

General

Molecular Aspects of Cartilage Microfracturation: Rehabilitation Insights.

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Cartilage microfracturation is a surgical technique specifically designed to address chondral defects, which are injuries to the cartilage that covers the ends of bones in joints. These defects can result from traumatic injuries, degenerative conditions such as osteoarthritis, or congenital abnormalities. The primary objective of microfracture surgery is to promote the regeneration of functional cartilage tissue, thereby restoring joint function, alleviating pain, and enhancing mobility. The procedure involves creating small, controlled perforations, or microfractures, in the subchondral bone plate beneath the damaged cartilage. This process, performed with precision to minimize damage to surrounding healthy tissue, penetrates the subchondral bone to reach the bone marrow, which is rich in mesenchymal stem cells (MSCs).

MSCs are multipotent cells capable of differentiating into various cell types, including chondrocytes, which are essential for cartilage production. The microfractures provide an access pathway for MSCs, allowing them to migrate from the bone marrow to the defect site. Once at the site, these stem cells differentiate into chondrocytes and initiate the formation of new cartilage tissue. This newly formed tissue helps fill the defect, aiming to restore the smooth surface of the cartilage and improve the joint's structural integrity.

Understanding the molecular processes involved in cartilage repair is crucial for optimizing rehabilitation strategies and improving clinical outcomes. These processes include the regulation of various signaling pathways that control stem cell migration, differentiation, and extracellular matrix (ECM) synthesis. Growth factors such as transforming growth factor-beta (TGF- β), bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs) play pivotal roles in these pathways, guiding MSCs in their transformation into functional cartilage cells. Additionally, the role of the ECM, composed primarily of collagen type II and proteoglycans like aggrecan, is paramount in providing structural support and biochemical signals. The ECM supports the newly differentiated chondrocytes and helps maintain the cartilage's mechanical properties. Matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), are crucial in ECM remodeling, ensuring the balance between synthesis and degradation of the matrix to support tissue repair and integrity.

Angiogenesis and vascularization are also critical aspects of the molecular repair process. The initial phase of

cartilage repair often includes transient vascularization facilitated by vascular endothelial growth factor (VEGF), ensuring that the newly formed tissue receives adequate nutrients and oxygen. However, as the repair tissue matures, it transitions back to an avascular state characteristic of healthy articular cartilage, which is essential for maintaining its mechanical properties and longevity.

Rehabilitation strategies following microfracture surgery are meticulously crafted to facilitate recovery and functional restoration. These strategies include early mobilization techniques, controlled mechanical loading, biological augmentation, nutritional support, and advanced therapeutic modalities. Early mobilization with gentle passive range-of-motion exercises promotes synovial fluid circulation within the joint, preventing adhesions and joint stiffness, and delivering essential nutrients and growth factors. Controlled mechanical loading through gradual weight-bearing activities helps condition the repaired tissue, stimulating chondrocyte proliferation and ECM production while ensuring proper integration and function.

Biological augmentation, including platelet-rich plasma (PRP) and hyaluronic acid (HA), supports cartilage repair by enhancing cellular activities and ECM formation. PRP, rich in growth factors, promotes cell proliferation and matrix synthesis, while HA injections improve joint lubrication, reduce pain, and provide a scaffold for new tissue growth. Nutritional support, including amino acids, vitamins, minerals, glucosamine, chondroitin sulfate, and antioxidants, plays a vital role in collagen synthesis and overall cartilage maintenance, protecting chondrocytes from oxidative stress and modulating inflammation.

Advanced therapeutic modalities such as low-intensity pulsed ultrasound (LIPUS), pulsed electromagnetic fields (PEMF), photobiomodulation therapy, and cryotherapy offer additional benefits by enhancing chondrocyte activity, reducing inflammation, and promoting ECM production. These modalities work through various molecular mechanisms, such as activating signaling pathways and modulating gene expression, to improve the quality and durability of the repaired cartilage.

By comprehensively understanding the molecular and practical aspects of cartilage repair, clinicians can develop more effective treatment plans that optimize cartilage healing and improve patient outcomes. The integration of molecular insights with advanced rehabilitation techniques holds the promise of revolutionizing cartilage repair, offering hope for those suffering from debilitating joint conditions. This review aims to bridge the gap between molecular biology and clinical practice, providing a roadmap for optimizing cartilage repair strategies and ensuring long-term success for patients.

INTRODUCTION

Cartilage microfracturation is a surgical intervention specifically designed to address chondral defects, which are damage to the cartilage that covers the ends of bones in joints. These defects can result from traumatic injuries, degenerative conditions such as osteoarthritis, or congenital abnormalities. The primary goal of microfracture surgery is to promote the regeneration of functional cartilage tissue, thereby restoring joint function, alleviating pain, and enhancing mobility.¹

The technique involves creating small, controlled perforations, or microfractures, in the subchondral bone plate, which lies just beneath the damaged cartilage. This process is meticulously performed using specialized surgical tools to ensure precision and minimize additional damage to the surrounding healthy tissue. The perforations penetrate the subchondral bone, reaching the bone marrow, which is rich in mesenchymal stem cells (MSCs).²

These MSCs are multipotent cells capable of differentiating into various cell types, including chondrocytes, which are the cells responsible for producing cartilage. The microfractures create an access pathway for the MSCs, allowing them to migrate from the bone marrow to the defect site. Once at the site, these stem cells undergo a process of differentiation into chondrocytes, initiating the formation of new cartilage tissue. This newly formed tissue helps to fill the defect, aiming to restore the smooth surface of the cartilage and improve the joint's structural integrity.³

Understanding the molecular processes involved in cartilage repair is crucial for optimizing rehabilitation strategies and improving clinical outcomes. These processes include the regulation of various signaling pathways that control stem cell migration, differentiation, and extracellular matrix synthesis. Growth factors such as transforming growth factor-beta (TGF- β), bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs) play pivotal

roles in these pathways, guiding the MSCs in their transformation into functional cartilage cells ([Figure 1](#)).

In addition to growth factors, the role of the extracellular matrix (ECM) in providing structural support and biochemical signals is paramount. The ECM, composed primarily of collagen type II and proteoglycans like aggrecan, offers a scaffold that supports the newly differentiated chondrocytes and helps maintain the cartilage's mechanical properties. Matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), are crucial in ECM remodeling, ensuring that the balance between synthesis and degradation of the matrix is maintained to support tissue repair and integrity.⁴

Another critical aspect of the molecular repair process involves angiogenesis and vascularization. The initial phase of cartilage repair often includes transient vascularization facilitated by vascular endothelial growth factor (VEGF), which ensures that the newly formed tissue receives adequate nutrients and oxygen. However, as the repair tissue matures, it transitions back to an avascular state, characteristic of healthy articular cartilage, which is essential for maintaining its mechanical properties and longevity.⁵

This review delves into these complex molecular mechanisms, providing a detailed exploration of the biological underpinnings of cartilage repair following microfracture surgery. Additionally, it offers insights into advanced rehabilitation protocols designed to enhance the healing process. Effective rehabilitation strategies are essential for maximizing the benefits of the microfracture procedure, ensuring that the newly formed cartilage integrates well with the surrounding tissue and can withstand the mechanical stresses of daily activities. Rehabilitation programs typically include controlled mechanical loading, passive motion exercises, and gradual weight-bearing activities to stimulate chondrocyte proliferation and extracellular matrix production.

Furthermore, the use of biological augmentation, such as platelet-rich plasma (PRP) and hyaluronic acid (HA), can significantly enhance the repair process by providing additional growth factors and creating a favorable environment for cell migration and differentiation. Nutritional support, including supplements like glucosamine, chondroitin sulfate, and antioxidants, also plays a vital role in promoting cartilage health and repair.⁶

By comprehensively understanding both the molecular and practical aspects of cartilage repair, clinicians can develop more effective treatment plans, ultimately improving patient outcomes and quality of life following microfracture surgery. The integration of molecular insights with advanced rehabilitation techniques holds the promise of revolutionizing cartilage repair, offering hope for those suffering from debilitating joint conditions. This review aims to bridge the gap between molecular biology and clinical practice, providing a roadmap for optimizing cartilage repair strategies and ensuring long-term success for patients.⁷

([Figure 1](#). Schematic of normal articular cartilage structure consisting of four zones: the superficial zone, the middle (transitional) zone, the deep zone, and the calcified

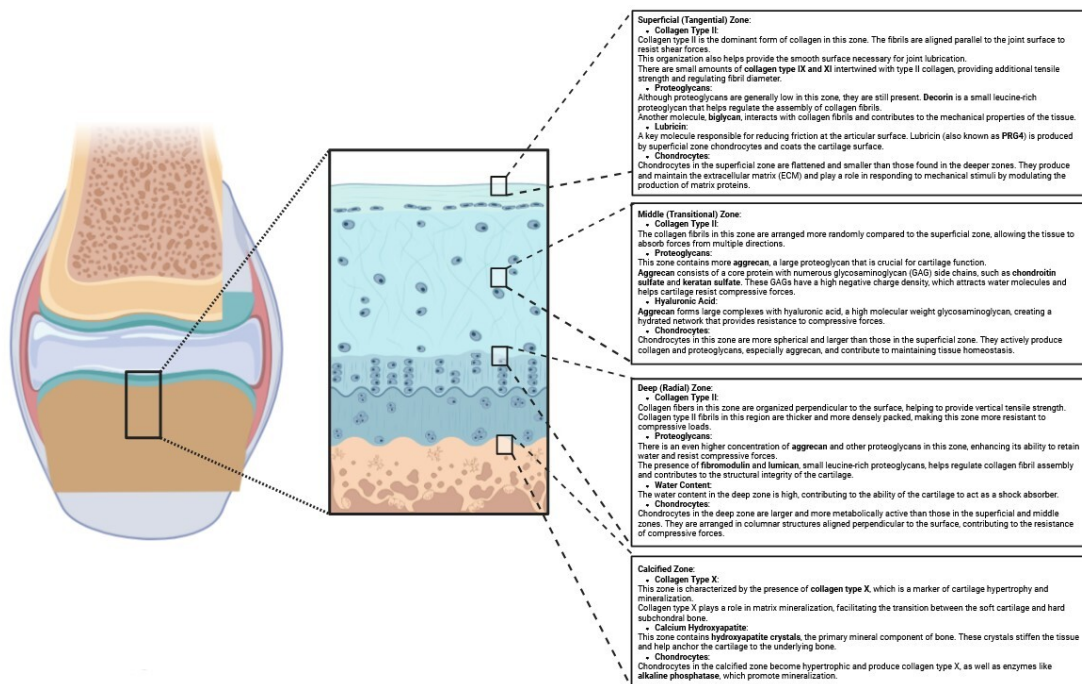


Figure 1. Schematic of normal articular cartilage structure consisting of four zones: the superficial zone, the middle (transitional) zone, the deep zone, and the calcified cartilage layer. Source: Schematic from Wei W, Dai H. Articular cartilage and osteochondral tissue engineering techniques: recent advances and challenges. *Bioactive Mater.* Dec 2021;6(12): 48304855.

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MOLECULAR MECHANISMS OF CARTILAGE REPAIR

The molecular mechanisms underlying cartilage repair hinge on a sophisticated network of cellular signals, transcriptional regulators, and extracellular matrix (ECM) remodeling events that collectively enable the restoration of damaged cartilage tissue.⁸ Although cartilage is often viewed as a relatively quiescent tissue with limited regenerative capacity, a deeper look into its molecular underpinnings reveals a dynamic process that can be harnessed to improve clinical interventions, such as microfracture surgery (Table 1). This expanded overview highlights additional molecular pathways and regulatory checkpoints that govern every stage of cartilage repair.

