Short Interfering RNA (siRNA)-Based Therapeutics for Cartilage Diseases

Libo Zhou, Lee E. Rubin, Chuanju Liu & Yupeng Chen

Regenerative Engineering and Translational Medicine

ISSN 2364-4133

Regen. Eng. Transl. Med.
DOI 10.1007/s40883-020-00149-z

Springer

Regenerative Engineering and Translational Medicine
https://doi.org/10.1007/s40883-020-00149-z

REVIEW

Short Interfering RNA (siRNA)-Based Therapeutics for Cartilage Diseases

Libo Zhou[1] · Lee E. Rubin[2] · Chuanju Liu[3] · Yupeng Chen[1]

Received: 23 July 2019 / Revised: 27 December 2019 / Accepted: 21 January 2020

Abstract

Articular cartilage injury, as a hallmark of arthritic diseases, is difficult to repair and causes joint pain, stiffness, and loss of mobility. Over the years, the most significant problems for the drug-based treatment of arthritis have been related to drug administration and delivery. In recent years, much research has been devoted to developing new strategies for repairing or regenerating the damaged osteoarticular tissue. The RNA interference (RNAi) has been suggested to have the potential for implementation in targeted therapy in which the faulty gene can be edited by delivering its complementary short interfering RNA (siRNA) at the posttranscriptional stage. The successful editing of a specific gene by the delivered siRNA might slow or halt osteoarthritic diseases without side effects caused by chemical inhibitors. However, cartilage siRNA delivery remains a challenging objective because cartilage is an avascular and very dense tissue with very low permeability. Furthermore, RNA is prone to degradation by serum nucleases (such as RNase H and RNase A) due to an extra hydroxyl group in its phosphodiester backbone. Therefore, successful delivery is the first and most crucial requirement for efficient RNAi therapy. Nanomaterials have emerged as highly advantage tools for these studies, as they can be engineered to protect siRNA from degrading, address barriers in siRNA delivery to joints, and target specific cells. This review will discuss recent breakthroughs of different siRNA delivery technologies for cartilage diseases.

Lay Summary

Articular cartilage breakdown is a hallmark of osteoarthritis, which is difficult to repair and treat. Currently, there is no disease-modifying therapeutic approved by FDA to treat osteoarthritis. In recent years, RNAi drugs have been suggested to have the potential for repairing or regenerating the damaged articular cartilage. However, the effective delivery of small RNAs remains a significant challenge. To overcome these obstacles, nanomaterial delivery systems including polymers, lipids, peptides, and oligonucleotide nanoparticles have been developed to enhance the effectiveness of RNAi drugs. Here, we review recent progress in using nanomaterials to deliver small RNAs for cartilage disease therapeutics. In the future, nanomaterials can be specifically designed to form small-sized delivery vehicles with excellent penetration properties. RNAi therapeutics based on these nanomaterials will have great promise to treat cartilage diseases.

Keywords Articular cartilage injury · Short interfering RNA · Nanomaterials · Delivery therapeutics

Introduction

Arthritis affects millions of people worldwide and is the most common cause of disability in the USA. This musculoskeletal condition causes significant impairment of mobility and has great social cost, especially in people elder than age 65 [1]. Articular cartilage breakdown, as a hallmark of the arthritic disease, is difficult to repair and causes joint pain, stiffness, and loss of mobility [2]. The most significant problems for drug-based treatment of arthritis have been related to drug administration and delivery. With oral medications, it is

✉ Yupeng Chen
yupeng.chen@uconn.edu

[1] Department of Biomedical Engineering, University of Connecticut, 260 Glenbrook Road, Unit 3247, Storrs, CT 06269-3247, USA

[2] Department of Orthopedics and Rehabilitation, Yale University School of Medicine, New Haven, CT, USA

[3] Department of Orthopedic Surgery and Cell Biology, New York University School of Medicine, New York, NY, USA

Published online: 29 January 2020

difficult to ensure sufficient enteral drug absorption allowing for transit of the medication into the bloodstream and ultimate drug penetration into the cartilage of the targeted joint. As a result, therapeutic effects in cell and matrix targets might not necessarily happen as desired [3]. Moreover, intravenous (IV) administration carries risk of off-target malfunction from systemic delivery and may need to be reserved for patients with generalized systemic diseases, as seen with current IV treatment regimens for rheumatoid arthritis and other autoimmune arthritis. The inherent risk of unintended side effects in other distant locations is a major reason why several potential osteoarthritis drugs have entered the stage of clinical practice [4]. The challenge of localized drug delivery to a single involved joint space (e.g., for treatment of cases of focal hip, knee, or shoulder osteoarthritis) likely means that precision-guided injections, using ultrasound or fluoroscopic techniques, will be a necessary part of the treatment regimen.

In recent years, much research has been devoted to developing new strategies for repairing or regenerating the damaged osteoarticular tissue. RNA interference (RNAi) has been suggested to have the potential for implementation in targeted therapy in which the faulty gene can be edited by delivering its complementary short interfering RNA (siRNA) at the post-transcriptional stage [5, 6]. The successful knock down of a specific gene by the delivered siRNA might slow or halt osteoarthritic diseases without side effects caused by chemical inhibitors [7]. However, cartilage siRNA delivery remains a challenging objective because cartilage is an avascular and very dense tissue with very low permeability [8]. Furthermore, RNA is prone to degradation by serum nucleases (such as RNase H and RNase A) due to an extra hydroxyl group in its phosphodiester backbone [5]. Therefore, successful delivery is the first and most crucial requirement for efficient RNAi therapy. There is sufficient need to develop carrier vehicles for siRNA delivery that ideally overcomes challenges of internalization, degradation, and cell-specific targeting. Figure 1 demonstrated a general siRNA delivery process. Currently, the most commonly studied chemical carriers include cationic polymers and lipids, which have previously demonstrated potential in various forms of drug delivery [9, 10]. Other biomolecules, such as cell-penetrating peptides and oligonucleotide nanoparticles have also been introduced as promising candidates for gene delivery.

