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REVIEW ARTICLE

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Targeted nanomedicines for the treatment of bone disease and regeneration

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Abstract

Targeted delivery by either passive or active targeting of therapeutics to the bone is an attractive treatment for various bone related diseases such as osteoporosis, osteosarcoma, multiple myeloma, and metastatic bone tumors. Engineering novel drug delivery carriers can increase therapeutic efficacy and minimize the risk of side effects. Developmnet of nanocarrier delivery systems is an interesting field of ongoing studies with opportunities to provide more effective therapies. In addition, preclinical nanomedicine research can open new

Abbreviations: ^{99m}Tc-HEDP, hydroxyethylidene diphosphonate; ^{99m}Tc-HMDP, hydroxyl methylene diphosphonate; ^{99m}Tc-MDP, technetium-99m methylene diphosphonate; Asp_8, poly-aspartic acid; ATP, adenosine triphosphate; BLI, bioluminescent imaging; BMP, bone morphogenetic protein; BMP-2, bone morphogenetic protein-2; BMSC, bone marrow stromal cell; BONJ, bisphosphonate-associated osteonecrosis of the jaws; BP, bisphosphonate; FDA, Food and Drug Administration; FGF, fibroblast growth factor; FPPS, farnesyl pyrophosphate synthase; GF, growth factor; Glu8, poly glutamic acid; GO, graphene oxide; HA, hydroxyapatite; hMSC, human mesenchymal stem cell; IGF, insulin-like growth factor; MM, multiple myeloma; MSC, mesenchymal stem cell; MTD, maximum tolerated dose; NPs, nanoparticles; OVX, ovariectomized; PBLG, poly-γ-benzyl-L-glutamate; PBLG-b-PGlu, copolymer poly(γ-benzyl-glutamate)-block-poly (glutamic acid) (PBLG-b-PGlu); PDGF, platelet-derived growth factor; PEG, polyethylene glycol; PLGA, poly-lactic-co-glycolic acid; Pox, poly-2-oxazoline; PSMA, prostate-specific membrane antigen; siRNA, small interfering RNA; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

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opportunities for preclinical bone-targeted drug delivery; nevertheless, further research is needed to progress these therapies towards clinical applications. In the present review, the latest advancements in targeting moieties and nanocarrier drug delivery systems for the treatment of bone diseases are summarized. We also review the regeneration capability and effective delivery of nanomedicines for orthopedic applications.

KEYWORDS

bone regeneration, nanomedicine, orthopedics, targeted drug delivery

1 | INTRODUCTION

Nanomedicine, the medical application of nanotechnology, has attracted a great deal of consideration in the drug delivery research field. The growing attention in nanomedicine is driven by its potential to improve the treatment of prevalent global diseases such as cancer, cardiovascular disorders, rheumatoid arthritis, and diabetes—by minimizing systemic toxicity and more effectively targeting therapies.[1–3] A variety of nanomedicines including polymeric nanoparticles (NPs), liposomes, micelles, and dendrimers have been proposed as drug delivery vehicles in the recent decades.[4,5] Doxil®, a PEGylated liposomal doxorubicin formulation, which was approved in 1995, paved the way for the development and FDA-approval of current nanomedicines for clinical application.[6]

An essential design factor in engineering advanced nanodrug delivery systems is a balance between therapeutic efficacy and adverse side effects.[7] To this end, nanocarrier mediated drug delivery offers numerous advantages for treatment of bony diseases. For example, it can carry the therapeutic agents to the target sight while keeping the drug concentrated. This improves pharmacokinetics and biodistribution of the therapeutics. In addition, the use of nanocarriers can protect therapeutic molecules from degradation, improve the circulation with a stable drug retention time in the body, and enhance the solubility of hydrophobic drugs due to the high specific surface area of the NPs. Previous studies have shown that the use of nanocarriers can also improve tolerability and therapeutic effects of drugs and facilitate drug delivery throughout the biological barriers such as endothelial as well as epithelial barriers, with minimal immunogenicity.[1,8–10]

There are two kinds of targeted delivery, namely passive and active targeting. These strategies are aimed to deliver NPs along with their therapeutic loads to diseased organs while minimizing their accumulation in healthy tissues.[11] In the bone, passive targeting utilizes bone-marrow capillaries' fenestrations.[12] In passive targeting, nanocarriers are taken in by the macrophages and accumulated in bone tissues. Some particles, generated by these macrophages, allow the small circulating cells to go into the bone marrow.[13] On the other hand, active targeting applies specific interactions with osteoid or takes advantage of ligand–receptor binding, which enhances selective accumulation to targeted sites and thus differentiates between the diseased and healthy tissues.[12,14] For an efficient NP accumulation in diseased tissue, longer circulation time in blood is desirable, to allow multiple passes of the nanomedicines by the target site.[8,15] The circulation time is dictated by intrinsic physicochemical characteristics of the nanomedicines including shape, size, charge, solubility, rigidity, and surface modifications.[1,16] Upon administration into the blood, nanomedicines interact with blood proteins and cells and are rapidly cleared from the blood circulatory system by the mononuclear phagocytic system. One of the first strategies to prolong circulation time is decorating the surface of nanomedicines by hydrophilic polymers, which could impart a stealth

character against the immune system.[17] Due to its high hydrophilicity and low toxicity, polyethylene glycol (PEG) is extensively served as stealth coating on NPs' surfaces specially in drug delivery field.[18]

However, some drawbacks such as transient nature of the effect, compromised interactions between the particle and target, activation of the immune system, and decreased cellular uptake of PEGylated nanomedicines have been reported.[19–21] Poly (2-oxazoline) (POx) is a nontoxic polymer with similar stealth properties as PEG. It is a potential alternative class of stealth polymer which has numerous advantages such as superb biocompatibility, thermo-responsiveness, and high stability.[18,22] Accordingly, several other approaches including modification of particle morphology (e.g., shape and size modification), modulation of mechanical properties (e.g., elasticity), and hitchhiking on red blood cells have been used to extend nanomedicine circulation time in blood.[20,21,23]

To improve the selective delivery of NPs to particular cells or tissues, targeting ligands (i.e., aptamers,[24] antibodies,[25] small molecules,[26] or peptides[27,28] that are attached to specific cell surface receptors) bind to the surface of NPs. Active targeting or ligand-mediated targeting permits preferential accumulation of the NPs in particular cells or tissues.[29,30] A few ligand-mediated targeting NPs have reached human clinical trials. For example, Bind-014 is a chemically coated NP functionalized with the tumor prostate-specific membrane antigen (PSMA)-specific ligand. Bind-014 is a docetaxel encapsulated polymeric NP for solid tumor treatment.[31] In phase I clinical trials, Bind-014 was well tolerated, with expected and controllable toxicity and a specific pharmacokinetic profile in comparison with conventional docetaxel.[32] CALAA-01, a transferrin targeted polymeric NP containing siRNA, MBP-426, a transferrin targeted liposomal oxaliplatin, and SGT-53, a transferrin targeted liposome composed of plasmid DNA encoding p53 are some of the other examples of targeted nanomedicines which are under clinical investigations.[33,34]

Although ligand-mediated targeting is ideal, all currently FDA-approved nanomedicines for clinical use such as *Ostim*®, *NanOss*®, *Vitoss*®, *OsSatura*®,[35,36] *Doxil*®,[6] *Abraxane*®,[37] *Taxol*,[38] and *Genexol-PM*[39] are passively targeted without targeting ligands. Due to inadequate knowledge in the nano-bio interface, the fate of nanomedicines in vivo, and also complicated processes to achieve a controllable and reproducible synthesis of targeted nanomedicines, the clinical translation of nanomedicine is slow.[1,40] Here, we present a summary of the latest advances in the use of drug nanocarriers for the treatment of bone diseases. Furthermore, we introduce bone targeting agents in the regeneration/repair of bone tissue.