A key event in cartilage regeneration is the mobilization of mesenchymal stem cells (MSCs) from the bone marrow. Chemotactic gradients—often established by cytokines, chemokines, and damage-associated molecular patterns—guide MSCs to the injury site, where they differentiate into chondrocytes. Several growth factors drive this differentiation process. Transforming growth factor-beta (TGF- β) activates SMAD2/3, which work in concert with the transcription factor SOX9 to upregulate chondrogenic genes, including COL2A1 (collagen type II) and ACAN (ag-

grecan). Bone morphogenetic proteins (BMPs) engage SMAD1/5/8, promoting further expression of collagen type II and proteoglycans. TGF- β and BMPs can also synergize with other pathways—for example, members of the Wnt/ β -catenin and Notch families—ensuring that MSCs commit fully to a chondrogenic fate rather than differentiating along osteogenic or adipogenic lineages. Meanwhile, insulin-like growth factor-1 (IGF-1) supports anabolic metabolism by binding to its receptor (IGF-1R) and activating downstream AKT and ERK signaling cascades, which collectively stimulate matrix protein synthesis.⁹

Once MSCs adopt a chondrogenic identity, they synthesize a specialized ECM primarily composed of collagen type II fibrils woven into a dense network with proteoglycans like aggrecan. This matrix arrangement confers tensile strength and compressive stiffness, properties essential for normal joint function. At the molecular level, the assembly, turnover, and maintenance of the ECM depend on a delicate balance between anabolic and catabolic processes.¹⁰ Anabolic processes involve the synthesis of collagen, proteoglycans, and cartilage-linking proteins, driven by the concerted action of growth factors, transcription factors, and epigenetic regulators. Catabolic enzymes, including a family of matrix metalloproteinases (MMPs)—notably MMP-13—and aggrecanases (ADAMTS-4 and ADAMTS-5), degrade components of the ECM. This degradation is not purely destructive; by removing damaged matrix, these enzymes create space for new collagen and proteoglycan deposition. However, cartilage homeostasis requires tight control of enzyme activity through tissue inhibitors of metalloproteinases (TIMPs). Dysregulation, for instance via ex-

cessive MMP production, can shift this equilibrium and accelerate cartilage breakdown, underscoring the importance of molecular checkpoints that prevent excessive tissue degradation.

Mechanical signals represent another vital input that shapes chondrocyte behavior and ECM metabolism.¹¹ Chondrocytes sense and respond to loading through mechanoreceptors, such as integrins, which connect the ECM to the cytoskeleton at focal adhesion complexes. The resulting activation of focal adhesion kinase (FAK) can trigger multiple downstream pathways, including the ERK1/2 and p38 MAPK cascades. These cascades promote chondrocyte proliferation, matrix synthesis, and cytoskeletal reorganization. Additional mechanosensory elements, such as ion channels (e.g., TRPV4) and primary cilia, also modulate the response to mechanical stress, reinforcing the notion that healthy mechanical loading can accelerate tissue repair. Mechanical signals further stimulate anabolic cytokines like BMP-2 and BMP-7, amplifying collagen and proteoglycan production. Thus, well-designed rehabilitation protocols that optimize mechanical loading on joint surfaces represent a critical facet of successful cartilage repair (Figure 2).

Inflammation provides a nuanced layer of control over cartilage healing.¹² In the acute phase of injury, pro-inflammatory mediators—including interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- α)—help marshal the immune response and assist in the recruitment of MSCs. However, when inflammation persists, it can become detrimental: IL-1 and TNF- α can upregulate MMPs and aggrecanases, provoking excessive ECM breakdown and inhibiting anabolic signaling. The NF- κ B pathway is central in propagating these catabolic signals, as it amplifies the transcription of various inflammatory mediators. Consequently, therapeutic strategies that mitigate chronic inflammation—be it through anti-inflammatory drugs, biologics targeting IL-1 or TNF- α , or even nutritional and exercise-based interventions—can shift the microenvironment back toward one that favors cartilage repair rather than destruction.

In parallel, the subchondral bone is now recognized as a critical player in promoting or hindering cartilage regeneration. The bone beneath articular cartilage not only provides mechanical support but also secretes factors that diffuse upward, influencing chondrocyte metabolism. Abnormalities in subchondral bone architecture, whether due to microdamage, sclerosis, or altered vascularization, can contribute to persistent inflammation, destabilizing the cartilage above. As a result, treatments that target the bone compartment—such as microfracture techniques, subchondral bone grafting, or advanced biomaterials—are being explored to restore a favorable niche for newly forming cartilage.

Cutting-edge approaches in molecular biology and bioengineering offer additional avenues to bolster cartilage repair. Gene therapy seeks to increase the local availability of pro-chondrogenic molecules (e.g., TGF- β , IGF-1) by delivering expression constructs directly to the joint or to chondrocytes in vitro. Some strategies also aim to knock

down catabolic mediators, leveraging RNA interference or genome editing technologies like CRISPR/Cas9.¹³ Stem cell therapy, similarly, brings a replenishable source of chondrogenic cells to the defect. By genetically modifying these stem cells to overexpress desired growth factors (or silence catabolic factors), researchers can push the local environment toward sustained regeneration. Other molecular techniques, such as microRNA-based therapies, could further fine-tune chondrocyte gene expression and bolster cartilage resilience.

In conclusion, cartilage repair reflects a highly orchestrated interplay among stem cell recruitment and differentiation, growth factor-mediated signaling pathways, finely tuned ECM remodeling, mechanotransduction, and inflammation regulation.¹⁴ By delving deeper into these molecular complexities, scientists and clinicians alike are developing targeted therapies—from gene-based interventions to sophisticated scaffolds and rehabilitation protocols—that foster sustained tissue repair. Ongoing research continues to refine our understanding of these intricate processes, increasing the likelihood of restoring functional cartilage and improving quality of life for individuals affected by cartilage injuries and degenerative joint diseases.

1. STEM CELL RECRUITMENT AND DIFFERENTIATION

The recruitment and differentiation of mesenchymal stem cells (MSCs) following microfracture surgery rely on an increasingly appreciated network of molecular and biomechanical signals that collectively drive MSCs toward the chondrocyte lineage. When the subchondral bone is perforated, damage-associated molecular patterns (DAMPs) and other marrow-derived cues establish localized chemotactic gradients that steer MSCs into the defect region.¹⁵ Upon arrival, MSCs encounter elevated concentrations of growth factors such as transforming growth factor-beta (TGF- β), bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs), which bind to their respective receptors and initiate a series of intracellular cascades that orchestrate chondrogenesis at the transcriptional and epigenetic levels.

TGF- β interacts with type I (TGF- β RI, often referred to as ALK5) and type II (TGF- β RII) receptors, promoting the phosphorylation of SMAD2 and SMAD3. These SMADs then heterodimerize with SMAD4 and translocate to the nucleus, where they bind to SMAD-binding elements in genes critical for cartilage matrix production, such as COL2A1 (collagen type II) and ACAN (aggrecan).¹⁶ This canonical TGF- β /SMAD pathway not only drives chondrogenic gene expression but also prevents chondrocyte hypertrophy by down-regulating hypertrophic markers like COL10A1. TGF- β can further signal through non-canonical pathways—such as TAK1 (TGF- β -activated kinase 1), p38 MAPK, or Rho-like GTPases—amplifying or modulating chondrogenic outcomes, especially under varying mechanical stresses. Meanwhile, BMPs, particularly BMP-2 and BMP-7, bind to BMP receptor type I (often ALK3 or ALK6) and type II (BMPRII) complexes, inducing the phosphorylation of SMAD1/

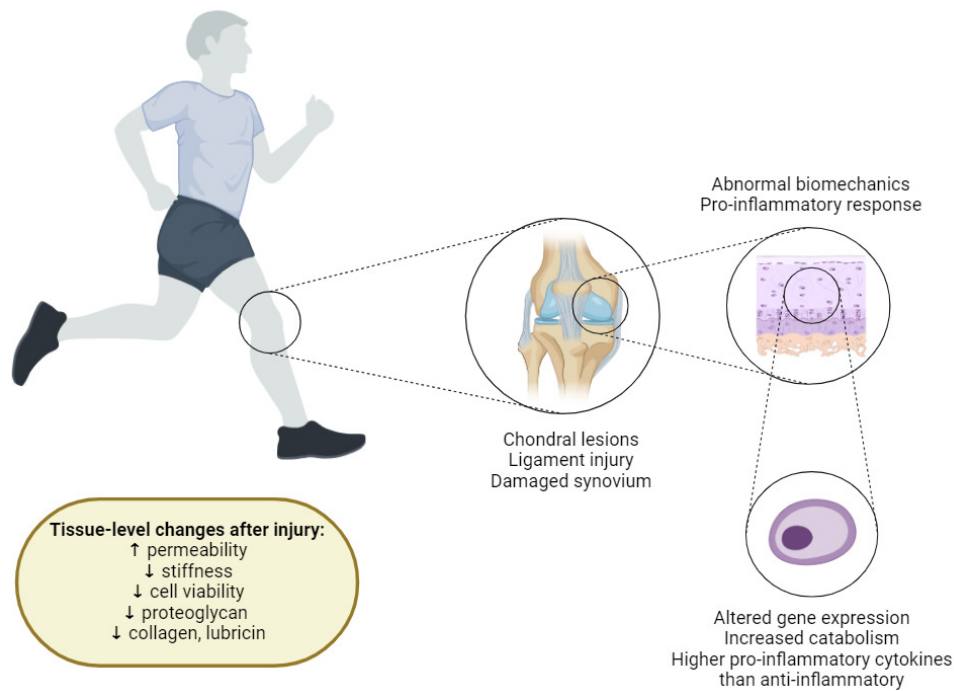


Figure 2. Overview of cartilage degradation mechanisms triggered by a joint injury. Joint injuries can result in lesions on articular cartilage surfaces, ligament tears, and synovium damage. These injuries collectively create a catabolic environment within the joint, characterized by abnormal biomechanical loading patterns and the diffusion of pro-inflammatory cytokines into the cartilage. Abnormal biomechanical loading can lead to locally elevated mechanical strains or stresses, which are suggested to cause cell death, collagen network damage, and proteoglycan (PG) loss. This mechanical stress can also result in the release of reactive oxygen species (ROS) and cell death through necrosis (acute) and apoptosis (persistent abnormal loading). The presence of pro-inflammatory cytokines further exacerbates cartilage degradation by upregulating catabolic and downregulating anabolic gene expression in chondrocytes. As a result, injured cartilage shows a loss of PG and collagen content, decreased cell viability, reduced stiffness, and increased permeability compared to healthy cartilage. These changes significantly impair the structural and functional integrity of the cartilage, leading to progressive joint degeneration.

5/8. These phosphorylated SMADs form transcriptionally active complexes with SMAD4 that synergize with TGF- β –SMAD2/3 signaling, further boosting the production of type II collagen, proteoglycans, and ancillary ECM molecules vital for cartilage's structural integrity.¹⁷ Because unchecked BMP signaling can lead to excessive tissue maturation or osteogenesis, inhibitory SMADs (SMAD6 and SMAD7) and soluble BMP antagonists (noggin, chordin, follistatin) form critical feedback loops that help maintain the equilibrium required for robust yet controlled chondrogenesis.

FGFs, especially FGF-18, act through tyrosine kinase receptors (FGFRs) to initiate downstream RAS–RAF–MEK–ERK signaling, thereby enhancing MSC proliferation and early chondrocyte expansion.¹⁸ FGF-18 has been particularly associated with increased cartilage matrix deposition and cell survival, as it elevates ERK-dependent transcription of genes that underlie proteoglycan and collagen synthesis. Crosstalk among these pathways adds further complexity—FGF- and BMP-mediated signals can converge on shared targets or influence SMAD activity, while TGF- β can modulate FGFR expression, collectively

fine-tuning the balance between proliferation, differentiation, and matrix assembly.¹⁹

Beyond soluble growth factors, local mechanical and biochemical properties contribute to MSC fate decisions. The stiffness and viscoelasticity of the cartilage defect region can modulate integrin clustering and focal adhesion assembly, which in turn affect the activity of focal adhesion kinase (FAK) and downstream Rho GTPases, including RhoA and Rac1.²⁰ These molecules orchestrate cytoskeletal reorganization, nuclear positioning, and gene expression patterns that reinforce chondrogenic differentiation under appropriate mechanical loads. Simultaneously, the cartilage's characteristic hypoxic microenvironment promotes the stabilization of hypoxia-inducible factors (HIF-1 α and HIF-2 α), driving transcriptional programs that support collagen type II and proteoglycan synthesis and inhibiting angiogenesis, thus preserving the low-oxygen milieu optimal for chondrocyte function.²¹ ECM constituents—particularly collagen type II, chondroadherin, and proteoglycans—reinforce this hypoxic niche by creating a dense matrix that limits oxygen diffusion and shields maturing chondrocytes from vascular invasion.

