Non-viral gene delivery systems for osteoarthritis therapy

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ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease marked by periarticular bony overgrowth and the degradation of articular cartilage, leading to severe pain, impaired joint function, and reduced quality of life for those affected. Current OA treatments, including pharmacotherapy, physical therapy, and joint replacement surgery, often provide limited therapeutic benefits and are associated with various side effects. As a result, there is a pressing need for alternative treatment options. Gene therapy has emerged as a promising approach for achieving longer-lasting benefits by repairing or modulating the molecular and cellular mechanisms within the joint. Specifically, gene therapy for OA involves either suppressing the expression of detrimental genes or enhancing the expression of therapeutic genes. The success of these approaches, however, significantly depends on the safe and efficient delivery platforms used. Given the risks of insertional mutations and high production costs associated with viral vectors, considerable efforts have been made to develop non-viral systems as safer and more cost-effective alternatives for gene delivery. Over the past few decades, a variety of innovative non-viral vectors with integrated functions have been proposed, successfully overcoming the challenges of gene delivery. The substantial progress made in the rational design of these vectors, along with their enhanced performance in OA gene therapy, warrants a comprehensive and timely review. This article aims to summarize these advancements, starting with a discussion of representative therapeutic gene targets for OA treatment. We then review the innovative non-viral vectors used in OA gene therapy, including lipids, extracellular vesicles, natural and synthetic polymers, inorganic nanoparticles, and protein/peptide carriers. Finally, we address key aspects that need further optimization to facilitate the design of non-viral vectors and promote their therapeutic application in OA treatment.

Keywords:
Osteoarthritis; Gene therapy; Delivery systems; Non-viral vector; Gene target

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1. Introduction

Osteoarthritis (OA) is a degenerative joint disease involving periarticular bony overgrowth and damage to articular cartilage, which is associated with severe pain, loss of joint function, and reduced quality of life for patients.[1,2] OA is caused by cumulative wear and tear of joint surfaces or genetic predisposition and affects approximately 250 million people, who suffer from pain and disability.[3,4] In its pathogenesis, the degradation of cartilage components triggers a foreign body reaction by synovial cells, inducing synovitis. This, in turn, leads to the production of inflammatory factors and metalloproteinases, as well as synovial angiogenesis, which exacerbates cartilage destruction.[5-7] Thus, cartilage destruction remains the primary hallmark of OA.[8]

At present, treatments for OA include mainly pharmacologic and non-pharmacologic therapies.[9] For pharmacological treatment, small-molecule corticosteroids and oral non-steroidal anti-inflammatory drugs, such as ibuprofen and diclofenac, are widely used to relieve pain and control inflammation in patients with OA.[10] While effective for patients with

moderate-to-severe OA, pharmacologic treatment often provides limited relief of pain and fails to protect cartilage.[4] Moreover, the long-term use of these medications poses significant risks to the gastrointestinal tract and cardiovascular system.[11] Non-pharmacologic treatments mainly involve surgical interventions, such as arthroplasty, which are suitable for patients in the advanced stages of OA.[12] Although surgical treatment can significantly improve the motor function of patients with OA, it is associated with risks, including post-operative bleeding, infection, and surgical trauma.[13] Given the limitations of the established treatments, developing alternative therapeutic approaches for OA treatment is highly desirable.

In this context, gene therapy has emerged as an innovative therapeutic approach for the treatment of OA, offering considerable promise for clinical translation.[14,15] Gene therapy involves the introduction of specific RNA or DNA into targeted cells to regulate gene and protein expression, thereby preventing or treating various diseases.[16-18] Specifically, gene therapy for OA involves either downregulating the expression of inflammatory biomolecules using small interfering RNA (siRNA) or enhancing the expression of anti-inflammatory proteins through DNA or messenger RNA (mRNA).[19] The multiple regulatory pathways and abundant targets make gene therapy highly advantageous, offering high specificity, excellent potency, and low toxicity, thereby demonstrating its significant therapeutic potential in improving treatment outcomes for OA.[20] With continued advancements and refinements in related technologies, gene therapy is poised to play a pivotal role in the future management of OA, offering transformative therapeutic solutions. However, naked nucleic acids are inherently fragile macromolecules with a negative charge. Their direct administration leads to degradation by nucleases in serum, immune responses, and poor cellular uptake.[21,22] Therefore, the development of safe and efficient delivery systems is crucial to enhance the bioavailability and therapeutic efficacy of nucleic acids.[23]

To date, a variety of gene delivery vectors have been developed, which can be categorized into viral and non-viral carriers. While viral vectors demonstrate superior delivery efficiency, their high production costs, challenges in large-scale manufacturing, and potential for bio-toxicity limit their widespread clinical application. As a result, significant efforts have been made to develop non-viral systems as safer, more cost-effective alternatives for gene delivery. These non-viral vectors offer several advantages, including ease of preparation and modification, low immunogenicity, and favorable biocompatibility. They encompass a wide range of types, including lipids, exosomes, natural and synthetic polymers, inorganic nanoparticles, and protein or peptide carriers, each designed with integrated functions that enable precise gene delivery and efficient transfection, thereby enhancing OA treatment.

Gene therapy for OA focuses on overexpressing therapeutic genes or downregulating the expression of harmful genes facilitated by delivery vectors. This review begins with a brief discussion of representative therapeutic gene targets for OA therapy, including transcription factors, growth factors, and inflammation-related cytokines. The review then highlights non-viral vectors designed for the delivery of these therapeutic genes, such as lipids, extracellular vesicles (EVs), natural and synthetic polymers, inorganic nanoparticles, and protein and peptide carriers, along with recent progress and research achievements in this area. Finally, the review proposes key aspects that need further optimization to facilitate the design of non-viral vectors and promote their therapeutic applications in OA treatment.

We performed a search in the Web of Science database using keywords such as "osteoarthritis," "gene therapy," "delivery systems," "non-viral vectors," and "gene targets," focusing on articles published between 2017 and 2024. During the selection process, we prioritized more recent publications or those from highly regarded journals that addressed similar topics.

2. Targets of gene therapy for OA

The success of gene therapy in OA depends on selecting the appropriate targets to intervene in or repair the pathological processes underlying the disease. Research indicates that targeting transcription factors, growth factors, and inflammation-related cytokines hold considerable promise for OA gene therapy. For example, OA gene therapy may involve upregulating beneficial targets such as transcription factors such as SRY-related HMG-box (Sox) 9, transforming growth factor β (TGF-β), and the anti-inflammatory cytokine interleukin (IL)-4. Conversely, it may also involve downregulating harmful targets such as hypoxia-inducible factor (HIF)-1α, tumor necrosis factor (TNF)-α, and IL-1β. This section provides a brief overview of the key targets proposed for OA gene therapy, as outlined in **Figure 1** and **Table 1**.

2.1. Upregulated targets

Transcription factors implicated in the progression of OA primarily include runt-related transcription factors (Runx1, Runx2, Runx3), the sex-determining region Y-containing cassette family, and HIF-1α, HIF-2α.[24] Upregulating Runx1 expression can help mitigate the progression of OA in mice, including the formation of bone regrowth and the prevention of cartilage destruction.[25] However, Runx2, a transcription factor essential for normal chondrocyte maturation and bone formation, induces the expression of catabolic factors in the cartilage extracellular matrix. Runx2 is upregulated in both human osteoarthritic cartilage and mouse articular cartilage following joint injury.[26] Runx3 functions similarly to Runx2 in regulating chondrocyte hypertrophy, although its effect is

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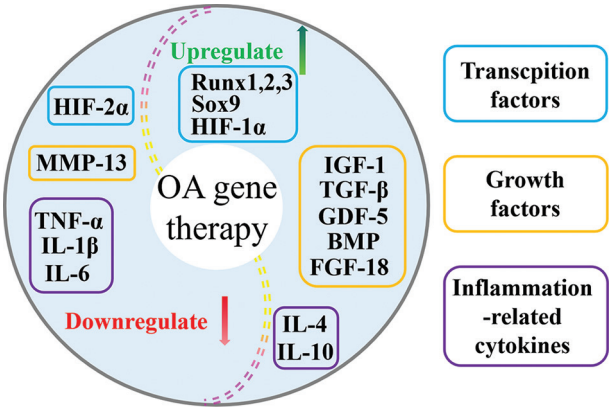

Figure 1. Schematic representation of targets in OA gene therapy, including transcription factors, growth factors, and inflammation-related cytokines. Figure created by the authors.
Abbreviations: BMP: Bone morphogenetic proteins; FGF-18: Fibroblast growth factor 18; GDF-5: Growth/differentiation factor 5; HIF: Hypoxia-inducible factor; IGF-1: Insulin-like growth factor 1; IL: Interleukin; MMP-13: Matrix metalloproteinase 13; OA: Osteoarthritis; Runx: Runt-related transcription factors; Sox9: SRY-related HMG-box 9; TGF-β: Transforming growth factor β; TNF-α: Tumor necrosis factor.

less pronounced than that of Runx2 (**Figure 2A**).[27] Sox9, an essential transcription factor for cartilage formation, promotes type 2 collagen expression. The delivery of the Sox9 gene has consistently promoted the repair of chondrocyte injury in sheep bone and mitigated the progression of OA in neighboring joints.[28] HIF-1α protects articular cartilage by enhancing chondrocyte differentiation, preserving chondrocyte viability, and facilitating metabolic adaptations to hypoxic conditions. Moreover, HIF-1α can promote chondrogenesis by upregulating Sox9 expression.[29]

