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Non-surgical Osteoarthritis Therapy, Intra-Articular Drug Delivery Toward Clinical Applications

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Abstract

Osteoarthritis (OA) is a common orthopedic disease in middle-aged and aged people. To date, no disease-modifying drug is available to prevent the progression of OA. Surgical treatment of OA has complications such as pain and high costs with increased risk of post-operative infections. An intra-articular drug delivery is a conservative treatment method to apply therapeutic composites directly into the OA joint cavity. This method has an advantage to improve the bioavailability of therapeutics and hence is a widely preferred choice to test novel disease-modifying drug targets for OA. Herein, we summarized and discussed the current status of intra-articular therapy for OA treatment as well as outlined drug delivery of small molecular, protein and gene delivery for OA therapy. Currently, new targeted nano-based drug delivery systems, including nanoparticles, exosomes and hydrogel formulations under investigation for OA treatment via intra-articular injection are also addressed. The emerging trend demonstrates that intra-articular drug delivery has vast prospects for the clinical selective treatment of OA. The rational application of intra-articular injection of drugs and biological agents will be of great significance for
alleviating the patients with OA, improving their quality of life, delaying surgery, and reducing the disease burden of OA.

**Keywords:** Intra-articular injection; Drug delivery; Nanomaterials; Target delivery; Osteoarthritis; Gene therapy

**Background**

Osteoarthritis (OA) is a degenerative joint disorder that commonly affects older people. It is mainly characterized by chronic pain, immobility of joints, and joint instability. The main pathological characteristics include articular cartilage damage, osteophyte formation, and subchondral osteosclerosis. The current prevalence of worldwide OA was approximately 303.1 million, with over 50% persons aged 50 years or older [1]. Surgical treatments are the only option for the end stage of OA and only performed on strict surgical indications with high treatment cost. Therefore, hip and knee replacement surgery should not be the first choice of OA treatment [2, 3]. Current drug intervention mainly relieves pain and provides anti-inflammatory effects, working as symptom-directed treatments, such as systematic use of nonsteroidal anti-inflammatory drugs (NSAIDs) and local intra-articular delivery of hyaluronic acid.

Articular cartilage is mainly composed of chondrocytes and extracellular matrix, without blood vessels or nerves. As a highly differentiated cell, chondrocytes primarily respond by synthesizing and secreting the cartilage matrix, which plays an essential role in maintaining the metabolic balance of cartilage tissue. Type II collagen, proteoglycan, and water are the main component of the cartilage matrix. In the early stage of OA, cartilage surface shows a hypertrophic-like changes and chondrocyte apoptosis. Simultaneously, chondrocyte hypertrophy-like changes produce matrix-degrading enzymes, including aggreganases and matrix...
metalloproteinases (MMPs), which cause protease-mediated degradation of the extracellular matrix of cartilage and loss of proteoglycans [4]. This degradation process is accompanied by synovial tissue inflammation, leading to the release of inflammatory factors such as Interleukin-1β (IL-1β) and tumor necrosis factor (TNFα). These factors, in turn, enhance the generation of cartilage matrix-degrading enzymes, which eventually exacerbate cartilage catabolism and lead to progressive OA [5]. Therefore, according to OA's pathological mechanisms, OA can be treated by inhibiting the production of inflammatory factors, reversing cartilage matrix degradation, and promoting extracellular matrix synthesis. Currently, early OA can be alleviated with physical therapy and drug for pain relief. When drug treatments become ineffective, surgical alternatives, such as joint replacement, or stem cell therapy, may be chosen. With the development and maturity of gene therapy technology, an increasing number of gene therapy methods have been applied for OA treatment.

