovió, en comparación con los grupos RLS o HA, una regeneración de cartílago hialino más maduro en lo que respecta a los colágenos tipo II y V, con características mecánicas y estructurales mejoradas en relación con el colágeno tipo VI, y una regeneración de cartílago más consistente y menos susceptible a la degradación en relación con los colágenos tipo IX y X. Además, no se observó formación de cartílago hipertrófico con el tratamiento PACI+PRP.

Conclusión 4

Los resultado obtenidos empleando el tratamiento PACI+PRP para la reparación de lesiones focales de cartílago articular informan de que se trata de una terapia completamente autóloga de aplicación sencilla y bajo coste, que puede realizarse en un solo paso quirúrgico y de forma segura, resultando en una nueva opción terapéutica de relevancia por conseguir regenerar cartílago hialino articular, con aplicabilidad clínica tanto en medicina veterinaria como humana.

IX. CONCLUSIONES - CONCLUSIONS

Conclusion 1 (Objective 1 – Chapters 1 and 2)

The major and minor collagens, as well as proteoglycans, play a crucial role as potential biomarkers of interest, enabling a precise histological assessment of repaired hyaline cartilage. These molecules are essential for early diagnostic procedures, the evaluation of the quality and characteristics of repaired cartilage, and the evaluation of treatment efficacy post chondral injury.

Conclusion 2 (Objective 2 – Chapters 3)

The regenerated cartilaginous tissue resulting from a treatment based on PACI+PRP showed a macroscopic morphology, histological structure, and chondrocyte-level repair comparable to the surrounding healthy cartilage after 9 and 18 months of the regenerative process. The PACI+PRP groups showed statistically significant differences in the percentage of defect repair, as well as in the cellular area, morphology, and number of chondrocytes within the repaired cartilaginous tissue when comparing the results obtained at 18 months with those at 9 months.

Conclusion 3 (Objective 3 – Chapters 4)

The immunohistochemical analysis revealed that the PACI+PRP compared with the RLS or HA groups, facilitated the development of mature hyaline cartilage, as correlated with collagen types II and V. This enhancement was more evident in the structural and mechanical characteristics associated with collagen type VI, resulting in a more consistent cartilaginous tissue that is less susceptible to degradation, linked to collagen type IX and X. Furthermore, the absence of hypertrophic cartilage formations was observed, persisting even after an 18-month recovery period, specifically in relation to collagen type X.

Conclusion 4

The results obtained with the PACI+PRP treatment for repairing focal lesions in articular cartilage suggest that it is a completely autologous, easily applicable, and cost-effective treatment that can be performed in a single surgical step. Therefore, it emerges as a primary therapeutic choice for regenerating articular hyaline cartilage, with clinical relevance in both veterinary and human medicine.

X. REFERENCIAS

(1) Anitua-Aldecoa E., Sánchez M. Un nuevo enfoque biológico de la cirugía ortopédica y medicina del deporte*,* 1ª ed.; Teamwork Media España S.L: Vitoria-Gasteiz Álava, España, 2012.

(2) Sophia Fox A.J., Bedi A., Rodeo S.A. The basic science of articular cartilage: Structure, composition, and function. Sports Health. 2009;1:461–468. doi: 10.1177/1941738109350438.

(3) Heir S., Nerhus T.K., Røtterud J.H., Løken S., Ekeland A., Engebretsen L., Aroen A. Focal cartilage defects in the knee impair quality of life as much as severe osteoarthritis: A comparison of knee injury and osteoarthritis outcome score in 4 patient categories scheduled for knee surgery. Am J Sports Med. 2010;38:231-237. doi: 10.1177/0363546509352157.

(4) Decker R.S. Articular cartilage and joint development from embryogenesis to adulthood. Semin. Cell Dev. Biol. 2017;62:50–56. doi: 10.1016/j.semcdb.2016.10.005.

(5) Medvedeva E.V., Grebenik E.A., Gornostaeva S.N., Telpuhov V.I., Lychagin A.V., Timashev P.S., Chagin A.S. Repair of damaged articular cartilage: Current approaches and future directions. Int J Mol Sci. 2018;18:2366. doi: 10.3390/ijms19082366.

(6) Vinod E., Boopalan P.R.J.V.C., Sathishkumar S. Reserve or Resident Progenitors in Cartilage? Comparative Analysis of Chondrocytes versus Chondroprogenitors and Their Role in Cartilage Repair. Cartilage. 2018;9:171–182. doi: 10.1177/1947603517736108.

(7) Zhang S., Hu B., Liu W., Wang P., Lv X., Chen S., Liu H., Shao Z. Articular cartilage regeneration: The role of endogenous mesenchymal stem/progenitor cell recruitment and migration. Semin. Arthritis Rheum. 2020;50:198–208. doi: 10.1016/j.semarthrit.2019.11.001.

(8) Domínguez-Pérez J.M., Fernández-Sarmiento J.A., García D., Granados-Machuca M.M., Morgaz-Rodríguez J., Navarrete-Calvo R., Pérez-Arévalo J., Carrillo-Poveda J.M., Alentorn-Geli E., Laiz-Boada P., et al. Cartilage regeneration using a novel autologous growth factors-based matrix for full-thickness defects in sheep. Knee Surg. Sports Traumatol. Arthrosc. 2019;27:950–961. doi: 10.1007/s00167-018-5107-z.

(9) Richter D.L., Schenck R.C., Wascher D.C., Treme G. Knee Articular Cartilage Repair and Restoration Techniques: A Review of the Literature. Sports Health. 2016;8:153–160. doi: 10.1177/1941738115611350.