Insulin-like growth factor (IGF)-1, a key growth factor in cartilage formation and maintenance, stimulates chondrocyte proliferation and extracellular matrix synthesis while inhibiting chondrocyte apoptosis and matrix degradation (**Figure 2B**). Under OA conditions, chondrocytes produce excessive amounts of IGF receptor-binding proteins, and IGF-1 is also degraded at an accelerated rate in an inflammatory environment. Therefore, in most cases, direct intra-articular injection of IGF-1 may not have the desired effect.[30] However, implanting IGF-1-overexpressing chondrocytes at the injury site can enhance type 2 collagen production and promote articular cartilage repair.[31] TGF-β is another growth factor involved in cartilage biology and OA. The TGF-β superfamily consists of nearly 40 ligands, with key members of this superfamily in cartilage including TGF-β, bone morphogenetic protein (BMP), and growth/differentiation factor-5 (GDF-5). These growth factors are essential for normal joint development and homeostasis *in vivo* and have been linked to the pathogenesis of OA.[32,33] TGF-β benefits cartilage by stimulating chondrocytes and promoting the production of type 2 collagen and proteoglycans. In addition, TGF-β inhibits catabolism induced by TNF-α and IL-1β.[34] BMP is considered to have a protective role in articular cartilage; however, it has also been implicated in chondrocyte hypertrophy and extracellular matrix degradation. Steinert *et al.*[35] introduced the BMP gene into human bone marrow mesenchymal stem cells (MSCs) and observed an upregulation of several markers associated with cartilage hypertrophy and maturation. These findings indicate that BMP should be cautiously considered a target for gene therapy in OA.[36] Meanwhile, GDF-5 stimulates the expression of Sox9 and aggrecan in human chondrocytes, promoting anabolic processes. Fibroblast growth factor 18, a member of the fibroblast growth factor family, plays a regulatory role in cartilage and acts as an anabolic factor, promoting the formation and repair of articular cartilage.[37,38]

Gene therapy aimed at upregulating anti-inflammatory factors presents a promising strategy for OA treatment.[39] IL-4 and IL-10 (**Figure 2C**) are key anti-inflammatory factors in OA, exerting significant protective effects on cartilage and reducing chondrocyte apoptosis.[40] For example, spherical MSCs expressing the IL-4 gene attenuated pain and provided durable protection of cartilage in rat OA.[41] Intra-articular administration of IL-10 resulted in prolonged local IL-10 expression and a significant reduction in the levels of IL-1β and IL-6.[42]

With growing research on microRNAs (miRNAs), long non-coding RNAs (ncRNAs), and circular RNAs in joints and body fluids, ncRNAs have been found to play crucial regulatory roles in the pathogenesis of OA.[43] ncRNAs regulate various physiological processes, including inflammation, aging, oxidative stress, cartilage differentiation, autophagy, and methylation, as well as key mediators (fibroblast growth factor 18, Sox9, Sox5, Hedgehog) and signaling molecules (TGF-β, nuclear factor kappa-light-chain-enhancer of activated B cells [NF-κB], Wnt-β-catenin). These ncRNAs influence the inflammatory response, extracellular matrix production, and cell death either directly or indirectly in the pathogenesis of OA.[44] Several ncRNAs can suppress TNF-α and IL-1β-induced inflammatory responses in chondrocytes by modulating both upstream and downstream components of the NF-κB pathway or related molecules.[45,46] For example, Zhu *et al.*[47] developed an *in situ* imine crosslinked nanocomplexes (PAR) consisting of polyethyleneimine (PEI) and aldehyde-modified hyaluronic acid (HA), which efficiently loaded and delivered the small ncRNA (microRNA [miR]-140) to chondrocytes, effectively attenuating OA progression in mice (**Figure 2D**).

2.2. Downregulated targets

In contrast to HIF-1α, HIF-2α, as a transcription factor, directly promotes chondrocyte catabolism, induces chondrocyte apoptosis, and regulates autophagy in mature chondrocytes.[48] Therefore, downregulating the expression of HIF-2α is favored as a treatment for OA. In addition, GDF-5 inhibits the expression of matrix metalloproteinase 13, an extracellular matrix-degrading enzyme, which is beneficial for OA gene therapy.[49]

Moreover, dysregulation of cytokine homeostasis is a major contributor to the pathogenesis of OA. This occurs primarily because OA promotes the production of pro-inflammatory

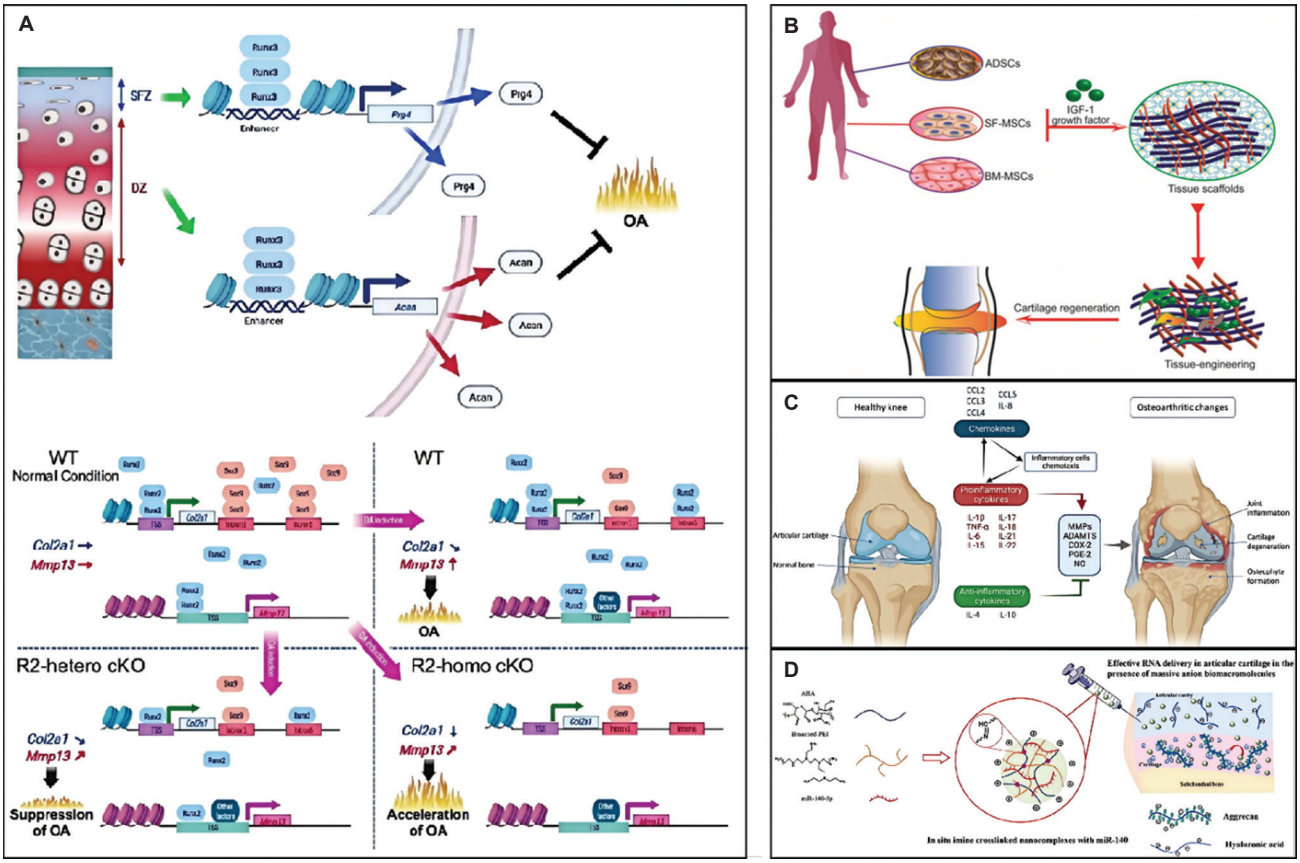

Figure 2. Schematic illustration of OA gene therapy through various targets. (A) Schematic diagrams representing molecular pathways through which Runx3 and Runx2 regulate articular cartilage during OA development. Reprinted with permission from Nagata *et al.*[27] Copyright 2022 Springer Nature. (B) Tissue engineering-based IGF-1 delivery involves seeding mesenchymal stem cells, such as ADSCs, SF-MSCs, and BM-MSCs, onto scaffolds. The introduction of IGF-1 stimulates chondrogenic differentiation of mesenchymal stem cells, enhancing cartilage tissue formation, glycosaminoglycan accumulation in the cartilage matrix, and type II collagen production, thereby supporting cartilage regeneration and repair of cartilage defects. Reprinted with permission from Wen *et al.*[30] Copyright 2021 Springer Nature. (C) Schematic representation of key inflammatory processes and factors in OA pathogenesis. Reprinted from Molnar *et al.*[50] Copyright 2021 Authors. (D) Schematic illustration of imine crosslinked nanocomplexes of RNA delivery to cartilage in the presence of ubiquitous anion biomacromolecules such as HA and CS. Reprinted with permission from Zhu *et al.*[47] Copyright 2021 Elsevier.

