

Anti-Inflammatory Strategies in Cartilage Repair

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Cartilage defects are normally concomitant with posttraumatic inflammation and pose a major challenge in cartilage repair. Due to the avascular nature of cartilage and its inability to surmount an inflammatory response, the cartilage is easily attacked by proinflammatory factors and oxidative stress; if left untreated, osteoarthritis may develop. Suppression of inflammation has always been a crux for cartilage repair. Pharmacological drugs have been successfully applied in cartilage repair; however, they cannot optimally work alone. This review article will summarize current pharmacological drugs and their application in cartilage repair. The development of extracellular matrix-based scaffolds and preconditioned tissue-specific stem cells will be emphasized because both of these tissue engineering components could contribute to an enhanced ability not only for cartilage regeneration but also for anti-inflammation. These strategies could be combined to boost cartilage repair under inflammatory conditions.

Introduction

JOINT INJURIES ARE COMMON in the young and active population and often result in cartilage or osteochondral lesions. If left untreated, these defects might lead to joint swelling and pain, eventually progressing toward osteoarthritis (OA). Over 27 million Americans are affected by OA, introducing huge clinical and socioeconomic burdens.¹ Traditional cartilage repair methods include the transplantation of osteochondral grafts,² microfracturing, and autologous chondrocyte implantation³; however, none of these cartilage repair strategies have generated long-lasting hyaline cartilage that meets functional demands. The causes of cartilage impairment are diverse, including inflammation, hypertrophy, and senescence (Fig. 1).^{4,5} Excessive mechanical surface contact stress can directly damage articular cartilage and subchondral bone and adversely alter chondrocyte function,⁶ while the disruption of the homeostasis of chondrocytes may gradually develop into OA.⁷ In the early phase of OA, disease-modifying interventions targeting inflammatory processes might be most efficacious for the prevention and treatment of OA.⁸ To this end, many anti-inflammatory strategies have been discovered, such as growth factor applications, exertion of anticytokines or anti-inflammatory drugs, and stem cell-based therapies.

Growth factors are utilized to improve clinical cartilage repair by altering the local biological environment at the site of cartilage damage. Inflammation at the damage site may disrupt the balance between catabolic and anabolic factors; growth factors that target specific catabolic proinflammatory mediators, such as cytokines or nitric oxide synthase (NOS), or affect anabolism are potential candidates in slowing down

the structural progression of the disease. The production and activities of many proinflammatory factors, such as cytokines, chemokines, growth factors, and various immune response regulators, are controlled by different signaling systems, such as nuclear factor-kappa B (NF- κ B), mitogen-activated protein kinases (MAPK), and Janus kinase/signal transducers and activators of transcription (JAK/STAT). Novel small-molecule regulators targeting specific signal pathways as well as related precursor molecules have received a great deal of attention as potential candidates for treatment of inflammatory diseases (Fig. 2).

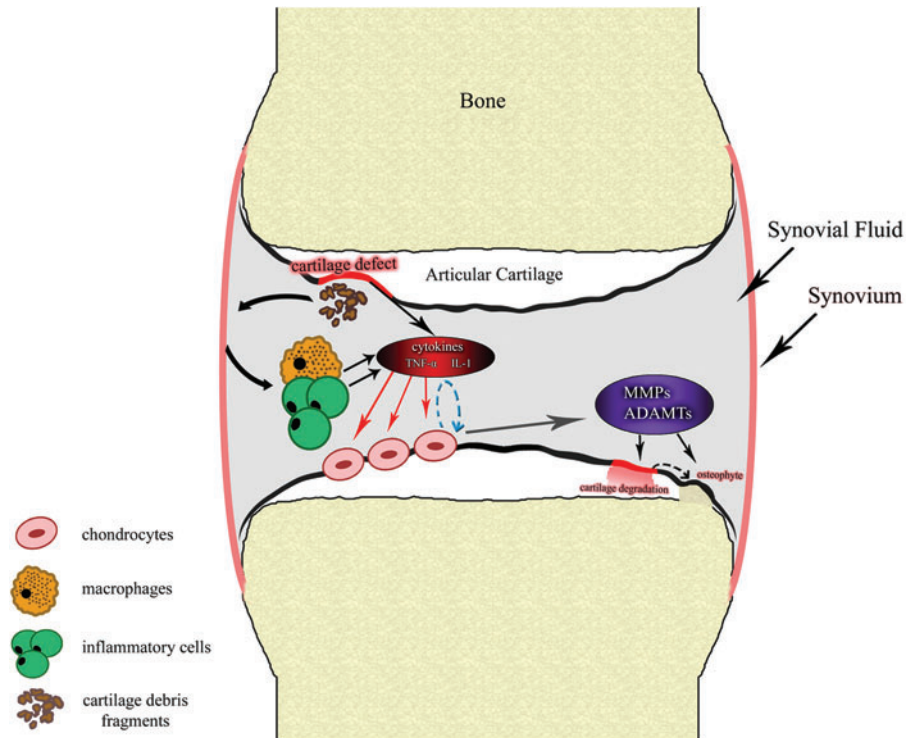
The development of stem cell technology provides the possibility of biotherapy for cartilage repair (Fig. 3). The availability of large quantities of mesenchymal stem cells (MSCs) and their multilineage differentiation potential, especially for chondrogenic differentiation, has made MSCs the ideal progenitor source for cartilage engineering and regeneration. Cell-based therapies using undifferentiated or prechondrogenic stem cells in biodegradable three-dimensional (3D) scaffolds for transplantation into focal lesions could regenerate hyaline-like cartilage.^{9,10} Since bioengineered cartilage constructs will eventually be transplanted into arthritic joints in which elevated levels of proinflammatory cytokines exist, it is especially important to select scaffolds that support the stability of bioengineered cartilage in an inflammatory environment. To this end, many extracellular matrix (ECM)-based scaffolds are hypothesized to meet the requirements of cartilage repair (Fig. 3).