Different bone diseases such as osteosarcoma, osteoporosis, multiple myeloma, and metastatic bone tumors constitute a major public health problem.[41–45] One of the most prevalent bone diseases is osteoporosis, marked by low level of bone mineral density and architectural degradation of bone tissue, resulting in increased susceptibility to fracture.[46] By the rapid increase in aging population, the prevalence of osteoporosis is steadily rising.[47] It is expected that the number of patients (over 50 years) suffering from osteoporosis will increase by 10.4 million (19%) from 2010 to 2020, and by 17.2 million (32%) from 2010 to 2030 in the United States alone.[48] Osteoporotic fractures impose heavy social and financial burdens, including direct medical costs and indirect costs caused by the decreased quality of life, loss of productivity, and morbidity.[49] For instance, the mortality rate after a hip fracture is 20%–30% in the first year and more than half of these individuals cannot return to their previous ambulatory status. In addition, the health care costs from osteoporosis surpass $15 billion annually in the United States alone.[42] Established therapies for the osteoporosis treatment currently focus on the bone resorption prevention (through bisphosphonates [BPs], calcitonin, denosumab, and selective oestrogen receptor modulators),[50] or directly increasing bone mineral density (through parathyroid hormone analogues).[42]

Bone lesions remain one of the most common sites of the prostate (65%–75%), breast (65%–75%), thyroid (60%), lung (30%–40%), and kidney metastases (20%–25%).[51,52] Tumor invasion into bone tissue can result in osteolysis, which indirectly causes spinal cord/intervertebral nerve compression, anemia, severe pain, fractures, and life-threatening hypercalcemia.[41] Currently, there are limited clinical treatments for bone metastases, and conventional chemotherapy and radiation treatments are palliative and relatively ineffective in many patients.[53] The use of adjuvant treatment with BPs or subcutaneous inhibitors of receptor activator of nuclear factor κB ligand (RANKL; also known as TNFSF11) antibodies can considerably slow down the progression of bone metastases and enhance survival.[53]

The infiltration of malignant plasma cells into the bone marrow, which takes place in skeletal disorder, is known as multiple myeloma (MM). In 2012, the annual prevalence was 89,650 patients, with an annual occurrence of 6.3 new cases per 100,000 individuals in the United States.[54] The 5-year overall survival remains only 48.5%.[55] The introduction of new generation proteasomes (e.g., bortezomib), immunomodulatory drugs (such as thalidomide and lenalidomide), monoclonal antibodies, and autologous stem-cell transplantation has greatly improved the clinical outcome of patients with multiple myeloma [54,56] with 3-year survival rates of 75%–80%.[55]

Osteosarcoma is another bone disease representing a primary bone malignancy in most children and adolescents, with a worldwide occurrence of approximately one to three cases annually per million.[44] Surgery is the mainstay of treatment, with survival rates of 15%–17%.[44] Adjuvant chemotherapy with high-dose methotrexate and vincristine followed by folic acid makes surgical resection easier and triple rates of survival for patients suffering from nonmetastatic diseases. Nevertheless, survival rate for patients with metastatic or relapsed osteosarcoma is low (a total 5-year survival rate of about 20%).[44]

As effective medications for many skeletal diseases are rare, developing new medicines and drug delivery systems is a necessity for effective and safe treatments. The current review summarizes the most recent efforts on modifying nanomedicines using bone-seeking moieties to increase their localization at the site of bone tissue and improve their drug release kinetics. First, several bone-seeking moieties such as oligopeptides, BPs, and tetracycline, which have a high affinity to hydroxyapatite (HA) are introduced. Then, surface modifications of nanomedicines including polymeric particles, liposomes, micelles, and dendrimers with these bone-targeting moieties are discussed.

2 | BONE MICROENVIRONMENT

The bone skeleton is a dynamic and multifunctional connective tissue which plays a significant role in shaping a structural framework. Bone skeleton supports the entire body, protects internal organs, and maintains mineral homeostasis and acid/base balance. It serves as a source of cytokines and growth factors (GFs), and provides the environment for hematopoiesis inside the marrow.[57,58] As a complex living tissue, bone consists of a mineralized extracellular matrix (ECM) with cellular components including osteoblasts, osteoclasts, and osteocytes.[50] Osteoblasts, originally derived from mesenchymal stem cells (MSCs), are bone cells with a single nucleus that generate the bone matrix and mineralize it during bone formation and remodeling.[59] Bone is comprised of a combination of extracellular proteins such as bone sialoprotein, osteocalcin, osteopontin, alkaline phosphatase (ALP), and a considerable amount of collagen (type I) which are produced by osteoblasts.[44,60] While most osteoblasts turn into osteocytes, some of them develop into lining cells.[61] Osteocytes are the most common and long-lived cell type in bone, which remain trapped within the bone matrix and eventually stop generating osteoid, but have a main role in coordinating the activities of osteoblast and osteoclast cells in remodeling processes.[58] For example, osteocytes can secrete sclerostin, which activates osteoclasts and inhibits osteoblastic bone formation.[61] Furthermore, osteocytes contribute to the endocrine functions of bone tissue by secreting hormones that affect other tissues and also regulate mineral homeostasis and hematopoiesis.[62] Osteocytes are stellate shaped cells carrying dendritic processes that are connected to each other and with osteoblasts, osteoclast precursors, and lining cells within a canalicular network.[50] Osteoclasts are derived from hematopoietic stem cells (HSCs) in the myeloid lineage and carry out the process of bone resorption.[63] Osteoclasts formation is controlled by the receptor activator of nuclear factor-kappa B ligand and macrophage colony-stimulating factor, which are produced by the osteoblast lineage of the cells.[64] The dynamic nature of the bone is achieved by a process called “remodeling.” During the remodeling process, damaged or old bone tissue is removed by monocyte lineage and/or osteoclasts of the hematopoietic macrophage, and new mineralized matrix of bone tissue is formed by osteoblasts of the mesenchymal lineage [65] (Figure 1). Bone remodeling is well-coordinated in healthy people, thus, the microarchitecture integrity and bone mass are kept in a steady state. Any disturbances of this balance can result in skeletal disorders.[66]

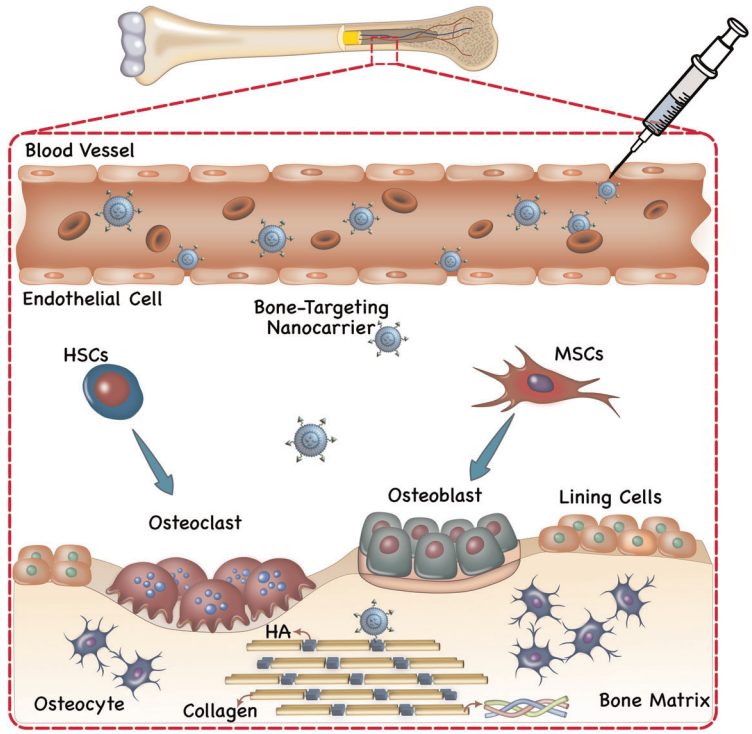

FIGURE 1 Cellular structure in bone and active targeting of hydroxyapatite as a distinctive feature of bone tissue. Bone remodeling involves resorption of either old or damaged tissues by osteoclasts of the hematopoietic myeloid lineage and replacement by osteoblasts descended from the mesenchymal stem cells. While most osteoblasts turn into osteocytes, some of them develop into lining cells. HSC, hematopoietic stem cell; MSC, mesenchymal stem cell [Color figure can be viewed at wileyonlinelibrary.com]

The osteocytes, derived from matrix-producing osteoblasts, express different markers including CD105, Stro1, CD29, and CD166. Also, matrix-producing osteoblasts express markers such as Cbfa1 and Osterix for differentiation of osteoblast, and then collagen and ALP for the creation of osteoid. Several specific cells begin to locate in osteoid and spread dendritic projections, keeping connections with other embedded cells as well as the bone surface cells through mechanisms which are not known well. Furthermore some molecules including E11/gp38 and MT1-MMP are effective in dendrite/canaliculi generation, whereas other molecules, like CapG and destrin, control the cytoskeleton.[67] Additionally, the biomineralization is regulated by PHEX, MEPE, and DMP-1, and FGF-23, controlling the renal phosphate secretion.[68] In addition, Sclerostin, known as a marker of the mature osteocyte, negatively controls the bone formation[69] and its viability is preserved by ORP150 in a hypoxic environment.[70]

