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Bone Specific Drug Delivery for Osteoporosis and Rare Skeletal Disorders

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Abstract

Purpose of review: The skeletal system provides an important role to support body structure and protect organs. The complexity of its architecture and components makes it challenging to deliver the right amount of the drug into bone regions, particularly avascular cartilage lesions. In this review, we describe the recent advance of bone-targeting methods using bisphosphonates, polymeric oligopeptides, and nanoparticles on osteoporosis and rare skeletal diseases.

Recent findings: Hydroxyapatite (HA), a calcium phosphate with the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is a primary matrix of bone mineral that includes a high concentration of positively charged calcium ion and is found only in bone. This unique feature makes HA a general targeting moiety to the entire skeletal system. We have applied bone-targeting strategy using acidic amino acid oligopeptides into lysosomal enzymes, demonstrating the effects of bone-targeting enzyme replacement therapy and gene therapy on bone and cartilage lesions in inherited skeletal disorders. Virus or no-virus gene therapy using techniques of engineered capsid or nanomedicine has been studied preclinically for skeletal diseases.

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Conflict of Interest

The authors declare no conflict of interest.

Summary: Efficient drug delivery into bone lesions remains an unmet challenge in clinical practice. Bone targeting therapies based on gene transfer can be potential as new candidates for skeletal diseases.

Keywords

bone-targeting; osteoporosis; metabolic rare skeletal disorders; acidic amino acid oligopeptide; nanoparticles

1. Introduction

Bone has many functions in the body, such as supporting the body structure and movement, protecting organs, regulating mineral homeostasis, and providing bone marrow cells which are differentiated to leukocytes, red blood cells, and platelets. The skeletal organ system consists of inorganic and organic materials, as well as bone cells which synthesize, remodel, and maintain bone [1]. Common skeletal and genetic disorders include osteoporosis, osteoarthritis, bone cancer, metabolic skeletal dysplasia, and infectious bone disease. The increasing number of patients with osteoporosis is a serious problem in an aging society. For instance, osteoporosis is a disease that is characterized by the disruption of bone mass and microarchitecture, which causes nearly 1.3 million fractures each year [2]. Population with osteoporosis and low bone mass based on bone mineral density is estimated at over 50 million older adults in the USA [3]. In addition, patients with unique, inherited skeletal diseases have severe osteoporosis since childhood. However, bone diseases can be difficult to treat, mainly because of a complicated anatomical nature, particularly in the avascular cartilage region [4]. After the administration of a drug, visceral organs will capture most of it, and a limited amount will be available for bone and cartilage distribution. Although high doses can improve the delivery of drugs into skeletal lesions, it may induce adverse effects in multiple organs [4][5]. Thus, effective bone-targeting systems are still required to improve the benefits of drugs on skeletal lesions.

Hydroxyapatite (HA) is a calcium phosphate with the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, which is found only in both bone and teeth. This unique feature makes HA be an ideal therapeutic target [6] [7], which has led to the development of several bone-targeting techniques to HA. Modification of drugs with bisphosphonate (BP) or acidic amino acid oligopeptide is the major method of bone-targeted drug delivery to the skeletal system. Nanoparticles (NPs) are also used for targeted drug delivery since NPs increase drug stability, improve its solubility, and prevent its degradation [4] [8]. Thus, nanoparticle-based drugs can be delivered to avascular lesions of bone and cartilage more effectively than non-targeted drugs. These specific tissue targeting systems may decrease the systemic side effects by reducing the dose amount or off-target effect.

In this review article, we summarize bone-targeting approaches using bisphosphonate, acidic oligopeptide, and nanoparticles for osteoporosis and related metabolic skeletal disorders. We also describe the effects of bone-targeting therapies on these bone disorders in recent preclinical studies.

