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Recent advances in targeted drug delivery for treatment of osteoarthritis

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Abstract

Purpose of review—Osteoarthritis is associated with severe joint pain, inflammation, and cartilage degeneration. Drugs injected directly into intra-articular joint space clear out rapidly providing only short-term benefit. Their transport into cartilage to reach cellular targets is hindered by the tissue's dense, negatively charged extracellular matrix. This has limited, despite strong preclinical data, the clinical translation of osteoarthritis drugs. Recent work has focused on developing intra-joint and intra-cartilage targeting drug delivery systems (DDS) to enable long-term therapeutic response, which is presented here.

Recent findings—Synovial joint targeting hybrid systems utilizing combinations of hydrogels, liposomes, and particle-based carriers are in consideration for pain-inflammation relief. Cartilage penetrating DDS target intra-cartilage constituents like aggrecans, collagen II, and chondrocytes such that drugs can reach their cellular and intra-cellular targets, which can enable clinical translation of disease-modifying osteoarthritis drugs including gene therapy.

Summary—Recent years have witnessed significant increase in both fundamental and clinical studies evaluating DDS for osteoarthritis. Steroid encapsulating polymeric microparticles for longer lasting pain relief were recently approved for clinical use. Electrically charged biomaterials for intra-cartilage targeting have shown promising disease-modifying response in preclinical models. Clinical trials evaluating safety of viral vectors are ongoing whose success can pave the way for gene therapy as osteoarthritis treatment.

Keywords

cartilage targeting; drug delivery; nanoparticles; osteoarthritis; pain and function treatment

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Musculoskeletal diseases, such as osteoarthritis (OA), rheumatoid arthritis (RA), and low back pain represent the second leading cause of disability globally, imposing a significant physiologic and economic burden on society [1,2]. Such diseases are characterized by tissue degeneration and inflammatory activity that can cause chronic pain and severe joint damage [3]. Specifically, osteoarthritic joints are most affected by articular cartilage degradation and synovial inflammation because of their load-bearing nature, which over time result in loss of joint function and mobility. Overexpression of biological factors, such as inflammatory cytokines [e.g. interleukin (IL)-1, IL-6, tumor necrosis factor α (TNFα)] and degradative enzymes [e.g. matrix metalloproteinase (MMP)13, a disintegrin metalloproteinase with thrombospondin motifs 5 (ADAMTS5)] accelerate progression to osteoarthritis, especially in case of joint injury [4]. The avascular nature of cartilage limits its self-regenerative capacity; timely therapeutic intervention is thus needed to repair the tissue and inhibit further disease progression [5].

In the early stages of osteoarthritis, patients usually experience mild pain and stiffness after performing routine activities, which is typically treated by either topical or oral nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics [6]. As the disease progresses to its mid-stage, joint space begins to narrow and shows signs of osteophyte formation and cartilage damage whereas chondrocytes begin to experience a hypertrophic state in an effort to restore tissue damage [6]. At this stage, interventions, such as intra-articular (IA) injections of high-dose corticosteroids or viscosupplements like hyaluronic acid are often recommended for relieving some of the pain and inflammation [7]. However, the aforementioned methods only provide temporary relief and fail to initiate any disease-modifying effect. As the disease progresses to end-stage osteoarthritis, surgical interventions using tissue engineering approaches [8], microfracture, and joint arthroplasty may be considered but eventually total joint replacement is required [7]. Early-stage intervention with disease-modifying osteoarthritis drugs (DMOADs) has the potential to slow down osteoarthritis progression and restore joint structure and function [9] but no such drugs have translated to clinical practice, in part because of a lack of effective delivery systems that can penetrate through the dense meshwork of cartilage to target chondrocytes and provide controlled low drug doses over a period of time with minimal off target effects [10,11■].

Most small molecule drugs clear out rapidly from the synovial joint (with half-lives of 1–4 h) following their intra-articular administration because of fast exchange of synovial fluid requiring multiple injections of high drug doses that cause toxicity [12]. In order to prolong joint residence times and provide sustained drug release intended for pain and inflammation relief, delivery systems like hydrogels, micelles, polymeric particles are in consideration owing to their large size or viscous nature [13] (Fig. 1). These systems can only target the synovium or the synovial fluid and use high drug doses, thus are only useful for providing pain relief. To achieve cartilage protection – that is to inhibit catabolism and stimulate regeneration, DMOADs must penetrate through the full thickness of cartilage and reach chondrocytes and other matrix target sites, a majority of which lie within the tissue deep zone [10]. Therefore, nanosized carriers that can penetrate into the cartilage and bind within to provide sustained drug release are under consideration [11■].

This review presents recent basic science and clinical developments in nanoparticle-based delivery systems for prolonging drug residence time within the joint space for pain-inflammation relief and targeting specific intra-cartilage components to restore joint structure and function for osteoarthritis therapy.

INTRA-JOINT DELIVERY

In the native knee, the primary source of pain arises from intra-joint components, such as the synovium, outer-third of meniscus, and osteochondral junction [14]. This is because the capillary network present in these regions begins to multiply (angiogenesis) in osteoarthritis, and contributes towards synovitis (hypertrophy of synovial macrophages and fibroblast-like synoviocytes), osteochondral damage, and osteophyte formation [14]. Thus, current efforts in the design of drug delivery systems (DDS) are focused on prolonging intra-joint residence time of intra-articular administered pain and inflammation relievers to enable efficacy over an extended period of time with a single low-dose administration. We have discussed recent advances in intra-joint DDS under three categories (Table 1): hybrid systems, smart environment responsive systems, and systems with specificity to intra-joint components, such as synoviocytes and vasculature.

Hybrid systems combine a variety of particle-based and hydrogel-based DDS to leverage their advantages. For example, micelles formed by antioxidant, eicosapentaenoic acid (EPA) encapsulated within a gelatin hydrogel enabled controlled drug release over 4 weeks in mouse joints [15■]. Following intra-articular injection of EPA hydrogels into DMM (destabilization of medial meniscus) mice, significantly greater suppression of glycosaminoglycan (GAG) loss and IL-1β and MMP13 expression was observed at 8 weeks' time compared with EPA alone [15■]. Another study tagged gold nanoparticles (possessing antioxidant activity) with fish oil protein (antiarthritic and anti-inflammatory) to create a hydrophilic structure, which was then encapsulated within a hydrophobic dipalmitoyl phosphatidyl-choline (DPPC) liposome (~295 nm diameter) to increase joint retention time and provide lubrication [16■]. The liposomal encapsulation of nanoparticles led to further suppression of NF-κB and iNOS among other synovial fluid catabolic markers obtained from osteoarthritis mouse knees compared with fish oil-tagged gold nanoparticles alone at 15 days following treatment [16■]. Another hybrid system incorporated Kartogenin (KGN), a chondrogenic drug, within a liposome loaded into a photo-crosslinkable Gelatin methacryloyl (GelMA) matrix in order to improve the drug stability and release from liposomes [17■]. The microinjectable hydrogel composite system (GelMa@Lipo@KGN, 100 μm diameter) was retained within rat DMM joints for over 5 weeks (compared with 2 weeks for liposomes), contributing to 75% KGN release over 25 days and resulting in reduction of osteophyte formation [17■].

