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Engineering gene-activated bioprinted scaffolds for enhancing articular cartilage repair

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ABSTRACT

Untreated articular cartilage injuries often result in severe chronic pain and dyskinesia. Current repair strategies have limitations in effectively promoting articular cartilage repair, underscoring the need for innovative therapeutic approaches. A gene-activated matrix (GAM) is a promising and comprehensive therapeutic strategy that integrates tissue-engineered scaffold-guided gene therapy to promote long-term articular cartilage repair by enhancing gene retention, reducing gene loss, and regulating gene release. However, for effective articular cartilage repair, the GAM scaffold must mimic the complex gradient structure of natural articular cartilage. Three-dimensional (3D) bioprinting technology has emerged as a compelling solution, offering the ability to precisely create complex microstructures that mimic the natural articular cartilage. In this review, we summarize the recent research progress on GAM and 3D bioprinted scaffolds in articular cartilage tissue engineering (CTE), while also exploring future challenges and development directions. This review aims to provide new ideas and concepts for the development of gene-activated bioprinted scaffolds with specific properties tailored to meet the stringent requirements of articular cartilage repair.

1. Introduction

Articular cartilage injury, a common chronic progressive articular disease in orthopedic clinical practice, can cause pain, swelling, and joint dysfunction, significantly affecting the motor ability and quality of life of patients [1]. Articular cartilage injury is the primary pathological feature of osteoarthritis (OA) and can be caused by trauma, falls, age, and overuse [2,3]. Unlike other tissues, articular cartilage lacks the distribution of blood vessels, nerves, and lymph and features a high ratio of extracellular matrix (ECM) to cells, leading to poor self-repair ability after injury owing to insufficient cells, repair factors, and nutrients [4–6]. Therefore, it is imperative to develop effective treatment strategies for articular cartilage injury. Unfortunately, current clinical treatment strategies for cartilage repair exhibit limitations, such as trauma and fibrocartilage formation, resulting in unsatisfactory clinical applicability [7]. Notably, the continuous development of gene therapy and cartilage tissue engineering (CTE) has promising prospects for the application of a gene-activated matrix (GAM) in articular cartilage repair [8–10].

Gene therapy promotes articular cartilage repair by providing a continuous supply of therapeutic genes. To date, several gene therapy approaches have progressed to clinical trials for articular cartilage repair [1,11]. Gene vectors are used to deliver genes to specific cells, while preventing their enzymatic degradation; however, there is no guarantee that the accumulation and sustained release of genes will occur at the site of cartilage injury. To address these challenges, a GAM, prepared by incorporating gene complexes into tissue-engineered scaffolds, can ensure the retention of gene complexes at the injury site for an extended period, reducing the diffusion and loss of therapeutic genes *in vivo*. Additionally, it enables the controlled and sustained release of gene complexes at the injury site, thereby realizing long-term promotion of articular cartilage repair (Fig. 1) [8,12,13].

In recent years, extensive research has been conducted on CTE scaffolds, with some scaffolds transitioning toward clinical applications [14]. Among the many CTE scaffolds, biomedical hydrogels have a high-water content and porosity, facilitating nutrient transfer and waste removal. Their exceptional biocompatibility, biodegradability, and tunable mechanical properties support cell adhesion, proliferation, migration, and differentiation, rendering them ideal for cartilage repair [15,16]. However, hydrogels face challenges in mimicking the gradient

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structure and mechanical properties of natural cartilage. Compared to traditional tissue-engineering approaches, bioprinted scaffolds manufactured using three-dimensional (3D) printing technology have promising applications by accurately mimicking the intricate microstructure of natural cartilage tissue [17]. Advancements in 3D printing technology have led to an increase in the number of studies demonstrating the profound reparative effects of 3D biological scaffolds on cartilage damage [18,19]. For GAMs in cartilage repair, 3D bioprinted scaffolds play a crucial role in matrix optimization. These scaffolds can effectively mimic the structure of natural articular cartilage, thereby enhancing the efficiency of articular cartilage repair. The fundamental components of gene-activated bioprinted scaffolds are the therapeutic genes, gene vectors, and bioprinted matrices.

In this review, we provide a concise overview of the composition and gradient structure of the articular cartilage. Subsequently, we examine the promotion of cartilage repair factors and viral and non-viral gene vectors for cartilage damage repair, highlighting their respective advantages, disadvantages, and developmental progress. To ensure the effective retention and sustained release of gene complexes within the joint cavity, this review introduces hydrogels commonly used in gene activation matrices, such as hyaluronic acid (HA), gelatin, and sodium alginate. Moreover, we discuss the current advancements in 3D bioprinted hydrogel scaffolds in CTE, aiming to better mimic the intricate natural structure of cartilage. Additionally, we address the research progress in gene-activated bioprinted scaffolds. Finally, we examine the challenges and prospects of transitioning scientific research into clinical practice for cartilage repair, based on which the future challenges of gene-activated bioprinted scaffolds for cartilage repair are proposed, and the development direction of cartilage repair is anticipated.

2. Structure and composition of articular cartilage

The cartilage is composed of the perichondrium and cartilage tissue. The perichondrium surrounds the surface of the cartilage tissue, provides nutrition and vascular supply to the underlying cartilage tissue, and maintains the normal metabolism and activity of chondrocytes [20]. Cartilage tissue, a special type of soft tissue, is composed of chondrocytes and the cartilage ECM. Chondrocytes are special cell types that exists only in cartilage and are derived from mesenchymal stem cells (MSCs). Chondrocytes mainly secrete the cartilage ECM, the morphology of which changes with cartilage depth [21]. Based on the different fibrous components of the cartilage ECM, the cartilage tissue is categorized into hyaline cartilage, fibrocartilage, and elastic cartilage [22]. Hyaline cartilage, which is predominantly composed of type II collagen (COL II), is the most widespread type of cartilage in the body and is found mainly in the articular cartilage, costal cartilage, and nasal septum cartilage [23]. Fibrocartilage, which mainly consists of type I collagen (COL I), is primarily distributed in the intervertebral disc, temporomandibular joint, and meniscus [24]. Elastic cartilage, which is primarily composed of elastic fibers, is predominantly found in the ear and epiglottis [25]. Notably, articular cartilage and fibrocartilage lack the perichondrium covering the cartilage tissue [20].

Articular cartilage, which is situated on the bone surface within joints, plays a crucial role in maintaining lubrication, wear resistance,

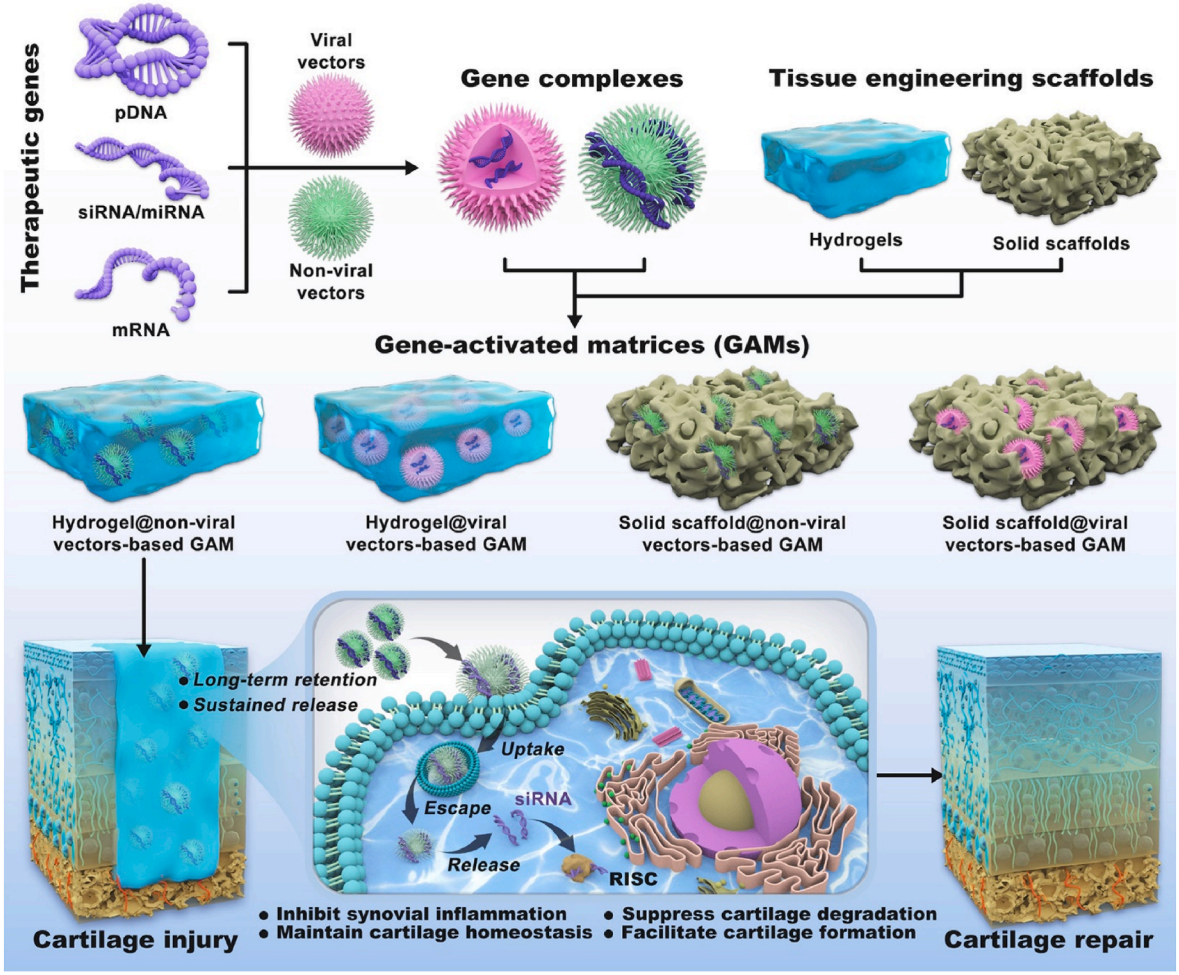

Fig. 1. Schematic illustration showing the preparation of GAMs for enhancing articular cartilage repair [8].

low friction, and facilitating smooth joint movement. The gradient structure of the natural articular cartilage tissue and its main individual components are shown in Fig. 2. The ECM of articular cartilage primarily consists of type I, II, IV, V, VI, IX, and XI collagen, aggrecan, and approximately 75 % water [22,26]. COL II, the predominant collagen type in hyaline cartilage, is vital for maintaining the normal physical and mechanical properties of articular cartilage. Its network structure provides tensile strength and prevents swelling of the interwoven aggrecan. Aggrecan exhibits a molecular structure resembling a "bottle brush," and aggrecan subunits, primarily formed by covalently linking glycosaminoglycans (GAGs) to core proteins, are attached to the main chain of HA through non-covalent bonds, enabling them to withstand compression. GAGs predominantly include chondroitin sulfate (CS), keratan sulfate, and dermatan sulfate, which impart a negative charge on the cartilage surface [21,22]. Collagen fibers and aggrecan are organized in a mesh-like pattern, ensuring nutrient exchange within the articular cartilage by allowing fluid flow through pressure changes induced by joint movement. The effective pore size of healthy cartilage is approximately 6 nm, whereas the mesh sizes of the collagen fiber and GAG networks are 50–100 nm and approximately 5 nm, respectively [27].

Normal human articular cartilage typically ranges from 2 to 4 mm in thickness and has a complex layered structure [27]. According to the arrangement of collagen fibers and the distribution of chondrocytes, articular cartilage is generally divided into four layers from the surface to the interior: the superficial (tangential) layer (10–20 %), middle (transitional) layer (40–60 %), deep (radial) layer (30–40 %), and calcified layer [21,22]. In the superficial layer, chondrocytes are characterized by flattened shapes and a single distribution, whereas collagen fibers exhibit an arched arrangement in parallel. Transitioning to the middle layer, the chondrocytes display a rounded and disorganized arrangement, with collagen fibers thickening and assuming an oblique distribution. Within the deep layer, the chondrocytes are larger, round/oval, and arranged in columns, with the thickest collagen fibers aligned perpendicular to the articular surface. The calcified layer signifies the zone in which the cartilage ECM undergoes calcification, linking it to the subchondral bone through internal COL II fibers [21,22,28]. The subchondral bone plays a critical role in providing stability and mechanical support to the joint, with its internal blood vessels delivering essential nutrients and oxygen to the chondrocytes. ECM homeostasis is primarily regulated by osteoblasts and osteoclasts, and its deterioration is a key pathological hallmark of OA [21,29].

Regrettably, the absence of blood vessels, nerves, and lymph tissue in articular cartilage, coupled with a high ratio of ECM to chondrocytes, leads to a scarcity of cells, repair factors, and nutrients necessary for effective post-injury repair. Additionally, articular cartilage injury initiates post-traumatic inflammation, chondrocyte death, and ECM degradation, resulting in a vicious cycle between inflammation and articular cartilage injury [30]. Therefore, it is paramount yet challenging to develop effective repair strategies for articular cartilage injury to enhance the quality of life of patients and alleviate the societal burden.

3. Gene-activated matrix for cartilage repair

3.1. Growth factors for cartilage repair

In CTE, MSCs have been extensively studied for their anti-inflammatory, immunoregulatory, and regenerative properties. However, there is uncontrollable proliferation and differentiation of MSCs after delivery to the body through the carrier. Therefore, measures must be taken to guide the directional differentiation of MSCs [31]. At present, researchers commonly utilize the method of adding growth factors to the carrier to induce the chondrogenic differentiation of MSCs, highlighting the crucial role growth factors play in the cartilage repair process. By strategically adding specific growth factors at appropriate concentrations, MSCs can be effectively guided toward chondrogenic differentiation, thereby enhancing the therapeutic efficacy of CTE. The key growth factors involved in CTE include fibroblast growth factor 2 (FGF-2), insulin-like growth factor 1 (IGF-1), transforming growth factor β (TGF-β), and bone morphogenetic protein (BMP). The main signaling pathways of these growth factors in articular cartilage are shown in Fig. 3. Moreover, SRY-box transcription factor 9 (SOX-9), a pivotal transcription factor, plays an important role in CTE [32]. These factors stimulate cell proliferation and differentiation, and contribute to maintaining the stability, integrity, and developmental status of the cartilage tissue [33,34].

FGF-2, an endogenous growth factor, binds to heparan sulfate proteoglycan in the ECM of articular cartilage. FGF-2 regulates cell proliferation, differentiation, and apoptosis by specifically binding to tyrosine kinase receptors and activating the FGF/FGFR signaling pathway, thus playing an important role in cartilage metabolism [35,36]. Notably, FGF-2 exerts two opposite effects on cartilage repair by binding to specific receptors. When combined with FGFR1, FGF-2 expedites cartilage degradation through the Raf-MEK1/2-ERK1/2 cascade reaction. This cascade reaction is triggered by two key signaling mediators, Ras and PKCδ, leading to the activation of two critical transcription factors, ELK1 and RUNX2, which in turn upregulate the expression of MMP-13, ADAMTS4, ADAMTS5, COL10A1, and vascular endothelial growth

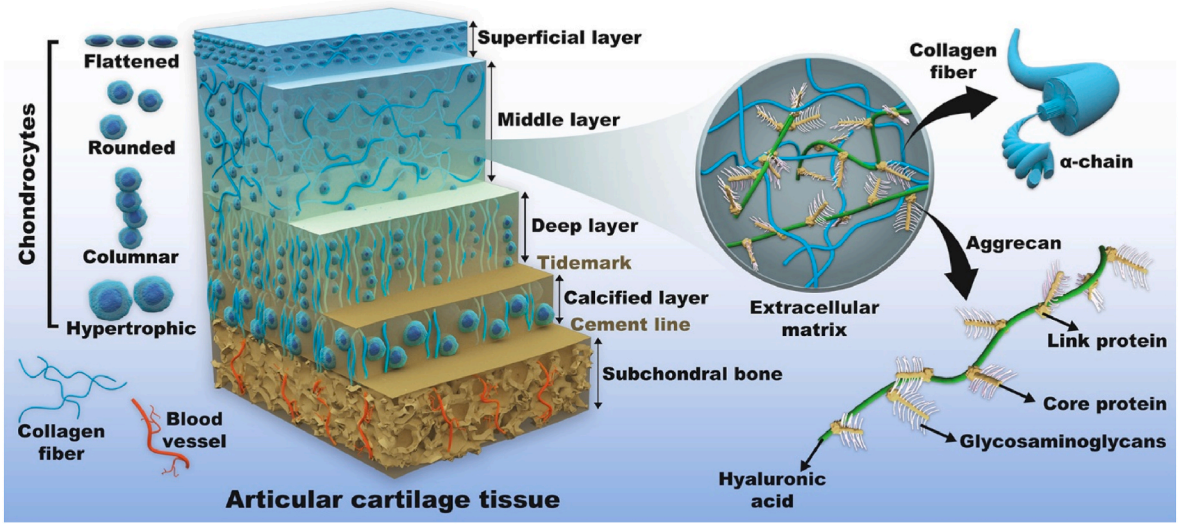

Fig. 2. Schematic illustration showing the gradient structure of natural articular cartilage tissue and its main individual components [22,28].

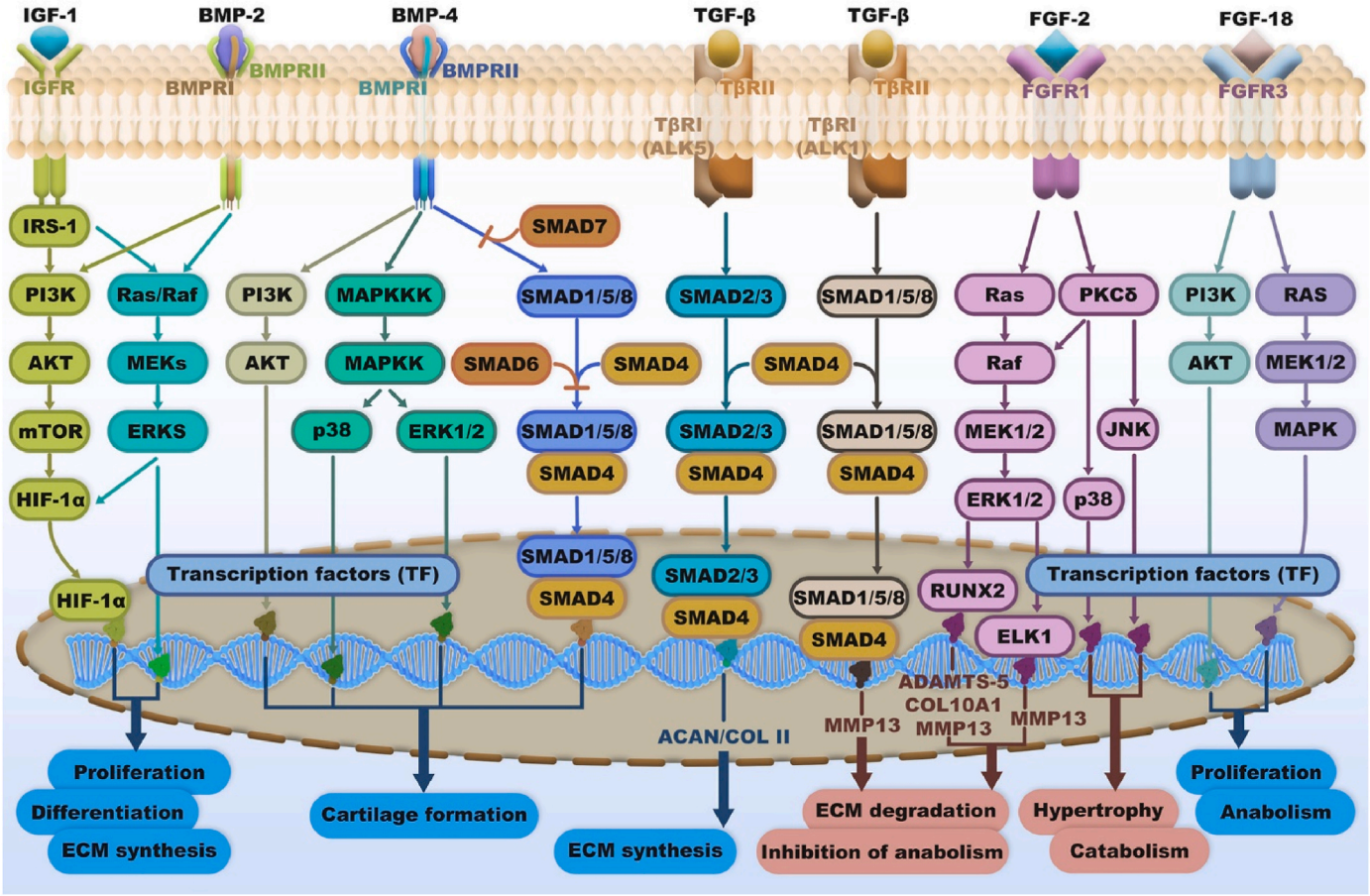

Fig. 3. Schematic illustration showing the main growth factors signaling pathways in articular cartilage [37,42,44,45,56,64].

factor (VEGF). Moreover, the activated PKCδ can also stimulate the p38 and JNK signal pathways, hastening cartilage degradation while inhibiting anabolic activity [36,37]. In contrast, FGF-2 may promote cartilage repair when combined with FGFR3. However, the effectiveness of this regulation remains controversial because different outcomes have been observed in murine and human chondrocytes [38]. Notably, FGF-2 plays a role in regulating subchondral bone to promote cartilage repair [39]. In addition, FGF-2 can promote cartilage repair by regulating various factors such as SOX-9, TGF-β2, VEGF, BMP-2, and BMP-4 [40,41]. In contrast to the contentious role of FGF-2 in cartilage repair, when combined with FGFR, FGF-18 has been demonstrated to stimulate cartilage anabolism by promoting ECM formation and chondrocyte differentiation [42]. Through PI3K-AKT signaling, FGF-18 enhances the proliferation and migration of chondrocytes, decreases the generation of reactive oxygen species (ROS), attenuates cell apoptosis, and restores mitochondrial function [43].

IGF-1 is a polypeptide with a molecular structure similar to that of insulin. IGF-1 can promote chondrocyte proliferation and cartilage ECM deposition while inhibiting chondrocyte apoptosis and plays a crucial role in regulating cartilage tissue by promoting anabolism and inhibiting catabolism. Additionally, IGF-1 can upregulate hypoxia-inducible factor 1 alpha (HIF-1α) to protect cartilage tissue and enhance aggrecan and COL II levels, thereby promoting cartilage repair. The signal pathways of IGF-1 upregulating HIF-1α protein levels involve PI3K/Akt/mTOR and Ras/Raf/MEK/ERK [44–46]. Notably, proteoglycan synthesis by IGF-I-stimulated chondrocytes requires activation of the PI3K pathway rather than ERK/MAPK [47]. Furthermore, IGF-1 inhibits chondrocyte apoptosis via the PI3K pathway [48]. It is important to note that high doses of IGF-1 favor cartilage formation, whereas low doses of IGF-1 are conducive to subchondral bone reconstruction [49]. In rabbit and equine cartilage defect models, the transplantation of chondrocytes overexpressing IGF-1 has been shown to effectively promote cartilage formation *in vivo* [50,51].

TGF-β plays a crucial role in regulating the proliferation and differentiation of chondrocytes, promoting the deposition of cartilage ECM such as aggrecan and COL II, and maintaining cartilage homeostasis [52–54]. The interaction between TGF-β and Smad3 molecules enhances the SOX-9-dependent transcription on chromatin, thereby inducing chondrogenesis [55]. TGF-β can induce Smad2/3 signaling to activate an anabolic response when interacting with ALK5 [56]. Moreover, the interaction between TGF-β and Smad2/3 molecules can be mediated by the MEK/ERK and p38 MAPK signaling pathways to regulate the chondrogenesis of MSCs. Inhibition of the MEK/ERK pathway increases TGF-β-induced Smad2/3 gene transcription levels, upregulating the expression of chondrocyte-specific genes, whereas inhibition of the p38 MAPK pathway decreases TGF-β-induced Smad2/3 gene transcription levels, downregulating the expression of chondrocyte-specific genes [57]. However, TGF-β can induce Smad1/5/8 signaling to activate a catabolic response when interacting with ALK1. Excessive TGF-β activation can cause synovial fibrosis and osteophyte formation, impacting its further application to cartilage repair [56]. Co-transfection with Smad7 not only promotes cartilage repair but also blocks the synovial fibrosis induced by TGF-β [58]. TGF-β stimulation can affect the expression of p38 in MSCs, and the Wnt/β-catenin inhibitor XAV939 can significantly inhibit the level of Smad1/5, which together lead to a reduction in RUNX2 expression, effectively inducing hyaline cartilage formation and inhibiting chondrocyte hypertrophy to prevent endochondral ossification [59].