Typically, adult bone is composed of about 50%–70% mineralized components, 20%–40% organic matrix, 5%–10% water, and 1%–5% lipids.[71] The bone mineral phase is a nonstoichiometric carbonated apatite with nanometer size and low crystallinity, which is structurally similar to geologic HA ($Ca_{10}(PO_4)_6(OH)_2$).[72] The organic matrix contains type I collagen, which includes about 90% of bone proteins, and the remaining 10% are a wide assortment of noncollagenous proteins such as sialoprotein, phosphoproteins, osteonectin, osteopontin, osteocalcin, blood proteins, and GFs which are involved in bone mineralization.[73]

To date, many therapeutics have been reported for the treatment of bone diseases including simvastatin,[74,75] strontium ranelate,[76] icariin,[77,78] and prostaglandin E_2 (PGE_2).[79] However, their delivery to the bone tissue is a complicated process owing to the complex microenvironment, structure, and architecture of the native bone tissue. For instance, the bone membrane, consisting of lining cells that cover the bone surfaces, works as a marrow–blood

barrier which limits the availability of large exogenous substances to the bone tissue surface.[80] Bone lining cells are mature passive flat shaped osteoblasts which cover the bone tissue surface to inhibit the bone resorption and formation.[63] High mineral contents, lack of natural biological target receptors for most bone diseases, low-blood flow (0.05~0.2 ml/min/g), and poor permeability are other obstacles for delivering therapeutic agents to bones via conventional modes of administration.[71,81,82] On the other hand, most of the therapeutic agents do not have specificity to the bone tissue. Therefore, they highly accumulate at nontarget sites such as the liver and kidneys. This insufficient uptake of therapeutics by bone can limit their efficacy and severely affect the treatment outcomes.[83,84] However, the presence of HA, which is a distinctive anatomic feature of bone, offers a highly specific target [84] (Figure 1), that will be discussed in more details in this review article.

3 | NOVEL DRUG DELIVERY SYSTEMS FOR TREATMENT OF BONE DISEASES

There are two innovative approaches for delivery of therapeutics to the bone: local implantable drug delivery systems and systemic targeted drug delivery (Figure 2). In local implantable drug delivery approaches, therapeutic agents such as drugs or bone GFs are encapsulated in implantable bone graft substitutes, scaffolds, hydrogels, cement, or bone implant coatings and applied to the bone defects.[85–88] Growing evidence has demonstrated the efficiency of these drug delivery systems to promote osteointegration and avoid bone infections.[89,90] For instance,

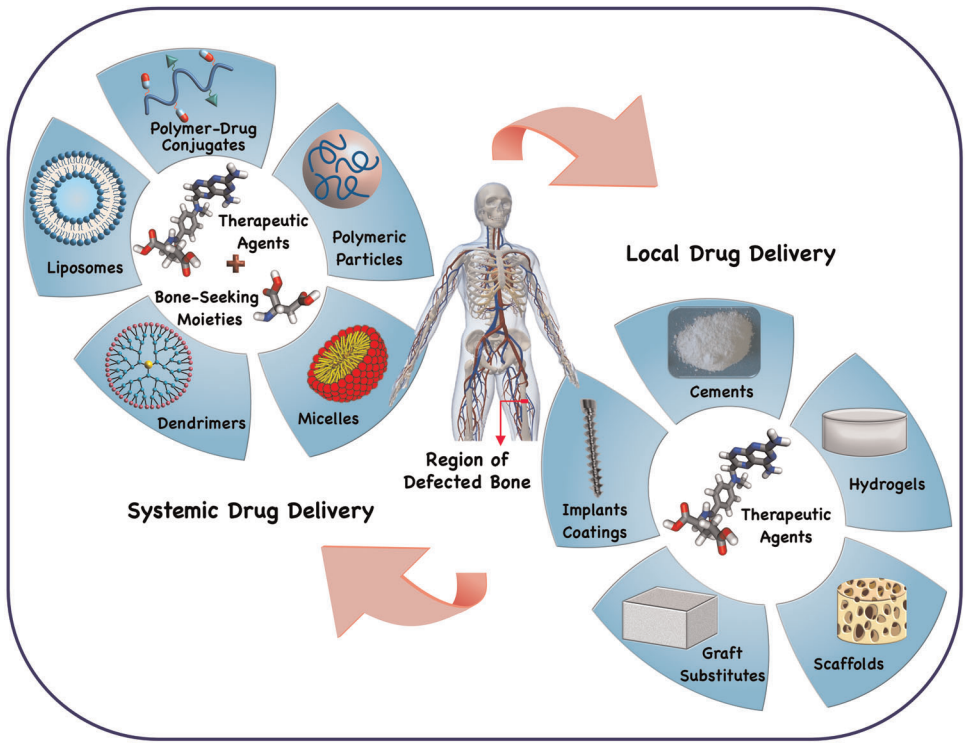

FIGURE 2 Schematic of drug delivery systems used in the treatment of bone diseases. There are two innovative approaches for delivery of therapeutic agents to the bone. Local implantable drug delivery (local drug administration at a site of interest) and targeted systemic drug delivery (administration of therapeutics into the circulatory system) [Color figure can be viewed at wileyonlinelibrary.com]

chitosan matrix coatings containing bioactive glass particles,[91] laponite nanoplatelets,[92] graphene oxide (GO) nanosheets,[93] and gelatin nanospheres[94] have been utilized for temporary and local delivery of antibiotics to prevent infections at the site of implant. These coatings serve as the source of the antibiotic and release it over a prolonged period of time.[90] While the multifunctional antibiotic-loaded coatings can inhibit bacterial infections, they simultaneously promote osteointegration.[89,90] Moreover, the local administration provides the therapeutics in a high concentration at the desired target tissue, providing several advantages such as improved bioavailability, reduced systemic exposure, and fewer off-target impacts.[80,95] However, despite great progress in implantable drug delivery systems for bone tissue, these strategies are not appropriate for most patients. This is primarily because of their invasive nature, wide diversity of bone diseases, patient conditions, and difficulties in the management and control of the post-implantation drug concentration.[80]

Another approach to improve the biodistribution of therapeutic agents in bone tissue is bone-targeted drug delivery systems. Similar to local drug delivery carriers, targeted drug delivery can enhance therapeutic indices, while minimizing the possibility of adverse side effects.[96] Moreover, systemic targeted drug delivery methods are less invasive routes compared to local implantable drug delivery systems.[46]

It has been shown that 99% of the calcium inside the human body is in the form of HA in bone tissues, and the remaining 1% is distributed in other parts of the body.[97] Thus, active targeting of HA, which is a distinctive feature of bone, may open new therapeutic opportunities to treat bone diseases. The idea of bone-targeted drug delivery based on of targeting HA dates back to 1980s.[98] In one of the earliest studies, tetracycline was conjugated to acetazolamide, a carbonic anhydrase inhibitor, with adipoyl dichloride and its high affinity to HA was shown in vitro.[98] Since then the development of compounds targeted specifically to HA for the treatment of bone diseases has been significantly progressed. Among all bone-seeking moieties, BPs, oligopeptides, and tetracycline have gained considerable interest.[71]

4 | BONE TARGETING AGENTS

Bone-targeting agents are known as a class of small molecules (such as BPs, oligopeptides, and tetracycline) with high affinity to bone tissue, which selectively deposit in bone for prolonged periods of time with less off-targeted effects.[99] In this section, the most well-known bone-targeting agents with a high affinity to a calcified matrix are discussed. Also, advantages and disadvantages of using these bone-seeking moieties are highlighted.