Polymers

Various cationic polymers have been identified to have high potential for clinical applications. Due to their ability to encapsulate the negatively charged nucleic acids, the resulting polyelectrolyte complexes not only allow the RNA to cross the cellular membrane but also protect the therapeutic material from enzymatic degradation [5, 11, 12]. Polymers are enticing delivery vehicles, because they are easily modifiable and can be constructed with specific physiological and physicochemical properties to optimize gene encapsulation, internalization, and cell-targeting [11, 13–15]. Several cationic polymers have demonstrated gene binding and complexation capabilities both in vitro and in vivo, including poly (L-lysine) (PLL), polyethylenimine (PEI), and chitosan.

Poly (L-Lysine) (PLL)

Poly (L-lysine) was one of the initial cationic polymers employed for gene delivery. Synthesized via polymerization of *N*-carboxy-anhydride of lysine, PLL is a linear polypeptide of repeating units of amino acid lysine, thereby being biodegradable [12, 16]. When applied alone, PPL has limited clinical prospects due to its inadequate transfection efficiency, highlighting its tendency to rapidly associate with plasma proteins and to quickly be removed from circulation via excretion [16]. To improve the prospects of PLL-mediated gene delivery, various adaptations have been made to improve RNA delivery and uptake.

Chemical modifications, including addition of synthetic peptides or pH-sensitive entities to polymers, are often utilized to assist endosomal escape. PLL conjugated with cholic acid, a hydrophobic moiety, and poly(ethylene glycol) (PEG),

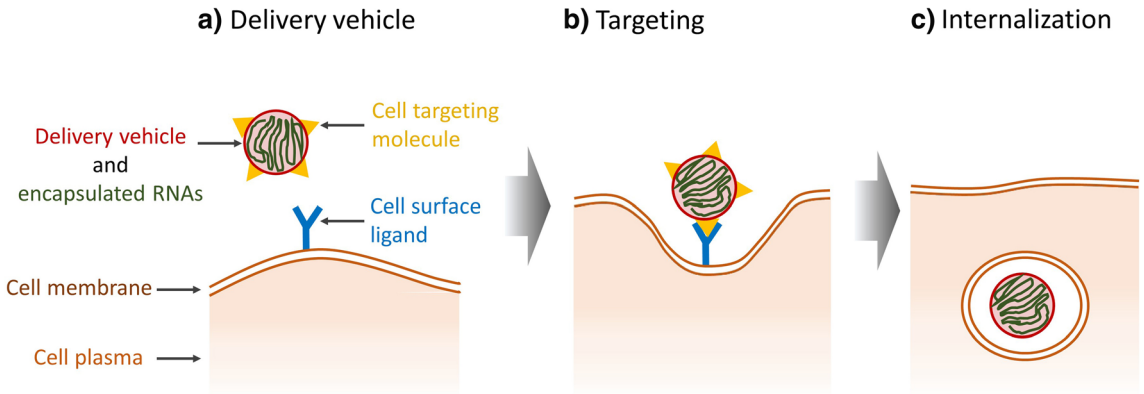

Fig. 1 A schamatic demostration of delivery vehicles and their cell targeting and uptaking processes

demonstrated improved delivery potential along with reduced cytotoxicity [17]. These results are likely due to the masking effect of PEG upon the cationic charge density, thereby reducing toxicity and protecting against plasma enzymatic degradation. It is fairly easy to take advantage of the easily amendable nature of PLL in terms of structure, molecular size, and surface. Studies have even complexed PLL with silica nanoparticles or have used dendritic PLL to enhance siRNA uptake and transfection while reducing cytotoxic effects [18, 19].

Polyethylenimine (PEI)

PEI is one of the most potent gene carrier systems due to its ability to buffer at virtually any pH, thereby appropriately earning the term, "proton sponge" [16, 20, 21]. This addresses the obstacle of endonuclease degradation because the pH buffer helps the carrier to bypass the endosomal barrier and avert lysosomal degradation, a critical step in achieving successful gene transfection [11]. PEI has been identified as a promising gene delivery agent for systemic application, capable of complexation with siRNA to induce c-erbB2/neu (HER-2) downregulation [22]. Unfortunately, one of the disadvantages of PEI is its limited degradability – leading to high levels of cytotoxicity that can interfere with transfection activity [23, 24]. Studies have suggested that this toxicity depends on properties of PEI itself, including molecular weight and structure. Although cytotoxicity decreases with increasing linearity of PEI, transfection efficiency also suffers [11, 25]. Consequently, researchers have designed chemical modifications to PEI by conjugating a variety of degradable crosslinkers to help facilitate intracellular degradation either by hydrolysis, enzymatic function, or cytosolic reductive action by glutathione [11]. Furthermore, although gene transfer efficiency increases for higher molecular weight varieties of PEI, cytotoxicity is also enhanced with larger polymer size. Therefore, a reasonable approach to PEI modification is to facilitate RNA delivery via low cytotoxicity and low molecular weight (LMW) PEI linked to biodegradable conjugates of suitable higher molecular weights [26].