**Status of intra-articular therapy for osteoarthritis**

Knees and hips are critical active joints in the human body, especially the knee joint, which is the largest of the weight-bearing joint; thus, knee arthritis incidence is very high. Currently, the most suitable route for local drug delivery of OA is intra-articular injection. A direct intra-articular injection is advantageous over systemic administration as drugs can be chosen that deliver to the diseased site and bypass the physiological barriers during transport in the body. The benefits of intra-articular injection of drugs for OA therapy include promoting bioavailability, reducing the adverse risk of systemic toxicity, and reducing the drugs' total cost. A wide range of drugs has been developed to treat OA, including OA, including chondroitin sulfate (CS), and hyaluronic acid, which have been approved by the US Food and Drug Administration (FDA) and the European Medicines Quality Agency for clinical application. Other new treatments, such as intra-articular ozone injections, platelet-rich plasma, and mesenchymal stem cell transplantation, are in clinical trials
Overall, currently, there is no clinical convincing and practical method to reverse joint cartilage destruction, indicating the search for new therapeutics is still in an urgent need.

**Advantages and challenges of intra-articular drug delivery platforms for OA treatment**

The choice of a suitable drug delivery system for intra-articular delivery drugs is the key to OA therapy's success. Drug delivery parameters should be considered, such as distribution, size, penetration efficiency, retention time, and release property [9]. Most of the drug formulations currently used for intra-articular injection are dissolved in solution. After injection, the drugs quickly diffuse into the systemic circulation, resulting in rapid clearance of the therapeutic drugs in the joint cavity and short retention time. The drug has the effect which dramatically limits the efficacy of the OA treatment. The main reason for the short retention time of the drugs in the joint cavity is the rapid turnover of synovial fluid through the synovial venules and lymphatic vessels [10, 11]. Generally, molecules smaller than 10 kDa are thought to be cleared through capillaries, while larger macromolecules are considered removed by the lymphatic system [12]. Especially in the OA joint's inflammatory environment, many secreted cells, including lymphocytes and macrophages, and enzymes enter the synovium, which may lead to a greater flow of synovial fluid in the lymphatic system and exacerbated drug degradation. Frequent injections are required to maintain therapeutic efficacy; however, this results in an increased risk of local pain, joint swelling, and infection.

On the other hand, the cartilage layer is a dense structure filled with collagen and aggregcan, which hinders drug penetration. Notably, it contains highly negatively charged glycosaminoglycan (GAG) chains, which block the penetration of most large molecules, making it difficult for the drug to reach the chondrocytes embedded in the deep areas of the affected cartilage tissue. As OA is a chronic disease, it is necessary
to establish a long-term sustained drug release delivery system to attenuate its progression. Therefore, it is vital to develop cartilage drug delivery material that can penetrate the cartilage surface after intra-articular administration. The delivery vehicle can quickly transfer the drug throughout the cartilage's thickness and prolong the joint's residence time as much as possible to achieve therapeutic efficiency. Current drug delivery systems, such as polymer particles, liposomes, micelles, and hydrogel formulations, have been used for intra-articular injection, widely re-engineered disease-modifying OA drugs (DMOADs) (Table 1). Viral vectors can penetrate intact tissue surface regions, but immunogenicity and safety issues hinder their clinical translation.

The emergence of microspheres and nanoparticles has provided a new drug delivery system for joint cavity injection therapy. These particles not only meet the requirements of good biocompatibility and degradability but can also modify and regulate the release of biological macromolecules. In addition, these particles are often modified by proteins, lipids, and other biomolecules during the synthesis process to improve the efficacy of these drug-loading systems.

Previous studies have shown that cationic nanocarriers smaller than 15 nm can overcome the biological barrier of joints by binding and penetrating negatively charged cartilage tissue. Their action is faster than their clearance from the joint space. Amine terminal polyamidoamine (PAMAM) dendrimers with dense surface functional groups consisting of 64 and 256 primary cationic amines have been used for intra-articular drug delivery. Based on polyamide-based dendrimers, nanocarriers were developed to deliver IGF-1 to chondrocytes at the joint site. By adjusting the nanocarriers' surface charge, dendrimer delivered IGF-1 penetrated bovine cartilage and persisted in reaching the full cartilage ex vivo. Cartilage-penetrating nanocarriers injected into the joints were retained in the OA model's rat knee and decelerated cartilage degeneration [13]. Tethering interleukin-1 receptor antagonist (IL-IRa) into self-assembled nanoparticles enhanced the IL-1Ra half-life in cartilage. In this research, they assemble amphiphilic block copolymer into nanoparticles, and the
IL-1ra protein were covalent conjugated to the nanoparticle surface with a diameter of 300 nm with a protein-bound part for surface. In vivo imaging showed that IL-1Ra drug delivered by nanoparticles remained in rat joints for more than 14 days. Moreover, The IL-1Ra-conjugated nanoparticles can maintain the structure and composition of cartilage.