2. Bone targeting strategy

2.1 Bisphosphonates (BPs)

BPs are a group of bone-seeking compounds that have been traditionally utilized for preventing the loss of bone density to treat osteoporosis and similar bone disorders since the effects of BPs on osteoporosis were described in 1968-1969 [9]. As a mechanism of the action of BPs, this chemical can strongly inhibit osteoclastic bone resorption and simultaneously enhance osteoblast differentiation. All BPs, and associated chemicals, share two terminal phosphate bonds (P-C-P) structure in common (Figure 1A). These side chains or R groups can influence binding interactions with bone. BPs have a strong affinity with calcium phosphate in HA by charge, which is a major bone matrix. Nitrogen-containing BPs (N-BPs), such as alendronate (ALN), zoledronic acid, and pamidronate, showed a higher binding affinity with HA than non-nitrogen containing ones [10]. Non-nitrogen-containing BPs are metabolized to cytotoxic ATP analogs and lead to osteoclast apoptosis. On the other hand, N-BPs are not metabolized and inhibit farnesyl pyrophosphate synthase, which is required for osteoclast function [11]. Due to the preferential binding to the bone, BPs considered very useful in the development of targeting moieties aimed at treating diseases with skeletal symptoms, particularly alveolar bone loss. However, BPs still have risks of adverse side effects of atrial fibrillation and gastrointestinal problems.

The modification of proteins with BPs should be useful to target skeletal lesions since most protein drugs seeking the bone do not have a high affinity with HA in physiological conditions. Previous studies showed that BP modification improved the bone-targeting delivery of osteoprotegerin, which is a decoy receptor for activator of nuclear factor κ B ligand (RANKL) inhibitor that prevents osteoclastogenesis in a rat model [12]. Intravenous administration of BP-modified bovine catalase suppressed bone metastasis in mice [13]. Furthermore, Katsumi et al. showed that ALN modification of bovine serum albumin and glutathione reductase, high molecular-weight protein, successfully achieved bone targeting and also demonstrated that approximately 3 to 4 ALN per protein is an optimal degree of modification for bone targeting of these proteins [14].

2.2 Acidic amino acid oligopeptide

HA is an ideal bone-targeted site since HA is a major inorganic component in bone and does not exist in soft tissues. Bone noncollagenous proteins, osteopontin and bone sialoprotein, bind to HA, and these proteins have a repeated sequence of acidic amino acid, aspartic acid (Asp) and glutamic acid (Glu) in their structure [15][16][17]. Based on these reports, Fujisawa et al. showed that poly glutamic peptides (six Glu) preferentially bound to HA [18]. Thus, acidic amino acid-conjugates have been developed as bone-targeted drugs to enhance therapeutic effects (Figure 1B).

The number of residues of Asp and Glu favors the affinity with HA, and, therefore, the *in vivo* study showed that the oligopeptides with six and more residues long attached to fluorescent probe 9-fluorenylmethylchloroformate (Fmoc) were detected primarily in bone [19]. Additionally, *in vitro* experiments showed that the acidic oligopeptide affinity for HA did not depend on the isoforms L-peptides or D-peptides; however, D-isoform showed better

compatibility with the bone and a low immune system recognition [20] [21] [19]. Taking advantage of biocompatible features of acidic oligopeptides, the studies in various fields have been performed with the conjugate of D- and L-peptides for selective delivery to the bone. These included 1) enzymes used for enzyme replacement therapy (ERT), 2) hormones, 3) antibiotics, 4) small interfering RNAs (siRNAs), and 5) gene therapy [4][22][23][24] [25] [26].

Oligopeptides conjugated with si-RNA treatment showed a successful target for osteoclasts located in bone surfaces. In 2012, Zhang et al. treated an osteoporotic rat model by using a delivery system form by dioleoyl trimethylammonium propane (DOTAP)-based cationic liposomes attached to oligopeptide (AspSerSer)₆ as specific bone moiety. The small interfering RNA (siRNA) against casein kinase-2 interacting protein-1 (Plekho1), an inhibitor of bone formation, was delivered to the bone [22][27]. The results of immunohistochemistry and flow cytometry showed that (AspSerSer)₆ –liposome allowed delivery of the Plekho1-siRNA and knockout of Plekho1 in osteogenic cells, even in healthy rats and that liposome plus (AspSerSer)₆ moiety improved the longevity and activated of osteoblasts [22][27][28]. These results proved the acidic oligopeptides as a system delivery for siRNA that is specific to osteogenic cells in the bone.

In 2012, Bo et al. used the acidic oligopeptide with D6 (L-Asp hexapeptide; Asp₆) as bone target moiety in combination with a RGD7 (R-G-D-D-D-D-D-D) like a tumor target for direct delivery of the anti-cancer drug, 5-fluorouracil, to the bone cancer cells [29]. Both *in vitro* and *in vivo* results showed the stability and release of the active drug as well as the high affinity to the bone. Oligopeptides, as a ligand or receptor, demonstrated the affinity and selectivity for the bone. This strategy would be applied to treat different bone lesions like cancer or osteoporosis [29].