Exploiting the modifiable nature of the polymers allows researchers to explore cell-targeting or biodegradable ligands to enhance the delivery mechanism by producing a more sterically stabilized carrier vehicle. Similar to PLL modifications, charge-neutralizing agents such as poly(ethylene glycol) (PEG) have been conjugated to PEI and have exhibited improved transfection with lower toxicity levels compared to non-PEGylated PEI polyplexes [16, 26].These modifications can mitigate the excessive net positive charge of cationic polymers that make them more capable of associating with anionic serum proteins. Other approaches have included linking degradable polyglutamic acid derivative backbones to PEI or crosslinking with disulfide bonds, both of which exhibited increased degradability and target gene downregulation [25, 26] .

Interleukin (IL)-15 plays an important role in the pathogenesis of rheumatoid arthritis (RA). Systemic knockdown of IL-15 receptor (IL-15R) might be an effective way for a reduction in inflammation at local sites. The IL-2/15Rβ siRNA, which can target the β chain of IL-15R and IL-2, was formulated with the commercial cationic polymer, in vivo-jetPEI, to be PEI-complexed siRNA nanoparticles. The nanoparticles can easily accumulate in arthritic paws of rats and are then taken up by immunocytes. IL-2/15Rb siRNA polyplexes exhibit an inhibitory effect on disease progression of RA assessed through swelling and arthritis scores indicating successful delivery of IL-2/15Rb siRNA to the arthritic joints [27].

Chitosan

First implicated in gene delivery in 1995 by Mumper et al., chitosan is a biocompatible and resorbable cationic aminopolysaccharide of glucosamine units[16]. As high toxicity exhibited by cationic polymers has limited their clinical application [28], greater interest has been focused on natural biodegradable polymers like chitosan. It was not until 2006 that chitosan was used for in vitro delivery of siRNA, which again emphasized the importance of proper chitosan-siRNA composition and formulation techniques, such as simple complexation, siRNA entrapment via ionic gelation, or siRNA adsorption onto chitosan nanoparticles [29, 30]. Additional studies have identified a variety of critical elements influencing physicochemical properties and transfection efficiency, such as composition and formulation of the chitosan-nucleic acid [31].

As with other polymers mentioned, chitosan is also subject to modifications aimed at improving therapeutic utility. The therapeutic application of chitosan is limited due to its insolubility in neutral pH; however, chitosan oligomers – short chains of chitosan produced by depolymerization of chitosan with nitrous acid – have demonstrated favorable pharmaceutical characteristics including solubility in water and reduced viscosity [31]. Another modification to yield improved transfection efficiency is to use trimethylated chitosan in which primary amine groups are replaced with quaternary amino groups. By introducing a quaternization degree, solubility significantly increased with increased transfection efficiency in fibroblast cell lines [31]. Shi et al. developed another modified chitosan gene nanocarrier which consists of folic acid, diethylethylamine, and polyethylene glycol. The nanocarrier was utilized to deliver siRNA capable of silencing tumor necrosis factor-alpha (TNF α), a pro-inflammatory cytokine playing a role in the pathophysiology of rheumatoid arthritis. This novel nanocarrier not only provides good protection for the siRNA against nuclease destruction but also facilities siRNA uptake by folate ligands. The successful siRNA delivery can control inflammation as well as bone and cartilage destruction in rheumatoid arthritis [29].

Lipids

Cationic Lipids

Cationic lipids have been utilized in gene therapy since *N*-[1-(2,3,-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA) was first introduced in 1987 by Felgner et al [32]. Identified as one of the more promising non-viral systems for gene therapy, cationic lipids have previously been implicated as delivery vectors for DNA-based drugs. Liposome-based carriers are attractive for gene delivery, as they can be formulated to about 100 nm in size and have generally inert metabolic products [33–35]. Additionally, liposomes are able to protect encapsulated oligonucleotides from nuclease degradation and renal clearance [36]. Cationic lipids execute three important roles in siRNA delivery. First, by electrostatic association, cationic lipids encapsulate and condense siRNA. Next, the net positive charge of the lipid-based nanoparticle enhances interaction with the negatively charged cell surfaces, thus promoting internalization. Finally, the resulting destabilized endosomal membrane facilitates cytoplasmic siRNA delivery [33, 37]. Unlike polymers, cationic lipids destabilize endosomal membrane by electrostatically interacting with anionic lipids to form ion pairs, forming an inverted hexagonal (H_{II}) phase. The H_{II} phase is an intermediate structure that forms during the fusion between the cationic and anionic lipids. This step is not only crucial for membrane destabilization but also for nanoparticle disassembly resulting in cytoplasmic cargo delivery [33]. In aqueous solutions, DOTMA aggregates spontaneously with anionic nucleic acid, forming gene-encapsulated liposomes.

Various cationic lipids have been developed, including an analog of DOTMA known as DOTAP (1,2-diacyl-3-trimethylammonium propane), a family of lipids containing ester as linker bonds. DOTAP-based liposomes have been applied in vivo to address metabolic skeletal disorders by silencing bone formation inhibitory genes in osteogenic lineage cells. With the conjugation of a targeting moiety consisting of 6 repetitive sequences of aspartate, serine, and serine $(AspSerSer)_6$, DOTAP-mediated siRNA delivery demonstrated high binding affinity to the bone-forming surface, along with downregulation of its target gene, Plekho1, thus providing a potential point of access to the clinical translation of RNAi-based arthritis therapy [8].