Smart DDS incorporate pH or thermoresponsive materials. For example, to utilize the acidic osteoarthritic environment within synovial fluid, a hydrophobic drug, Celastrol, was loaded into pH-responsive, chitosan-coated, hollow, 275 nm sized mesoporous silica nanoparticles (CSL@HMSNs-Cs) [18■]. Greater than three-fold higher drug release was observed in acidic condition (pH 6) compared with a neutral pH 7 environment over 24 h *in vitro*. Following intra-articular administration, CSL@HMSNs-Cs led to greater improvements in

paw withdrawal threshold and reduced cartilage erosion at 8 weeks following MIA (monosodium iodoacetate) induction in rats compared with free Celastrol and drug-free nanoparticles [18■]. Similarly, polylactic co-glycolic acid (PLGA) nanoparticles were encapsulated with ammonium bicarbonate for pH sensitivity and hyaluronic acid, yielding sustained hyaluronic acid release over 10 days at pH 5. They were retained within the knee joints of DMM mice for 35 days and suppressed the incidence of osteophyte formation significantly greater than non-pH-responsive nanoparticles [19■■]. Metal-organic frameworks (MOF) possessing pH sensitivity (23% drug release at pH 5.6 compared with 13% at pH 7.4) were modified with hyaluronic acid and loaded with an anti-inflammatory drug, leading to enhanced reductions in synovial inflammation and inflammatory marker expression measured at 8 weeks in ACLT (anterior cruciate ligament transection) rats compared with free drug [20■]. Acid-activatable poly-beta-amino-ester (PBAE) curcumin nanoparticles, because of protonable tertiary amine groups present on its backbone, were shown to enhance drug release over 7 days in acidic conditions and decreased inflammatory cytokine production greater than unmodified curcumin in osteoarthritis mice joints over 28 days [21■]. Thermoresponsive DDS, chitosan-modified MoS_2 (molybdenum disulfide), was shown to release a small molecule drug, dexamethasone (Dex) inside the mouse knee joint cavity, when near-infrared (NIR) light was applied on the joint from outside the body [22■]. NIR triggered photothermal conversion of MoS_2 to provide controlled site-specific drug delivery and resulted in greater suppression of joint TNFα, IL-1β and IL-8 expression levels for 28 days following treatment compared with free Dex or NIR-free Dex-loaded nanoparticles [22■]. Hemoglobin, a molecule possessing photothermal properties, was recently conjugated with nitric oxide (NO) and Notch1 siRNA prior to encapsulation within a PLGA-polyethylene glycol (PEG) vesicle (NHsPP) [23■■]. Application of NIR light at 650 nm wavelength triggered a 24 h NO release *in vitro* and was able to further inhibit inflammatory cytokine expression in osteoarthritis mice for 36 days compared with nanoparticles without drug [23■■]. Deloney *et al.* [24■■] utilized the thermosensitive property of N-isopropyl acrylamide (NIPAm) to generate ‘hollow’ core nanoparticles; preparation of these particles at 4°C (below the lower critical solution temperature) allows for the structure to swell, facilitating removal of noncrosslinked cores to increase the drug-loading capacity. These ‘hollow’ core nanoparticles were capable of loading and releasing significantly higher amounts of MK2 (mitogen-activated protein kinase-activated protein kinase 2) inhibiting peptides compared with solid nanoparticles, contributing to enhanced suppression of IL-1β-stimulated IL-6 production in chondrocytes [24■■]. Additionally, these particles possessed the ability to reduce their size to 200 nm in diameter at 37 °C because of deswelling, preventing any inflammatory response often seen with larger-sized particles [24■■]. These nanoparticle were shown to be retained within the rat knee joints for 7 days following their intra-articular administration [24■■].

DDS design has also focused on targeting macrophages, fibroblast-like synoviocytes (FLS), microvasculature endothelium (MVE) and angiogenesis, all of which are overexpressed in an inflamed joint. For example, a positively charged peptide dendrimer nanogel (PDN, constituted of crosslinked polyhedral oligomeric silsesquioxane core-based generation 3 poly (L-lysine) dendrimers), was constructed by physically encapsulating carbon monoxide (CO) release molecules and tagging their surface with folic acid-modified hyaluronic acid to

target macrophages [25■]. A macrophage-targeted and pH-responsive zeolitic imidazolate framework (ZIF)-8 was modified with anti-CD16/32 antibody, resulting in a prolonged synovial macrophage and intra-joint retention [26■■]. Another study reported the design and preparation of dextran sulfate–triamcinolone acetonide conjugate (DS-TCA) nanoparticles for treating osteoarthritis by specifically targeting scavenger receptor class A (SR-A) on activated macrophages leading to alleviation of cartilage damage for 3 weeks [27■]. Surface modification of polylactic acid (PLA)-PEG nanoparticles with adenosine, via binding to A2A adenosine receptor to stimulate cAMP production to prevent or treat osteoarthritis, was designed to target both the macrophages and chondrocytes to exhibit an anti-inflammatory effect in both *in vitro* and *in vivo* [28].

FLS targeting has previously been achieved using peptide SFHQFARATLAS (HAP-1) [29]. In a recent study, HAP-1-modified microgels containing PLGA nanoparticles were found to be bound to rat and human synoviocytes *in vitro* and were retained within the synovial membrane and joint space of rats for 3 weeks without inducing degenerative activity [30■■]. Peptide sequence CKSTHDRLC coated on a PLA, polycaprolactone and PEG nanoparticle, specifically homed to the synovial MVE over 7 days following intravenous injection and suppressed arthritic activity in rats upon delivery of peptide-nanoparticle encapsulated methotrexate [31■]. Another MVE-targeting peptide, CKPFDRALC was coated onto Dex-encapsulated liposomes, leading to effective inhibition of arthritis progression in rats over a period of 3 weeks [32].

Hybrid systems, smart, environmentally sensitive and synovium-targeting methods, thus have the potential to increase intra-joint residence time of drugs, providing controlled drug release and enabling long-term therapeutic benefit. However, these strategies require complex formulation processes that can hinder their clinical translatability. Additionally, as these carriers cannot penetrate into the cartilage deep zones where most of the target sites reside, their use is limited to pain and inflammation relief.

INTRA-CARTILAGE DELIVERY

Articular cartilage is a dense, avascular tissue constituting of a meshwork of a high density of negatively charges aggrecans (35% dry weight), collagen II (50–60% dry weight) and a low density of chondrocytes (<5% dry weight), which together contribute to the tissue's structure and function [11■]. Aggrecans contain several highly sulfated GAG side chains conferring high negative fixed charge density (FCD) to the tissue that provides hydration, swelling pressures and compressive stiffness [11■,33]. As joints are loaded, increased electrostatic repulsion between the intra-cartilage negatively charged groups helps resist deformation enabling the tissue to re-swell and regain back its original shape [33]. Although these negatively charged aggrecans are critical for tissue function, they make penetration and drug delivery into cartilage extremely difficult; it is imperative that drugs and drug carriers reach the tissue deep zones as a majority of cells and matrix target sites reside there [10,34■]. Below, we present a variety of intra-cartilage nano-carrier-based drug delivery systems designed to target either the aggrecans, collagen II or chondrocytes enabling multistage drug delivery at tissue, cellular and intra-cellular levels (Table 2).