BMP is a member of the TGF-β superfamily known for its osteoinductive and chondrogenic abilities [60]. Unlike TGF-β signal

transduction mediated by Smad2/3, BMP signal transduction occurs mainly through Smad1/5/8. Moreover, BMP-induced chondrogenesis is associated with the p38 MAP kinase pathway [54]. Among the BMPs studied in CTE, BMP-2, BMP-4, BMP-6, and BMP-7 are the most prominent. These BMPs can effectively prevent cartilage damage resulting from inflammation or trauma and facilitate cartilage regeneration by binding to specific receptors to activate distinct intracellular signaling pathways [61]. However, the application of BMP-2 in cartilage repair remains debatable. BMP-2 not only stimulates chondrocyte proliferation and chondrogenic differentiation of MSCs but also triggers hypertrophic differentiation and endochondral ossification of MSCs, thereby disrupting the chondrogenic phenotype. Silencing Smad7 enhances BMP-2-induced chondrogenic differentiation and impedes endochondral ossification [62]. BMP-2, which is mediated by Smad1/5, enhances the expression of RUNX-2, subsequently upregulating the transcription of the type X collagen (COL X) gene and modulating chondrocyte hypertrophy. Conversely, BMP-4 suppresses COL X expression, thereby preventing chondrocyte hypertrophy [54]. BMP-4 promotes chondrocyte proliferation, ECM deposition, and cartilage maturation *in vivo*, while also inducing redifferentiation of dedifferentiated chondrocytes [63,64]. BMP-6 functions as an autocrine factor that promotes chondrocyte differentiation and maturation. The inhibition of its expression via a parathyroid hormone-related peptide (PTHrP) can prevent maturation [65]. BMP-6 is capable of inducing chondrogenic differentiation of human adipose-derived stem cells (ADSC) by upregulating the expression of chondrogenic genes and downregulating the expression of chondrocyte hypertrophy and endochondral ossification genes [66]. Additionally, BMP-7 exhibits pro-anabolic and anti-catabolic effects on cartilage tissue repair [67]. It can suppress inflammation in arthritis, inhibit cartilage degeneration, and delay degeneration associated with intense excessive running [68,69]. Notably, unlike BMP-2, which triggers hypertrophic differentiation during the chondrogenic differentiation of progenitor cells, BMP-7 suppresses hypertrophic differentiation [70].

SOX-9 plays a pivotal role in regulating chondrogenic differentiation and maintaining cartilage morphology and function (Fig. 4) [71]. SOX-9 promotes the expression of cartilage-related genes, such as collagen type II alpha 1 (COL2A1), cartilage oligomeric matrix protein (COMP), and aggrecan (ACAN), and facilitates the synthesis of the cartilage ECM, which is crucial for the maintenance of cartilage integrity and function [72,73]. Furthermore, SOX-9 is critical for chondrocyte hypertrophy, which can maintain the lineage fate of chondrocytes to allow hypertrophic maturation and block osteoblastic differentiation by reducing β-catenin and RUNX2 activity [74]. SOX-9 can inhibit Wnt/β-catenin signals by competing with β-catenin binding to LEF/TCF proteins [75]. SOX-5, SOX-6, and SOX-9 are collectively referred to as the SOX trio. SOX-5 and SOX-6 can synergistically enhance the stability and efficiency of SOX-9 binding to its target regions [76]. Activation of the SOX trio promotes chondrogenesis and inhibits endochondral ossification of MSCs [77]. Notably, the SOX trio can upregulate PTHrP expression to negatively regulate chondrocytes maturation and calcification [78]. Moreover, TGF-β, BMP-2, and related miRNA play significant roles in regulating the transcription and expression of SOX-9 [55,71,73]. Additionally, mechanical stimulation and zinc-finger protein 145 enhance the regulatory function of SOX-9 in the chondrogenic differentiation of MSCs [79,80].

Other factors also play vital roles in cartilage repair. Indian Hedgehog (IHH) has the potential to induce cartilage repair, but high levels of IHH can lead to hypertrophic differentiation of chondrocytes. Notably, the negative feedback effect between IHH and PTHrP can inhibit the hypertrophic differentiation of chondrocytes [81,82]. Enhanced Sonic Hedgehog (SHH) activity can boost the chondrogenic differentiation of MSCs [83]. SHH promotes the redifferentiation of dedifferentiated chondrocytes by stimulating the expression of SOX-9, BMP-2, and IGF-1, thereby improving cartilage repair [84]. Soluble FLT-1, acting as a VEGF antagonist, blocks VEGF signal transduction, preventing blood vessel and bone invasion, promoting the chondrogenic potential of mouse skeletal muscle-derived stem cells, and improving articular cartilage repair persistence [85]. Connective tissue growth factors can be used to encourage articular cartilage regeneration and inhibit the mineralization of articular chondrocytes [86]. Increased expression of HIF-2α in the chondrocytes of OA can induce chondrocyte apoptosis and cartilage ECM degradation. Therefore, alleviating the progression of OA can be achieved by silencing the expression of the HIF-2α gene [87].

3.2. Gene vectors for cartilage repair

Extensive research has been conducted on growth factors in CTE. Nevertheless, *in vivo*, growth factors are swiftly eliminated, lack effective targeting of specific cells, and exhibit short half-lives, resulting in limited therapeutic efficacy [88,89]. To address this challenge, gene therapy has emerged as a potential solution for modulating cell behavior. By introducing specific genes into cells, sustained and controlled expression of growth factors can be achieved, preventing the

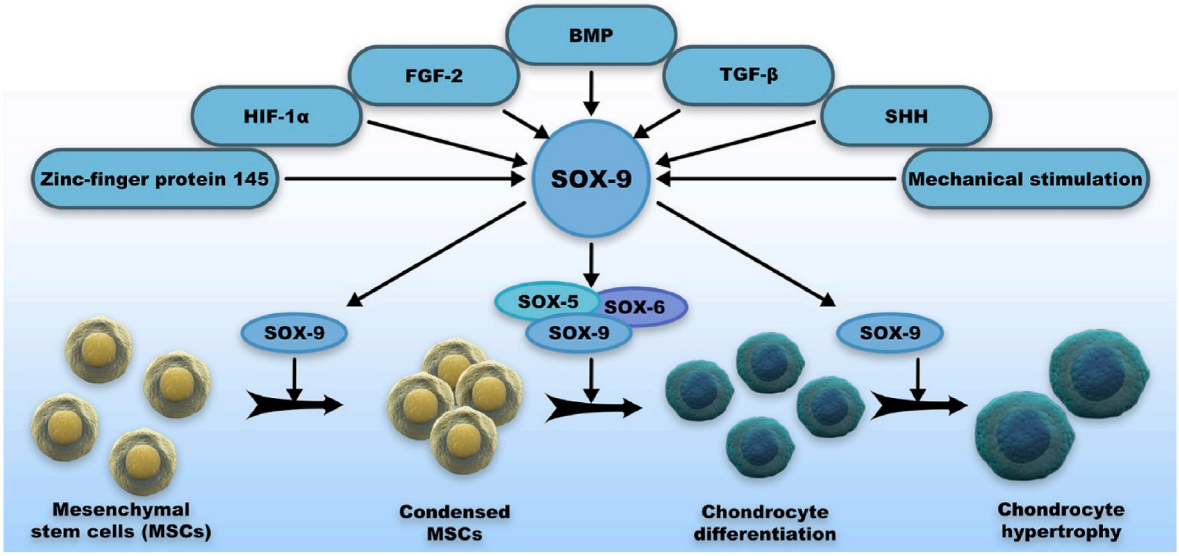

Fig. 4. Schematic illustration showing the sequential actions of SOX-9 on chondrogenesis of MSCs [71].

rapid degradation and uncontrolled release of growth factors [90,91]. Gene therapy has the potential to enhance cartilage repair by continuously producing therapeutic gene products, thus promoting cartilage repair. This approach presents a novel strategy for CTE that offers precise regulation of cell behavior to foster cartilage regeneration and repair [31,92]. However, gene therapy has several challenges, primarily involving the enzymatic degradation of gene nucleases, cellular uptake, and endosomal escape [93]. Moreover, articular cartilage is avascular and characterized by a dense negatively charged ECM, the low permeability of which presents a barrier to efficient gene transfer [94,95]. Therefore, it is essential to develop efficient, non-toxic, and targeted gene vectors to facilitate cartilage repair effectively. An ideal gene vector should possess qualities such as safety, efficacy, targeting specificity, high loading capacity, stability, and ease of large-scale production [96,97].

Currently, the commonly utilized gene vectors consist of both viral and non-viral vectors (Table 1). Viral gene carriers, such as lentiviruses, adenoviruses (AVs), and adeno-associated viruses (AAVs), demonstrate remarkable gene delivery and transfection efficiency. However, their potent immunogenicity and carcinogenic potential hinder clinical

Table 1
The commonly utilized gene vectors for CTE.

Vectors Type	Vectors Name	Genes	Transfected Cells	Biofunctions	Ref.
Viral vector	Lentivirus	shRNA targeting aggrecanase-1 and -2 transcripts	Chondrocytes	Inhibiting chondrocyte aggrecanases; Promoting cartilage formation;	[99]
	Lentivirus	siRNA targeting ADAMTS-5	chondrocytes	Downregulating ADAMTS-5 protein; Preventing the degradation of articular cartilage in rats with OA;	[100]
	Lentivirus	BMP-2	BMSCs	promoting cartilage and bone repair;	[101]
	Adenoviral	BMP-2 cDNA or IGF-1 cDNA	Mesenchymal cells	Stimulating the regeneration of hyaline articular cartilage;	[102]
	rAAV	FGF-2, IGF-1, or SOX-9	MSCs and chondrocytes	Promoting cartilage repair;	[104–106]
	Carbon dots-guided rAAV	SOX-9 or TGF-β	MSCs	Promoting cell proliferation and cartilage ECM deposition; Inhibits COL I and COL X accumulation;	[107]
Non-viral vectors	Lipofectamine 2000	TGF-β1	BMSCs	Promoting cartilage ECM deposition; Reducing the levels and activity of MMP1 and MMP3 protein;	[110]
	Peptide-modified ionizable lipid NPs	IGF mRNA	Chondrocytes	Exhibiting superior penetrability and retention; Inhibiting chondrocyte apoptosis and cartilage ECM degradation; Maintaining interfacial cellularity;	[48]
	Cationic niosomes	SOX-9 pDNA	MSCs	Promoting chondrogenic differentiation; Reducing hypertrophic differentiation;	[72]
	Non-liposomal lipid	IGF-I cDNA	Chondrocytes	Upregulating IGF-1 gene expression; Promoting articular cartilage repair;	[111]
	Cationic carbon dots	SOX-9 pDNA	MEFs	Promoting chondrogenic differentiation;	[113]
	Modified QDs	RUNX2 siRNA	MSCs	Suppressing hypertrophic differentiation	[114]
	Peptidic NPs	NF-κB siRNA	Chondrocytes	Reducing early chondrocyte apoptosis and reactive synovitis; Maintaining cartilage homeostasis;	[115]
	CuS@PC NPs	TGF-β1 pDNA	MSCs	Facilitating cartilage repair;	[116]
	HA-shielded PEI	SOX-9 pDNA	MSCs	Mitigating the cytotoxicity of PEI; Enhancing the uptake of gene complexes and chondrogenesis of MSCs;	[118]
	HA-PEI	RUNX2 pDNA and SOX trio pDNA	/	Promoting osteochondral tissue regeneration;	[119]
	HA-ss-PEI	endostatin pDNA	MSCs	Enhancing the secretion of anti-angiogenic protein;	[120]
	CAP-PEI	HIF-2α siRNA	Chondrocytes	Preventing synovial inflammation and cartilage degradation; Maintaining cartilage integrity;	[121,122]
	PEI-modified PLGA NPs	SOX-9 pDNA and Cbfa-1 siRNA	MSCs	Promoting the chondrogenesis;	[123,124]
	PEI-modified polysaccharide	SOX-9 pDNA	MEFs	Promoting the reprogramming of MEFs into chondrocytes	[126]
	PEI-coupled with dexamethasone	SOX trio pDNA	MSCs	Upregulating chondrogenic factors; Downregulating osteogenic factors;	[127]
	G5-AHP	miR-140	Chondrocytes	Promoting COL II expression; Inhibiting ADAMTS-5 and MMP-13 expression; Maintaining cartilage ECM homeostasis;	[132]
	G5-AHP	miR-224-5p	Chondrocytes	Suppressing cartilage degradation and synovial inflammation; Restoring homeostasis and synergistically alleviating OA;	[133]
	Phosphorus dendrimers	HIF-2α siRNA	ATDC5 cells	Reducing the level of MMP-13 protein by downregulating HIF-2α	[134]
	pSP-modified CTS	miR-140, IL-1Ra pDNA, and IGF-1 pDNA	Chondrocytes	Enhancing chondrocyte proliferation and ECM deposition; Inhibiting inflammatory responses, chondrocyte apoptosis, and nitric oxide synthesis	[136–138]
	CS-modified CTS	MMP-13 siRNA	chondrocytes	Downregulating the expression of MMP-13	[139]
	Transferrin-modified PLL and cationic liposomes	pDNA carrying the marker β-gal, PTHrP and TGF-β1 genes	primary chondrocytes	Facilitating the repair of articular cartilage defects	[145]
	PEO-modified PLL	TGF-β1 pDNA	MSCs	Upregulating expression of chondrogenesis-related genes	[146]

application [98]. Lentivirus-mediated shRNA significantly reduces aggrecanase transcripts levels in chondrocytes. This reduction leads to increased chondrocyte proliferation and enhanced deposition of GAGs and total collagen, ultimately promoting cartilage formation [99]. Moreover, lentivirus-mediated siRNA inhibits a disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5), effectively preventing articular cartilage degradation in rats with OA [100]. Furthermore, the combined delivery of lentivirus-mediated BMP-2 into bone MSCs (BMSCs) and platelet-rich plasma (PRP) has proven to be an effective strategy for promoting cartilage and bone repair in cartilage defects [101]. The delivery of BMP-2 cDNA or IGF-1 cDNA using an AV vector can stimulate the regeneration of hyaline articular cartilage by enhancing the deposition of cartilage ECM components, such as COL II and aggrecan, and inhibiting the production of COL I. Notably, compared with IGF-1 cDNA, BMP-2 cDNA-transfected cells are accompanied by the formation of osteophytes [102]. Recombinant AAV (rAAV) vector-mediated gene therapy targeting FGF-2, IGF-1, or SOX-9, has been proven effective in promoting cartilage repair [103–106]. Engineered carbon dot-guided rAAV vectors have demonstrated significant enhancements in delivering SOX-9 or TGF-β, thereby stimulating cell proliferation and promoting the deposition of cartilage ECM. Simultaneously, this strategy effectively inhibits the accumulation of COL I and COL X in the cartilage ECM [107].

In contrast, non-viral gene vectors such as lipofectamine, nanoparticles (NPs), and cationic polymers provide advantages such as lower immunogenicity, minimal toxicity, and ease of production [108,109]. Lipofectamine 2000 has shown efficacy in delivering TGF-β1 into MSCs, thereby promoting the deposition of cartilage ECM and reducing the levels and activities of MMP1 and MMP3 proteins. MSCs modified with TGF-β1 loaded onto a polysaccharide-based scaffold can effectively facilitate the repair of hyaline cartilage in the rabbit knee joint [110]. A cartilage-targeted gene delivery complex (IGF mRNA-cLNP) was prepared by combining messenger RNA (mRNA) encoding IGF-1 with lipid NPs modified with a peptide capable of binding to aggrecan in the cartilage. The IGF mRNA-cLNP demonstrated superior penetrability and retention in the cartilage, effectively inhibiting chondrocyte apoptosis. *In vivo*, the IGF mRNA-cLNP maintained their interfacial cellularity and inhibited ECM degradation [48]. Compared with lipofectamine, niosomes are known for their enhanced stability and lower toxicity. Cationic niosomes were developed using a combination of polysorbate 80 and poloxamer 407, which acted as non-ionic surfactants. Niosomes efficiently deliver the SOX-9 plasmid (pDNA), thereby promoting effective chondrogenic differentiation and reducing the hypertrophic differentiation of MSCs [72]. Furthermore, utilizing a non-liposomal lipid formulation for the delivery of IGF-I cDNA leads to the upregulation of IGF-1 gene expression in chondrocytes, effectively promoting the repair of articular cartilage and inhibiting OA [111].

Inorganic NPs play a crucial role in gene delivery for CTE [112]. Research has revealed that utilization of arginine and glucose through microwave-assisted pyrolysis produces cationic carbon dots with low cytotoxicity, high photoluminescence, and improved transfection efficiency of SOX-9 pDNA, which can effectively induce chondrogenesis in mouse embryonic fibroblasts (MEFs) [113]. Quantum-dot (QD) NPs vectors are also used for gene delivery in CTE. The modification of β-cyclodextrin and RGD peptides on QD nanocarriers enables the efficient co-delivery of kartogenin (KGN) and RUNX2 siRNA, which can promote chondrogenic differentiation and suppress hypertrophic differentiation of hMSCs. Moreover, QD nanocarriers exhibit excellent photostability and allow prolonged tracking *in vivo* [114]. Peptidic NPs efficiently deliver NF-κB siRNA to reduce early chondrocyte apoptosis and reactive synovitis, while boosting AMPK signaling and suppressing mTORC1 and Wnt/β-catenin activities to maintain cartilage homeostasis. The NPs can deeply penetrate the cartilage and remain localized for extended durations, overcoming challenges in gene delivery to chondrocytes [115]. Compared to lipofectamine 3000, biomimetic copper sulfide@phosphatidylcholine (CuS@PC) NPs (CTPs) exhibit higher transfection efficiency for TGF-β1 pDNA in MSCs. The CTP-engineered MSCs enhance cell migration, promote chondrogenesis, and inhibit ECM degradation in the cartilage. In OA mice, the intraarticular injection of CTP-engineered MSCs significantly facilitates cartilage repair [116].

Cationic polymers primarily interact with genes via electrostatic interactions and offer excellent safety, ease of modification, and stable physical and chemical properties. Moreover, these polymers can effectively overcome the negative electrostatic barrier of the cartilage ECM and reversibly bind to enhance gene availability. Therefore, cationic polymers are considered promising gene vectors for cartilage repair [12,93,96].

Polyethyleneimine (PEI) is a commonly used non-viral gene vector known for its high transfection efficiency and ease of modification. However, significant cytotoxicity restricts its further development for clinical applications [117]. Hence, the combination of PEI and other polymers enhances the gene transfection efficiency and mitigates cytotoxicity. Utilizing HA to shield the PEI/SOX-9 pDNA gene complex has been proven effective for mitigating cytotoxicity linked to PEI. Moreover, the specific interaction between HA and CD44 receptors on the surface of MSCs not only enhances the uptake of gene complexes by cells, but also facilitates the chondrogenesis of MSCs [118]. The HA-PEI vector can effectively deliver RUNX2 pDNA and SOX trio pDNA, and then be embedded in the hydrogel scaffold in layers, effectively promoting osteochondral tissue regeneration [119]. Additionally, a stimuli-responsive HA-ss-PEI vector was developed by integrating disulfide bonds into HA-PEI, which exhibited redox- and pH-responsive properties for endostatin pDNA release to enhance the secretion of anti-angiogenic proteins without affecting the chondrogenic differentiation of MSCs [120]. Moreover, by modifying PEI with a chondrocyte-affinity peptide (CAP), the CAP-PEI vector can acquire cartilage-specific targeting capabilities, thereby enhancing transfection efficiency [121]. Subsequently, the CAP-PEI vector delivers HIF-2α siRNA to the joint, markedly enhancing the local concentration and prolonging the residence time of siRNA in cartilage. This vector can specifically target the delivery of HIF-2α siRNA into chondrocytes, efficiently preventing synovial inflammation and cartilage degradation in OA mice by inhibiting the expression of inflammatory factors and catabolic proteins, thereby maintaining cartilage integrity [122]. Additionally, the application of PEI-modified poly(lactide-co-glycolic acid) (PLGA) NPs can significantly improve the cellular uptake of SOX-9 pDNA, effectively promoting MSC chondrogenesis [123]. PEI-modified PLGA NPs facilitate the co-delivery of SOX-9 pDNA and Cbfa-1 siRNA to allow MSC chondrogenesis by upregulating the expression of cartilage-related genes [124]. Similarly, PEI-modified PLGA NPs can induce chondrogenesis in MSCs by co-delivering SOX-9 protein and Cbga-1 siRNA [125]. Compared to pure PEI, the vector prepared using the PEI-modified polysaccharide exhibits a higher SOX-9 pDNA transfection efficiency and lower cytotoxicity, which can effectively promote the reprogramming of MEFs into chondrocytes [126]. Additionally, the vector formed by coupling PEI and dexamethasone demonstrates superior transfection efficiency of SOX trio pDNA, facilitating the chondrogenic differentiation of MSCs by upregulating chondrogenic factors and downregulating osteogenic factors [127].

Polyamidoamine (PAMAM) dendrimers are hyperbranched polymers with unique 3D structures and nanoscale sizes. The numerous cationic groups present on the surface enable efficient gene binding, making them a widely studied gene vector [128,129]. Polyethylene glycol (PEG)-modified PAMAM possesses adjustable surface charge characteristics and improved penetration of cartilage tissue, which can significantly prolong the residence time of IGF-1 in the joints, suppress cartilage degradation, and enhance the therapeutic effect for OA [130]. Further introduction of CAP can endow PEG-PAMAM with enhanced cellular uptake and cartilage-targeting ability, rendering it an ideal vector for targeting articular cartilage [131]. Furthermore, the amino acid-functionalized generation 5 PAMAM dendrimer (G5-AHP) exhibits

enhanced stability, cellular uptake, and transfection efficiency, while sustaining the long-term biological activity of the gene. The G5-AHP vector can efficiently carry miR-140 to promote the expression of COL II and inhibit the expression of ADAMTS-5 and MMP-13, thereby maintaining ECM homeostasis. Loading into the hydrogel microsphere can prevent the degradation of articular cartilage and alleviate the progression of OA [132]. Compared with lipofectamine 3000, the G5-AHP vector loaded with miR-224-5p exhibits enhanced cellular uptake and transfection efficiency, along with the protection of miR-224-5p from RNase degradation. In an OA mouse model, the PAMAM/miR-224-5p complex effectively suppresses cartilage degradation and synovial inflammation, thereby restoring homeostasis and synergistically alleviating OA [133]. Compared with PAMAM, phosphorus dendrimers are heteroatom dendritic polymers with a more robust spatial structure and enhanced transfection efficiency. The phosphorus dendrimer prepared using hexachlorocyclotriphosphonitrile as the core demonstrates proficiency in effectively loading HIF-2α siRNA. This vector also shows responsiveness to the inflammatory microenvironment, facilitating deep penetration and efficient enrichment of HIF-2α siRNA within the cartilage ECM. Simultaneously, it reduces the level of MMP-13 protein by downregulating HIF-2α. Additionally, encapsulation within methacrylated HA (HA-MA) hydrogel microspheres effectively delays the progression of OA by inhibiting cartilage ECM degradation and subchondral bone changes [134].

