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Immunomodulatory Strategies for Cartilage Regeneration in Osteoarthritis

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Osteoarthritis (OA) is the most prevalent musculoskeletal disorder and a leading cause of disability globally. Although many efforts have been made to treat this condition, current tissue engineering (TE) and regenerative medicine strategies fail to address the inflammatory tissue environment that leads to the rapid progression of the disease and prevents cartilage tissue formation. First, this review addresses in detail the current anti-inflammatory therapies for OA with a special emphasis on pharmacological approaches, gene therapy, and mesenchymal stromal cell (MSC) intra-articular administration, and discusses the reasons behind the limited clinical success of these approaches at enabling cartilage regeneration. Then, we analyze the state-of-the-art TE strategies and how they can be improved by incorporating immunomodulatory capabilities such as the optimization of biomaterial composition, porosity and geometry, and the loading of anti-inflammatory molecules within an engineered structure. Finally, the review discusses the future directions for the new generation of TE strategies for OA treatment, specifically focusing on the spatiotemporal modulation of anti-inflammatory agent presentation to allow for tailored patient-specific therapies.

Keywords: osteoarthritis, immunomodulation, anti-inflammatory, cartilage tissue engineering

Impact Statement

Osteoarthritis (OA) is a prevalent and debilitating musculoskeletal disorder affecting millions worldwide. Despite significant advancements in regenerative medicine and tissue engineering (TE), mitigating inflammation while simultaneously promoting cartilage tissue regeneration in OA remains elusive. In this review article, we discuss current anti-inflammatory therapies and explore their potential synergy with cutting-edge cartilage TE strategies, with a special focus on novel spatiotemporal and patient-specific anti-inflammatory strategies.

Introduction

Osteoarthritis (OA) is the most common form of arthritis affecting >10% of the population over age 60 worldwide.[1] OA is a degenerative disease resulting in gradual loss of cartilage and subchondral bone in a joint. These changes in cartilage homeostasis result in reduced protection and lubrication between the connecting ends of bones. OA progression causes significant joint stiffness and pain, and can lead to loss of function and disability.

Owing to the high incidence of OA and its degenerative nature, disease management represents a significant economic and social burden. In 2013, treatments costed $18.4 billion in the United States and, although effective in

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reducing pain, they fail to provide cartilage regeneration.[2] Therefore, regenerative strategies to treat OA are highly sought after to achieve functional healing.

The pro-inflammatory environment plays a key role in OA progression. In early stages, the increased production of inflammatory cytokines shifts the anabolic–catabolic equilibrium within the joint toward catabolism, that is, matrix remodeling and destruction. This shift results in breakdown of aggrecan and collagen type II, the main components of hyaline cartilage (Fig. 1). In addition, hypertrophic differentiation of chondrocytes leads to collagen type X production and calcification of the cartilage matrix. These changes significantly affect the mechanical function of cartilage by increasing stiffness and shear modulus.[3–5]

The main cytokines involved in inflammation are interleukin-1β (IL-1β), tissue necrosis factor α (TNF-α), and interleukin-6 (IL-6). IL-1β and TNF-α act in a similar manner through the upregulation of matrix metalloproteinases (MMPs), in particular MMP-1 and MMP-13 (Fig. 1). These MMPs cause degradation of collagen type II and aggrecan by binding the catalytic domain of the enzyme to the cleavage site of these matrix components.[6–8]

IL-1β and TNF-α also upregulate aggrecanases, such as a disintegrin and metalloproteinase (ADAM) with thrombospondin type I motif 4 (ADAMTS4), which cleave aggrecan more effectively than MMPs.[9] In addition to direct cartilage degradation, IL-1β and TNF-α elicit the production of IL-6,[10] which also contributes to type II collagen and aggrecan degradation through the stimulation of MMP-13 production.[11]

In addition to cartilage extracellular matrix (ECM) degradation, IL-1 and TNF-α also inhibit cell migration by impairing recruitment of chondrogenic progenitor cells from their niches and reducing cell proliferation by overexpressing different factors such as miR-216b.[12,13] Inflammatory cytokines additionally induce apoptosis of articular cartilage chondrocytes.[14,15] Reduced cell migration and proliferation and increased chondrocyte apoptosis prevent the secretion of new cartilage ECM, thus aggravating the pathology of OA.

Different therapies have been explored to target the OA inflammatory environment. The most commonly employed treatments include analgesic drugs such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and cyclooxygenase-2 (COX-2) inhibitors.[16] Although these treatments are able to manage pain and limit disease progression, long-term use can become ineffective and may result in off-target effects.[17]

As the disease progresses to late stages, it becomes necessary to carry out surgical treatment such as joint arthroscopy or arthroplasty.[18] Surgical treatments initially appear promising, with patients reporting significant improvements in pain and mobility.[19] However, over time these methods have limitations, with some patients experiencing a significant decline in quality-of-life only 3 years after surgical