(10) Musumeci G., Castrogiovanni P., Leonardi R., Trovato F.M., Szychlinska M.A., Di Giunta A., Loreto C., Castorin S. New perspectives for articular cartilage repair treatment through tissue engineering: A contemporary review. World J. Orthop. 2014;5:80–88. doi: 10.5312/wjo.v5.i2.80.

(11) Campbell A.B., Pineda M., Harris J.D., Flanigan D.C. Return to Sport after Articular Cartilage Repair in Athletes' Knees: A Systematic Review. Arthroscopy. 2016;32:651–668. doi: 10.1016/j.arthro.2015.08.028.

(12) Wu P.I.K., Diaz R., Borg-Stein J. Platelet-Rich Plasma. Phys Med Rehabil Clin N Am. 2016;27:825-853. doi: 10.1016/j.pmr.2016.06.002.

(13) Xue Y., Su X., Jiang M., Yu Z., Yang H., Qin L., Giannoudis P.V. Pure platelet-rich plasma facilitates the repair of damaged cartilage and synovium in a rabbit hemorrhagic arthritis knee model. Arthritis Res Ther. 2020;22:68. doi: 10.1186/s13075-020-02155-6.

(14) Milano G, Sanna-Passino E, Deriu L, Careddu G, Manunta L, Manunta A, Saccomanno M F, Fabbriciani C (2010) The effect of platelet rich plasma combined with microfractures on the

treatment of chondral defects: An experimental study in a sheep model. Osteoarthritis Cartilage 18:971-980.

(15) Cugat R., Alentorn-Geli E., Steinbacher G., Álvarez-Díaz P., Cuscó X., Seijas R., Barastegui D., Navarro J., Laiz P., García-Balletbó M. Treatment of knee osteochondral lesions using a novel clot of autologous plasma rich in growth factors mixed with healthy hyaline cartilage chips and intra-articular injection of PRGF. Case Rep. Orthop. 2017;2017:8284548. doi: 10.1155/2017/8284548.

(16) Farr J., Cole B.J., Sherman S., Karas V. Particulated articular cartilage: CAIS and DeNovo NT. J Knee Surg. 2012;25:23–29.

(17) Cugat R., Alentorn-Geli E., Navarro J., Cuscó X., Steinbacher G., Seijas R., Álvarez-Díaz P., Barastegui D., Laiz P., Samitier G., et al. A novel autologous-made matrix using hyaline cartilage chips and platelet-rich growth factors for the treatment of full-thickness cartilage or osteochondral defects: Preliminary results. J. Orthop. Surg. 2019;28:2309499019887547. doi: 10.1177/2309499019887547.

(18) Luria A., Chu C.R. Articular Cartilage Changes in Maturing Athletes: New Targets for Joint Rejuvenation. Sports Health. 2014;6:18–30. doi: 10.1177/1941738113514369.

(19) Thorp H., Kim K., Kondo M., Maak T., Grainger D.W., Okano T. Trends in Articular Cartilage Tissue Engineering: 3D Mesenchymal Stem Cell Sheets as Candidates for Engineered Hyaline-Like Cartilage. Cells. 2011;10:643. doi: 10.3390/cells10030643.

(20) Sun Y., Yan L., Chen S., Pei M. Functionality of decellularized matrix in cartilage regeneration: A comparison of tissue versus cell sources. Acta Biomater. 2018;74:56-73. doi: 10.1016/j.actbio.2018.04.048.

(21) Hu H., Liu W., Sun C., Wang Q., Yang W., Zhang Z.C., Xia Z., Shao Z., Wang B. Endogenous Repair and Regeneration of Injured Articular Cartilage: A Challenging but Promising Therapeutic Strategy. Aging Dis. 2021;12:886-901. doi: 10.14336/AD.2020.0902.

(22) Heir S., Nerhus T.K., Røtterud J.H., Løken S., Ekeland A., Engebretsen L., Aroen a. Focal cartilage defects in the knee impair quality of life as much as severe osteoarthritis: a comparison of knee injury and osteoarthritis outcome score in 4 patient categories scheduled for knee surgery. Am J Sports Med. 2010;38:231-237. doi: 10.1177/0363546509352157.

(23) Patel J.M., Saleh K.S., Burdick J.A., Mauck R.L. Bioactive factors for cartilage repair and regeneration: Improving delivery, retention, and activity. Acta Biomater. 2019;93:222–238. doi: 10.1016/j.actbio.2019.01.061.

(24) Bhosale A.M., Richardson J.B. Articular cartilage: Structure, injuries and review of management. Br. Med. Bull. 2008;87:77–95. doi: 10.1093/bmb/ldn025.

(25) Phull A.R., Eo S.H., Abbas Q., Ahmed M., Kim S.J. Applications of Chondrocyte-Based Cartilage Engineering: An Overview. BioMed Res. Int. 2016;2016:1879837. doi: 10.1155/2016/1879837.

(26) Alford J.W., Cole B.J. Cartilage restoration, part 1: basic science, historical perspective, patient evaluation, and treatment options. Am J Sports Med. 2005;33:295-306. doi: 10.1177/0363546504273510.

(27) Ulrich-Vinther M., Maloney M.D, Schwarz E.M., Rosier R., O'Keefe R.J. Articular cartilage biology. J Am Acad Orthop Surg. 2003;11:421-430. doi: 10.5435/00124635-200311000-00006.

(28) Adkisson H.D., Martin J.A., Amendola R.L., Milliman C., Mauch K.A., Katwal A.B., Seyedin M., Amendola A., Streeter P.R., Buckwalter J.A. The potential of human allogeneic juvenile

chondrocytes for restoration of articular cartilage. Am J Sports Med. 2010;38:1324-1333. doi: 10.1177/0363546510361950.

(29) Cohen N.P., Foster R.J., Mow V.C. Composition and dynamics of articular cartilage: Structure, function, and maintaining healthy state. J Orthop Sports Phys Ther. 1998;28:203215.