Chitosan (CTS) is a biocompatible and biodegradable polysaccharide that has been widely studied as a non-viral gene vector. Studies have shown that high-molecular weight (MW) CTS can form stable NPs, whereas low-MW CTS enhances transfection efficiency. Furthermore, low-MW oligomeric CTS with high deacetylation was found to increase gene transfection efficiency without affecting cell viability [108]. However, the transfection efficiency of CTS is relatively low under physiological conditions. To address this challenge, researchers have explored chemical structure modifications to enhance the gene transfection efficiency of CTS. For instance, the conjugation of phosphorylatable short peptides (pSP) has been found to improve the pDNA transfection efficiency of CTS [135]. The pSP-modified CTS vector facilitates the co-transfection of specific genes including IL-1Ra/miR140, IL-1Ra/IGF-1, and IGF-1/miR140, resulting in enhanced chondrocyte proliferation, ECM deposition, and inhibition of inflammatory responses, chondrocyte apoptosis, and nitric oxide synthesis, ultimately promoting the repair of full-thickness cartilage defects in rabbits [136–138]. Additionally, CS-modified CTS can effectively downregulate MMP-13 expression in chondrocytes by loading and delivering MMP-13 siRNA, which can be used as a gene vector to target articular cartilage tissue [139].

Poly-L-lysine (PLL) is a cationic peptide that induces early chondrogenic gene expression, thereby promoting the chondrogenic differentiation of MSCs. Additionally, PLL may regulate chondrogenic differentiation by altering the biosynthesis and distribution of GAGs, the cross-linking of aggrecans with cells, and cell morphologys [140,141]. Compared with PLL, star-shaped PLL and triblock PLL-based polymers with intermediate hydrophobic chains exhibit higher pDNA transfection efficiency [142,143]. ε-PLL (EPL) is a safe vector that can interact with the cartilage ECM through electrostatic interactions, enhancing the permeation and retention of genes and drugs within the cartilage, making it suitable for treating OA [144]. The combination of a transferrin-modified PLL vector and cationic liposomes demonstrates great efficiency in delivering pDNA carrying the marker β-galactosidase (β-gal), PTHrP, and TGF-β1 genes into primary chondrocytes and perichondrial cells, enabling targeted gene product expression to facilitate the repair of articular cartilage defects [145]. Poly (ethylene oxide) (PEO)-modified PLL has been shown to successfully deliver TGF-β1 pDNA into MSCs, leading to upregulated expression of chondrogenesis-related genes. Additionally, a PLGA scaffold filled with PEO-PLL/TGF-β1 pDNA, fibrin gel, and MSCs can promote the deposition of GAGs and COL II, thereby promoting cartilage restoration *in vivo* [146].

Currently, there are limitations associated with surgery and autologous chondrocyte implantation (ACI) for cartilage repair, such as the formation of fibrocartilage and its limited effectiveness [147,148]. In contrast, gene therapy shows promise in enhancing cartilage repair by maintaining the expression of targeted gene products directly at the site of injury. However, therapeutic genes tend to degrade quickly enzymatically in the body and cannot be retained at the injury site for an extended period, resulting in suboptimal therapeutic outcomes [12,149]. The use of gene vectors has demonstrated significant benefits in delivering genes to specific cells while protecting them from enzymatic degradation. However, achieving sustained release and accumulation of these genes at the injury site remains a challenge, particularly because of dilution by the synovial fluid. Furthermore, the nonspecific diffusion of genes can lead to adverse side effects, including immune responses and synovial chondrogenesis. The integration of biological scaffolds offers a promising solution, enabling the precise spatiotemporal release of gene products and facilitating long-term cartilage repair treatment. According to the approach of gene therapy for CTE, these scaffolds not only facilitate the delivery of gene-transfected cells but also serve as a platform for loading gene complexes to generate GAMs (Fig. 5) [12,31].

3.3. Gene-transfected cells for cartilage repair

Delivery of gene-transfected cells for CTE is shown in Table 2. Commonly used bioactive scaffolds are primarily composed of collagen, HA, gelatin, and CTS because of their biocompatibility, capacity to promote cartilage repair, and compositional similarity to the cartilage tissue. The ADSCs have been modified to overexpress TGF-β1 by transfecting TGF-β1 pDNA using poly (β-amino ester). The transfected cells were then encapsulated in an injectable ECM-mimicking hydrogel composed of thiolated HA (HA-SH) and COL I. The hydrogel can reduce cell death during injection, enhance the paracrine effect of OA-like chondrocytes, reduce the level of TNF-α, and promote the deposition of the cartilage ECM. Moreover, the hydrogel effectively alleviates OA progression by reducing cartilage degeneration and inhibiting inflammation [150]. SOX-9 pDNA-transfected MSCs/chondrocytes via nucleofection were embedded in polysaccharide capsules, which significantly increased the cartilage-like ECM and promoted chondrogenesis [151]. The transfected cells were prepared using the commercially available TurboFect vector to transfer BMP-7 pDNA into primary chondrocytes and then encapsulated into a gelatin-oxidized dextran cryogel, which promoted the growth of chondrocytes and cartilage repair [152]. Notably, MSCs transfected with SOX-9, TGF-β1, or BMP-2 using AV were able to differentiate into cartilage tissues with varying degrees of hypertrophy in collagen hydrogel. Compared with TGF-β1 and BMP-2, MSCs transfected with SOX-9 displayed the least hypertrophy in the differentiated cartilage tissue [153]. Several miRNAs, including miR-30a, miR-488, miR-221, and miR-410, play crucial roles in the regulation of chondrogenic differentiation. The miR-410 transfected MSCs with lipofectamine were embedded in methacrylated gelatin (Gel-MA) scaffolds, which enhanced their migration, proliferation, and differentiation, thereby achieving rabbit cartilage defect repair [154].

3.4. Biological scaffolds-based gene-activated matrices for cartilage repair

Compared to directly delivering gene-transfected cells, GAMs can ensure the long-term retention of gene complexes at the site of injury. This can reduce the diffusion of gene complexes *in vivo* and provide a continuous and controllable release of gene complexes *in situ* to achieve long-term promotion of cartilage repair. Moreover, GAMs can overcome the limitations of long-term expression caused by the migration and apoptosis of gene-transfected cells [12]. Increasing evidence suggests that the GAM is a promising approach for CTE (Table 3). However, there are still some challenges in preparing GAMs with high biocompatibility,

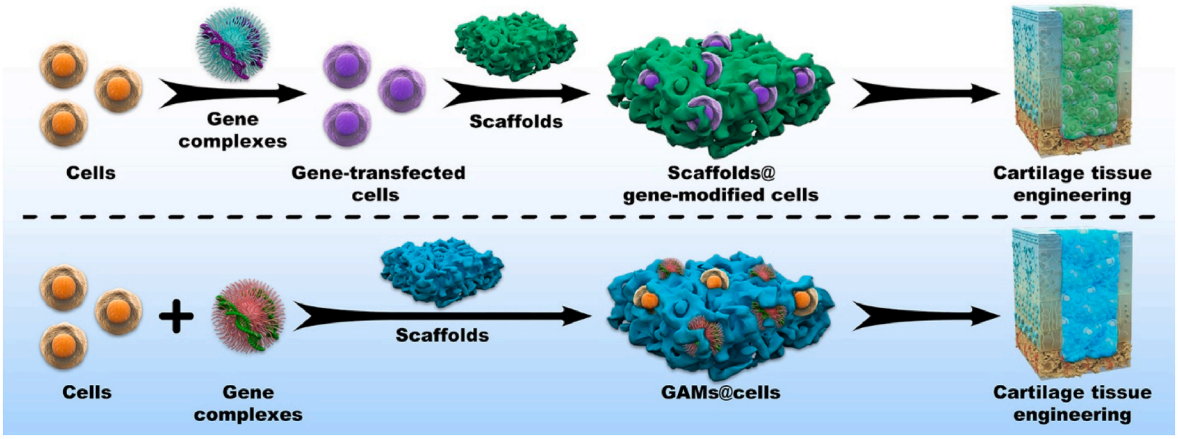

Fig. 5. Schematic illustration showing the delivery of gene-transfected cells or gene complexes/cells using scaffolds for CTE.

Table 2
The gene-transfected cells used for CTE.

Gene complexes	Modified Cells	Scaffolds	Biofunctions	Ref.
Poly (β-amino ester)/TGF-β1 pDNA	ADSCs	HA-SH/COL I	Promoting cartilage ECM deposition; Alleviating the progression of OA;	[150]
Nucleofector system/SOX-9 pDNA	MSCs/ Chondrocytes	Polysaccharide capsules	Increasing the cartilage-like ECM; Promoting chondrogenesis;	[151]
Turbofect/ BMP-7 pDNA	Primary chondrocytes	Gelatin-oxidized dextran cryogel	Promoting the growth of chondrocytes and cartilage repair;	[152]
AV/SOX-9, TGF-β1, or BMP-2	MSCs	Collagen	Differentiating into cartilage tissues with varying degrees of hypertrophy	[153]
Lipofectamine/ miR-410	MSCs	Gel-MA	Enhancing the migration, proliferation, and differentiation of MSCs;	[154]

high transfection efficiency, and long-term gene delivery efficiency. Ideally, biological scaffolds should have appropriate mechanical properties and controllable biodegradability, maintain cell activity, promote cell proliferation and chondrogenic differentiation, and enhance the deposition of cartilage ECM components, such as COL II and aggrecan. Currently, biological scaffolds mainly include solid scaffolds and hydrogels [8,12].

3.4.1. Solid scaffold-based gene-activated matrices for cartilage repair

Solid scaffolds are characterized by high porosity, which facilitates cell migration and infiltration from the surrounding tissues [155]. Commonly used solid scaffolds comprise natural polymers such as CTS and collage and synthetic polymers such as polycaprolactone (PCL) and PLGA. The GAM prepared by CTS-based solid scaffolds loaded with a TGF-β1 pDNA complex can promote the proliferation of chondrocytes and chondrogenic differentiation of MSCs [156–158]. An innovative miR-activated scaffold was developed by incorporating GAG-binding enhanced transduction system NPs (GET NPs) encapsulating an miR-221 inhibitor into a collagen/HA scaffold, which effectively inhibited miR-221 for up to 28 d to enhance MSC-mediated chondrogenesis and cartilage repair [159]. Solid scaffolds based on poly (L-glutamic acid) and CTS were designed to drive the formation of adipose stem cell (ASC) spheroids, thereby promoting hyaline cartilage-specific gene expression and enhancing cartilage ECM deposition, while minimizing fibrous matrix deposition [160]. Compared with MSCs, MSC spheroids demonstrate enhanced regeneration of hyaline cartilage. However, the limited cell surface area of MSC spheroids results in a low gene transfection efficiency. Enhancing the stiffness of a solid scaffold can promote cell spreading, and hence improve gene transfection efficiency. Solid scaffolds based on poly (L-glutamic acid) facilitate cell attachment and detachment through reversible binding to fibronectin, followed by the formation of spheroids *in situ*. Moreover, incorporating the N,N,N-trimethyl CTS chloride/TGF-β1 pDNA complex effectively mediates gene transfection during cell adhesion and significantly enhances chondrogenic differentiation [161]. Compared with the direct loading of IGF-1 pDNA onto collagen-based scaffolds, chemical cross-linking of IGF-1 pDNA onto the scaffolds using a lipid transfection reagent effectively prevented the passive bolus release of pDNA. This approach not only prolonged IGF-1 expression but also significantly enhanced chondrogenesis in chondrocytes [162].

Although natural polymer-based scaffolds exhibit excellent degradability and biocompatibility, they often exhibit weak compressive properties and inadequate shape retention. In contrast, synthetic polymer-based scaffolds can provide the mechanical properties necessary to promote cartilage formation and restore joint function during cartilage repair [12]. A pocket-type porous microcarrier was created using F127 and PLGA to deliver SOX-9 pDNA-loaded gold NPs, dexamethasone, and MSCs, effectively promoting the chondrogenic differentiation of stem cells [163]. PCL is a promising choice for CTE matrices because of its biocompatibility, slow degradation rate, low acidic degradation products, controllable anisotropy, and nonlinear mechanical properties [12,164]. PCL-based scaffolds can effectively sustain the release of pDNA-loaded silica NPs, thereby facilitating successful cell transfection [165]. PLL can immobilize viral vectors onto PCL films via electrostatic interactions. Leveraging this, the immobilization of rAAV-mediated SOX-9 or TGF-β complexes onto PCL films can sustain gene expression for up to 21 d while promoting the deposition of the cartilage ECM. Additionally, grafting PCL membranes with poly(sodium styrene sulfonate) (pNaSS) enhances the chondrogenic differentiation activity in human bone marrow aspirates [166,167].

Combining of natural and synthetic polymers is an appealing strategy for creating functionally superior scaffolds. This approach takes advantage of the enhanced mechanical properties offered by synthetic polymers, while also benefiting from the viscoelastic characteristics and maintenance of cell viability provided by natural polymers [168].

Table 3
The GAM used for CTE.

Gene vector	Gene	Scaffolds	Biofunctions	Ref.
HA/CTS	TGF-β1 pDNA	Porous CTS	Promoting chondrocytes proliferation;	[157]
CTS/gelatin	TGF-β1 pDNA	CTS/gelatin	Promoting chondrogenic differentiation; Improving rabbit cartilage repair;	[158]
GET NPs	miR-221 inhibitor	Collagen/HA	Enhancing MSC-mediated chondrogenesis and cartilage repair;	[159]
N,N,N-trimethyl chitosan chloride	TGF-β1 pDNA	poly (L-glutamic acid)	Driving the formation of ASC spheroids Enhancing chondrogenic differentiation;	[160,161]
Lipid transfection reagent	IGF-1 pDNA	collagen	Prolonging IGF-1 expression; Enhancing chondrogenesis of chondrocytes	[162]
Gold NPs	SOX-9 pDNA	F127/PLGA	Promoting the chondrogenic differentiation of stem cells	[163]
Silica NPs	pDNA	PCL	Facilitating successful cell transfection	[165]
rAAV	SOX-9 or TGF-β	pNaSS-grafted PCL	Promoting cartilage ECM deposition Enhancing chondrogenic differentiation	[166,167]
PEO-PLL; CTS-derivative; Lipofectamine	TGF-β1 pDNA	Fibrin/PLGA	Promoting neo-cartilage formation; Achieving cartilage repair;	[146,171, 172]
/	TGF-β1 pDNA and BMP-2 pDNA	CTS/gelatin; HAP-doped CTS/gelatin	Supporting the regeneration of articular cartilage and subchondral bone	[174]
Layered double hydroxides	siRNA	pNIPAAM	Achieving efficient gene transfection efficiency	[176]
rAAV	IGF-1	Alginate	Promoting long-term cartilage repair;	[177]
Niosomes	SOX-9 pDNA	HA-GMA	Promoting chondrogenic differentiation; Reducing the expression of fibrocartilage and hypertrophic markers	[178]
AAV	TGF-β1	Fibrin glue	Upregulating the expression of cartilage-specific genes	[179]
Lipofectamine	antimiR-211	Fibrin/HA hydrogel	Blocking the expression of miR-211 to enhance cartilage regeneration by endogenous cells	[180]
Peptide NPs	Klotho pDNA	Stem cell-homing peptide	Improving senescent microenvironment Enhancing cartilage regeneration	[182]
Lipid NPs	VEGFa siRNA	Gel-MA	Facilitating cartilage formation by suppressing angiogenesis	[185]
AV	BMP-2 pDNA/TGF-β3 pDNA	Demineralized bone matrix	Facilitating the pig cartilage defect repair	[186]
nHAP	BMP-2 pDNA/TGF-β3 pDNA	Alginate	Promoting the hypertrophy differentiation of MSCs	[187]
3DFectIN	SOX-9 pDNA	Col I/alginate	Promoting chondrogenic differentiation; Inhibiting osteogenic differentiation	[188]
3DFectIN	SOX-9 mRNA	Fibrin	Promoting chondrogenic differentiation	[189]
rAAV	SOX-9 cDNA	F127	Promoting cartilage repair *in vivo*	[190]
Microcapsules	LOX pDNA	Gel-MA	Reducing inflammation; Enhancing cartilage regeneration	[191]

Compared with pure PCL scaffolds, incorporating a cartilage ECM into PCL scaffolds can enhance their mechanical properties and wettability, significantly promoting chondrocyte proliferation *in vitro* and facilitating cartilage regeneration *in vivo* [169]. In a composite scaffold based on CTS and PCL, PCL provides basic mechanical support, whereas CTS recruits tetrahedral framework nucleic acids (TFNA), which are injected into the joint cavity through electrostatic interactions. Recruitment of TFNA promotes proliferation and chondrogenic differentiation of encapsulated synovium-derived MSCs (SMSCs), thereby facilitating cartilage regeneration *in vivo* [170]. Moreover, a composite scaffold prepared by filling the PLGA sponge with fibrin gel can better mimic cartilage tissue and realize the repair of cartilage defects by loading BMSCs and different vector/gene complexes, including the PEO-PLL/TGF-β1 pDNA, CTS-derivative/TGF-β1 pDNA, and lipofectamine/TGF-β1 pDNA complexes. The three GAMs can promote neo-cartilage formation with a similar amount and distribution of COL II and GAGs as hyaline cartilage, and integrate well with surrounding tissues and subchondral bone, which achieves cartilage repair of full-thickness rabbit cartilage defects [146,171,172]. Notably, a composite scaffold, designed to mimic the cartilage tissue, may be more suitable as a matrix for CTE. An osteochondral scaffold was fabricated by successively filling bilayer PCL scaffolds featuring gradient pore sizes with a silk fibroin (SF) hydrogel encapsulating the B2A peptide and an alginate hydrogel encapsulating the E7 peptide. The degradation rate of alginate was higher than that of SF. Consequently, the burst release of the E7 peptide promoted BMSC migration, whereas the sustained release of the B2A peptide supported BMSC proliferation and facilitated osteogenic and chondrogenic differentiation, which enhanced the regeneration of cartilage and subchondral bone in rabbit osteochondral defects [173]. A bilayer GAM was developed using a CTS/gelatin scaffold. The upper layer consisted of a CTS/gelatin scaffold loaded with TGF-β1 pDNA, whereas the lower layer featured a hydroxyapatite (HAP)-doped CTS/gelatin scaffold loaded with BMP-2 pDNA. This dual-layer scaffold effectively guided MSCs in each layer to differentiate into chondrocytes and osteoblasts, thereby supporting the regeneration of articular cartilage and subchondral bone in a rabbit knee osteochondral defect model [174].

3.4.2. *Hydrogel-based gene-activated matrices for cartilage repair*

Compared with solid scaffolds, biomedical hydrogels are commonly used biological scaffolds that possess a 3D porous network structure and an ECM-mimicking microenvironment. Moreover, hydrogels exhibit controllable physicochemical properties including water content, permeability, and mechanical properties, making them widely studied for use in cartilage repair [5,175]. An injectable thermosensitive hydrogel prepared using poly (N-isopropylacrylamide) (pNIPAAM) and layered double hydroxides can enhance siRNA delivery into chondrocytes to achieve efficient gene transfection [176]. In a full-thickness minipig cartilage defect model, alginate hydrogel loaded with the gene complex prepared by rAAV and IGF-1 can promote long-term cartilage repair and inhibit perifocal OA, achieving articular cartilage protection [177]. Currently, hydrogels commonly used in CTE are based on HA, gelatin, alginate, and fibrin [16].

HA is a polysaccharide naturally present in cartilage and synovial fluid and has been widely studied in CTE because it promotes cell adhesion and proliferation, regulates inflammation, and promotes cartilage regeneration [4]. A novel GAM was developed using glycidyl methacrylated HA (HA-GMA) cryogels encapsulating the niosome/SOX-9 pDNA complex, which can effectively enhance *in situ* gene transfection of MSCs to promote chondrogenic differentiation, restore cartilage ECM, and reduce the expression of fibrocartilage and hypertrophic markers [178]. Fibrin glue hydrogel can effectively

upregulate the expression of cartilage-specific genes in MSCs by loading the AAV/TGF-β1 complex. The hydrogel exhibits controlled pore size, fiber thickness, and dissolution rate by regulating fibrin concentration [179]. The GAM prepared by the fibrin/HA hydrogel loaded lipofectamine/antimiR-211 complex can effectively improve gene transfection efficiency and block the expression of miR-211 to enhance cartilage regeneration by endogenous cells [180]. A bioactive self-assembling peptide nanofiber hydrogel modified by a stem cell-homing peptide can achieve the loading and sustained delivery of miR-29b-5p, thereby effectively inhibiting the senescence and metabolic imbalance of chondrocytes and alleviating OA progress. Furthermore, the hydrogel can promote the recruitment and chondrogenic differentiation of endogenous SMSCs, enhancing cartilage repair without the need for exogenous MSCs [181]. Additionally, a cell-homing peptide hydrogel was used to encapsulate the Klotho-expressing pDNA-based complex and Tanshinone IIA, which can attenuate OA progression by improving the senescent microenvironment and enhancing cartilage regeneration. The GAM effectively recruits endogenous BMSCs and induces their differentiation into chondrocytes [182]. Regrettably, the conventional GAM used in CTE often requires the embedding of cells within the scaffold before implantation to more effectively enhance cartilage repair. A porous cell-free composite scaffold composed of HA, fibrin, nanohydroxyapatite (nHAP), and graphene oxide can effectively create a microenvironment that supports host cell adhesion, proliferation, and differentiation. This scaffold can promote osteochondral repair by upregulating the expression of specific genes associated with osteogenesis and chondrogenesis and promoting mineralization and ECM deposition [183]. In addition, a cell-free CTE scaffold was developed using a CTS and SF composite hydrogel incorporating stromal cell-derived factor-1 (SDF-1) and KGN-loaded microspheres designed for sequential drug release. Initially, SDF-1 was released from the hydrogel to facilitate BMSCs recruitment. Subsequently, KGN was gradually released from the microspheres within the hydrogel to promote the chondrogenic differentiation of MSCs. This approach spatiotemporally regulates the recruitment and chondrogenic differentiation of endogenous MSC, thereby accelerating cartilage repair [184].

The inhibition of angiogenesis can promote cartilage formation owing to the avascular nature of cartilage. A lipid NP/VEGFa siRNA complex was encapsulated into Gel-MA hydrogel along with chondrocytes and ADSC, which effectively upregulated the expression of HIF-1α, SOX-9, COL-II, and ACAN and suppressed angiogenesis, thus facilitating cartilage formation [185]. The AV-mediated BMP-2 pDNA and TGF-β3 pDNA were encapsulated along with BMSCs into the demineralized bone matrix to effectively induce the chondrogenic differentiation of BMSCs and promote the deposition of COL II, facilitating the repair of pig cartilage defects [186]. Furthermore, nHAP-mediated BMP-2 pDNA and TGF-β3 pDNA were encapsulated along with MSCs into the alginate hydrogel, which also promoted the deposition of GAGs and COL II to facilitate chondrogenesis. Notably, compared with the co-delivery of BMP-2 pDNA and TGF-β3 pDNA, individual delivery can promote the hypertrophy differentiation of MSCs. Therefore, the chondrogenic and osteogenic differentiation pathways of MSCs can be effectively directed by controlling the delivery mode of BMP-2 pDNA and TGF-β3 pDNA [187]. In addition, the mechanical properties of the hydrogels have an important impact on directing the chondrogenic differentiation of MSCs. Commercially available 3DFectIN vector-mediated SOX-9 pDNA was encapsulated along with MSCs into COL I/alginate hydrogels with tunable stiffness and adhesion properties. Increasing the stiffness and cell-adhesive ligand density of the hydrogel effectively enhanced complex uptake and SOX-9 pDNA transfection, promoting chondrogenic differentiation, while inhibiting the osteogenic differentiation of MSC [188]. Compared with pDNA, mRNA exhibited better gene expression efficiency. The 3DFectIN vector-mediated SOX-9 mRNA was encapsulated along with MSCs into the fibrin hydrogel, which showed better gene expression induction and chondrogenic differentiation of MSCs than that of 3DFectIN vector-mediated SOX-9 pDNA [189]. In minipig models of full-thickness cartilage defects, an injectable and thermosensitive hydrogel formulated with F127 was utilized to encapsulate the rAAV vector-based complex that overexpressed SOX-9. This demonstrated a significant enhancement in cartilage repair, exhibiting collagen fiber orientation similar to that of normal cartilage, while also providing protection to the subchondral bone [190]. Lysyl oxidase (LOX), a copper ion-dependent amine oxidase, plays a vital role in regulating cartilage regeneration. LOX pDNA was encapsulated in interleukin 4 (IL-4)-modified self-assembled SF microcapsules, which were embedded in a Gel-MA hydrogel. The release of IL-4 promoted macrophage polarization toward the M2 phenotype, thereby reducing inflammation. Simultaneously, the release of LOX pDNA enhanced cartilage ECM deposition and significantly enhanced cartilage regeneration [191].