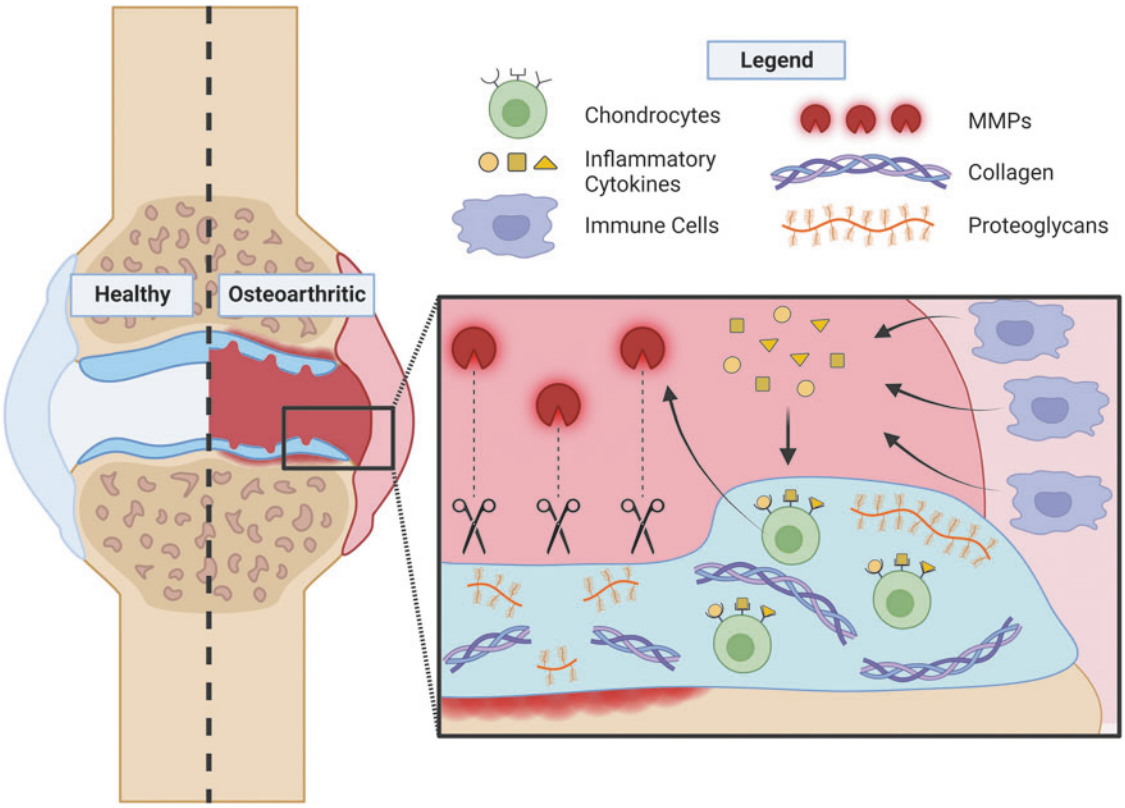

FIG. 1. Process osteoarthritis progression in the joint. In the osteoarthritic joint, the inflammatory cytokines secreted by immune cells in the synovium stimulate articular chondrocytes to express catabolic enzymes (i.e., metalloproteases and matrix metalloproteases), which degrade the collagen and aggrecan present in the ECM. ECM, extracellular matrix.

intervention due to associated comorbidities, musculoskeletal pain in locations other than the implant site, and additional mental health decline due to unmanaged expectations.[20]

In more recent years, focus has shifted to developing regenerative medicine techniques to treat and prevent OA through the repair of damaged cartilage. Techniques include allogenic chondrocyte transplantation, mesenchymal stromal cell (MSC) implantation, cell-loaded scaffolds, and cell-free scaffolds.[21–25] However, these techniques fail to address the inflammatory conditions associated with OA that may limit regeneration.

For example, OA immune cytokines can cause apoptosis of implanted chondrocytes and MSCs, destruction of ECM in *in vitro* engineered cartilage, limit implant integration with the host tissue, and prevent cell migration into cell-free scaffolds. Addressing the inflammatory environment associated with OA is essential to advance future technologies to effectively treat the condition and achieve full regeneration.

This review outlines current anti-inflammatory strategies to treat OA alongside tissue engineering (TE) strategies that are being developed to provide a regenerative solution to OA. It also discusses future directions in the field of biomaterials and TE to both tackle the problem of inflammatory conditions in OA treatment and promote effective cartilage healing.

Current Anti-Inflammatory Strategies

Anti-inflammatory strategies to treat OA have become a major focus due to the increased understanding of the inflammatory characteristics of OA and their effects on disease progression. Several therapies have been developed to target and counteract the action of the inflammatory cytokines upregulated in OA. This section outlines different anti-inflammatory therapies that have undergone clinical trials and their limitations.