Another lipid-mediated gene delivery of interest is via stable nucleic acid lipid particle (SNALP). Of the lipid nanoparticle (LNP) family, SNALP is a popularly utilized approach to in vivo delivery of siRNA [38] . SNALP favorably generates uniformly sized nanoparticles with high siRNA encapsulation efficiency and has progressed to clinical trials since achieving gene silencing in nonhuman primates [39]. Recently, a novel positively charged LNP-siRNA delivery system was developed by mixing a lipid mixture ethanol stream with a siRNA aqueous stream. The system was used to knock down the cartilage-specific gene Indian Hedgehog (*Ihh*) in vivo. The increased activity of *Ihh* has been considered closely related to osteoarthritis progression in humans. This study demonstrated that siRNA could be delivered by LNPs into the knee joint to knock down *Ihh* and inhibit cartilage degeneration [7].

Although laboratory use of commercial cationic lipids such as lipofectin, lipofectamine, or transfectam is well-established, their use may not be effective for clinical application. Newer lipid-based gene delivery strategies have focused on polyvalent cationic head groups containing long carbon chains connected by either glycosidic, diether, or disulfide groups [16]. The performance of various lipid-mediated siRNA delivery has also been investigated to identify the structural motifs that could achieve high levels of knockdown, and many of these elements focused on the amines per head unit and acyl chain properties [40].

Shielding Lipids

As with polymers, PEGyation serves multiple beneficial purposes for liposomal RNA delivery, mainly prolonging circulation half-life by reducing opsonization [41]. Steric hindrance due to the high chain motility of PEG limits adsorption of proteins, such as opsonins. Subsequently, activation of the complement pathway is avoided, as is the recognition and clearance of the liposome by macrophages [42]. However, the shielding effect mediated by PEG has shown to hinder membrane fusion between the liposome and endosomal membrane, reducing cellular uptake by target cells and decreasing efficacy in vitro and in vivo [43]. Two strategies have been introduced to improving PEGylated nanoparticle efficacy. One involves conjugation of PEG to the liposome by acid-sensitive bonds, such as oxime linkers, which were shown to have improved siRNA release at pH 5.5 and enhanced gene silencing in vivo [44]. Another method is to utilize pH-sensitive modified PEG linked to liposomes via ionic interactions. Although the modified PEG (covalently linked to HEMA-histidine-methacrylic acid) has a net negative charge at physiological pH, the methacrylic acid becomes protonated in the endosome, thus changing the net charge of PEG to positive. The reversion of the net charge causes the release of PEG from the lipid core, exposing the cationic liposomal membrane and allowing fusion with the endosome [45].

Peptides

Cell-Penetrating Peptides

Cell-penetrating peptides (CPPs) have been utilized as delivery vehicles for various molecular cargos, including fluorescent dyes, proteins, and oligonucleotides. With the discovery that

the full-length HIV-1 TAT protein alone was able to penetrate the cell membrane and induce trans-activation of the HIV-1 promoter, the peptide fragment conferring cell permeability was identified [46] The TAT peptide, as it is known, is characterized by a high density of basic residues (e.g., lysine or arginine) that are proposed to mediate cellular uptake by interacting with the anionic surface of the plasma membrane and enhancing peptide internalization [47–49]. Since this initial observation, there have been more CPP identified, such as the MPG, a new peptide-based gene delivery system, and synthetic chimeric peptides such as model amphipathic peptide (MAP) [50, 51].

Two main approaches to CPP-mediated siRNA delivery have been identified: non-covalent CPP/siRNA complexation and covalent attachment of CPP to siRNA via disulfide bonds. The non-covalent complexation utilizes the dense cationic charge of CPPs to condense siRNAs into nanoparticles with a net positive charge. The covalent linkage of CPP to siRNA is the preferred method, as this yields small, monomeric CPP/siRNA molecules that can evade various in vivo complications associated with cationic condensation of nucleic acids [48, 52–54]. However, covalently linking the negatively charged siRNA to CPP neutralizes the overall charge of the CPP/siRNA complex, jeopardizing internalization by reducing ability to bind to the cell surface.

CPP-mediated RNA delivery in mammalian cells was achieved with the MPG peptide–based system. The MPG peptide is a fusion of the HIV-1 gp41 peptide domain with the nuclear localization signal (NLS) of the SV40 large T antigen [50, 55]. Through non-covalent complexes with nucleic acids, the MPG peptide demonstrated plasmid DNA delivery via endosomal pathway-independent cell entry with intact nuclear targeting. By disrupting the NLS through a point mutation, nuclear delivery is impaired; therefore, MPG is applicable for cytoplasmic delivery of siRNA. Application of the MPG-siRNA system elicited rapid siRNA release with downregulation of the target mRNA [45].