4. Bioprinted hydrogel scaffolds for cartilage repair

Bioprinted scaffolds have recently received significant attention in CTE owing to their precise and controllable geometric shape and microstructure [192,193]. Ideally, the bioprinting ink should have excellent mechanical properties and printability and the scaffold should exhibit good biocompatibility, suitable porosity, and appropriate biodegradation rate, as well as effectively maintaining cell activity and inducing chondrogenic differentiation of encapsulated cells (Fig. 6) [194]. Hydrogels have been widely studied as bioprinting inks and used in CTE, exhibiting many advantages, including good biocompatibility, bioactivity, bionic structure, controllable mechanical properties, biodegradability, and enhanced cartilage regeneration. Currently, the most commonly used hydrogel-based bioprinted scaffolds for CTE are based on HA, gelatin, alginate, collagen, and SF (Fig. 7 and Table 4) [19, 195–197].

4.1. HA-based bioprinted hydrogel scaffolds

Numerous studies have shown that functionalized HA-based hydrogels can regulate the inflammatory microenvironment and promote cartilage tissue repair and regeneration; therefore, they have been widely investigated as bioprinting inks. We systematically review the recent progress in the multifunctional design of HA-based hydrogels and their application in CTE [4]. HA-MA hydrogel has been the most widely studied bioprinted scaffold owing to its controllable pore size, mechanical properties, degradation rate, and self-polymerization. A mixture of functionalized gelatin, collagen, and ECM can improve the physicochemical properties and biological activity of HA-MA scaffolds to meet the complex structural requirements of cartilage tissue. A bioprinted photocurable scaffold was prepared using HA-MA and Gel-MA, which could mimic the structural and mechanical properties of natural cartilage, match the degradation rate with cartilage regeneration, and stimulate the deposition of cartilage ECM and chondrogenesis [198, 199]. The addition of collagen enhanced the chondrogenesis of the encapsulated MSCs in the HA-MA bioprinted scaffold [200]. The mechanical properties of the HA-MA bioprinted scaffold were significantly improved by adding decellularized ECM (dECM) to meet the requirements of bioprinting, thereby, providing a good microenvironment for maintaining cell proliferation and activity [201]. Norbornene-modified HA (HA-Nor), another bioprinting ink, was used to prepare a bioprinted photocurable scaffold by reacting it with DL-dithiothreitol (DTT), which effectively maintained the activity and homogenous distribution of encapsulated MSCs while facilitating chondrogenesis [202]. In addition to bioprinted photocurable scaffolds, HA-SH-based bioprinted scaffolds have been developed using the thiol-ene Michael addition reaction, which can promote the deposition of the cartilage ECM. Notably, the bioprinted scaffolds with high-MW HA-SH exhibit a homogenous distribution of cartilage ECM and enhanced construct stiffness [203,204]. Moreover, HA-SH and dECM particles can be used to fabricate a bioprinted scaffold by forming disulfide bonds, exhibiting mechanical properties and layered structure

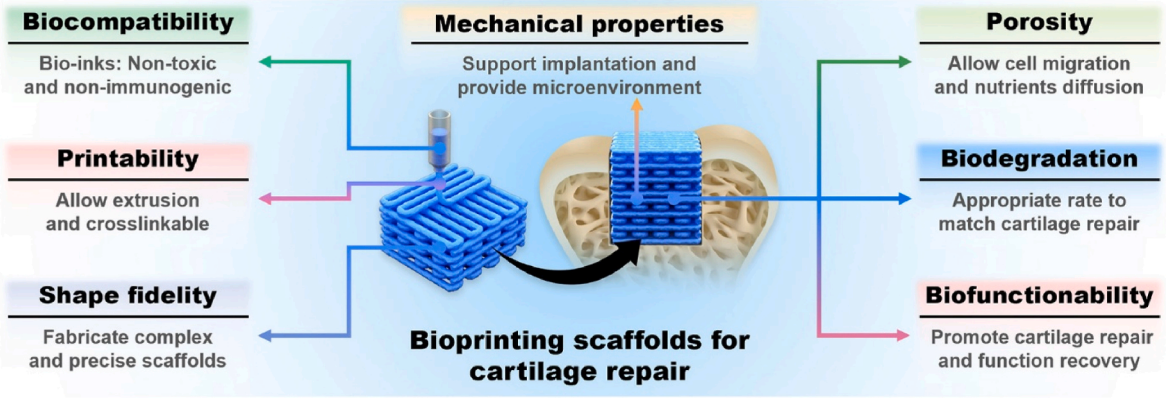

Fig. 6. Schematic illustration showing the requirements of ideal bioprinted scaffold for CTE.

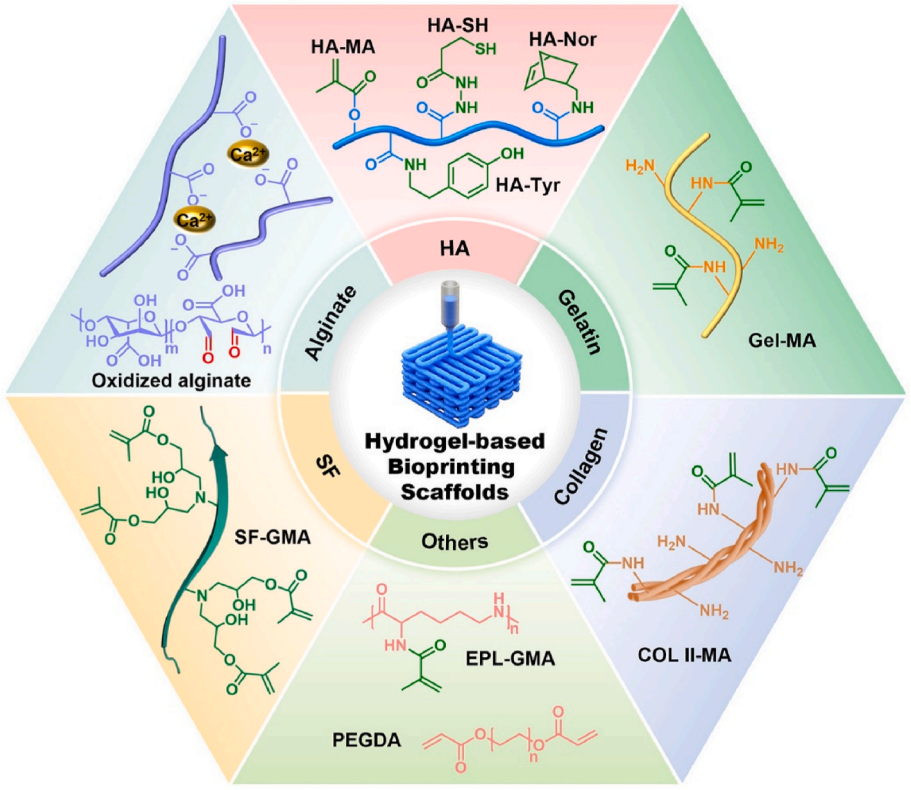

Fig. 7. The commonly used hydrogel-based bioprinted scaffolds in CTE.

similar to natural cartilage, while improving encapsulated cell activity [205]. Tyramine-grafted HA (HA-Tyr) can be used to develop an enzymatically crosslinked bioprinted scaffold with outstanding printability and adjustable porosity, effectively maintaining the activity and differentiation of encapsulated chondrocytes [206].

4.2. Gelatin-based bioprinted hydrogel scaffolds

Gel-MA has been extensively investigated as a bioprinting ink in CTE owing to its simple synthesis, low cost, controllable mechanical properties, good biocompatibility, excellent printability, and favorable shape fidelity [207,208]. A biphasic scaffold based on Gel-MA demonstrated exceptional cartilage regeneration capabilities through the co-culture of BMSCs and costal chondrocytes. Notably, the regenerated cartilage exhibited a Young's modulus comparable to that of natural cartilage [209]. However, Gel-MA bioprinted scaffolds can support the formation of fibrocartilage-like tissues containing COL I and COL II because of the lack of tissue-specific induction activity, which limits their further application in cartilage repair [210]. The addition of HA-MA can significantly enhance chondrogenesis over Gel-MA bioprinted scaffolds [211]. An ECM-mimicking bioprinted photocurable scaffold was developed using Gel-MA, HA-MA, and methacrylated CS (CS-MA), which induced the aggregation, proliferation, and differentiation of encapsulated SMSCs through TGF-β1 loading, thereby enhancing cartilage ECM deposition and promoting cartilage regeneration and articular function recovery [212]. Wharton's jelly, which is sourced from the human umbilical cord, is a promising material for cartilage repair because it contains several chondrogenic factors. The addition of methacrylated acellular Wharton's jelly (AWJ-MA) can improve the physicochemical properties and biological activity of Gel-MA bioprinted scaffolds. Additionally, this cartilage-mimicking scaffold is beneficial for enhancing the proliferation, migration, and chondrogenic

Table 4
The commonly utilized hydrogel-based bioprinted scaffolds for CTE.

Polymers	Modified polymers	Bioprinted scaffolds	Biofunctions	Ref.
Hyaluronic acid (HA)	HA-MA	HA-MA/Gel-MA	Exhibiting bionic structure and mechanical properties, and appropriate degradation rate;	[198, 199]
		HA-MA/COL II	Enhancing chondrogenesis of encapsulated MSCs;	[200]
		HA-MA/dECM	Providing a good microenvironment for maintaining cell proliferation and activity	[201]
	HA-Nor	HA-Nor/DTT	Maintaining the activity and homogenous distribution of encapsulated MSC;	[202]
	HA-SH	HA-SH/ Allylated polymers	Promoting the deposition of cartilage ECM and chondrogenic differentiation of MSCs;	[203, 204]
		HA-SH/dECM particles	Mimicing mechanical properties and layered structure to natural cartilage;	[205]
	HA-Tyr	HA-Tyr	Maintaining the activity and differentiation of encapsulated chondrocytes;	[206]
Gelatin	Gel-MA	Gel-MA	Exhibiting appropriate modulus; Supporting fibrocartilage formation;	[209, 210]
		Gel-MA/HA-MA	Enhancing chondrogenesis over Gel-MA;	[211]
		Gel-MA/HA-MA/CS-MA	Promoting cartilage regeneration and articular function recovery;	[212]
		Gel-MA/AWJ-MA	Achieving the full-thickness articular cartilage repair;	[213]
		Gel-MA/CTS-MA	Guiding MSC aggregates for enhanced chondrogenesis and cartilage regeneration;	[214]
		Gel-MA/ECM-MA/PEO/PCL	Facilitating cartilage-like tissue regeneration	[215]
	/	SF-gelatin	Upregulating the chondrogenic specific genes and downregulating the hypertrophic specific gene;	[216, 217]
Alginate	/	Alginate/ $CaCl_2$	Facilitating the development of hyaline-like cartilage;	[220]
		Alginate/COL I/$CaCl_2$	Improving mechanical strength and bioactivity; Suppressing dedifferentiation of chondrocytes and maintain the phenotype;	[221]
		Alginate/MC/ $CaCl_2$	Facilitating the chondrogenesis of chondrocytes;	[222]
		Alginate/ Gellan gum/ Silk NPs/$CaCl_2$	Enhancing the mechanical properties and chondrogenesis of MSCs;	[223]
		Alginate/ Xanthan gum/ $CaCl_2$	Enhancing chondrogenic differentiation of encapsulated MSCs spheroids;	[224]
		Alginate/ dECM/Gum arabic/PVA/ $CaCl_2$	Providing a more physiologically relevant environment for chondrocytes;	[225]
		Alginate/HA/ PLA/$CaCl_2$	Facilitating the chondrogenesis of encapsulated chondrocytes;	[226]
		Alginate/HA/ gelatin/ fibronectin/ $CaCl_2$	Stimulating neo-cartilage regeneration with layered structures resembling natural cartilage;	[227]
	Oxidized alginate	Oxidized alginate/ Thermal pre-treated gelatin/$CaCl_2$	Enhancing printability to fabricate hierarchical bioprinted scaffold structures;	[228]
	Alginate-Cys/ Alginate-Nor	Alginate-Cys/ Alginate-Nor/ $CaCl_2$	Exhibiting stronger mechanical properties, long-term stability, and improved printability;	[229]
Collagen	/	COL I/TGF-β1/ PLA-TMC	Promoting the chondrogenic differentiation of BMSCs	[231]
	/	COL I/COL II/ HA	Enhancing the chondrogenic differentiation of MSCs	[232]
	COL II-MA	COL II-MA	Inducing chondrogenic differentiation; Inhibiting hypertrophy;	[233]
		Cartilage ECM-MA	Promoting cartilage generation rich in sulfated GAGs and collagen	[234]
Silk fibroin (SF)	/	SF/gelatin/E7	Providing optimized structure and function for cartilage repair;	[235]
		SF/dECM	Enhancing chondrogenesis of BMSCs;	[236]
		SF/HPMC-MA	Increasing mechanical properties;	[237]
	SF-GMA	SF-GMA	Building highly complex organ structures with excellent structural stability and reliable biocompatibility;	[238]
		SF-GMA/ gelatin/ PEGDA	Facilitating the cartilage ECM deposition	[239]
Others	PEGDA	PEGDA/Cobalt nanowires	Constructing a hypoxic microenvironment to promote chondrogenic differentiation;	[240]
	EPL-GMA	EPL-GMA	Possessing intrinsic antibacterial activity;	[241]
	NAR-MA	NAR-MA/Gel-MA	Enhancing the shape fidelity and cartilage repair efficiency;	[242]

differentiation of encapsulated BMSCs, thereby achieving the repair of full-thickness articular cartilage defects [213]. Compared with individual stem cells, stem cell aggregates encapsulated in bioprinted hydrogels exhibit enhanced chondrogenic differentiation. CTS promotes stem cell aggregations to maintain long-term proliferation and high efficiency of chondrogenic differentiation. A bioprinted scaffold with high cell adhesion and aggregation properties was prepared using methacrylated

CTS (CTS-MA) and Gel-MA, which had controllable mechanical properties and could promote the chondrogenic differentiation of encapsulated BMSCs, effectively facilitating hyaline cartilage repair [214]. A microporous bioprinted scaffold was prepared using methacrylated ECM (ECM-MA), Gel-MA, PEO, and PCL to facilitate cartilage-like tissue regeneration. PEO served as a porogen to create a microporous structure for nutrient exchange, whereas PCL enhanced the mechanical properties to enhance the shape fidelity of the scaffold [215]. Compared to the Gel-MA scaffold, the SF-gelatin bioprinted scaffold enhanced the chondrogenesis of the encapsulated BMSCs by upregulating chondrogenic specific genes and downregulating hypertrophic specific genes. Additionally, the scaffold regulated the Wnt/β-catenin and TGF-β signaling pathway in the chondrogenesis process [216,217].

4.3. Alginate-based bioprinted hydrogel scaffolds

Compared with the Gel-MA scaffold, the alginate-based bioprinted scaffold can promote the deposition of COL II and facilitate the development of hyaline-like cartilage, revealing significant application prospects in CTE [210,218,219]. Alginate-based hydrogels are typically prepared by ionic crosslinking between alginate and calcium ions, which results in poor biological stability owing to the fast degradation rates of the hydrogel [17,19]. Moreover, the viscosity of the alginate bioprinting ink affects its printability, mechanical properties, and encapsulated cell viability. High-viscosity alginate enhances printability but may decrease cell activity, whereas low-viscosity alginate promotes cell activity but hinders printability [220]. Therefore, alginate-based bioprinted scaffolds prepared by adding other natural polymers exhibit enhanced mechanical properties and biological activities.

The addition of COL I or agarose can improve the mechanical strength of alginate-based bioprinted scaffolds. Compared with agarose, the addition of COL I significantly accelerates cell adhesion and proliferation and upregulates the expression of cartilage-specific genes such as SOX-9, ACAN, and COL2A1, while downregulating the expression of fibrocartilage genes such as COL1 alpha 1 (COL1 A1) [221]. Methylcellulose (MC), as a thickener, can increase the viscosity during the printing process to improve the shape fidelity of alginate-based bioprinted scaffolds. Additionally, a combination of alginate and MC can stimulate ECM deposition and facilitate chondrogenesis of encapsulated chondrocytes [222]. The addition of gellan gum can improve the printability and structural stability of alginate-based bioprinted scaffolds, and further incorporation of silk NPs can enhance their mechanical properties and chondrogenic ability [223]. A bioprinted scaffold prepared with alginate and xanthan gum effectively ensured faster and more effective chondrogenic differentiation of encapsulated MSCs spheroids, accompanied by abundant ECM deposition [224]. Incorporating articular cartilage dECM, gum arabic, and polyvinyl alcohol (PVA) into an alginate-based bioprinted scaffold sustained chondrocyte viability and promoted chondrogenic activity, as the scaffold had a structure similar to that of natural tissue and could provide a more physiologically relevant environment for chondrocytes [225]. The addition of HA to an alginate-based bioprinted scaffold co-printed with polylactic acid (PLA) can facilitate chondrogenesis of encapsulated chondrocytes by upregulating chondrogenic genes and increasing cartilage ECM deposition [226]. Bioprinted scaffold prepared with a combination of alginate/HA/gelatin composite hydrogel and fibronectin plays a crucial role in promoting the chondrogenic differentiation of encapsulated chondrogenic progenitor cells by upregulating the expression of COL II, SOX-9, and especially PRG4. Furthermore, in rat cartilage defect, bioprinted modified autologous matrix-induced chondrogenesis technology can effectively stimulate neo-cartilage regeneration with typical layered structures resembling natural hyaline cartilage [227].

Functionally modified alginate is another effective method for preparing bioprinted scaffolds with improved mechanical properties. A bioprinted scaffold prepared from oxidized alginate and thermally pretreated gelatin exhibited enhanced printability for fabricating hierarchical bioprinted scaffold structures with shape stability and fidelity, providing a valuable bioprinted scaffold for CTE [228]. A double crosslinked alginate-based bioprinted scaffold was developed using L-cysteine-modified alginate (alginate-Cys) and norbornene-modified alginate (alginate-Nor). Compared with the single ionic crosslinked scaffold, the double crosslinked scaffold exhibited stronger mechanical properties, long-term stability, and improved printability, and promoted the chondrogenic differentiation of encapsulated MSCs [229].

4.4. Collagen-based bioprinted hydrogel scaffolds

Collagen has been extensively studied in CTE owing to its excellent biocompatibility and cell adhesion. However, it is difficult to use collagen alone as a bioprinted scaffold because of its low viscosity, weak mechanical properties, and uncontrollable degradation properties [17,19]. Additionally, low-concentration (1 %) collagen does not support high-precision bioprinting. Conversely, the inherent porosity of high-concentration (4 %) collagen bioprinted scaffolds is insufficient to provide oxygen and nutrient to the encapsulated cells, leading to the ineffective maintenance of cell survival [230]. Hence, the preparation of collagen-based scaffolds primarily involves the combination of collagen and other biomaterials, or the functional modification of collagen. A COL I hydrogel loaded with TGF-β1 was previously filled into a poly (D, L-lactic acid-co-trimethylene carbonate) (PLA-TMC) bioprinted scaffold, which was shown to maintain the proliferation and activity of BMSCs and promote the chondrogenic differentiation of BMSCs [231]. The addition of COL II and HA can enhance the chondrogenic differentiation of MSCs on COL I scaffolds while preserving the essential mechanical properties of the scaffolds [232]. Notably, cartilage ECM is more abundant in COL II than in COL I. Studies have been conducted on bioprinted scaffolds using functionally modified COL II. Methacrylated COL II (COL II-MA) can be used to prepare a photocurable mono-component hydrogel with controllable mechanical properties for fabricating complex 3D architectures. Compared with COL I, the COL II-MA hydrogel has been shown to enhance cartilage ECM deposition and chondrogenic differentiation of encapsulated BMSCs while inhibiting hypertrophy [233]. Furthermore, cartilage ECM-MA derived from COL II-MA can be used to develop a photocurable bioprinted scaffold, that can maintain the viability and enhance chondrogenesis of encapsulated BMSCs post-bioprinting, while promoting cartilage generation rich in sulfated GAGs and collagen [234].

4.5. SF-based bioprinted hydrogel scaffolds

SF, an emerging bioprinting ink, has also been extensively investigated in CTE owing to its superior elasticity, compatible degradation rates, and low immune reactions [17,19]. A combination of SF and gelatin was used to prepare a bioprinted scaffold with an optimized structure and function by adding the BMSC affinity peptide E7. The scaffold could retain adequate BMSCs through efficient recruitment and provide conducive mechanical protection and a microenvironment for the proliferation and chondrogenic differentiation of BMSCs [235]. The addition of dECM or methacrylated hydroxypropyl MC (HPMC-MA) enhances the mechanical properties and degradation rate of SF-based bioprinted scaffolds. The scaffolds also exhibit good printability and can maintain the proliferation and chondrogenic differentiation of the encapsulated BMSCs [236,237]. Moreover, research has been conducted on bioprinted scaffolds using functionally modified SF. Glycidyl methacrylated SF (SF-GMA) can be used to build highly complex photocurable mono-component organ structures, including the heart, vessels, brain, trachea, and ear, with excellent structural stability and reliable biocompatibility. High-concentration (30 %) SF-GMA bioprinted scaffolds exhibit a higher compressive elastic modulus than the Gel-MA scaffold at the same concentration and provide a suitable microenvironment for the proliferation and chondrogenesis of encapsulated

chondrocytes [238]. In contrast, low-concentration SF-GMA bioprinted scaffolds have been developed via blending with other polymers. A photocurable bioprinted scaffold prepared with SF-GMA (3 %), gelatin, and PEG diacrylate (PEGDA) exhibited the desired printability, stable structure and mechanical properties, as well as suitable degradation rate. The scaffold promoted the activity and maturation of chondrocytes while facilitating cartilage ECM deposition [239].

4.6. Other bioprinted hydrogel scaffolds

In addition to natural polymer-based bioprinted scaffolds, synthetic polymer-based bioprinted scaffolds have also been widely used in CTE owing to their unique mechanical properties and biological activities [17,19]. A bioprinted hypoxia-mimicking PEGDA scaffold was fabricated by incorporating cobalt nanowires as hypoxia-inducing agents, which exhibited enhanced bioprinting fidelity and mechanical properties. Moreover, without the addition of conventional growth factors, the scaffold could promote chondrogenic differentiation and inhibit hypertrophic or osteogenic differentiation of encapsulated MSCs by constructing a hypoxic microenvironment and activating the HIF-1α pathway [240]. A glycidyl methacrylated EPL (EPL-GMA) bioprinted scaffold possessed intrinsic antibacterial activity to avoid the risk of bacterial infection in the application of CTE, while showing high efficiency of cartilage-like tissue regeneration after encapsulating chondrocytes [241]. Naringin (NAR), a flavonoid, has been suggested to promote chondrogenesis of MSCs and increase cartilage ECM deposition. Methacrylated NAR (NAR-MA) and Gel-MA have been used to develop bioprinted scaffolds with enhanced shape fidelity. The scaffold maintains chondrocytes activity and regulates the transcription of genes involved in chondrogenesis and against oxidative stress, facilitating cartilage regeneration [242].