(30) Cumming M.H., Hall B., Hofman K. Isolation and characterisation of major and minor collagens from hyaline cartilage of hoki (Macruronus novaezelandiae) Mar. Drugs. 2019;17:223. doi: 10.3390/md17040223.

(31) Alcaide-Ruggiero L., Molina-Hernández V., Granados M.M., Domínguez J.M. Main and minor types of collagens in the articular cartilage: The role of collagens in repair tissue evaluation in chondral defects. Int. J. Mol. Sci. 2021;22:13129. doi: 10.3390/ijms222413329.

(32) Eyre D. Collagen of articular cartilage. Arthritis Res. 2002;4:30-35. doi: 10.1186/ar380.

(33) Luo Y., Sinkeviciute D., He Y., Karsdal M., Henrotin Y., Mobasheri A., Önnerfjord P., Bay-Jensen A. The minor collagens in articular cartilage. Protein Cell. 2017;8:560–572. doi: 10.1007/s13238-017-0377-7.

(34) Wu L., Petrigliano F.A., Ba K., Lee S., Bogdanov J., McAllister D.R., Adams J.S., Rosenthal A.K., Van Handel B., Crooks G.M., et al. Lysophosphatidic acid mediates fibrosis in injured joints by regulating collagen type I biosynthesis. Osteoarthr. Cartil. 2015;23:308–318. doi: 10.1016/j.joca.2014.11.012.

(35) Buckwalter J.A., Pita J.C., Muller F.J., Nessler J. Structural differences between two populations of articular cartilage proteoglycan aggregates. J Orthop Res. 1994;12:144-148. doi: 10.1002/jor.1100120118.

(36) Maroudas A., Wachtel E., Grushko G., Katz E.P., Weinberg P. The effect of osmotic and mechanical pressures on water partitioning in articular cartilage. Biochim Biophys Acta. 1991;1073:285-294.

(37) Torzilli P.A. Influence of cartilage conformation on its equilibrium water partition. J Orthop Res. 1985;3:473-483.

(38) Mow V.C., Ratcliffe A., Poole A. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. Biomaterials. 1992;13:67–97. doi: 10.1016/0142-9612(92)90001-5.

(39) Setton L.A., Zhu W., Mow V.C. The biphasic poroviscoelastic behavior of articular cartilage: Role of the surface zone in governing the compressive behavior. J Biomech. 199326:581–92. doi: 10.1016/0021-9290(93)90019-b.

(40) Ateshian G.A. The Role of Interstitial Fluid Pressurization in Articular Cartilage. J Biomech. 2009;42:1163-1176. doi: 10.1016/j.jbiomech.2009.04.040

(41) Soltz M.A., Ateshian G.A. A conewise linear elasticity mixture model for the analysis of tension-compression nonlinearity in articular cartilage. J Biomech Eng. 2000;122:576-586. doi: 10.1115/1.1324669.

(42) Chan D.D., Cai L., Butz K.D., Trippel S.B., Nauman E.A., Neu C.P. In vivo articular cartilage deformation: noninvasive quantification of intratissue strain during joint contact in the human knee. Sci Rep. 2016;6:19220. doi: 10.1038/srep19220.

(43) Woo S.L.Y., Simon B.R., Kuei S.C., Akeson W.H. Quasi-linear viscoelastic properties of normal articular cartilage. J Biomech Eng. 1980;102:85-90. doi: 10.1115/1.3138220

(44) Mostakhdemin M., Nand A., Ramezani M. Articular and Artificial Cartilage, Characteristics, Properties and Testing Approaches - A Review. Polymers. 2021;13:2000. doi: 10.3390/polym13122000.

(45) Maroudas A.I. Balance between swelling pressure and collagen tension in normal and degenerate cartilage. Nature. 1976;260:808–809. doi: 10.1038/260808a0.

(46) Venäläinen M.S., Mononen M.E., Salo J., Räsänen L.P., Jurvelin J.S., Töyräs J., Virén Y., Korhonen R.K. Quantitative Evaluation of the Mechanical Risks Caused by Focal Cartilage Defects in the Knee. Sci Rep. 2016;6:1–11. doi: 10.1038/srep37538.

(47) Barbour K.E., Helmick C.G., Boring M., Brady T.J. Vital Signs: Prevalence of Doctor-Diagnosed Arthritis and Arthritis-Attributable Activity Limitation - United States, 2013 - 2015. MMWR Morb Mortal Wkly Rep. 2019;66:246–253. doi: 10.15585/mmwr.mm6609e1.

(48) Lopa S., Madry H. Bioinspired scaffolds for osteochondral regeneration. Tissue Eng Part A. 2014;20:2052-2076. doi: 10.1089/ten.tea.2013.0356.

(49) Wei W., Dai H. Articular cartilage and osteochondral tissue engineering techniques: Recent advances and challenges. Bioact Mater. 2021;6:4830-4855. doi: 10.1016/j.bioactmat.2021.05.011.

(50) Wu X., Cai Y., Lu S., Xu K., Shi X., Yang L., Huang Z., Xu P. Intra-articular Injection of Chloramphenicol Reduces Articular Cartilage Degeneration in a Rabbit Model of Osteoarthritis. Clin Orthop Relat Res. 2019;477:2785-2797. doi: 10.1097/CORR.0000000000001016.

(51) Johnson-Nurse C., Dandy D.J. Fracture-separation of articular cartilage in the adult knee. J Bone Joint Surg Br. 1985;67:42-43. doi: 10.1302/0301-620X.67B1.3968141.

(52) Cahill B.R. Osteochondritis Dissecans of the Knee: Treatment of Juvenile and Adult Forms. J Am Acad Orthop Surg. 1995;3:237-247. doi: 10.5435/00124635-199507000-00006.