Overall, CPP' are a promising class of delivery vectors due to their ability to overcome notable barriers to delivery, including condensing nucleic acids into compact particles, targeting specific cell receptors, and promoting endosomal escape. However, the mechanism of entry is still yet to be fully elucidated, as they may differ based on specific conditions such as the type of CPP, type of cargo, and the type of cell [47, 55]

Oligonucleotide Nanoparticles

Three-dimensional oligonucleotide nanostructures have been explored for encapsulation of cargo, ranging from imaging to therapeutic delivery applications [56–59]. Oligonucleotide nanoparticles (ONPs) formed via programmable self-assembly of short DNA and RNA fragments with therapeutic siRNA have shown to induce gene-silencing activity. Unlike conventional delivery nanoparticles (NP's) that are heterogeneous in size, composition, and surface chemistry resulting in suboptimal performance and even cytotoxicity, ONPs have a defined size, structure, and stoichiometry [60, 61]. A self-assembled tetrahedron composed of six DNA strands forms six edges of 30 base pairs long, with a centrally located nick that is complementary to the siRNA overhang. Therefore, six siRNA molecules can be bound per nanoparticle. Other than natural nucleic acids, modified molecules were also used to build nanotubes and other nanostructures for therapeutic delivery [58, 59, 62–65]. As shown in Fig. 2, Janus base molecules were synthesized mimicking DNA base pairs. Six Janus bases can self-assemble into a rosette plane via hydrogen bonds, and these rosette planes can further stack into therapeutic delivery ONPs via π-π interactions [58, 59, 62]. Moreover, the nucleic acid composition of ONPs can be easily manipulated, allowing for specific hybridization of siRNA sequences. Folate-modified ONPs have demonstrated gene knockdown in mice expressing folate-receptor positive tumor xenografts [60]. However, clinical application of DNA NPs can be limited by their relative structural and functional simplicity; therefore, other RNA-based nanostructures have been developed. Bacteriophage phi29 packaging RNA (pRNA) is widely used for RNA nanoparticle assembly due to its structural features. These RNA NPs can then be functionalized by siRNA. Different oligomers can be formed to include interlocking loops or palindromic sequences to control unpredictable off-target effects. Additionally, RNA NPs are already highly soluble and not prone to aggregation and do not need to be linked to PEG or serum albumins [61].

Limitations

While many siRNA options listed within this review have great clinical promise, the clinical applications of DNA- or RNA-based delivery vehicles are currently limited for a number of reasons. An ideal method of precise drug delivery to affected joint(s) limits the capability of various therapeutic options, and the potential risk of systemic side effects is of clinical concern for any type of oral or IV medication at this time. In addition, for any drug utilized, there are current gaps in understanding the pharmacokinetics and toxicology associated with clearance, plasma kinetics, and systemic distribution [66, 67]. Furthermore, sensitivity to RNase degradation and possible dissociation due to non-covalent linkage of self-assembled RNA quaternary structure, which can be improved by RNA ribose ring modification, still need to be further elucidated [61]

Discussion and Conclusion

Physical impairment due to arthritis is common, debilitating, and expensive [68]. In many cases, therapeutic treatment is

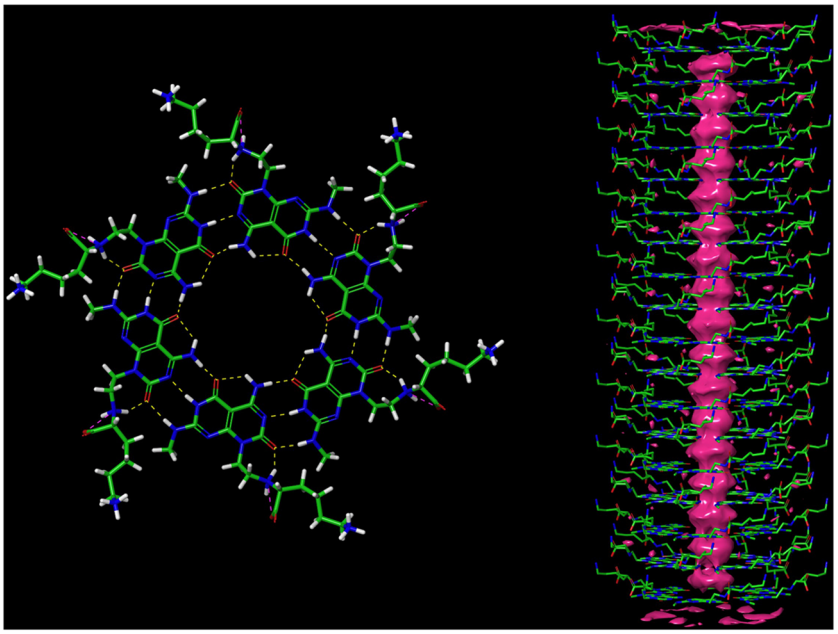

Fig. 2 The rosette plane (left) and the nanotube vehicle (right) formed by Janus base molecules

imperfect because it is difficult to achieve meaningful drug penetration and transport into cartilage. Gene-based therapy, specifically RNAi, has been implicated in therapeutic applications due to its versatility and specificity. In the context of arthritic disease, RNAi might improve repair and regeneration at sites of injury by enabling the local, sustained, and potentially regulated expression of therapeutic gene products, including morphogens, growth factors, and anti-inflammatory proteins [69]. In addition, enhanced capability of drug penetration into cartilage to facilitate stimulation of chondrocytes through utilizing targeted methods will also have the potential to usher in a meaningful new era of "Regenerative Medicine" and "Cartilage Restoration." However, to achieve the clinical potential of RNAi, delivery materials are required to transport siRNA into the cells of target tissues considering the easily enzymatically degraded siRNA. SiRNA-based treatment against cartilage and joint diseases is still very new. Currently, there was no clinical trial on any of these therapeutics. With the development and perfection of RNA delivery technologies, we believe siRNA will successfully translate into clinical settings in the near future.