Concerning gene therapy, the use of viral vectors can successfully transfec genes into chondrocytes. There have been many preclinical studies on using viral vectors to carry therapeutic genes for OA treatment. The application of viral vectors has certain limitations, such as host immune and inflammatory responses, limiting their application in treating chronic OA disease. Nonviral gene delivery systems for OA therapy are nonimmunogenic and generally have low toxicity, but have limitations in low transfection efficiency, which restricts these systems’ use.

**Intra-articular delivery of gene therapy for osteoarthritis**

IL-1β is a crucial inflammatory cytokine in the pathogenesis of OA. Studies have shown that the elevated IL-1β in OA patients not only stimulates the secretion of the chondrocyte protease to accelerate the degradation of cartilage collagen but also indirectly causes the inflammation of osteoarthritic synoviocytes [14]. IL-1RA is a physiological inhibitor of the IL-1 signaling pathway that can compete with IL-1α and IL-1β to block the IL-1 receptor. Anakinra (IL1-RA) is an approved rheumatoid arthritis drug that can alleviate the disease, but it has not passed a phase II clinical trial for OA. Chevalier *et al.* found that the insufficient delivery of protein drugs is the key factor that leads to non-significant improvements in OA symptoms; for example, a single intra-articular injection of anakinra could not penetrate cartilage, and the joint half-life of anakinra was only four hours [15, 16]. Thus, enhancing the retention time in cartilage is essential. Intra-articular gene therapy is a promising technology that has provided a new method for IL1-RA-based treatment. Recent studies using animal models have confirmed that the viral vector can efficiently express the IL1-RA
transgene for OA treatment. It has been reported that adenovirus vectors carrying cDNA encoding horse IL-1RA were used to treat horse models of OA by local joint cavity injection[17, 18]. The results showed that the IL-1RA gene transferred using viral vectors was continuously expressed in the joint for 28 days and was influential in cartilage preservation. The treatment of IL-1RA carried by adeno-associated virus sc-rAAV2.5 also confirmed its effectiveness and safety in MIA-induced rat OA models [19]. Currently, phase 1 clinical trials are being conducted using the IL-1RA gene therapy method with humans (ClinicalTrials.gov Identifier: NCT04119687).

Nuclear factor-kappa B p65 (NF-KBp65) was reported to be involved in the OA inflammatory process. Activated NF-KBp65 not only accelerates extracellular matrix (ECM) degradation by stimulating ECM-degrading enzymes, including ADAMTS and MMP-13 but also boosts the secretion of several inflammatory factors. Thus, OA could be alleviated by blocking the NF-κB (p60/p65) signaling pathway, which has become an attractive gene therapy target for OA. Adenovirus-mediated or nanoparticle-based NF-κBp65-specific siRNA inhibited inflammatory signaling pathways when intra-articularly injected in a rat OA model delaying OA articular cartilage degeneration [20, 21]. HIF-2a is an important factor that is upregulated in cartilage catabolism, which may induce the production of matrix metabolic enzymes of MMPs and Adamts-4 [22]. Suppression of HIF-2a expression by injecting siRNA into the joint cavity reduced articular cartilage catabolism and facilitated cartilage tissue repair [20]. MMP-13 is the most crucial degradation enzyme in cartilage matrix catabolism, directly destroying the collagen II embedded in the cartilage matrix. A previous study found that conditional gene knockout of MMP-13 in mice (Mmp13Col2ER) prevented and decelerated cartilage degradation. Both intra-articular short interfering RNA-targeting MMP-13 or CRISPR-based knockout of MMP-13 delayed the acquisition of an OA-like phenotype in a surgically induced OA model [23, 24].