Abbreviations: Acan: Aggrecan; ADAMTS: A disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif; ADSCs: Adipose-derived mesenchymal stem cells; AHA: Aldehyde-modified hyaluronic acid; BM-MSCs: Bone marrow-derived mesenchymal stem cells; CCL: Chemokine (C-C motif) ligand; Col2a1: Type II collagen; COX-2: Cyclooxygenase-2; CS: Chitosan; DZ: Deeper zone; GAG: Glycosaminoglycans; HA: Hyaluronic acid; IGF-1: Insulin-like growth factor 1; IL: Interleukin; MMP: Matrix metalloproteinase; MSCs: Mesenchymal stem cells; NO: Nitric oxide; OA: Osteoarthritis; PGE2: Prostaglandin E2; Prg4: Proteoglycan 4; RNA: Ribonucleic acid; Runx: Runt-related transcription factors 3; R2-Hetero cKO: *Col2a1-Cre*^{ERT2}; *Runx2*$^{fl/+}$; SFZ: Superficial zone; SF-MSCs: Synovial fluid mesenchymal stem cells; Sox9: SRY-related HMG-box 9; TNF-α: tumor necrosis factor; TSS: Transcriptional start sites.

cytokines that degrade cartilage and other intra-articular structures by activating catabolic enzymes. The three cytokines, TNF-α, IL-1β, and IL-6, are the predominant inflammatory mediators in the pathogenesis of OA.[50] Downregulating the expression of TNF-α and IL-1β in the joints has been shown to reduce extracellular matrix degradation and chondrocyte apoptosis, thereby alleviating the progression of OA.[51]

3. Non-viral carriers for OA gene therapy

Building on the aforementioned targets, researchers have developed a variety of efficient delivery vectors with a strong emphasis on non-viral systems. These non-viral vectors offer several advantages, including ease of preparation and modification, low immunogenicity, and favorable biocompatibility. In addition, they exhibit a broad range of functionalities, making them highly adaptable for optimized gene delivery and enhanced therapeutic outcomes in OA treatment. This section introduces several representative non-viral vectors and their applications in gene therapy for OA, including lipids, exosomes, natural and synthetic polymers, inorganic nanoparticles, as well as proteins and peptides. **Table 2** provides a summary of the advantages and limitations of the non-viral vectors discussed.

3.1. Lipids

Lipid-based delivery systems, including liposomes, lipid-based nanoparticles, and other lipid nanomaterials, have been extensively applied in gene delivery.[71] Liposomes are water-nucleated vesicles surrounded by a lipid bilayer that can encapsulate hydrophilic drugs.[72,73] Modified liposomes are

Table 1. Targets in OA gene therapy

Target type	Genes	Nucleic acid type	Expression	Outcome	References
Transcription factors	*Run×1*	mRNA	Upregulated	Enhanced expression of cartilage-anabolic markers and proliferation	25
	HIF2A	siRNA	Downregulated	Downregulated the expression of OA-related catabolic markers and upregulated cartilage-specific markers in chondrocytes	48
Growth factors	*IGF1*	mRNA	Upregulated	Reduced histological OARSI score and decreased loss of cartilage extracellular matrix	52
	MMP	siRNA	Downregulated	Prevented matrix degradation and supported extracellular matrix homeostasis in articular cartilage	53
Inflammation-related cytokines	*IL1B*	siRNA	Downregulated	Attenuated chondrocyte apoptosis and maintained cartilage homeostasis	54
	IL1RA	pDNA	Upregulated	Reduced the inflammatory effects stimulated by IL-1β	55
	IL4	pDNA	Upregulated	Improved cartilage protection and pain relief function	40
	IL1RA+IL10	pDNA	Upregulated	Inhibited cartilage breakdown	56
Non-coding RNAs	miR-224-5p	miRNA	Upregulated	Inhibited joint space narrowing, reduced subchondral osteosclerosis, and ameliorated synovitis	57
	miR-200c-3p	miRNA	Upregulated	Reduced expression levels of inflammatory factors	58
	miR-25-3p	miRNA	Upregulated	Reduced chondrocyte apoptosis	59
	miR-140	miRNA	Upregulated	Inhibited cartilage-degrading proteases and alleviated OA progression	60
	circRNA.33186	circRNA	Downregulated	Increased type 2 collagen expression and decreased MMP-13 expression	61

Abbreviations: circRNA: Circular RNAs; HIF-2α: Hypoxia-inducible factor 2α; IGF-1: Insulin-like growth factor 1; IL: Interleukin; IL-1Ra: Interleukin-1 receptor antagonist; miR: microRNA; miRNA: microRNAs; MMP: Matrix metalloproteinase; mRNA: messenger RNA; OA: Osteoarthritis; OARSI: Osteoarthritis Research Society International; pDNA: Plasmid deoxyribonucleic acid; Run×1: Runt-related transcription factor 1; siRNA: Small interfering RNA; *IL1RA*: Interleukin-1 receptor antagonist.

Table 2. Non-viral vectors for osteoarthritis gene therapy

Non-viral vectors	Advantages	Limitations	Delivery system	Nucleic acid type	References
Lipids	Low immunogenicity; good biocompatibility; versatility	Low stability; short half-time in the bloodstream; potential toxicity	Cationic liposomes	miRNA	62
			Lipid nanoparticle	siRNA	63
Extracellular vesicles/ exosome	Enhanced targeting; good biocompatibility; versatility; reduced immunogenicity	Complexity; limited loading capacity; high cost	Exosomes from umbilical cord mesenchymal stem cells	miRNA	64
			Extracellular vesicles derived from fibroblast-like synoviocytes	miRNA	59
			Exosomes targeting chondrocytes	miRNA	65
Natural polymer	Good biocompatibility; low toxicity; cationic charge; ability to be modified with active targeting ligands	Poor water solubility; poor targeting ability; charge deduction under physiological conditions; premature release in the cytoplasm	Chondroitin sulfate/hyaluronic acid/ chitosan nanoparticles	miRNA	66
			CS/HA nanoparticles	pDNA	55
Synthetic polymer	High transfection efficiency; pH buffering capacity; endosomal escape capacity; ability to transfect non-dividing cells	Potential toxicity; inflammatory response	Multifunctional polyamidoamine dendrimers with amino acids	miRNA	57
			Cationic nanoparticles (AcPEI-NPs)	siRNA	53
			PEG-b-P (Asp (DET))	mRNA	67
			In situ PEI crosslinked nanocomplexes	miRNA	47
Inorganic nanoparticles	Biocompatibility; magnetic properties; high surface area; biodegradability; imaging capability	Limited loading capacity; instability; limited specificity	Metal-organic skeleton ZIF-8 with cytotoxic-free zinc (II) as a metal coordination center	miRNA	58
			Metal-organic framework	siRNA	68
Protein and peptide	Biodegradability; low toxicity; high stability; specificity for delivery; moderate transfection efficiencies	Potential toxicity; instability; limited loading capacity	Engineered cationic amphoteric peptides	siRNA	69
			Self-assembled peptide nanoparticles	siRNA	70

Abbreviations: AcPEI-NPs: Acetylated PEI-poly (lactic-co-glycolic acid) nanoparticles; CS: Chitosan; HA: Hyaluronic acid; miRNA: microRNAs; pDNA: Plasmid deoxyribonucleic acid; PEG-b-P (Asp (DET)): PEG-b-polyaspartamide having 1,2-diaminoethane side chain; PEI: Polyethyleneimine; siRNA: Small interfering RNA; ZIF-8: Zeolitic imidazolate framework-8.

composed of cationic lipids combined with neutral auxiliary lipids and ionizable cationic amino head groups. These modifications help reduce cytotoxicity and enhance transfection efficiency.[74] Cationic liposomes can form complexes with nucleic acids through electrostatic interactions.[75,76] This method involves encapsulating plasmid DNA (pDNA), mRNA, and siRNA in spheres with hydrophilic polar groups and hydrophobic tails.[77] Lipid nanoparticles (LNPs), which are typically composed of lipids and polymers, are primarily utilized for the delivery of RNA therapeutics, such as mRNA vaccines. LNPs enhance the stability of RNA molecules through encapsulation and facilitate their intracellular delivery.[78,79] For example, Zhang *et al.*[80] developed novel inhaled LNPs targeting intercellular adhesion molecule-1 receptors on the apical surface of airway epithelial cells. This delivery system improved the targeted delivery efficiency of siRNA in airway epithelial cells, effectively inhibiting the expression of pro-inflammatory cytokines and alleviating associated symptoms (**Figure 3**). Cai *et al.*[81] reported a type of reactive oxygen species-responsive LNP based on a thioketal moiety. This particle selectively released mRNA inside tumor cells, leading to enhanced gene expression and tumor inhabitation. Kim *et al.*[82] introduced an efficient approach for incorporating tumor-targeting peptides into LNPs. In their study, programmed death-ligand 1 (PD-L1) binding peptides were conjugated to polyethylene glycol (PEG)ylated lipids, followed by the construction of PD-L1-incorporated LNP composites (Pep LNPs). The resulting Pep LNPs exhibited strong interaction with PD-L1 proteins, facilitating their uptake into PD-L1-overexpressing cancer cells, both *in vitro* and *in vivo* (**Figure 4**).