5. Gene-activated bioprinted scaffolds for cartilage repair

GAMs have been extensively studied in CTE. However, conventional GAM cannot simulate the complex gradient structure of the natural cartilage. Bioprinted scaffolds can produce complex structures that mimic natural cartilage, thereby broadening the applications of hydrogels in CTE. Conventional bioprinted scaffolds face significant challenges in CTE, primarily because of their inefficient cartilage repair capabilities. This issue stems from the inadequate and discontinuous supply of essential repair factors to the damaged area, which adversely affects cell proliferation, chondrogenic differentiation, and ECM distribution. Consequently, cartilage repair outcomes are suboptimal. Despite advancements in structural design and manufacturing, bioprinted scaffolds lack biocompatibility, functionality, and biological activity, thereby compromising the scaffold performance and hindering the quality of cartilage regeneration [17,243]. Gene therapy can effectively address the problem of insufficient repair factors by continuously delivering therapeutic gene products directly to the site of injury. However, the application of gene delivery vectors in CTE encounters difficulties such as poor *in situ* retention and rapid diffusion of genes [12]. To address these challenges, gene-activated bioprinted scaffolds that incorporate gene complexes can ensure the long-term retention and sustained release of genes at the injury site.

Gene-activated bioprinted scaffolds are an emerging therapeutic technology that synergizes the advantages of bioprinted scaffolds and gene therapy, to efficiently promote the regeneration and repair of cartilage tissue. During the bioprinting process, embedding gene complexes prepared from specific genes and gene vectors, into the scaffolds enables the on-demand release of therapeutic gene products at the cartilage injury site, thereby enhancing cell growth, differentiation, and functional recovery. In contrast to conventional bioprinted scaffolds, gene-activated bioprinted scaffolds offer physical support and create a favorable microenvironment for cartilage regeneration. By regulating the release of therapeutic gene products, the gene-activated bioprinted scaffolds can significantly enhance the survival and activity of cells, effectively facilitating cartilage repair. Moreover, the integration of bioprinted scaffolds with gene therapy shows great potential for bridging the gap between laboratory research and clinical practice, paving the way for new treatment options for cartilage injuries [12,244].

Alginate-based bioprinted scaffolds can effectively facilitate pDNA transfection and enhance the expression of target proteins [245]. An alginate bioprinting ink with controllable porosity was developed by adding MC as a porogen, which could achieve spatiotemporally defined pDNA delivery in bioprinted tissues and promote the transfection of encapsulated MSCs. Subsequently, a bilayered osteochondral-mimicking gene-activated bioprinted scaffold was fabricated by layered deposition of the two different bioprinting inks into the PCL scaffold. The lower bioprinting inks were formed by loading the nHAP/BMP-2 pDNA complex onto alginate, and the upper bioprinting inks were formed by loading the amphiphilic peptide/TGF-β3 pDNA/BMP-2 pDNA/SOX-9 pDNA complex onto alginate/MC. The bilayered scaffold could induce targeted differentiation of encapsulated MSCs, achieving successful osteochondral regeneration [246]. Another gene-activated bioprinted scaffold was developed by loading the GET NPs/SOX-9 pDNA complex and MSC onto a collagen/HA/PCL bioprinted scaffold. Compared with the gene-free bioprinted scaffold, the gene-activated bioprinted scaffold exhibited SOX-9 mRNA expression two orders of magnitude higher and a significant increase in its downstream mRNA expression, including COL2A1 and ACAN. Moreover, the scaffold exhibited increased deposition and spatial distribution of the cartilage ECM, facilitating healthy hyaline cartilage regeneration [247]. Previous reports have indicated that gene-activated bioprinted scaffolds exhibit superior efficacy in promoting the regeneration of high-quality hyaline cartilage compared to conventional bioprinted scaffolds. Both GAM and bioprinted scaffolds have been extensively explored in CTE. However, research on gene-activated bioprinted scaffolds remains relatively limited.

Compared to gene-activated bioprinted scaffolds, bioprinted scaffolds have also been studied for the delivery of growth factors in CTE. A microenvironmentally optimized bioprinted scaffold with favorable mechanical strength was co-printed using an ECM, Gel-MA, and PCL to deliver PLGA microspheres loaded with TGF-β3. The scaffold could promote cartilage-like tissue regeneration with fewer fibrotic components and abundant aggrecan through the sustained release of TGF-β3 to direct endogenous stem/progenitor cell migration and differentiation [248]. The pore size of porous scaffolds has a regulatory effect on the chondrogenic differentiation of MSCs. Scaffolds with small pore sizes can promote chondrogenesis but inhibit osteogenesis and angiogenesis. Therefore, cartilage regeneration requires the development of a biomimetic scaffold that mimics the gradient pore size structure. A bioprinted gradient-structured scaffold was developed using gradient spacing PCL fiber and a BMSC-laden hydrogel composed of gelatin, fibrinogen, HA, and glycerol. The gradient scaffold was demonstrated to activate the HIF1α/FAK signaling axis in the pore-size-dependent scenario, facilitating anisotropic cartilage regeneration [249]. Furthermore, a dual-factor releasing and gradient bioprinted scaffold was prepared using the above gradient scaffold to encapsulate PLGA microspheres loaded with TGF-β3 or BMP-4 hierarchically. The gradient scaffold exhibited a compressive Young's modulus similar to that of natural cartilage, providing a favorable microenvironment for the proliferation, migration, and chondrogenic differentiation of BMSCs. In the rabbit knee cartilage defect model, the gradient scaffold induced neo-cartilage regeneration with a structure similar to that natural articular cartilage, including superficial articular cartilage and deep hypertrophic cartilage [250]. Adapters are short nucleotide fragments capable of specifically recognizing targets. NPs based on the HM69 adapter have demonstrated the effective capture and enrichment of MSCs. The HM69 adapter-modified dECM, Gel-MA, and PCL were combined to fabricate a difunctional bioprinted scaffold for *in situ*

cartilage regeneration. The scaffold specifically recognized and recruited the endogenous MSCs and provided a microenvironment for their adhesion and proliferation, simultaneously facilitating their chondrogenic differentiation by the sustained release of encapsulated TGF-β3 [251]. Compared with the non-covalent incorporation of growth factors, the covalent binding of growth factors in bioprinted scaffolds can further enhance the chondrogenic differentiation of encapsulated MSCs, and promote the deposition and homogenous distribution of cartilage ECM [252].

In CTE, bioprinted scaffolds frequently improve cartilage repair by incorporating growth factors. However, the effectiveness of these growth factors is limited by their short biological half-lives and limited diffusion distances. Consequently, it is essential to explore alternative solutions to effectively address these limitations [253]. Gene-activated bioprinted scaffolds are an emerging technology that has shown great promise for overcoming the limitations associated with traditional growth factors. Unlike growth factors, these scaffolds can continuously deliver therapeutic gene products, thereby promoting cell growth and differentiation and ultimately enhancing cartilage repair. Gene-activated bioprinted scaffolds typically comprise bioprinted scaffolds, gene vectors, and therapeutic genes. To create an ideal gene-activated bioprinted scaffold, it is essential to optimize the material selection, enhance the gene delivery efficiency, and facilitate clinical translation. To effectively support cell growth and tissue regeneration, the design of bioprinted scaffolds must meet two key requirements: 1) The internal architecture of the scaffold, including pore size and porosity, should be precisely engineered to facilitate cell and tissue infiltration, nutrient transport, oxygen supply, and metabolic waste removal. The external structure and overall shape of the scaffold should align with the specific requirements of cartilage regeneration. 2) The scaffold should exhibit a biodegradation rate corresponding to the rate of cartilage repair to ensure that it is gradually replaced throughout the healing process. Therefore, when selecting scaffolds, hydrogels stand out because of their excellent biocompatibility, high water content, and outstanding permeability, all of which contribute to the creation of a favorable microenvironment for cells and facilitate cartilage regeneration. Moreover, the degradation rate of hydrogels can be precisely controlled by adjusting the degree and mode of crosslinking, allowing them to align with the growth rate of new tissue formation. This versatility makes hydrogels promising materials for CTE applications [254]. Gene vectors are generally classified into two categories: viral and non-viral vectors. Viral vectors can present challenges such as immunogenicity and toxicity, whereas non-viral vectors often encounter issues related to low transfection efficiency. Furthermore, both vector types may exhibit off-target effects and have limitations in term of efficiency and purity [243]. The majority of currently marketed gene therapy products are still based on viral vectors, whereas research on non-viral vectors remains largely in the biological experimental or preclinical stages. Therefore, optimizing non-viral gene vectors to maximize transfection efficiency, while ensuring good biocompatibility and cost-effectiveness, remains a critical focus in gene therapy research [255].

6. Conclusion and further prospects

Articular cartilage injury, a common clinical disease in orthopedics, is prevalent worldwide. The unique structure of cartilage tissue presents significant limitations and challenges for articular cartilage repair [12,22]. Among the various clinical surgical treatments, ACI is the only true restorative method for repairing or regenerating damaged cartilage tissue. Based on ACI, matrix-induced ACI (MACI) was developed by implanting synthetic matrices loaded with autologous isolated and enriched chondrocytes onto the cartilage injury site. Notably, MACI can effectively facilitate cartilage repair by optimizing the biological activity and mechanical properties of the matrices [256]. Recently, applicable matrices have been extensively developed by fabricating suitable tissue engineered scaffolds. The bioprinted scaffolds provide the possibility for preparing biomimetic tissue engineered scaffolds with intricate structures, and can effectively enhance cartilage repair by loading cells and growth factors [9,17–19]. Unfortunately, the application of growth factors in cartilage repair is hindered by limitations such as rapid clearance, short half-life, denaturation during storage, and limited targeting. Compared to growth factors, gene therapy loaded with genes encoding specific growth factors holds promise as a potential solution to overcome these limitations [88,90]. Recently, gene therapy has garnered significant attention in CTE, with several gene therapy approaches progressing to clinical trials for cartilage repair [1]. However, therapeutic genes face obstacles in effectively penetrating and enriching the deep layers of cartilage, owing to the high-density structure and negative charge of cartilage tissue. These obstacles render genes unable to sustain long-term retention at the injury site, making them susceptible to enzymatic degradation *in vivo* [134]. Therefore, the key to achieving efficient gene therapy is the selection of optimal gene vectors. Currently, viral gene vectors continue to dominate as the primary focus of gene therapy products in the market, whereas non-viral gene vectors are in the biological testing or preclinical phase. Notably, viral gene vectors have high transfection efficiency, but exhibit drawbacks such as insertional mutagenesis, immune response, enhanced inflammation, and high cost. Conversely, non-viral gene vectors have advantages such as easy preparation, easy modification, high reproducibility, and good biosafety. However, low transfection efficiency significantly hinders their progress toward clinical transformation [255]. Hence, the primary challenge in gene therapy lies in optimizing non-viral gene vectors to significantly enhance transfection efficiency, while maintaining excellent biocompatibility and low cost. Additionally, non-viral gene vectors should effectively achieve gene penetration and enrichment in the deep layer of the cartilage, and accurately spatiotemporally release genes to enhance cartilage repair [12,134].In the future, we believe that many highly promising and advantageous non-viral gene vectors will continue to emerge to propel the development of gene therapy.

Cartilage repair *in vivo* must ensure effective retention and sustained release of gene complexes at the articular cartilage injury site. GAMs, prepared by embedding gene complexes in biological scaffolds, can ensure long-term retention and sustained controlled release of gene complexes *in situ* to minimize their diffusion and loss *in vivo*, achieving long-term promotion of cartilage repair [12,96]. Among the various biological scaffolds, biomedical hydrogels possess a porous network structure and controllable physicochemical properties that can provide a biomimetic microenvironment similar to the ECM and enable the controlled release of gene complexes. These characteristics have led to extensive research into the application of hydrogels in cartilage repair [257,258]. Unfortunately, it is difficult to simulate the complex gradient structure of natural cartilage tissue effectively using conventional hydrogels, which limits their further application in CTE. Bioprinted scaffolds provide the possibility of preparing customizable biomimetic cartilage scaffolds with complex gradient structures [18]. The selection of the bioprinting ink is the foundation for preparing applicable bioprinted scaffolds, and this ink should exhibit excellent printability and biocompatibility. Bioprinted scaffolds can maintain the adhesion, proliferation, migration, and differentiation of the encapsulated cells and exhibit excellent shape fidelity, mechanical properties, biomimetic structures, and appropriate biodegradation [194,259]. Furthermore, joint injury and inflammation form a vicious cycle, in which injury causes inflammation and vice versa. Complete regeneration of injured cartilage requires gene-activated bioprinted scaffolds to provide immediate mechanical support for cell deposition, proliferation, and differentiation, sustained controlled release of genes to enhance chondrogenesis and inhibition of the inflammatory microenvironment [27]. Therefore, in response to the complex physiological characteristics of the cartilage, the development of appropriate gene-activated bioprinted scaffolds to guide chondrogenesis and achieve true cartilage regeneration is highly desirable. Appropriate gene-activated bioprinted

scaffolds can be fabricated by optimizing the physicochemical structure, particle size, surface charge, and active targeting ability of gene vectors, as well as the printability, mechanical properties, biocompatibility, biodegradability, anti-inflammatory and bioactive activities of bioprinted scaffolds. These scaffolds can effectively overcome high-density cartilage ECM barriers and inhibit the inflammatory microenvironment to improve gene permeability and bioavailability, thereby achieving efficient repair of cartilage injury. Unfortunately, research on gene-activated bioprinted scaffolds is relatively scarce compared to that on GAMs. Hence, the development of ideal gene-activated bioprinted scaffolds is at the forefront of future research on cartilage repair.

In this review, we first briefly introduce the composition and structure of articular cartilage. Then, we review the growth factors that facilitate chondrogenic differentiation and maintain cartilage homeostasis, including FGF-2, IGF-1, TGF-β, and BMP, in detail. Additionally, we offer a detailed overview of the gene vectors used in cartilage repair, including viral vectors such as lentivirus, AV, and AAV, as well as non-viral vectors such as lipofectamine, NPs, and cationic polymers. Notably, cationic polymer-based gene complexes can effectively deliver genes by overcoming the negative electrostatic barrier of the cartilage ECM to improve gene utilization, which have been widely used in CTE. Common cationic polymers include PEI, PAMAM, CTS, and PLL. However, gene complexes require appropriate biological scaffolds for loading to ensure effective retention and sustained release within the cartilage injury sites. Biomedical hydrogels exhibit a 3D porous structure mimicking the cartilage ECM and have been extensively researched as biological scaffolds. This article provides a detailed review of the hydrogels commonly used in GAMs, such as HA, gelatin, alginate, and fibrin. However, conventional hydrogels make it difficult to simulate the complex gradient structures of natural cartilage tissues. Bioprinted scaffolds have emerged as an effective solution to this challenge. Finally, we provide an overview of bioprinted scaffolds and gene-activated bioprinted scaffolds for cartilage repair. Unfortunately, research on gene-activated bioprinted scaffolds is currently scarce. In summary, the convergence of gene therapy and bioprinted scaffolds represents a promising avenue for advancing cartilage repair strategies, offering potential solutions to existing limitations in promoting effective and sustained cartilage repair. However, numerous biomaterials face challenges in their actual clinical translational applications owing to their intricate structures and compositions. Therefore, the future challenges and prospects of gene-activated bioprinted scaffolds for cartilage repair include the following: 1) Delving into the molecular mechanisms of cartilage repair to provide more effective therapeutic genes for cartilage repair, investigating the optimal duration and delivery method of therapeutic genes, and assessing the long-term effects and potential risks associated with gene therapy. 2) Developing non-viral gene vectors with efficient transfection activity, excellent biocompatibility, optimal particle size and surface charge, and active targeting ability can achieve gene penetration and enrichment in the deep layer of cartilage, and facilitate cartilage repair by accurately spatiotemporally releasing genes. 3) Developing bioprinting inks with easily synthesizable, simple components and good biocompatibility can prepare multifunctional bioactive bioprinted scaffolds that mimic the complex gradient structures of natural cartilage tissues, enable controlled and precise release of cells, genes, and drugs, enhance the integration of scaffolds with surrounding tissues, facilitate cartilage repair, and inhibit an inflammatory microenvironment. 4) Exploring optimal therapeutic genes, gene vectors, and bioprinted scaffolds can realize gene-activated bioprinted scaffolds to strengthen their application in cartilage repair. 5) Researching the molecular mechanisms, metabolic pathways, and clinical translation of existing biomaterials can result in more targeted functional modifications and promote clinical applications. The evolving landscape of gene therapy and bioprinted scaffolds anticipates a positive trajectory for addressing the multifaceted challenges of articular cartilage injury, thereby offering hope for improved treatment outcomes. Therefore, we believe that gene-activated bioprinted scaffolds will emerge as products with significant potential for clinical applications in cartilage repair.

CRediT authorship contribution statement

Min Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jiachen Wang:** Writing – review & editing, Investigation. **Xin Xu:** Writing – review & editing. **Erliang Li:** Writing – review & editing. **Peng Xu:** Supervision, Conceptualization.

Ethics approval and consent to participate

The review article does not contain the animal or human experiment. No ethics approval and consent are required.

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

No data was used for the research described in the article.

References

[1] S. Muthu, J.V. Korpershoek, E.J. Novais, G.F. Tawy, A.P. Hollander, I. Martin, Failure of cartilage regeneration: emerging hypotheses and related therapeutic strategies, Nat. Rev. Rheumatol. 19 (7) (2023) 403–416.
[2] M. Wang, S.H. Li, L.Y. Zhang, J. Tian, J.P. Ma, B. Lei, P. Xu, Injectable bioactive antioxidative one-component polycitrate hydrogel with anti-inflammatory effects for osteoarthritis alleviation and cartilage protection, Adv. Healthcare Mater. 13 (2) (2024) 2301953.
[3] R.Y. Chen, J.S. Pye, J.R. Li, C.B. Little, J.J. Li, Multiphasic scaffolds for the repair of osteochondral defects: outcomes of preclinical studies, Bioact. Mater. 27 (2023) 505–545.
[4] M. Wang, Z.X. Deng, Y. Guo, P. Xu, Designing functional hyaluronic acid-based hydrogels for cartilage tissue engineering, Mater. Today Bio 17 (2022) 100495.
[5] J.W. Wu, Q. Chen, C. Deng, B.P. Xu, Z.Y. Zhang, Y. Yang, T.L. Lu, Exquisite design of injectable hydrogels in cartilage repair, Theranostics 10 (21) (2020) 9843–9864.
[6] X.Q. Guo, L.L. Xi, M.Y. Yu, Z.L. Fan, W.Y. Wang, A.D. Ju, Z. Liang, G.D. Zhou, W. J. Ren, Regeneration of articular cartilage defects: therapeutic strategies and perspectives, J. Tissue Eng. 14 (2023) 20417314231164765.
[7] Z.H. Gu, J.Y. Wang, Y. Fu, H. Pan, H.Y. He, Q. Gan, C.S. Liu, Smart biomaterials for articular cartilage repair and regeneration, Adv. Funct. Mater. 33 (10) (2023) 2212561.
[8] M. Cucchiarini, H. Madry, Biomaterial-guided delivery of gene vectors for targeted articular cartilage repair, Nat. Rev. Rheumatol. 15 (1) (2019) 18–29.
[9] M.K. Wang, Y. Wu, G.F. Li, Q.S. Lin, W.C. Zhang, H. Liu, J.C. Su, Articular cartilage repair biomaterials: strategies and applications, Mater. Today Bio 24 (2024) 100948.
[10] H. Kwon, W.E. Brown, C.A. Lee, D.A. Wang, N. Paschos, J.C. Hu, K.A. Athanasiou, Surgical and tissue engineering strategies for articular cartilage and meniscus repair, Nat. Rev. Rheumatol. 15 (9) (2019) 550–570.
[11] M.W. Grol, The evolving landscape of gene therapy strategies for the treatment of osteoarthritis, Osteoarthritis Cartilage 32 (4) (2024) 372–384.
[12] R. Yang, F. Chen, J. Guo, D. Zhou, S. Luan, Recent advances in polymeric biomaterials-based gene delivery for cartilage repair, Bioact. Mater. 5 (4) (2020) 990–1003.
[13] Y.S. Kim, A.G. Mikos, Emerging strategies in reprogramming and enhancing the fate of mesenchymal stem cells for bone and cartilage tissue engineering, J. Contr. Release 330 (2021) 565–574.
[14] A. Zelinka, A.J. Roelofs, R.A. Kandel, C. De Bari, Cellular therapy and tissue engineering for cartilage repair, Osteoarthritis Cartilage 30 (12) (2022) 1547–1560.
[15] B.H. Shan, F.G. Wu, Hydrogel-based growth factor delivery platforms: strategies and recent advances, Adv. Mater. 36 (5) (2024) e2210707.