MicroRNA(miRNAs) are 20- to 23-nucleotide-long single-stranded non-coding RNA molecules that act as transcriptional silencers by binding to the 3’ untranslated
region of the target messenger RNA (mRNA). miRNAs play an important regulatory role in the pathogenesis of OA. Tardif et al. found that miR-140 was significantly decreased in human OA chondrocytes, and the upregulation of miR-140 significantly inhibited cartilage degradation [25]. We also revealed that miRNA-140 is critical involved in estrogen modulating ECM degradation [26, 27]. Thus, intra-articular injection of miR-140 in rat models of OA regulated extracellular matrix homeostasis and delayed OA progression [28, 29].

Gene therapy can also be used to treat OA by delivering various essential growth factors involved in cartilage synthesis. Table 1 have summarized some drug delivery through intra-articular injection for cartilage repair. Due to the limited regeneration capacity of cartilage, gene transfection of growth factors such as TGF-β or Insulin-like growth factor (IGF-1) is useful for enhancing the cartilage matrix restoration. InvossaTM is a TGF-β1 transfection agent carried by a retrovirus [30]. Multiple animal experiments have confirmed its effectiveness in repairing cartilage defects. Phase I and II clinical trials have also verified the safety and efficacy of InvossaTM in treating severe arthritis patients. They have shown that it has good performance in relieving pain and improving motor function. Recently, gene therapy was approved in Korea for the treatment of moderate OA. Phase III clinical testing has been carried out in the United States for the treatment of arthritis [31]. IGF-1 was chosen as an import growth factor to regenerate cartilage of arthritis. The intra-articular injection of adenovirus-mediated IGF-1 can promote articular cartilage proteoglycan synthesis without producing significant toxic or other side effects [32]. bFGF has also been used to stimulate the proliferation and differentiation of chondrocytes. In vitro studies have shown that, after adenovirus-mediated bFGF infection of chondrocytes in an OA context, the chondrocyte proliferation significantly increased. The matrix synthesis of proteoglycans and type II collagen was much improved. Injection of a recombinant adenoviral vector carrying bFGF in an OA-affected cavity in a rabbit knee joint promoted cartilage repair of OA-induced damage by promoting proteoglycan and extracellular matrix synthesis [33].
Gene-mediated cartilage regeneration promotes cartilage formation, inhibits cartilage matrix breakdown, and suppresses the anti-inflammatory response, which can significantly improve OA therapy [34].

**Intra-articular drug delivery targeting cartilage**

The ideal drug delivery system should act precisely on or around the lesion to improve the drug's efficacy and reduce the extent of undesired side effects. Targeted drug delivery to cartilage sites is one of the ultimate goals of OA drug delivery. Targeting cartilage delivery determines the ability of the drug-carrying system to enter and remain in the cartilage matrix. Currently, targeting drugs to the cartilage layer has represented a promising strategy in OA therapy. The articular cartilage can be divided into four layers: the superficial layer, the transition layer, the deep layer, and the calcified layer (Figure 1). The chondrocytes in the transitional (middle) zone have apparent damage in OA, making it the most effective target site for OA treatment. Collagen is the primary component of cartilage ECM; thus, many studies have designed anti-arthritis drug delivery systems for collagen-specific targeting (Table 2).

Affinity peptides are selected as target ligands for cell-specific drug delivery. Previously, a peptide was identified with a high affinity for collagen II α1 that also showed a high affinity for collagen II α1. Then, the short peptide, with the sequence WYRGRL, was conjugated to polysulfide propylene nanoparticles’ surface. In vivo experiments showed that this functional nanoparticle successfully targeted the cartilage matrix with a retention time of 71-fold that of none targeted nanoparticles [35]. Affinity selection with a phage display of a peptide library was used to determine the cartilage-targeting polypeptide (CAP, sequence: DWRVIIPPRPSA). The CAP-modified drug delivery vector showed perfect cartilage-targeting properties; for example, CAP-PEI nanoparticles specifically facilitated hypoxia-inducible factor-2a (HIF-2) siRNA delivery to chondrocytes with high local concentrations in the cartilage seven days after injection [36], indicating that the CAP-PEI targeted drug-loading system may be suitable for OA gene therapy.
The inflammatory response of OA is mainly caused by activated macrophages. Activated inflammatory cells produce various reactive oxygen species (ROS), including H$_2$O$_2$, and overflow into the extracellular matrix, eventually destroying the articular cartilage's microenvironment. Thus, ROS-responsive carrier material could be used for targeted delivery [37]. The PLGA-designed hollow microspheres contained the anti-inflammatory drug dexamethasone (Dex). Under the action of H$_2$O$_2$ in the local inflammatory environment, Fe$^{2+}$ and sodium bicarbonate initiated a cascade of chemical reactions, generating CO$_2$ bubbles to destroy the microspheres and release the anti-inflammatory drugs inside the microspheres [38]. In vivo and in vitro experiments have proven that the ROS-reactive microsphere can be used to target anti-inflammatory drugs to inflame tissue and attenuate osteoarthritic changes.