Integrin-mediated cell–ECM interactions refine this process even further. Once integrins (e.g., $\alpha 5 \beta 1$, $\alpha v \beta 3$) engage ECM ligands, intracellular adaptor proteins such as paxillin, talin, and vinculin assemble at focal adhesions, linking extracellular mechanical and biochemical cues to signaling networks like ERK, p38 MAPK, and Akt. These signaling axes not only govern chondrocyte proliferation and ECM secretion but also modulate epigenetic regulators, including histone acetyltransferases (e.g., p300/CBP) and microRNAs (e.g., miR-140), which stabilize the chondrocyte phenotype by suppressing catabolic genes (e.g., MMP13) and reinforcing anabolic gene expression.

2. EXTRACELLULAR MATRIX (ECM) SYNTHESIS AND REMODELING

The extracellular matrix (ECM) of articular cartilage undergoes continuous turnover and remodeling to preserve the tissue's structural and functional integrity, and this dynamic process is even more critical following microfracture surgery. Newly differentiated chondrocytes derived from mesenchymal stem cells (MSCs) ramp up their synthesis of core ECM constituents, predominantly collagen type II and large proteoglycans such as aggrecan, to reconstitute a matrix capable of withstanding mechanical loads in the joint.²² Collagen type II molecules assemble into fibrils with a characteristic triple-helical structure, forming a robust scaffold that confers tensile strength, while aggrecan and its attached glycosaminoglycan (GAG) side chains imbue the tissue with compressive resilience by trapping water and creating a hydrated gel-like environment.

ECM remodeling is regulated by a tightly controlled interplay between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs).^{23,24} MMP-13, for example, selectively cleaves type II collagen helices at specific sites, facilitating the removal of damaged matrix or allowing for local reorganization. Aggrecanases (e.g., ADAMTS-4 and ADAMTS-5) degrade the proteoglycan components, ensuring that damaged or excess matrix can be cleared when necessary. However, excessive enzymatic activity leads to an over-degradation of newly synthesized matrix, weakening the repair tissue. TIMPs, by binding MMPs and limiting their proteolytic function, maintain a balance that enables appropriate matrix turnover while preventing destructive breakdown. This MMP–TIMP equilibrium is sensitive to a spectrum of signals, including cytokines, mechanical forces, and local growth factors, which can tilt ECM homeostasis toward either anabolism (matrix buildup) or catabolism (matrix breakdown).

Beyond collagen and aggrecan, small leucine-rich proteoglycans (SLRPs) such as decorin, biglycan, and fibromodulin play critical roles in modulating the architecture and organization of the collagen network.^{25,26} These molecules bind collagen fibrils at specific intervals, orchestrating proper fibril spacing and diameter. Their presence helps stabilize and strengthen the collagen network, preserving the biomechanical properties of the tissue. Chondrocytes, by regulating SLRP synthesis, further refine ECM morphol-

ogy in response to local requirements for tensile strength, compressive resistance, or matrix repair.

Mechanistically, integrins on the chondrocyte surface—particularly $\alpha 5 \beta 1$, $\alpha v \beta 3$, and $\alpha 1 \beta 1$ —mediate adhesion to ECM components such as fibronectin, collagen, and proteoglycans, triggering intracellular signaling cascades that govern cell shape, cytoskeletal arrangement, and ultimately gene expression profiles critical for ECM maintenance.²⁷ These integrin-dependent signals often converge on focal adhesion kinase (FAK), which couples to pathways involving Rho GTPases, ERK1/2, and p38 MAPK. Through this network, chondrocytes sense matrix density, composition, and stiffness, adjusting their synthetic activity and proteolytic enzyme release accordingly. Integrin–FAK signaling thus fine-tunes the rate of ECM synthesis, ensures proper fibril assembly, and coordinates ECM breakdown when needed for remodeling.

Cytokines, notably interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α), are potent regulators of ECM turnover and can either support or undermine the repair process depending on their concentration and timing.²⁸ High levels of these pro-inflammatory cytokines activate the NF- κ B pathway, driving up the expression of MMPs and aggrecanases and shifting the balance away from cartilage preservation. If not tightly regulated, this catabolic shift accelerates matrix breakdown, disrupts collagen architecture, and hinders new matrix deposition. In contrast, controlled, low-grade inflammation can promote beneficial remodeling by clearing damaged tissue and facilitating the integration of newly formed cartilage.

Mechanical stimuli also exert a profound impact on ECM homeostasis, driving a process known as mechanotransduction.²⁹ When the repaired cartilage bears weight or experiences shear forces, integrins and mechanosensitive ion channels (e.g., TRPV4) transmit physical cues to the cytoskeleton, triggering activation of MAPK/ERK pathways that boost anabolic activity and ECM synthesis. Moderate, well-distributed mechanical loading—such as that achieved through tailored rehabilitation exercises—reinforces the chondrocyte phenotype and promotes collagen type II and aggrecan production, improving the overall resilience of the repaired tissue.³⁰ Conversely, excessive or uneven mechanical stress can induce a surge in MMP and aggrecanase activity, fracturing new matrix and impeding long-term repair success.

Additionally, the interplay between biochemical and mechanical factors influences post-translational modifications of collagen, such as hydroxylation and crosslinking (mediated by lysyl oxidase), which further bolster tensile strength. Local availability of oxygen and nutrients—a function of joint vascularization and synovial fluid flow—shapes these biochemical events, ensuring that newly synthesized collagen fibrils and proteoglycans are adequately stabilized. Such coordinated regulation of ECM biosynthesis, assembly, and remodeling underscores the complexity required to achieve robust, hyaline-like cartilage within a defect site.

3. ANGIOGENESIS AND VASCULARIZATION

Angiogenesis and vascularization during the early stages of microfracture-driven cartilage repair involve a series of tightly regulated molecular events that initially support tissue regeneration but must later subside to reestablish the avascular nature of healthy articular cartilage. When the subchondral bone is perforated, growth factors, damage-associated molecular patterns (DAMPs), and cytokines accumulate at the defect site, creating conditions that favor endothelial cell recruitment and vessel formation.³¹ Among these molecules, vascular endothelial growth factor (VEGF) is a primary driver of neovascularization. VEGF binds to its tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), on endothelial cells, triggering a cascade of intracellular signals—including the PI3K–Akt and RAF–MEK–ERK pathways—that promote endothelial cell proliferation, migration, and tubulogenesis. The nascent blood vessels formed via this process supply critical nutrients and oxygen, while removing metabolites and waste, thereby fostering an environment conducive to cartilage repair.^{32,33}

VEGF upregulation is strongly tied to hypoxic conditions at the repair site. The low-oxygen environment stabilizes hypoxia-inducible factor-1 α (HIF-1 α) by preventing its proteasomal degradation. HIF-1 α then dimerizes with HIF-1 β , binding to hypoxia-responsive elements (HREs) in target genes, including VEGF. This transcriptional upregulation of VEGF ensures that newly forming tissue has immediate access to vascular support, allowing for robust cell proliferation and ECM synthesis. However, because cartilage ultimately requires an avascular milieu, the repair tissue must transition back to a state of minimal vascular infiltration once the early regenerative phase concludes.^{34,35}

A critical part of this transition is governed by the interaction of angiopoietins (Ang-1 and Ang-2) with the Tie-2 receptor on endothelial cells.³⁶ Angiopoietin-1 (Ang-1) promotes vessel stabilization by recruiting pericytes and smooth muscle cells, reinforcing vessel walls and making them less susceptible to regression. In contrast, Angiopoietin-2 (Ang-2) can destabilize vessels in the absence of sufficient VEGF signaling, effectively pruning or regressing any unneeded vasculature and facilitating the shift toward an avascular environment. The balance between Ang-1 and Ang-2 is pivotal for regulating the timing and extent of vessel maturation, as well as subsequent vessel regression.

Other angiogenic factors play supporting roles in modulating the vascular response. Platelet-derived growth factor (PDGF) recruits pericytes, which wrap nascent endothelial tubes and impart mechanical and trophic support, while basic fibroblast growth factor (bFGF) stimulates endothelial cell proliferation and induces the expression of proteolytic enzymes like matrix metalloproteinases (MMPs), which degrade the surrounding basement membrane to allow capillary sprouting.³⁷ At the same time, tissue inhibitors of metalloproteinases (TIMPs) and natural anti-angiogenic molecules such as thrombospondins (e.g., TSP-1) and endostatin (a fragment of collagen XVIII) restrict unchecked

vascular expansion by blocking pro-angiogenic signals or inhibiting endothelial cell migration.^{38,39}

Because persistent vascularization beyond the early repair stage can lead to fibrocartilage formation rather than the desired hyaline cartilage, local signaling shifts to promote vessel regression.⁴⁰ Reduced expression of VEGF—driven by diminished hypoxia, altered local cytokine levels, or direct repression by anti-angiogenic factors—contributes to vessel pruning. Concomitantly, increased Ang-2 and decreased Ang-1 can destabilize nascent vessels, hastening their regression. HIF-1 α levels also drop as oxygen availability improves, further tempering the production of pro-angiogenic genes. This orchestrated return to avascularity ensures that the engineered or regenerating cartilage retains its hallmark feature of nutrient diffusion from synovial fluid rather than direct vascular perfusion.

On a molecular level, this avascular transition is also influenced by interplay between integrin-mediated signaling and mechanical cues. As the nascent ECM matures, collagen type II and proteoglycan deposition creates a dense, low-oxygen environment that stabilizes established chondrocytes. Chondrocyte integrins, such as $\alpha 1\beta 1$ and $\alpha 5\beta 1$, respond to matrix composition changes by modulating intracellular signaling pathways—FAK, Rho GTPases, ERK1/2—that can fine-tune angiogenic factor secretion or repression. Mechanical stimuli, if well-regulated, reinforce the chondrocyte phenotype and reduce pro-angiogenic signals by maintaining a biochemical milieu that favors ECM production over vascular invasion.⁴¹

By carefully balancing the early angiogenic demands of regenerating cartilage against the eventual need for an avascular environment, researchers and clinicians can optimize the microfracture procedure and other cartilage repair techniques. Harnessing and refining these molecular processes has the potential to increase the likelihood of forming robust, load-bearing hyaline cartilage while mitigating the risks of fibrocartilage formation and long-term joint deterioration.

1. EARLY MOBILIZATION AND LOADING

Early mobilization and progressive mechanical loading after microfracture surgery trigger a series of intricate molecular cascades that reinforce the chondrogenic potential of repaired cartilage. During the initial postoperative phase, passive range-of-motion exercises enhance synovial fluid flow across the cartilage surface, delivering nutrients, growth factors, and cytokines crucial for healing.⁴² This circulation not only supplies anabolic factors—such as transforming growth factor-beta (TGF- β) and insulin-like growth factor-1 (IGF-1)—but also aids in removing catabolic cytokines (e.g., interleukin-1, tumor necrosis factor-alpha) that would otherwise upregulate matrix metalloproteinases (MMPs) and degrade the nascent cartilage matrix.^{43,44}

TGF- β binds to TGF- β type I and II receptors on chondrocytes (TGF- β RI/TGF- β RII), activating receptor-regulated SMAD2/3. These phosphorylated SMADs form heteromeric complexes with SMAD4, which translocate to the nucleus and upregulate genes like COL2A1 (type II colla-

Table 1. Summary of Molecular Mechanisms of Cartilage Repair.