(53) Mankin H.J. The Reaction of Articular Cartilage to Injury and Osteoarthritis. N Engl J Med. 1974;291:1285-1292. doi: 10.1056/NEJM197412122912406.

(54) Guettler J.H., Demetropoulos C.K., Yang K.H., Jurist K.A. Osteochondral defects in the human knee: influence of defect size on cartilage rim stress and load redistribution to surrounding cartilage. Am J Sports Med. 2004;32:1451-1458. doi: 10.1177/0363546504263234.

(55) Gratz K.R., Wong B.L., Bae W.C., Sah R.L. The effects of focal articular defects on cartilage contact mechanics. J Orthop Res. 2009;27:584-592. doi: 10.1002/jor.20762.

(56) Quinn T.M., Allen R.G., Schalet B.J., Perumbuli P., Hunziker E.B. Matrix and cell injury due to sub-impact loading of adult bovine articular cartilage explants: effects of strain rate and peak stress. J Orthop Res. 2001;19:242-249. doi: 10.1016/S0736-0266(00)00025-5.

(57) Ewing J.W. Articular cartilage and knee joint function : basic science and arthroscopy´, 1ª ed.; Raven Press: Universidad de Michigan, 1990.

(58) Huey D.J., Hu J.C., Athanasiou K.A. Unlike bone, cartilage regeneration remains elusive. Science. 2012;338:917-921. doi: 10.1126/science.1222454.

(59) Camp C.L., Stuart M.J., Krych A.J. Current Concepts of Articular Cartilage Restoration Techniques in the Knee. Sports Health. 2014;6:265-273. doi: 10.1177/1941738113508917.

(60) Martin R., Jakob R.P. Review of K.H. Pridie (1959) on “A method of resurfacing osteoarthritic knee joints.” J ISAKOS. 2022;7:39–46. doi: 10.1016/j.jisako.2021.11.001.

(61) Sevillano R.C. Desbridamiento artroscópico y artroplastia por abrasión. Posibilidades actuales. Revista Española de Cirugía Ortopédica y Traumatología. 2002;46:451-457.

(62) Moseley J.B., O´Malley K., Petersen N.J., Menke T.J., Brody B.A., Kuykendall D.H., Hollingsworth J.C., Asthon C.M., Wray.A.P. A controlled trial of arthroscopic surgery for osteoarthritis of the knee. N Engl J Med. 2002;347:81-88. doi: 10.1056/NEJMoa013259.

(63) Jackson R.W., Dieterichs C. The results of arthroscopic lavage and debridement of osteoarthritic knees based on the severity of degeneration: A 4- to 6-year symptomatic follow-up. Arthroscopy. 2003;19:13-20. doi: 10.1053/jars.2003.50022.

(64) McNickle A.G., Provencher M.T., Cole B.J. Overview of existing cartilage repair technology. Sports Med Arthrosc. 2008;16:196–201. doi:10.1097/JSA.0B013E31818CDB82.

(65) Harwin S.F. Arthroscopic debridement for osteoarthritis of the knee: Predictors of patient satisfaction. Arthrosc -J Arthrosc Relat Surg 1999;15:142–146. doi:10.1053/AR.1999.V15.015014.

(66) Mankin H.J. The reaction of articular cartilage to injury and osteoarthritis (first of two parts). N Engl J Med. 1974;291:1285–1292. doi:10.1056/NEJM197412122912406.

(67) Mithoefer K, Hambly K, Villa S Della, Silvers H, Mandelbaum BR. Return to sports participation after articular cartilage repair in the knee: scientific evidence. Am J Sports Med. 2009;37:1:167S-176S. doi:10.1177/0363546509351650.

(68) Johnson L.L. Arthroscopic abrasion arthroplasty historical and pathologic perspective: present status. Arthroscopy. 1986;2:54–69. doi:10.1016/S0749-8063(86)80012-3.

(69) Hangody L., Füles P. Autologous osteochondral mosaicplasty for the treatment of full-thickness defects of weight-bearing joints: ten years of experimental and clinical experience. J Bone Joint Surg Am. 2003;2:25–32. doi:10.2106/00004623-200300002-00004.

(70) Bentley G., Biant L.C., Carrington R.W.J., Akmal M., Goldberg A., Williams A.M., Skinner J.A., Pringle J. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. J Bone Joint Surg Br. 2003;85:223–230. doi:10.1302/0301-620X.85B2.13543.

(71) Demange M., Gomoll A.H. The use of osteochondral allografts in the management of cartilage defects. Curr Rev Musculoskelet Med. 2012;5:229-235. doi:10.1007/S12178-012-9132-0.

(72) Lai W.C., Bohlen H.L., Fackler N.P., Wang D. Osteochondral Allografts in Knee Surgery: Narrative Review of Evidence to Date. Orthop Res Rev. 2022;14:263-274. doi:10.2147/ORR.S253761.

(73) Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of Deep Cartilage Defects in the Knee with Autologous Chondrocyte Transplantation. N Engl J Med. 1994;331:889–895. doi:10.1056/NEJM199410063311401.

(74) Kwon H., Brown W.E., Lee C.A., Wang D., Paschos N., Hu J.C., Athanasiou K.A. Surgical and tissue engineering strategies for articular cartilage and meniscus repair. Nat Rev Rheumatol. 2019;15:550-570. doi:10.1038/S41584-019-0255-1.

(75) Thompson C.L., Plant J.C.T., Wann A.K.T., Bishop C.L., Novak P., Mitchison H.M., Beales P.L., Chapple J.P., Knight M.M. Chondrocyte expansion is associated with loss of primary cilia and disrupted hedgehog signalling. Eur Cell Mater. 2017;34:128–141. doi:10.22203/ECM.V034A09.