As cartilage is an anionically charged tissue, electrostatic attraction using cationic peptide carriers (CPCs) was utilized to develop a new charge-based intra-cartilage penetrating drug delivery system overcomes the biological obstacle created by the dense, negatively charged cartilage matrix [39]. An avidin protein with a high negative charge (net charge +20) was highly efficient in penetrating and binding to the middle and deep layers of cartilage. In another study, they designed a charge-based intracartilage drug delivery nanostructure-multi-arm avidin (mAv), which contains 28 covalent binding sites for small molecule drug by using a hydrolysable ester linkers, the cationic multi-arm avidin (mAv) nanostructure can quickly penetrate the entire thickness of the negatively charged cartilage, and realize the continuous delivery and release of the small molecule dextamethasone to the chondrocytes. A single-low dose of mAv-Dex could attenuate the IL-1α-induced GAG loss and shown much significantly potential than that of Dex alone. Thus, the avidin-based delivery of Dex compounds was able to suppress catabolic effects in posttraumatic OA [40]. Super positively charged GFP could electrostatically interact with cartilage tissue and penetrate full-thickness cartilage [41], also suggesting that an engineered supercharged protein-coupled with drugs can be utilized for functional intra-articular delivery. Overall, this charge-based drug delivery material provides the easiest way to
restart drugs that clinical trial failures reasons come for lack intra-articular targeting or appear systemic side effects. Also, we can reuse the FDA approved rheumatic drugs by these drug delivery materials to achieve OA therapy.

In addition, antibody-mediated delivery might provide alternative delivery options. The type II collagen antibody could benefit drug delivery to the cartilage matrix and enhance drug retention at disease sites. Nanoparticles were modified to generate type II collagen antibody NPs (mAbCII-siNPs) that reside in the extracellular matrix to enhance siRNA activity. mAbCII-siNP conjugated with MMP13 siRNA (mAbCII-siNP/siMMP13) had a higher binding capacity for porcine cartilage 17-fold increase compared to that of the untargeted control NP. In a mouse model of acute mechanical injury caused by PTOA, mAbCII-18 siNP/siMMP13 inhibited the expression level of mMP13 by almost 80% [42]. This matrix-targeted nanoplex provides a novel nano-platform for gene target therapy. The current research on drug delivery biomaterials for osteoarthritis therapy should take full account of biological safety, controllability, target orientation, etc. In addition, basic research on the diameter of drug delivery material need more deeply understood, which can provide guidance for the design of ideal drug carrier.

**Intra-articular delivery of extracellular vesicle-based drugs for OA treatment**

In recent years, extracellular vesicles (EVs) have attracted significant attention, not only because of their fundamental biological properties but also because of their potential therapeutic applications. EVs are cell-derived nanoparticle that can be collected by almost all cell types. They consist of a phospholipid bilayer to encapsulate cargo, such as miRNA, mRNA, and protein. EVs can selectively and specifically transfer their biological contents between donor cells and recipient cells, acting over short and long distances. Although the mechanism of EV targeting and entry into receptor cells is not fully understood, an increasing body of evidence has demonstrated the potential value of these natural carriers as drug delivery vehicles for the treatment of various diseases. Exosomes originated from mesenchymal stem cells have been reported to attenuate cartilage degradation and enhance cartilage
regeneration. Intra-articular administration of primary chondrocyte-derived exosomes restored mitochondrial dysfunction. However, fewer studies have reported the use of extracellular vesicles as drug delivery vehicles for OA therapeutics. EV-based OA drug delivery requires exosomes to cross the cartilage extracellular matrix. However, the size of an EV is usually larger than the ECM mesh size, so it is unclear how they pass through dense ECM.