4.1 | Bisphosphonates

Bisphosphonates (BPs) are a widely utilized category of drugs that suppress bone resorption and are usually prescribed for skeletal disorder treatment such as hypercalcemia, osteoporosis, and Paget's disease.[100,101] Moreover, growing evidence has shown the antitumor potency of BPs and their efficacy in inhibiting proliferation, and inducing cell cycle changes and/or apoptosis in various types of human tumor cells.[102] Prolonged administration of BPs for at least one year reduces the skeletal morbidity rate in bone metastatic breast cancer patients up to 30%–40%, as well as multiple myeloma patients with bone metastases up to 50%.[103]

BPs are nonmetabolizable endogenous analogs of the naturally occurring pyrophosphates (P-O-P) of the bone matrix, in which the oxygen atom (between the two phosphate groups) is replaced by a carbon atom (P-C-P) as shown in Figure 3.[104] BPs can chelate Ca^{2+} in the HA structure through electrostatic interaction,[105] and as a result bind to bone mineral in vivo.[106] BPs' affinity to HA depends on their structures.[107] While the two phosphonate groups of BPs mainly mediate their binding affinity to HA, other properties such as a hydroxyl group or an amine group at R_1 can trigger tridentate binding to HA crystals, and the nature of the R_2 substituent can affect their antiresorptive capacity and pharmacological properties.[108] BPs are divided into two groups: nonnitrogenous BPs

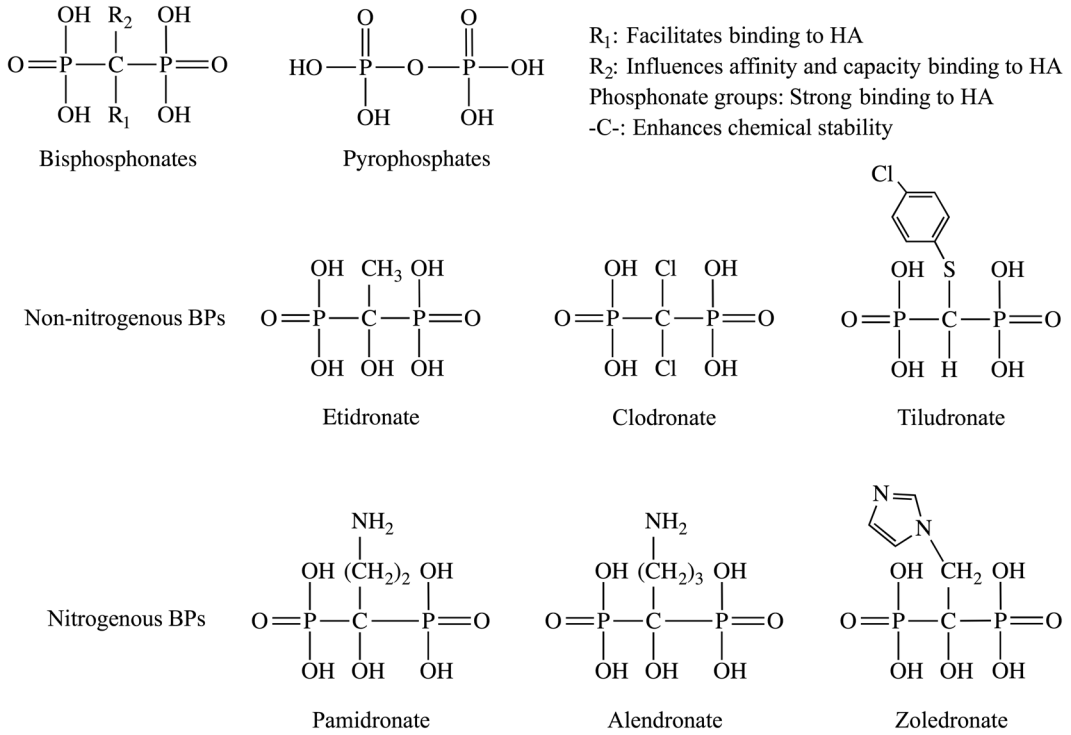

FIGURE 3 Chemical structure of the BPs. BP, bisphosphonate; HA, hydroxyapatite

including etidronate, clodronate, and tiludronate; and nitrogenous BPs such as alendronate, pamidronate, and zoledronate.[109]

The mechanism of action behind the antiresorptive ability of a BP is identified by the presence of a nitrogen molecule in the side chain. The nonnitrogenous BPs, due to their close structural similarity to pyrophosphates, can be intracellularly metabolized into methylene-incorporating analogues of adenosine triphosphate (ATP) using aminoacyl-tRNA synthetase enzymes.[106]

BPs can be controlled either intravenously or orally. However, because of low bioavailability via the oral administration, most BPs are usually used through intravenous route. Following intravenous administration, BPs quickly disappear from plasma, displaying a half-life of 1–2 h based on renal clearance along with bone uptake.[110] BPs bind to bone mineral around resorbing osteoclasts and result in very high local concentrations of BPs in the resorption lacunae (up to 1000 μM).[111] The release of BPs from bone tissue is slow and is governed by the rate of bone remodeling.[99] Therefore, BPs shows prolonged half-life from 1 to 10 years, depending on the rate of bone turnover.[110]

Besides being prescribed as drugs, BPs are widely investigated for targeted drug delivery to the bone tissue, such as the administration of drugs,[102] radiopharmaceuticals,[80] and imaging agents.[99] The application of BPs as radiopharmaceuticals and bone scanning agents mainly relates to their high affinity for calcified matrix as well as their capability to bind with a gamma-emitting technetium isotope.[112] Administration of bone-seeking radiopharmaceuticals is a well-tolerated and effective treatment in 60–80% of the patients with painful bone metastases.[113] Several radioactive agents including strontium (^{89}Sr), samarium (^{153}Sm), rhenium (^{186}Re) have been clinically used to control bone pain in cancer patients.[99] While strontium (^{89}Sr) is a natural bone-seeker, others should be conjugated to proper bone seeking moieties such as BPs to selectively deposit in bone skeleton, where particulate radiation is emitted and absorbed by bone cells.[99] Bone imaging agents such as technetium-99m

methylene diphosphonate (^{99m}Tc-MDP), hydroxyl methylene diphosphonate (^{99m}Tc-HMDP), and hydroxyethylidene diphosphonate (^{99m}Tc-HEDP) are commonly used for investigation of skeletal disorders such as metastatic bone cancer, osteoporotic, and Paget's disease.[99] Several review papers have summarized the progress (pharmaceutically and clinically) in development of bone-targeting radiopharmaceuticals for treatment of bone diseases.[99,113,114]

Ideally, for targeted drug delivery to the bone tissue, there should be stable bonds between the drug molecules and BP, which survive during systemic circulation. On the other hand, these bonds should be unstable at the bone site to release the drug.[108] To this end, Hochdörffer et al. designed two types of chemotherapeutic prodrugs containing a BP bone-targeting moiety for the treatment of bone metastases.[51] They decorated doxorubicin, a potent anticancer drug, with a BP group as a bone-targeting moiety, and investigated the effect of acid sensitive and/or cathepsin B cleavable bonds on the release profile of doxorubicin. They observed effective bonding of the prodrugs with HA and also reported fast release of the drug with enough stability during several hours in human body plasma.

Besides their advantages for bone treatment, BPs also have some common side effects. One of the most important adverse effects of BPs usage is BP-associated osteonecrosis of the jaws (BONJ), mainly associated with exposed bone for a prolonged time (>6 weeks) and a high doses of BPs.[115]

4.2 | Oligopeptides

Acidic amino acid homopeptides such as poly(aspartic acid) (Asp_8) and poly(glutamic acid) (Glu_8) oligopeptides (as eight residues sequential of aspartic acid and glutamic acid, respectively) have shown significant affinities to HA.[82] The concept of using acidic amino acid homopeptides depends on the physical characteristics of some non-collagenous proteins of bone, mainly osteocalcin, osteopontin, and bone sialoprotein, which have a repetitive acidic amino acids sequence (L-Asp or L-Glu) and can bind to HA.[116,117] In 2000, Kasugai et al. showed that upon systemic administration, a small peptide of acidic amino acid (L-Asp or L-Glu) could selectively deliver drugs to the bone and maintain in the bone tissue.[118] They also demonstrated that estradiol conjugated with an acidic oligopeptide (L-Asp-hexapeptide) could be selectively targeted to the bone, where the parent drug was gradually regenerated, leading to improvement of bone mineral density without the systemic adverse effects of estradiol.[119]

Oligopeptide conjugates can alter the pharmacokinetics and biodistribution of therapeutic agents. The increased hydrophilicity of drug conjugates can change distribution to visceral organs, blood clearance, and biological activities of therapeutics.[120] In contrast to the P-C-P bond of BPs, the oligopeptides do not have longterm adverse impacts, do not form colloids with metallic ions, and also are enzymatically degradable.[82] Another advantage of using oligopeptides as bone targeting moieties is that no synthetic or unnatural chemicals are needed for designing a bone-targeted drug delivery system.[82]