Mechanism	Key Molecules & Pathways	Description / Biological Effects	Role in Cartilage Repair	References
1. Stem Cell Recruitment and Differentiation	- MSCs, TGF-β, BMPs (BMP-2, BMP-7), FGFs (FGF-18) - SMAD2/3, SMAD1/5/8, SOX9 - IGF-1, AKT, ERK - Wnt/β-catenin, Notch (crosstalk)	MSCs migrate in response to chemotactic gradients (cytokines, DAMPs). TGF- β activates SMAD2/3 and SOX9 for chondrogenesis, while BMPs engage SMAD1/5/8 to further induce collagen type II and proteoglycan synthesis. FGFs, especially FGF-18, promote chondrocyte proliferation and matrix production. IGF-1 supports anabolic activity.	Ensures that MSCs home to the injury site and differentiate into chondrocytes, laying down cartilage-specific extracellular matrix (ECM). Balances chondrocyte lineage commitment over osteogenic or adipogenic fates.	8,9,15-19
2. ECM Synthesis and Remodeling	- Collagen type II, Aggrecan - MMPs (MMP-13), ADAMTS-4/5 - TIMPs - SLRPs (Decorin, Biglycan) - Integrins (α5β1, αvβ3)	Newly differentiated chondrocytes produce collagen type II fibrils and proteoglycans (e.g., aggrecan). MMPs and aggrecanases degrade damaged ECM, while TIMPs inhibit excessive ECM breakdown. SLRPs orchestrate collagen fibril organization. Integrin signaling regulates cytoskeletal changes and gene expression for matrix upkeep.	Maintains mechanical integrity (tensile strength, compressive resilience). Balanced remodeling removes damaged ECM, integrates new matrix, and preserves healthy cartilage structure. Excessive catabolism (e.g., high MMP levels) or insufficient remodeling impairs repair outcomes.	10,23-30
3. Angiogenesis and Vascularization	- VEGF, VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) - HIF-1α (hypoxia) - Angiopoietins (Ang-1, Ang-2), Tie-2 receptor - PDGF, bFGF (FGF-2) - Thrombospondins, Endostatin (anti-angiogenic)	Early vascularization provides nutrients and oxygen; VEGF upregulation under hypoxic conditions activates new vessel formation via PI3K-Akt, RAF-MEK-ERK. Ang-1 and Ang-2 regulate vessel stability and regression. Anti-angiogenic factors (e.g., endostatin) ensure eventual return to an avascular cartilage phenotype.	Supports initial nutrient delivery and waste removal to regenerating tissue. Transition to avascular state is crucial for hyaline cartilage formation. Persistent vascularization drives fibrocartilage production instead of stable, load-bearing cartilage.	31-41
4. Growth Factor Synergy and Crosstalk	- TGF-β/BMP cross-regulation - Wnt/β-catenin, Notch - FGF, IGF-1 - SMAD inhibitory loops (SMAD6/7), Noggin, Chordin, Follistatin	TGF- β and BMPs can activate overlapping SMAD pathways. Crosstalk with Wnt and Notch pathways influences cell fate decisions. Inhibitory SMADs and BMP antagonists prevent excessive cartilage or bone formation, ensuring balanced chondrogenesis. IGF-1 and FGFs add anabolic signals, promoting matrix deposition and cell proliferation.	Harmonizes the rate and extent of chondrocyte differentiation, preventing cartilage from undergoing premature ossification or from forming suboptimal fibrocartilage. Ensures stable, hyaline-like cartilage matrix over the course of repair.	9,17,19
5. Mechanotransduction	- Integrins (e.g., α5β1, αvβ3) - FAK (focal adhesion kinase), RhoA, Rac1 - TRPV4 (ion channel) - MAPK (ERK1/2, p38)	Chondrocytes sense mechanical loads through integrins at focal adhesions. FAK activation leads to downstream MAPK/ERK and p38 signaling, modulating cytoskeletal dynamics and gene transcription. Mechanosensitive ion channels like TRPV4 also trigger anabolic responses	Appropriate mechanical stress enhances chondrocyte proliferation, ECM synthesis (collagen type II, proteoglycans), and matrix organization. Excessive or abnormal loading can upregulate catabolic enzymes,	11,20,29,30, 41

Mechanism	Key Molecules & Pathways	Description / Biological Effects	Role in Cartilage Repair	References
		under physiological loading conditions.	hindering repair.	
6. Inflammation Regulation	- IL-1, TNF- α , MMPs, ADAMTS - NF- κ B pathway	Acute inflammation aids MSC recruitment and early remodeling. Persistent inflammation upregulates catabolic enzymes (MMPs, ADAMTS), degrading ECM and inhibiting chondrocyte metabolism. NF- κ B amplifies cytokine production. Calibrating the immune response is critical for functional repair.	Moderates ECM destruction and promotes chondrocyte activity. Chronic inflammation disrupts cartilage integrity; controlled inflammation balances matrix turnover and new tissue integration.	12,27,28
7. Subchondral Bone Interaction	- Bone marrow cues, microdamage - TGF- β , BMPs (released from bone) - Cross-talk via vascular channels and cytokines	Subchondral bone provides mechanical support and releases molecular signals that diffuse into cartilage. Microdamage or sclerosis alters these signals, contributing to chronic inflammation and suboptimal cartilage regeneration.	A healthy subchondral bone niche fosters improved cartilage repair by stabilizing the tissue and providing growth factors that enhance chondrogenesis. Abnormal bone remodeling can degrade cartilage or impede regeneration.	10,12
8. Gene and Cell-Based Influences	- Viral/non-viral vectors (for TGF- β , IGF-1, SOX9) - RNAi, CRISPR/Cas9 to silence catabolic mediators - MicroRNA-based regulation (miR-140)	Gene transfer methods can increase local chondrogenic factor expression or block cartilage-degrading genes. Engineered stem cells may overexpress cartilage-specific signals or remain resistant to catabolic stimuli. MicroRNAs fine-tune anabolic/catabolic gene expression.	Enhances endogenous cartilage repair, directs MSCs toward stable chondrocyte differentiation, and limits ECM breakdown. Potential to produce more robust and durable cartilage akin to native tissue.	13,14

gen) and ACAN (aggrecan)—key structural components of articular cartilage. In parallel, IGF-1 engages its receptor (IGF-1R) and signals through the PI3K/Akt/mTOR axis, increasing chondrocyte proliferation and protein synthesis. This dual interplay of TGF- β /SMAD and IGF-1/PI3K/Akt pathways underpins the anabolic state necessary for robust extracellular matrix (ECM) synthesis and early stabilization of the repair tissue.⁴³

As partial weight-bearing activities commence, mechanical forces further modulate the chondrogenic environment by stimulating integrin-mediated mechanotransduction. Integrins such as $\alpha 5 \beta 1$, $\alpha \nu \beta 3$, and $\alpha 1 \beta 1$ bind ECM ligands (e.g., collagen type II, fibronectin) and cluster at focal adhesions, where focal adhesion kinase (FAK) initiates downstream effectors like the Rho GTPases (RhoA, Rac1) and MAPKs (e.g., ERK1/2, p38). These signaling cascades reorganize the actin cytoskeleton, upregulate matrix genes, and enhance the chondrocyte's anabolic profile. Mechanical stimuli also induce intracellular calcium influx through mechanosensitive ion channels (e.g., TRPV4), activating calcineurin–NFAT signaling or additional MAPK pathways that boost ECM assembly.^{45,46}

Mechanical loading not only drives integrin–FAK signaling but also augments local expression of bone morpho-

genetic proteins (BMP-2 and BMP-7). These BMPs bind to their respective type I (ALK3/ALK6) and type II receptors (BMPRII), phosphorylating SMAD1/5/8, which form active complexes with SMAD4. The SMAD1/5/8 arm intersects with the TGF- β –SMAD2/3 axis, promoting synergistic activation of cartilage-specific genes. This BMP–TGF- β crosstalk amplifies collagen type II and aggrecan production, reinforcing the matrix's mechanical integrity.⁴⁷ Non-SMAD (e.g., TAK1, p38) BMP pathways can additionally reshape chondrocyte gene expression, fostering a balanced tissue that resists premature hypertrophy or osteogenic conversion.

Rehabilitation protocols capitalize on these molecular mechanisms by carefully dosing activity and rest. Early stages often feature passive motion to maintain joint flexibility and synovial fluid turnover without imposing high compressive loads on the newly formed cartilage.⁴⁸ Gradual transitions to active exercises (light cycling, gentle weight-bearing) elicit higher chondrocyte metabolic activity, supporting ECM remodeling and more extensive collagen fibril crosslinking. Proprioceptive exercises integrated into rehabilitation regimens heighten neuromuscular control, reducing shear forces that can damage immature cartilage.^{49, 50} At the molecular level, improved proprioception may

boost the release of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), which fortify neuromuscular pathways and contribute to joint stability.⁵¹

Progressive resistance exercises further augment muscle strength surrounding the joint, reducing direct load on the healing cartilage. As muscles contract under higher resistance, they release myokines (e.g., irisin) that can modulate inflammation and maintain an anabolic microenvironment.⁵²⁻⁵⁴ Irisin specifically dampens the activity of IL-1 and TNF- α , thus minimizing MMP-driven cartilage erosion, while simultaneously cooperating with PI3K/Akt to boost collagen and proteoglycan synthesis.⁵⁵ With increased mechanical stimuli, chondrocytes respond by activating additional calcium-dependent pathways that intensify matrix production and refine the collagen–proteoglycan network.

Epigenetic regulation also influences the chondrocyte's response to mechanical load. Mechanical stress, for instance, can alter chromatin accessibility via histone acetylation or DNA methylation, modulating transcription factors like SOX9 and RUNX2 that direct chondrocyte lineage decisions. MicroRNAs (e.g., miR-140) may be upregulated in response to mechanical signals, tuning the expression of catabolic mediators (e.g., MMP-13) while preserving anabolic pathways. These epigenetic modifications enable chondrocytes to adapt dynamically to changing mechanical and biochemical stimuli during the rehabilitation process.

Overall, early mobilization and progressive mechanical loading harness a symphony of growth factors, cytokine gradients, integrin-mediated signaling, and epigenetic adjustments to mold the developing ECM into a resilient, hyaline-like cartilage. By strategically sequencing rest, passive mobility, active exercises, proprioception drills, and resistance training, clinicians optimize the molecular environment for cartilage repair, ensuring durable mechanical strength and improved long-term function for patients recovering from microfracture surgery.⁴⁶

2. BIOLOGICAL AUGMENTATION

Biological augmentation harnesses an array of molecular and cellular strategies to boost the innate processes of cartilage repair and regeneration. Platelet-rich plasma (PRP) provides a concentrated source of growth factors, notably transforming growth factor-beta (TGF- β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), each of which activates specific intracellular pathways that stimulate chondrocyte proliferation, extracellular matrix (ECM) synthesis, and tissue remodeling.⁵⁶ When injected into the injured site, PRP-derived platelets degranulate, releasing not only these growth factors but also various chemokines that help orchestrate cell migration, homing, and angiogenesis. For example, TGF- β binds to its type I and II receptors on chondrocytes—TGF- β RI/ALK5 and TGF- β RII—initiating SMAD2/3 phosphorylation and SMAD4 translocation to the nucleus, thereby upregulating critical chondrogenic genes such as COL2A1 (type II collagen) and ACAN (aggrecan). PDGF engages PDGF receptors on chondrocytes and synovial fibroblasts, activating the RAS–RAF–MEK–ERK cascade, which fosters cell pro-

liferation and proteoglycan production. VEGF, meanwhile, spurs the formation of nascent blood vessels to ensure that newly forming tissue has an adequate supply of oxygen and nutrients, a key requirement during the early stages of repair.⁵⁷ Concurrently, PRP reduces synovial levels of pro-inflammatory cytokines—primarily interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- α)—by inducing the expression of naturally occurring antagonists or soluble receptors. This dampens matrix metalloproteinases (MMPs) and ADAMTS (aggrecanases) that might otherwise degrade the nascent cartilage matrix, thereby preserving a protective microenvironment for chondrogenic activity.

Hyaluronic acid (HA) supplementation offers a complementary mechanism of action, acting as both a biomechanical lubricant and a biochemical modulator.⁵⁸ On the mechanical side, HA's high viscoelasticity buffers compressive forces and maintains smoother joint articulation, reducing friction that can otherwise lead to microdamage of the healing tissue. On the molecular front, HA interacts with chondrocyte surface receptors—most notably CD44 and RHAMM—initiating a chain of intracellular signals that frequently involve Rho GTPases and MAPK pathways, ultimately boosting the expression of ECM components such as collagen type II and aggrecan. These signals also help stabilize the cytoskeleton and promote chondrocyte survival, mitigating cell apoptosis under mechanical or inflammatory stress. By upregulating genes involved in ECM formation, HA not only preserves existing cartilage but also stimulates reparative processes that restore tissue tensile strength and compressive resilience. Additionally, HA can bind and concentrate growth factors (including TGF- β) in the local extracellular space, prolonging their anabolic effects on chondrocytes and prolonging the early window of high metabolic activity essential for robust repair.

Clinical investigations report that PRP and HA, when administered in conjunction with microfracture surgery, measurably enhance cartilage repair outcomes, including improved tissue fill, superior histological appearance, and faster symptomatic relief.^{59,60} PRP's anti-inflammatory properties help maintain a balanced environment in which catabolic enzymes remain suppressed, supporting stable cartilage formation. HA, meanwhile, sustains synovial fluid viscosity and ensures that mechanical loads are more evenly distributed across the lesion, further minimizing deleterious shear or compressive stresses.