Recently, the decellularized ECM deposited by stem cells (DSCM) has attracted attention due to its excellent rejuvenation of expanded stem cells' chondrogenic potential, which has been reviewed.¹¹ Furthermore, DSCM-expanded

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FIG. 1. Inflammation in cartilage repair. Abnormally high contact stresses such as mechanical overload transmitted to focal areas of articular cartilage result in cartilage defects and release cartilage fragments. This process stimulates the synovial membrane, leading to the activation of macrophages and inflammatory cells such as T cells, which produce interleukin-1 β (IL-1 β) and tumor necrosis factor alpha (TNF α). In an autocrine–paracrine manner, these activated inflammatory factors may stimulate chondrocytes to secrete degradative enzymes like proteinases, such as matrix metalloproteinases (MMPs) and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS), which are directly involved in degradation of type II collagen and aggrecans in cartilage matrix. In the meantime, chondrocytes can change phenotype and size in response to stimulation from inflammatory factors and undergo hypertrophy, which is an essential step in the endochondral ossification process. Color images available online at www.liebertpub.com/teb



human synovium-derived stem cells (SDSCs) demonstrated *in vitro* antioxidant and anti-inflammatory capabilities.¹² A study in mini pigs successfully proved its resurfacing effect on partial-thickness cartilage defects after intra-articular injection of DSCM-expanded allogeneic SDSCs.¹³ In this review article, the strategies using pharmacologic drugs, biomechanical stimulation, tissue-specific stem cells, and ECM-based scaffolds are summarized. An emerging strategy using a DSCM preconditioning approach to rejuvenate tissue-specific stem cells in both cell amount and chondrogenic potential is emphasized for its vital role in cartilage repair and anti-inflammation (Fig. 3). Future goals include the utilization of 3D ECM scaffolds, in conjunction with other combined strategies, including the use of preconditioned tissue-specific stem cells in a more favorable microenvironment to reduce the inflammation in cartilage repair.

Pharmacologic Strategy

Growth factors

In articular cartilage, numerous growth factors work in concert throughout life to regulate the development and homeostasis of articular cartilage.¹⁴ Disruption in the balance of regulatory factors may hinder tissue maintenance and repair, ultimately resulting in reduced synthesis of ECM, tissue degeneration, and consequently, an accelerated erosion of the articular surface.¹⁵ Bioactive growth factors are considered promising candidates for enhanced healing of chondral injuries and modification of the arthritic disease process. Members of the transforming growth factor beta (TGF- β)/bone morphogenetic protein family, insulin-like

growth factor-I (IGF-I), and fibroblast growth factors (FGFs) are considered to be major anabolic factors for cartilage formation. They may stimulate chondrocyte synthesis of proteoglycans, aggrecan, and type II collagen, inducing cell proliferation, driving stem cell chondrogenic differentiation, and decreasing the catabolic effects of cytokines.^{16,17} Overexpression of the growth factor progranulin (PGRN), for example, has been implicated in the stimulation of chondrocyte proliferation; PGRN also acts as a physiological antagonist of tumor necrosis factor alpha (TNF α) signaling and disturbs the binding of TNF α and TNF receptor,¹⁸ potentially inhibiting cartilage degradation.¹⁹ Cytokines such as TNF α can stimulate vascular endothelial growth factor expression.²⁰ Therefore, some anti-inflammatory strategies inhibit inflammation-induced angiogenesis although it remains unclear to what extent angiogenesis inhibition mediates their therapeutic effects.²¹

By exerting influence over inflammation, growth factors can modulate the microenvironment of chondrocytes to improve the local residence and provide a more ideal atmosphere for cartilage regeneration. For instance, growth factors such as platelet-derived growth factor (PDGF) and TGF- β obtained from platelet-rich plasma (PRP) decreased interleukin 1 beta (IL-1 β)-induced NF- κ B activation, a major pathway involved in the pathogenesis of OA (Fig. 2).^{22,23} Furthermore, in activated PRP, Bendinelli *et al.* observed increases in hepatocyte growth factor (HGF), IL-4, and TNF α ; HGF and TNF α , by disrupting NF- κ B-transactivating activity through the enhanced cellular I κ B α expression, were important for the anti-inflammatory function of activated PRP.²⁴ Paradoxically, platelet lysate (PL), a PRP derivative,