4.3 | Tetracycline

Tetracycline is a yellow crystalline amphoteric material generated by the metabolites of the actinomycete *Streptomyces rimosus*, which was introduced as an antibiotic in 1948.[121] Tetracycline represents a class of polycyclic naphthacene carboxamides compounds, with a widespread range of antibacterial activities against either Gram-positive or Gram-negative bacteria.[66,122,123] Tetracyclines are protein synthesis inhibitors which prevent aminoacyl-tRNA from binding to the A-site of ribosome over peptide elongation.[124] Soon after its application in medicine, the presence of tetracycline in bone was reported. In 1957, Rall et al. discovered a bright yellow fluorescence under ultraviolet light in the skeletons of animals that had received tetracycline.[125] The high affinity of tetracycline to HA causes discoloration of the primary and permanent dentitions [126] and also inhibits skeletal

formation in children.[127] Due to the high affinity of tetracycline to HA, some research groups have studied the potential of tetracycline as a potent compound for bone targeting.[128,129] For instance, Orme et al. designed a conjugated tetracycline h-estradiol as a bone-seeking estrogen prodrug and demonstrated its high affinity binding to the bone tissue in vitro.[130] In another work, estrogen was conjugated to a tetracycline-derived bone-targeting agent, and it was subcutaneously administered to an ovariectomized (OVX) rat osteoporosis model. The results exhibited a partial skeletal separation effects of estradiol from the uterine effects.[130]

Despite the advantages of tetracycline as a bone-targeting agent (such as oral bioavailability and fairly non-toxicity), its clinical application is limited. In addition, the complicated chemical structure along with low stability of tetracycline over chemical modifications limit its usage as a bone-targeting agent.[128]

4.4 | Growth factors

Bone healing procedure is physiologically complex, involving the cells and cytokines interactions acting in concert with different GFs. GFs are polypeptides which locally modulate the cellular activities. These polypeptides induce an intracellular signal transduction through binding to target cell receptors and regulate various biological responses. In the case of bone repair, different GFs including bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs), fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) represent a significant role.[131]

Delivery of different GFs with proper doses to the target site is very challenging. Due to the complexity of the bone regeneration process, recent studies have focused on the dual/multiple GFs delivery. It has been shown that treatments by multiple GFs have synergistic effects on bone formation. Dual or multi-GF delivery profiles require timely and sequential release of the individual compartments, which may complicate the design of the biomaterials.[132]

NPs incorporated scaffold has been widely used for delivery of GF for bone tissue regeneration. For example, proteins can be encapsulated in the NPs and then embedded in the scaffolds. The scaffolds containing these NPs allow for controlled and sustained release regime of GF, depending on the application.[133] The NPs incorporated scaffolds offer several advantages. First, the incorporation of NPs can increase the mechanical properties of the scaffolds, which is a crucial parameter for bone tissue regeneration.[133] Furthermore, studies have been shown that the presence of NPs could enhance osteoconduction, osteointegration, as well as osteoinduction. These could be due to their capability to mimic the native bone environment along with providing a suitable milieu for cell adhesion and proliferation.[134] Additionally, high drug-loading efficiency of small size NPs (high specific surface area), is another advantage of these nanoscale structures. Nanocarriers with high encapsulating capability can protect proteins from enzymatic degradation in vivo, allowing for protein preservation.[135] In the study conducted by Wang et al., BMP-2 loaded polyelectrolyte complex NPs containing chitosan/chondroitin sulfate were fabricated. The engineered NPs were incorporated in a biphasic calcium phosphate scaffold. The response of bone marrow stromal cell (BMSCs) in vitro along with in vivo ectopic bone creation were studied, confirming that the scaffolds were osteoconductivite and supported bone formation.[133]

Additionally, multimodal delivery of GF (one or multiple) by incorporation of GF-loaded nano- and micro-scale particles into hydrogel structures is reported in different studies. For example, Yilgor et al. prepared a multiple GFs incorporated system.[136] In this study, they prepared wet-spun chitosan scaffolds and then incorporated poly-lactic-co-glycolic acid (PLGA) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanocapsules into it. PLGA and PHGV nanocapsules were developed using double emulsion solvent evaporation and loaded by BMP-2 and MP-7, respectively. In vitro studies showed that the nanocapsules allowed for the delivery of BMP-2 and then BMP-7 sequentially. This sequential delivery improved the ALP activity of bone marrow derived MSCs. In another work, Subbiah et al. produced a dual GF delivery carrier for BMP2 and VEGF simultaneously to improve osteogenesis and angiogenesis.[137] To this end, the PLGA NPs loaded by BMP2 were fabricated through double emulsion evaporation

process. Afterward the obtained NPs were mixed with VEGF-encapsulating alginate microcapsules by a electro-dropping method. The in vivo results showed high bone formation in rat calvarial defects, which were treated with these scaffolds, demonstrating the synergistic effect of the co-delivery system.

5 | TARGETED DRUG DELIVERY SYSTEMS

The use of polymeric particles, liposomes, micelles, and dendrimers has been extensively studied for their potential application in the treatment of bone diseases. Compared to drug conjugates, nanomedicines can control drug release and protect the active compound in the human body. In this section, besides the general requirements of nanomedicines for systemic drug delivery, we specially review the application of bone targeted nanomedicines with a focus on HA targeting. We also discuss the use of various kinds of targeting moieties and nanocarriers for targeted delivery applications with their clinical potential for the treatment of bone diseases. Table 1 shows the nanocarriers used in bone diseases and their applications.

5.1 | Polymeric nanoparticles

Polymeric NPs have been produced using different techniques, including emulsification-solvent evaporation,[159] nanoprecipitation,[139] solvent-displacement,[154] and emulsification solvent diffusion.[160] The choice of synthesizing technique is dependent on their applications and the type of encapsulated therapeutic agents inside NPs.[161] Biodegradable polymeric NPs, either synthetic or naturally occurring, have revealed promising results in drug delivery systems because of providing a controlled/sustained drug release, degrading into nontoxic, absorbable subunits, and no need for a follow-up surgical removal after depletion of the drug supply.[162]

Among the different polymers utilized to formulate polymeric NPs, PLGA is widely used due to its favorable characteristics including biocompatibility, biodegradability, along with FDA and European Medicine Agency approval.[163] PLGA NPs reduce the therapeutic degradation and enhance the bioavailability of therapeutic agents with poor water solubility.[164]

Bone-targeted PLGA NPs loaded with anticancer therapeutics offer an innovative way to deliver a concentrated amount of drugs in a controlled and target-specific manner to treat and prevent bone metastases. In vitro studies confirmed that alendronate decorated PLGA NPs represented an appropriate blood compatibility, did not result in hemolysis and changing the plasmatic phase of coagulation, and had no cytotoxic effect on BMSCs, endothelial cells, and trabecular osteoblasts.[165,166] In vivo investigation of alendronate-conjugated PLGA NPs loaded with potent anticancer drugs such as doxorubicin,[154] bortezomib,[16] and coencapsulated curcumin and bortezomib[151] also exhibited a significantly enhanced survival and decreased the incidence of metastases as well as tumor burden and growth. For example, Salerno et al. observed that alendronate decorated PLGA NPs loaded with doxorubicin had a greater or at least the same efficacy than free drug, in the reduction of tumor area and prevention of osteolytic bone metastases. Likewise, Pignatello et al. revealed that doxorubicin-loaded PLGA NPs could significantly decrease the incidence of metastases in comparison to unloaded drug in a mouse model of bone metastases, most likely as a consequence of alendronate antiosteolytic properties.[167] To assure if alendronate persevered its antiosteoclastic activities after the conjugation with PLGA NPs, Cenni et al. designed an in vitro experiment on human osteoclast cells.[167] Surprisingly, they observed that not only alendronate decorated PLGA NPs but also pure PLGA NPs caused apoptosis, disruption of actin ring, and prevented degradation of collagen in human osteoclasts.[167] In conclusion, alendronate decorated PLGA NPs can be used as appropriate drug carriers for the treatment of osteolytic bone metastases. The synergistic effect of alendronate, PLGA, and anticancer drugs can inhibit the disease-associated bone degradation.[167]