In summary, the oligopeptide system presents advantages and disadvantages; the authors describe the great advantages of this system are the high biocompatible to the bone, the efficacy and specificity delivery of drugs [4] [20] [30] [29]. Additionally, *in vitro* and *in vivo* results concluded that the oligopeptide system has low immune system recognition, thus reducing the undesired side effects [21]. For those reasons, the L- and D-peptides isoforms are a potential system to treat osteoporosis and bone diseases. However, oligopeptide-based delivery to bone have some problems to tendency of aggregation and/or low stability [5][31].

2.3 Nanoparticle

Nanotechnology is being used as another alternative method for bone-targeted treatment. Because of its small size feature and similarity in components found in tissues [32], the nanoparticle materials can be delivered to specific tissues, organelles or cells where the medicine will be released [33]. The advantage of nanoparticles provides a large capacity of the drug concerning size [34] [35], improves solubility [36], gives stability to the drug [37], reduces adverse effects [38], and improves transport to internalize in specific organelles [39]. Another advantage is that the nanoparticles, which are made of calcium phosphate, gold, and nanodiamonds, can help to activate functions in the cells, improve the mineralization, and stimulate bone growth [38]. Thus, nanotechnology can overcome the limitations of conventional bone therapy, such as adverse effects and poor penetration to

skeletal lesions. Moreover, adding targeting ligands such as peptides, aptamers, or small molecules can improve bone-targeting ability [40].

Nanoparticles can be classified into two groups: organic and inorganic. Organic nanoparticles are solid particles composed of organic compounds such as polymers, protein, peptide, or lipid [41]. The advantage of organic nanoparticles is that they have low toxicity and improve passive and active transport to target lesions. Inorganic nanoparticles are composed of silica, silicon materials, metals (gold, titanium, silver), Oxides (ZnO, TiO₂, SiO₂) semiconductor metals (CdS and CdSe -quantum dots-) carbon (nanodiamond, nanotubes) and iron oxides or other magnetic elements as nickel or cobalt (magnetic nanoparticles). For certain applications, there are several advantages of inorganic nanoparticles over organic ones, including high stability and precise control over shape and size [42]. These two types of nanoparticles have been applied to bone-targeting methods, and the effects of this strategy on bone disorders have been reported in many preclinical studies (Table 1).

BP conjugates nanoparticles have been developed because this bone target moiety can act as a useful drug delivery system to bone. Poly (D, L-lactic-co-glycolic acid) (PLGA) nanoparticles modified with both, polyethylene glycol (PEG) and ALN, showed a strong absorption with increased ALN concentration [43]. Zoledronate tagged PLGA NPs conjugated with docetaxel also showed prolong blood circulation and higher retention at the bone site [44]. Doxorubicin conjugated with PLGA and ALN demonstrated a significant reduction of incidence of bone metastases compared with the free drug [45] [46]. Doxorubicin-loaded liposomes modified with ALN and an aqueous polyelectrolyte, poly (acrylic acid) showed the prolonged presence of doxorubicin in osteosarcoma tumor and significantly improved efficacy in a xenograft mouse model [47]. Swami et al. demonstrated the effect of bortezomib conjugated with PLGA, PEG, and BPs in mouse models of multiple myeloma. These targeted NPs enhanced survival and decreased tumor burden in the model mice more efficiently than the free drug [48].

3 Bone targeting for diseases

3.1 Osteoporosis

Osteoporosis is a common health problem, especially in older women, leading to the loss of bone mineral density (BMD) and the increase in the risk of fractures. Some of risk factors can increase the likelihood of developing osteoporosis, including age, hormone imbalance, life-style, and medical conditions. The reduction of estrogen level at menopause in women or testosterone level in men is a strong risk factor for this disease. Hormonal changes during the menopause cause the elevation of follicle-stimulating hormone (FSH) level and the reduction of estradiol (E₂) level, resulting in a progressive decline in BMD [23] [49][50]. Treatment for the regeneration of bone in postmenopausal women was initiated with estrogen replacement therapy; however, this treatment could develop endometritis and breast and uterus cancer if used for a long period [4] [23]. Therefore, development of bone-targeting treatment for osteoporosis should be required to enhance therapeutic efficacy and to alleviate the side effects of conventional therapy.