Compared to other types of vectors, lipid nanomaterials offer unique advantages, such as high delivery efficiency, low immunogenicity, and versatility.[83] Lipid-based nanoparticles have demonstrated potential in preclinical studies and are currently being evaluated in clinical trials for gene therapy.[84-86] However, limitations such as low stability, short half-life, and high concentration toxicity must be considered.[87,88] Researchers have developed various strategies to optimize the stability, delivery efficiency, and safety of lipid vectors, which have facilitated the design of diverse lipid-based nanoparticles. Advances in nanotechnology and lipid chemistry are anticipated to drive the development of more sophisticated lipid-based nanoparticles, potentially enabling more precision

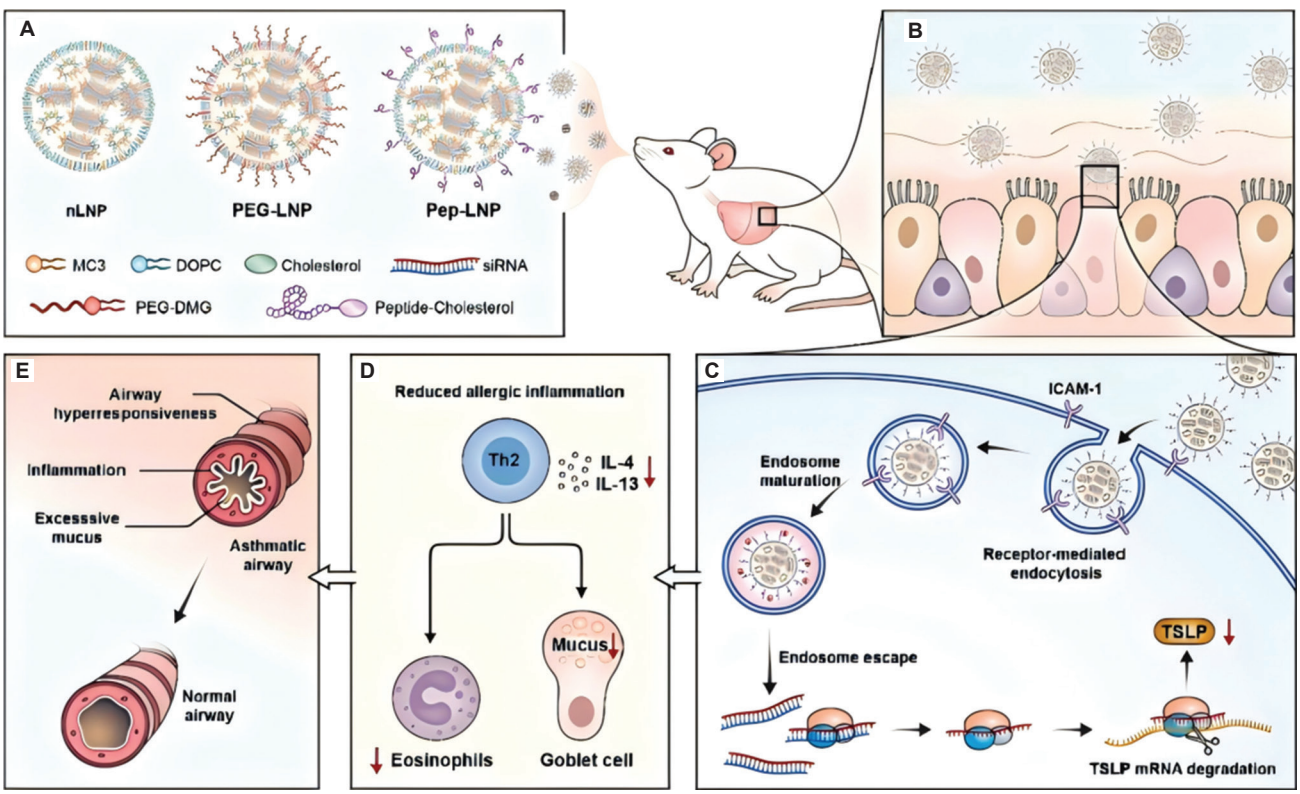

Figure 3. Schematic illustration of airway epithelial cell-specific delivery of LNPs-siTSLP to alleviate allergic asthma through pulmonary administration. (A) Different formulations of LNPs-siRNA were used in this study. (B) Deposition of LNPs-siRNA in the airways following pulmonary administration. (C) ICAM-1 receptor-mediated endocytosis of Pep-LNPs-siTSLP by airway epithelial cells and subsequent RNA interference action. (D) Inhibition of T-helper (Th) 2 cytokine production, eosinophil infiltration, and excessive mucin secretion. (E) Reduced airway inflammation in allergic asthma. Reprinted with permission from Zhang *et al.*[80] Copyright 2022 Elsevier.

Notes: Pep-LNPs are cyclic peptides that mimic a segment of the rhinovirus capsid protein and bind to ICAM-1 receptors. These peptides were first conjugated with cholesterol and then combined with ionizable cationic lipids to assemble the LNPs.

Abbreviations: DOPC: Dioleoylphosphatidylcholine; ICAM-1: Intercellular adhesion molecule-1; IL: Interleukin; LNPs: Lipid nanoparticles; MC3: DLin-MC3-DMA; mRNA: Messenger RNA; nLNP: Nanolipid nanoparticles; PEG: Polyethylene glycol; PEG-DMG: 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol; siRNA: Small interfering RNA; siTSLP: siRNA against thymic stromal lymphopoietin; TSLP: Thymic stromal lymphopoietin.

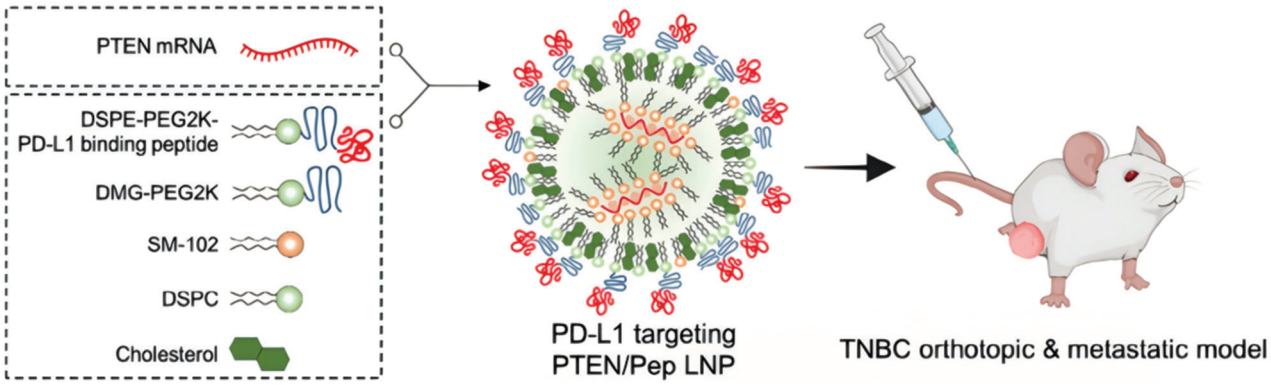

Figure 4. Schematic diagram of the assembly of Pep LNPs with mRNA. Reprinted with permission from Kim *et al.*[82] Copyright 2024 Wiley.
Note: Pep LNPs: PD-L1 binding peptides are conjugated to PEGylated lipids through a copper-free click reaction and subsequently integrated into the LNP formulation.
Abbreviations: DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine; DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; mRNA: Messenger RNA; PD-L1: Programmed death-ligand 1; PEG: Polyethylene glycol; PTEN: Phosphatase and tensin homolog; SM-102: Amino cationic lipid; TNBC: Triple-negative breast cancers.

and efficient delivery of nucleic acids to gene therapy targets for OA.[89,90] For example, He *et al.*[62] prepared clonoxicam cationic liposomes (Lnxc-CL) through the thin-film dispersion method, where (miR-140) and clonoxicam were co-loaded in cationic liposomes (Lnxc-CL/miR-140). The Lnxc-CL/miR-140 complex effectively delivered miR-140 into chondrocytes, upregulating the expression of miR-140 and COL2A1 mRNA. In subsequent *in vivo* tests, Lnxc-CL/miR-140 demonstrated effective treatment of OA by reducing joint inflammation and promoting the repair of damaged chondrocytes. Similarly, Wang *et al.*[63] developed a novel LNP for siRNA delivery, which effectively inhibited cartilage degeneration by silencing specific genes. In a rat model of OA, intra-articular injection of LNP-Indian Hedgehog siRNA slowed the progression of OA. This study highlights the significant chondroprotective potential of the LNP-RNA interference delivery system in mitigating cartilage degeneration, suggesting its promise as a therapeutic approach for cartilage diseases by targeting specific genes.

3.2. Engineered vesicles/exosomes

EVs are lipid membrane-enclosed nanoparticles of cellular origin, capable of encapsulating diverse nucleic acids such as pDNA, mRNA, and siRNA.[91] EVs play a pivotal role in intercellular communication and are secreted by nearly all cell types within an organism.[92] As a result, EVs can be isolated from various body fluids, including blood, urine, saliva, amniotic fluid, and synovial fluid, through ultracentrifugation.[93] In general, EVs are categorized into three groups according to their size and biogenesis: exosomes (40 – 120 nm), originating from the endolysosomal pathway; microvesicles/microparticles (50 – 1,000 nm), formed through plasma membrane protrusions; and apoptotic vesicles (1 – 5,000 nm), generated through plasma membrane vesiculation.[94] EVs offer considerable therapeutic potential for non-viral gene delivery, attributed to their inherent biocompatibility, low immunogenicity and cytotoxicity, improved targeting ability, and versatile engineering possibilities.[95] For instance, Zhang *et al.*[96] reported the development of light-activatable silencing natural killer-derived exosomes (LASNEO), which were engineered by incorporating hydrophilic siRNA and the hydrophobic photosensitizer chlorin e6 (Ce6) into exosomes derived from natural killer cells (**Figure 5A**). The proposed LASNEO demonstrated notable anti-tumor efficacy through the recruitment of various immune cell types.