Anti-inflammatory molecules

Owing to the limitations of NSAIDs and analgesic drugs, disease-modifying OA drugs (DMOADs) have been explored to alleviate OA symptoms and improve the disease state (i.e., delay or halt disease progression). Currently, there are several DMOADs in Phase II or III of clinical trials pending FDA approval for clinical use. To receive FDA approval as DMOADs, drugs must be proven to be safe in clinical use, improve physical OA symptoms (i.e., relieve pain and improve function), and slow down progression of joint deterioration.[26,27]

Although several of the drugs discussed in this section have been proven safe and shown to relieve OA symptoms, none have demonstrated reduction in the loss of joint space width (an indirect measure of cartilage thickness) and, therefore, have not been approved for clinical use in OA treatment.[28,29]

The most clinically researched DMOADs are anti-inflammatory agents (Table 1).[30–37] These are drugs that target OA-specific inflammatory cytokines (IL-1, TNF, and IL-6). One of the earliest approved anti-inflammatory agents is Anakinra, which acts as a recombinant receptor antagonist for IL-1 (IL-1RA). This drug was approved to treat rheumatoid arthritis by the FDA in 2001 after efficacy was proven across five clinical trials.[38–42] Despite safety approval, Anakinra was not shown to be effective for treating OA when delivered intra-articularly to locally target IL-1.[30] Similarly, other anti-inflammatory (AI) drugs, such as diacerein, adalimumab, and tocilizumab, have been developed to target IL-1, TNF, and IL-6, but have also failed to meet efficacy requirements.[32,33,36]

Although there is extensive research focused on the ability of DMOADs to inhibit inflammation, there are several risks associated with suppressing inflammation systemically, such as reducing a patient's ability to fight infection, and developing autoimmune diseases such as

Table 1. Anti-Inflammatory Disease-Modifying Osteoarthritis Drugs Strategies Explored in Clinical Trials

Strategy	*Target*	*Delivery method*	*Clinical trial*	*References*
Anakinra	IL-1 receptor antagonist	IA	Phase II Clinically for RA used but not approved for OA	30
AMG108	Inhibits IL-1	S/C	Phase II	31
Diacerein	Inhibits IL-1 production	Oral	Phase III	32
Adalimumab	Anti-TNF	S/C	Phase II Clinically used but not approved for OA	33
Etanercept	Anti-TNF	S/C	Phase III Clinically used but not approved for OA	34
Infliximab	Anti-TNF	IA	Phase IV Clinically used but not approved for OA	35
Tocilizumab	Anti-IL-6	IV	Phase III	36
GLPG1972/ S201086	ADAMTS5 inhibitor	Oral	Phase II	37

IA, intra-articular; IL, interleukin; IV, intravenous; OA, osteoarthritis; RA, rheumatoid arthritis; S/C, subcutaneous; TNF, tissue necrosis factor.

lupus and vasculitis.[43] Furthermore, excess inhibition of these cytokines can interfere with the native tissue regeneration and repair processes.[44] As an alternative to cytokine inhibition, different DMOADs have been designed to target matrix-degrading collagenases (MMPs) and aggrecanases (ADAMTS) (Table 1).[45] An example of a protease-inhibiting drug is GLPG1972/S201086, an ADAMTS5 inhibitor; however, it showed no significant improvement in cartilage thickness in a Phase-II clinical trial.[37]

Clinical trial failure of the discussed strategies can be due to different factors. Efficiency of the drug delivery system has been identified as an important challenge that needs to be addressed for successful clinical translation. The traditional oral delivery of DMOADs can cause systemic effects and failure to locally target the affected joint, resulting in reduced therapeutic effect.[46] Intra-articular drug delivery increases local drug availability in the joint. However, the short half-life of DMOADs results in low residency times and, therefore, reduced therapeutic effect.[46]

Furthermore, the accurate placement of these injections to the target area and the ability of the drug to penetrate through the dense matrix in the joint represents an additional challenge to achieve optimal effect at the target location.[47] In addition, the biggest concern with intra-articular drug delivery is infection as repeated injections can lead to sepsis, tissue morbidity, and mortality in severe cases.[47,48]

Gene therapy

Gene therapy is a promising alternative to the delivery of anti-inflammatory cytokines and factors that mediate cartilage tissue repair and homeostasis.[49] Gene transfer allows for sustained synthesis of a protein of interest at the injury site for a prolonged period of time, addressing the limitations of drug administration. In addition, local synthesis of gene products with native conformations and true post-translational modifications offers a more physiological option in comparison with the injection of bacteria-produced recombinant proteins.[49] Anti-inflammatory inhibitors such as IL-1RA have been overexpressed through viral and non-viral gene delivery to suppress OA inflammation.[50,51]

Recombinant adeno-associated gene transfer of *IL-1RA* through intra-articular injection is currently in Phase I clinical trials in patients with moderate knee OA.[52] The overexpression of IL-10 is another focus within OA gene therapy that reduces the production of inflammatory cytokines.[53] IL-10 has also been proven to reduce the production of MMPs and prevent chondrocyte apoptosis.[54] There is currently a Phase II clinical trial surrounding the safety and efficacy of a single injection of XT-150, a nonviral therapy containing plasmid DNA encoding for *IL-10*.[55]

Intra-articular injection of MSCs

MSCs have been a focus of musculoskeletal regenerative medicine due to their ability to differentiate into osteogenic, adipogenic, and chondrogenic lineages and their immunomodulatory and pro-regenerative properties.[56,57] Recently, local intra-articular delivery of MSCs has been investigated to promote repair and regeneration of cartilage tissue. MSCs secrete cytokines, growth factors, and anti-inflammatory factors that promote chondrocyte proliferation and ECM protection in OA.[58]

In 2018, Zhou et al. reported that direct intra-articular delivery of MSCs reduced the production of pro-inflammatory cytokines and protected chondrocytes from apoptosis.[59] The paracrine action of MSCs is a promising alternative for OA treatment, and several MSC-based therapies have been tested in clinical trials (Table 2).[60–63] In all cases described in Table 2, cells were expanded under good manufacturing practice (GMP) conditions and harvested at passages lower than or equal to four. Before harvesting, cells were characterized to confirm phenotype, cell number, and viability, and to ensure no contamination was present.