TABLE 1 Nanocarriers used in bone diseases and their application

Nanocarriers	Application	Outcome	References
Porous silicon NPs	PTX delivery	Drug delivery to bone-marrow improved	Mann et al.[138]
PLGA NPs	ZOL delivery	Targeting ability of ZOL enhanced by strong affinity to bone	Ramanlal Chaudhari et al.[139]
TC-PLGA NPs	SIM delivery	The curative effects of SIM on the recovery of bone mineral density improved	Wang et al.[129]
PLGA-PEG NPs	DTX delivery	DTX delivery to bone was increased	Ramanlal Chaudhari et al.[139]
DEX–ADM–PEI NPs	DXR delivery	Drug efficacy enhanced	Sun et al.[140]
Chitosan NPs	ß-gal DNA plasmid delivery	It was suggested as gene carrier for bone formation	Corsi et al.[141]
Magnetic arsenic trioxide NPs	ATO delivery	It had inhibition effect on osteosarcoma	Li et al.[142]
mPEG-PLGA NPs	Teicoplanin delivery	It was effective for treating osteomyelitis	Peng et al.[143]
Chitosan NPs	IL-1 Ra DNA delivery	A significant reduction was noted in the severity of histologic cartilage lesions	Zhang et al.[144]
Cationic polymeric NPs	Dextran delivery	The retention of therapeutic agents in articular joints (e.g., osteoarthritis) was increased	Morgen et al.[145]
PLGA-CaP	Tigecycline delivery	Calcium phosphate filled a bone defect	Ignjatović et al.[146]
PLGA scaffold	Doxycycline delivery	Doxycycline was effectively delivered in a controlled fashion with prolonged duration	Feng et al.[147]
Au NPs	Cartilage and bone repair	Differentiation and mineralization of primary osteoblasts was stimulated	Zhang et al.[148]
PLGA-b-PEG NPs	Bortezomib delivery	Tumor (myeloma cells) growth slowed down	Swami et al.[149]
Platinum NPs	Photothermal therapy	Tumor growth and osteolysis in a bone metastasis model was inhibited	Wang et al.[150]
PLGA NPs	Curcumin and bortezomib delivery	Tumor growth rate slowed down	Thamake et al.[151]
Zoledronic-anchored bimodal mesoporous silica-covered gadolinium (III) NPs	Plumbagin delivery	Tumoriogenesis and osteoclastogenesis were attenuated significantly	Qiao et al.[152]

TABLE 1 (Continued)

Nanocarriers	Application	Outcome	References
PLGA NPs	PTX delivery	Progression of bone metastasis slowed down and bone loss was inhibited	Adjei et al.[153]
PLGA NPs	DXR delivery	The incidence of metastases reduced	Salerno et al.[154]
DXR-hyd-PEG-ALN self-assembled micelles	DXR delivery	Antitumor activity improved and bone destruction reduced	Ye et al.[155]
Dextran NPs	DXR delivery	It showed pronounced antiproliferative effects against osteosarcoma cell lines	Susa et al.[156]
Chitosan NPs	Dz13 (a DNA enzyme) delivery	It was efficacious against osteosarcoma	Tan et al.[157]
Polymerized liposomal NPs	DXR delivery	Growth inhibition of osteosarcoma cells was enhanced	Federman et al.[158]

Abbreviations: ALN, alendronate; ATO, arsenic trioxide; Au, gold; CaP, calcium phosphate; DEX-ADM, dextrane-adriamycin; DTX, docetaxel; DXR, doxorubicin; mPEG, poly (ethylene glycol) monomethyl ether; NP, nanoparticle; PEG, polyethylene glycol; PLGA, poly lactic-co-glycolic acid; PTX, paclitaxel; SIM, simvastatin; TC-PLGA, tetracycline-based poly(lactic-co-glycolic acid); ZOL, zoledronate.

Swami et al. demonstrated that the bortezomib-loaded bone targeting NPs, as a pretreatment regimen, could inhibit myeloma growth in mouse models.[149] They pretreated mice with bortezomib-alendronate-PLGA NPs for 3 weeks, three times a week, before injecting myeloma cells. The in vivo results showed that pre-treatment with bortezomib-loaded alendronate decorated PLGA NPs resulted in slower growth of myeloma as shown with remarkably lower bioluminescent imaging (BLI) signal in comparison to the free drug and alendronate-empty-NP groups (Figure 4A and 4C). As shown in Figure 4B, the survival time was increased to 41 days in mice receiving targeted bortezomib compared to 34–36 days in mice receiving free drug, and alendronate-empty-NP groups, respectively.[149]

Protein absorption on the surface of PLGA NPs promotes the opsonization and rapid clearance from the bloodstream during systemic delivery.[168] Surface modifications of PLGA NPs with PEG is a common method to prevent the clearance of NPs by the reticuloendothelial system.[169] PEG is a nonionic, hydrophilic polymer with excellent biocompatibility.[170] PEGylation, which is coating the surface of the particles using PEG, enhances the half-life of blood circulation by several orders of magnitude (Figure 5A).[171] PEGylation also alters the physicochemical properties and consequently the pharmacological behavior of drug conjugates.[172] For instance, PEGylated alendronate-conjugated PLGA NPs showed excellent retention, accumulation, and bone homing.[16] Similarly, the zoledronate-conjugated PLGA-PEG NPs exhibited prolonged half-life of blood circulation, decreased liver uptake, and notably prolonged maintenance at the bone site (Figure 5B).[139] In these examples, while BPs act as bone-targeting moieties, a layer of PEG coating can significantly reduce immune recognition and prolong blood circulation of NPs.[16] The molecular weight, conformation, and surface chain density of PEG macromolecules are the main factors, which affect the stealth characteristics of PEGylated NPs.[171] Moreover, the molecular weight of PEG in PEGylated PLGA NPs was shown to influence binding to HA, where particles decorated with PEG 2000 had lower potency of alendronate than those bearing PEG 500.[173]

As discussed, the other candidates used as bone-targeting elements are acidic oligopeptides, which are negatively-charged molecules at physiological pH with a strong affinity to bone mineral HA.[176,177] In vitro HA binding assays confirmed that poly(aspartic acid) conjugated PLGA NPs had high affinity to HA gel and to

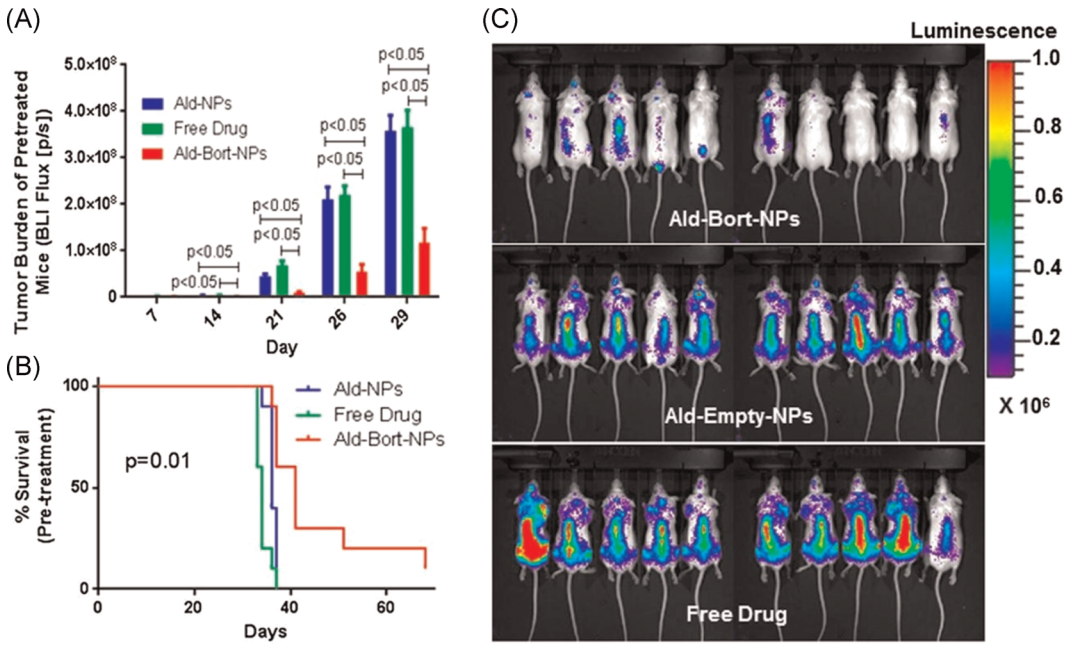

FIGURE 4 Effect of free drug and drug-loaded PLGA NPs on metastases inhibition. First mice were pre-treated using free drug, Ald-Empty-NPs, or Ald-Bort-NPs for 3 weeks followed by injection of GFP + Luc+MM1S cells. (A) BLI flux was highly low in Ald-Bort-NPs in comparison to Ald-Empty-NPs or free drug groups based on daily imaging. (B) A significant improvement in survival was obtained in the Ald-Bort-NPs–pre-treated group. (C) The BLI signal displayeda decrease in tumor burden in mice pre-treated with Ald-Bort-NPs (n = 10) at day 29.[149] Copyright 2014, National Academy of Sciences. BLI, bioluminescent imaging; NP, nanoparticles; PLGA, poly-lactic-co-glycolic acid [Color figure can be viewed at wileyonlinelibrary.com]

mineralized matrices made by human mesenchymal stem cells (hMSCs) and mouse BMSCs, without having cytotoxic effects on human osteoblast-like MG63 cells.[159] *In vivo* bone targeting potential of the PLGA-PEG NPs in zebrafish and rats assured that conjugating aspartic acid oligopeptide to the NPs would increase bone-targeting efficiency (Figure 5C).[174]