Bone targeting therapy using the oligopeptide with BPs and NPs has been developed in preclinical studies. Yokogawa et al. used the oligopeptide, L-Asp-hexapeptide (3D6), attached with E2 for specific delivery to the bone in ovariectomized (OVX) mice [24] [51] [52]. The weekly administration of E2-3D₆ (0.11 to 1.1 mmol/kg-sc) showed a high affinity for bone and specific delivery of E2 to the osteoblasts. Consequently, an increase of bone matrix proteins as osteopontin, type I collagen α , and sialoprotein after 4 hours of injection was detected [51]. In addition, this treatment increased the BMD and improved the architecture of bone. Moreover, E2-3D₆ showed a low affinity to ER α and ER α and decreased the risk of cancer and endometritis [51] [52]. These results postulate the acidic oligopeptides as a right candidate for specific delivery to the osteoporotic bone.

BP-modified drugs for osteoporosis have been developed in preclinical studies. PEG-conjugated hormones such as parathyroid hormone (PTH) or calcitonin were prepared, and BP-mediated conjugates were synthesized. The effects of these hormones mediated by BP were tested in animal models, and these bone targeting drugs showed significant improvement of bone strength and formation of osteoporosis model rats [53] [54]. BP-conjugated prodrugs of prostaglandin E2 and 17 β -estradiol were also developed [55][56]. Repeated administration of either of these conjugated compounds in animal models of osteoporosis showed specific delivery to bone and improvement of BMD compared with that of free compounds.

3.2 Inherited metabolic diseases causing osteoporosis

3.2.1 Mucopolysaccharidoses (MPS IVA)—Mucopolysaccharidoses (MPS) are a group of inherited lysosomal metabolic diseases caused by the deficiency of lysosomal enzymes, leading to excessive accumulation of glycosaminoglycans (GAGs) in multiple tissues, including bone and cartilage. Types of GAG are dermatan sulfate (DS), chondroitin sulfate (CS), heparan sulfate (HS), keratan sulfate (KS), and/or hyaluronan. In general, clinical symptoms do not appear in newborn patients with MPS, and growth impairments start during the first 2 to 4 years of age [57][58]. Skeletal deformities are common symptoms in any types of patients with MPS, including kyphoscoliosis, lumbar gibbus, flaring of the rib cage, pectus carinatum, abnormal joint mobility (rigidity or laxity of joint), short stature, and genu valgum.

MPS IVA (also called Morquio syndrome type A) is caused by a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), leading to KS and chondroitin-6-sulfate (C6S) accumulation. This disease shows the most severe skeletal dysplasia among all types of MPS with incomplete ossification and successive thin cortical bone. Most patients with MPS IVA show marked low bone mineral density and osteoporotic status [59][60]. This skeletal disorder often causes airway compromise such as trachea obstruction and respiratory infection and sometimes provides the risks of dying of sleep apnea and related complications. ERT and hematopoietic stem cell transplantation (HSCT) are available for patients with MPS IVA. We have followed the therapeutic efficacy of conventional ERT and HSCT on growth, activity of daily living (ADL), and frequency of surgery [61]. Doherty et al. showed that conventional ERT did not have an impact on bone growth of MPS IVA patients even if ERT started at 2 years of age [62]. We also showed that HSCT is likely to

have more effects on bone and cartilage lesions of patients with this disease than ERT since HSCT improved an ADL and a surgical frequency compared to ERT [49]. However, HSCT did not provide a significant impact on the bone growth of patients with MPS IVA, even if HSCT started at 4 years of age [63][64]. Nevertheless, it remains unknown if ERT and HSCT may provide a benefit in bone growth if they start before one year of age. Therefore, the approach for bone and cartilage lesions of MPS IVA is still an unmet challenge.

We have applied bone-targeting approaches using the acidic amino acid oligopeptide to ERT and have examined the effects of this bone-targeted ERT on bone and cartilage lesions of murine models, including MPS IVA and MPS VII [65][66]. The human GALNS enzyme was bioengineered with N-terminal extension conjugated by hexa-glutamate sequence (E6), and this modified enzyme was administered to the MPS IVA mouse. Half-life time of this enzyme was markedly prolonged compared with a native enzyme, and the enzyme activity in bone was also retained longer in bone after a single administration of enzymes, indicating that the modified enzyme was circulated for a longer time and was selectively delivered into bone lesions. After weekly administration of 1 mg/kg of this enzyme for 12 or 24 weeks, the pathological findings in this mouse model showed substantial clearance of storage materials from chondrocyte in the growth plate and improvement of column structure [65]. These improvements of skeletal lesions were also seen when we administered E6- β -glucuronidase into MPS VII mice [66].