Unlike the trials surrounding DMOADs, MSC therapies have demonstrated efficacy as well as safety. Despite the

Table 2. Clinical Trials Involving the Intra-Articular Injection of Mesenchymal Stromal Cells for Osteoarthritis Treatment

Cell type	*Cell dosage*	*Clinical trial*	*Trial outcome*	*References*
Allogenic bone marrow-derived MSCs	Varying doses 25×10^6, 50×10^6, 75×10^6, and 150×10^6	Phase II	Biggest improvement seen in 25×10^6 group. Higher doses caused adverse events such as knee pain and swelling.	60
Autologous bone marrow-derived MSCs	30.5×10^6	Phase I/II	No adverse events observed. Increased knee cartilage thickness.	61
Allogenic umbilical cord-derived MSCs	20×10^6	Phase I/II	No adverse events observed. Treated patients experienced reduced pain and improved function.	62
Autologous adipose-derived MSCs	Varying doses 1.0×10^7, 5.0×10^7, and 1.0×10^8	Phase I/II	No adverse events observed. The lower dosage groups appeared promising initially, but improvements deteriorated after 1 year.	63

MSC, mesenchymal stromal cell.

promising results, direct delivery of MSCs raises similar concerns to the limitations with DMOADs. MSC-secreted factors have limited ability to penetrate the dense cartilage matrix, and the injected cells may also rapidly die or migrate to other tissues after delivery, limiting their local therapeutic effect.[64–66]

The high cell number required presents a further shortcoming of MSC therapy due the extended time and cost associated with cell expansion. It is also important to note that MSCs lose their ability to differentiate as the passage number increases; therefore, a large number of cells must be harvested to expand to the correct number of cells that still express MSC characteristics, further increasing the costs.[67]

Tissue Regeneration Strategies to Treat OA

Anti-inflammatory strategies are critical for mitigating pain and improving mobility in patients with OA. However, these strategies do little to address the lost or damaged cartilage tissue, which negatively impacts joint function.[68–70] Regenerating native cartilage is difficult because this avascular aneural tissue has limited capacity for self-healing.[68,71–74] A wide range of approaches, including surgical interventions, biomaterial and/or cell implantation, and immunomodulation, have been explored to treat tissue damage caused by OA.[69,71,75]

Despite decades of research and clinical studies, these early-intervention treatments still fail to regenerate fully functional durable cartilage when used independently.[68,70,75–77] The newly formed tissue is typically mechanically inferior compared with native cartilage and integrates poorly with the surrounding tissue.[76,78] To address these limitations, TE is a promising strategy capable of incorporating multifaceted approaches to restore tissue function by regenerating native-like cartilage tissue through techniques involving the implantation of biomaterial scaffolds into the defect site.[68,69,79–82] This section describes clinical and state-of-the-art TE approaches to regenerate cartilage to treat OA.

Clinical strategies for cartilage repair and regeneration

Current clinical regenerative treatments for early-stage OA include microfracture, mosaicplasty, cellular implantation, and biomaterial implantation.[16,69,71,73] Although microfracture recruits progenitor cells from bone marrow directly into the defect site,[83] other techniques such as mosaicplasty transplant healthy tissue from a non-load-bearing site into the affected joint.[84]

Another alternative is autologous chondrocyte implantation (ACI) where patient's cells are harvested, expanded *in vitro*, and then implanted into the defect site several weeks later.[69] However, sourcing autologous cells and tissues can result in donor site morbidity.[69] To avoid tissue damage, cells and tissues can be obtained from a cadaveric allogeneic donor, but this approach introduces risk for immune rejection.[69,71]

Cellular-based strategies can be improved upon by seeding cells onto a biomaterial scaffold, which provides support to the cells during and after implantation. For example, in matrix-induced autologous chondrocyte implantation (MACI), autologous chondrocytes are seeded onto collagen scaffolds before implantation.[85,86] Biomaterials can also be implanted acellularly, and different options have been approved by the FDA in recent years. Agili-C™ is a porous biphasic aragonite-hyaluronate scaffold designed for off-the-shelf use to treat osteochondral defects.[87]

Similarly, MaioRegen is a triphasic scaffold composed of type I collagen and hydroxyapatite to mimic the composition of chondral and subchondral layers.[88] Both MaioRegen and Agili-C have demonstrated capacities for reduced adverse events and mild clinical improvements in patients postimplantation in the short term.[89] However, their ability to regenerate native-like cartilage and complete tissue integration, which is necessary for long-term clinical success, remains to be demonstrated in clinical studies over extended periods of time.[87,88]