In parallel, breakthroughs in stem cell therapy have opened new molecular avenues for cartilage repair.⁶¹ Mesenchymal stem cells (MSCs), derived either autologously or allogeneically, possess the capacity to differentiate into chondrocytes under TGF- β and bone morphogenetic protein (BMP) stimulation. Beyond their direct chondrogenic contribution, MSCs secrete a milieu of anti-inflammatory factors such as IL-10 and TGF- β , which reduce inflammation-driven matrix breakdown and tip the balance toward anabolic tissue remodeling. Strategies like preconditioning MSCs with specific growth factors (e.g., IGF-1) or genetic modifications (e.g., SOX9 overexpression) can amplify their chondrogenic potency, thereby generating more stable and durable repair cartilage. These cells also release exosomes

laden with microRNAs (e.g., miR-140) that regulate cartilage-specific gene expression, effectively transferring regenerative signals to resident chondrocytes.

Gene therapy extends these chondrogenic effects by localizing anabolic or anti-inflammatory gene products at the repair site.⁶² Viral vectors, such as adenovirus and lentivirus, or non-viral systems like plasmid DNA and liposomes, are used to deliver genes coding for factors like insulin-like growth factor-1 (IGF-1), BMP-2, or TGF- β , providing a sustained anabolic stimulus to chondrocytes. Conversely, genes encoding IL-1 receptor antagonist (IL-1Ra) or soluble TNF receptors blunt the catabolic and pro-inflammatory cascades that degrade cartilage matrix. With CRISPR-Cas9 gene editing on the horizon, fine-tuning the expression of key regulatory genes (e.g., MMP-13) may further improve cartilage repair fidelity and durability.

Meanwhile, tissue engineering efforts center on biomimetic scaffolds designed to recapitulate the structural and biochemical architecture of native cartilage.⁶³ Fabricated from materials such as collagen, HA, polylactic acid, or composite polymers, these scaffolds provide spatial cues that guide cell attachment, integrin signaling, and cytoskeletal rearrangement. Their porosity, stiffness, and degradation profile can be finely tuned to optimize chondrocyte or MSC function. Incorporating growth factors into the scaffold matrix enables controlled, localized release over time, while sensor-triggered or stimulus-responsive designs can deliver anabolic factors in response to mechanical loading, pH changes, or enzymatic activity.⁶⁴ This dynamic approach helps maintain a stable environment in which the newly differentiating chondrocytes are constantly receiving the necessary signals to build, remodel, and reinforce the ECM.

Overall, biological augmentation represents a multi-pronged molecular strategy that targets both the anabolic and anti-catabolic facets of cartilage repair. PRP and HA address immediate requirements—ranging from inflammation control to matrix assembly—while stem cells, gene therapy, and advanced biomimetic scaffolds sustain long-term regenerative capacity. Combining these modalities with established procedures such as microfracture and well-structured rehabilitation protocols can substantially elevate the quality, resilience, and functionality of newly formed cartilage. As research in molecular biology, regenerative medicine, and bioengineering converges, the prospects for highly personalized, durable, and effective cartilage repair continue to broaden, promising better outcomes and improved quality of life for patients with joint injuries and degenerative conditions.

3. NUTRITIONAL SUPPORT

Nutritional support plays a pivotal role in the maintenance, repair, and regeneration of cartilage tissue, acting through a variety of molecular and cellular pathways that collectively support chondrocyte function, extracellular matrix (ECM) synthesis, and overall joint integrity. Adequate intake of specific amino acids, vitamins, minerals, antioxidants, and other nutrients supplies the biochemical build-

ing blocks and cofactors needed to construct and maintain a robust cartilage matrix, while also modulating inflammatory and oxidative processes that can undermine tissue health.

Amino acids are fundamental to collagen synthesis and other aspects of cartilage maintenance. Cartilage relies heavily on collagen type II and proteoglycans, which together form the structural backbone that enables the tissue to resist tensile and compressive forces. Collagen is made up of peptide chains rich in specific amino acids such as glycine, proline, and lysine. Glycine residues occupy every third position in the collagen triple helix, allowing the chains to pack tightly. Proline and lysine are also crucial, as they undergo post-translational modifications that are essential for collagen stability. Proline is converted to hydroxyproline by prolyl hydroxylase, while lysine is converted to hydroxylysine by lysyl hydroxylase. These hydroxylations require vitamin C as a cofactor and enable the formation of intra- and intermolecular hydrogen bonds, stabilizing the triple-helical collagen structure and increasing its tensile strength. Thus, a diet with sufficient protein and vitamin C supports these enzymatic modifications and bolsters cartilage resilience.^{65,66}

Beyond their role in collagen formation, amino acids can modulate various cell signaling pathways involved in cartilage repair. For example, arginine can be converted into nitric oxide (NO) via nitric oxide synthase, with NO acting in controlled concentrations as a signaling molecule that may influence chondrocyte proliferation and matrix synthesis. Meanwhile, glutamine is essential for cellular energy metabolism, and it can indirectly support chondrocyte viability by fueling the TCA cycle and maintaining redox balance. Adequate protein intake overall ensures that the body can generate and replace chondrocytes and supporting cells at the defect site, maintaining an environment conducive to continuous cartilage remodeling and repair.

Vitamins also play multiple molecular roles. Vitamin C, aside from being a cofactor for hydroxylase enzymes, acts as a potent antioxidant, neutralizing reactive oxygen species (ROS) that can damage chondrocytes and ECM macromolecules. Excess ROS can degrade proteoglycans and collagen fibers, leading to compromised mechanical properties. Vitamin E similarly protects lipid membranes in chondrocytes from peroxidative damage, a process that can trigger inflammation and cell death. By preserving membrane integrity, vitamin E helps maintain stable chondrocyte metabolism and signaling, which is particularly important for mechanotransduction processes that require intact cell membranes and ion channels.

Minerals such as zinc, copper, and magnesium further influence cartilage repair at the enzymatic and transcriptional levels.⁶⁷ Zinc is vital for the structural integrity of numerous proteins, including transcription factors and enzymes like collagenase, which remodels collagen during tissue repair. Inadequate zinc can impair DNA replication and protein synthesis, slowing down chondrocyte proliferation and ECM turnover. Copper is a cofactor for lysyl oxidase, the enzyme responsible for creating covalent cross-links within collagen and elastin fibers. These cross-links give

cartilage its indispensable tensile strength and elasticity, allowing it to withstand repetitive loading and stress. Magnesium, meanwhile, is involved in over 300 enzymatic reactions, including those that modulate ATP production, protein synthesis, and ion channel regulation. Ensuring sufficient magnesium intake can help stabilize ATP-dependent reactions that drive protein assembly and chondrocyte energy metabolism.

Glucosamine and chondroitin sulfate are widely recognized as supportive supplements for cartilage structure, working through molecular mechanisms closely tied to glycosaminoglycan (GAG) synthesis.⁶⁵ Glucosamine serves as a precursor for the formation of key GAGs, including chondroitin sulfate and keratan sulfate, which combine with proteins to form proteoglycans. Proteoglycans, in turn, enhance cartilage's compressive resistance by binding water molecules within the matrix. Chondroitin sulfate is itself a major GAG component that helps retain water in the tissue and preserve elasticity. By bolstering proteoglycan content, these supplements may slow cartilage breakdown and encourage a more favorable environment for tissue repair. Additionally, some research suggests that glucosamine may downregulate the activity of cartilage-degrading enzymes such as MMPs and aggrecanases, while also suppressing pro-inflammatory mediators like IL-1 and TNF- α .

Omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exert significant anti-inflammatory effects that can protect cartilage from excess catabolic signaling.⁶⁸ EPA and DHA can modulate the production of inflammatory eicosanoids, reducing prostaglandin E2 (PGE2) and leukotriene synthesis, both of which amplify cartilage degradation. Omega-3s also serve as precursors to specialized pro-resolving mediators (SPMs), such as resolvins and protectins, that actively help resolve chronic inflammation. By mitigating the prolonged inflammatory response, these fatty acids protect chondrocytes against oxidative and enzymatic damage, promoting a microenvironment more conducive to regeneration.

Sulfur-containing compounds like methylsulfonylmethane (MSM) also support collagen cross-linking and proteoglycan formation by supplying sulfur for disulfide bond formation.⁶⁹ These sulfur bonds reinforce the quaternary structure of collagen, improve resilience, and contribute to the stabilization of proteoglycans. Sulfur is also integral to glutathione synthesis, enhancing the chondrocyte's antioxidant capacity. Through these routes, MSM helps maintain cartilage homeostasis and fosters a robust protective framework against mechanical wear and inflammatory insults.

Adequate hydration is similarly critical at the molecular level. Cartilage depends on proteoglycans to retain water, conferring the tissue with its gel-like properties that buffer compressive forces and enable smooth joint articulation. Dehydration can reduce chondrocyte metabolism, hamper nutrient diffusion, and alter synovial fluid viscosity, collectively degrading the environment necessary for sustained ECM turnover. Proper hydration supports the sulfated glycosaminoglycan network's capacity to bind water and maintain appropriate osmotic pressure within the cartilage

matrix, directly impacting cartilage mechanics and overall joint function.

Micronutrients like vitamin D and vitamin K can also influence cartilage health by modulating bone-cartilage cross-talk and preventing pathological calcification of cartilage. Vitamin D regulates calcium and phosphate homeostasis, contributing to subchondral bone quality, which indirectly affects cartilage integrity. Vitamin K is required for the γ -carboxylation of matrix Gla-protein (MGP), which inhibits ectopic calcification within articular cartilage, thus preserving flexibility and resilience.⁶⁹ These factors highlight how nutrition must be considered holistically, encompassing both direct effects on chondrocytes and indirect effects mediated through bone remodeling and joint fluid properties.

When combined, these nutritional and molecular interventions have been shown to improve biochemical and biomechanical aspects of repaired cartilage.⁶⁶ By supplying the raw materials and cofactors essential for collagen synthesis, glycosaminoglycan assembly, antioxidant defense, and inflammatory modulation, a comprehensive nutritional strategy enhances the body's natural repair mechanisms. This synergy may be particularly beneficial following cartilage repair procedures such as microfracture, autologous chondrocyte implantation, or stem cell-based therapies, where the proliferative and synthetic demands on chondrocytes are elevated.

Overall, integrating molecular insights from nutrition with clinical cartilage repair strategies can substantially improve patient outcomes. Tailored supplementation of amino acids, vitamins, minerals, antioxidants, fatty acids, and other nutrients, in conjunction with balanced hydration and controlled inflammation, supports the delicate balance of catabolic and anabolic activities within cartilage. Such approaches complement surgical and rehabilitative measures, facilitating more robust matrix deposition, stronger collagen networks, and healthier chondrocyte populations, thereby enhancing the durability and function of the repaired tissue for the long term.

4. ADVANCED THERAPEUTIC MODALITIES

Advanced therapeutic modalities such as low-intensity pulsed ultrasound (LIPUS), pulsed electromagnetic fields (PEMF), photobiomodulation therapy (PBMT), cryotherapy, and extracorporeal shockwave therapy (ESWT) influence cartilage repair through a complex interplay of molecular signaling pathways that bolster chondrocyte metabolism, enhance extracellular matrix (ECM) assembly, and modulate inflammation. When integrated alongside standard rehabilitation strategies and regenerative medicine approaches, these modalities provide a multifaceted framework that optimizes the chondrogenic microenvironment and accelerates tissue regeneration.

LIPUS harnesses acoustic waves to stimulate mechanotransductive pathways within chondrocytes, increasing the production of collagen type II and proteoglycans.⁷⁰ Mechanistically, these sound waves exert mechanical stress on the cell membrane and integrin complexes, activating

focal adhesion kinase (FAK) and downstream MAPK/ERK and p38 pathways. These kinases in turn upregulate key chondrogenic genes, including those encoding for matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), ensuring a balanced remodeling of the ECM. LIPUS also enhances the release of growth factors such as TGF- β , IGF-1, and FGF-2 that synergize to promote chondrocyte proliferation and inhibit apoptosis. Additionally, by dampening pro-inflammatory mediators like IL-1 and TNF- α , LIPUS helps create a local milieu conducive to stable tissue growth.⁷¹

PEMF therapy applies electromagnetic fields that penetrate joint tissues, modulating intracellular and transmembrane potential differences. At the molecular level, PEMF increases the synthesis of endogenous anabolic factors such as BMPs, PDGF, and basic fibroblast growth factor (bFGF), driving chondrocyte proliferation and differentiation.⁷² Simultaneously, it enhances the Wnt/ β -catenin pathway, a critical regulator of cartilage lineage commitment, and activates the A2A adenosine receptor, leading to elevated intracellular cAMP and protein kinase A (PKA) signaling. These cascades collectively boost proteoglycan and collagen deposition while counteracting catabolic processes mediated by NF- κ B. By suppressing inflammatory cytokines, PEMF promotes a shift toward an anti-inflammatory profile, stabilizing the ECM and protecting nascent cartilage from enzymatic breakdown.