The latest published research shows that, in contrast to synthetic nanoparticles, EVs easily penetrate nanoporous ECM. Targeted delivery to specific lesion cells or tissues is the key to the successful clinical application of nucleic acid drugs. In particular, the delivery of microRNA-140 (miR-140) through the dense avascular cartilage matrix to chondrocytes remains a significant challenge. We recently genetically engineered exosomes by chondrocyte-affinity peptide (CAP) fusion with lam2p that display on CAP on exosomes' external surface to achieve targeted chondrocytes. Compared with non-targeted natural exosomes, CAP exosomes intra-articularly injected can remain in the joint cavity for a long time. CAP exosomes also deliver miR-140 to the deep cartilage area through the dense cartilage matrix, inhibit cartilage degrading proteases, thus inhibit the osteoarthritis deterioration in the rat model, which provides a potential cell-free therapy for OA [43]. An ex vivo assay demonstrating the chondrocyte targeted exosome could be an uptake in cartilage (Figure 2).

The use of engineered hydrogels demonstrates that the matrix's mechanical properties under restricted conditions mediate the transportation of exosomes, resulting in free diffusion and rapid transportation [44]. This evidence further suggests the free transport of EVs in cartilage matrix. The ideal drug-loading system is capable of accurately acting on the disease or around the disease area, which not only improves the therapeutic efficiency of the drug but also reduces its side effects. Therefore, targeted exosomes can be used as a powerful tool to deliver drugs to articular cartilage.
Conclusions and future perspectives

Overall, intra-articular injection of drugs and biologics for therapy has shown great promise to relieve the clinical symptoms experienced by osteoarthritis patients, which will improve the quality of life and delay surgery of OA patients, therefore, reduce the disease burden of osteoarthritis to the society.

The emergence of nanotechnology will overcome the obstacles and challenges of intra-articular targeted drug delivery. The current development in intra-articular drug delivery platforms not only enable OA drugs to pass the ECM barrier but also improve their targeting efficiency and bioavailability, which will become critical for the effective clinical translation of potential therapeutics in OA therapy. However, some challenges persist. Thus, future studies will be needed to confirm the safety of intra-articular delivery nanovehicles. The combination of target therapy with nanotechnology will provide a rationale for promoting OA cartilage repair via intra-articular injection.

Abbreviations

CS: Chondroitin sulfate
DMOADs: Disease-modifying OA drugs
ECM: Extracellular matrix
EVs: Extracellular vesicles
FDA: Food and Drug Administration
FGF: Fibroblast growth factor
GAG: Glycosaminoglycan
MMPs: Matrix metalloproteinases
miRNA: MicroRNA
NSAIDs: Nonsteroidal anti-inflammatory drugs

IGF-I: Insulin-like growth factor-I

IL-IRα: Interleukin-1 receptor antagonist

IL-1β: Interleukin-1β

OA: Osteoarthritis

TNFα: Tumor necrosis factor-α

Ethics approval and consent to participate

Cartilage samples were collected from donors at Shenzhen Second People’s Hospital upon the approval from the Ethics Committee of Shenzhen Second People’s Hospital. (exemption dated Sep 10, 2020)

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare no competing interests regarding the publication of this paper.

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Authors' contributions

YJL prepared the manuscript. IP, YX and DL contributed to the manuscript review and editing. XX and XLM contributed to the manuscript discussion. DPW, DL and JX supervised the study. All authors read and approved the final manuscript.

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Table 1. Intra-articular drug delivery for osteoarthritis therapy

Table 2: Developed ligand for cartilage-targeting drug delivery

Figure 1. Nanomaterials were developed for intra-articular drug delivery in osteoarthritis therapy. Structural of articular cartilage and composition of articular cartilage include a dense extracellular matrix with a distribution of highly specialized chondrocytes which is the target cell for drug treatment.