State-of-the-art cartilage TE approaches

State-of-the-art cartilage TE approaches focus on improving the organization and integration of newly regenerated tissue to restore native-like function. It is worth noting, however, that regeneration of functional cartilage tissue is severely hindered by lack of integration with surrounding osteochondral tissue. This necessitates that cartilage TE strategies address recapitulating the osteochondral unit for true regeneration of tissue. These strategies include cell-laden hydrogels,[90,91] mechanical stimulation of tissue constructs,[91] and biomaterials with spatially organized bioactive cues.[92–94]

The majority of TE approaches focuses on the use of hydrogels composed of natural and synthetic materials because they mimic the high-water content found in cartilage and their physical and biochemical properties can be easily modified to promote cartilage formation.[25,91,92,95] For example, the cross-linking density of hyaluronic acid-based hydrogels can be tuned to increase collagen II and glycosaminoglycan (GAG) production (Fig. 2A).[90] However, hydrogels are relatively soft (0.5–40 kPa) compared with native cartilage (500–900 kPa) and often require mechanical reinforcement to withstand physiological loads.[91,96–98]

To produce mechanically relevant tissues, hydrogels can be combined with three-dimensional (3D)-printed biocompatible polymeric resins and mechanically loaded, creating spatial mechanical properties that induce stratified tissue formation *in vitro* (Fig. 2B).[91] Hydrogels also allow for easy incorporation of bioactive cues to guide cell responses. For example, MSC-laden alginate hydrogels used for localized gene delivery of chondrogenic and osteogenic growth factors promoted spatial osteochondral tissue formation *in vitro* (Fig. 2C).[92] Similarly, 3D-printed biomaterials can be spatially functionalized with osteogenic and chondrogenic peptides to influence local MSCs differentiation and matrix formation (Fig. 2D).[93]

Although these approaches are promising, the pro-inflammatory environment created by OA has deleterious effects on cell response, tissue formation, and tissue integration. OA inflammation will likely impair the regenerative potential of tissue engineered strategies.[70,72,74] Effective treatments must, therefore, include strategies that reduce inflammation while simultaneously promoting functional tissue formation.[70,74,80]