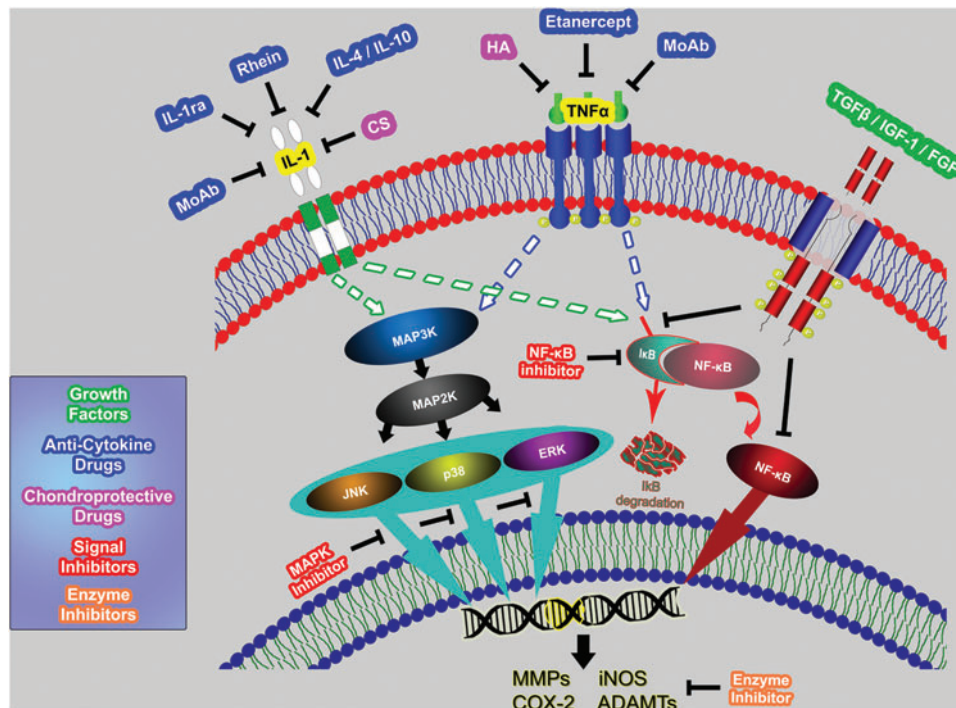


FIG. 2. Schematic representation of key signaling pathways in the inflammatory process and potential strategies for inflammatory inhibition in cartilage repair. Use of anti-cytokine and chondroprotective drugs against IL-1, TNF α , or against their receptors is a direct and effective approach in the suppression of inflammation. Strategies targeting disturbance or intervention of key signal pathways in cytosol have been developed such as blocking the mitogen-activated protein kinases (MAPK) signal pathways or the nuclear factor-kappa B (NF- κ B) pathway. Using inhibitors for downstream products of degradative enzymes, such as MMPs, cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), and ADAMTS, can also effectively protect cartilage from degradation and inflammation. Growth factors such as fibroblast growth factor (FGF)-2, insulin-like growth factor (IGF)-I, or transforming growth factor (TGF)- β may interfere with inflammation by blocking the associated signal pathways. Use of growth factors is a possible anti-inflammatory strategy in cartilage repair despite the fact that growth factors can promote neocartilage formation. Color images available online at www.liebertpub.com/teb

was thought to play a role as a proinflammatory agent, acting synergistically with the canonical proinflammatory cytokines such as IL-1 α , thus enhancing the initial inflammatory response; surprisingly, PL also contributes to the downregulation of the NF- κ B signal pathway and cyclooxygenase 2 (COX-2) expression, thus triggering the resolution of the inflammation.²⁵ The anti-inflammatory role of PRP was also demonstrated in an *in vivo* study. In the antigen-induced arthritis porcine model, the intra-articular injection of PRP attenuated the subsequent inflammatory response.²⁶ The use of PRP may also improve the integration of an osteochondral graft at the cartilage interface and decreased degeneration in an *in vivo* rabbit model.²⁷

The strategy combining growth factors and 3D scaffolds demonstrated a huge impact on tissue regeneration. Because of PRP's prominent role in anti-inflammation and fewer immunogenic as well as more biocompatible characteristics, the cell-free polyglycolic acid (PGA)-hyaluronan scaffold combined with PRP led to cartilage repair and improved patient-reported outcomes (the Knee injury and Osteoarthritis Score, KOOS) during 12 months of follow-up.²⁸ Moreover, TGF- β , delivered together with calcium alginate to the sites of osteochondral defects, improved the repair of osteochondral defects in the rabbit knee.²⁹ However, the local production of osteophytes has been observed in clinical trials; therefore, caution is advised because some growth

factors favor the dedifferentiation of stem cells and promote the endochondral ossification process.³⁰

Anticytokine therapy

Like growth factors, cytokines can also be produced in joint tissues and released into the synovial fluid; they influence the surrounding cells in an autocrine-paracrine manner (Fig. 1). Low levels of factors are necessary for normal homeostasis; however, inflammatory or oxidative stress conditions may disrupt normal homeostasis, driving the pathogenesis of OA. Among the vast number of cytokines, IL-1 β and TNF α seem quite prominent and of major importance to cartilage destruction.³¹ Treatment strategies targeting major inflammatory factors have been developed (Fig. 2). Specific inhibitors of production/activity of IL-1, such as recombinant human IL-1 receptor antagonist (IL-1ra), can block the actions of IL-1 without any detectable agonist activity.³² The use of monoclonal antibodies against IL-1 or type I IL-1 receptor (IL-1RI) represents another possible approach in neutralization of the cytokine.³³ Pharmacologic reagents, which downregulate the production and activity of active proinflammatory and procatabolic IL-1 β , are also a feasible approach.³⁴ One such example is to apply the rhein, an active metabolite of the semisynthetic anthraquinone derivative diacerein, to downregulate the production and activity