(76) Dunkin B.S., Lattermann C. New and Emerging Techniques in Cartilage Repair: MACI. Oper Tech Sports Med. 2013;21:100-107. doi:10.1053/J.OTSM.2013.03.003.

(77) Roberts S., McCall I.W., Darby A.J., Menage J., Evans H., Harrison P.E., Richardson J.B. Autologous chondrocyte implantation for cartilage repair: monitoring its success by magnetic resonance imaging and histology. Arthritis Res Ther. 2003;5:R60-73. doi:10.1186/AR613.

(78) Benthien J.P., Behrens P. Autologous Matrix-Induced Chondrogenesis (AMIC): Combining Microfracturing and a Collagen I/III Matrix for Articular Cartilage Resurfacing. Cartilage. 2010;1:65-68. doi:10.1177/1947603509360044.

(79) Hinckel B.B., Thomas D., Vellios E.E., Hancock K.J., Calcei J.G., Sherman S.L., Eliasberg C.D., Fernandes T.L., Farr J., Lattermann C., Gomoll A.H. Algorithm for Treatment of Focal Cartilage Defects of the Knee:Classic and New Procedures. Cartilage. 2021;13:473S-495S. doi:10.1177/1947603521993219.

(80) Marx R.E. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 2001;10:225–228. doi:10.1097/00008505-200110000-00002.

(81) Kingsley C.S. Blood coagulation; evidence of an antagonist to factor VI in platelet-rich human plasma. Nature. 1954;173:723–724. doi:10.1038/173723A0.

(82) Matras H. [Effect of various fibrin preparations on reimplantations in the rat skin]. Osterr Z Stomatol. 1970;67:338-359.

(83) Knighton D.R., Ciresi K.F., Fiegel V.D., Austin L.L., Butler E.L. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg. 1986;204:322–330. doi:10.1097/00000658-198609000-00011.

(84) Marx R.E., Carlson E.R., Eichstaedt R.M., Schimmele S.R., Strauss J.E., Georgeff K.R. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85:638–646. doi:10.1016/S1079-2104(98)90029-4.

(85) Le A.D.K., Enweze L., DeBaun M.R., Dragoo J.L. Current Clinical Recommendations for Use of Platelet-Rich Plasma. Curr Rev Musculoskelet Med. 2018;11:624. doi:10.1007/S12178-018-9527-7.

(86) O'Connell B., Wragg N.M., Wilson S.L. The use of PRP injections in the management of knee osteoarthritis. Cell Tissue Res. 2019;376:143–152. doi:10.1007/S00441-019-02996-X.

(87) Anitua E., Nurden P., Prado R., Nurden A.T., Padilla S. Autologous fibrin scaffolds: When platelet- and plasma-derived biomolecules meet fibrin. Biomaterials. 2019;192:440–460. doi:10.1016/J.BIOMATERIALS.2018.11.029.

(88) Boswell S.G., Cole B.J., Sundman E.A., Karas V., Fortier L.A. Platelet-rich plasma: a milieu of bioactive factors. Arthroscopy. 2012;28:429–439. doi:10.1016/J.ARTHRO.2011.10.018.

(89) Deppermann C., Kubes P. Start a fire, kill the bug: The role of platelets in inflammation andinfection. Innate Immun. 2018;24:335-348. doi:10.1177/1753425918789255.

(90) Pham T.A.V., Tran T.T.P., Luong N.T.M. Antimicrobial Effect of Platelet-Rich Plasma against Porphyromonas gingivalis. Int J Dent. 2019;2019:7329103. doi:10.1155/2019/7329103.

(91) Qian Y., Han Q., Chen W., Song J., Zhao X., Ouyang Y., Yuan W., Fan C. Platelet-Rich Plasma Derived Growth Factors Contribute to Stem Cell Differentiation in Musculoskeletal Regeneration. Front Chem. 2017;5:89. doi:10.3389/FCHEM.2017.00089.

(92) Mazzocca A.D., McCarthy M.B.R., Chowaniec D.M., Dugdale E.M., Hansen D., Cote M.P., Bradley J.P., Romeo A.A., Arciero R.A., Beitzel K. The positive effects of different platelet-rich plasma methods on human muscle, bone, and tendon cells. Am J Sports Med. 2012;40:1742–1749. doi:10.1177/0363546512452713.

(93) Abrams G.D., Frank R.M., Fortier L.A., Cole B.J. Platelet-rich plasma for articular cartilage repair. Sports Med Arthrosc. 2013;21:213–219. doi:10.1097/JSA.0b013e3182999740.

(94) Mariani E., Pulsatelli L. Platelet Concentrates in Musculoskeletal Medicine. Int J Mol Sci. 2020;21:1328. doi:10.3390/IJMS21041328.

(95) Zhao J., Huang H., Liang G., Zeng L.F., Yang W., Liu J. Effects and safety of the combination of platelet-rich plasma (PRP) and hyaluronic acid (HA) in the treatment of knee osteoarthritis: a systematic review and meta-analysis. BMC Musculoskelet Disord. 2020;21:224. doi:10.1186/s12891-020-03262-w.

(96) Cugat R., Samitier G., Vinagre G., Sava M., Alentorn-Geli E., García-Balletbó M., Cuscó X., Seijas R., Barastegui D., Navarro J., Laiz P. Particulated Autologous Chondral–Platelet-Rich Plasma Matrix Implantation (PACI) for Treatment of Full-Thickness Cartilage Osteochondral Defects. Arthrosc Tech. 2021;10:e539-e544. doi:10.1016/J.EATS.2020.10.038.