Aggrecan targeting

Bajpayee *et al.* [35] showed that particles have to be smaller than 10 nm in hydrodynamic diameter to be able to penetrate through the full thickness of normal cartilage; larger sized particles are sterically hindered and get trapped within the tissue's superficial zones. They showed that the high negative FCD of tissues can be converted from a barrier to drug entry into a drug depot by modifying drugs with optimally charged cationic domains such that the weak-reversible nature of electrostatic interactions can enhance their intra-cartilage transport, uptake and retention [35–38]. Avidin, a cationic glycoprotein, because of its optimal size (<10 nm in hydrodynamic diameter) and net charge (between +6 and +20) penetrated through the full thickness of rabbit cartilage in high concentrations and was present within the tissue even after 2 weeks of its intra-articular administration in a rabbit ACLT model of posttraumatic osteoarthritis (PTOA) [38]. Avidin was conjugated to four moles of a broad spectrum glucocorticoid, Dexamethasone (Dex), using hydrolysable ester linkers and administered in a single low-dose intra-articular injection 1 week following ACLT in a rabbit model [39]. Avidin-Dex suppressed injury-induced joint inflammation, synovitis and reduced incidence and severity of osteophyte formation significantly greater than free Dex [39]. To increase the drug-loading content of this delivery system, recently multiarm Avidin (mAv) constituting of branched PEG chains was developed providing 28 sites for covalent conjugation of small molecule drugs [Fig. 2a(i)]) [40■■]. Similar to Avidin, mAv also penetrated through the full thickness of healthy and osteoarthritis cartilage explants [Fig. 2a(ii)] [40■■]. mAv was conjugated to Dex (mAv-Dex) using a combination of hydrolysable ester linkers enabling controlled Dex release over a period of 2 weeks [41]; its single low-dose suppressed IL-1-induced GAG loss [Fig. 2a(iii)] and chondrocyte death in cartilage explants significantly more effectively than free Dex [40■■]. Avidinbased targeting strategies have also been used for targeting other negatively charged and aggrecan-rich tissues, such as the nucleus pulposus of intervertebral disks. Wagner *et al.* [42■] designed Avidingrafted dextran nanostructures with multiple drug conjugation sites to enable electrostatic binding with aggrecans, providing a month-long intra-discal retention. Using short-length arginine and lysine-rich cationic peptide carriers (CPCs, ~3 kDa) of varying net charge, it was recently shown that there exists an optimal net cationic charge that a drug of given size should possess to target a tissue of known negative FCD to rapidly penetrate through the full thickness of tissue in high concentrations [43■■]. Intra-cartilage uptake did not monotonically increase with net charge of CPCs; CPC +14 had the highest uptake (400× higher than an uncharged solute), greater than both CPC +8 and CPC +20, all of which had similar sizes [43■■]. CPC +8 and CPC +14 penetrated through the full cartilage thickness whereas CPC +20 did not, owing to stronger binding interactions with negatively charged aggrecans that hindered its penetrability and uptake [43■■]. This work highlighted that the optimal net charge on a carrier should be chosen to take advantage of Donnan partitioning-induced enhanced transport, such that charge interactions are weak enough for the carriers to rapidly get past the tissue superficial zones but strong enough to bind within tissue-deep zones for long-term retention [10,11■,43■■]. Another key finding was that short-range binding interactions like hydrophobic and H-bonds can synergistically stabilize long-range charge interactions, and thus can enhance drug retention within arthritic tissues, which have lost a majority of GAGs, and thus have lower negative FCD [43■■]; a feature that can be incorporated in carrier design.

Sangar *et al.* [44■■] recently identified cationic cysteine-dense peptide, CDP-11R, as a carrier that accumulates within cartilage because of its distribution of positive charge and disulfide-bonded tertiary structure, even when administered systemically via intravenous injection in healthy mice [Fig. 2b(i and ii)]. Upon conjugation of CDP-11R with triamcinolone acetonide (TCA), ankle joint inflammation [Fig. 2b(iii)] and off-target toxicities in RA rats were suppressed for 4 days following treatment [44■■]. Another study explored the use of variously charged green fluorescent proteins for enhancing cartilage penetration and retention as well [45■]. Sharma and co-workers modified PLGA nanoparticles (260–290 nm) with didodecyldimethylammonium bromide (DMAB) containing a quaternary ammonium cation and showed six-fold greater retention in healthy bovine cartilage explants because of binding with the tissue superficial zone compared with anionic polyvinyl alcohol (PVA)-modified PLGA nanoparticles. However, retention of cationic nanoparticles was reduced two-fold in presence of negatively charged synovial fluid and 2.9-fold in arthritic tissue, indicating the charge-dependency of nanoparticle retention [46]. They also demonstrated cationic PLGA nanoparticles for delivering KGN, a chondrogenic drug, coupled with a cartilage-binding bioadhesive to improve retention [47]. A more recent study focused on the scavenging of reactive oxygen species (ROS) utilized cationic manganese dioxide (MnO_2) nanoparticles for full depth cartilage penetration and chondrocyte targeting, leading to suppression of IL-1β-induced GAG loss and NO release from cartilage explants [48■]. In-vivo rat studies revealed intra-joint residence for over 1 week with the nanoparticle accumulating on chondral surfaces [48■].

PBAEs have also been considered as they are inexpensive, biocompatible, cationic and can be end-capped with therapeutics [49]. Perni *et al.* recently chemically modified and optimized PBAE components (amine, acrylate and end-capping) for enhancing their ability to target and bind with cartilage. The optimized PBAE chain conjugated to Dex showed an eight-fold increase in cartilage uptake compared with free Dex [49,50], likely owing to adsorption within the cartilage superficial zones. This DDS resulted in significantly reduced IL-1-induced cartilage degradation compared with free drug *in vitro* [49]. Positively charged sixth generation polyamidoamine (PAMAM) dendrimers (6.7 nm in diameter) have also been utilized for enhancing intra-cartilage penetration and retention of insulin-like growth factor-1 (IGF-1) in rat knee joints. PAMAM-IGF-1 significantly suppressed cartilage degeneration and osteophyte formation compared with untreated control and unmodified IGF-1 in a rat ACLT model at 4 weeks postsurgery [51].

Collagen II targeting

Using phage display, a collagen II-binding peptide sequence, WYRGRL was discovered and it has been widely used for cartilage targeting [52]. This peptide was shown to be retained within the deep zones of healthy and GAG-depleted osteoarthritis cartilage for 48 h, whereas cationic chitosan suffered from a significant drop in retention in GAG-depleted cartilage compared with that in normal tissue [53■]. Following conjugation to Dex using ester linkers, the collagen-targeting prodrug demonstrated a drug-release half-life of 35.8 ± 9.0 h in presence of PBS that remained unchanged in low concentration esterase solution [53■]. The prodrug was able to significantly reduce IL-1β induced GAG loss in an in-vitro bovine cartilage explant model [53■]. In another study, WRYGRL was genetically displayed onto an