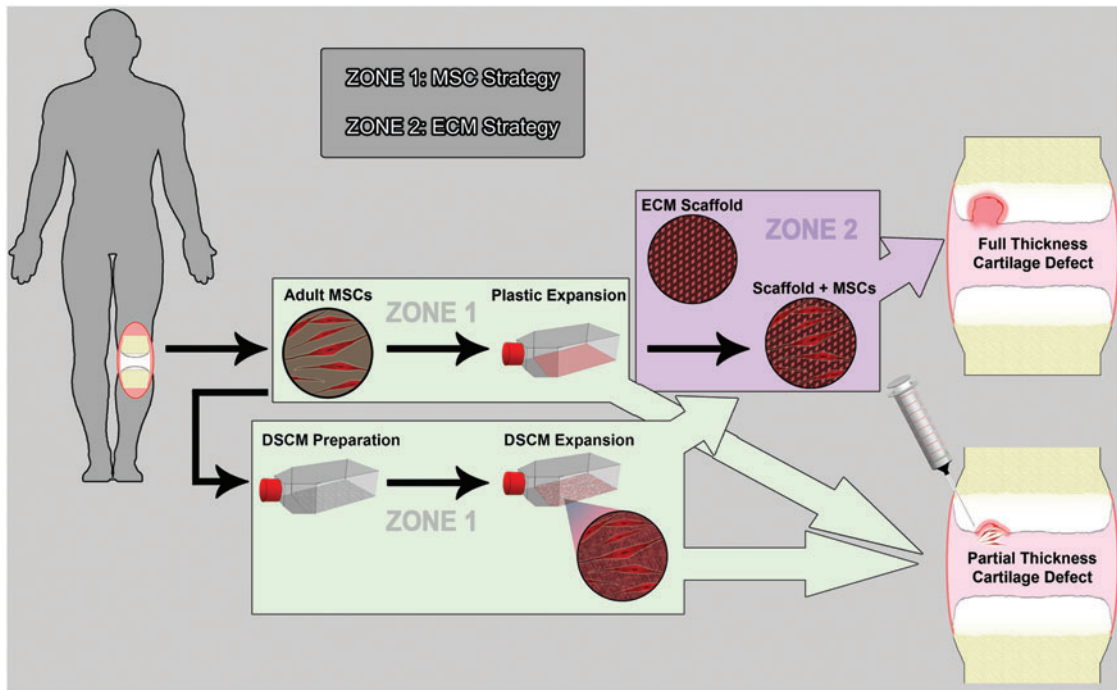


FIG. 3. Therapeutic strategies for cartilage damage utilizing preconditioned stem cell and three-dimensional (3D) matrix. After *ex vivo* expansion of adult stem cells extracted from the human body, two strategies can be used for the treatment of cartilage defects: direct intra-articular injection or transplant of cells in a 3D scaffold. For full-thickness cartilage defects, the second strategy is normally used. Three-dimensional scaffolds can be made of extracellular matrix (ECM) materials. A structurally and mechanically stable scaffold allows for infiltration and attachment of bioactive molecules (IGF-I, FGF-2, and/or TGF- β), which can exert their anti-inflammatory effects on the surrounding environment. For partial-thickness cartilage defects, direct intra-articular injection of expanded stem cells is an option. Mesenchymal stem cells can secrete growth factors, which may exert their anti-inflammatory and antioxidative effects in the microenvironment where mesenchymal stem cells (MSCs) reside. These growth factors also benefit tissue regeneration. A recent finding suggests that decellularized stem cell matrix (DSCM) could rejuvenate expanded MSCs in proliferation and chondrogenic potential; this process may provide a large quantity of high-quality MSCs for cell-based cartilage regeneration. DSCM-expanded MSCs exhibiting an enhanced capacity against oxidation and inflammation may represent a promising anti-inflammatory strategy in the near future. Color images available online at www.liebertpub.com/teb

of IL-1 β in both the cartilage layer and synovial membrane.³⁵ Etanercept, a recombinant soluble p75 TNF receptor, has a high affinity for TNF α , preventing it from binding with its receptor.³⁶ A recent report indicates that etanercept enhanced preservation of osteochondral allograft viability.³⁷ An *in vivo* study showed that subcutaneous injection of etanercept promoted repair of osteochondral defects in the rabbit knee.³⁸ Application of the anti-TNF α monoclonal antibody in polyarthritic transgenic mice demonstrated reversal of cartilage degradation in the young mice.³⁹

Besides IL-1 β and TNF α , proinflammatory cytokines, such as IL-6,⁴⁰ leukemia inhibitory factor,⁴¹ and the chemokine IL-8,⁴² may modulate the direct catabolic effects of some cytokines. They usually synergize with TNF α or IL-1 β in the catabolic process of cartilage.⁴³ Interference with those proinflammatory factors is another direction for anti-inflammatory therapy in cartilage repair. A number of anti-inflammatory cytokines, such as IL-4, IL-10, IL-11, and IL-13, were found in increased levels in the synovial fluid of OA patients.⁴⁴ They decrease the production and/or activity of the proinflammatory cytokines *in vitro*⁴⁵ and, thus, have been classified as anti-inflammatory cytokines. Endogenous IL-4 and IL-10 have been shown not only to reduce local IL-

1 but may also have direct stimulatory or protective effects on chondrocyte metabolism.^{46,47} Since the early phase of OA is the most effective period to inhibit inflammation, administration of cytokine antagonists following cartilage repair is a safe strategy for not only promotion of cartilage integration but also the prevention of inflammation occurrence.

Chondroprotective drugs

Among pharmacological treatments, symptomatic slow-acting drugs have been largely studied over the last decade. Recently, there has been an increase in the use of symptomatic slow-acting/chondroprotective drugs such as glucosamine sulfate (GS), chondroitin sulfate (CS), hyaluronic acid (HA), and diacerein. Glucosamine decreased the activation of NF- κ B in rat chondrocytes when treated with IL-1 β .⁴⁸ In normal human articular chondrocytes, GS inhibited IL-1 β and TNF α -induced nitric oxide (NO) production, which is a major contributor for the inflammatory reaction in arthritis.⁴⁹ Natural sulfated glycosaminoglycan (GAG), in particular CS, seemed to have beneficial effects on the pathophysiology of OA by reducing blood markers of inflammation and the activity of destructive proteases like matrix metalloproteinases

(MMPs), diminishing pain as well as improving the function of the affected joint.^{50–52} In chondrocytes, CS diminished IL-1 β -induced increases in p38 MAPK and extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation, and decreased NF- κ B nuclear translocation and as a consequence, reduced the formation of proinflammatory cytokines, IL-1 β and TNF α , and proinflammatory enzymes, such as phospholipase A2 (PLA2), COX-2, and inducible NOS (iNOS).⁵³ However, other reports suggested that chondroitin-4-sulfate inhibited the enhanced expression of COX-2 and prostaglandin E (PGE) synthases 1, but had no effect on the IL-1 β -induced decrease of I κ B α and nuclear translocation of NF- κ B⁵⁴; the extent of sulfation influenced the responsiveness of inflammation.⁵⁵