Photobiomodulation therapy, also known as low-level laser therapy (LLLT), uses light of specific wavelengths (often in the red or near-infrared spectrum) to enhance mitochondrial activity in chondrocytes.⁷³ When absorbed, the photons elevate cytochrome c oxidase activity, increasing the mitochondrial membrane potential and ATP generation. The surplus ATP fuels anabolic pathways and drives the synthesis of proteins essential for ECM maintenance, including collagen and aggrecan. PBMT also modulates reactive oxygen species (ROS) balance by upregulating endogenous antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase, thereby reducing oxidative damage to both chondrocytes and ECM macromolecules. Moreover, by influencing transcription factors like NF- κ B, PBMT can downregulate pro-inflammatory gene expression, aiding in tissue preservation and improved repair.⁷⁴

Cryotherapy involves the application of cold temperatures to modulate local blood flow, cellular metabolism, and inflammation.⁷⁵ At reduced temperatures, the activity of certain inflammatory mediators—including prostaglandins and leukotrienes—is diminished, which can lower local edema and joint effusion. Cryotherapy also affects heat shock proteins (HSPs) within chondrocytes, some of which function as molecular chaperones that help refold damaged proteins or prevent protein aggregation. By adjusting the expression and activity of these HSPs and inflammatory cytokines, cryotherapy stabilizes the microenvironment around repairing cartilage, conserving cellular energy for matrix synthesis rather than inflammation.⁷⁶

Extracorporeal shockwave therapy (ESWT) directs high-energy acoustic pulses into the joint, inciting localized mechanical stress that stimulates angiogenesis and tissue re-

pair.⁷⁷ On a cellular level, ESWT prompts nitric oxide (NO) release, which elevates blood perfusion and fosters an environment rich in nutrients and oxygen, essential for robust chondrocyte activity. Mechanotransductive pathways—particularly ERK1/2 and p38 MAPK—are triggered in response to these shockwaves, upregulating genes that encode for collagen type II, aggrecan, and other ECM constituents.⁷⁸ NO additionally modulates local inflammatory cells, helping restrain chronic inflammatory processes that degrade cartilage. This synergy of increased nutrient delivery, lowered inflammation, and heightened anabolic signaling paves the way for superior cartilage regeneration.

As regenerative medicine evolves, combining these modalities with stem cell therapy and gene therapy offers an even greater molecular advantage.⁷⁹⁻⁸¹ Mesenchymal stem cells (MSCs) exposed to LIPUS or PEMF exhibit heightened expression of chondrogenic transcription factors, such as SOX9, and produce more ECM. Meanwhile, gene therapy provides a means to introduce or edit genes for anabolic factors (e.g., TGF- β , IGF-1) or anti-inflammatory agents (e.g., IL-1Ra, soluble TNF receptors), maintaining a long-term balance that fosters stable cartilage formation.^{82,83} Such integrative regimens leverage the biological synergy between mechanically induced cell signaling and genetic modifications, propelling cartilage repair beyond traditional limitations.

In clinical practice, these advanced modalities are often paired with progressive loading and rehabilitation exercises for maximal benefit.⁸⁴ Patients receiving LIPUS or PEMF show improved pain relief, faster chondrocyte proliferation, and a stronger, more organized matrix. Early mobilization catalyzed by these modalities also encourages proper ECM alignment, ensuring that collagen fibrils and proteoglycans are deposited in orientations conducive to load-bearing. Furthermore, the synergy between mechanical stimulation and biologically active fields can optimize the microenvironment for stem cell engraftment, gene expression, and scaffold integration.

In summary, advanced therapeutic modalities exert significant molecular impact on chondrocytes and their surrounding milieu. LIPUS, PEMF, PBMT, cryotherapy, and ESWT enhance the anabolic signals necessary for ECM regeneration while mitigating the catabolic processes that threaten newly formed cartilage.⁸⁵ By activating key pathways—ranging from integrin-mediated mechanotransduction to Wnt/ β -catenin and NF- κ B modulation—these non-invasive or minimally invasive interventions accelerate tissue repair, reduce pain and inflammation, and support durable cartilage outcomes. As research in biophysics, cell signaling, and regenerative medicine continues to deepen, these modalities will likely become increasingly refined, further improving patient outcomes and broadening the possibilities for restoring joint function in cases of cartilage injury or degeneration.

5. BLOOD FLOW RESTRICTION TRAINING

Blood flow restriction (BFR) training offers a multidimensional molecular framework that can be harnessed in car-

tilage microfracture rehabilitation by driving controlled inflammation, hypoxia-mediated gene expression, anabolic hormone secretion, and fibrinolysis—all while preventing excessive mechanical stress on newly forming cartilage [118]. During low-load resistance exercises with partial venous occlusion, the localized hypoxia and metabolite accumulation trigger a cascade of intracellular signals that benefit not only skeletal muscle but also the cartilage matrix, tendons, and surrounding soft tissues [119].

Acute inflammation is induced by elevated shear stress upon reperfusion, which prompts the release of pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) [120]. IL-6 binds its IL-6R/gp130 receptor complex and activates Janus kinase (JAK1/JAK2/Tyk2), phosphorylating signal transducer and activator of transcription 3 (STAT3) [121]. Phosphorylated STAT3 translocates to the nucleus and induces genes that promote tissue repair, including processes relevant to chondroprogenitor cell recruitment. TNF- α , though harmful in chronic excess, can transiently assist cartilage healing by activating the I κ B kinase (IKK) complex, freeing nuclear factor kappa B (NF- κ B) to upregulate immune cell recruitment and debris clearance [122]. Critically, macrophage populations eventually shift toward an M2 anti-inflammatory state, supporting a more regenerative and less degradative environment conducive to cartilage matrix deposition and remodeling [123].

Simultaneously, reduced venous outflow stabilizes hypoxia-inducible factor-1 α (HIF-1 α). Under sufficient hypoxia, prolyl hydroxylase domain (PHD) enzymes are inhibited, preventing HIF-1 α degradation by the von Hippel-Lindau (VHL) pathway. Stabilized HIF-1 α forms heterodimers with HIF-1 β , binding hypoxia-responsive elements (HREs) to induce transcription of vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) [124]. VEGF promotes endothelial cell proliferation and vascular sprouting, potentially benefiting the subchondral bone region that supplies nutrients to the microfracture repair site. eNOS-derived nitric oxide (NO) augments local vasodilation, supporting blood flow within the vicinity of the forming cartilage. HIF-1 α signaling is also central to chondrocyte viability in the low-oxygen niche of repaired cartilage, safeguarding ECM integrity while coordinating collagen and proteoglycan synthesis [125].

Metabolite buildup—particularly lactate and hydrogen ions—heightens sympathetic drive and fosters a systemic endocrine response that amplifies growth hormone (GH) and insulin-like growth factor 1 (IGF-1) release. IGF-1, whether liver-derived or produced locally in skeletal muscle (e.g., mechano growth factor, MGF), binds the IGF-1 receptor to activate phosphoinositide 3-kinase (PI3K) and downstream AKT/mTORC1 signaling [126]. This pathway phosphorylates p70 S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), unleashing cap-dependent translation of anabolic mRNAs [127]. For cartilage microfracture patients, the net effect is heightened protein synthesis in periarticular musculature as well as tendon and possibly joint tissues, reinforcing both skeletal support and the local environment in which new chondral tissue forms.

IGF-1 further bolsters collagen gene transcription in tenocytes, which supports improved tendon stiffness—particularly valuable in joint stabilization during rehabilitation [128].

BFR preferentially recruits type II muscle fibers at loads well below those usually necessary for fast-twitch activation, an advantage for patients with arthrogenic muscle inhibition (AMI) or limited tolerance for high-intensity exercise [129] [130]. The hypoxic and metabolite-rich milieu triggers group IV afferent fibers, boosting central motor drive and promoting the contraction of higher-threshold motor units. For microfracture rehabilitation, preserving or increasing type II fiber mass helps stabilize the joint and shield the fragile chondral repair from undue forces while allowing functional gains in strength [131].

Alongside these anabolic and immunomodulatory processes, partial occlusion stimulates tissue plasminogen activator (tPA) release from Weibel-Palade bodies in the vascular endothelium [132]. tPA catalyzes the conversion of plasminogen to plasmin, sustaining fibrinolysis and mitigating thrombotic concerns, while also degrading extraneous fibrin that could hinder nutrient diffusion or ECM organization in the microfracture site [133]. This safeguard, in concert with muscle contractions, maintains adequate fibrin turnover and limits the risk of deep vein thrombosis (DVT) [134].

Overall, BFR training weaves together transient inflammation, hypoxia-driven angiogenesis, robust anabolic signaling, and fibrinolytic activity to facilitate muscle and connective tissue repair while posing minimal mechanical stress on the microfractured cartilage [135]. This molecular synergy—manifested in chondrocyte preservation, regulated macrophage activation, enhanced growth factor profiles, and stable clot remodeling—makes BFR an innovative option in designing comprehensive and effective rehabilitation programs for cartilage microfracture patients [136] [137] [138].

BOUNDING NATURAL PROCESS OF CARTILAGE HEALING WITH REHABILITATION STRATEGIES

The natural process of cartilage healing involves a dynamic continuum of cellular and molecular events that unfold in three overlapping phases—namely, the inflammation, fibroblastic, and remodeling stages. Each phase draws on a complex network of signaling pathways, transcription factors, and extracellular mediators that coordinate tissue repair. By aligning rehabilitation strategies with the underlying molecular processes, clinicians can more effectively promote functional cartilage regeneration.

During the inflammation stage, vasodilation and increased vascular permeability bring platelets and immune cells—neutrophils, monocytes, and macrophages—to the injury site.⁸⁶ This recruitment is orchestrated by molecular signals such as histamine, bradykinin, and prostaglandin E2 (PGE2), which elevate the local concentration of pro-inflammatory cytokines and reactive oxygen species (ROS).⁸⁷,⁸⁸ Nuclear factor kappa B (NF- κ B) is a pivotal transcription factor in this phase: when activated (for instance, by IL-1

Table 2. Summary of Rehabilitation Strategies.