Current research on exosomes/EVs has primarily focused on naturally occurring, non-engineered EVs and their interaction with target cells and tissues.[97] Cao *et al.*[64] employed exosomes derived from umbilical cord MSCs (UCMSC-EXOs) with chondrocyte-targeting capabilities and a controlled release mechanism for the treatment of OA by rejuvenating senescent chondrocytes. To enhance the therapeutic efficacy and *in vivo* retention duration of UCMSC-EXOs, these exosomes were modified with a tailored chondrocyte-targeting polymer and encapsulated within thiolated HA microgels, which effectively promoted cartilage regeneration in a rat model of OA (**Figure 5B**). Liu *et al.*[98] loaded exogenous miR-223 into EVs derived from human UCMSC (hUC-EV) through electroporation. In addition, they genetically engineered a collagen II-targeting peptide (onto the surface of hUC-EV to enhance the targeted and efficient delivery of cartilage RNA, aiming to ameliorate OA. Wang *et al.*[59] demonstrated that cell-derived fibroblast-like synoviocytes EVs successfully delivered miR-25-3p into chondrocytes, leading to an upregulation of miR-25-3p expression within the cells. This, in turn, suppressed cytoplasmic polyadenylation element-binding protein 1 transcription and downregulated the expression levels of IL-18, IL-1β, LR family pyrin domain containing 3, cleaved caspase-1, and N-terminal domain of gasdermin D, consequently mitigating the degeneration of OA chondrocytes.

Moreover, enhancing the targeting efficacy of exosome vectors is essential for realizing the broad clinical applications of exosomes. Liang *et al.*[65] engineered a chondrocyte affinity peptide (CAP) fused with lysosome-associated membrane glycoprotein 2b on exosomal surfaces, yielding CAP-exosomes proficient in encapsulating miR-140. These specialized

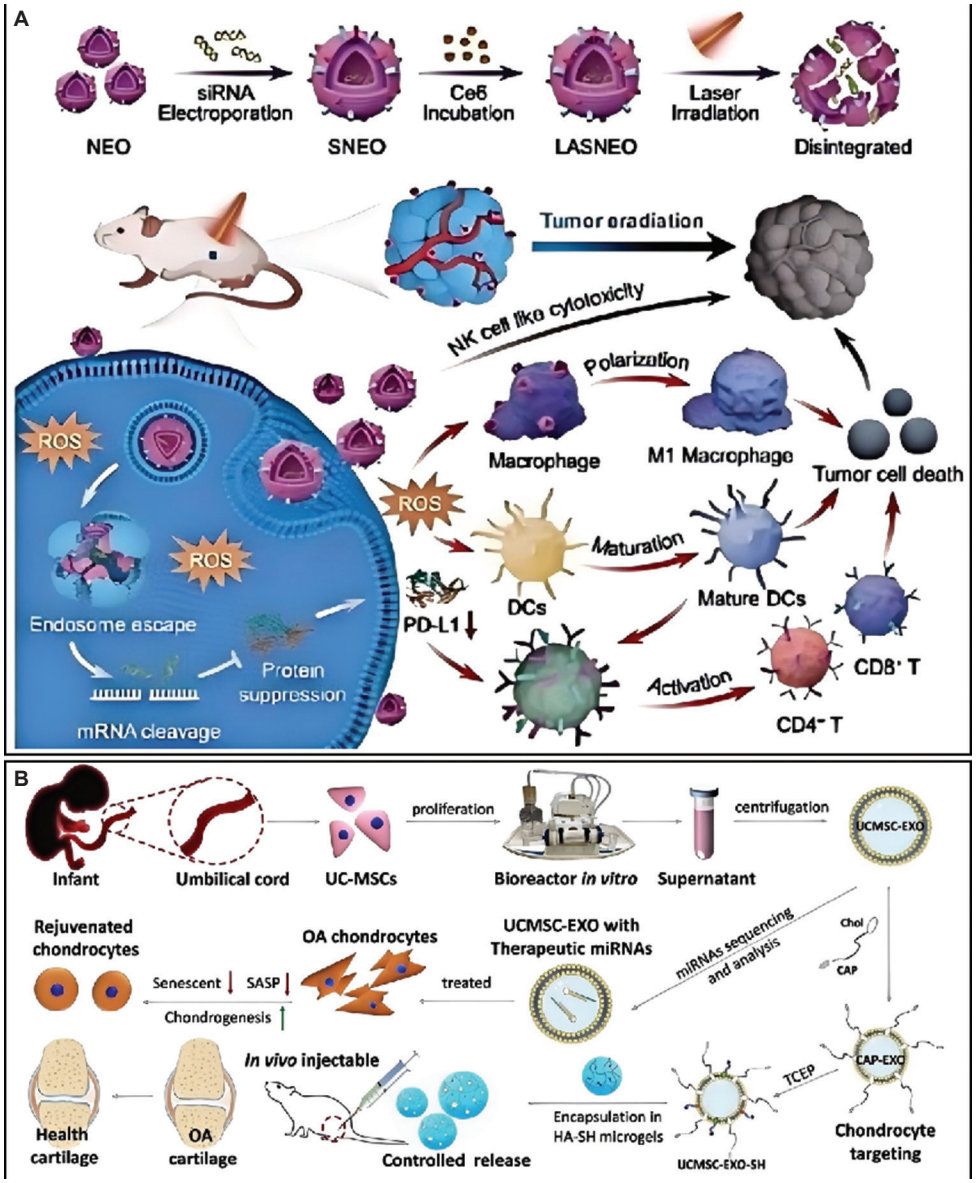

Figure 5. Schematic illustration of extracellular vesicle-mediated gene therapy. (A) Schematic illustration of LASNEO-mediated synergetic tumor eradication. Reprinted with permission from Zhang *et al.*[96] Copyright 2022 Wiley. (B) Preparation of UCMSC-EXOs targeting chondrocytes for the sustained release of therapeutic microRNAs to rejuvenate OA chondrocytes *in vivo*. Reprinted with permission from Cao *et al.*[64] Copyright 2023 American Chemical Society.

Abbreviations: CAP: chondrocyte affinity peptide; CD: Cluster of differentiation; Ce6: Chlorin e6; Chol: Cholesterol; DCs: Dendritic cells; HA: Hyaluronic acid; LASNEO: Light-activatable silencing natural killer-derived exosomes; mRNA: Messenger RNA; NEO: Exosomes derived from natural killer cells; NK: Natural killer; OA: Osteoarthritis; PD-L1: Programmed-death ligand 1; ROS: Reactive oxygen species; SASP: Senescence-associated secretory phenotype; SH: Microgels is thiolated; siRNA: Small interfering RNA; sNEO: siRNA-loaded NEO; TCEP: Tris (2-carboxyethyl)phosphine; UCMSC-EXOs: Exosomes derived from umbilical cord mesenchymal stem cells.

exosomes exhibited targeted entry into chondrocytes and effective cargo delivery *in vitro*. Moreover, CAP-exosomes facilitated the delivery of miR-140 across dense mesenchymal cartilage to deeper cartilage regions, where they suppressed cartilage-degrading proteases and mitigated OA progression in a rat model.

Although EVs have attracted considerable research interest in OA treatment, further studies are needed to explore their population heterogeneity, differences in isolation techniques, and reproducibility before they can be effectively translated into clinical applications as gene-delivery vehicles. These investigations are crucial for ensuring the broad implementation of EVs in gene therapy for OA.

3.3 Natural polymer

Natural polymers encompass various plant-derived polysaccharides with linear or branched structures characterized by both positive and negative charges.[99] In contrast to synthetic polymers, natural polymers exhibit anti-inflammatory properties.[100] Furthermore, the intrinsic antioxidant and anticoagulant properties of natural polymers contribute to their low immunogenicity.[101] The two most commonly applied natural polymer vectors in gene therapy are chitosan

(CS) and HA.[102,103] Their primary advantages as gene delivery vectors include biodegradability, biocompatibility, chemical diversity, and the presence of modifiable active sites, which enhance physicochemical properties for various biological applications.[104] For example, Zhou *et al.*[105] identified a novel long ncRNA TMEM44-AS1 associated with 5-fluorouracil (FU) resistance and developed a novel nanocarrier termed CS-gelatin-EGCG (CGE). This nanocarrier demonstrated superior gene silencing efficacy compared to Lipo2000, facilitating the delivery of si-TMEM44-AS1 to efficiently silence TMEM44-AS1 expression. This approach synergistically reversed 5-FU resistance in gastric cancer, significantly enhancing the therapeutic response to 5-FU in a xenograft mouse model of gastric cancer (**Figure 6A**). Nevertheless, a key limitation associated with the utilization of natural polymers as vector materials is the batch-to-batch variability due to their derivation from natural sources, resulting in inconsistent composition. This issue can be addressed through two primary strategies: First, by selecting natural polymers of high purity to ensure consistent sourcing, thereby reducing batch-to-batch variability, and second, by employing chemical modifications of the natural polymers to enhance their stability and optimize their drug carrier properties, thus minimizing the effects of batch-to-batch discrepancies.[106]

At present, natural polymers have received growing interest in OA gene therapy due to their inherent advantages and potential efficacy. Çelik *et al.*[66] synthesized miRNA-loaded chondroitin sulfate (HA/CS) nanoparticles designed for the concurrent delivery of therapeutic genes and cartilage matrix constituents to stem cells, aiming to promote cartilage regeneration while simultaneously suppressing pathological markers linked to OA. The findings demonstrated effective transfection of miR-149-5p, leading to the downregulation of its target gene fucosyltransferase 1. Furthermore, due to their high polysaccharide content, the nanoparticles were able to stimulate the synthesis of chondrogenic markers and enhance