Synthetically sulfated HA was at first characterized as an inhibitor of TNF α .⁵⁶ HA is an anionic nonsulfated GAG that acts as a crucial structural component of the ECM and as an important mediator of leukocyte adhesion and migration.⁵⁷ HA is widely used in the treatment of OA and exerts significant chondroprotective effects. An *in vitro* report showed that HA altered the profile of inflammatory mediators, shifting the balance between cell matrix synthesis and degradation.⁵⁸ An *in vivo* rabbit study showed that three weekly intra-articular injections of HA had a positive effect on the repair tissue that formed within the chondral defect at the early follow-up time point.⁵⁹ CS, diacerein, GS, and HA demonstrated pain reduction and physical function improvement with very low toxicity in OA treatment and could be of potential interest for the symptomatic management of OA.⁶⁰ Slow-acting drugs avoid interrupting the normal cellular function and toxicity to cells and, thus, could provide an anti-inflammatory strategy for cartilage repair (Fig. 2). However, high concentrations of chondroprotective drugs are not presumably attained through oral administration; intra-articular injections may represent a feasible and effective approach.⁶¹ With the application of tissue engineering in cartilage repair, slow-acting drugs serve as a basic unit of a 3D scaffold and, together with stem cells, demonstrated the potential of modulating inflammatory chemokines/receptors and catabolic/inhibiting factors.⁶²

Small molecules targeting inflammation signals

Strategies for generating functional small molecules of synthetic and natural products targeting inflammatory signals have been considered effective in the suppression of inflammation.⁶³ Primary proinflammatory factors IL-1 β and TNF α have the capacity to activate a diverse array of intracellular signaling pathways; upon activation, they can induce phosphorylation-dependent signaling pathways, such as NF- κ B, p38 MAPK, and c-Jun N-terminal kinase (JNK), which regulate the synthesis of several inflammatory cytokines and MMPs, many of which play major roles in the process of OA formation (Fig. 2).⁶⁴

The NF- κ B signaling pathway mediates critical events in the inflammatory response by chondrocytes, leading to progressive ECM damage and cartilage destruction. There are an increasing number of reports about NF- κ B inhibitors, such as glucocorticoids, cyclosporine A, and tacrolimus (FK-506).⁶⁵ I κ B kinase beta (IKK β) has also become a particularly appealing target because of its crucial role in the activation of the NF- κ B pathway.⁶⁶ A recent report showed

that a novel butanoylated GlcNAc derivative, 3,4,6-O-Bu3GlcNAc, an inhibitor of NF- κ B activity, has the potential to stimulate new tissue production and reduce inflammation in IL-1 β -induced chondrocytes with utility for OA and other forms of inflammatory arthritis.⁶⁷ One concern about inhibiting several of these components of the NF- κ B pathway is the specificity of such drugs. For example, the proteasome, which is responsible for I κ B degradation, has many other important functions. Thus, inhibition of proteasome activity could potentially cause severe side effects. Also, it may not be feasible to block the NF- κ B pathway for prolonged periods, since NF- κ B plays an important role in the maintenance of host defense responses.⁶⁸

Two MAPKs, p38 MAPK and JNK, are frequently activated by a wide range of environmental stresses and cytokines to induce inflammation and joint destruction, hence the name stress-activated protein kinases (SAPKs). p38 MAPK positively regulates the expression of many genes involved in inflammation, such as those coding for TNF α , IL-1 β , IL-6, IL-8, COX-2, and collagenase-1 and -3.⁶⁹ Many small-molecule inhibitors of p38 MAPK such as Cannabidiol,⁷⁰ SB203580, Doramapimod (BIRB-796),⁷¹ VX-702,⁷² RO-3201195,⁷³ and SB-242235⁷⁴ have demonstrated positive anti-inflammatory effects. Among all these p38 MAPK inhibitors currently in clinical trials, SB203580 appears to be the most potent compound in terms of anti-inflammatory activity. For instance, inhibition of the p38 MAPK signaling pathway with SB203580 showed anti-inflammatory effects in both cartilage explants⁷⁵ and animal models.⁷⁶ However, there is a conflicting report showing that inhibition of the p38 MAPK pathway using SB203580 leads to OA-like changes in a rat animal model.⁷⁷ Similarly, the JNKs are activated in macrophages after stimulation with lipopolysaccharides.⁷⁸ Inhibition of the JNK signaling pathway has shown both preventive effects with regard to bone and cartilage destruction in rheumatoid arthritis⁷⁹ and downregulation of IL-1-induced MMP-13 expression in OA chondrocytes.⁸⁰

Small molecules targeting specific enzymes

Small molecules, which target specific enzymes involved in OA, have been developed as a possible way to alleviate inflammation in cartilage repair (Fig. 2). MMPs are synthesized and secreted by chondrocytes in response to stimulants such as IL-1 and TNF.⁸¹ Inappropriate expression of MMP activity constitutes part of the pathogenic mechanism associated with the destruction of cartilage and bone in OA. Possible strategies include impeding the production of MMPs, blocking the active site of MMPs, and increasing the endogenous production of tissue inhibitors of metalloproteinases (TIMPs).⁸²

Another representative member of cyclooxygenase, the COX-2 enzyme, is primarily associated with inflammation. Cytokines and growth factors increase the expression of COX-2, mainly at inflammatory sites, producing prostaglandins that mediate inflammation, pain, and fever.⁸³ Targeting cyclooxygenase, nonsteroidal anti-inflammatory drugs (NSAIDs) such as celecoxib play a major role in the management of inflammation and pain caused by arthritis.^{84,85} However, nonselective NSAIDs cause gastrointestinal complications in a significant number of patients, and