MMP13 and pH-responsive ferritin nanocage for delivery of anti-inflammatory drug hydroxychloroquine (HCQ) to cartilage [54▪]. This HCQ nanostructure was retained for 14 days in osteoarthritis mice cartilage, resulting in suppression of synovial inflammation [54▪]. Ferritin nanocages functionalized with this peptide have also been used for metformin delivery [55]. A recent study utilized this peptide for delivering microgels containing PLGA nanoparticles tagged with rhodamine B to healthy and osteoarthritis rats, leading to significant binding with articular cartilage as well as increased residence time (up to 26 days) compared with free dye [30▪▪]. Bedingfield *et al.* [56▪] recently utilized a monoclonal antibody that specifically targets type II collagen (mAbCII) for delivering MMP13 siRNA. In a mouse PTOA model, significantly higher MMP13 silencing was achieved compared with noncollagen-targeting siRNA, contributing towards improved OARSI scores [56▪]. A newly devised strategy for targeting type II collagen is the use of Avimers, which are small derivations of cell surface protein A-domains involved in protein–protein interactions [57▪▪]. Avimer M26 displayed high collagen II specificity, allowing for 1 month intra-joint and intra-cartilage retention following intra-articular injection into rat knees [Fig. 2c(i)] [57▪▪]. Further-more, intra-articular delivery of IL-1Ra fused M26 suppressed IL-1 induced IL-6 expression more significantly than free IL-1Ra [Fig. 2c(ii)] [57▪▪]. It should be noted that unlike charge interactions, strong binding of nanocarriers with collagen II can hinder their transport and penetration through the full thickness of normal or early-stage osteoarthritis cartilage [11▪]. Most of the above discussed work has utilized mouse or rat models that have very thin cartilage, and thus the transport data from these studies should be interpreted cautiously as these models are not appropriate for studying intra-articular transport kinetics and drug delivery [37,38]. Solutes penetrate much faster through thin cartilage than thick, as the diffusion time scales as the square of tissue thickness [38]. It is imperative to validate these results using larger animal models with thicker cartilage more like that of humans as the performance of the DDS not only depends on its size and surface properties but also on the biophysical properties of the animal joint [10,38]. Therefore, targeting collagen II for drug binding should be considered especially in later stages of the disease where a majority of aggrecans in cartilage have been degraded and results should be validated in large animal models [11▪].

Chondrocyte targeting

As the drug ultimately has to be delivered to cell receptors, drug carriers are functionalized with chondrocyte- targeting motifs in combination with aggrecan and collagen-targeting strategies to facilitate multistage drug delivery [58,59,60▪]. A chondrocyte affinity peptide, DWRVIIPPRPSA, discovered by Pi *et al.* [59] was recently functionalized on hesperetin-loaded $GD_2(CO_3)_3^-$ nanoparticles [61▪]. In ACLT mice, the construct exhibited strong cartilage specificity, alleviating cartilage degradation and IL-1-induced apoptosis and inflammation [61▪]. Melittin-derived positively charged peptide VLTTGLPALISWIRRRHRRHC (p5RHH) was previously shown to have strong chondrocyte and cartilage-penetrating ability (up to 700 μm depth) [62]; it was recently modified for delivery of NF-κB p65 siRNA to IL-1 treated cartilage explants, resulting in suppression of p65 for 3 weeks and attenuating cell death [63▪].

Gene delivery

Gene therapy has emerged as a promising strategy for manipulation of expression levels of diseaseassociated genes via a controlled and targeted mechanism [64]. Genetic materials are introduced into cells via viral or nonviral vectors to induce long-term overexpression or silence a selected gene, thereby creating a prolonged therapeutic response. This technique is especially advantageous for slow-progressing diseases like osteoarthritis, where ablation of catabolic (ADAMTS5, MMP13) and pro-inflammatory (IL-1, TNFα) genes or introduction of anabolic [IGF-1, transforming growth factor β (TGFβ)] and anti-inflammatory [IL-1 receptor antagonist (*IL-1Ra*), *IL-4*] genes have the potential to prevent further cartilage damage while promoting tissue regeneration [64,65].

Early gene therapy strategies utilized recombinant adenoviruses to encode human IGF-1 into rabbit knees, resulting in increased matrix synthesis from the joint cartilage [66]. Frisbie *et al.* [67] used an adenoviral vector to overexpress intra-articular IL-1Ra in an equine osteoarthritis model and showed elevated expression for 28 days leading to significant improvements in pain, cartilage preservation and synovial membrane histological parameters. However, the most successful strategy to date has been the use of adeno-associated (AAV) or helper-dependent adeno-viruses (HDAV) as carriers for genes for in-vivo transduction. These are small (20–25 nm diameter), nonenveloped, single-stranded DNA viruses that are dependent upon a helper virus – either adenovirus or herpesvirus, for replication [68]. Therefore, despite their limited transgene capacity (<4.8 kb), the absence of all viral-coding sequences makes AAVs less immunogenic and a more attractive option for a well tolerated gene delivery system [69]. Recently, Tang *et al.* [70▪] showed that AAV-mediated delivery of follistatin – a protein involved in enhancing muscle formation by neutralizing members of the TGF-β superfamily, to mice prior to medial meniscus destabilization prevented posttraumatic osteoarthritis like changes within the joint. In a similar mouse model, Ashraf *et al.* delivered Ras homolog enriched in brain (*RHEB*) gene via intra-articular injection, resulting in suppression of ADAMTS5 and MMP13 and overexpression of COL2A1 immunohistochemical staining, while reducing apoptosis [71▪]. It was recently suggested that a combination of genes, such as IL-1Ra with proteoglycan 4 (PRG4) on individual HDAV carriers can provide enhanced therapeutic benefit over monotherapy when delivered to mice suffering from PTOA [72▪]. Recent work has incorporated promoters within the delivery systems to ensure that only diseased cells express and secrete the desired gene [72▪,73].

Gene editing

A new development in osteoarthritis therapy is the use of CRISPR/Cas9 technology to ablate disease-causing genes. An efficient gene-editing technique, this strategy employs a complex of Cas9 proteins and an engineered single guide RNA, which recognize and introduce a double-stranded break in the target DNA [74▪▪]. The DNA undergoes a repair process, which causes insertions or deletions resulting in disruption, thereby eliminating gene expression [74▪▪]. Zhao *et al.* [74▪▪] delivered an AAV expressing CRISPR/Cas9 to mice via intra-articular injection to target genes encoding MMP13, IL-1β and nerve growth factor (NGF). NGF ablation was able to mitigate pain induced by partial meniscectomy while disruption of MMP13 and IL-1β reduced the expression levels of cartilage-degrading

enzymes [74■■]. Similarly, Seidl *et al.* [75] demonstrated reduced MMP13 levels and enhanced type II collagen accumulation in healthy and osteoarthritis human articular chondrocytes when administered ribonucleoprotein complexes containing CRISPR/Cas9 technology targeting the *MMP13* gene. RNA interference (RNAi) is another strategy for inhibiting gene expression or translation via targeting of mRNA molecules. Small interfering RNA (siRNA) – double-stranded noncoding RNA molecules of 20–25 bp length, targeting the silencing of the *p66shc* gene were encapsulated within PLGA nanoparticles and delivered via intra-articular injection to osteoarthritic mice [76■]. Silencing of the *p66shc* gene, which is implicated in the generation of mitochondrial reactive oxygen species (mtROS), resulted in alleviation of cartilage damage and pain behavior as well was suppression of IL-1β, TNFα and Cyclooxygenase 2 (COX2) expression levels [76■]. Using the same PLGA-based nanoparticle system, Shin *et al.* [77] also silenced *p47phox* to reduce ROS-induced chondrocyte damage in an osteoarthritis rat model.

On the basis of the success shown in preclinical models and its current development in clinical trials, AAV and HDAV seem to be the most well tolerated and promising carriers for genes for osteoarthritis therapy. Overexpression of anabolic and anti-inflammatory genes have the potential to prevent further cartilage damage and related catabolic activity within the joint, while gene ablation and silencing through CRISPR/Cas9 and RNAi approaches remain interesting areas of future research.

OSTEOARTHRITIS DRUG DELIVERY SYSTEMS IN CLINICAL TRIALS

Particle-based delivery systems

Taiwan Liposome Company (TLC) developed TLC599, a Dex-sodium phosphate incorporated liposome (~130 nm), for intra-articular delivery for knee osteoarthritis patients (Table 3). The hydrophobic surface of liposome particles enhances their binding within the hydrophobic synovial fluid and their large size prevents them from exiting via the lymphatics. In a phase II clinical trial (NCT03005873), intra-articular injection of 12 mg TLC599 resulted in greater suppression of pain from week 1 through week 24 compared with placebo [78■]. Currently, there are two ongoing clinical trials targeting knee osteoarthritis with TLC599. NCT03754049 is a 90 participant phase II study focused on pharmacokinetic evaluation [79] whereas NCT04123561 is a 500 participant phase III study focused on efficacy [80].