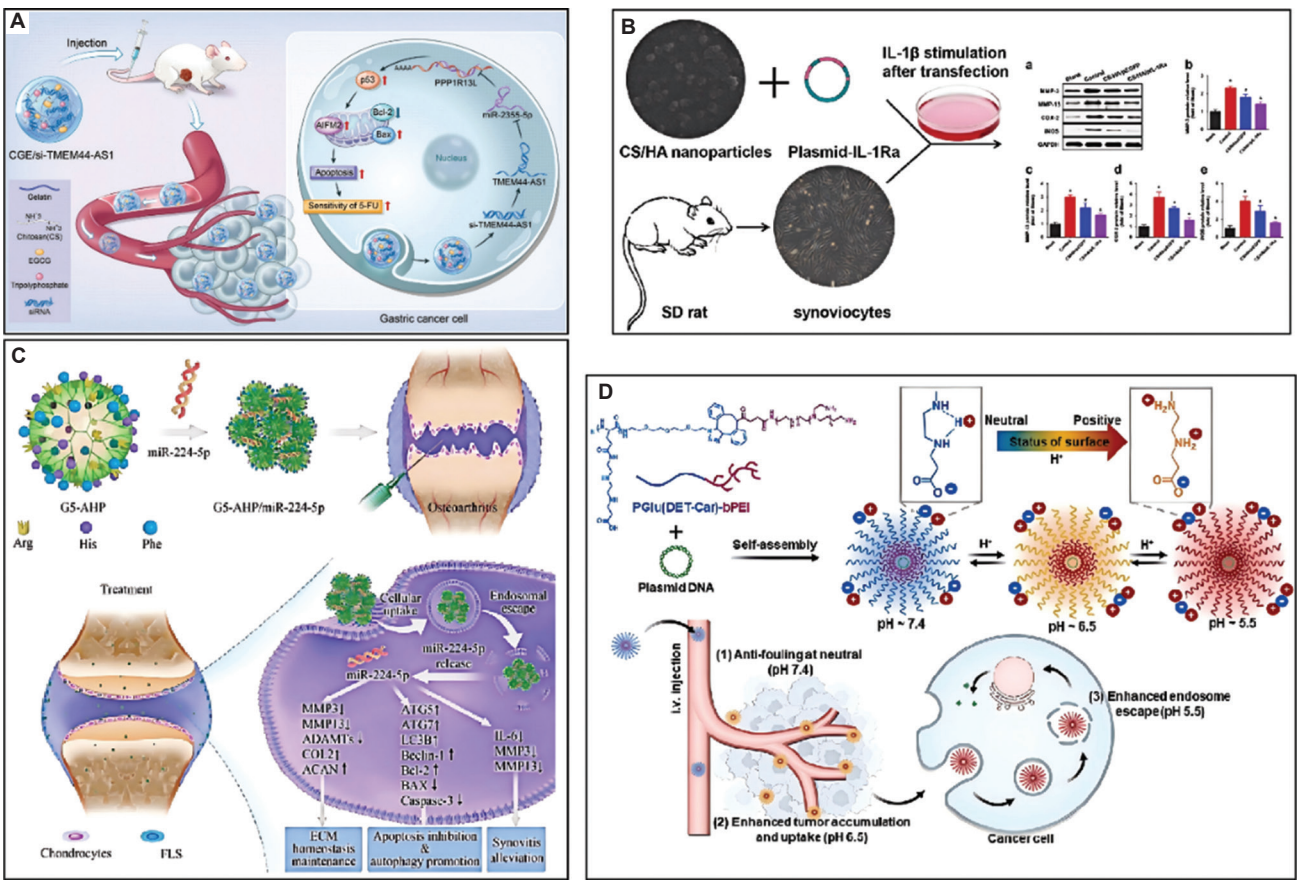

Figure 6. Schematic illustration of polymer-mediated gene therapy. (A) Schematic diagram of the mechanism by which CGE/si-TMEM44-AS1 complexes enhance the sensitivity of gastric cancer cells to 5-FU. Reprinted with permission from Zhou *et al.*[105] Copyright 2022 Wiley. (B) Schematic overview of chitosan/hyaluronic acid/plasmid DNA nanoparticles encoding IL-1 receptor antagonist (IL-1Ra), which attenuate inflammation in synoviocytes induced by IL-1β. Reprinted with permission from Deng *et al.*[55] Copyright 2018 Springer Nature. (C) G5-AHP combined with miR-224-5p for gene therapy in OA. Reprinted with permission from Chen *et al.*[57] Copyright 2023 Elsevier. (D) Stepwise pH-triggered charge conversion of PGDC PM for effective gene delivery. Reprinted with permission from Shen *et al.*[107] Copyright 2023 Elsevier. Abbreviations: ADAMTS: A disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif; ACAN: Aggrecan; AIFM2: Apoptosis-inducing factor 2; Arg: Arginine; Bax: Bcl-2-associated X protein; Bcl-2: B-cell CLL/lymphoma 2; CGE: Chitosan-gelatin-EGCG; COX-2: Cyclooxygenase-2; CS: Chitosan; ECM: Extracellular matrix; EGCG: Epigallocatechin gallate; FLS: Fibroblast-like synoviocytes; G5-AHP: Arginine, histidine, and phenylalanine-modified 5th-generation polyamines; HA: Hyaluronic acid; His: Histidine IL: Interleukin; iNOS: Inducible nitric oxide synthase; miR: MicroRNA; MMP: Matrix metalloproteinase; pEGFP: Plasmid enhanced green fluorescent protein; PGDC PM: PGlu(DET-Car)-coated polyplex micelle; Phe: Phenylalanine; p53: Tumor protein P53; SD: Sprague Dawley; siRNA: Small interfering RNA; TMEM44-AS1: a novel lncRNA; 5-FU: 5-fluorouracil.

the expression of associated genes. In addition, Deng *et al.*[55] fabricated CS/HA/pIL-1Ra nanoparticles through electrostatic interactions. *In vitro,* transfection assays validated the suitability of the CS/HA complex as a gene carrier in primary synoviocytes. Moreover, CS/HA nanoparticles loaded with the interleukin-1 receptor antagonist (*IL1RA*) gene exhibited significant functionality in synoviocytes, effectively mitigating the inflammatory responses induced by IL-1β. These findings suggest that CS/HA/pIL-1Ra nanoparticles hold considerable promise for therapeutic intervention against IL-1β-induced inflammation in synoviocytes (**Figure 6B**).

3.4. Synthetic polymer

Synthetic polymers, such as PDMAEMA (poly[2(dimethylamino) ethyl methacrylate]), poly(β-amino esters), polyamidoamine (PAMAM) dendrimers, and PEI, have found extensive application in gene delivery due to their cationic nature. For instance, PDMAEMA can be easily synthesized to produce various homopolymers and copolymers of different lengths, which serve as polycationic vectors. The tertiary amino groups in PDMAEMA have a pKa value of around 7.5.[108] This property enables efficient loading of anionic nucleic acids through electrostatic interactions, facilitating cellular uptake and endosome escape.[109] Poly(β-amino esters) contain degradable ester linkages in their polymer backbone, which facilitate cargo release.[110,111]. In addition, cationic polymers can be further tailored to regulate their affinity for genetic material, ensuring efficient delivery of nucleic acids while offering robust protection against enzymatic degradation.[112] Recently, Chen *et al.*[57] synthesized fifth-generation polyamines (arginine, histidine, and phenylalanine-modified 5th-generation polyamines [G5-AHP]), a multifunctional PAMAM dendrimer modified with arginine, histidine, and phenylalanine, to facilitate the efficient delivery of miR-224-5p into cells, ensuring its protection against degradation. Intra-articular injections of G5-AHP/miR-224-5p nanoparticles mitigated joint space narrowing, diminished subchondral osteosclerosis, and alleviated synovitis, thereby overcoming the principal constraints associated with miR-224-5p in local gene therapy for OA (**Figure 6C**). Li *et al.*[60] synthesized G5-AHP and innovatively formulated G5-AHP/miR-140 nanoparticles through complexation with miR-140. These nanoparticles effectively attenuated the progression of OA by suppressing the expression of MMP-13 and ADAMTS5 in chondrocytes. Zhu *et al.*[47] devised PAR, composed of PEI, aldehyde-modified HA, and small ncRNAs (miR-140). These PAR nanocomplexes are easy to prepare and are notably distinguished by their high stability in RNA embedding. They demonstrated efficient transfer of small regulatory RNAs into chondrocytes, overcoming strong interference from CS and HA, which are prevalent anionic biomolecules in and around cartilaginous tissues. This capability enabled effective mitigation of OA in mice. While synthetic polymer-carriers such as PAMAM dendrimers and PEI exhibit efficacy in delivering nucleic acids, their substantial positive charge can result in non-specific interactions with negatively charged phospholipid cell membranes following systemic delivery.[113]

Furthermore, the introduction of functional groups can further enhance environment-responsive ability, resulting in effective cellular uptake and endosomal escape.[114-116] For example, Shen *et al.*[107] devised a stepwise pH-responsive polyplex micelle for the delivery of pDNA, featuring a surface adorned with ethylenediamine-based polycarboxybetaines. This polyplex micelle underwent a charge transition from neutral at pH 7.4 to positive under tumorous and endo/lysosomal pH conditions (i.e., pH 6.5 and 5.5, respectively), thereby improving cellular uptake and promoting endosomal escape, ultimately facilitating efficient gene transfection (**Figure 6D**).