(97) Anitua E., Sanchez M., Nurden A.T., Zalduendo M., De La Fuente M., Orive G., Azofra J., Andia I. Autologous fibrin matrices: A potential source of biological mediators that modulate tendon cell activities. J Biomed Mater Res - Part A. 2006;77:285–293. doi:10.1002/jbm.a.30585.

(98) Weyrich A.S., Lindeaaann S., Zimmerman G.A. The evolving role of platelets in inflammation. J Thromb Haemost. 2003;1:1897–1905. doi:10.1046/J.1538-7836.2003.00304.X.

(99) Nurden A.T. Platelets, inflammation and tissue regeneration. Thromb Haemost. 2011;105:13–33. doi:10.1160/THS10-11-0720.

(100) Anitua E., Sánchez M., Orive G. Potential of endogenous regenerative technology for in situ regenerative medicine. Adv Drug Deliv Rev. 2010;62:741–752. doi:10.1016/J.ADDR.2010.01.001.

(101) Anitua E., Andia I., Ardanza B., Nurden P., Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004;91:4–15. doi:10.1160/TH03-07-0440/ID/JR0440-11.

(102) Reed G.L. Platelet secretory mechanisms. Semin Thromb Hemost. 2004;30:441–450. doi:10.1055/S-2004-833479.

(103) Kiritsy C.P., Lynch S.E. Role of growth factors in cutaneous wound healing: a review. Crit Rev Oral Biol Med. 1993;4:729–760. doi:10.1177/10454411930040050401.

(104) Hsu C., Chang J. Clinical implications of growth factors in flexor tendon wound healing. J Hand Surg Am. 2004;29:551–563. doi:10.1016/j.jhsa.2004.04.020.

(105) Fioravanti C., Frustaci I., Armellin E., Condò R., Arcuri C., Cerroni L. Autologous blood preparations rich in platelets, fibrin and growth factors. Oral Implantol. 2016;8:96–113. doi:10.11138/ORL/2015.8.4.096.

(106) Li Y., Foster W., Deasy B.M., Chan Y., Prisk V., Tang Y., Cummins J., Huard J. Transforming growth factor-beta1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis. Am J Pathol. 2004;164:1007–1019. doi:10.1016/S0002-9440(10)63188-4.

(107) Shen W., Li Y., Zhu J., Schwendener R., Huard J. Interaction between macrophages, TGF-beta1, and the COX-2 pathway during the inflammatory phase of skeletal muscle healing after injury. J Cell Physiol. 2008;214:405–412. doi:10.1002/JCP.21212.

(108) Taylor M.A., Norman T.L., Clovis N.B., Blaha J.D. The response of rabbit patellar tendons after autologous blood injection. Med Sci Sports Exerc. 2002);34:70–73. doi:10.1097/00005768-200201000-00012.

(109) Gebert W.A., Rose W.J., Gran H.S. Evaluation of the Effects of City of Middleton Stormwater-Management Practices on Streamflow and Water-Quality Characteristics of Pheasant Branch, Dane County, Wisconsin, 1975-2008. USGS science for a chaning world. 2012.

(110) Molloy T., Wang Y., Murrell G.A.C. The roles of growth factors in tendon and ligament healing. Sports Med. 2003);33:381–394. doi:10.2165/00007256-200333050-00004.

(111) Murata S., Ohkohchi N., Matsuo R., Ikeda O., Myronovych A., Hoshi R. Platelets promote liver regeneration in early period after hepatectomy in mice. World J Surg. 2007;31:808–816. doi:10.1007/S00268-006-0772-3.

(112) Anitua E., Andía I., Sanchez M., Azofra J., del Mar Zalduendo M., de la Fuente M., Nurden P., Nurden A.T. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. J Orthop Res. 2005;23:281–286. doi:10.1016/J.ORTHRES.2004.08.015.

(113) Zhang J., Middleton K.K., Fu F.H., Im H.J., Wang J.H.C. HGF mediates the anti-inflammatory effects of PRP on injured tendons. PLoS One. 2013;8:e67303. doi:10.1371/JOURNAL.PONE.0067303.

(114) Bendinelli P., Matteucci E., Dogliotti G., Corsi M.M., Banfi G., Maroni P., Desiderio M.A. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: Mechanisms of NF-κB inhibition via HGF. J Cell Physiol. 2010;225:757–766. doi:10.1002/JCP.22274.

(115) Lind M. Growth factors: possible new clinical tools. A review. Acta Orthop Scand. 1996;67:407–417. doi:10.3109/17453679609002342.

(116) Tsubone T., Moran S.L., Amadio P.C., Zhao C., An K.N. Expression of growth factors in canine flexor tendon after laceration in vivo. Ann Plast Surg. 2004;53:393–397. doi:10.1097/01.SAP.0000125501.72773.01.

(117) Canalis E. Clinical review 35: Growth factors and their potential clinical value. J Clin Endocrinol Metab. 1992;75:1–4. doi:10.1210/JCEM.75.1.1618994.

(118) Grageda E. Platelet-rich plasma and bone graft materials: a review and a standardized research protocol. Implant Dent. 2004;13:301–309. doi:10.1097/01.ID.0000148555.91063.06.

(119) Bennett N.T., Schultz G.S. Growth factors and wound healing: biochemical properties of growth factors and their receptors. Am J Surg. 1993;165:728–737. doi:10.1016/S0002-9610(05)80797-4.

(120) Kurtz C.A., Loebig T.G., Anderson D.D., DeMeo P.J., Campbell PG. Insulin-like growth factor I accelerates functional recovery from Achilles tendon injury in a rat model. Am J Sports Med. 1999;27:363–369. doi:10.1177/03635465990270031701.

(121) Albrecht F.H. Der verschluss gelenkknorpel-defekten mittels knorpelfragmenten und fibrinkleber. Fortschr Med. 1983;101:1650–1652.