Recently, intra-joint sustained release formulation of TCA encapsulated within micron-sized PLGA particles (Flexion Therapeutics product FX006, Zilretta) received clinical approval for osteoarthritis pain relief as it showed prolonged synovial fluid joint residency for 12 weeks, owing to its large micron size (20–100 μm), following a single intra-articular injection in patients with knee osteoarthritis [81]. The efficacy of FX006 in patients with unilateral knee osteoarthritis was evaluated in a phase III study with results showing significant improvements in WOMAC and ADP (average-daily-pain) scores compared with saline and free drug over 24 weeks [82■■]. Following approval, this product has been under review in clinical trials for 24-week synovial inflammation (NCT03529942) [83■] and performance measures in bilateral knee osteoarthritis patients (NCT03895840) [84]. Another microparticle product, EP-104IAR (60–150 μm) developed by Eupraxia Pharmaceuticals,

formulated fluticasone propionate and PVA for intra-articular treatment of osteoarthritis [85]. A 238 patient phase II study evaluating safety, efficacy and pharmacokinetics is currently in the prerecruitment stage (NCT04120402) [86].

Hydrogel-based delivery systems

Cingal, a chemically cross-linked hyaluronic acid gel loaded with triamcinolone hexacetonide is currently under review in a phase III trial for pain relief evaluation at 26 weeks (NCT04231318) [87■]. A clinical trial for knee osteoarthritis pain, NCT03209362, evaluated intra-articular injections of SI-613, an injectable hyaluronic acid-based formulation incorporating diclofenac, however, results are still pending [88]. A similar DDS, encapsulating polynucleotides, was administered to knee osteoarthritis patients, however, no significant differences in WOMAC score were reported at 6 months time point (NCT02417610) [89].

Gene delivery systems

Clinically, IL-1Ra (interleukin-1 receptor antagonist) is most commonly employed osteoarthritis therapeutic for gene delivery; currently there are two ongoing trials for intra-articular delivery using an AAV (NCT02790723) [90■] and an HDAV (NCT04119687) [91■]. A phase I study delivering XT-150 – a plasmid DNA with a variant of IL-10, was recently completed, however, results have not yet been published [92]. Use of most other types of viral and nonviral vectors for osteoarthritis therapy have faced difficulty in transducing chondrocytes in their in-vivo environment [93]. Thus, their use in ex-vivo approaches, where patient cells can be extracted and then transduced with a gene prior to depositing them back into the joint space, is of interest [93]. This strategy has been commonly employed with synoviocytes to deliver IL-1Ra and IL-10 genes in experimental models of osteoarthritis [94,95]. Invossa, a product combining TGF-β1 transduced and nontransduced chondrocytes for intra-articular delivery has been approved for treating osteoarthritis in South Korea and is currently under review in a phase III clinical trial (NCT03203330) [96■]. A complete list of ongoing clinical trials evaluating drug delivery systems for treatment of knee osteoarthritis can be found in Table 3.

CONCLUSION

Synovial joint and cartilage-targeting strategies can enable clinical translation of a variety of osteoarthritis drugs that despite strong preclinical evidence have not translated to practice yet. Recent years have witnessed significant increase in both basic science and clinical studies evaluating drug delivery systems for osteoarthritis treatment. Steroid-encapsulating polymeric micron particles for providing longer lasting pain relief were recently approved for clinical use. Electrically charged biomaterials for intra-cartilage targeting and delivery of DMOADs have shown promising results in preclinical models warranting studies with larger animal models. With ongoing clinical trials, gene delivery has the potential to become an effective therapy especially if disease biomarkers at various stages of osteoarthritis can be detected and targeted at early timepoint to prevent further disease progression.

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KEY POINTS

- Drug delivery for osteoarthritis therapy remains a challenge because of rapid joint clearance following intra-articular administration and the inability to penetrate through the dense cartilage matrix to reach target cells.
- To prolong joint residence times and provide sustained drug release intended for pain and inflammation relief, delivery systems like hydrogels, micelles, polymeric particles are in consideration owing to their large size or viscous nature.
- Steroid encapsulating polymeric micron sized particles for providing longer lasting pain relief were recently approved for clinical use.
- To restore joint structure and function, osteoarthritis drugs must penetrate through the full thickness of cartilage to reach their cellular and intra-cellular targets; electrically charged carriers targeting negatively charged aggrecans have shown promise in preclinical models.
- Current clinical trials are evaluating the safety of viral-vectors whose success can pave the way for gene therapy as osteoarthritis treatment.