However, it is worth noting that the stability of complexes generated by synthetic polymers is significantly affected by the molecular weight of these polymers. Complexes formed by lower molecular weight polymers are prone to instability, especially under physiological conditions, due to their limitations in resisting interference from serum proteins. This instability manifests as disassembly, degradation, and subsequent clearance of the molecular cargo.[117] This issue can be mitigated by implementing targeted surface modifications, such as PEG or poly(lactic-co-glycolic acid) coupling, to enhance spatial stability and minimize undesired interactions with salts and other charged or neutral particles circulating in the system.[118]

For example, Wang *et al.*[119] reported fast adenosine triphosphate-depleting micellar system activated by intracellular redox for the co-delivery of the anticancer drug paclitaxel and siRNA targeting polo-like kinase1 to inhibit tumor growth *in vivo*. The micelles were self-assembled from redox-responsive amphiphilic polymers, denoted as bPEG-SS-P123-PEI, comprising biocompatible branched PEG with eight arms (bPEG), adenosine triphosphate-depleted Pluronic P123 (P123), and cationic low molecular weight PEI blocks. In addition, Deng *et al.*[67] employed mRNA encoding an IL-1Ra to administer anti-inflammatory treatment in a rat model of temporomandibular joint OA. The delivery of the anti-inflammatory protein through the polymeric carrier PEG-b-P(Asp(DET)) (PEG-b-polyaspartamide having 1,2-diaminoethane side chain) effectively ameliorates OA symptoms. This carrier exhibited excellent tissue penetration and minimal immunogenicity, thus fostering advancements in mRNA therapy. Shin *et al.*[120] discovered that inhibition of p66shc through siRNA delivered by poly(lactic-co-glycolic acid)-based nanoparticles alleviated pain behaviors, cartilage damage, and inflammatory cytokine production in the knee joints of rats with monosodium iodoacetate-induced OA. p66shc plays a pivotal role in cartilage degeneration associated with OA. Delivery of nanoparticles loaded with p66shc siRNA to the knee joints of OA subjects resulted in a substantial reduction in cartilage damage induced by mitochondrial dysfunction.

3.5. Inorganic nanoparticles

Various inorganic materials, encompassing metal-organic frameworks (MOFs), calcium phosphate (CaP), gold nanoparticles, silica, magnesium phosphates, carbon nanotubes, and magnetic nanomaterials (e.g., iron oxides), have demonstrated efficacy in delivering nucleic acids into cells.[121] Inorganic carriers offer numerous advantages, including high biocompatibility, magnetism, large specific surface area, and

imaging capabilities, which have garnered significant attention from researchers.[122] For instance, CaP particles have attracted considerable interest in bone regeneration due to their ability to enhance structural strength and stiffness. When combined with short hairpin RNA, CaP nanoparticles have been shown to effectively promote bone formation in human osteoblasts.[123] In addition, nano-hydroxyapatite carriers can efficiently deliver pDNA encoding vascular endothelial growth factor and BMP 2 MSCs, significantly accelerating bone healing and facilitating tissue vascularization.[124] However, the limitations of inorganic materials as gene delivery vectors include their restricted loading capacity, instability, and limited specificity.[125]

Recently, Yang *et al.*[58] selected the MOF zeolitic imidazolate framework-8 (ZIF-8), featuring a non-cytotoxic zinc(II) metal coordination center, as a delivery vehicle for miRNA. They synthesized miR-200c-3p@ZIF-8 in a single step using a Y-shaped microfluidic chip, achieving intracellular release with low toxicity, scalability, and high efficiency of cellular uptake. As a proof of concept, the synthesized miR-200c-3p@ZIF-8 demonstrated efficacy in treating OA (**Figure 7A**). Meanwhile, Zhang *et al.*[68] developed a pH-responsive MOF, MIL-101-NH_2, for the co-delivery of the anti-inflammatory drug curcumin and siRNAs targeting HIF-2α. The curcumin and siRNA were loaded through the encapsulation and surface-liganding

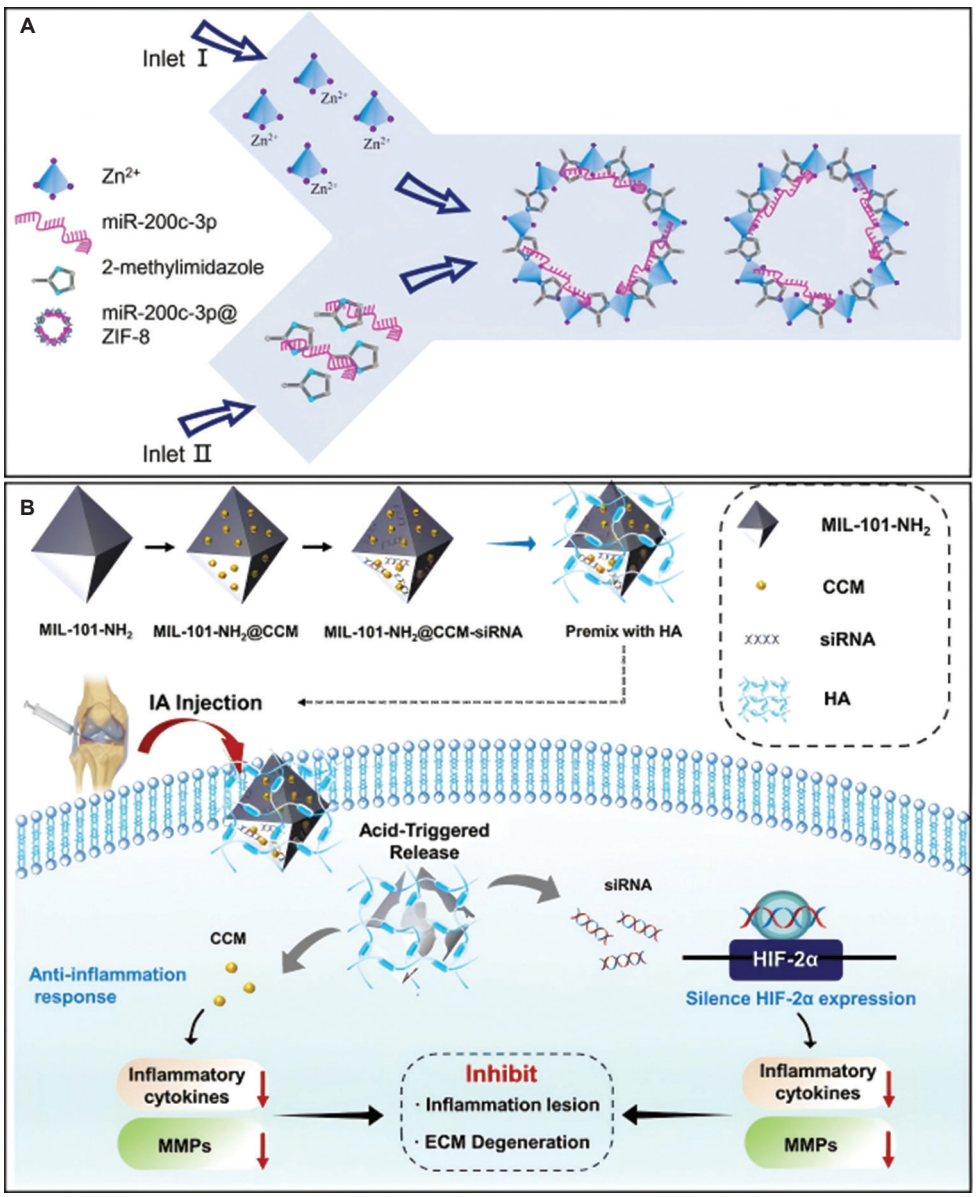

Figure 7. Schematic illustration of inorganic nanoparticles-mediated gene therapy. (A) A schematic diagram of the one-step microfluidic fabrication process for miR-200c-3p@ZIF-8. Reprinted with permission from Yang *et al.*[58] Copyright 2023 FRONTIERS MEDIA SA. (B) The preparation process of MIL-101-NH_2@CCM-siRNA nanoparticles, along with a schematic representation of these nanoparticles (premixed with HA solution) for osteoarthritis therapy. Reprinted with permission from Zhang *et al.*[68] Copyright 2023 Springer.

Notes: MIL-101-NH_2: A pH-responsive metal-organic framework. ZIF-8: A metal-organic framework with non-cytotoxic zinc (II) as the metal coordination center.

Abbreviations: CCM: Curcumin; ECM: Extracellular matrix; HA: Hyaluronic acid; HIF-2α: Hypoxia-inducible factor 2α; miR: MicroRNA; MMP: Matrix metalloproteinase; siRNA: Small interfering RNA; ZIF-8: Zeolitic imidazolate framework-8; Zn^{2+}: Zinc ion.

capabilities of MIL-101-NH_2. This hybrid material showed promising therapeutic potential for OA in both *in vitro* and *in vivo* studies, providing an effective strategy for OA treatment utilizing MOF as an inorganic carrier (**Figure 7B**).