In this review, we summarized the commonly used delivery vehicles for naked siRNA, including cationic polymers, cationic lipids, peptides, and oligonucleotide nanoparticles. Significant improvements to these nanomaterials, namely, chemical modifications, have been implemented to address barriers in siRNA delivery to joints. By successful delivery of siRNA into the joints, decreasing the progression of inflammation and cartilage degeneration can be achieved. As we presented in the review, the siRNA-based therapeutics provide great promise for arthritic disease therapy, but the clinical application of these advanced scientific concepts remains elusive. Further translational research will help to set the stage for longitudinal assessment of the effects of these various classes of medication. Once the drug delivery, cartilage membrane penetration, and cellular modification steps have been elucidated, this unique class of medications has a potentially large impact on the lessening the future impairment of patients due to musculoskeletal disease.

Funding Information This work is funded by 7R01AR072024 and 7R03AR069383 from DHHS/NIH/NIAMS and the CAREER award (1905785) from NSF.

Compliance with Ethical Standards

Conflict of Interest Dr. Yupeng Chen is co-founder of NanoDe Therapeutics.

References

1. Felson DT, Zhang YQ. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. Arthritis Rheum. 1998;41(8):1343–55.
2. Strombeck B, Petersson IF, Vlieland TPMV, Grp ENW. Health care quality indicators on the management of rheumatoid arthritis and osteoarthritis: a literature review. Rheumatology. 2013;52(2):382–90.
3. Bajpayee AG, Grodzinsky AJ. Cartilage-targeting drug delivery: can electrostatic interactions help? Nat Rev Rheumatol. 2017;13(3):183–93.
4. Rothenfluh DA, Bermudez H, O'Neil CP, Hubbell JA. Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage. Nat Mater. 2008;7(3):248–54.
5. Gary DJ, Puri N, Won YY. Polymer-based siRNA delivery: perspectives on the fundamental and phenomenological distinctions