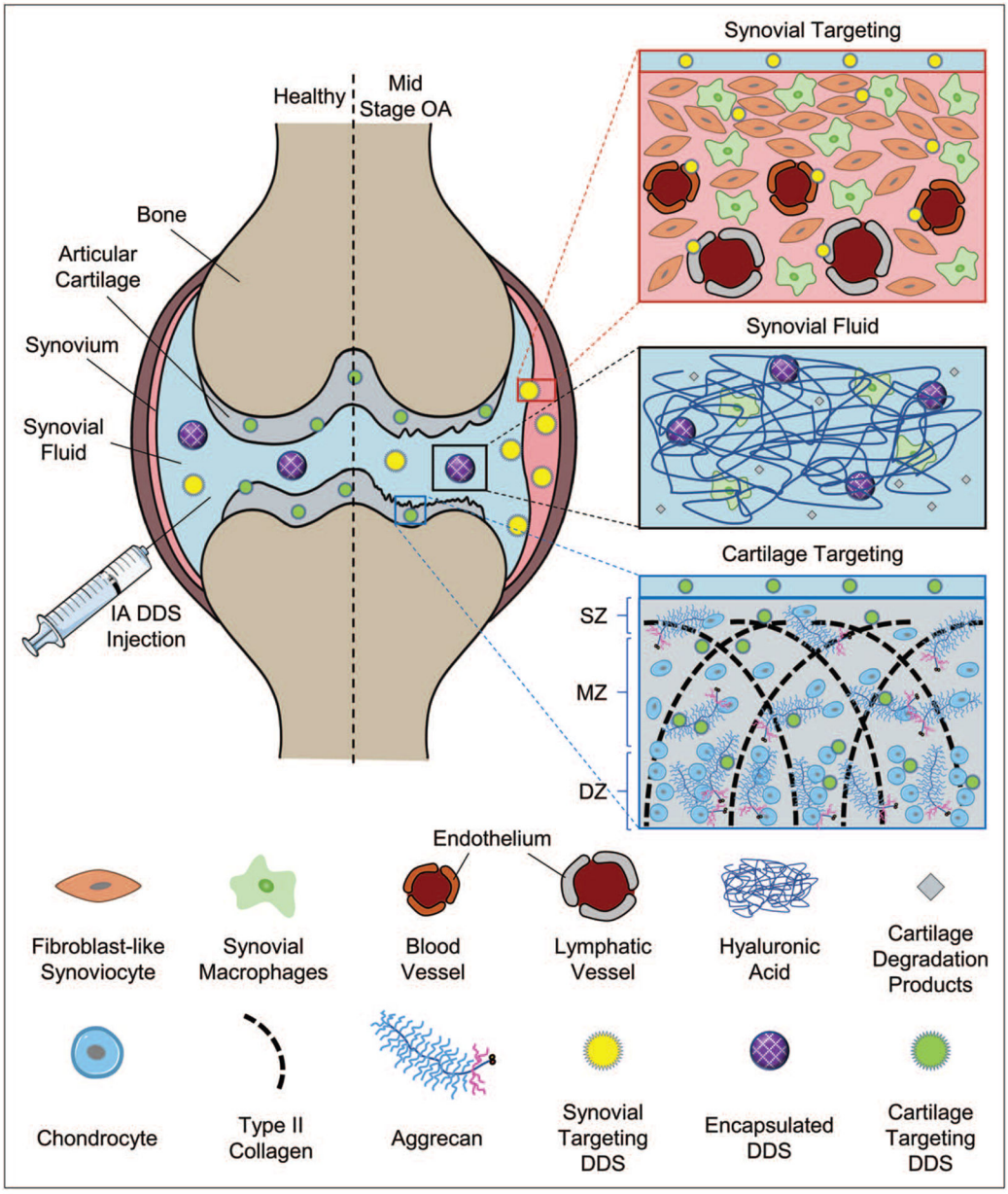

FIGURE 1.

Schematic showing healthy and mid-stage osteoarthritis of the knee. In osteoarthritis, the synovium undergoes hypertrophy with an increase in synovial macrophages and fibroblast-like synoviocytes (FLS) accompanied by an outgrowth of blood and lymphatic vessels (angiogenesis), which contribute towards significant pain and inflammation. The environment of the synovial fluid becomes acidic and infiltrated by macrophages and cartilage degradation products. Cartilage and its matrix components (aggrecan and collagen II) begin to degrade, while chondrocytes enter a hypertrophic and apoptotic state. To prolong drug residence and provide long-term osteoarthritis therapy, drugs can be administered via intra-articular injection and modified in the form of drug delivery systems (DDS) to specifically target intra-joint components as shown in the synovium (top), synovial fluid

(middle) and cartilage (bottom) insets. DDS can be designed for targeting the synovium (FLS, macrophages, microvasculature endothelium), prolonging synovial fluid residence or targeting the cartilage (aggrecan, collagen II, chondrocytes).

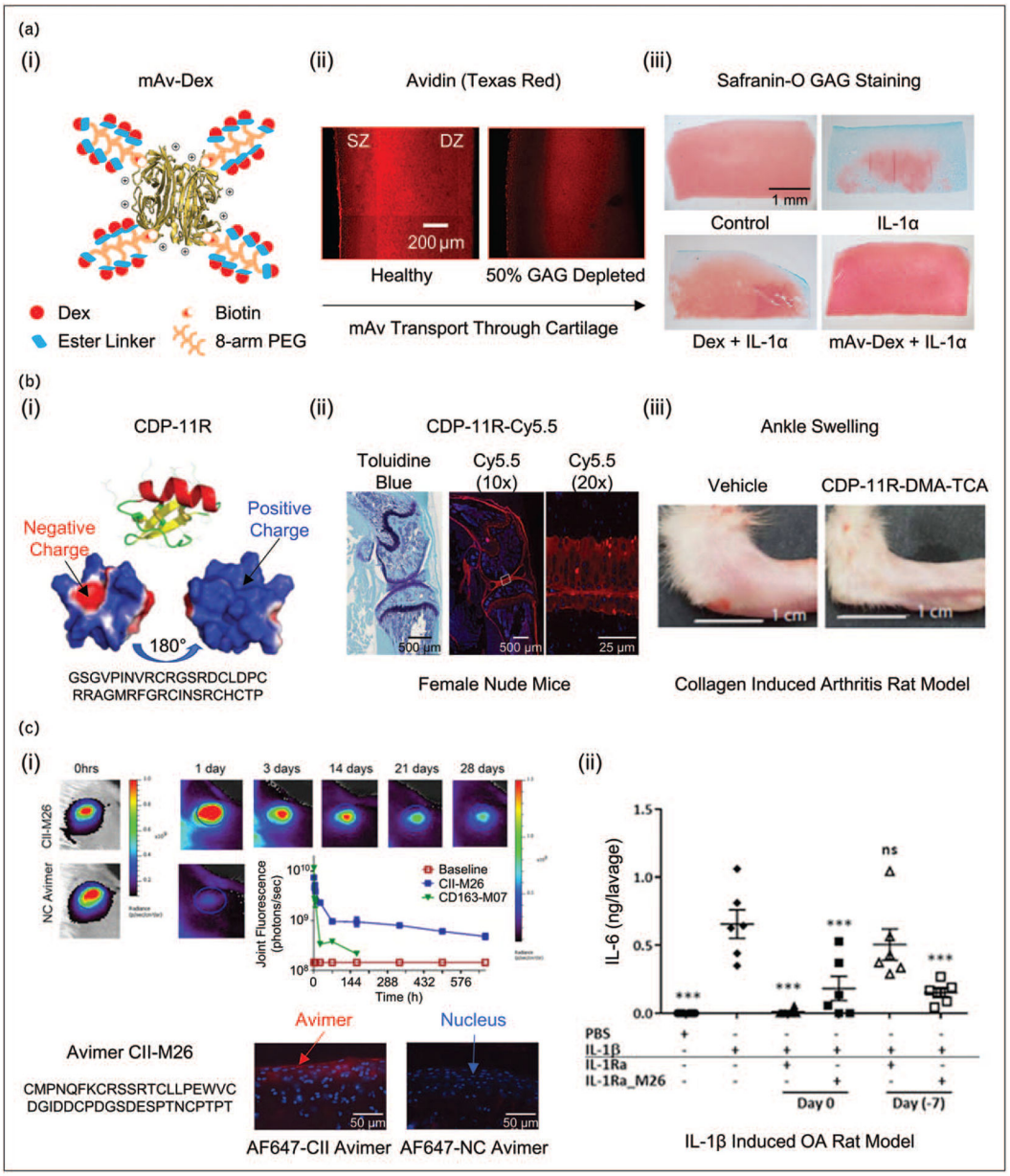

FIGURE 2.

(a, I) Multiarm Avidin conjugated to Dex (mAv-Dex) via controlled release ester linkers. (ii) Confocal imaging showing full thickness penetration from superficial zone (SZ) to deep zone (DZ) of mAv-Dex in healthy and GAG-depleted cartilage explants within 24 h. (iii) A single low dose of mAv-Dex suppressed IL-1-induced GAG loss significantly greater than free Dex at 16 days as shown by Safranin-O/fast green staining of cartilage explants. Adapted with permission from reference [40■■]. (b, I) X-ray crystallography showing structure ribbon (top) and molecular surface representations (bottom) of CDP-11R (α-helices: red; β-strands: yellow; random coil: green; disulfide bonds: gold). Positive (blue) and negative (red) electrostatic potentials are also shown. (ii) Mouse knee joint stained with Toluidine Blue (left). Cy5.5 and DAPI channel fluorescent images of CDP-11R-Cy5.5 (red)

localized to articular cartilage following intravenous injection. (iii) Suppression of CIA rat ankle joint swelling following intravenous injection of CDP-11R–DMA–TCA on treatment day 4. Adapted with permission from reference [44■■]. (c, I) IVIS imaging showing collagen II-binding Avimer (CII-M26) being retained within rat knee joint for 28 days after intra-articular injection compared with negative control (NC), which was cleared within 1 day. Region of interest of each image is plotted as fluorescence vs. time. DAPI (nucleus: blue) and AF657 staining (Avimer: red) of articular cartilage confirms intra-tissue presence of CII-M26 for 28 days through confocal imaging. (ii) IL-1Ra-M26 [given either at the same time as (day 0) or 7 days prior to IL-1β intra-articular injection) suppresses IL-6 expression in synovial fluid of rat knees at 4 h following treatment. ***P 0.001 or less. Adapted with permission from from reference [57■■].