Section	Focus / Key Concepts	Molecular Pathways & Mechanisms	Cellular/ECM Outcomes & Clinical Impact	References
1. Early Mobilization and Loading	Passive Motion & Synovial Fluid Circulation	- Anabolic signals (TGF- β → SMAD2/3; IGF-1 → PI3K/Akt/mTOR) - Catabolic mediators (IL-1, TNF- α → ↑ MMPs)	- Improved nutrient delivery and removal of catabolic cytokines. - Early ECM stabilization and chondrocyte support.	42_44
	TGF- β & IGF-1 Signaling	- TGF- β binds TGF- β RI/II → SMAD2/3-SMAD4 complex → ↑ COL2A1, ACAN - IGF-1 engages IGF-1R → Akt/mTOR → ↑ protein synthesis	- Robust production of type II collagen and aggrecan. - Establishment of anabolic milieu critical for early cartilage repair.	43
	Integrin-Mediated Mechanotransduction	- Integrins (α 5 β 1, α v β 3, α 1 β 1) → FAK → RhoA, Rac1 → MAPKs (ERK1/2, p38) - TRPV4-dependent Ca ²⁺ influx → calcineurin-NFAT	- Cytoskeletal rearrangement boosts matrix gene expression. - Reinforcement of chondrocyte anabolic profile under partial weight-bearing.	45,46
	BMP-TGF- β Crosstalk	- BMP-2, BMP-7 → SMAD1/5/8, intersecting with TGF- β -SMAD2/3 - Non-SMAD pathways (TAK1, p38)	- Amplified cartilage-specific gene expression (type II collagen, aggrecan). - Balanced matrix remodeling and avoidance of hypertrophic or osteogenic fates.	47
	Rehabilitation Protocols & Progressive Exercises	- Passive → Active exercise progression - Proprioception: BDNF release - Resistance training: myokines (irisin)	- Enhanced ECM crosslinking and chondrocyte metabolic activity. - Neuromuscular control reduces shear forces on immature cartilage. - Irisin mitigates IL-1/TNF- α impact and elevates collagen/proteoglycan synthesis.	48_55
	Epigenetic Regulation	- Histone acetylation, DNA methylation - microRNAs (miR-140)	- SOX9/RUNX2 transcriptional balance shapes chondrocyte lineage decisions. - microRNA-mediated repression of catabolic enzymes (MMP-13) while reinforcing anabolic genes.	46
2. Biological Augmentation	Platelet-Rich Plasma (PRP)	- TGF- β , PDGF, VEGF released upon platelet degranulation - SMAD2/3, RAS-RAF-MEK-ERK pathways - Anti-inflammatory milieu (↓ IL-1, TNF- α)	- Chondrocyte proliferation and ECM synthesis (COL2A1, ACAN). - Early angiogenesis via VEGF. - Suppression of MMP/ADAMTS activity, guarding new cartilage from degradation.	56,57
	Hyaluronic Acid (HA)	- CD44, RHAMM receptor binding - Rho GTPases, MAPKs → Upregulation of collagen II, aggrecan - Local growth factor depot (TGF- β)	- HA's viscoelasticity protects healing cartilage from compressive stress. - Enhanced chondrocyte survival and reduced apoptosis. - Prolonged anabolic signaling fosters robust ECM assembly and mechanical resilience.	58
	Stem Cell Therapy	- MSCs: TGF- β → SMAD2/3; BMP →	- Direct chondrogenic differentiation and anti-inflammatory secretome.	61

Section	Focus / Key Concepts	Molecular Pathways & Mechanisms	Cellular/ECM Outcomes & Clinical Impact	References
		SMAD1/5/8 - Paracrine factors: IL-10, TGF- β - Exosomes with microRNAs (miR-140)	- Stabilization of ECM through reduced matrix breakdown. - Genetically modified/preconditioned MSCs can further enhance repair cartilage durability.	
	Gene Therapy	- Viral / non-viral vectors (adenovirus, lentivirus, plasmids) - IGF-1, BMP-2, TGF- β (anabolic); IL-1Ra, soluble TNF receptors (anti-inflammatory) - CRISPR-Cas9 editing	- Localized, sustained expression of chondrogenic or anti-catabolic factors. - Potential fine-tuning of MMP-13 or other catabolic genes. - Enhanced ECM formation and reduced chronic inflammation.	62
	Biomimetic Scaffolds	- Materials: collagen, HA, polylactic acid, composites - Scaffold design: porosity, stiffness, degradation rate - Stimulus-responsive factor release	- Guidance for cell attachment, integrin signaling, and cytoskeletal reorganization. - Controlled, localized delivery of growth factors. - High-fidelity replication of native cartilage architecture, promoting stable repair.	63,64
3. Nutritional Support	Amino Acids & Collagen Synthesis	- Proline, Lysine $\rightarrow$ Hydroxylation (vitamin C-dependent) - Glutamine, Arginine modulating chondrocyte viability and NO signaling	- Stabilized collagen triple helix and improved tensile strength. - Adequate protein intake supports continuous cartilage cell turnover and ECM remodeling.	65,66
	Vitamins & Antioxidants	- Vitamin C: cofactor for hydroxylases, antioxidant - Vitamin E: membrane antioxidant - Vitamin D/K: subchondral bone-cartilage cross-talk	- Preservation of proteoglycan-collagen matrix from ROS damage. - Maintenance of normal chondrocyte membrane and bone-cartilage interface. - Inhibition of ectopic calcification within cartilage.	67,69
	Minerals (Zinc, Copper, Magnesium)	- Zinc: collagenase function, transcription factor stability - Copper: lysyl oxidase (collagen cross-linking) - Magnesium: ATP-dependent enzymatic reactions	- Proper collagen/elastin cross-linking essential for mechanical strength. - Support for DNA replication, chondrocyte proliferation, and ECM turnover. - Key metabolic co-factors for robust repair processes.	67
	Glucosamine & Chondroitin	- Precursors for GAGs (chondroitin sulfate, keratan sulfate) - Potential suppression of MMPs, IL-1, TNF- α	- Increased proteoglycan content confers compressive resilience. - Encouragement of a more anabolic cartilage environment.	65
	Omega-3 Fatty Acids (EPA, DHA)	- Anti-inflammatory eicosanoid modulation - Precursor to resolvins, protectins	- Attenuation of chronic inflammation and catabolic signaling, protecting chondrocytes. - Preservation of ECM from oxidative/enzyme-mediated damage.	68
	Sulfur-Containing Compounds (MSM)	- MSM supplies sulfur for disulfide bonds in collagen/proteoglycans - Aids glutathione synthesis	- Strengthened collagen networks and antioxidant capacity. - Protection against mechanical wear and inflammatory damage.	69
	Hydration &	- Proteoglycan-water	- Adequate water supports	66,69

Section	Focus / Key Concepts	Molecular Pathways & Mechanisms	Cellular/ECM Outcomes & Clinical Impact	References
	Micronutrients	interactions - Vitamin K (MGP γ -carboxylation)	shock absorption and nutrient diffusion. - Vitamin K prevents ectopic calcification, maintaining cartilage elasticity.	
4. Advanced Therapeutic Modalities	Low-Intensity Pulsed Ultrasound (LIPUS)	- Acoustic waves $\rightarrow$ integrin/FAK $\rightarrow$ MAPK/ERK, p38 - Upregulation of TGF- β , IGF-1, FGF-2 - Balanced MMP/TIMP expression	- Improved synthesis of collagen type II and proteoglycans. - Reduced pro-inflammatory factors (IL-1, TNF- α), supporting stable tissue growth.	70,71
	Pulsed Electromagnetic Fields (PEMF)	- Electromagnetic fields $\rightarrow$ transmembrane potential alterations - $\uparrow$ Wnt/ β -catenin, A2A receptor (cAMP, PKA) - $\uparrow$ BMPs, PDGF, bFGF	- Amplified chondrocyte proliferation and ECM deposition. - NF- κ B suppression leading to anti-inflammatory environment. - Sturdier cartilage matrix shielding nascent tissue from degradation.	72
	Photobiomodulation Therapy (PBMT/LLLT)	- Red/Near-infrared light $\rightarrow$ $\uparrow$ cytochrome c oxidase, ATP - $\uparrow$ Antioxidants (SOD, GPx) - $\downarrow$ NF- κ B-driven inflammation	- Improved mitochondrial function fueling ECM protein synthesis. - Diminished ROS levels safeguarding cartilage matrix. - Downregulation of inflammatory mediators, aiding chondrocyte preservation.	73,74
	Cryotherapy (pressotherapy)	- Cold exposure $\rightarrow$ $\downarrow$ prostaglandins, leukotrienes - Regulation of heat shock proteins (HSPs)	- Lowered edema/inflammation, protecting energy reserves for ECM repair. - HSP-mediated stabilization of protein folding and chondrocyte viability.	75,76
	Extracorporeal Shockwave Therapy (ESWT)	- High-energy acoustic pulses $\rightarrow$ NO release - ERK1/2, p38 MAPK activation - $\uparrow$ Angiogenic (VEGF), ECM (collagen II, aggrecan)	- Stimulated neovascularization and nutrient delivery. - Triggering of anabolic gene programs for robust cartilage regeneration. - Suppressed chronic inflammation and improved remodeling.	77,78
	Synergy with Stem Cells/Gene Therapy	- MSCs + LIPUS/PEMF $\rightarrow$ $\uparrow$ SOX9, ECM - Gene edits for TGF- β , IGF-1, IL-1Ra	- Enhanced chondrogenesis under mechanical/electromagnetic stimulation. - Long-term, targeted expression of anabolic or anti-inflammatory factors.	79_83
5. Blood Flow Restriction (BFR) Training	Localized Hypoxia & Metabolic Stress	- Venous occlusion $\rightarrow$ hypoxia, metabolite buildup (lactate, H $^+$) - Inflammatory surge: IL-6, TNF- α $\rightarrow$ JAK-STAT, NF- κ B - M2 macrophage shift	- Controlled inflammatory environment clears debris, fosters ECM remodeling. - Enhanced anabolic cytokines promote chondrocyte survival and matrix synthesis. - M2 macrophages protect new cartilage from excessive MMP activity.	[118]-[123]
	HIF-1 α Stabilization & Angiogenesis	- PHD inhibition under hypoxia $\rightarrow$ HIF-1 α accumulation - $\uparrow$ VEGF, $\uparrow$ eNOS $\rightarrow$	- Encourages vascular responses benefiting subchondral bone flow. - Shields chondrocytes in avascular cartilage zones,	[124], [125]

Section	Focus / Key Concepts	Molecular Pathways & Mechanisms	Cellular/ECM Outcomes & Clinical Impact	References
		angiogenesis, vasodilation - HIF-1 α also maintains chondrocyte viability in low O ₂	coordinating collagen/ proteoglycan production.	
	Anabolic Hormone Secretion & PI3K-AKT-mTORC1	- IGF-1 (including mechano growth factor, MGF) $\rightarrow$ IGF-1R - Activation of PI3K $\rightarrow$ Akt $\rightarrow$ mTORC1 - Phosphorylation of p70 S6 kinase, 4E-BP1 $\rightarrow$ $\uparrow$ cap-dependent translation	- Heightened protein synthesis in muscle, tendon, and possibly joint tissues. - Reinforced skeletal support around the microfracture site; improved local environment for cartilage repair.	[126]-[128]
	Type II Fiber Recruitment & Strength Preservation	- Hypoxia + metabolite accumulation $\rightarrow$ activation of group IV afferent fibers - Augmented central motor drive recruits higher-threshold motor units	- Helps maintain or grow fast-twitch muscle fibers under low-load conditions. - Shields fragile cartilage from high-intensity forces while preserving strength gains.	[129]-[131]
	Fibrinolysis & Endothelial Protection	- tPA release from Weibel-Palade bodies - Plasmin formation degrades fibrin - Limits DVT risk and clears provisional matrices	- Active fibrinolysis prevents excessive clot formation while aiding tissue remodeling. - Balanced vascular homeostasis with minimal thrombotic complications.	[132]-[138]

or TNF- α binding to their respective receptors), NF- κ B translocates to the nucleus and upregulates genes involved in inflammation, immune cell chemotaxis, and early tissue breakdown (e.g., matrix metalloproteinases, MMPs). ROS act both as signaling molecules and antimicrobial agents; however, excessive ROS can degrade collagen and proteoglycans if not balanced by endogenous antioxidants. Cryotherapy with compression helps to temper the vasodilatory response and reduce the production of pro-inflammatory mediators, while NSAIDs (when not contraindicated) can dampen NF- κ B-driven pathways.⁸⁹ Manual therapy at this stage often focuses on pain control and maintenance of joint mobility, preventing deleterious adhesions and facilitating subsequent phases of healing.

As the process advances to the fibroblastic stage, molecular cues pivot toward growth and proliferation. Key growth factors—Transforming Growth Factor-beta 1 (TGF- β 1), Bone Morphogenetic Proteins (BMPs), and Connective Tissue Growth Factor (CTGF)—are central players in activating fibroblasts and chondroprogenitor cells.⁹⁰ When TGF- β 1 binds its type I/II serine/threonine kinase receptors, it triggers phosphorylation of SMAD2/3, which then forms a transcriptionally active complex with SMAD4 in the nucleus. This SMAD complex promotes genes linked to cell cycle progression, proteoglycan synthesis (e.g., aggrecan), and collagen deposition. BMPs likewise engage SMAD1/5/8, as well as non-SMAD pathways (such as p38 MAPK), further stimulating fibroblastic proliferation and matrix production.⁹¹ Vascular endothelial growth factor (VEGF), which is

also upregulated, drives angiogenesis, ensuring that regenerating tissues gain sufficient oxygen and nutrients. The provisional ECM, consisting mainly of fibrin, fibronectin, collagen type III, and other structural proteins, provides the scaffold into which fibroblasts migrate and deposit new matrix components, particularly collagen type I in early scar formation. Rehabilitation interventions—such as electrical stimulation, laser therapy, ultrasound, pulsed electromagnetic field therapy (PEMF), extracorporeal shock wave therapy (ESWT), and isometric or blood flow restriction (BFR) exercise—enhance fibroblastic activity through various mechanisms. For example, ultrasound and ESWT can intensify integrin-mediated mechanotransduction, boosting local release of TGF- β and BMPs, while BFR harnesses localized hypoxia and metabolic stress to drive an anabolic hormonal response.⁹² These interventions collectively enhance matrix deposition and fiber alignment, crucial for achieving a mechanically robust tissue as healing progresses.