from polymer-based DNA delivery. J Control Release. 2007;121(1–2):64–73.
6. Fire A, Xu SQ, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998;391(6669):806–11.
7. Wang SW, Wei XC, Sun XJ, Chen CW, Zhou JM, Zhang G, et al. A novel therapeutic strategy for cartilage diseases based on lipid nanoparticle-RNAi delivery system. Int J Nanomedicine. 2018;13:617–31.
8. Zhang G, Guo BS, Wu H, Tang T, Zhang BT, Zheng LZ, et al. A delivery system targeting bone formation surfaces to facilitate RNAi-based anabolic therapy. Nat Med. 2012;18(2):307–14.
9. Abdallah B, Hassan A, Benoist C, Goula D, Behr JP, Demeneix BA. A powerful nonviral vector for in vivo gene transfer into the adult mammalian brain: Polyethylenimine. Hum Gene Ther. 1996;7(16):1947–54.
10. Trubetskoy VS, Torchilin VP, Kennel SJ, Huang L. Use of N-terminal modified poly(L-lysine) antibody conjugate as a carrier for targeted gene delivery in mouse lung endothelial-cells. Bioconjug Chem. 1992;3(4):323–7.
11. Cho C-S. Design and development of degradable polyethylenimines for delivery of DNA and small interfering RNA: an updated review. ISRN Mater Sci. 2012;2012:1–24.
12. Cao HQ, Jiang X, Chai C, Chew SY. RNA interference by nanofiber-based siRNA delivery system. J Control Release. 2010;144(2):203–12.
13. Liu L, Zheng M, Librizzi D, Renette T, Merkel OM, Kissel T. Efficient and tumor targeted siRNA delivery by polyethylenimine-graft-polycaprolactone-block-poly(ethylene glycol)-folate (PEI-PCL-PEG-Fol). Mol Pharm. 2016;13(1):134–43.
14. Feng CL, Han YX, Guo HH, Ma XL, Wang ZQ, Wang LL, et al. Self-assembling HA/PEI/dsRNA-p21 ternary complexes for CD44 mediated small active RNA delivery to colorectal cancer. Drug Deliv. 2017;24(1):1537–48.
15. Sezlev Bilecen D, Uludag H, Hasirci V. Development of PEI-RANK siRNA complex loaded PLGA nanocapsules for the treatment of osteoporosis. Tissue Eng Part A. 2019;25(1–2):34–43.
16. Zhang SB, Xu YM, Wang B, Qiao WH, Liu DL, Li ZS. Cationic compounds used in lipoplexes and polyplexes for gene delivery. J Control Release. 2004;100(2):165–80.
17. Guo JF, Cheng WP, Gu JX, Ding CX, Qu XZ, Yang ZZ, et al. Systemic delivery of therapeutic small interfering RNA using a pH-triggered amphiphilic poly-L-lysine nanocarrier to suppress prostate cancer growth in mice. Eur J Pharm Sci. 2012;45(5):521–32.
18. Hartono SB, Gu WY, Kleitz F, Liu J, He LZ, Middelberg APJ, et al. Poly-L-lysine functionalized large pore cubic mesostructured silica nanoparticles as biocompatible carriers for gene delivery. ACS Nano. 2012;6(3):2104–17.
19. Inoue Y, Kurihara R, Tsuchida A, Hasegawa M, Nagashima T, Mori T, et al. Efficient delivery of siRNA using dendritic poly(L-lysine) for loss-of-function analysis. J Control Release. 2008;126(1):59–66.
20. Boussif O, Lezoualch F, Zanta MA, Mergny MD, Scherman D, Demeneix B, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in-vivo - polyethylenimine. P Natl Acad Sci USA. 1995;92(16):7297–301.
21. Rodriguez M, Lapierre J, Ojha CR, Kaushik A, Batrakova E, Kashanchi F, et al. Intranasal drug delivery of small interfering RNA targeting Beclin1 encapsulated with polyethylenimine (PEI) in mouse brain to achieve HIV attenuation. Sci Rep. 2017;7(1):1862.
22. Urban-Klein B, Werth S, Abuharbeid S, Czubayko F, Aigner A. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. Gene Ther. 2005;12(5):461–6.
23. Lacave JM, Fanjul A, Bilbao E, Gutierrez N, Barrio I, Arostegui I, et al. Acute toxicity, bioaccumulation and effects of dietary transfer of silver from brine shrimp exposed to PVP/PEI-coated silver nanoparticles to zebrafish. Comp Biochem Physiol C Toxicol Pharmacol. 2017;199:69–80.
24. Israel LL, Lellouche E, Ostrovsky S, Yarmiayev V, Bechor M, Michaeli S, et al. Acute in vivo toxicity mitigation of PEI-coated maghemite nanoparticles using controlled oxidation and surface modifications toward siRNA delivery. ACS Appl Mater Interfaces. 2015;7(28):15240–55.
25. Breunig M, Hozsa C, Lungwitz U, Watanabe K, Umeda I, Kato H, et al. Mechanistic investigation of poly(ethylene imine)-based siRNA delivery: disulfide bonds boost intracellular release of the cargo. J Control Release. 2008;130(1):57–63.
26. Wen YT, Pan SR, Luo X, Zhang X, Zhang W, Feng M. A biodegradable low molecular weight polyethylenimine derivative as low toxicity and efficient gene vector. Bioconjug Chem. 2009;20(2):322–32.
27. Zhang TT, Bai XH, Mao XH. Systemic delivery of small interfering RNA targeting the interleukin-2/15 receptor beta chain prevents disease progression in experimental arthritis. PLoS One. 2013;8(11):e78619.
28. Lv H, Zhang S, Wang B, Cui S, Yan J. Toxicity of cationic lipids and cationic polymers in gene delivery. J Control Release. 2006;114(1):100–9.
29. Shi Q, Rondon-Cavanzo EP, Dalla Picola IP, Tiera MJ, Zhang XL, Dai KR, et al. In vivo therapeutic efficacy of TNF alpha silencing by folate-PEG-chitosan-DEAE/siRNA nanoparticles in arthritic mice. Int J Nanomedicine. 2018;13:387–402.
30. Howard KA, Rahbek UL, Liu X, Damgaard CK, Glud SZ, Andersen MO, et al. RNA interference in vitro and in vivo using a novel chitosan/siRNA nanoparticle system. Mol Ther. 2006;14(4):476–84.
31. Raftery R, O'Brien FJ, Cryan SA. Chitosan for gene delivery and orthopedic tissue engineering applications. Molecules. 2013;18(5):5611–47.
32. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, et al. Lipofection - a highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci U S A. 1987;84(21):7413–7.
33. Kesharwani P, Gajbhiye V, Jain NK. A review of nanocarriers for the delivery of small interfering RNA. Biomaterials. 2012;33(29):7138–50.
34. Xue HY, Guo P, Wen WC, Wong HL. Lipid-based nnocarriers for RNA delivery. Curr Pharm Des. 2015;21(22):3140–7.
35. Parashar D, Rajendran V, Shukla R, Sistla R. Lipid-based nanocarriers for delivery of small interfering RNA for therapeutic use. Eur J Pharm Sci. 2020;142:105159.
36. Xu YH, Szoka FC. Mechanism of DNA release from cationic liposome/DNA complexes used in cell transfection. Biochemistry-Us. 1996;35(18):5616–23.
37. Wittrup A, Ai A, Liu X, Hamar P, Trifonova R, Charisse K, et al. Visualizing lipid-formulated siRNA release from endosomes and target gene knockdown. Nat Biotechnol. 2015;33(8):870–6.
38. Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. Chem Biol. 2012;19(1):60–71.
39. Semple SC, Akinc A, Chen JX, Sandhu AP, Mui BL, Cho CK, et al. Rational design of cationic lipids for siRNA delivery. Nat Biotechnol. 2010;28(2):172-U18.
40. Schroeder A, Levins CG, Cortez C, Langer R, Anderson DG. Lipid-based nanotherapeutics for siRNA delivery. J Intern Med. 2010;267(1):9–21.
41. Huang L, Liu Y. In vivo delivery of RNAi with lipid-based nanoparticles. Annu Rev Biomed Eng. 2011;13:507–30.
42. Tseng YC, Mozumdar S, Huang L. Lipid-based systemic delivery of siRNA. Adv Drug Deliv Rev. 2009;61(9):721–31.