Simvastatin is a commonly prescribed type of cholesterol-lowering drug used for prevention and treatment of cardiovascular diseases.[178] Based on recent studies, it can also enhance the expression of BMP-2 and VEGF, leading to an increase in bone formation rate and bone mineral density, and also decrease the risk of bone fracture.[179] However, it has limited applications in the treatment of bone diseases due to its low water solubility and lack of distribution to the skeleton.[180] One promising approach to improve the pharmacokinetics and biodistribution of simvastatin to promote bone formation is its delivery by utilizing nanoparticulate carriers. To this end, Wang et al. encapsulated simvastatin in PLGA NPs.[129] To enhance the targeted drug delivery ability of NPs, they conjugated the NPs with tetracycline, as a bone-seeking element. Their results in OVX rats showed that the simvastatin-loaded tetracycline decorated PLGA NPs enhanced the curative effects of the drug on the bone mineral density recovery in comparison to simvastatin-loaded PLGA NPs and/or free simvastatin.[129] PLGA has other advantages such as well-studied drug release kinetics, ease of synthesis and modification, and also cost-efficacy.[181] Importantly, the kinetic of polymer degradation could be optimized to obtain an ideal therapeutic agent release profile.[181]

Apart from PLGA NPs, poly(γ-benzyl-L-glutamate) (PBLG), as a synthetic polypeptide, has attracted great attention due to its biocompatibility, biodegradability, good solubility in most organic solvent, and modifiable ester side chains.[182,183] Ozcan et al. conjugated alendronate to PEGylated PBLG NPs and studied the potential of the

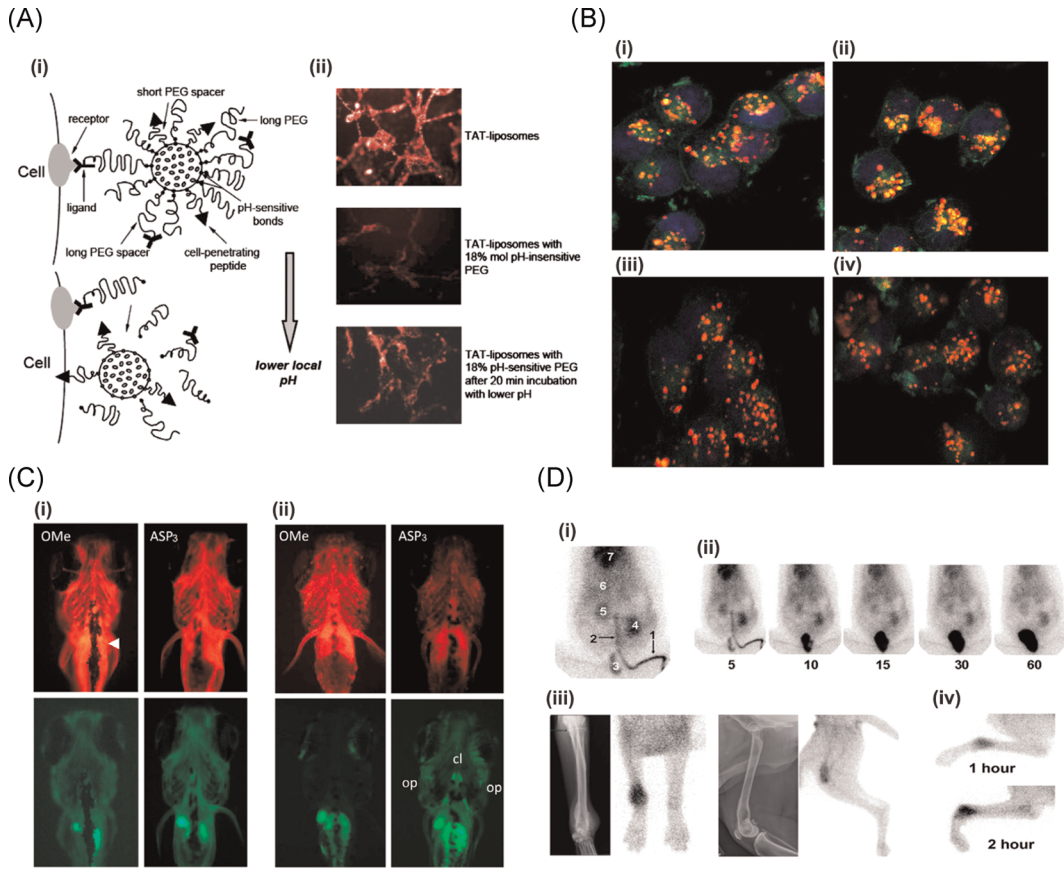

FIGURE 5 Bone targeted nanocarriers. (A) Dual-targeted nanocarrier with temporarily "hidden" properties. (i) Schematic of direct interacting between the cell-penetrating functions of the carrier and tumor cells. (ii) Representative fluorescence images displaying the efficiently uptake of Rhodamine-labeled liposomes by cells.[171] Copyright 2010, Elsevier. (B) Confocal microscopic images showing uptake of PLGA-PEG20 NPs and PLGA-PEG-ZOL NPs (i and ii) PLGA-PEG20 NPs images and (iii and iv) PLGA-PEG-ZOL NPs images. The lysosomal compartment is shown by yellow color.[139] Copyright 2012, Elsevier. (C) The colloidal NPs injected in Zebrafish larvae which were containing of synthetic fluorescence tracers including FITC-labeled hybrid NPs (green) and TexasRed (red) at the common cardinal vein (white arrowhead) and observed at (i) 0.5 hpi and (ii) 24 hpi reveal the potential of NPs to specific target and accumulate in the zebrafish larvae bone.[174] Copyright 2014, Elsevier. (D) Biodistribution of ^{99m}Tc-labeled pamidronate-doxorubicin-NPs in pet dogs with osteosarcoma. (i) Dynamic overlay images of ^{99m}Tc-labeled Pam-Doxo-NPs infused into a pet dog with osteosarcoma. Images of ^{99m}Tc-labeled Pam-Doxo-NPs in different sites including: (1) intravenous infusion; (2) descending caudal vena cava; (3) urinary bladder; (4) left kidney; (5) right kidney; (6) liver; and (7) heart. (ii) Images after infusion of ^{99m}Tc-labeled Pam-Doxo-NPs from 5 to 60 min showing extended dwell time in highly vascular visceral organs. (iii) Localized 99mTc-labeled Pam-Doxo-NPs in focal malignant osteolysis for two dogs with osteosarcoma (dog 1 with distal radial osteosarcoma [left] and dog 2 with distal femoral osteosarcoma [Right]) approved by colocalization of changes in pathologic radiographic and ^{99m}Tc activity. (iv) Images of progressive ^{99m}Tc-labeled Pam-Doxo-NPs accumulation over focal areas of malignant osteolysis in different time points: 1 and 2 h after injection.[175] Copyright 2016, National Academy of Sciences. NP, nanoparticle; PLGA, poly-lactic-co-glycolic acid
[Color figure can be viewed at wileyonlinelibrary.com]