In the remodeling stage, the newly formed scar tissue undergoes a finely tuned transformation into a more mature and stable matrix. This phase depends on the interplay of matrix metalloproteinases (MMPs) and their antagonists, tissue inhibitors of metalloproteinases (TIMPs), to selectively degrade disorganized matrix components while preserving and refining well-aligned collagen fibrils.^{93,94} On a molecular level, fibroblasts and myofibroblasts continue to produce collagen (particularly collagen type I transitioning toward type II in chondral repair), proteoglycans, and

other ECM molecules. The downregulation of inflammatory markers (e.g., NF- κ B, IL-1, TNF- α) during this phase aligns with an upregulation of chondrogenic genes such as COL2A1 (collagen type II) and ACAN (aggrecan), which are indispensable for restoring the functional properties of hyaline cartilage.⁹⁵ Epigenetic factors can also play a role, as post-translational modifications of histones (e.g., acetylation, methylation) modulate the expression of tissue-specific genes. Proper mechanical loading is imperative in guiding fiber realignment and tensile strength development; insufficient load can yield disorganized matrix, whereas excessive load may induce secondary inflammation or mechanical failure.

Therapeutic strategies in the remodeling stage focus on safely reloading the joint through manual therapy (joint and soft tissue mobilization) and progressively challenging neuromuscular function, flexibility, and cardiovascular fitness.⁹⁶ Exercises that increase range of motion (ROM), proprioception, and motor control are integrated to ensure the maturing cartilage adapts to physiologic demands without overloading. Advanced biologic interventions—such as platelet-rich plasma (PRP) injections or stem cell therapy—may supply additional anabolic signals (e.g., TGF- β , PDGF, IGF-1) and immunomodulatory factors (e.g., IL-10, HGF), accelerating the remodeling process and potentially improving the longevity of the repaired cartilage.⁹⁷ These cellular therapies can also upregulate SOX9, a transcription factor critical for chondrocyte differentiation and maintenance of cartilage-specific gene expression.

Overall, the integration of molecular biology with rehabilitation strategies provides the roadmap for orchestrating inflammation, promoting ECM synthesis, and achieving meaningful cartilage regeneration. Each stage hinges on a balance of pro-inflammatory versus anabolic cues: mild to moderate inflammation is vital for debris clearance and immune surveillance, whereas growth factors and mechanical stimuli drive fibroblasts and chondrocytes to rebuild a durable matrix.⁹⁸ By recognizing the molecular hallmarks—NF- κ B and ROS activity in inflammation, SMAD-mediated gene expression in fibroblastic proliferation, and MMP-TIMP equilibrium during remodeling—clinicians can tailor modalities to support tissue progression at every step. Such a targeted approach maximizes healing outcomes, conferring more robust structural integrity and better long-term function of the regenerated cartilage.

CONCLUSION

A comprehensive understanding of the molecular mechanisms underlying cartilage repair following microfracture is essential for developing effective rehabilitation strategies. Cartilage repair is a complex and multifaceted process that involves a delicate balance of cellular activities, signaling pathways, and extracellular matrix (ECM) remodeling. Early mobilization, biological augmentation, nutritional support, and advanced therapeutic modalities collectively contribute to the successful formation of functional cartilage.⁹⁹⁻¹⁰¹

Early mobilization and controlled mechanical loading are crucial for stimulating the biological processes involved in cartilage repair. Mechanical forces activate mechanotransduction pathways, such as integrins and ion channels, which convert physical stimuli into biochemical signals. These signals promote chondrocyte proliferation and ECM synthesis, essential for the structural integrity of the newly formed cartilage. Understanding the molecular pathways involved in mechanotransduction, such as the ERK1/2 and p38 MAPK pathways, allows clinicians to design effective rehabilitation programs that optimize cartilage healing and improve patient outcomes.¹⁰²

Biological augmentation with agents like platelet-rich plasma (PRP) and hyaluronic acid (HA) enhances the local biochemical environment, promoting chondrocyte activity and ECM production. PRP contains a high concentration of growth factors, including TGF- β and IGF-1, which stimulate cell proliferation and matrix synthesis.^{103,104} HA provides a supportive scaffold for cell migration and ECM formation, mimicking the natural cartilage environment and promoting chondrogenesis. These biological therapies modulate the expression of key genes and signaling pathways involved in cartilage repair, creating a conducive environment for tissue regeneration.⁹¹

Nutritional support plays a vital role in cartilage health and repair. Adequate intake of amino acids, vitamins, and minerals is essential for collagen synthesis and overall cartilage maintenance. Nutritional supplements like glucosamine and chondroitin sulfate provide the building blocks necessary for the synthesis of new cartilage. Antioxidants such as vitamins C and E protect cartilage cells from oxidative stress, while omega-3 fatty acids modulate the inflammatory response, reducing damage to the cartilage and supporting its repair. Understanding the molecular mechanisms through which these nutrients influence cartilage biology can inform dietary recommendations and supplementation strategies to enhance the outcomes of cartilage repair procedures.¹⁰⁵

Advanced therapeutic modalities such as low-intensity pulsed ultrasound (LIPUS), pulsed electromagnetic fields (PEMF), photobiomodulation therapy, and cryotherapy offer promising adjunctive treatments for cartilage repair. These modalities influence cellular activities and signaling pathways that promote tissue regeneration and reduce inflammation. For example, LIPUS and PEMF activate pathways such as the ERK1/2 and Wnt/ β -catenin signaling pathways, enhancing chondrocyte proliferation and ECM synthesis. Photobiomodulation therapy improves mitochondrial function and ATP production, while cryotherapy modulates inflammatory cytokines and heat shock proteins. Integrating these advanced therapies into rehabilitation programs can optimize the healing environment and enhance the regenerative processes initiated by the microfracture technique.¹⁰⁶

Ongoing research and clinical trials are essential for refining these approaches and developing new strategies for cartilage repair. Advances in molecular biology and bioengineering are continually expanding our understanding of the mechanisms underlying cartilage regeneration.

Table 3. Bounding natural process of cartilage healing with rehabilitation strategies.

Healing Stage	Cellular Phase	Biophysical Characteristics	Therapeutic Intervention
Inflammation Stage	Vasodilation & Immune Cell Influx: Platelets, neutrophils, monocytes, and macrophages migrate to the injury site under the influence of chemical signals such as histamine, bradykinin, prostaglandin E2 (PGE2), and interleukin-1 (IL-1). These mediators elevate local blood flow and vascular permeability. NF-κB Activation: Inflammatory cytokines (IL-1, TNF-α) activate NF-κB, which upregulates genes involved in further immune recruitment (e.g., chemokines) and matrix metalloproteinases (MMPs) for initial debris clearance. Platelet-Derived Growth Factor (PDGF): Platelets release PDGF upon degranulation, promoting local fibroblast and chondrocyte activation, setting the stage for subsequent repair phases.	Clinical Symptoms: Swelling, erythema, warmth, and pain arise from vasodilation and increased vascular permeability. Provisional Clot Formation: A fibrin-rich clot fills the defect, stabilizing the injury site and temporarily bridging the damaged cartilage. Microenvironment: Elevated cytokines, ROS, and growth factors that prime local tissues for immune surveillance and early tissue debridement.	- Cryotherapy with Compression: Reduces local inflammation and edema by constricting vessels, mitigating excessive NF-κB activation, and limiting extravascular fluid accumulation. - NSAIDs (if not contraindicated): Dampen cyclooxygenase activity and reduce PGE2 synthesis, partially modulating the inflammatory cascade. - Manual Therapy and Isometrics Loading: Light soft tissue mobilization or gentle joint mobilization with isometric mechanical loading to maintain mobility while avoiding stress on the nascent fibrin clot.
Fibroblastic Stage	Growth Factor-Mediated ECM Synthesis: TGF-β1, Bone Morphogenetic Proteins (BMPs), and Connective Tissue Growth Factor (CTGF) prime fibroblasts and chondroprogenitor cells. - TGF-β1 activates SMAD2/3 → SMAD4 translocation to the nucleus, driving gene transcription for collagen, fibronectin, and proteoglycan synthesis. - BMPs engage SMAD1/5/8 and sometimes MAPKs (p38, ERK), further enhancing fibroblast proliferation and early cartilage matrix production. - VEGF fosters angiogenesis, improving blood supply. Inflammatory Markers: Although inflammation is generally subsiding, cytokines and chemokines still exist at moderate levels, helping regulate fibroblast and chondrocyte activity.	ECM Deposition: Rapid collagen (type I predominantly at first), fibronectin, and proteoglycans accumulate, providing structural scaffolding. Increased Tensile Strength: Scar tissue begins to gain mechanical strength through better-organized collagen fibers. Transition from Inflammatory to Anabolic: The local environment remains partially inflamed but increasingly dominated by anabolic signals promoting robust tissue formation.	- Manual Therapy: Gentle passive and active range-of-motion (ROM) exercises, soft tissue mobilization, and joint mobilization to ensure adequate fiber alignment and prevent adhesions. - Biophysical Modalities: Electrical stimulation, laser therapy, ultrasound, PEMF (pulsed electromagnetic field therapy), ESWT (extracorporeal shock wave therapy), and BFR (blood flow restriction) training. These techniques augment fibroblastic proliferation and ECM synthesis (e.g., ultrasound and ESWT amplify growth factor release via mechanotransduction). - Therapeutic Exercises: Slow eccentric or isometric exercises to stimulate collagen alignment and enhance muscle support around the healing cartilage without excessive load.
Remodeling Stage	Collagen Maturation & Myofibroblast Activity: Fibroblasts and myofibroblasts refine and reorganize ECM components (transitioning collagen type I → type II in chondral repair) under the influence of balanced MMP-TIMP activity. - MMPs degrade disorganized ECM, while TIMPs inhibit excessive MMP-driven breakdown.	Mechanical Properties: The evolving scar/matrix becomes stiffer and more resilient, with increasingly aligned collagen fibrils. Tissue Maturation: Transition toward functional integrity of the cartilage with improved load-	- Manual Therapy: Patient-specific protocols for joint mobilization, scar mobilization, and comprehensive assessments to address residual stiffness or malalignment. - Therapeutic Exercises: Emphasis on progressive active ROM, flexibility, muscle strength and endurance, proprioception, motor control, and cardiovascular fitness. Exercises are often advanced to dynamic weight-bearing and function-specific training. - Advanced Biologics (PRP, Stem Cells): Can be

Healing Stage	Cellular Phase	Biophysical Characteristics	Therapeutic Intervention
	- Downregulation of pro-inflammatory cytokines (IL-1, TNF-α) and upregulation of cartilage-specific genes (e.g., COL2A1, ACAN) define the shift to stable cartilage matrix. Reduced Inflammatory Markers: The environment becomes more anabolic as catabolic signals wane and chondrocytes produce definitive ECM.	bearing capacity and tensile strength. Potential Residual Pain: Pain at this stage often indicates either an underlying degenerative process (e.g., osteoarthritis), re-damage, or delayed-onset muscle soreness (DOMS) rather than acute inflammation.	used to intensify anabolic signaling (e.g., TGF-β, PDGF) and foster late-stage cartilage regeneration and mechanical resilience if indicated.

Techniques such as stem cell therapy and gene editing offer new possibilities for enhancing cartilage repair by providing a source of progenitor cells or modulating the expression of therapeutic genes. As these technologies evolve, they hold the potential to significantly improve the efficacy of cartilage repair interventions and patient outcomes.^{107, 108}

In conclusion, a multidisciplinary approach that incorporates early mobilization, biological augmentation, nutritional support, and advanced therapeutic modalities is critical for optimizing cartilage repair following microfracture surgery. By leveraging insights from molecular biology,

clinicians can develop targeted and effective treatment plans that enhance the body’s natural healing processes. This integrated approach not only addresses the immediate needs of the repair process but also promotes long-term joint health and function. Continued research and innovation in this field will pave the way for improved therapies and better quality of life for patients undergoing cartilage repair procedures.

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