Figure 2. Human cartilage penetration assay show chondrocyte target delivery of nanovesicles. (A)Cartilage explants were harvested from human knee joints with a diameter of 6mm. (B)Schematic demonstrating the exosome delivery in cartilage. (C) Explant slices shown CAP-exosome enter inside the explants. Cartilage explants were placed in a 48-well plate and incubated with DilC-labeled CAP-exosomes for 24 h. After washes with PBS, the cartilage explants were embedded in Optimal Cutting Temperature (O.C.T.) compound, the sections (14 μm) were imaged using confocal microscopy.
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| Categorize          | Drugs     | Drug delivery system                                      | Reference                                   |
|---------------------|-----------|----------------------------------------------------------|---------------------------------------------|
| Anti-inflammatory   | IL1RA     | Adeno-Associated Virus                                   | (Watson et al., 2018)[14]                   |
| therapy             | IL1RA     | copolymer particle                                       | (Whitmire et al., 2012)                     |
|                     | Dexamethasone | Multi-arm cationic nano-construct of Avidin (mA)       | (He et al., 2020) [17]                      |
|                     | curcumin  | Acid-activatable polymer micelles                         | (Kang et al., 2020)[18]                     |
| Anti-matrix          | siRNA Hif-2a| Chondrocyte-homing nanoparticles                         | (Pi et al., 2015) [19]                      |
| proteases           | miR-140   | Chondrocyte-targeted exosome                             | (Liang et al., 2020) [20]                   |
|                     | siRNA MMP-13| Matrix-targeted Nanoparticles                             | (Bedingfield et al., 2020) [21]            |
|                     | siRNA NF-κBp65| Peptide-siRNA nanocomplexes                              | (Yan et al., 2016)[22]                      |
| Chondrogenic        | bFGF      | adenovirus                                               | (Chen et al., 2010)[23]                     |
|                     | IGF       | Cartilage-penetrating PAMAM dendrimer                    | (Geiger et al., 2018) [13]                  |
|                     | KGN       | MSC-targeted exosome ; thermo-responsive polymeric nanospheres ; chitosan nano/microparticles ; upconversion nanoparticles ; | (Xu et al., 2020) [24], (Kang et al., 2016)[25], (Kang et al., 2014) [26], (Li et al., 2016) [27] |
| Targeting moieties                  | Binding partner                | Drugs                        | Applications                              | Reference                      |
|------------------------------------|--------------------------------|------------------------------|-------------------------------------------|--------------------------------|
| DWRVIIPPRPSA                       | Human/rabbit/mouse/rat chondrocytes | Hif-2 siRNA                  | Plasmid DNA delivery and siRNA delivery   | (Pi et al., 2011)               |
| WYRGRL                             | collagen II α1                  | N/A                          | target the cartilage matrix              | (Rothenfluhet al., 2008)       |
| RLDPTSYLRTFW and HDSQLEALIKFM       | aggrecan                        | N/A                          | Chondrocyte-Binding                       | (Cheung et al., 2013)          |
| LRELHNLNNNC                        | type I collagen                 | α-TNF, α – TGF-β             | inflammation-targeted therapeutic         | (Katsumata et al., 2019)       |
| Cationic peptide                   | negatively charged cartilage tissue | N/A                          | cartilage penetrating and binding        | (Armin et al., 2019)           |
| PAMAM dendrimer                    | negatively charged cartilage tissue | IGF-1                        | Improve tissue binding, penetration, and residence time | (Geiger et al., 2018)          |
| Avidin (net charge +20)            | negatively charged cartilage tissue | dexamethasone                | Anti-catabolic in post-traumatic OA       | (Bajpayee et al., 2014)        |
| GFP (net charge +36)               | negatively charged cartilage extracellular matrix | NO                           | specifically target cartilage             | (Bajpayee et al., 2016)        |
| type II collagen antibody          | cartilage matrix (collage II)    | MMP13 siRNA                  | prevent Post-Traumatic OA                | (Bedingfield, 2020)            |
| ROS-Responsive Gas-Generating Carrier | inflamed tissues               | Dexamethasone                | site-specific delivery of anti-inflammatory drug to inflamed tissues | (Chung et al., 2015)          |