(122) Bonasia D.E., Marmotti A., Rosso F., Collo G., Rossi R. Use of chondral fragments for one stage cartilage repair: A systematic review. World J Orthop. 2015;6:1006. doi:10.5312/WJO.V6.I11.1006.

(123) Christensen B.B., Olesen M.L., Hede K.T.C., Bergholt N.L., Foldager C.B., Lind M. Particulated Cartilage for Chondral and Osteochondral Repair: A Review. Cartilage. 2021;13:1047S. doi:10.1177/1947603520904757.

(124) Lu Y., Dhanaraj S., Wang Z., Bradley D.M., Bowman S.M., Cole B.J., Binette F. Minced cartilage without cell culture serves as an effective intraoperative cell source for cartilage repair. J Orthop Res. 2006;24:1261–1270. doi:10.1002/JOR.20135.

(125) Frisbie D.D., Lu Y., Kawcak C.E., Dicarlo E.F., Binette F., Mcilwraith C.W. In vivo evaluation of autologous cartilage fragment-loaded scaffolds implanted into equine articular defects and compared with autologous chondrocyte implantation. Am J Sports Med. 2009;37:71S-80S. doi:10.1177/0363546509348478.

(126) Marmotti A., Bruzzone M., Bonasia D.E., Castoldi F., Von Degerfeld M.M., Bignardi C., Mattia S., Maiello A., Rossi R., Peretti G.M. Autologous cartilage fragments in a composite scaffold for one stage osteochondral repair in a goat model. Eur Cell Mater. 2013;26:15–32. doi:10.22203/ECM.V026A02.

(127) Zhang C., Zhao X., Ao Y., Cao J., Yang L., Duan X. Proliferation ability of particulated juvenile allograft cartilage. J Orthop Surg Res. 2021;16: doi:10.1186/S13018-020-02199-Z.

(128) Grawe B., Burge A., Nguyen J., Strickland S., Warren R., Rodeo S., Shubin Stein B. Cartilage Regeneration in Full-Thickness Patellar Chondral Defects Treated with Particulated Juvenile Articular Allograft Cartilage: An MRI Analysis. Cartilage. 2017;8:374. doi:10.1177/1947603517710308.

(129) Saltzman B.M., Lin J., Lee S. Particulated Juvenile Articular Cartilage Allograft Transplantation for Osteochondral Talar Lesions. Cartilage. 2017;8:61. doi:10.1177/1947603516671358.

(130) Farr J., Yao J.Q. Chondral Defect Repair with Particulated Juvenile Cartilage Allograft. Cartilage. 2011;2:346. doi:10.1177/1947603511405838.

(131) Riboh J.C., Cole B.J., Farr J. Particulated articular cartilage for symptomatic chondral defects of the knee. Curr Rev Musculoskelet Med. 2015;8:429-435. doi:10.1007/S12178-015-9300-0

(132) Delman C., Wuellner J., Kreulen C., Lundeen G., Giza E. Particulated Autograft Cartilage Implantation for the Treatment of Osteochondral Lesions of the Talus: A Novel Technique. Foot Ankle Spec. 2018;11:365–371. doi:10.1177/1938640018770277.

(133) Goodfriend B., Essilfie A.A., Jones I.A., Thomas Vangsness C. Fresh osteochondral grafting in the United States: the current status of tissue banking processing. Cell Tissue Bank. 2019;20:331–337. doi:10.1007/S10561-019-09768-5.

(134) Wu K., Yong K.W., Ead M., Sommerfeldt M., Skene-Arnold T.D., Westover L., Duke K., Laouar L., Elliott J.A.W., Jomha N.M. Vitrified Particulated Articular Cartilage for Joint Resurfacing: ASwine Model. Am J Sports Med. 2022;50:3671. doi:10.1177/03635465221123045.

(135) Levinson C., Cavalli E., Sindi D.M., Kessel B., Zenobi-Wong M., Preiss S., Salzmann G., Neidenbach P. Chondrocytes From Device-Minced Articular Cartilage Show Potent Outgrowth Into Fibrin and

Collagen Hydrogels. Orthop J Sport Med. 2019;7:2325967119867618. doi:10.1177/2325967119867618.

(136) Hunziker E.B. The Elusive Path to Cartilage Regeneration. Adv Mater. 2009;21:3419-3424. doi:10.1002/adma.200801957.

(137) Madry H., van Dijk C.N., Mueller-Gerbl M. The basic science of the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18:419–433. doi:10.1007/S00167-010-1054-Z.

(138) Ahern B.J., Parvizi J., Boston R., Schaer T.P. Preclinical animal models in single site cartilage defect testing: a systematic review. Osteoarthr Cartil. 2009;17:705–713. doi:10.1016/J.JOCA.2008.11.008.

(139) Chu C.R., Szczodry M., Bruno S. Animal Models for Cartilage Regeneration and Repair. Tissue Eng Part B Rev. 2010;16:105–115. doi:10.1089/TEN.TEB.2009.0452.

(140) Proffen B.L., McElfresh M., Fleming B.C., Murray M.M. A comparative anatomical study of the human knee and six animal species. Knee. 2012;19:493–499. doi:10.1016/J.KNEE.2011.07.005.

(141) Madry H., Ochi M., Cucchiarini M., Pape D., Seil R. Large animal models in experimental knee sports surgery: focus on clinical translation. J Exp Orthop. 2015;2:1–12. doi:10.1186/S40634-015-0025-1.

(142) Mora G., Alvarez E., Ripalda P., Forriol F. Articular cartilage degeneration after frozen meniscus and Achilles tendon allograft transplantation: Experimental study in sheep. Arthrosc - J Arthrosc Relat Surg. 2003;19:833–841. doi:10.1016/S0749-8063(03)00731-X.