Nanomedicine should be another candidate treatment option for MPS IVA. Alvarez et al. developed nanostructured lipid carriers (NLC) containing the GALNS enzyme. They studied its stability, efficacy, and distribution pattern of the enzyme immobilized in NLC by both *in vitro* and *in vivo* experiments. The results showed that the NLC stabilized the GALNS enzyme, reached to the lysosome, and reduced GAG accumulation in cartilage cells. After the intravenous administration of the NLC, the GALNS enzyme was also distributed to hard-to-accessible organs such as bone and cartilage regions [67][68].

3.2.2 Hypophosphatasia (HPP)—Hypophosphatasia (HPP) is characterized by defective mineralization of bone and/or teeth in the presence of low activity of serum and bone alkaline phosphatase (TNSALP). HPP comprises six clinical forms usually recognized based on age at diagnosis and severity of features. HPP is caused by mutations in the *ALPL* gene [69][70][71]. Perinatal and infantile HPP are inherited in an autosomal recessive manner. The milder forms, especially adult forms and odontohypophosphatasia, may be inherited in an autosomal recessive or autosomal dominant manner. Morin et al. have shown that the more frequent mutation within HPP patients is a missense mutation and that patients with several phenotypes had less activity while patients with the mid phenotypes had low levels of enzymatic activity [72]. TNSALP is expressed in bone, liver, kidney, adrenal tissues, and teeth, and this enzyme hydrolyzes inorganic pyrophosphate (PPi) in the bone. Unmetabolized PPi inhibits the growth of the HA crystals, which are important molecules for calcification of chondrocytes with the correct bone mineralization [69][70][71]. As a result of the accumulation of PPi in the bone, HPP causes low bone mineralization, leading to variable clinical manifestations such as osteoporosis, premature loss of dentition, rickets, calcific arthropathies during adulthood, hypercalcemia and severe

hypomineralization, respiratory failure due to lung hypoplasia lung, hypercalcemia, vitamin-B6-dependent seizures and death in utero or few days after the birth [69][70][71][73].

ERT has been a potential to treat HPP since this metabolic disease is caused by a single enzyme deficiency. The study of the therapeutic efficacy of the ERT for HPP started since the 1990s with an enzyme purified from human tissues [74][70][71]. These studies showed an improvement in visceral organs and normalization of the enzyme level in blood circulation; however, the outcomes from these studies did not show any significant improvement in bone mineralization [69][70][71][75][76][74]. In 2006, Nishioka et al. reported the improvement in bone mineralization after the administration of the C-terminal-anchorless TNSALP enzyme with an oligopeptide. This modified enzyme works as a motif to allow the specific delivery to bone in *in vitro* affinity experiments. The *in vitro* assays showed that labeled enzymes with L-Asp had a 30-fold higher affinity to HA, and *in vivo* biodistribution analysis revealed that the labeled-enzyme was retained in bone of AKP^{-/-} mice after 18 hours intravenous (IV) administration. Additionally, *in vitro* assays in bone marrow from HPP patients showed that the TNSALP-L-Asp enzyme could mineralize the bone marrow. Taken together, Nishioka et al. showed that TNSALP-L-Asp enzyme was delivered to the bone with high specificity and with the bioactivity to mineralize the bone [69]. Based on further investigation of the TNSALP combined with the acidic oligopeptide, clinical trials of ERT for HPP were conducted, and asfotase alfa (human TNSALP-Strensiq^{TM}) was approved in 2015 in Japan, Canada, United States, and Europe Union for perinatal, infants, and childhood (under 18 ages) HPP patients [77] [70][71]. Asfotase alfa is composed of TNSALP, FC fragment of IgG₁, and (ASP)₁₀ (a deca-aspartate) motif [70][71]. The IV administration of asfotase alfa in preclinical and clinical studies showed normalization of TNSALP in blood and organs, improvement of clinical complications related to skeletal and dental defects, and reduction of the accumulation of PPi in lung and muscle [70][71][77][78].