COX-2 inhibitors have recently raised concerns regarding cardiovascular side effects/risks.⁸⁶

The possible roles of NO in OA pathophysiology have been supported by a study showing that selective inhibition of the iNOS could reduce the progression of structural changes in experimental OA in dogs, partly related to a reduction in the levels of synovial inflammation.⁸⁷ Many of these small-molecule compounds have already been launched on the market as drugs acting against inflammatory conditions. For example, leflunomide was found to significantly inhibit IL-4- and IL-13-enhanced production of chemokine (C-C motif) ligand (CCL)-26.⁸⁸

Biomechanical Strategy

Biomechanical factors play an important role in the health of diarthrodial joints. Under normal physiological loading, moderate mechanical loading exhibits little or no wear over decades of use because articular cartilage provides a nearly frictionless surface for the transmission and distribution of joint loads. In reality, moderate loading is necessary to maintain healthy articular cartilage. Through a variety of mechanisms, including ion channels and integrin-mediated connections to the ECM that involve membrane, cytoskeletal, and intracellular deformation, chondrocytes in cartilage may perceive physical signals from the outside environment. Altered joint loading, which might be associated with obesity, malalignment, trauma, or joint instability, is a critical risk factor for joint degeneration and OA occurrence.

Biomechanical influences on cartilage are complex. Depending on the way cartilage is loaded, the effects on cartilage homeostasis can vary. An overload of mechanical stress may lead to acute joint injury such as posttraumatic OA, which is also caused by gradual onset of structural damage and cartilage compositional degradation due to chronic overloading of injured joints.⁸⁹ Cartilage explants subjected to static compression exhibit a significant suppression of metabolic activity that is dependent on the magnitude of applied stress.⁹⁰ A high-magnitude mechanical strain is proinflammatory and initiates cartilage destruction while inhibiting matrix synthesis, both of which are involved in the NF- κ B-related signal pathway.^{91,92}

It has been reported that a low-magnitude mechanical strain inhibits inflammation by suppressing IL-1 β and TNF α -induced transcription of multiple proinflammatory mediators involved in cartilage degradation.⁹¹ It is noteworthy, however, that the influences of mechanical stress are not independent; many mechanical and physiochemical factors that are known to affect chondrocytes are inextricably coupled to one another within the cartilage ECM. Ramachandran *et al.* reported that treatment with C-type natriuretic peptide and dynamic compression increased anabolic activities and blocked catabolic effects induced by IL-1 β .⁹³ For cyclic loading, however, the cartilage reaction is different; low frequencies and amplitudes do not appear to affect biosynthesis rates. Some studies suggested that, above a certain threshold frequency, cyclic loading increases the synthesis of components of ECM, such as aggrecans, cartilage oligomeric matrix protein, and fibronectin.⁹⁰

There is increasing evidence showing that muscle function is closely related to OA. The muscle, lying anatomically adjacent to cartilage, provides the cartilage with a

biomechanical stimulation that promotes nutrient distribution and maintains homeostasis.⁹² Muscle loss⁹⁴ and reduced muscle strength⁹⁵ have been shown to be risk factors for knee OA. On the other hand, patients with knee OA were found to have impaired muscle function; quadriceps, hamstring, and hip muscles are significantly impaired in subjects with knee OA compared with age-matched controls.⁹⁶ Recent studies showed that a rat chondrocyte cell line cocultured with muscle cells or cultured in muscle cell-conditioned medium in a monolayer demonstrated enhanced resistance to proinflammatory factors such as IL-1 β and TNF α , suggesting that nonloading biochemical effects of muscle cells have a significant influence on cartilage homeostasis and a preventative role in OA formation.⁹⁷ Mechanistically, it is known that the proinflammatory cytokines IL-1 β and TNF α are major inflammation initiators in cartilage by inducing the expression of MMPs, promoting chondrocyte hypertrophy, and reducing the synthesis of cartilage matrix genes. However, muscle cell-derived factors or myokines inhibited the mRNA expression of MMPs as well as hypertrophic markers in bioengineered cartilage; it could also rescue IL-1 β -induced chondrocyte growth arrest through regulating the cell cycle. The beneficial role of muscle cells on OA indicated that muscle strengthening exercises could be a potential intervention for OA.⁹⁸

MSC Strategy

Inherent anti-inflammatory properties

Despite being the most advanced and promising approach for cartilage repair, chondrocyte-based cartilage repair has some disadvantages, such as morbidity caused by damage to the donor-site articular surface and cell senescence during *ex vivo* expansion.⁴ Furthermore, the inflammatory synovial fluid microenvironment triggers human chondrocytes to actively take part in inflammatory processes, particularly during the initiation and progression of inflammatory joint diseases and in the disruption of cartilage repair mechanisms resulting in cartilage degradation.⁹⁹ Unlike chondrocytes, MSCs are not limited by an intrinsic tendency to lose their phenotype and dedifferentiate during expansion.¹⁰⁰ MSCs also play an important role in immunomodulation and tissue regeneration by secretion of soluble factors.^{101,102} In an inflammatory environment, MSCs secrete factors which cause multiple anti-inflammatory effects and influence matrix turnover in synovium and cartilage explants.¹⁰³ MSCs have also been revealed as robust sources of TIMP-mediated MMP-inhibition, capable of protecting the perivascular niche from high levels of destructive MMPs, even under pathological conditions.¹⁰⁴

Due to their potential to modulate the local microenvironment via anti-inflammatory and immunosuppressive functions, MSCs have an additional advantage for allogeneic application (Fig. 3). Moreover, by secreting various bioactive soluble factors, MSCs can protect the cartilage from further tissue destruction and facilitate regeneration of the remaining progenitor cells *in situ*.¹⁰⁵ The joint resurfacing function of MSCs was also demonstrated in *in vivo* studies. After intra-articular injection of autologous MSCs into a caprine OA model (complete excision of the medial meniscus and resection of the anterior cruciate ligament), there was evidence of marked regeneration of the medial meniscus and implanted

cells were detected in the newly formed tissue. Degeneration of the articular cartilage, osteophytic remodeling, and subchondral sclerosis were reduced in cell-treated joints compared with joints treated with vehicle alone without cells.¹⁰⁶ A clinical case report demonstrated that, 24 weeks after the injection of autologous MSCs into a knee with symptomatic and radiographic degenerative joint disease, there was significant cartilage growth, decreased pain, and increased joint mobility in the patient.¹⁰⁷ As a result, MSCs could be used in cartilage repair as a potential anti-inflammatory strategy especially in the context of allogeneic transplantation.