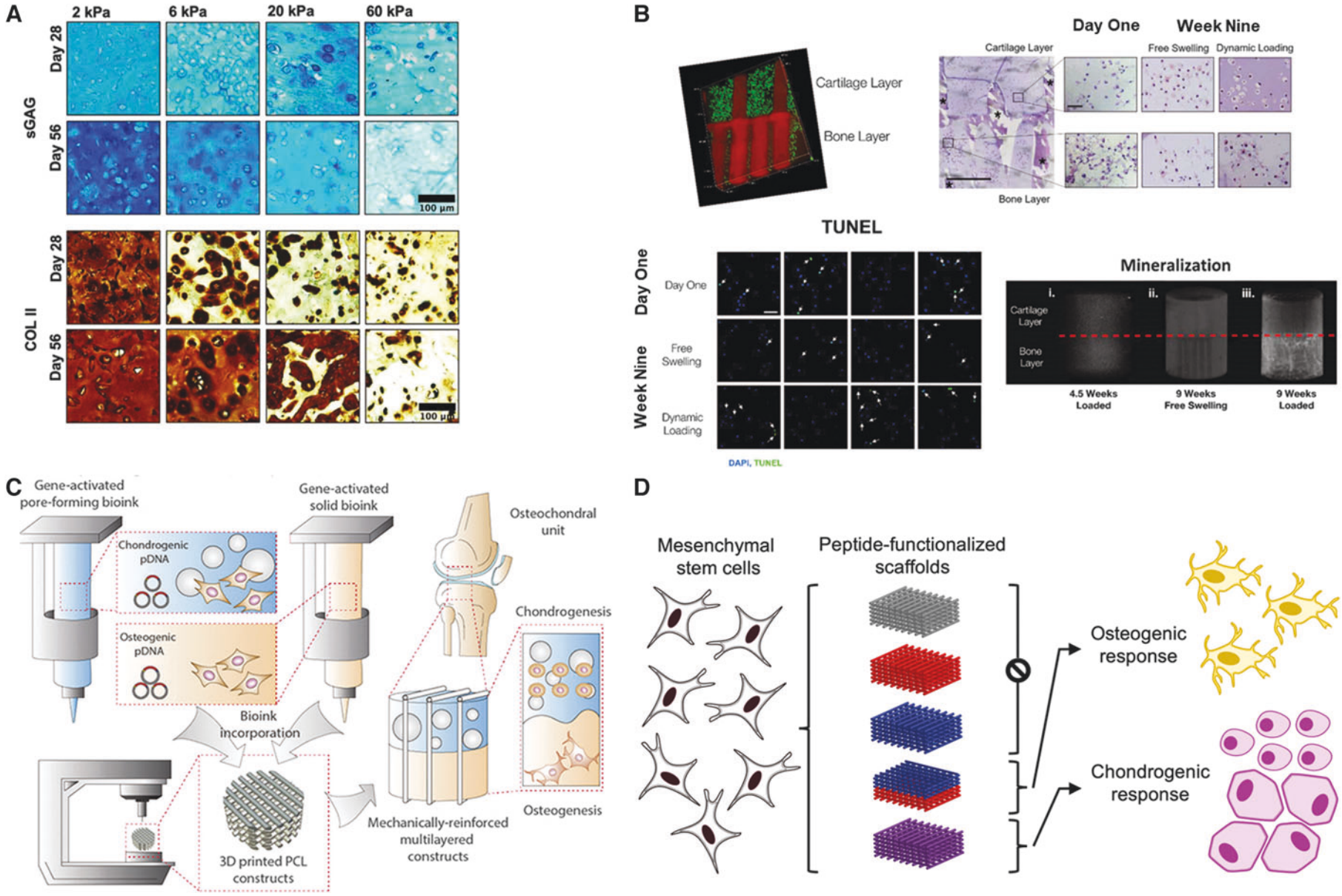

FIG. 2. Current state-of-the-art tissue engineering strategies for osteochondral repair. **(A)** MSCs seeded in hyaluronic acid-based hydrogels with lower stiffness due to lower cross-linking density produced higher amounts of sGAG and COL II. **(A)** Adapted with permission from Ref.[90] Copyright 2021, *Biofabrication*. **(B)** Dynamic loading of reinforced hydrogels with spatial mechanical properties resulted in stratified tissue formation of cartilage and bone layers. **(B)** Adapted with permission from Ref.[91] Copyright 2021, *Biofabrication*. **(C)** Localized delivery of osteogenic and chondrogenic genes to MSCs in PCL-reinforced alginate hydrogels drove spatial tissue formation *in vitro*. **(C)** Adapted with permission from Ref.[92] Copyright 2019, *Journal of Controlled Release*. **(D)** Spatial organization of bone-promoting and cartilage-promoting peptides on the surface of 3D-printed PCL scaffolds influenced MSC differentiation toward osteogenesis and chondrogenesis. **(D)** Adapted with permission from Ref.[93] Copyright 2021, *Biomaterials Science*. 3D, three-dimensional; COL II, collagen II; MSC, mesenchymal stromal cell; PCL, poly(ε-caprolactone); sGAG, sulfated glycosaminoglycan.

TE Strategies for Immunomodulation

The pro-inflammatory conditions associated with OA compromise the therapeutic success of cellular and cell-free TE strategies. In cell-laden scaffolds, inflammatory cytokines such as IL-1 and TNF-α have been shown to reduce MSC chondrogenesis and to impair the integration of the newly engineered cartilage with native cartilage tissue.[99] Cell-free scaffolds may have reduced healing potential because inflammatory conditions suppress the migration of MSCs and chondrocytes to the scaffold, therefore reducing its healing potential.[12]

It is essential to regulate the OA inflammatory environment to improve the overall success of TE. Different strategies aiming to modulate inflammation while providing cartilage regeneration have been previously explored.[100] The most prevalent are the use of immunomodulatory materials, promotion of MSC immunomodulatory potential through material properties, and incorporation of anti-inflammatory molecules into biomaterial scaffolds (Fig. 3).

Immunomodulatory materials

Biomaterials can have specific biochemical or physical properties that provide intrinsic immunomodulatory effects, which can be exploited to enhance TE strategies. For example, materials containing sulfated GAGs such as chondroitin sulfate or materials mimicking the cartilage GAG-rich composition such as sulfated alginate have been reported to display anti-inflammatory and anti-catabolic properties.[101,102] Macrophages have become immune cells of great interest for immunomodulation, especially for cartilage regeneration due to their prominent role in the pathophysiology of OA.

Pro-inflammatory macrophage activation results in the inflammatory environment and tissue destruction associated with OA.[15,16] Alternatively, pro-regenerative polarization of macrophage phenotype counteracts this inflammation and aids in tissue repair. Stiff biomaterials have been reported to drive macrophage polarization toward a pro-inflammatory (M1) phenotype, whereas softer materials prime cells toward an anti-inflammatory (M2) phenotype.[103]

In contrast, Xue et al. reported that macrophage expression of inflammatory cytokines increased as poly(ε-caprolactone) (PCL)/EUG scaffold stiffness decreased.[104] Owing to these conflicting results, the role of material stiffness on macrophage polarization requires further investigation. In addition, research on the effects of collagen scaffold stiffness on macrophage polarization using chemical cross-linkers to vary stiffness suggest that it is not only physical hydrogel properties that determines macrophage polarization but also chemical composition.[105]

Material geometry and topography are also key regulators of immune response. Surface topography affects macrophage attachment and phenotype; however, the relationship between the two is not yet fully understood.[106] Aligned fibers promote M2 macrophage phenotype, whereas randomly aligned fibers promote M1 polarization and the production of inflammatory cytokines such as TNF-α and IL-1β.[107] Fiber and pore dimensions also influence macrophage polarization, with increased pore and fiber size increasing M2 marker expression.[108]

Enhancing MSC anti-inflammatory potential through substrate properties

The anti-inflammatory properties of MSCs can be further enhanced by seeding them in biomaterials with specific