[16] W. Wei, Y. Ma, X. Yao, W. Zhou, X. Wang, C. Li, J. Lin, Q. He, S. Leptihn, H. Ouyang, Advanced hydrogels for the repair of cartilage defects and regeneration, Bioact. Mater. 6 (4) (2020) 998–1011.
[17] X. Yang, S. Li, Y. Ren, L. Qiang, Y.H. Liu, J.W. Wang, K.R. Dai, 3D printed hydrogel for articular cartilage regeneration, Composites, Part B 237 (2022) 109863.
[18] M.Y. Li, D.C. Sun, J. Zhang, Y.M. Wang, Q.H. Wei, Y.N. Wang, Application and development of 3D bioprinting in cartilage tissue engineering, Biomater. Sci. 10 (19) (2022) 5430–5458.
[19] Q. Liang, Y. Ma, X. Yao, W. Wei, Advanced 3D-printing bioinks for articular cartilage repair, Int. J. Bioprint. 8 (3) (2022) 511.
[20] D. Gvaramia, J. Kern, Y. Jakob, M. Zenobi-Wong, N. Rotter, Regenerative potential of perichondrium: a tissue engineering perspective, Tissue Eng., Part B 28 (3) (2022) 531–541.
[21] N.A. Chartrain, K.H. Gilchrist, V.B. Ho, G.J. Klarmann, 3D bioprinting for the repair of articular cartilage and osteochondral tissue, Bioprinting 28 (2022) e00239.
[22] W.Y. Wei, H.L. Dai, Articular cartilage and osteochondral tissue engineering techniques: recent advances and challenges, Bioact. Mater. 6 (12) (2021) 4830–4855.
[23] C. Chiu, K.W. Zheng, M.X. Xue, D.J. Du, Comparative analysis of hyaline cartilage characteristics and chondrocyte potential for articular cartilage repair, Ann. Biomed. Eng. 52 (4) (2024) 920–933.
[24] Y.R. Wang, Q.L. Li, H.H. Li, X.N. Yang, H. Fang, R.Y. Bi, S.S. Zhu, Heterogeneous characteristics of the CD90^{+} progenitors in the fibrocartilage of different joints, Cartilage (2023) 19476035231200359.
[25] B.J. Bielajew, J.C. Hu, K.A. Athanasiou, Collagen: quantification, biomechanics and role of minor subtypes in cartilage, Nat. Rev. Mater. 5 (10) (2020) 730–747.
[26] C.D. DiDomenico, M. Lintz, L.J. Bonassar, Molecular transport in articular cartilage-what have we learned from the past 50 years? Nat. Rev. Rheumatol. 14 (7) (2018) 393–403.
[27] H.Q. Zhou, Z. Zhang, Y.L. Mu, H. Yao, Y. Zhang, D.A. Wang, Harnessing nanomedicine for cartilage repair: design considerations and recent advances in biomaterials, ACS Nano 18 (16) (2024) 10667–10687.
[28] L. Yu, S. Cavelier, B. Hannon, M. Wei, Recent development in multizonal scaffolds for osteochondral regeneration, Bioact. Mater. 25 (2023) 122–159.
[29] D.L. Yang, J.W. Xu, K. Xu, P. Xu, Skeletal interoception in osteoarthritis, Bone Res. 12 (1) (2024) 22.
[30] M.Z. Li, H. Yin, Z.E. Yan, H.Y. Li, J. Wu, Y. Wang, F. Wei, G.Z. Tian, C. Ning, H. Li, C.J. Gao, L.W. Fu, S.P. Jiang, M.X. Chen, X. Sui, S.Y. Liu, Z.W. Chen, Q.Y. Guo, The immune microenvironment in cartilage injury and repair, Acta Biomater. 140 (2022) 23–42.
[31] Y. Liu, M. Wang, Y. Luo, Q. Liang, Y. Yu, F. Chen, J. Yao, Enhancing stem cell therapy for cartilage repair in osteoarthritis-a hydrogel focused approach, Gels 7 (4) (2021) 263.
[32] A. Lolli, L. Penolazzi, R. Narcisi, G.J.V.M. van Osch, R. Piva, Emerging potential of gene silencing approaches targeting anti-chondrogenic factors for cell-based cartilage repair, Cell. Mol. Life Sci. 74 (19) (2017) 3451–3465.
[33] M. Mazor, E. Lespessailles, R. Coursier, R. Daniellou, T.M. Best, H. Toumi, Mesenchymal stem-cell potential in cartilage repair: an update, J. Cell Mol. Med. 18 (12) (2014) 2340–2350.
[34] L. Chen, J.X. Liu, M. Guan, T.Q. Zhou, X. Duan, Z. Xiang, Growth factor and its polymer scaffold-based delivery system for cartilage tissue engineering, Int. J. Nanomed. 15 (2020) 6097–6111.
[35] J. Zhang, Z.M. Liu, Y.W. Li, Q. You, J.B. Yang, Y. Jin, G. Zou, J.F. Tang, Z. Ge, Y. Liu, FGF2: a key regulator augmenting tendon-to-bone healing and cartilage repair, Regener, Med 15 (9) (2020) 2129–2142.
[36] Y.L. Xie, A. Zinkle, L. Chen, M. Mohammadi, Fibroblast growth factor signalling in osteoarthritis and cartilage repair, Nat. Rev. Rheumatol. 16 (10) (2020) 547–564.
[37] H.Z. Li, J.L. Zhang, D.L. Yuan, W.Q. Xie, C.H. Ladel, A. Mobasheri, Y.S. Li, Role of signaling pathways in age-related orthopedic diseases: focus on the fibroblast growth factor family, Mil. Med. Res. 11 (1) (2024) 40.
[38] X. Li, M.B. Ellman, J.S. Kroin, D. Chen, D.Y. Yan, K. Mikecz, K.C. Ranjan, G. Z. Xiao, G.S. Stein, S.G. Kim, B. Cole, A.J. van Wijnen, H.J. Im, Species-specific biological effects of FGF-2 in articular cartilage: implication for distinct roles within the FGF receptor family, J. Cell. Biochem. 113 (7) (2012) 2532–2542.
[39] W.Y. Yang, Y.T. Cao, Z. Zhang, F.C. Du, Y.P. Shi, X.M. Li, Q.Q. Zhang, Targeted delivery of FGF2 to subchondral bone enhanced the repair of articular cartilage defect, Acta Biomater. 69 (2018) 170–182.
[40] S.L. Shi, C.R. Wang, A.J. Acton, G.J. Eckert, S.B. Trippel, Role of Sox9 in growth factor regulation of articular chondrocytes, J. Cell. Biochem. 116 (7) (2015) 1391–1400.
[41] X. Li, G. Su, J. Wang, Z. Zhou, L. Li, L. Liu, M. Guan, Q. Zhang, H. Wang, Exogenous bFGF promotes articular cartilage repair via up-regulation of multiple growth factors, Osteoarthritis Cartilage 21 (10) (2013) 1567–1575.
[42] M.B. Ellman, D. Yan, K. Ahmadinia, D. Chen, H.S. An, H.J. Im, Fibroblast growth factor control of cartilage homeostasis, J. Cell. Biochem. 114 (4) (2013) 735–742.
[43] X.D. Yao, J.M. Zhang, X.Z. Jing, Y.P. Ye, J.C. Guo, K. Sun, F.J. Guo, Fibroblast growth factor 18 exerts anti-osteoarthritic effects through PI3K-AKT signaling and mitochondrial fusion and fission, Pharmacol. Res. 139 (2019) 314–324.
[44] C.N. Wen, L.M. Xu, X. Xu, D.P. Wang, Y.J. Liang, L. Duan, Insulin-like growth factor-1 in articular cartilage repair for osteoarthritis treatment, Arthritis Res. Ther. 23 (1) (2021) 277.
[45] K. Gelse, C. Muehle, K. Knaup, B. Swoboda, M. Wiesener, F. Hennig, A. Olk, H. Schneider, Chondrogenic differentiation of growth factor-stimulated precursor cells in cartilage repair tissue is associated with increased HIF-1α activity, Osteoarthritis Cartilage 16 (12) (2008) 1457–1465.
[46] M. Sani, R. Hosseinie, M. Latifi, M. Shadi, M. Razmkhah, M. Salmannejad, H. Parsaei, T. Talaei-Khozani, Engineered artificial articular cartilage made of decellularized extracellular matrix by mechanical and IGF-1 stimulation, Biomater. Adv. 139 (2022) 213019.
[47] B.G. Starkman, J.D. Cravero, M. DelCarlo, R.F. Loeser, IGF-I stimulation of proteoglycan synthesis by chondrocytes requires activation of the PI 3-kinase pathway but not ERK MAPK, Biochem. J. 389 (2005) 723–729.
[48] X.N. Yu, T.J. Xu, H.M. Shi, J.Q. Hong, X.Q. Jin, L. Cao, J.J. Wang, Y.T. Lin, Z. Y. Pan, S.H. Wang, J.H. Fang, K.W. Xu, H.Y. Song, Z.X. Zhou, S.A. Zhu, J. Yin, Y. Y. Qi, X.S. Dai, Cartilage-targeting mRNA-lipid nanoparticles rescue perifocal apoptotic chondrocytes for integrative cartilage repair, Chem. Eng. J. 465 (2023) 142841.
[49] Z. Zhang, L. Li, W. Yang, Y. Cao, Y. Shi, X. Li, Q. Zhang, The effects of different doses of IGF-1 on cartilage and subchondral bone during the repair of full-thickness articular cartilage defects in rabbits, Osteoarthritis Cartilage 25 (2) (2017) 309–320.
[50] H. Madry, G. Kaul, M. Cucchiarini, U. Stein, D. Zurakowski, K. Remberger, M. D. Menger, D. Kohn, S.B. Trippel, Enhanced repair of articular cartilage defects *in vivo* by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I), Gene Ther. 12 (15) (2005) 1171–1179.
[51] L.R. Goodrich, C. Hidaka, P.D. Robbins, C.H. Evans, A.J. Nixon, Genetic modification of chondrocytes with insulin-like growth factor-1 enhances cartilage healing in an equine model, J. Bone Jt. Surg., Br 89b (5) (2007) 672–685.
[52] E.N. Blaney Davidson, P.M. van der Kraan, W.B. van den Berg, TGF-β and osteoarthritis, Osteoarthritis Cartilage 15 (6) (2007) 597–604.
[53] A.S. Patil, R.B. Sable, R.M. Kothari, An update on transforming growth factor-β (TGF-β): sources, types, functions and clinical applicability for cartilage/bone healing, J. Cell. Physiol. 226 (12) (2011) 3094–3103.
[54] H. Kwon, N.K. Paschos, J.C. Hu, K. Athanasiou, Articular cartilage tissue engineering: the role of signaling molecules, Cell. Mol. Life Sci. 73 (6) (2016) 1173–1194.
[55] T. Furumatsu, T. Ozaki, H. Asahara, Smad3 activates the Sox9-dependent transcription on chromatin, Int. J. Biochem. Cell Biol. 41 (5) (2009) 1198–1204.
[56] C. Cherifi, S. Monteagudo, R.J. Lories, Promising targets for therapy of osteoarthritis: a review on the Wnt and TGF-β signalling pathways, Ther. Adv. Musculoskeletal Dis. 13 (2021) 1759720X211006959.
[57] J. Li, Z. Zhao, J. Liu, N. Huang, D. Long, J. Wang, X. Li, Y. Liu, MEK/ERK and p38 MAPK regulate chondrogenesis of rat bone marrow mesenchymal stem cells through delicate interaction with TGF-β1/Smads pathway, Cell Prolif. 43 (4) (2010) 333–343.
[58] E.N.B. Davidson, E.L. Vitters, W.B. van den Berg, P.M. van der Kraan, TGFβ-induced cartilage repair is maintained but fibrosis is blocked in the presence of Smad7, Arthritis Res. Ther. 8 (3) (2006) R65.
[59] Y.H. Deng, G.H. Lei, Z.X. Lin, Y.H. Yang, H. Lin, R.S. Tuan, Engineering hyaline cartilage from mesenchymal stem cells with low hypertrophy potential via modulation of culture conditions and Wnt/β-catenin pathway, Biomaterials 192 (2019) 569–578.
[60] A. Hoffmann, G. Gross, BMP signaling pathways in cartilage and bone formation, Crit. Rev. Eukaryot. Gene Expr. 11 (1–3) (2001) 23–45.
[61] Z.H. Deng, Y.S. Li, X. Gao, G.H. Lei, J. Huard, Bone morphogenetic proteins for articular cartilage regeneration, Osteoarthritis Cartilage 26 (9) (2018) 1153–1161.
[62] P.C. Xiao, Z.L. Zhu, C.C. Du, Y.S. Zeng, J.Y. Liao, Q. Cheng, H. Chen, C. Zhao, W. Huang, Silencing Smad7 potentiates BMP2-induced chondrogenic differentiation and inhibits endochondral ossification in human synovial-derived mesenchymal stromal cells, Stem Cell Res. Ther. 12 (1) (2021) 132.
[63] L. Lin, C.Y. Zhou, X.L. Wei, Y. Hou, L.H. Zhao, X. Fu, J.Y. Zhang, C. Yu, Articular cartilage repair using dedifferentiated articular chondrocytes and bone morphogenetic protein 4 in a rabbit model of articular cartilage defects, Arthritis Rheum. 58 (4) (2008) 1067–1075.
[64] Y.Q. Pan, Z.W. Jiang, Y.R. Ye, D.J. Zhu, N. Li, G.L. Yang, Y. Wang, Role and mechanism of BMP4 in regenerative medicine and tissue engineering, Ann. Biomed. Eng. 51 (7) (2023) 1374–1389.
[65] C.D. Grimsrud, P.R. Romano, M. D'Souza, J.E. Puzas, P.R. Reynolds, R.N. Rosier, R.J. O'Keefe, BMP-6 is an autocrine stimulator of chondrocyte differentiation, J. Bone Miner. Res. 14 (4) (1999) 475–482.
[66] B.T. Estes, A.W. Wu, F. Guilak, Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6, Arthritis Rheum. 54 (4) (2006) 1222–1232.
[67] H.J. Kim, M.A. Han, J.Y. Shin, J.H. Jeon, S.J. Lee, M.Y. Yoon, H.J. Kim, E.J. Choi, S.H. Do, V.C. Yang, H.N. He, Y.I. Yang, Intra-articular delivery of synovium-resident mesenchymal stem cells BMP-7-loaded fibrous PLGA scaffolds for cartilage repair, J. Contr. Release 302 (2019) 169–180.
[68] T. Takahashi, T. Muneta, K. Tsuji, I. Sekiya, BMP-7 inhibits cartilage degeneration through suppression of inflammation in rat zymosan-induced arthritis, Cell Tissue Res. 344 (2) (2011) 321–332.
[69] I. Sekiya, T. Tang, M. Hayashi, T. Morito, Y.J. Ju, T. Mochizuki, T. Muneta, Periodic knee injections of BMP-7 delay cartilage degeneration induced by excessive running in rats, J. Orthop. Res. 27 (8) (2009) 1088–1092.
[70] M.M. Caron, P.J. Emans, M.M. Coolsen, D.A. Surtel, A. Cremers, L.W. van Rhijn, T.J. Welting, Hypertrophic differentiation during chondrogenic differentiation of progenitor cells is stimulated by Bmp-2 but suppressed by Bmp-7, Osteoarthritis Cartilage 21 (2013) 604–613.

[71] H. Song, K.H. Park, Regulation and function of SOX9 during cartilage development and regeneration, Semin. Cancer Biol. 67 (2020) 12–23.
[72] N. Carballo-Pedrares, C. Sanjurjo-Rodriguez, J. Senaris, S. Diaz-Prado, A. Rey-Rico, Chondrogenic differentiation of human mesenchymal stem cells via SOX9 delivery in cationic niosomes, Pharmaceutics 14 (11) (2022) 2327.
[73] M.R. Iaquinta, C. Lanzillotti, C. Mazziotta, I. Bononi, F. Frontini, E. Mazzoni, L. Oton-Gonzalez, J.C. Rotondo, E. Torreggiani, M. Tognon, F. Martini, The role of microRNAs in the osteogenic and chondrogenic differentiation of mesenchymal stem cells and bone pathologies, Theranostics 11 (13) (2021) 6573–6591.
[74] P. Dy, W.H. Wang, P. Bhattaram, Q.Q. Wang, L. Wang, R.T. Ballock, V. Lefebvre, Sox9 directs hypertrophic maturation and blocks osteoblast differentiation of growth plate chondrocytes, Dev. Cell 22 (3) (2012) 597–609.
[75] Y. Tamamura, T. Otani, N. Kanatani, E. Koyama, J. Kitagaki, T. Komori, Y. Yamada, F. Costantini, S. Wakisaka, M. Pacifici, M. Iwamoto, M. Enomoto-Iwamoto, Developmental regulation of Wnt/β-catenin signals is required for growth plate assembly, cartilage integrity, and endochondral ossification, J. Biol. Chem. 280 (19) (2005) 19185–19195.
[76] Y. Han, V. Lefebvre, L-Sox5 and Sox6 drive expression of the aggrecan gene in cartilage by securing binding of Sox9 to a far-upstream enhancer, Mol. Cell Biol. 28 (16) (2008) 4999–5013.
[77] R.M. Raftery, A.G.G. Vazquez, G. Chen, F.J. O'Brien, Activation of the SOX-5, SOX-6, and SOX-9 trio of transcription factors using a gene-activated scaffold stimulates mesenchymal stromal cell chondrogenesis and inhibits endochondral ossification, Adv. Healthcare Mater. 9 (10) (2020) 1901827.
[78] K. Amano, K. Hata, A. Sugita, Y. Takigawa, K. Ono, M. Wakabayashi, M. Kogo, R. Nishimura, T. Yoneda, Sox9 family members negatively regulate maturation and calcification of chondrocytes through up-regulation of parathyroid hormone-related protein, Mol. Biol. Cell 20 (21) (2009) 4541–4551.
[79] T. Kanazawa, T. Furumatsu, M. Hachioji, T. Oohashi, Y. Ninomiya, T. Ozaki, Mechanical stretch enhances COL2A1 expression on chromatin by inducing SOX9 nuclear translocalization in inner meniscus cells, J. Orthop. Res. 30 (3) (2012) 468–474.
[80] T.M. Liu, X.M. Guo, H.S. Tan, J.H. Hui, B. Lim, E.H. Lee, Zinc-Finger Protein 145, Acting as an upstream regulator of SOX9, improves the differentiation potential of human mesenchymal stem cells for cartilage regeneration and repair, Arthritis Rheum. 63 (9) (2011) 2711–2720.
[81] J.T. Sieker, M. Kunz, M. Weissenberger, F. Gilbert, S. Frey, M. Rudert, A. F. Steinert, Direct bone morphogenetic protein 2 and Indian hedgehog gene transfer for articular cartilage repair using bone marrow coagulates, Osteoarthritis Cartilage 23 (3) (2015) 433–442.
[82] E. Mariani, L. Pulsatelli, A. Facchini, Signaling pathways in cartilage repair, Int. J. Mol. Sci. 15 (5) (2014) 8667–8698.
[83] B.E. Walczak, H.L. Jiao, M.S. Lee, W.J. Li, Reprogrammed synovial fluid-derived mesenchymal stem/stromal cells acquire enhanced therapeutic potential for articular cartilage repair, Cartilage 13 (2_Suppl) (2021) 530s–543s.
[84] L. Lin, Q. Shen, T. Xue, X.N. Duan, X. Fu, C.L. Yu, Sonic hedgehog improves redifferentiation of dedifferentiated chondrocytes for articular cartilage repair, PLoS One 9 (2) (2014) e88550.
[85] S. Kubo, G.M. Cooper, T. Matsumoto, J.A. Phillippi, K.A. Corsi, A. Usas, G.H. Li, F. H. Fu, J. Huard, Blocking vascular endothelial growth factor with soluble Flt-1 improves the chondrogenic potential of mouse skeletal muscle-derived stem cells, Arthritis Rheum. 60 (1) (2009) 155–165.
[86] S.S. Zhu, B. Zhang, C. Man, Y.Q. Ma, X.W. Liu, J. Hu, Combined effects of connective tissue growth factor-modified bone marrow-derived mesenchymal stem cells and NaOH-treated PLGA scaffolds on the repair of articular cartilage defect in rabbits, Cell Transplant. 23 (6) (2014) 715–727.
[87] Z.J. Zhang, Y.K. Hou, M.W. Chen, X.Z. Yu, S.Y. Chen, Y.R. Yue, X.T. Guo, J. X. Chen, Q. Zhou, A pH-responsive metal-organic framework for the co-delivery of HIF-2α siRNA and curcumin for enhanced therapy of osteoarthritis, J. Nanobiotechnol. 21 (1) (2023) 18.
[88] R. Censi, A. Dubbini, P. Matricardi, Bioactive hydrogel scaffolds-advances in cartilage regeneration through controlled drug delivery, Curr. Pharmaceut. Des. 21 (12) (2015) 1545–1555.
[89] J.Y. Guan, J.W. Feng, F. Lu, Recent advances in the hydrogel-based biomolecule delivery system for cartilage tissue engineering, Adv. Mater. Sci. Eng. 2022 (2022) 1899400.
[90] Y. Esmaeili, E. Bidram, A. Bigham, M. Atari, R. Nasr Azadani, M. Tavakoli, S. Salehi, M. Mirhaj, A. Basiri, Z. Mirzavandi, M. Boshtam, M. Rafienia, A. Zargar Kharazi, S. Karbasi, L. Shariati, A. Zarrabi, Exploring the evolution of tissue engineering strategies over the past decade: from cell-based strategies to gene-activated matrix, Alex. Eng. J. 81 (2023) 137–169.
[91] Y.F. Han, L.H. Cao, G.F. Li, F.J. Zhou, L. Bai, J.C. Su, Harnessing nucleic acids nanotechnology for bone/cartilage regeneration, Small 19 (37) (2023) 2301996.
[92] A. Santhagunam, C. Madeira, J.M.S. Cabral, Genetically engineered stem cell-based strategies for articular cartilage regeneration, Biotechnol. Appl. Biochem. 59 (2) (2012) 121–131.
[93] H. Yin, R.L. Kanasty, A.A. Eltoukhy, A.J. Vegas, J.R. Dorkin, D.G. Anderson, Non-viral vectors for gene-based therapy, Nat. Rev. Genet. 15 (8) (2014) 541–555.
[94] A. Vedadghavami, E.K. Wagner, S. Mehta, T.F. He, C.Z. Zhang, A.G. Bajpayee, Cartilage penetrating cationic peptide carriers for applications in drug delivery to avascular negatively charged tissues, Acta Biomater. 93 (2019) 258–269.
[95] L.B. Zhou, L.E. Rubin, C.J. Liu, Y.P. Chen, Short interfering RNA (siRNA)-based therapeutics for cartilage diseases, Regener. Eng. Transl. Med.d 7 (3) (2020) 283–290.
[96] T. Gonzalez-Fernandez, D.J. Kelly, F.J. O'Brien, Controlled non-viral gene delivery in cartilage and bone repair: current strategies and future directions, Adv. Ther. 1 (7) (2018) 1800038.
[97] A. Saraf, A.G. Mikos, Gene delivery strategies for cartilage tissue engineering, Adv. Drug Deliv. Rev. 58 (4) (2006) 592–603.
[98] W. Zhu, T. Niu, Z. Wei, B. Yang, X. Weng, Advances in biomaterial-mediated gene therapy for articular cartilage repair, Bioengineering 9 (10) (2022) 502.
[99] Z.H. Wang, Z.Q. Yang, X.J. He, B.E. Kamal, Z. Xing, Lentivirus-mediated knockdown of aggrecanase-1 and -2 promotes chondrocyte-engineered cartilage formation *in vitro*, Biotechnol. Bioeng. 107 (4) (2010) 730–736.
[100] X. Chu, H. You, X. Yuan, W. Zhao, W. Li, X. Guo, Protective effect of lentivirus-mediated siRNA targeting ADAMTS-5 on cartilage degradation in a rat model of osteoarthritis, Int. J. Mol. Med. 31 (5) (2013) 1222–1228.
[101] S. Ruan, J. Deng, L. Yan, W. Huang, Evaluation of the effects of the combination of BMP-2-modified BMSCs and PRP on cartilage defects, Exp. Ther. Med. 16 (6) (2018) 4569–4577.
[102] K. Gelse, K. von der Mark, T. Aigner, J. Park, H. Schneider, Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells, Arthritis Rheum. 48 (2) (2003) 430–441.
[103] D.C. Kelly, R.M. Raftery, C.M. Curtin, C.M. O'Driscoll, F.J. O'Brien, Scaffold-based delivery of nucleic acid therapeutics for enhanced bone and cartilage repair, J. Orthop. Res. 37 (8) (2019) 1671–1680.
[104] Y.P. Morscheid, J.K. Venkatesan, G. Schmitt, P. Orth, D. Zurakowski, S. Speicher-Mentges, M.D. Menger, M.W. Laschke, M. Cucchiarini, H. Madry, rAAV-mediated human FGF-2 gene therapy enhances osteochondral repair in a clinically relevant large animal model over time *in vivo*, Am. J. Sports Med. 49 (4) (2021) 958–969.
[105] M. Cucchiarini, H. Madry, Overexpression of human IGF-I via direct rAAV-mediated gene transfer improves the early repair of articular cartilage defects, Gene Ther. 21 (9) (2014) 811–819.
[106] M. Cucchiarini, P. Orth, H. Madry, Direct rAAV SOX9 administration for durable articular cartilage repair with delayed terminal differentiation and hypertrophy *in vivo*, J. Mol. Med. 91 (5) (2013) 625–636.
[107] W. Meng, A. Rey-Rico, M. Claudel, G. Schmitt, S. Speicher-Mentges, F. Pons, L. Lebeau, J.K. Venkatesan, M. Cucchiarini, rAAV-Mediated overexpression of SOX9 and TGF-β via carbon dot-guided vector delivery enhances the biological activities in human bone marrow-derived mesenchymal stromal cells, Nanomaterials 10 (5) (2020) 855.
[108] R.M. Raftery, D.P. Walsh, I.M. Castano, A. Heise, G.P. Duffy, S.A. Cryan, F. J. O'Brien, Delivering nucleic-acid based nanomedicines on biomaterial scaffolds for orthopedic tissue repair: challenges, progress and future perspectives, Adv. Mater. 28 (27) (2016) 5447–5469.
[109] S. Raisin, E. Belamie, M. Morille, Non-viral gene activated matrices for mesenchymal stem cells based tissue engineering of bone and cartilage, Biomaterials 104 (2016) 223–237.
[110] C.A. Guo, X.G. Liu, J.Z. Huo, C. Jiang, X.J. Wen, Z.R. Chen, Novel gene-modified-tissue engineering of cartilage using stable transforming growth factor-β1-transfected mesenchymal stem cells grown on chitosan scaffolds, J. Biosci. Bioeng. 103 (6) (2007) 547–556.
[111] H. Madry, G. Kaul, D. Zurakowski, G. Vunjak-Novakovic, M. Cucchiarini, Cartilage constructs engineered from chondrocytes overexpressing Igf-I improve the repair of osteochondral defects in a rabbit model, Eur. Cell. Mater. 25 (2013) 229–247.
[112] Q.Q. Liu, Y.J. Kim, G.B. Im, J.T. Zhu, Y.Z. Wu, Y.J. Liu, S.H. Bhang, Inorganic nanoparticles applied as functional therapeutics, Adv. Funct. Mater. 31 (12) (2021) 2008171.
[113] X. Cao, J.P. Wang, W.W. Deng, J.J. Chen, Y. Wang, J. Zhou, P. Du, W.Q. Xu, Q. Wang, Q.L. Wang, Q.T. Yu, M. Spector, J.N. Yu, X.M. Xu, Photoluminescent cationic carbon dots as efficient non-viral delivery of plasmid SOX9 and chondrogenesis of fibroblasts, Sci. Rep. 8 (2018) 7057.
[114] J. Xu, J. Li, S. Lin, T. Wu, H. Huang, K. Zhang, Y. Sun, K.W.K. Yeung, G. Li, L. Bian, Nanocarrier-mediated codelivery of small molecular drugs and siRNA to enhance chondrogenic differentiation and suppress hypertrophy of human mesenchymal stem cells, Adv. Funct. Mater. 26 (15) (2016) 2463–2472.
[115] H. Yan, X. Duan, H. Pan, N. Holguin, M.F. Rai, A. Akk, L.E. Springer, S. A. Wickline, L.J. Sandell, C.T.N. Pham, Suppression of NF-κB activity via nanoparticle-based siRNA delivery alters early cartilage responses to injury, Proc. Natl. Acad. Sci. USA 113 (41) (2016) E6199–E6208.
[116] Y. Cai, C.X. Wu, Q.H. Ou, M.H. Zeng, S. Xue, J.L. Chen, Y. Lu, C.H. Ding, Enhanced osteoarthritis therapy by nanoengineered mesenchymal stem cells using biomimetic CuS nanoparticles loaded with plasmid DNA encoding TGF-β1, Bioact. Mater. 19 (2023) 444–457.
[117] P. Wu, H.J. Chen, R.H. Jin, T.T. Weng, J.K. Ho, C.G. You, L.P. Zhang, X.G. Wang, C.M. Han, Non-viral gene delivery systems for tissue repair and regeneration, J. Transl. Med. 16 (2018) 29.
[118] J.S. Park, S.W. Yi, H.J. Kim, K.H. Park, Receptor-mediated gene delivery into human mesenchymal stem cells using hyaluronic acid-shielded polyethylenimine/pDNA nanogels, Carbohydr. Polym. 136 (2016) 791–802.
[119] C.J. Needham, S.R. Shah, R.L. Dahlin, L.A. Kinard, J. Lam, B.M. Watson, S. Lu, F. K. Kasper, A.G. Mikos, Osteochondral tissue regeneration through polymeric delivery of DNA encoding for the SOX trio and RUNX2, Acta Biomater. 10 (10) (2014) 4103–4112.
[120] P.H. Yeh, J.S. Sun, H.C. Wu, L.H. Hwang, T.W. Wang, Stimuli-responsive HA-PEI nanoparticles encapsulating endostatin plasmid for stem cell gene therapy, RSC Adv. 3 (31) (2013) 12922–12932.
[121] Y.B. Pi, X. Zhang, J.J. Shi, J.X. Zhu, W.Q. Chen, C.G. Zhang, W.W. Gao, C.Y. Zhou, Y.F. Ao, Targeted delivery of non-viral vectors to cartilage *in vivo* using a