Acquired anti-inflammatory capacity

Treatment strategies focused on both reducing inflammation and increasing tissue production are necessary to effectively treat OA from a tissue-engineering perspective. Despite their multilineage differentiation potential and immunomodulatory properties as well as anti-inflammatory abilities, MSC survival after transplantation is still very low, thereby hindering their therapeutic efficacy.¹⁰⁸

Recent studies found that MSCs could be rejuvenated by preconditioning strategies, which enhance their post-transplantation survival and functionality.¹⁰⁹ The concept of preconditioning was established in 1986 by Murry *et al.*, who found that ischemic preconditioning of cardiomyocytes led to the activation of survival signaling.¹¹⁰ In theory, any factors that might influence the proliferation and differentiation of therapeutic cells can be an advantage. For instance, hypoxic preconditioning not only has pro-survival and cytoprotective effects, it also helps the cells maintain their stemness and promote proliferation and differentiation potential post-engraftment.¹¹¹ When referring to a cartilage engineering application, preconditioning of MSCs can be derived from microenvironment factors such as low oxygen,¹¹² growth factors,¹¹³ or potentially pharmacological substances targeting specific signal pathways involved in MSC chondrogenesis such as the MAPK^{114,115} and Wnt signal pathways.^{114,116}

DSCM-mediated stem cell preconditioning. The stem cell niche is a specialized microenvironment that helps sustain the stem cell pool within each tissue or organ system. It is hypothesized that stem cells can create their own microenvironment; in such a microenvironment, adult stem cells are expected to greatly expand, while retaining their stemness for a tissue-specific lineage. In 2009, He *et al.* utilized porcine SDSCs, tissue-specific stem cells for chondrogenesis,¹¹⁷ as a model to reconstruct an *in vitro* 3D stem cell microenvironment, in which expanded SDSCs produced a drastic increase in cell number and chondrogenic potential.¹¹⁸ Later on, this DSCM-mediated stem cell preconditioning was also demonstrated to be effective in rejuvenating human bone marrow stromal cells, in terms of enhanced proliferation and chondrogenic hypertrophy.¹¹⁹ Interestingly, this DSCM-mediated expansion system also works for primary chondrocytes, such as articular chondrocytes^{120,121} and nucleus pulposus cells.^{122,123}

The regulation of intracellular reactive oxygen species (ROS) is crucial for cell survival in a harsh environment and guarantees successful cell therapy.¹²⁴ DSCM preconditioning could decrease the expanded stem cell ROS level^{119,125} and protect human SDSCs from oxidative stress-induced cell senescence, as it applies to cell proliferation and dif-

ferentiation capacity.¹² Furthermore, our recent data showed that human SDSCs expanded on DSCM exhibited an increased chondrogenic potential as well as heightened protection against IL-1 β -induced inflammation.¹²⁶ The adaptive capacity in a harsh environment was also validated in a mini pig study, in which DSCM-expanded SDSCs exhibited an enhanced *in vivo* cartilage regeneration capacity postinjection, evidenced by intensely stained type II collagen and sulfated GAGs in the partial-thickness cartilage defects with negligible staining of type I collagen,¹³ indicating that DSCM preconditioned SDSCs have the ability to resist inflammation. In contrast, the existence of type I collagen in the regenerated cartilage from the plastic flask-expanded SDSC groups suggested fibrocartilage formation,¹³ which might be explained by dedifferentiation caused by inflammatory stress.¹²⁷

Low oxygen and FGF-2 contribute to DSCM-mediated stem cell preconditioning. Low oxygen tension (hypoxia) maintains undifferentiated states of MSC phenotypes and also influences proliferation and cell fate commitment.¹²⁸ There is increasing evidence suggesting that hypoxia (or, more appropriately, physiological hypoxia) can stimulate chondrogenesis.^{129,130} Hypoxic preconditioning can boost the expression of genes favoring MSC proliferation and growth prolongation by inducing the expression of pro-survival and proangiogenic markers in MSCs.¹³¹ Recently, hypoxia-inducible factors (HIFs) have been shown to activate specific signaling pathways such as Notch and the expression of transcription factors such as the octamer-binding transcription factor 4 (Oct4), which is responsible for controlling stem cell self-renewal and multipotency.¹³² This finding suggests that modulation of oxygen availability and HIF expression can influence stem cell fate. Our recent report suggested that the combination of hypoxia and FGF-2 significantly enhanced DSCM-expanded SDSC proliferation and chondrogenic potential.¹³³ This microecosystem apparently reconciled the contradiction of Quantity versus Quality, a predicament often met in stem cell-based cartilage repair. *In vivo*, numerous growth factors and morphogens are immobilized by directly binding to the ECM through specific heparin-binding domains, by direct binding to ECM molecules such as collagen, or by direct anchoring to cell membranes.¹³⁴ By immobilizing growth factors in a concentrated area, DSCM helps to amplify the effects of growth factors and reduce the inflammation reaction leading to improved proliferation and differentiation of stem cells.¹⁰²