The therapeutic efficacy of ERT in adults or in HPP patients with mild symptoms has not been studied in-depth since gene therapy carrying TNSALP has been explored in the animal model. Alternative treatment using stem cells and gene therapy is currently being investigated. The first studies have shown that the genetic correction in two childhood HPP patient-derived induced pluripotent stem cells recovers enzyme activity and calcification *in vitro* [70][77][79]. Several studies showed that a single injection of vectors with TNALP with deca-aspartates (D10) at the C-terminus in newborn AKP^{-/-} mice corrected the expression of TNALP level and improved some clinical manifestations [80][81][82].

In 2011, Matsumoto et al. reported that the AKP^{-/-} mice treated with single postnatal IV injection of adeno-associated virus 8 (AAV8) vectors carrying TNALP-D10 improved the survival rate with correct mineralization of the epiphyses. However, high levels of ALP activity detected in plasma decreased with time from 165.1 ± 79.8 U/ml in day 10 to 13.7 ± 1.1 U/ml on day 28. This consequence led to the interference of the treatment in bone mineralization [80].

Continuing with the studies of the efficacy of the AAV8-TNALP-D₁₀ vector and better clinical applicability, Ikeue et al. developed a self-complementary AAV8 (scAAV8) vector

that carrying TNALP-D₁₀ under the control of the muscle creatine kinase (MCK) promoter and they treated the scAAV8 vector with AKP2^{-/-} neonatal mice. In this study, 2.5×10^{12} vg/body of scAAV8-MCK-TNALP-D₁₀ vector was injected into the bilateral quadriceps and showed the effectivity of expression of the vector in bone and muscle [83]. The ALP activity in plasma increased over 1 U/ml during 90 days post-injection, and physical activity, facial appearance, and mineralization of dentin structure were improved. The bone structure also showed improvements evaluated by radiology analysis [81][83]. Nevertheless, the micro-CT analysis still showed low bone mineralization and abnormal bone architecture [81]. These data indicate that the vector used allowed the delivery safely to bone and muscle in AKP2^{-/-} mice. Still, the therapeutic effect remained incomplete in the improvement of bone mineralization and architecture. A further study on the effects of scAAV8-MCK-TNALP-D₁₀ showed a high doses of 4.5×10^{12} vg/body injected into the bilateral quadriceps in newborn AKP2^{-/-} mice could increase ALP activity to 20 U/ml in blood and improved the mineralization and bone structure analyzed by micro-CT and radiological analysis. In addition, the histological analysis in growth plate showed normal growth and well-organized cartilage arrangement of AKP2^{-/-} mice treated with 4.5×10^{12} vg/body [84].

4 Future directions

The recent results from different studies on the metabolic disorders showed that gene therapy as an excellent candidate to treat skeletal manifestations. In 2018, Almeciga et al. demonstrated a novel strategy for specific delivery of AAV vector to the bone by introducing an aspartic acid octapeptide (D8) in the N-terminal region of the VP2 capsid protein of AAV2 vector. After the administration into MPS IVA mice, the AAV2-D8 vector showed a high affinity to HA, a high number of copies of the viral vector, and an increased GALNS enzyme activity in bone after 3 months after IV injection [25][26] (Figure 3). These results showed that the bone-targeting peptide in the capsid vector was a possible retargeting of the AAV vector, leading to the treatment of bone diseases.

On the other hand, to lead the gene therapy to molecular and metabolic regulation levels, modified microRNA (miRNA) therapy started to treat bone diseases. In 2019, Sun et al. reviewed the potency of miRNA-base gene therapies for bone regeneration, improving the bone structure and preventing osteoporosis and its osteoporotic fracture. The miRNAs played an important role in a variety of cell processes like proliferation and differentiation in bone cells [85][86]. The miRNAs treatment provides two roles; 1) reduce the expression of pathological genes related to bone formation and 2) increase the expression of genes that lose the activity during pathological bone development. However, this kind of treatment could result in low stability in the organism, low specificity to bone, and off-target effects [85]. To resolve the limitations of specificity and efficacy delivery of miRNAs, the recent studies made the combination with the vector like AVV or Baculovirus vector increased the specificity of the delivery to bone. These kinds of systems are called Viral Vector- Based Systemic miRNA Delivery Systems (Figure 3) [85][86]. The miRNAs-based treatment needs to be investigated further with an excellent potency for bone treatment. For future directions in gene therapy, we have to generate clinically effective drugs for bone degeneration without the off-targeting effect.