chondrocyte-homing peptide identified by phage display, Biomaterials 32 (26) (2011) 6324–6332.
[122] Y. Pi, X. Zhang, Z. Shao, F. Zhao, X. Hu, Y. Ao, Intra-articular delivery of anti-Hif-2α siRNA by chondrocyte-homing nanoparticles to prevent cartilage degeneration in arthritic mice, Gene Ther. 22 (6) (2015) 439–448.
[123] J.H. Kim, J.S. Park, H.N. Yang, D.G. Woo, S.Y. Jeon, H.J. Do, H.Y. Lim, J.M. Kim, K.H. Park, The use of biodegradable PLGA nanoparticles to mediate SOX9 gene delivery in human mesenchymal stem cells (hMSCs) and induce chondrogenesis, Biomaterials 32 (1) (2011) 268–278.
[124] S.Y. Jeon, J.S. Park, H.N. Yang, D.G. Woo, K.H. Park, Co-delivery of SOX9 genes and anti-Cbfa-1 siRNA coated onto PLGA nanoparticles for chondrogenesis of human MSCs, Biomaterials 33 (17) (2012) 4413–4423.
[125] S.Y. Jeon, J.S. Park, H.N. Yang, H.J. Lim, S.W. Yi, H. Park, K.H. Park, Co-delivery of Cbfa-1-targeting siRNA and SOX9 protein using PLGA nanoparticles to induce chondrogenesis of human mesenchymal stem cells, Biomaterials 35 (28) (2014) 8236–8248.
[126] Q.T. Yu, Y. Wang, X. Cao, W.W. Deng, M.A. Frimpong, J.N. Yu, X.M. Xu, One-step formation of chondrocytes through direct reprogramming via polysaccharide-based gene delivery, Adv. Polym. Technol. 2019 (2019) 7632873.
[127] S.W. Yi, H.J. Kim, H.J. Oh, H. Shin, J.S. Lee, J.S. Park, K.H. Park, Gene expression profiling of chondrogenic differentiation by dexamethasone-conjugated polyethyleneimine with SOX trio genes in stem cells, Stem Cell Res. Ther. 9 (2018) 341.
[128] P. Kesharwani, S. Banerjee, U. Gupta, M.C.I.M. Amin, S. Padhye, F.H. Sarkar, A. K. Iyer, PAMAM dendrimers as promising nanocarriers for RNAi therapeutics, Mater. Today 18 (10) (2015) 565–572.
[129] A. Shaikh, P. Kesharwani, V. Gajbhiye, Dendrimer as a momentous tool in tissue engineering and regenerative medicine, J. Contr. Release 346 (2022) 328–354.
[130] B.C. Geiger, S. Wang, R.F. Padera, A.J. Grodzinsky, P.T. Hammond, Cartilage-penetrating nanocarriers improve delivery and efficacy of growth factor treatment of osteoarthritis, Sci. Transl. Med. 10 (469) (2018) eaat8800.
[131] Q. Hu, Q. Chen, X.Y. Yan, B.M. Ding, D.W. Chen, L.F. Cheng, Chondrocyte affinity peptide modified PAMAM conjugate as a nanoplatform for targeting and retention in cartilage, Nanomedicine 13 (7) (2018) 749–767.
[132] B. Li, F. Wang, F.Q. Hu, T. Ding, P. Huang, X. Xu, J. Liang, C.W. Li, Q. Zhou, M. Lu, L.F. Deng, L. Guo, W.G. Cui, Injectable "nano-micron" combined gene-hydrogel microspheres for local treatment of osteoarthritis, NPG Asia Mater. 14 (1) (2022) 1.
[133] H. Chen, F. Chen, F. Hu, Y. Li, M. Zhang, Q. Zhou, T. Ding, N. Tulufu, T. Ye, F. Wang, L. Guo, MicroRNA-224-5p nanoparticles balance homeostasis via inhibiting cartilage degeneration and synovial inflammation for synergistic alleviation of osteoarthritis, Acta Biomater. 167 (2023) 401–415.
[134] L. Chen, J. Zhang, J. Wang, J.W. Lin, X.J. Luo, W.G. Cui, Inflammation-regulated auto aggregated hydrogel microspheres via anchoring cartilage deep matrix for genes delivery, Adv. Funct. Mater. 33 (51) (2023) 2305635.
[135] R.L. Zhao, B. Sun, T.Y. Liu, Y. Liu, S.M. Zhou, A.J. Zuo, D.C. Liang, Optimize nuclear localization and intra-nucleus disassociation of the exogene for facilitating transfection efficacy of the chitosan, Int. J. Pharm. 413 (1–2) (2011) 254–259.
[136] R. Zhao, S. Wang, L. Jia, Q. Li, J. Qiao, X. Peng, Interleukin-1 receptor antagonist protein (IL-1Ra) and miR-140 overexpression via pNNS-conjugated chitosan-mediated gene transfer enhances the repair of full-thickness cartilage defects in a rabbit model, Bone Joint Res 8 (3) (2019) 165–178.
[137] R.L. Zhao, X.X. Peng, Q. Li, W. Song, Effects of phosphorylatable short peptide-conjugated chitosan-mediated IL-1Ra and IGF-1 gene transfer on articular cartilage defects in rabbits, PLoS One 9 (11) (2014) e112284.
[138] R.L. Zhao, X.M. Zhang, L.N. Jia, W. Song, Y.L. Sun, X.Y. Meng, X.X. Peng, pNNS-conjugated chitosan mediated IGF-1 and miR-140 overexpression in articular chondrocytes improves cartilage repair, BioMed Res. Int. 2019 (2019) 2761241.
[139] N.A. Moghadam, F. Bagheri, M.B. Eslaminejad, Chondroitin sulfate modified chitosan nanoparticles as an efficient and targeted gene delivery vehicle to chondrocytes, Colloids Surf., B 219 (2022) 112786.
[140] Y.S. Kim, A.J. Chien, J.L. Guo, B.T. Smith, E. Watson, H.A. Pearce, G.L. Koons, A. M. Navara, J. Lam, D.W. Scott, K.J. Grande-Allen, A.G. Mikos, Chondrogenesis of cocultures of mesenchymal stem cells and articular chondrocytes in poly(L-lysine)-loaded hydrogels, J. Contr. Release 328 (2020) 710–721.
[141] J. Lam, E.C. Clark, E.L.S. Fong, E.J. Lee, S. Lu, Y. Tabata, A.G. Mikos, Evaluation of cell-laden polyelectrolyte hydrogels incorporating poly(L-Lysine) for applications in cartilage tissue engineering, Biomaterials 83 (2016) 332–346.
[142] D.P. Walsh, R.M. Raftery, I.M. Castano, R. Murphy, B. Cavanagh, A. Heise, F. J. O'Brien, S.A. Cryan, Transfection of autologous host cells *in vivo* using gene activated collagen scaffolds incorporating star-polypeptides, J. Contr. Release 304 (2019) 191–203.
[143] B.Z. Chen, L. Yu, Z.B. Li, C. Wu, Design of free triblock polylysine-b-polyleucine-b-polylysine chains for gene delivery, Biomacromolecules 19 (4) (2018) 1347–1357.
[144] J.Q. Lin, W.C. Hu, T. Gao, B.B. Bao, X.W. Li, T.L. Huang, Y. Sun, J.J. Shen, H. N. Xu, K.Y. Zhu, H.Y. Zhu, Y.L. Yang, X.Y. Zheng, ε-Poly-l-lysine as an efficient cartilage penetrating and residing drug carrier with high intraarticular injection safety for treating osteoarthritis, Chem. Eng. J. 430 (2022) 133018.
[145] R.S. Goomer, L.J. Deftos, R. Terkeltaub, T. Maris, M.C. Lee, F.L. Harwood, D. Amiel, High-efficiency non-viral transfection of primary chondrocytes and perichondrial cells for ex-vivo gene therapy to repair articular cartilage defects, Osteoarthritis Cartilage 9 (3) (2001) 248–256.
[146] B. Li, J.Z. Yang, L. Ma, F.F. Li, Z.Y. Tu, C.Y. Gao, Fabrication of poly(lactide-co-glycolide) scaffold filled with fibrin gel, mesenchymal stem cells, and poly (ethylene oxide)-b-poly(L-lysine)/TGF-β1 plasmid DNA complexes for cartilage restoration *in vivo*, J. Biomed. Mater. Res., Part A 101 (11) (2013) 3097–3108.
[147] C. Lesage, M. Lafont, P. Guihard, P. Weiss, J. Guicheux, V. Delplace, Material-assisted strategies for osteochondral defect repair, Adv. Sci. 9 (16) (2022) 2200050.
[148] M.L. Redondo, A.J. Beer, A.B. Yanke, Cartilage restoration: microfracture and osteochondral autograft transplantation, J. Knee Surg. 31 (3) (2018) 231–238.
[149] K. Gelse, H. Schneider, Ex vivo gene therapy approaches to cartilage repair, Adv. Drug Deliv. Rev. 58 (2) (2006) 259–284.
[150] W. Yu, B. Hu, K.O. Boakye-Yiadom, W.L. Ho, Q.J. Chen, X.Y. Xu, X.Q. Zhang, Injectable hydrogel mediated delivery of gene-engineered adipose-derived stem cells for enhanced osteoarthritis treatment, Biomater. Sci. 9 (22) (2021) 7603–7616.
[151] J.C. Babister, R.S. Tare, D.W. Green, S. Inglis, S. Mann, R.O.C. Oreffo, Genetic manipulation of human mesenchymal progenitors to promote chondrogenesis using "bead-in-bead" polysaccharide capsules, Biomaterials 29 (1) (2008) 58–65.
[152] S. Odabas, G.A. Feichtinger, P. Korkusuz, I. Inci, E. Bilgic, A.S. Yar, T. Cavusoglu, S. Menevse, I. Vargel, E. Piskin, Auricular cartilage repair using cryogel scaffolds loaded with BMP-7-expressing primary chondrocytes, J. Tissue Eng. Regener. Med. 7 (10) (2013) 831–840.
[153] M. Weissenberger, M.H. Weissenberger, M. Wagenbrenner, T. Heinz, J. Reboredo, B.M. Holzapfel, M. Rudert, J. Groll, C.H. Evans, A.F. Steinert, Different types of cartilage neotissue fabricated from collagen hydrogels and mesenchymal stromal cells via SOX9, TGFB1 or BMP2 gene transfer, PLoS One 15 (8) (2020) e0237479.
[154] Z.J. Pei, M.Y. Gao, J.H. Xing, C.B. Wang, P.Q. Zhao, H.T. Zhang, J. Qu, Experimental study on repair of cartilage defects in the rabbits with GelMA-MSCs scaffold prepared by three-dimensional bioprinting, Int. J. Bioprint. 9 (2) (2023) 662.
[155] H. Madry, J.K. Venkatesan, N. Carballo-Pedrares, A. Rey-Rico, M. Cucchiarini, Scaffold-mediated gene delivery for osteochondral repair, Pharmaceutics 12 (10) (2020) 930.
[156] T. Guo, J.N. Zhao, J.B. Chang, Z. Ding, H. Hong, J.N. Chen, J.F. Zhang, Porous chitosan-gelatin scaffold containing plasmid DNA encoding transforming growth factor-β1 for chondrocytes proliferation, Biomaterials 27 (7) (2006) 1095–1103.
[157] H. Lu, L. Lv, Y. Dai, G. Wu, H. Zhao, F. Zhang, Porous chitosan scaffolds with embedded hyaluronic acid/chitosan/plasmid-DNA nanoparticles encoding TGF-β1 induce DNA controlled release, transfected chondrocytes, and promoted cell proliferation, PLoS One 8 (7) (2013) e69950.
[158] H.J. Diao, J.L. Wang, C. Shen, S.H. Xia, T. Guo, L. Dong, C.Y. Zhang, J.N. Chen, J. N. Zhao, J.F. Zhang, Improved cartilage regeneration utilizing mesenchymal stem cells in TGF-β1 gene-activated scaffolds, Tissue Eng., Part A 15 (9) (2009) 2687–2698.
[159] C. Intini, L.B. Ferreras, S. Casey, J.E. Dixon, J.P. Gleeson, F.J. O'Brien, An innovative miR-activated scaffold for the delivery of a miR-221 inhibitor to enhance cartilage defect repair, Adv. Ther. 6 (7) (2023) 2200329.
[160] K.X. Zhang, S.F. Yan, G.F. Li, L. Cui, J.B. Yin, In-situ birth of MSCs multicellular spheroids in poly(L-glutamic acid)/chitosan scaffold for hyaline-like cartilage regeneration, Biomaterials 71 (2015) 24–34.
[161] K.X. Zhang, H.W. Fang, Y.C. Qin, L.L. Zhang, J.B. Yin, Functionalized scaffold for in situ efficient gene transfection of mesenchymal stem cells spheroids toward chondrogenesis, ACS Appl. Mater. Interfaces 10 (40) (2018) 33993–34004.
[162] R.M. Capito, M. Spector, Collagen scaffolds for nonviral IGF-1 gene delivery in articular cartilage tissue engineering, Gene Ther. 14 (9) (2007) 721–732.
[163] H.J. Kim, J.M. Park, S. Lee, S.J. Hong, J.I. Park, M.S. Lee, H.S. Yang, J.S. Park, K. H. Park, In situ pocket-type microcarrier (PMc) as a therapeutic composite: regeneration of cartilage with stem cells, genes, and drugs, J. Contr. Release 332 (2021) 337–345.
[164] X.J. Yang, Y.T. Wang, Y. Zhou, J.Y. Chen, Q.B. Wan, The application of polycaprolactone in three-dimensional printing scaffolds for bone tissue engineering, Polymers 13 (16) (2021) 2754.
[165] V. Chernonosova, M. Khlebnikova, V. Popova, E. Starostina, E. Kiseleva, B. Chelobanov, R. Kvon, E. Dmitrienko, P. Laktionov, Electrospun scaffolds enriched with nanoparticle-associated DNA: general properties, DNA release and cell transfection, Polymers 15 (15) (2023) 3202.
[166] J.K. Venkatesan, W.K. Meng, A. Rey-Rico, G. Schmitt, S. Speicher-Mentges, C. Falentin-Daudré, A. Leroux, H. Madry, V. Migonney, M. Cucchiarini, Enhanced chondrogenic differentiation activities in human bone marrow aspirates via sox9 overexpression mediated by pNaSS-grafted PCL film-guided rAAV gene transfer, Pharmaceutics 12 (3) (2020) 280.
[167] J.K. Venkatesan, X.Y. Cai, W.K. Meng, A. Rey-Rico, G. Schmitt, S. Speicher-Mentges, C. Falentin-Daudré, A. Leroux, H. Madry, V. Migonney, M. Cucchiarini, pNaSS-grafted PCL film-guided rAAV TGF-β gene therapy activates the chondrogenic activities in human bone marrow aspirates, Hum. Gene Ther. 32 (17–18) (2021) 895–906.
[168] V. Thomas, J. Mercuri, *In vitro* and *in vivo* efficacy of naturally derived scaffolds for cartilage repair and regeneration, Acta Biomater. 171 (2023) 1–18.
[169] B. Feng, T.J. Ji, X.G. Wang, W. Fu, L.C. Ye, H. Zhang, F. Li, Engineering cartilage tissue based on cartilage-derived extracellular matrix cECM/PCL hybrid nanofibrous scaffold, Mater. Des. 193 (2020) 108773.
[170] P.X. Li, L.W. Fu, Z.Y. Liao, Y. Peng, C. Ning, C.J. Gao, D.X. Zhang, X. Sui, Y.F. Lin, S.Y. Liu, C.X. Hao, Q.Y. Guo, Chitosan hydrogel/3D-printed poly(ε-caprolactone) hybrid scaffold containing synovial mesenchymal stem cells for cartilage regeneration based on tetrahedral framework nucleic acid recruitment, Biomaterials 278 (2021) 121131.
[171] W. Wang, B. Li, Y.L. Li, Y.Z. Jiang, H.W. Ouyang, C.Y. Gao, *In vivo* restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with