Table 1.

Recent developments in intra-joint drug delivery systems

DDS	Drug	In-vivo model	Major outcomes		References
Hybrid systems					
Micelle and hydrogel	EPA	PTOA – mouse DMM	4-week sustained release; suppression of GAG loss, expression levels of IL-1β and MMP13 and NF-κB ÿignalling pathway for 8 weeks		[15■]
Gold NP and liposomes	Fish oil protein	OA – Mouse bacterial collagenase injection	Release over 24 h; suppression of expression levels of TNFα and IL-6 and NF-κB ÿignalling pathway over 15 days		[16■]
Liposome and hydrogel	Kartogenin	PTOA – Rat DMM	5-week joint retention; 75% sustained release over 25 days; reduction of osteophytes, lesser decrease in aggrecan and type II collagen expression		[17■]
Smart joint environnent responsive systems					
HMS NP modified with chitosan	Celastrol	OA – Rat MIA injection	pH responsive	68.9% release at pH 6.0, 21.7% release at pH 7.0 over 24 h; improvements in paw withdrawal threshold, articular surface erosion and joint effusion	[18■]
PLGA NP encapsulated with NH_4HCO_3	HA	PTOA – mouse DMM	pH responsive	Joint retention over 35 days, sustained drug release over 10 days at pH 5.0; reduced osteophyte formation, did not worsen OA progression over 35 days	[19■■]
MOF modified with HA	PCA	PTOA – rat ACLT	pH responsive	13% release at pH 7.4, 23% at pH 5.6 over 24 h; reduction in synovial inflammation, downregulation of inflammatory markers, promotion of cartilage-specific marker expression for 8 weeks	[20■]
PBAE modified with Curcumin	Curcumin	OA – mouse MIA injection	pH responsive	Sustained release over 7 days at pH 6.0; suppression of IL-1 and TNF-α production, improvements in articular surface erosion at 28 days	[21■]
MoS_2 nanosheet modified with chitosan	Dex	OA – mouse papain injection	NIR radiation triggered	Dex released on demand by controlling the NIR light source chitosan, prolonged residence time; attenuated cartilage erosion, reduced toxicity, suppression of MMP13, ADAMTS5 over 28 days	[22■]
Hemoglobin and PLGA-PEG NP	NO, Notch1 siRNA	OA – mouse papain injection	NIR radiation triggered	24 h joint retention; photothermal-triggered NO release; inhibition of pro-inflammatory cytokine expression, prevention of cartilage erosion	[23■■]
N-isopropyl acrylamide	MK2-inhibiting peptide	PTOA – IL-1β chondrocytes; healthy – rat	Temperature responsive	Joint retention time over 7 days, sustained release over 5 days at 37°C; suppression of IL-6 production for 4 days	[24■■]
Targeted systems					
PDN surfaced with FA-modified HA ligand	CORM-401	OA – rat MIA injection	Macrophage targeting	Suppression of IL-1β, IL-6, TNFα secretion, inhibition of CO release, depletes ROS in OA joints for 23 days	[25■]
ZIF-8 NP modified with anti-CD16/32 Ab	SMT, CAT	PTOA – mouse ACLT	Macrophage targeting	4-day joint retention; both drugs release rapidly at pH 5.4 and release steadily at pH 7.4 over 24 h; rescuing of mitochondrial function, inhibition of cartilage degradation for 4 weeks	[26■■]
Dextran sulfate-TCA	TCA	OA – mouse MIA injection	Macrophage targeting	24 h drug release; targeting specificity for SR-A on macrophages; alleviation of structural cartilage damage and pro-inflammatory cytokine expression for 3 weeks	[27■]
Microgel with PLGA, modified with HAP-1	–	PTOA – rat MMT	FLS Targeting	Specific binding to rat and human synoviocytes; trapped withing synovial membrane, 3-week intra-joint retention with no degenerative changes	[30■■]

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DDS	Drug	In-vivo model	Major outcomes		References
PLA-PCL-PEG NP modified with CKSTHDRLC	Methotrexate	RA – rat AIA and CIA	MVE targeting	Specific homing to MVE, accumulation in inflamed joints; prevented AIA and CIA at low dose and lower frequency, prevention of neoangiogenesis and synovial inflammation	[31■]
Liposome modified with CKPFDRALC	Dex	RA – rat MTB injection	MVE targeting	Enhanced endothelial cell binding, 6-day intra-joint retention; improvement in arthritis scores, no further adverse effects	[32]

Ab, Aantibody; ACLT, Anterior cruciate ligament transection; ADAMTS, a disintegrin and metalloproteinase with a thrombospondin motif; AIA, antigen-induced arthritis; CAT, catalase; CIA, collagen-induced arthritis; CORM, carbon monoxide release molecules; Dex, dexamethasone; DMM, destabilization of the medial meniscus; EPA, eicosapentanoic acid; FA, folic acid; FLS, fibroblast-like synoviocyte; GAG, glycosaminoglycan; HA, hyaluronic acid; HMS, hollow mesoporous silica; IL, interleukin; MIA, monosodium iodoacetate; MK2, mitogen-activated protein kinase-activated protein kinase 2; MMP, matrix metalloproteinase; MMT, medial meniscus transection; MOF, metal organic framework; MTB, Mycobacterium tuberculosis; MVE, microvasculature endothelium; NF-κB, nuclear factor κB; NIR, near infrared; NO, nitric oxide; OA, osteoarthritis; PBAE, poly-beta-amino-ester; PCA, protocatechuic acid; PDN, peptide dendrimer nanogel; PEG, polyethylene glycol; PLGA, poly-lactic co-glycolic acid; PTOA, posttraumatic OA; RA, rheumatoid arthritis; ROS, reactive oxygen species; SMT, S-methylisothiourea hemisulfate salt; SR-A, scavenger receptor class A; TCA, triamcinolone acetonide; TNFα, tumor necrosis factor α; ZIF, zeolitic imidazolate framework.

Table 2.

Recent developments in intra-cartilage nanoparticle-based drug delivery systems targeting aggrecans, collagen type II, chondrocytes for applications in gene delivery