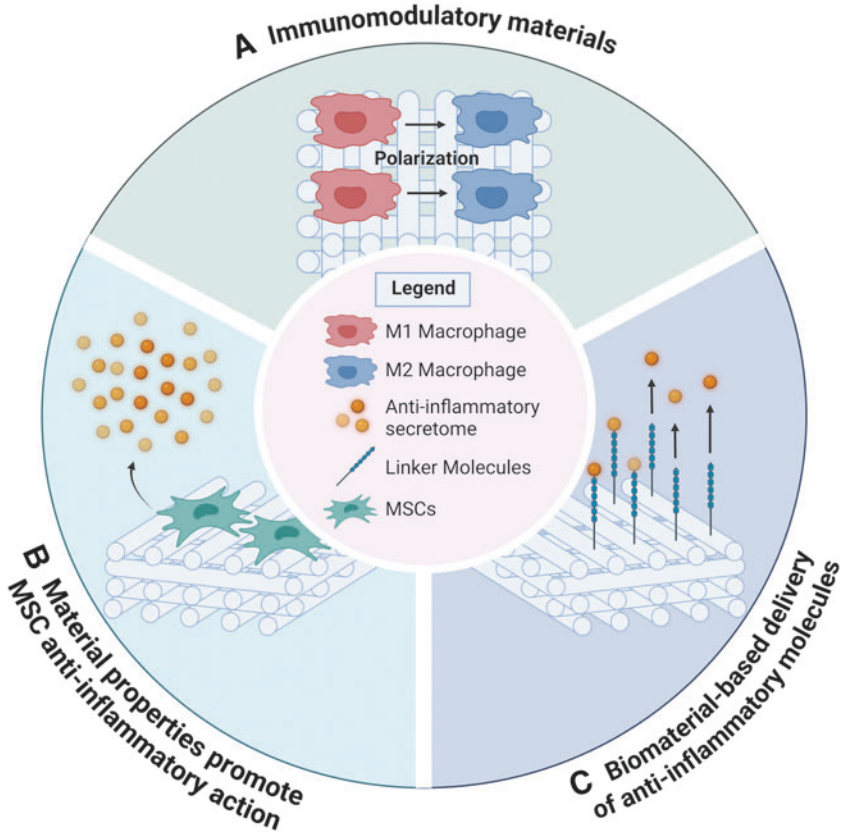

FIG. 3. Different material-based strategies for immunomodulation in osteoarthritis. **(A)** Immunomodulatory materials, **(B)** promotion of MSC immunomodulatory action through biomaterial properties, and **(C)** biomaterial-based delivery of anti-inflammatory molecules.

mechanical, physical, and chemical characteristics that are associated with immunomodulatory functions as described in the previous section. The combination of MSCs and hydrogels have reached Phase III clinical trials and perform significantly better than MSCs injected alone.[109] Owing to this, there has been a focus on using hydrogels as MSC carriers to increase cell viability.

Cell viability of 59% associated with cell injection was increased to 89% when cells were loaded in alginate hydrogels, with the increased presence of cells allowing for greater paracrine signaling by MSCs to modulate immune response and inflammation.[109] Although many studies have demonstrated the ability of scaffolds to improve MSC viability, Su et al. demonstrated that electrospun fibrous scaffolds have the ability to improve paracrine signaling of MSCs, with significantly higher levels of anti-inflammatory cytokines produced by the cells seeded on the fibrous scaffolds compared with those seeded on polystyrene 24-well plates.[110]

Similarly to macrophage polarization, substrate topography can also dictate MSC anti-inflammatory action. Kadir et al. investigated this effect by seeding MSCs on plates with different topographies leading to the production of a pro-regenerative secretome when cells were cultured on aligned fibers, in comparison with randomly oriented fibers.[111]

Biomaterial-based delivery of anti-inflammatory drugs

Although material properties have been shown to direct macrophage polarization and MSC fate, materials alone may not be sufficient to modulate the immune response associated with OA. To successfully suppress inflammation *in vivo*, the combination of tissue engineered constructs and the delivery of anti-inflammatory molecules has been established as a promising strategy. The most common anti-inflammatory molecule used in TE is IL-1RA, which targets IL-1, a key cytokine involved in OA.

Moutos et al. investigated the effect of binding doxycycline (dox)-inducible lentiviral vectors containing *IL-1RA* transgenes to PCL scaffolds.[98] Cells seeded in these constructs were transduced *in situ* and produced significant quantities of IL-1RA, which reduced MMP activity when treated with IL-1.[98] Inhibitors of TNF-α, such as etanercept, have also been incorporated into scaffolds to enhance cartilage repair.[112] Biomaterials can also be used to deliver pro-regenerative cytokines. Gong et al. demonstrated that an IL-4 loaded 3D-printed osteochondral scaffold led to enhanced MSC improved cartilage and subchondral bone regeneration in an *in vivo* rabbit model of OA by relieving the negative effects associated with inflammation.[113]

Future Directions and Conclusive Remarks

Tailoring the properties of biomaterial scaffolds can modulate undesired inflammatory responses. Whether it is incorporating natural biological components (e.g., chondroitin sulfate) into hydrogels, tuning substrate stiffness and topography, or delivering anti-inflammatory molecules, the fabricated substrates yield increased inflammatory suppression and/or pro-regenerative macrophage responses.[101–104] Despite the potential of these state-of-the-art strategies, there is a dire need to explore novel ways to couple suppression of inflammation with the regenerative action of materials and cells.