43. Kanasty R, Dorkin JR, Vegas A, Anderson D. Delivery materials for siRNA therapeutics. Nat Mater. 2013;12(11):967–77.
44. Kolli S, Wong SP, Harbottle R, Johnston B, Thanou M, Miller AD. pH-triggered nanoparticle mediated delivery of siRNA to liver cells in vitro and in vivo. Bioconjug Chem. 2013;24(3):314–32.
45. Lin SY, Zhao WY, Tsai HC, Hsu WH, Lo CL, Hsiue GH. Sterically polymer-based liposomal complexes with dual-Shell structure for enhancing the siRNA delivery. Biomacromolecules. 2012;13(3): 664–75.
46. Frankel AD, Pabo CO. Cellular uptake of the tat protein from human immunodeficiency virus. Cell. 1988;55(6):1189–93.
47. Endoh T, Ohtsuki T. Cellular siRNA delivery using cell-penetrating peptides modified for endosomal escape. Adv Drug Deliv Rev. 2009;61(9):704–9.
48. Meade BR, Dowdy SF. Exogenous siRNA delivery using peptide transduction domains/cell penetrating peptides. Adv Drug Deliv Rev. 2007;59(2–3):134–40.
49. Ullah I, Chung K, Beloor J, Kim J, Cho M, Kim N, et al. Trileucine residues in a ligand-CPP-based siRNA delivery platform improve endosomal escape of siRNA. J Drug Target. 2017;25(4):320–9.
50. Simeoni F, Morris MC, Heitz F, Divita G. Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. Nucleic Acids Res. 2003;31(11):2717–24.
51. Oehlke J, Scheller A, Wiesner B, Krause E, Beyermann M, Klauschenz E, et al. Cellular uptake of an alpha-helical amphipathic model peptide with the potential to deliver polar compounds into the cell interior non-endocytically. Bba-Biomembranes. 1998;1414(1–2):127–39.
52. Ye J, Liu E, Gong J, Wang J, Huang Y, He H, et al. High-yield synthesis of monomeric LMWP(CPP)-siRNA covalent conjugate for effective cytosolic delivery of siRNA. Theranostics. 2017;7(9): 2495–508.
53. Xie X, Yang Y, Lin W, Liu H, Liu H, Yang Y, et al. Cell-penetrating peptide-siRNA conjugate loaded YSA-modified nanobubbles for ultrasound triggered siRNA delivery. Colloids Surf B: Biointerfaces. 2015;136:641–50.
54. Lee SH, Kang YY, Jang HE, Mok H. Current preclinical small interfering RNA (siRNA)-based conjugate systems for RNA therapeutics. Adv Drug Deliv Rev. 2016;104:78–92.
55. Bolhassani A. Potential efficacy of cell-penetrating peptides for nucleic acid and drug delivery in cancer. Biochim Biophys Acta Rev Cancer. 2011;1816(2):232–46.
56. Bhatia D, Surana S, Chakraborty S, Koushika SP, Krishnan Y. A synthetic icosahedral DNA-based host-cargo complex for functional in vivo imaging. Nat Commun. 2011;2.
57. Keum JW, Ahn JH, Bermudez H. Design, assembly, and activity of antisense DNA nanostructures. Small. 2011;7(24):3529–35.
58. Chen YP, Song S, Yan ZM, Fenniri H, Webster TJ. Self-assembled rosette nanotubes encapsulate and slowly release dexamethasone. Int J Nanomedicine. 2011;6:1035–44.
59. Song S, Chen YP, Yan ZM, Fenniri H, Webster TJ. Self-assembled rosette nanotubes for incorporating hydrophobic drugs in physiological environments. Int J Nanomedicine. 2011;6:101–7.
60. Lee H, Lytton-Jean AK, Chen Y, Love KT, Park AI, Karagiannis ED, et al. Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery. Nat Nanotechnol. 2012;7(6):389–93.
61. Shu Y, Shu D, Haque F, Guo PX. Fabrication of pRNA nanoparticles to deliver therapeutic RNAs and bioactive compounds into tumor cells. Nat Protoc. 2013;8(9):1635–59.
62. Sun X, Chen Y, Yu H, Machan JT, Alladin A, Ramirez J, et al. Anti-miRNA oligonucleotide therapy for chondrosarcoma. Mol Cancer Ther. 2019;18(11):2021–9.
63. Webster TJ, Chen Q, Chen Y, Fenniri H, Hemraz UD. Nanotubes as carriers of nucleic acids into cells. US10364440B2, 2014.
64. Chen Q, Chen Y, Yu H, Ehrlich MG. Nanocarriers And Their Processing For Diagnostics And Therapeutics. US9775842B2, 2015.
65. Chen Q, Yu H, Chen Y. Nanomaterials Compositions, Synthesis, and Assembly. US20170362238A1, 2017.
66. Dobrovolskaia MA. Self-assembled DNA/RNA nanoparticles as a new generation of therapeutic nucleic acids: immunological compatibility and other translational considerations. DNA RNA Nanotechnol. 2016;3(1):1–10.
67. Nixon AJ, Goodrich LR, Scimeca MS, Witte TH, Schnabel LV, Watts AE, et al. Gene therapy in musculoskeletal repair. Ann N Y Acad Sci. 2007;1117:310–27.
68. Andersson G. The burden of musculoskeletal diseases in the United States: prevalence. In: Societal and Economic Cost. Rosemont: American Academy of Orthopaedic Surgeons; 2008.
69. Evans CH, Huard J. Gene therapy approaches to regenerating the musculoskeletal system. Nat Rev Rheumatol. 2015;11(4):234–42.