fibrin gel, bone marrow mesenchymal stem cells and DNA complexes, Biomaterials 31 (23) (2010) 5953–5965.
[172] B. Li, F.F. Li, L. Ma, J.Z. Yang, C.F. Wang, D.A. Wang, C.Y. Gao, Poly(lactide-co-glycolide)/fibrin gel construct as a 3D model to evaluate gene therapy of cartilage *in vivo*, Mol. Pharmaceutics 11 (7) (2014) 2062–2070.
[173] Y.F. Wang, C. Ling, J.L. Chen, H.Y. Liu, Q.Y. Mo, W. Zhang, Q.Q. Yao, 3D-printed composite scaffold with gradient structure and programmed biomolecule delivery to guide stem cell behavior for osteochondral regeneration, Biomater. Adv. 140 (2022) 213067.
[174] J.N. Chen, H.A. Chen, P. Li, H.J. Diao, S.Y. Zhu, L. Dong, R. Wang, T. Guo, J. N. Zhao, J.F. Zhang, Simultaneous regeneration of articular cartilage and subchondral bone *in vivo* using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds, Biomaterials 32 (21) (2011) 4793–4805.
[175] H. Zhang, S.L. Wu, W.K. Chen, Y. Hu, Z. Geng, J.C. Su, Bone/cartilage targeted hydrogel: strategies and applications, Bioact. Mater. 23 (2023) 156–169.
[176] H.Y. Yang, R.J. van Ee, K. Timmer, E.G.M. Craenmehr, J.H. Huang, F.C. Oner, W. J.A. Dhert, A.H.M. Kragten, N. Willems, G.C.M. Grinwis, M.A. Tryfonidou, N. E. Papen-Botterhuis, L.B. Creemers, A novel injectable thermoresponsive and cytocompatible gel of poly(N-isopropylacrylamide) with layered double hydroxides facilitates siRNA delivery into chondrocytes in 3D culture, Acta Biomater. 23 (2015) 214–228.
[177] J. Maihofer, H. Madry, A. Rey-Rico, J.K. Venkatesan, L. Goebel, G. Schmitt, S. Speicher-Mentges, X.Y. Cai, W.K. Meng, D. Zurakowski, M.D. Menger, M. W. Laschke, M. Cucchiarini, Hydrogel-guided, rAAV-mediated IGF-I overexpression enables long-term cartilage repair and protection against perifocal osteoarthritis in a large-animal full-thickness chondral defect model at one year *in vivo*, Adv. Mater. 33 (16) (2021) 2008451.
[178] N. Carballo-Pedrares, J. López-Seijas, D. Miranda-Balbuena, I. Lamas, J. Yáñez, A. Rey-Rico, Gene-activated hyaluronic acid-based cryogels for cartilage tissue engineering, J. Contr. Release 362 (2023) 606–619.
[179] H.H. Lee, A.M. Haleem, V. Yao, J. Li, X. Xiao, C.R. Chu, Release of bioactive adeno-associated virus from fibrin scaffolds: effects of fibrin glue concentrations, Tissue Eng., Part A 17 (15–16) (2011) 1969–1978.
[180] A. Lolli, K. Sivasubramaniyan, M.L. Vainieri, J. Oieni, N. Kops, A. Yayon, G. van Osch, Hydrogel-based delivery of antimiR-221 enhances cartilage regeneration by endogenous cells, J. Contr. Release 309 (2019) 220–230.
[181] J.J. Zhu, S.H. Yang, Y.D. Qi, Z. Gong, H.T. Zhang, K.Y. Liang, P.Y. Shen, Y. Y. Huang, Z. Zhang, W.L. Ye, L. Yue, S.W. Fan, S.Y. Shen, A.G. Mikos, X.M. Wang, X.Q. Fang, Stem cell-homing hydrogel-based miR-29b-5p delivery promotes cartilage regeneration by suppressing senescence in an osteoarthritis rat model, Sci. Adv. 8 (13) (2022) eabk0011.
[182] P. Wang, Z.B. Zhao, Z.Y. Li, X. Li, B.Z. Huang, X.Q. Lu, S.M. Dai, S.S. Li, Z.T. Man, W. Li, Attenuation of osteoarthritis progression via locoregional delivery of Klotho-expressing plasmid DNA and Tanshinon IIA through a stem cell-homing hydrogel, J. Nanobiotechnol. 22 (1) (2024) 325.
[183] S. Pathmanapan, A. Muthuramalingam, A.K. Pandurangan, N. Ayyadurai, S. K. Anandasadagopan, Hyaluronic acid-based cell-free composite scaffold promotes osteochondral repair in vitro by upregulating osteogenic- and chondrogenic-specific gene expressions, Adv. Eng. Mater. 26 (10) (2024) 2300815.
[184] Y.S. Dong, Y.F. Liu, Y.H. Chen, X. Sun, L. Zhang, Z.L. Zhang, Y.Y. Wang, C.X. Qi, S. F. Wang, Q. Yang, Spatiotemporal regulation of endogenous MSCs using a functional injectable hydrogel system for cartilage regeneration, NPG Asia Mater. 13 (1) (2021) 71.
[185] Y. Chen, W. Chen, Y. Ren, S. Li, M. Liu, J. Xing, Y. Han, Y. Chen, R. Tao, L. Guo, X. Sui, Q. Guo, S. Liu, Y. Han, Lipid nanoparticle-encapsulated VEGFa siRNA facilitates cartilage formation by suppressing angiogenesis, Int. J. Biol. Macromol. 221 (2022) 1313–1324.
[186] X. Wang, Y. Li, R. Han, C. He, G. Wang, J. Wang, J. Zheng, M. Pei, L. Wei, Demineralized bone matrix combined bone marrow mesenchymal stem cells, bone morphogenetic protein-2 and transforming growth factor-β3 gene promoted pig cartilage defect repair, PLoS One 9 (12) (2014) e116061.
[187] T. Gonzalez-Fernandez, E.G. Tierney, G.M. Cunniffe, F.J. O'Brien, D.J. Kelly, Gene delivery of TGF-β3 and BMP2 in an MSC-laden alginate hydrogel for articular cartilage and endochondral bone tissue engineering, Tissue Eng., Part A 22 (9–10) (2016) 776–787.
[188] A.M. Ledo, K.H. Vining, M.J. Alonso, M. Garcia-Fuentes, D.J. Mooney, Extracellular matrix mechanics regulate transfection and SOX9-directed differentiation of mesenchymal stem cells, Acta Biomater. 110 (2020) 153–163.
[189] A.M. Ledo, A. Senra, H. Rilo-Alvarez, E. Borrajo, A. Vidal, M.J. Alonso, M. Garcia-Fuentes, mRNA-activated matrices encoding transcription factors as primers of cell differentiation in tissue engineering, Biomaterials 247 (2020) 120016.
[190] H. Madry, L. Gao, A. Rey-Rico, J.K. Venkatesan, K. Müller-Brandt, X.Y. Cai, L. Goebel, G. Schmitt, S. Speicher-Mentges, D. Zurakowski, M.D. Menger, M. W. Laschke, M. Cucchiarini, Thermosensitive hydrogel based on PEO-PPO-PEO poloxamers for a controlled in situ release of recombinant adeno-associated viral vectors for effective gene therapy of cartilage defects, Adv. Mater. 32 (2) (2020) 1906508.
[191] S.J. He, X.J. Fu, L. Wang, Y.Y. Xue, L. Zhou, S.G. Qiao, J.Z. An, T.T. Xia, Self-assemble silk fibroin microcapsules for cartilage regeneration through gene delivery and immune regulation, Small 19 (40) (2023) 2302799.
[192] Y.Z. Liu, L.Q. Peng, L.L. Li, C.S. Huang, K.D. Shi, X.B. Meng, P.P. Wang, M.M. Wu, L. Li, H.J. Cao, K.F. Wu, Q.Q. Zeng, H.B. Pan, W.W. Lu, L. Qin, C.S. Ruan, X. L. Wang, 3D-bioprinted BMSC-laden biomimetic multiphasic scaffolds for efficient repair of osteochondral defects in an osteoarthritic rat model, Biomaterials 279 (2021) 121216.
[193] Y. Wu, P. Kennedy, N. Bonazza, Y. Yu, A. Dhawan, I. Ozbolat, Three-dimensional bioprinting of articular cartilage: a systematic review, Cartilage 12 (1) (2021) 76–92.
[194] D.G. O'Shea, C.M. Curtin, F.J. O'Brien, Articulation inspired by nature: a review of biomimetic and biologically active 3D printed scaffolds for cartilage tissue engineering, Biomater. Sci. 10 (10) (2022) 2462–2483.
[195] A.C. Daly, F.E. Freeman, T. Gonzalez-Fernandez, S.E. Critchley, J. Nulty, D. J. Kelly, 3D bioprinting for cartilage and osteochondral tissue engineering, Adv. Healthcare Mater. 6 (22) (2017) 1700298.
[196] P. Abdollahiyan, F. Oroojalian, A. Mokhtarzadeh, M. de la Guardia, Hydrogel-based 3D bioprinting for bone and cartilage tissue engineering, Biotechnol. J. 15 (12) (2020) 2000095.
[197] Q.J. Mei, J.D. Rao, H.P. Bei, Y.X. Liu, X. Zhao, 3D Bioprinting photo-crosslinkable hydrogels for bone and cartilage repair, Int. J. Bioprint. 7 (3) (2021) 367.
[198] L.K. Shopperly, J. Spinnen, J.P. Kruger, M. Endres, M. Sittinger, T. Lam, L. Kloke, T. Dehne, Blends of gelatin and hyaluronic acid stratified by stereolithographic bioprinting approximate cartilaginous matrix gradients, J. Biomed. Mater. Res. 110 (Part B) (2022) 2310–2322.
[199] H.T. Xia, D.D. Zhao, H.L. Zhu, Y.J. Hua, K.Y. Xiao, Y. Xu, Y.Q. Liu, W.M. Chen, Y. Liu, W.J. Zhang, W. Liu, S.J. Tang, Y.L. Cao, X.Y. Wang, H.H. Chen, G.D. Zhou, Lyophilized scaffolds fabricated from 3D-printed photocurable natural hydrogel for cartilage regeneration, ACS Appl. Mater. Interfaces 10 (37) (2018) 31704–31715.
[200] D.G. O'Shea, T. Hodgkinson, C.M. Curtin, F.J. O'Brien, An injectable and 3D printable pro-chondrogenic hyaluronic acid and collagen type II composite hydrogel for the repair of articular cartilage defects, Biofabrication 16 (1) (2024) 015007.
[201] C. Li, Z. Zheng, J. Jia, W. Zhang, L. Qin, W. Zhang, Y. Lai, Preparation and characterization of photocurable composite extracellular matrix-methacrylated hyaluronic acid bioink, J. Mater. Chem. B 10 (2022) 4242–4253.
[202] J.H. Galarraga, M.Y. Kwon, J.A. Burdick, 3D bioprinting via an *in situ* crosslinking technique towards engineering cartilage tissue, Sci. Rep. 9 (2019) 19987.
[203] J. Hauptstein, T. Boeck, M. Bartolf-Kopp, L. Forster, P. Stahlhut, A. Nadernezhad, G. Blahetek, A. Zernecke-Madsen, R. Detsch, T. Jungst, J. Groll, J. Tessmar, T. Blunk, Hyaluronic acid-based bioink composition enabling 3D bioprinting and improving quality of deposited cartilaginous extracellular matrix, Adv. Healthcare Mater. 9 (15) (2020) 2000737.
[204] J. Hauptstein, L. Forster, A. Nadernezhad, H. Horder, P. Stahlhut, J. Groll, T. Blunk, J. Tessmar, Bioink platform utilizing dual-stage crosslinking of hyaluronic acid tailored for chondrogenic differentiation of mesenchymal stromal cells, Macromol. Biosci. 22 (2) (2022) 2100331.
[205] J.E. Barthold, K.P. McCreery, J. Martinez, C. Bellerjeau, Y.F. Ding, S.J. Bryant, G. L. Whiting, C.P. Neu, Particulate ECM biomaterial ink is 3D printed and naturally crosslinked to form structurally-layered and lubricated cartilage tissue mimics, Biofabrication 14 (2) (2022) 025021.
[206] K. Flégeau, A. Puiggali-Jou, M. Zenobi-Wong, Cartilage tissue engineering by extrusion bioprinting utilizing porous hyaluronic acid microgel bioinks, Biofabrication 14 (3) (2022) 034105.
[207] K. Hölzl, M. Fürsatz, H. Göcerler, B. Schadl, S. Zigon-Branc, M. Markovic, C. Gahleitner, J. Van Hoorick, S. Van Vlierberghe, A. Kleiner, S. Baudis, A. Pauschitz, H. Redl, A. Ovsianikov, S. Nürnberger, Gelatin methacryloyl as environment for chondrocytes and cell delivery to superficial cartilage defects, J. Tissue Eng. Regener. Med. 16 (2) (2022) 207–222.
[208] N. Rajabi, A. Rezaei, M. Kharaziha, H.R. Bakhsheshi-Rad, H.R. Luo, S. RamaKrishna, F. Berto, Recent advances on bioprinted gelatin methacrylate-based hydrogels for tissue repair, Tissue Eng., Part A 27 (11–12) (2021) 679–702.
[209] Y.Y. Cao, P. Cheng, S.B. Sang, C. Xiang, Y. An, X.C. Wei, Y.Y. Yan, P.C. Li, 3D printed PCL/GelMA biphasic scaffold boosts cartilage regeneration using co-culture of mesenchymal stem cells and chondrocytes: in vivo study, Mater. Des. 210 (2021) 110065.
[210] A.C. Daly, S.E. Critchley, E.M. Rencsok, D.J. Kelly, A comparison of different bioinks for 3D bioprinting of fibrocartilage and hyaline cartilage, Biofabrication 8 (4) (2016) 045002.
[211] K. Martyniak, A. Lokshina, M.A. Cruz, M. Karimzadeh, R. Kemp, T.J. Kean, Biomaterial composition and stiffness as decisive properties of 3D bioprinted constructs for type II collagen stimulation, Acta Biomater. 152 (2022) 221–234.
[212] S. Sang, X. Mao, Y. Cao, Z. Liu, Z. Shen, M. Li, W. Jia, Z. Guo, Z. Wang, C. Xiang, L. Sun, 3D bioprinting using synovium-derived MSC-laden photo-cross-linked ECM bioink for cartilage regeneration, ACS Appl. Mater. Interfaces 15 (2023) 8895–8913.
[213] G.H. Hu, Z. Liang, Z.L. Fan, M.Y. Yu, Q.Q. Pan, Y. Nan, W. Zhang, L. Wang, X. S. Wang, Y.J. Hua, G.D. Zhou, W.J. Ren, Construction of 3D-Bioprinted cartilage-mimicking substitute based on photo-crosslinkable Wharton's jelly bioinks for full-thickness articular cartilage defect repair, Mater. Today Bio 21 (2023) 100695.
[214] Y. Liu, L. Du, H. Zhang, G. Li, Y. Luo, Z. Hu, R. Xu, J. Yao, Z. Shi, Y. Chen, C. Zhang, Z. Jin, C. Zhang, C. Xie, J. Fu, Y. Zhu, Y. Zhu, Bioprinted biomimetic hydrogel matrices guiding stem cell aggregates for enhanced chondrogenesis and cartilage regeneration, J. Mater. Chem. B 12 (2024) 5360–5376.
[215] L.T. Jia, Y.J. Hua, J.S. Zeng, W.S. Liu, D. Wang, G.D. Zhou, X. Liu, H.Y. Jiang, Bioprinting and regeneration of auricular cartilage using a bioactive bioink based on microporous photocrosslinkable acellular cartilage matrix, Bioact. Mater. 16 (2022) 66–81.

[216] J. Chakraborty, J. Fernández-Pérez, K.A. van Kampen, S. Roy, T. ten Brink, C. Mota, S. Ghosh, L. Moroni, Development of a biomimetic arch-like 3D bioprinted construct for cartilage regeneration using gelatin methacryloyl and silk fibroin-gelatin bioinks, Biofabrication 15 (3) (2023) 035009.
[217] S. Chameettachal, S. Midha, S. Ghosh, Regulation of chondrogenesis and hypertrophy in silk fibroin-gelatin-based 3D bioprinted constructs, ACS Biomater. Sci. Eng. 2 (9) (2016) 1450–1463.
[218] D.V. Krishna, M.R. Sankar, Extrusion based bioprinting of alginate based multicomponent hydrogels for tissue regeneration applications: state of the art, Mater. Today Commun. 35 (2023) 105696.
[219] A. Jahani, M.S. Nourbakhsh, M.H. Ebrahimzadeh, M. Mohammadi, D. Yari, A. Moradi, Biomolecules-loading of 3D-printed alginate-based scaffolds for cartilage tissue engineering applications: a review on current status and future prospective, Arch. Bone Jt. Surg. 12 (2) (2024) 92–101.
[220] I. Gorronogoitia, U. Urtaza, A. Zubiarrain-Laserna, A. Alonso-Varona, A. M. Zaldua, A study of the printability of alginate-based bioinks by 3D bioprinting for articular cartilage tissue engineering, Polymers 14 (2) (2022) 354.
[221] X.C. Yang, Z.H. Lu, H.Y. Wu, W. Li, L. Zheng, J.M. Zhao, Collagen-alginate as bioink for three-dimensional (3D) cell printing based cartilage tissue engineering, Mater. Sci. Eng., C 83 (2018) 195–201.
[222] D. Kilian, T. Ahlfeld, A.R. Akkineni, A. Bernhardt, M. Gelinsky, A. Lode, 3D Bioprinting of osteochondral tissue substitutes-*in vitro*-chondrogenesis in multi-layered mineralized constructs, Sci. Rep. 10 (1) (2020) 8277.
[223] J. Chakraborty, N. Majumder, A. Sharma, S. Prasad, S. Ghosh, 3D bioprinted silk-reinforced alginate-gellan gum constructs for cartilage regeneration, Bioprinting 28 (2022) e00232.
[224] M.C. Decarli, A. Seijas-Gamardo, F.L.C. Morgan, P. Wieringa, M.B. Baker, J.V. L. Silva, A.M. Moraes, L. Moroni, C. Mota, Bioprinting of stem cell spheroids followed by post-printing chondrogenic differentiation for cartilage tissue engineering, Adv. Healthcare Mater. 12 (19) (2023) 2203021.
[225] A.H. Aitchison, N.B. Allen, I.R. Shaffrey, C.N. O'Neill, B. Abar, A.T. Anastasio, S. B. Adams, Fabrication of a novel 3D extrusion bioink containing processed human articular cartilage matrix for cartilage tissue engineering, Bioengineering 11 (4) (2024) 329.
[226] C. Antich, J. de Vicente, G. Jiménez, C. Chocarro, E. Carrillo, E. Montañez, P. Gálvez-Martín, J.A. Marchal, Bio-inspired hydrogel composed of hyaluronic acid and alginate as a potential bioink for 3D bioprinting of articular cartilage engineering constructs, Acta Biomater. 106 (2020) 114–123.
[227] Y. Zhou, R. Qin, T. Chen, K.B. Zhang, J.C. Gui, 3D bioprinting modified autologous matrix-induced chondrogenesis (AMIC) technique for repair of cartilage defects, Mater. Des. 203 (2021) 109621.
[228] T. Kreller, T. Distler, S. Heid, S. Gerth, R. Detsch, A.R. Boccaccini, Physico-chemical modification of gelatine for the improvement of 3D printability of oxidized alginate-gelatine hydrogels towards cartilage tissue engineering, Mater. Des. 208 (2021) 109877.
[229] Y. Chu, L. Huang, W.P. Hao, T.T. Zhao, H.T. Zhao, W. Yang, X. Xie, L. Qian, Y. Y. Chen, J.W. Dai, Long-term stability, high strength, and 3D printable alginate hydrogel for cartilage tissue engineering application, Biomed. Mater. 16 (6) (2021) 064102.
[230] E.V. Isaeva, E.E. Beketov, V.V. Yuzhakov, N.V. Arguchinskaya, A.A. Kisel, E. P. Malakhov, T.S. Lagoda, N.D. Yakovleva, P.V. Shegai, S.A. Ivanov, A.D. Kaprin, The use of collagen with high concentration in cartilage tissue engineering by means of 3D-bioprinting, Cell Tissue Biol 15 (5) (2021) 493–502.
[231] C. Wang, H.B. Yue, W. Huang, X.D. Lin, X.Q. Xie, Z. He, X. He, S.B. Liu, L. Bai, B. H. Lu, Y. Wei, M. Wang, Cryogenic 3D printing of heterogeneous scaffolds with gradient mechanical strengths and spatial delivery of osteogenic peptide/TGF-β1 for osteochondral tissue regeneration, Biofabrication 12 (2) (2020) 025030.
[232] C. Intini, M. Lemoine, T. Hodgkinson, S. Casey, J.P. Gleeson, F.J. O'Brien, A highly porous type II collagen containing scaffold for the treatment of cartilage defects enhances MSC chondrogenesis and early cartilaginous matrix deposition, Biomater. Sci. 10 (4) (2022) 970–983.
[233] K. Yang, J. Sun, D. Wei, L. Yuan, J.R. Yang, L.K. Guo, H.S. Fan, X.D. Zhang, Photo-crosslinked mono-component type II collagen hydrogel as a matrix to induce chondrogenic differentiation of bone marrow mesenchymal stem cells, J. Mater. Chem. B 5 (44) (2017) 8707–8718.
[234] K. Behan, A. Dufour, O. Garcia, D. Kelly, Methacrylated cartilage ECM-based hydrogels as injectables and bioinks for cartilage tissue engineering, Biomolecules 12 (2) (2022) 216.
[235] W.L. Shi, M.Y. Sun, X.Q. Hu, B. Ren, J. Cheng, C.X. Li, X.N. Duan, X. Fu, J. Y. Zhang, H.F. Chen, Y.F. Ao, Structurally and functionally optimized silk-fibroin-gelatin scaffold using 3D printing to repair cartilage injury *in vitro* and *in vivo*, Adv. Mater. 29 (29) (2017) 1701089.
[236] X. Zhang, Y. Liu, C.Y. Luo, C.J. Zhai, Z.X. Li, Y. Zhang, T. Yuan, S.L. Dong, J. Y. Zhang, W.M. Fan, Crosslinker-free silk/decellularized extracellular matrix porous bioink for 3D bioprinting-based cartilage tissue engineering, Mater. Sci. Eng., C 118 (2021) 111388.
[237] T.Y. Ni, M. Liu, Y.J. Zhang, Y. Cao, R.J. Pei, 3D Bioprinting of bone marrow mesenchymal stem cell-laden silk fibroin double network scaffolds for cartilage tissue repair, Bioconjugate Chem. 31 (8) (2020) 1938–1947.
[238] S.H. Kim, Y.K. Yeon, J.M. Lee, J.R. Chao, Y.J. Lee, Y.B. Seo, M.T. Sultan, O.J. Lee, J.S. Lee, S.I. Yoon, I.S. Hong, G. Khang, S.J. Lee, J.J. Yoo, C.H. Park, Precisely printable and biocompatible silk fibroin bioink for digital light processing 3D printing, Nat. Commun. 9 (2018) 1620.
[239] A. Bandyopadhyay, B.B. Mandal, N. Bhardwaj, 3D bioprinting of photo-crosslinkable silk methacrylate (SilMA)-polyethylene glycol diacrylate (PEGDA) bioink for cartilage tissue engineering, J. Biomed. Mater. Res., Part A 110 (4) (2022) 884–898.
[240] S. Ravi, L.P.P. Chokkakula, P.S. Giri, G. Korra, S.R. Dey, S.N. Rath, 3D Bioprintable hypoxia-mimicking PEG-based nano bioink for cartilage tissue engineering, ACS Appl. Mater. Interfaces 15 (16) (2023) 19921–19936.
[241] Y. He, Z. Zhou, Y. Huang, W. Zhu, N. He, X. Zhu, X. Han, H. Liu, An antibacterial ε-poly-L-lysine-derived bioink for 3D bioprinting applications, J. Mater. Chem. B 10 (40) (2022) 8274–8281.
[242] Y. Huang, X. Meng, Z. Zhou, W. Zhu, X. Chen, Y. He, N. He, X. Han, D. Zhou, X. Duan, P.M. Vadgama, H. Liu, A naringin-derived bioink enhances the shape fidelity of 3D bioprinting and efficiency of cartilage defect repair, J. Mater. Chem. B 10 (36) (2022) 7030–7044.
[243] P. Shende, R. Trivedi, 3D printed bioconstructs: regenerative modulation for genetic expression, Stem Cell Rev. Rep. 17 (4) (2021) 1239–1250.
[244] Y. Xiang, Z. Zhong, E.J. Yao, W. Kiratitanaporn, M.T. Suy, S. Chen, 3D bioprinting of gene delivery scaffolds with controlled release, Bioprinting 31 (2023) e00270.
[245] M.A. Khvorostina, A.V. Mironov, I.A. Nedorubova, T.B. Bukharova, A.V. Vasilyev, D.V. Goldshtein, V.S. Komlev, V.K. Popov, 3D printed gene-activated sodium alginate hydrogel scaffolds, Gels 8 (7) (2022) 421.
[246] T. Gonzalez-Fernandez, S. Rathan, C. Hobbs, P. Pitacco, F.E. Freeman, G. M. Cunniffe, N.J. Dunne, H.O. McCarthy, V. Nicolosi, F.J. O'Brien, D.J. Kelly, Pore-forming bioinks to enable spatio-temporally defined gene delivery in bioprinted tissues, J. Contr. Release 301 (2019) 13–27.
[247] F.J. O'Brien, D.J. Kelly, J.E. Dixon, C. Intini, T. Hodgkinson, M. Joyce, Gene activated reinforced scaffolds for SOX9 delivery to enhance repair of large load bearing articular cartilage defects, Eur. Cell. Mater. 47 (2024) 91–108.
[248] Z. Yang, F. Cao, H. Li, S. He, T. Zhao, H. Deng, J. Li, Z. Sun, C. Hao, J. Xu, Q. Guo, S. Liu, W. Guo, Microenvironmentally optimized 3D-printed TGFβ-functionalized scaffolds facilitate endogenous cartilage regeneration in sheep, Acta Biomater. 150 (2022) 181–198.
[249] Y. Sun, Q. Wu, Y.X. Zhang, K.R. Dai, Y.Z. Wei, 3D-bioprinted gradient-structured scaffold generates anisotropic cartilage with vascularization by pore-size-dependent activation of HIF1α/FAK signaling axis, Nanomed. Nanotechnol. Biol. Med. 37 (2021) 102426.
[250] Y. Sun, Y. You, W. Jiang, B. Wang, Q. Wu, K. Dai, 3D bioprinting dual-factor releasing and gradient-structured constructs ready to implant for anisotropic cartilage regeneration, Sci. Adv. 6 (37) (2020) eaay1422.
[251] Z. Yang, T. Zhao, C. Gao, F. Cao, H. Li, Z. Liao, L. Fu, P. Li, W. Chen, Z. Sun, S. Jiang, Z. Tian, G. Tian, K. Zha, T. Pan, X. Li, X. Sui, Z. Yuan, S. Liu, Q. Guo, 3D-bioprinted difunctional scaffold for *in situ* cartilage regeneration based on aptamer-directed cell recruitment and growth factor-enhanced cell chondrogenesis, ACS Appl. Mater. Interfaces 13 (20) (2021) 23369–23383.
[252] J. Hauptstein, L. Forster, A. Nadernezhad, J. Groll, J. Tessmar, T. Blunk, Tethered TGF-β1 in a hyaluronic acid-based bioink for bioprinting cartilaginous tissues, Int. J. Mol. Sci. 23 (2) (2022) 924.
[253] I.Y. Bozo, R.V. Deev, I.V. Smirnov, A. Yu Fedotov, V.K. Popov, A.V. Mironov, O. A. Mironova, A. Yu Gerasimenko, V.S. Komlev, 3D printed gene-activated octacalcium phosphate implants for large bone defects engineering, Int. J. Bioprint. 6 (3) (2020) 275.
[254] T. Pan, W. Song, H. Xin, H. Yu, H. Wang, D. Ma, X. Cao, Y. Wang, MicroRNA-activated hydrogel scaffold generated by 3D printing accelerates bone regeneration, Bioact. Mater. 10 (2022) 1–14.
[255] V. Picanco-Castro, C.G. Pereira, D.T. Covas, G.S. Porto, A. Athanassiadou, M. L. Figueiredo, Emerging patent landscape for non-viral vectors used for gene therapy, Nat. Biotechnol. 38 (2) (2020) 151–157.
[256] X. Niu, N. Li, Z. Du, X. Li, Integrated gradient tissue-engineered osteochondral scaffolds: challenges, current efforts and future perspectives, Bioact. Mater. 20 (2023) 574–597.
[257] H.F. Li, J. Wang, G.M. Yang, X. Pei, X. Zhang, Advances of mussel-inspired hydrogels for Bone/Cartilage regeneration, Chem. Eng. J. 487 (2024) 150560.
[258] M. Ansari, A. Darvishi, A. Sabzevari, A review of advanced hydrogels for cartilage tissue engineering, Front. Bioeng. Biotechnol. 12 (2024) 1340893.
[259] X.R. Wu, X.L. Cheng, M.Y. Kang, R.P. Dong, J.W. Zhao, Y. Qu, Natural polysaccharide-based hydrogel bioprinting for articular cartilage repair, Front. Mater. 10 (2023) 1204318.