Targeted carrier	Drug	Model	Major outcomes	References
Aggrecan				
Multiarm Avidin	Dexamethasone	PTOA – bovine cartilage explants	Full depth cartilage penetration, 2-week sustained release; suppression of GAG loss, cell death, inflammation	[40■■]
Avidin grafted dextran	–	LBP – bovine nucleus pulposus explants	Month long intra-tissue retention through combined effects of size and charge	[42■]
Cationic peptide carriers (CPC)	–	Healthy and OA – bovine cartilage explants	Rapid full thickness penetration, high uptake and 7-day retention with CPC + 14; weak and reversible binding required for full depth penetration	[43■■]
Cysteine dense peptides (CDP)	Dexamethasone, TCA	RA – rat collagen-induced arthritis	Cartilage accumulation with CDP-11R following systemic IV injection; reduction of joint inflammation and off-target toxicities	[44■■]
Supercharged – green fluorescent proteins (S-GFP)	–	Healthy – human, bovine cartilage explants	High-uptake and fast transport through full thickness with S-GFP + 9 and S-GFP + 15	[45■]
MnO_2 NP	–	PTOA – bovine cartilage explants; healthy rats	Full depth cartilage and chondrocyte penetration, suppression of IL-1- induced GAG loss and NO release; less than 1-week intra-joint residence, accumulation on chondral surfaces	[48■]
Poly-beta-amino esters (PBAE)	Dexamethasone	PTOA – bovine cartilage explants	Increased dexamethasone uptake eight-fold, prevented IL-1-induced cartilage degradation	[49]
PAMAM	IGF-1	PTOA – rat ACLT	10-fold increase in joint residence time for 30 days; reduced cartilage degradation and osteophyte burden	[51]
Type II collagen				
WYRGRL	Dexamethasone	PTOA – bovine cartilage explants	Deep zone retention; reduced inflammatory markers, GAG loss	[53■]
WRYGRL	Hydroxychloroquine	OA – mice papain injection	14-day retention; suppression of synovial inflammation	[54■]
WRYGRL	Metformin	OA – mice papain injection	3–4-week retention; reduced inflammation	[55]
WYRGRL	–	PTOA – rat MMT	Specific binding to bovine articular cartilage, increased intra-joint half-life and retention *in vivo* for 26 days	[30■■]
mAbCII	MMP13 siRNA	PTOA – mice repetitive joint loading	Enhanced reduction in MMP13 expression; improved OARSI scores	[56■]
Avimer M26	IL-1Ra	PTOA – rat IL-1β injection	1-month retention; enhanced suppression of IL-6	[57■■]
Chondrocyte				
DWRVIIPPRPSA	Hesperetin	PTOA – mice ACLT	Alleviation of gradual degeneration of cartilage via TLR-2 inhibition	[61■]
p5RHH	NF-κB p65 siRNA	PTOA – bovine cartilage explants	3-week suppression of p65; attenuation of cell death	[63■]
Gene delivery				
AAV9	Follistatin	PTOA – high-fat diet mice DMM	Reduction in cartilage degeneration, synovitis, pro-inflammatory cytokine expression and mechanical algesia at 12 weeks; enhanced muscle growth	[70■]

AV	RHEB	PTOA – mouse DMM	Inhibition of OA progression at 8 weeks, regulation of ADAMTS5 and MMP13, reduction in apoptosis	[71■]
HDAV w/Ef1 or NF-κB promoter	PRG4, IL-1Ra	PTOA – mouse DMM or CLT	Enhanced preservation of articular cartilage volume, surface area, increased expression of cartilage matrix genes with combination therapy at 10 weeks	[72■]
HDAV w/NF-κB promoter	IL-1Ra	PTOA – mouse CLT	Lowered OA scores, increased cartilage volume and surface area	[73]
Targeted carrier	**Target gene**	**Model**	**Major outcomes**	**Referencess**
Gene editing				
AAV w/CRISPR-Cas9	MMP13, IL-1β, NGF	PTOA – mouse partial meniscectomy	Alleviation of pain but worsening of joint damage with NGF ablation, attenuation of structural damage with deletion of MMP13 and IL-1β; combination therapy mitigates adverse events of NGF ablation at 3 months	[74■■]
Ribonucleoprotein complexes w/ CRISP-Cas9	MMP13	Healthy and OA – human chondrocytes	Significant reduction in MMP13 secretion and activity levels, enhanced type II collagen accumulation for 7 days	[75]
PLGA NP w/siRNA	p66shc	OA – rat MIA injection	96.4% release in 48 h *in vitro;* attenuation of ROS production, amelioration of pain behavior, cartilage damage and IL-lβ, TNFα, COX2 production levels for 21 days	[76■]
PLGA NP w/siRNA	P47phox	OA – Rat MIA injection	53.2% burst release at 24 h; attenuation of oxidative stress, proteoglycan loss, articular cartilage calcification and apoptosis for 14 days	[77]

AAV, adeno-associated virus; ACLT, anterior cruciate ligament transection; AV, adenovirus; CDP, cysteine dense peptide; CLT, cruciate ligaments transection; CPC, cationic peptide carrier; DMAB, didodecyldimethylammonium bromide; DMM, destabilization of the medial meniscus; Ef1, elongation factor 1; GAG, glycosaminoglycan; HDAV, helper-dependent adenovirus; IGF-1, insulin-like growth factor-1; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; IV, intravenous; LBP, low back pain; MIA, monosodium Iodoacetate; MMP, matrix metalloproteinase; MnO_2, manganese dioxide; NF-κB, nuclear factor κB; NGF, nerve growth factor; NO, nitric oxide; OA, osteoarthritis; PAMAM, polyamidoamine; PBAE, poly-beta-amino-ester; PLGA, poly-lactic co-glycolic acid; PRG4, proteoglycan 4; PTOA, posttraumatic OA; RA, rheumatoid arthritis; RHEB, Ras homolog enriched in brain; S-GFP, supercharged green fluorescent protein; siRNA, small interfering RNA; TCA, triamcinolone acetonide; TNFα, tumor Necrosis Factor α.

Table 3.

Ongoing clinical trials evaluating drug delivery systems for treatment of knee osteoarthritis

Trial (start year)	Phase	Sponsor	Product	Drug	Delivery system	Clinical outcomes
Particle-based delivery systems						
NCT03754049 (2019)	II	Taiwan Liposome Company	TLC599	Dexamethasone	Liposome	**PK parameters**, AE
NCT04123561 (2019)	III					**WOMAC**, PGIC
NCT03529942 (2018)	III	Flexion Therapeutics	Zilretta (FX006)	Triamcinolone Acetonide	PLGA	**Synovial volume**
NCT03895840 (2018)	IV					**Chair standing test, Fast paced walking test, Stair climb**, KOOS, NRS for pain
NCT04120402 (2020)	II	Eupraxia Pharmaceuticals	EP-104IAR	Fluticasone propionate	PVA	**WOMAC**, OMERACT-OARSI
Hydrogel-based delivery systems						
NCT04231318 (2020)	III	Anika Therapeutics	Cingal	Triamcinolone Hexacetonide	Crosslinked HA hydrogel	**WOMAC**
NCT03209362 (2017)	II	Seikagaku Corporation	SI-613	Diclofenac	HA hydrogel	**WOMAC**
Gene delivery						
NCT02790723 (2019)	I	Mayo Clinic	sc-rAAV2.5 IL-1Ra	Interleukin-1 Receptor Antagonist (IL-1Ra)	Self-complementary recombinant adeno-associated virus	**AE**
NCT04119687 (2019)	I	Flexion Therapeutics	FX201	IL-1Ra	Helper-dependent adenoviral vector	**AE**, systemic biodistribution
NCT03769662 (2019)	I	Xalud Therapeutics	XT-150	Interleukin-10 (IL-10)	Plasmid DNA	**AE**, KOOS, Verbal Numeric Rating Score, Clinical Global Improvement
NCT03203330 (2018)	III	Kolon TissueGene	TissueGene-C	Transforming Growth Factor β-1 (TGF-β1)	Transduced and nontransduced chondrocytes	**WOMAC, VAS**, MRI, Physical Component Score, Health Assessment

Primary clinical outcomes bolded. AE, adverse events; HA, hyaluronic acid; KOOS, Knee Injury and Osteoarthritis Outcome Score; NRS, Numeric Rating Scale; OMERACT-OARSI, Outcome Measures in Rheumatology-Osteoarthritis Research Society International; PGIC, Patient Global Impression of Change; PK, pharmacokinetics; PLGA, poly-lactic-co-glycolic acid; PVA, polyvinyl alcohol; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.