Cartilage inflammation and repair is a highly dynamic process controlled by the temporal presentation of inflammatory cytokines. In healthy patients, the inflammatory cascade after injury may resolve physiologically. OA patients experience fluctuating osteoarthritic flares, which exacerbates pain and functional impairs articular cartilage.[114] Therefore, it is necessary to temporally modulate drug release kinetics in response to cyclic inflammatory stimuli.

Inflammation-responsive approaches have been explored to address the cyclic nature of OA. For example, Joshi et al. engineered a self-assembling triglycerol monostearate hydrogel sensitive to MMPs and arthritic serum, allowing for the temporal release of an anti-inflammatory corticosteroid and the attenuation of pulsating inflammatory arthritis.[115] Another strategy explored by Park et al. is the use of genipin-cross-linked gelatin microspheres.[116]

The microspheres electrostatically bind anti-inflammatory cytokines and are released in response to the proteolytic enzyme activity in the inflamed synovium.[116] This strategy resulted in a concentration-dependent response of anti-inflammatory cytokines and the reduction of inflammation.[116] However, these approaches are limited by the use of supraphysiological doses of these anti-inflammatory molecules (i.e., triamcinolone acetonide [10–40 mg/mL],[115] IL-1β [2 ng/mL],[116] and TNF-α [10 ng/mL][116]).

Alternative to the delivery of recombinant cytokines, the combined used of synthetic biology and gene therapy can control the cell-mediated production of anti-inflammatory molecules. For example, Choi et al. used CRISPR/Cas9 to genetically engineer induced pluripotent stem cells (iPSCs) into autoregulating feedback loops to attenuate inflammation in a stimuli-responsive way.[117] This genetic circuit is initiated by the presence of IL-1 and activation of the NF-κB signaling pathway, which results in downstream expression of IL-1RA.[117] However, the difficulty of *ex vivo* production of large numbers of edited cells limits the clinical translation of these approaches.

In addition to the temporal presentation of anti-inflammatory molecules, their spatial presentation is another key aspect that deserves consideration. Articular cartilage is a highly organized tissue that transitions from the superficial zone to the hypertrophic deep zone anchored to the subchondral bone.[118,119] As OA inflammation deteriorates articular cartilage, the degraded ECM exposes the subchondral bone, leaving it vulnerable to sclerosis, cysts, and bone marrow lesions.[120,121]

Inflammatory suppression is typically applied onto the cartilage surface due to the tissue's exposure to the synovial fluid, but further research is needed to explore the effects of inflammation suppression in the subchondral bone region due to pathological changes with bone marrow.[122] Therefore, understanding where to localize inflammatory suppressant molecules remains unclear. Some studies focus on the delivery of anti-inflammatory molecules only on the articular cartilage zone of bilayer scaffolds,[113,123] whereas others have incorporated anti-inflammatory molecules onto an entire osteochondral construct.[124]

Another limitation hindering the clinical translation of TE and anti-inflammatory strategies is the sexual differences in joint cartilage homeostasis. There is evidence that sex hormone receptors and chondrocyte signaling pathways are different between male and female OA patients.[125] Despite

women having a higher prevalence of OA, musculoskeletal research primarily emphasizes males in human and vertebrate animal studies.

The sexual dimorphism of cartilage production is evident in murine models; with males undergoing greater chondrogenic differentiation in *in vitro* and *in vivo* models.[126,127] However, there are limited studies exploring the effects of inflammatory OA and inflammatory suppressant molecules on human female chondrocytes. Therefore, the inclusion of both male and female donors is vital for understanding the pathogenesis of OA and in advancing personalized TE therapies.

Overall, the regeneration of cartilage and subchondral bone has proven to be a significant challenge. Anti-inflammatory agents such as DMOADs and gene transfer therapies are engineered to ameliorate OA inflammation, but further clinical testing is required to meet efficacy standards. Anti-inflammatory strategies involving intra-articular injections relieve joint pain and improve mobility, yet this method provides limited ability to restore osteochondral tissue functionality.

Therefore, TE approaches must design biomaterials to reinforce physiologically relevant mechanical properties and functionalize with biomimetic cues to drive osteochondral tissue regeneration. Ultimately, TE approaches need to enhance the repair of the osteochondral tissue while attenuating inflammation. The new generation of regenerative medicine strategies for OA treatment will have to provide a multifunctional approach with precise spatiotemporal control over pro-regenerative and anti-inflammatory stimuli presentation.

Authors' Contributions

Conceptualization, writing—original draft preparation, and review and editing by O.K. Conceptualization, writing—original draft preparation, review and editing, and visualization by A.K. and C.O. Conceptualization, supervision, and writing—review and editing by L.W.C. Conceptualization, supervision, writing—original draft preparation, and review and editing by T.G.-F. All authors reviewed and approved the final version of the article.

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