Small molecules targeting inflammation signals contribute to DSCM-mediated stem cell preconditioning. Widely used as an anti-inflammatory drug in cartilage repair, the p38 MAPK inhibitor also blocks chondrogenesis,¹³⁵ indicating that the p38 MAPK inhibitor has some disadvantages in the treatment of cartilage damage with concomitant inflammation. Considering its adverse effects for clinical treatment of OA, MSCs pretreated by SB203580 in the DSCM expansion phase were assumed to avoid this dilemma. Preconditioning using SB203580 significantly enhanced DSCM-expanded human SDSC chondrogenic potential; this rejuvenation of chondrogenic capacity, instilled in SDSCs by SB203580, gave the stem cell the ability to resist the influence of IL-1 β -induced inflammation.¹²⁶

ECM Strategy

Compared to natural biomaterials, synthetic polymers are more controllable and predictable in their chemical and physical properties, holding some promise of success in tissue engineering and regenerative medicine. Of the synthetic polymers, those derived from poly(α -hydroxy esters),¹³⁶ poly(propylene fumarates),¹³⁷ polyurethanes,¹³⁸ PGA,¹³⁹ and poly(lactide-co-glycolide)¹⁴⁰ have been used for cartilage regeneration; however, inflammatory reaction due to synthetic scaffolds that impaired the quality of regenerated cartilage has also been reported in some studies.^{141,142}

Natural matrix scaffolds

Naturally occurring polymers as scaffolds offer options for cartilage tissue engineering due to biocompatibility, biodegradability, low toxicity of degradation by-products, and plasticity in processing into a variety of material formats.¹⁴³ In an attempt to repair articular cartilage, allografted articular chondrocytes, embedded in collagen gel, were transplanted into full-thickness defects in rabbit articular cartilage; 24 weeks after implantation, the defects were filled with hyaline cartilage, synthesized type II collagen, and exhibited no signs of immunologic rejection and degeneration of the reparative tissue.^{144,145} Rahfoth *et al.* found that the transplantation of allograft chondrocytes embedded in agarose gel was a suitable method to repair articular cartilage defects in rabbits because there were no signs of graft-versus-host rejection or infiltration by immune cells.¹⁴⁶ Other natural polymers that have been explored as bioactive scaffolds for cartilage tissue engineering include, but are not limited to silk,¹⁴⁷ hyaluronan,¹⁴⁸ and chitosan.¹⁴⁹ In a recent study, the responses of articular chondrocytes under inflammatory conditions were compared after seeding within three polymeric scaffolding materials (silk, collagen, and polylactic acid [PLA]); Kwon *et al.* found that chondrocytes grown in the silk and collagen scaffolds exhibited higher levels of cartilage matrix gene expression than those in the PLA scaffolds.¹⁵⁰ When using a PGA scaffold for treatment of full-thickness defects, however, there appears to be a moderate immunoreaction as evidenced by lymphocytes in transplanted joints.^{9,151}

Chondrocyte sheets

A cell sheet technique developed in 1993¹⁵² shows promise in cartilage repair with concomitant inflammation. Hamahashi *et al.* reported that a layered chondrocyte sheet produced the most humoral factors, including PGE₂, which plays a key role in its anti-inflammatory function.¹⁵³ In chondrocyte sheets, catabolic factors such as MMP3, MMP13, and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) were also observed to decrease, while the expression of TIMP1 with antagonistic actions against MMP3 increased.¹⁵⁴ In a rabbit model with partial-thickness cartilage defects, layered chondrocyte sheets were able to maintain the cartilaginous phenotype and could be attached to the site of cartilage damage, acting as a barrier to prevent a loss of proteoglycan from these sites and to protect them from catabolic factors in the joint.¹⁵⁴ This technique might have great potential for OA treatment and inflammatory prevention in cartilage repair.

Decellularized tissue matrix

In contrast to the DSCM described above, which attempts to rejuvenate expanded stem cells' chondrogenic potential as well as anti-inflammatory capacity, the decellularized tissue matrix influences tissue engineering directly, providing a carrier (or scaffold) and chondrogenically induced growth factors as well as an anti-inflammatory function. The use of ECM derived from decellularized tissue is increasingly common in regenerative medicine and tissue engineering.¹⁵⁵ By virtue of physical, chemical, or enzymatic approaches, organ decellularization removes all cellular material without adversely affecting the composition, biologic activity, or mechanical integrity of the remaining 3D matrix.¹⁵⁶ The molecules that constitute ECM are largely and highly conserved across species and are well tolerated even by xenogeneic recipients. The effects of xenogeneic ECM on the innate immune response, specifically the responding macrophages, may elicit a necessary M2 phenotypic profile to support a constructive remodeling response for the scaffold.¹⁵⁷ In the field of cartilage regeneration, many decellularized tissue scaffolds showed positive effects and reparative capability for cartilage defects.¹⁵⁸ For example, the combined use of cells with a decellularized aortic scaffold was able to prevent the generation of a strong inflammatory response and improve the overall tracheal cartilage regeneration.¹⁵⁹ Compared with a PGA scaffold, an ECM scaffold derived from porcine cartilage not only strongly supported chondrogenic differentiation of rabbit MSCs but also helped maintain its phenotype *in vivo*.¹⁶⁰

Summary

The treatment of cartilage injury remains a clinical challenge despite the advancements in surgical procedures and techniques. An important hurdle is the concomitant inflammation during cartilage repair. Pharmacological drugs have been successfully applied in cartilage repair; however, they cannot optimally work alone.¹⁶¹ With the development of ECM-based scaffolds and preconditioned tissue-specific stem cells, both tissue engineering components could not only contribute to an enhanced ability in anti-inflammation but also to cartilage regeneration. All these strategies could combine to boost cartilage repair under inflammatory conditions.

Disclosure Statement

The authors declare no potential conflicts of interest.

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