5 Conclusion

Prevention and resolution of bone and cartilage lesions remain an unmet challenge in patients with skeletal diseases, causing osteoporosis. Bone-targeted therapy provides several advantages of delivering the right amount of drug to skeletal lesions, decreasing drug dosage, reducing adverse effects, and increasing therapeutic efficacy over non-targeting treatment. Bone-targeting approaches using polymeric oligopeptides and nanoparticles have been developed all over few decades; however, these achievements remain in the preclinical phase. Further studies should be required to move forward to the clinical level. Gene therapy combined with a bone-targeting strategy such as acidic amino acid oligopeptide and nanomedicine can be potential as the next generation of one-time therapy to correct the systemic bone diseases.

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Abbreviations:

AAV	adeno-associated virus
ADL	activity of daily living
ALN	alendronate
Asp	aspartic acid
BMD	bone mineral density
BPs	bisphosphonates
C6S	chondroitin-6-sulfate
D8	aspartic acid octapeptide
E2	estradiol
ERT	enzyme replacement therapy
GAG	glycosaminoglycans
GALNS	N-acetylgalactosamine 6-sulfate sulfatase
Glu	glutamic acid
HA	hydroxyapatite

HPP	hypophosphatasia
HS	heparan sulfate
HSCT	hematopoietic stem cell transplantation
IV	intravenous
KS	keratan sulfate
miRNA	microRNA
MPS	mucopolysaccharidosis
N-BPs	nitrogen-containing BPs
NLC	nanostuctured lipid carriers
NPs	nanoparticles
OVX	ovariectomized
PEG	polyethylene glycol
PLGA	poly (D,L-lactic-co-glycolic acid)
siRNA	small interfering RNA

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Figure 1. Binding of bisphosphonate and oligopeptide conjugated drug to bone.

(A) Chemical structure of bisphosphonates. Two side chains (R and R') determine the chemical properties of bisphosphonates. Bisphosphonates specifically bind to HA via coordination between two phosphate groups (and a hydroxyl group in R) and calcium ions of the crystal structure. (B) Negatively-charged acidic amino acid tagged drugs circulate in blood for longer time and delivered into bone more efficiently. These negatively-charged drugs bind with calcium site on HA. HA: hydroxyapatite.

Figure 2.

Different components for targeting organic and inorganic nanoparticles to the bone. There are more unspecific nanoparticles whose targets are the vesicles inside cells or receptors on the cell surface. Organic materials as a family of CaP are more specific for targeting bone. These materials bind with the ligands like BPs, acidic oligopeptides, and aptamers to target bone. Other organic materials as silica, nanotubes of carbon, and magnetic nanoparticles are alternatives binding with the ligands and targeting bone lesions.

Figure 3. Future directions for osteoporosis treatment

AAV gene therapy with acidic oligopeptides (aspartic octapeptide-D8) in the capsid protein increases the specificity of the treatment in bone lesions. Additionally, miRNAs treatment with the AAV vector system increases the expression of the underlying gene directly in the bone.

Table 1.

Different combinations of nanoparticle systems

Ligands	Materials	types drugs encapsulated	Therapeutic uses
BPs	CaP	Anti-inflammatories drugs Antibiotics drug Growth factors Genes Small molecules	Osteogenesis, inflammation, infections, post-surgery, osteoarthritis, rheumatoid arthritis, analgesics, osteoporosis, gene therapy, cancer
	Silica	Genes	Gene therapy
	Nanotubes	Implants Small molecules	Surgeries, bone repair, osteoporosis
	Metals		antimicrobial
	Nanodiamonds	Small molecules	osteoporosis
	Chitosan	Genes	Gene therapy
BPs Oligopeptides Aptamer	Polymers	Proteins Hormones Growth factors Small molecules Scaffold	Osteogenesis, bone repair, osteoarthritis, rheumatoid arthritis, osteoporosis, cancer.
BPs Oligopeptides Aptamer	Lipid	Proteins Hormones Growth factors Small molecules	Osteogenesis, mucopolysaccharidosis, osteoarthritis, rheumatoid arthritis, cancer.

Abbreviation

BPs, bisphosphonates; CaP, calcium phosphate