(143) Hoemann C.D., Sun J., Chrzanowski V., Buschmann M.D. A multivalent assay to detect glycosaminoglycan, protein, collagen, RNA, and DNA content in milligram samples of cartilage or hydrogel-based repair cartilage. Anal Biochem. 2002;300:1–10. doi:10.1006/abio.2001.5436.

(144) Shelburne K.B., Torry M.R., Pandy M.G. Contributions of muscles, ligaments, and the ground-reaction force to tibiofemoral joint loading during normal gait. J Orthop Res. 2006;24:1983–1990. doi:10.1002/JOR.20255.

(145) Taylor W.R., Poepplau B.M., König C., Ehrig R.M., Zachow S., Duda G.N., Heller M.O. The medial-lateral force distribution in the ovine stifle joint during walking. J Orthop Res. 2011;29:567–571. doi:10.1002/JOR.21254.

(146) Nesbitt R.J., Herfat S.T., Boguszewski D.V., Engel A.J., Galloway M.T., Shearn J.T. Primary and Secondary Restraints of Human and Ovine Knees for Simulated In Vivo Gait Kinematics. J Biomech. 2014;47:2022. doi:10.1016/J.JBIOMECH.2013.11.029.

(147) Brzezinski A., Ghodbane S.A., Patel J.M., Perry B.A., Gatt C.J., Dunn M.G. The Ovine Model for Meniscus Tissue Engineering: Considerations of Anatomy, Function, Implantation, and Evaluation. Tissue Eng Part C Methods. 2017;23:829. doi:10.1089/TEN.TEC.2017.0192.

(148) Lo Monaco M., Merckx G., Ratajczak J., Gervois P., Hilkens P., Clegg P., Bronckaers A., Vandeweerd J.M., Lambrichts I. Stem Cells for Cartilage Repair: Preclinical Studies and Insights in Translational Animal Models and Outcome Measures. Stem Cells Int. 2018;2018:9079538. doi:10.1155/2018/9079538.

(149) Lu Y., Markel M.D., Swain C., Kaplan L.D. Development of partial thickness articular cartilage injury in an ovine model. J Orthop Res. 2006;24:1974–1982. doi:10.1002/JOR.20249.

(150) Musumeci. G., Castrogiovanni P., Mazzone V., Szychlinska M.A., Castorina S., Loreto C. Histochemistry as a Unique Approach for Investigating Normal and Osteoarthritic Cartilage. Eur J Histochem. 2014;25:2371. doi: 10.4081/ejh.2014.2371.

(151) Musumeci G., Loreto C., Leonardi R., Castorina S., Giunta S., Carnazza M.L., Trovato F.M., Pinchler K., Weinberg A.M. The effects of physical activity on apoptosis and lubricin expression in articular cartilage in rats with glucocorticoid-induced osteoporosis. J Bone Miner Metab. 2013;31:274-284. doi: 10.1007/s00774-012-0414-9.

(152) Teti G., Salvatore V., Ruggeri A., Manzoli L., Orsini G., Falconi. In vitro reparative dentin: a biochemical and morphological study. Eur J Histochem. 2013;57:e23. doi: 0.4081/ejh.2013.e23.

(153) Pinchler K., Loreto C., Leonardi R., Reuber T., Weinberg A. M., Musumeci G. RANKL is downregulated in bone cells by physical activity (treadmill and vibration stimulation training) in rat with glucocorticoid-induced osteoporosis. Histol Histopathol. 2013;28:1185-1196. doi: 10.14670/HH-28.1185.

(154) Loreto C., Musumeci G., Castorina A., Loreto C., Martinez G. Degenerative disc disease of herniated intervertebral discs is associated with extracellular matrix remodeling, vimentin-positive cells and cell death. Ann Anat. 2011;193:156-162. doi: 10.1016/j.aanat.2010.12.001.

(155) Alcaide-Ruggiero L., Cugat R., Domínguez J.M. Proteoglycans in Articular Cartilage and Their Contribution to Chondral Injury and Repair Mechanisms. Int J Mol Sci. 2023;24:10824. doi: 10.3390/ijms241310824.

(156) Alcaide-Ruggiero L., Molina-Hernández V., Morgaz J., Fernández-Sarmiento J.J., Granados M.M., Navarrete-Calvo R., Pérez J., Quirós-Carmona S., Carrillo J.M., Cugat R., Domínguez J.M. Particulate cartilage and platelet-rich plasma treatment for knee chondral defects in sheep. Knee Surg Sports Traumatol Arthrosc. 2023;31:2944-2955. doi: 10.1007/s00167-022-07295-7.

(157) Bourgot I., Primac I., Louis T., Noël A., Maquoi E. Reciprocal interplay between fibrillar collagens and collagen-binding integrins: Implications in cancer progression and metastasis. Front. Oncol. 2020;10:1488. doi: 10.3389/fonc.2020.01488.

(158) Parsons P., Gilbert S.J., Vaughan-Thomas A., Sorrell D.A., Notman R., Bishop M., Haynes A.J., Mason D.J., Duance V.C. Type IX collagen interacts with fibronectin providing an important molecular bridge in articular cartilage. J. Biol. Chem. 2011;286:34986–34997. doi: 10.1074/jbc.M111.238188.

(159) He Y., Siebuhr A.S., Brandt-Hansen N.U., Wang J., Su D., Zheng Q., Simonsen O., Petersen K.K., Arendt-Nielsen L., Eskehave T., et al. Type X collagen levels are elevated in serum from human osteoarthritis patients and associated with biomarkers of cartilage degradation and inflammation. BMC Musculoskelet. Disord. 2014;15:309. doi: 10.1186/1471-2474-15-309.