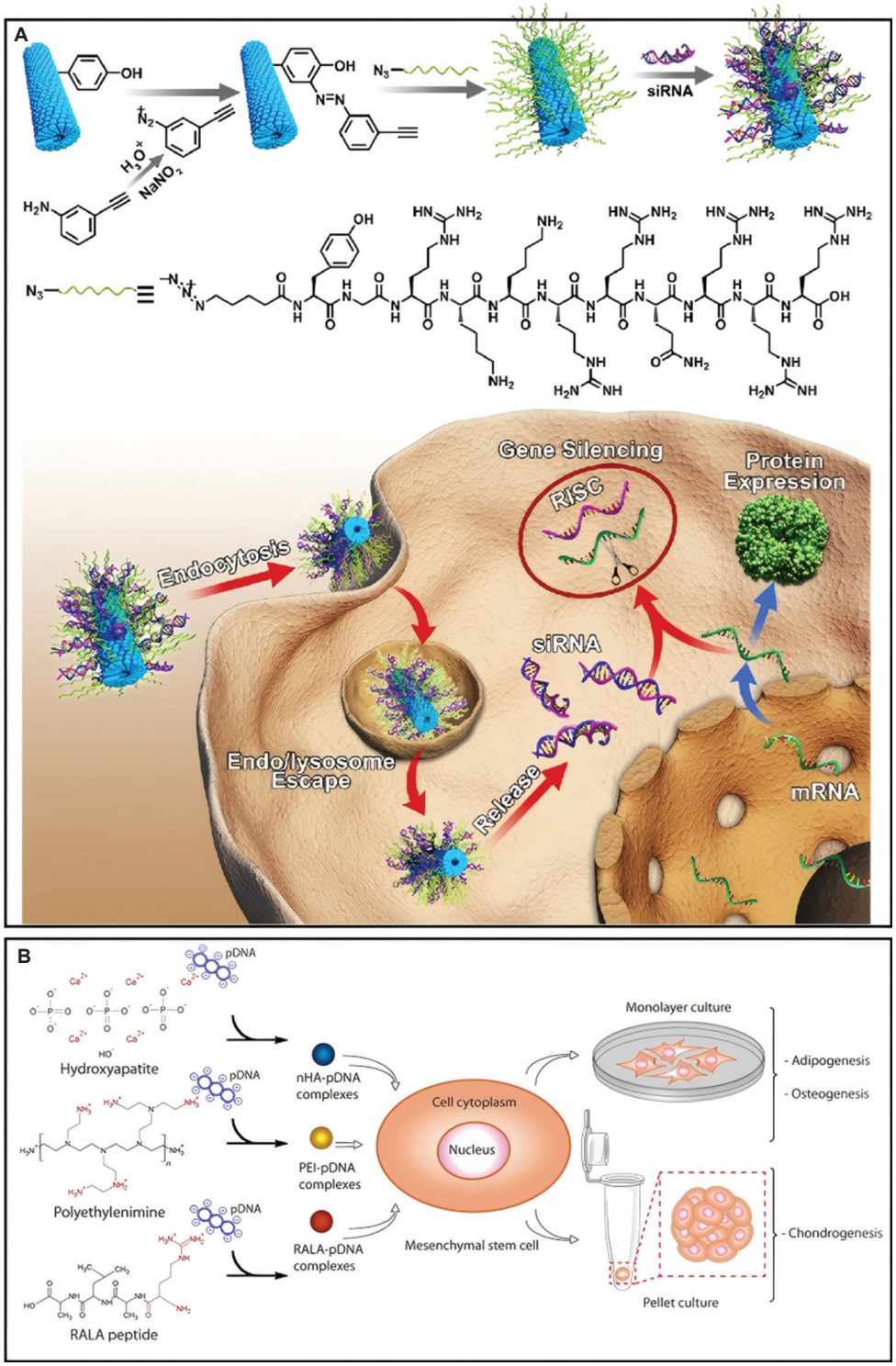

Figure 8. Schematic illustration of protein/peptide-mediated gene therapy. (A) Schematic diagram of transactivator of transcription-decorated tobacco mosaic virus and siRNA loading, along with the gene-silencing process. Reprinted with permission from Tian *et al.*[133] Copyright 2018 American Chemical Society. (B) MSCs were transfected using ceramic (nHA) cationic polymer (PEI) or amphipathic peptide (RALA) non-viral gene delivery vectors complexed with pDNA carrying reporter or therapeutic genes. After transfection, the adipogenic, osteogenic, and chondrogenic potentials of the treated MSCs were evaluated in both two-dimensional (monolayer culture in bipotent media) and three-dimensional (pellet culture) models to investigate the impact of the gene delivery nanomaterials on MSC differentiation. Reprinted with permission from Gonzalez-Fernandez *et al.*[134] Copyright 2017 Elsevier.
Abbreviations: mRNA: Messenger RNA; MSCs: Mesenchymal stem cells; nHA: Nano-hydroxyapatite; pDNA: plasmid DNA; PEI: Polyethylenimine; RALA: Ras-related protein Ral-A; RISC: RNA-induced silencing complex; siRNA: Small interfering RNA.

3.6. Protein and peptide carrier

Protein- and peptide-based nanocarriers are naturally derived polymers characterized by biodegradability, high stability, and binding capacity.[126] Cationic peptides, which contain basic amino acid residues such as lysine or arginine in their structure, bind nucleic acids to form nanocomplexes.[127-129] In addition, specific peptide sequences can be applied to target membrane receptors on specific cell types.[130,131] The release of nucleic acids into the cytoplasm is regulated by selective cleavage of nuclear endosomes and using nuclear localization sequences derived from viral origins.[132]

Recently, Tian *et al.*[133] developed a one-dimensional rod-like gene-silencing vector based on the plant virus tobacco mosaic virus (TMV), modified with a transactivator of transcription (TAT) peptide to enhance gene transfer efficiency and minimize side effects. The TAT modification allows tunable isoelectric points (~3.5 – ~9.6), depending on the TAT dose, thereby improving cell internalization. The TMV-TAT vector also facilitates endo/lysosomal escape without damaging lysosomes, ensuring high efficiency and low cytotoxicity. *In vitro*, siRNA-loaded TMV-TAT successfully knocked down green fluorescent protein expression in mouse epidermal stem cells by more than 85% whereas maintaining nearly 100% cell viability. *In vivo*, treatment with siRNA@TMV-TAT reduced green fluorescent protein expression in highly metastatic hepatocellular carcinoma tumors by 80.8% (**Figure 8A**). Gonzalez-Fernandez *et al.*[134] utilized a self-assembling peptide nanoparticle platform incorporating a cell-penetrating peptide complexed with NF-κB p65 siRNA. Their study demonstrated efficient penetration into human cartilage, enabling the delivery of siRNA cargo to a depth of at least 700 μm, which could potentially attenuate cartilage degeneration progression (**Figure 8B**). Despite these promising advantages, limitations exist in utilizing proteins and peptides as gene delivery vectors, such as potential toxicity and limited loading capacity.[135]

4. Limitations

Although this review offers a comprehensive overview of the current state of research on non-viral gene delivery systems for the treatment of OA, several limitations should be acknowledged. First, the scope of the references may be constrained by the database and search terms employed, which could lead to the exclusion of key studies, thereby affecting the completeness of the review and its coverage of existing knowledge and technological advancements. Second, given the rapid pace of development in non-viral vector-mediated gene therapy for OA, maintaining timeliness poses a challenge. The reviewed literature may not encompass the most recent developments in the field, potentially limiting the relevance of the information presented and the accuracy of evaluating the current state and future applications of this area. Finally, the review may lack sufficient clinical data on non-viral vector-mediated gene therapy for OA. The absence of reliable clinical data concerning the efficacy and safety of gene therapy for OA complicates a thorough evaluation of the practical significance of non-viral vector technologies and their potential for clinical translation. In conclusion, while this review highlights the progress and potential of non-viral gene delivery systems in OA treatment, the aforementioned limitations must be carefully considered when interpreting the findings and making future projections. Therefore, to ensure the continued relevance and reliability of this review in the rapidly evolving field of non-viral gene delivery systems for OA, it is essential to broaden the literature search, incorporate the latest research, and enhance the collection of clinical data.

5. Conclusions and perspectives

Gene therapy for OA is an emerging therapeutic approach to ameliorate the symptoms and effects of OA by modulating the expression of specific genes. The primary modality of gene therapy for OA involves the introduction of therapeutic genes into target sites using gene delivery vectors to restore or improve joint tissue function.[136] Current research has focused on gene therapy strategies to modulate inflammatory factors, promote chondrocyte proliferation and differentiation, inhibit cartilage degradation, and improve joint lubrication.[137] Gene therapy has the potential to reduce pain, improve joint function, promote cartilage regeneration, and slow down disease progression, offering potential benefits for the treatment of OA. So far, research has proposed various types of delivery systems based on lipids, engineered vesicles/exosomes, natural polymers, synthetic polymers, inorganic nanoparticles, and protein and peptide vectors. These delivery vectors, with their designed structures and functionalities, have achieved significant success in gene delivery for OA therapy, as summarized in the current work.

Despite these advancements, challenges remain in enhancing the performance of delivery vectors and OA treatment. For example, non-viral vectors typically exhibit suboptimal delivery efficiency in specific cells or tissues. Future research efforts should prioritize the optimization of the physicochemical properties of these vectors to improve cellular uptake and membrane penetration. In addition, the development of targeted delivery systems capable of selectively recognizing and binding to target cells is essential to minimize non-specific delivery and associated side effects.[138] While non-viral vectors generally exhibit lower immunogenicity, high doses or repeated administrations may still provoke immune responses.[139] Therefore, it is crucial to utilize materials with low immunogenic potential and minimal toxicity while employing surface modifications to reduce immune system recognition. Furthermore, exploring localized gene therapy delivery may help mitigate systemic immune responses and potential toxicities. These strategies are expected to enhance both the safety and efficacy of non-viral vector-based gene therapies, facilitating their successful transition into clinical practice.

Finally, the scalability of vector production and quality control is another critical aspect. Innovative fabrication methods and techniques must be further optimized to enhance production control and cost efficiency, thus facilitating the widespread adoption and application of gene therapy. In addition, regulatory bodies should implement standardized manufacturing protocols and stringent quality control measures to ensure consistent batch quality. This includes improving the

validation of raw material suppliers and production processes to maintain manufacturing stability.

Despite these remaining challenges, we are confident that continued optimization of design, technological advancements, interdisciplinary collaboration, and improvements in production processes will play a crucial role in advancing vector design and enhancing their therapeutic applications in OA gene therapy. We firmly believe that gene therapy holds immense promise in offering more effective and personalized treatment options for OA patients. Given the rapid evolution of gene delivery technologies, it is anticipated that gene therapy will quickly expand its scope to encompass OA therapy and potentially extend to a broader field of biomedical applications.

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