NPs in bone targeting in vitro and in an animal model.[184] As expected, PBLG NPs conjugated with alendronate exhibited higher accumulation in bone tissue compared with equivalent nontargeted NPs.[184] Moreover, de Miguel et al. observed that the multivalency of the alendronate decorated PBLG-PEG NPs led to a 4000-fold stronger interaction with HA, in comparison to the monovalent interaction of free alendronate molecules with HA.[169] Their group also studied the dual effects of poly(glutamic acid) as a bone targeting moieties and as a coordinating metal-based anticancer drugs (i.e., cisplatin).[185] They synthesized amphiphilic copolymer poly(γ-benzyl-glutamate)-block-poly(glutamic acid) (PBLG-b-PGlu) NPs using a nanoprecipitation approach. Then, high cisplatin payloads (6.2% w/w) were coordinated to carboxylate groups of the PGlu in preformed NPs.[186] The in vitro drug release studies showed that physiological concentrations of chloride ions triggered cisplatin release with a near zero-order kinetics profile over 14 days.[187] The high affinity binding of cisplatin-loaded PBLG-b-PGlu NPs to calcified matrix was also approved by an in vitro HA binding assay.[187]

Gelatin is another candidate which has been used as a carrier for bone targeted drug delivery. Gelatin is biocompatible, biodegradable, nontoxic, and -inexpensive polymer. Also, it has various active groups for binding targeting molecules, which facilities its chemical modification.[188,189] Recently, Farbod et al. showed the potential of alendronate conjugated gelatin NPs for targeted drug delivery to bone tissue.[190] Conjugated bone-targeting alendronate to gelatin NPs exhibited a high affinity to mineralized tissues.[190]

Finally, in a recent study, Yin et al. developed pamidronate functionalized polylactide NPs loaded with doxorubicin for targeted treatment of malignant skeletal tumors.[175] Pamidronate functionalized NPs showed increased bone tumor accumulation and extended maintenance in comparison to non-targeted NPs. Notably, they studied the efficacy of drug-loaded bone targeting NPs in a clinical evaluation of osteosarcoma tumor targeting in dogs, in which the tumors were anatomically and physiologically comparable to those in humans.[175] The results of clinical trial of phase I in pet dogs with osteosarcoma are shown in Figure 5D. When bone-targeted NPs labeled with ^{99m}Tc were administered via intravenous infusion, they quickly spread into the major visceral organs such as kidney, heart, liver, and spleen with ultimate removal into the urinary bladder as seen in Figure 5D(i and ii). Importantly, within 1–2 h after administration, the NPs were mainly localized and accumulated in the localized bone tumor microenvironment as shown in Figure 5D(iii). Moreover, the activity of ^{99m}Tc preferentially increased by time, indicating the dynamic concentration as well as accumulation of the bone-targeted NPs over the microenvironment of bone tumor (Figure 5D(iv)). Moreover, the authors demonstrated that the repeat dosing of doxorubicin-loaded NPs decorated with pamidronate in dogs with osteosarcoma was well-tolerated nonhematologic, nonhematologic, and cardiac toxicities.[175]

One of the promising aspects of nanomedicine is enhancing bone-targeting delivery and modulating the biodistribution of small interfering RNAs (siRNA) at both cellular and tissue levels. siRNA, as a helix containing 20–25 nucleotides, interferes with gene expression by the cleavage of messenger RNA (mRNA) through forming a protein/RNA complex known as RNA-induced silencing complex (RISC). The siRNA selectively suppresses the expression of target proteins whose mRNA contains a sequence similar to the sense strand of the siRNA.[191] Molecular therapy via siRNA has demonstrated high therapeutic potential for diseases, resulting from mutation or unusual gene overexpression. However, the efficient siRNA delivery and high stability in vivo were challenging to the use of siRNA therapeutics.[192] siRNAs are large, negative charge, and hydrophobic molecules, which are not stable in serum and are prone to nuclease degradation.[193] Besides, other major limitations of delivery naked siRNAs include reticuloendothelial system clearance, short half-life, insufficient accumulation at targeted tissue or cells, limited penetration across the capillary endothelium, and cellular internalization, and off-target effects.[194,195] Therefore, developing an effective and specific delivery of siRNA therapeutics is very challenging. Several comprehensive reviews have outlined the obstacles and recent efforts to deliver siRNAs in vivo.[194,196,197] Today, various phase I clinical trials are in progress which are using nanotechnology-based delivery of siRNA such as CALLA 01 (utilizing cyclodextrin NPs attached to transferrin, and coated with PEG for solid tumors); Atu027 (using cationic phospholipids to form lipoplexes directed against Protein Kinase N3(PKN3), a main factor in metastasis and cancer progression); TKM 080301 (targeting polo-kinase of solid tumors by using lipid NPs).[192]

Regarding bone tissue, successful siRNA delivery requires a delivery carrier such as polymeric NPs or liposomes, and a bone-targeting moiety to specifically deliver siRNAs to bone tissue. In a recent study, Zhang and coworkers developed bone targeted D-Asp_8-HPMA (*N*-(2-hydroxypropyl) methacrylamide copolymer) NPs for siRNA molecules delivery to inhibit *Semaphorin 4D*(*sema4D*) expression, which modulates the bone modeling cycle by suppressing osteoblast maturation.[198] As expected because of D-Asp_8 bone-targeting moiety, the resultant NPs were thoroughly and evenly distributed in bone skeleton after intravenous administration. Interestingly, the weekly intravenous injection of the siRNA loaded NPs significantly increased the active osteoblasts at the bone site, leading in higher bone volume in an ovariectomy-induced osteoporosis animal model.[198]

5.2 | Liposomes

Among the nanocarriers, liposomes are the most clinically accepted drug delivery systems that are widely applied to deliver drugs, genes, vaccines, and imaging agents.[199] Liposomes are self-assembled colloidal vesicles containing of amphiphilic phospholipids and cholesterol molecules that can be loaded with either hydrophilic or hydrophobic therapeutic molecules.[200,201] Liposomal nanocarriers have demonstrated a variety of favorable properties including biocompatibility, biodegradability, ease surface modifications, and improving the solubility, pharmacokinetics, as well as pharmacodynamics of therapeutic agents.[202,203] In addition, liposomal encapsulation strongly reduces the volume of distribution of the loaded drug, decreases drug toxicity, extends the presence of the drug in circulation, and enhances tumor accumulation.[204,205] One of the first clinically approved chemotherapeutic nanomedicine is Doxil®, which approved by FDA in 1995.[6] The blood circulation of Doxil is hundred times longer than free doxorubicin. Moreover, this PEGylated liposomal formulation of doxorubicin decreases the cardiotoxicity of doxorubicin.[206] The other clinically approved liposomal formulation of doxorubicin is Myocet™ which was approved in Europe and Canada in 2000.[207] Another FDA-approved liposome for treatment of acute lymphoblastic leukemia is Marqibo® which contains vincristine sulfate.[208] Success of FDA-approved liposomes in cancer therapy opens new avenues for other therapeutic applications of liposomes. For instance, BPs conjugated liposomes showed great potential to treat bone diseases.[209,210]

One strategy to enhance the therapeutic effect of drugs is to release them at the pathological site of disease. One of the advantages of liposomes is the ability to design liposome-based thermo-sensitive nanocarriers that can release their therapeutics at the sites of elevated temperature.[211,212] Thermally sensitive liposomes change structure when heated to 40–45°C, releasing their therapeutics directly at the site of the elevated temperature. ThermoDox is one example of thermo-sensitive liposomes which contains doxorubicin. The combination of ThermoDox with hyperthermia or radiofrequency ablation, is in phase II trials for the treatment of breast cancer and liver metastasis, as well as in phase III trials for the treatment of hepatocellular carcinoma.[211] Bone is a common site for metastasis in cancer patients. So, targeted delivery of chemotherapeutic with BP-conjugated thermo-sensitive liposomes seems a good therapeutic opportunity. To this end, Song et al. designed thermo-sensitive liposomes containing doxorubicin and conjugated them with pamidronate.[213] As expected the pamidronate conjugated liposomes exhibited stronger binding affinity to HA compared to nonconjugated ones. They also showed that doxorubicin was completely released when liposomes were heated up to 42°C either alone or in combination with HA.[213]

Radiolabeled antibiotics are fast emerging therapeutics for the specific diagnosis and detection of infectious lesions, such as osteomyelitis.[214,215] To this end, Ferreira et al. engineered alendronate conjugated PEGylated liposomes containing ^{99m}TC-radiolabeled ceftizoxime.[216] The resultant liposomes showed higher uptake in regions of septic inflammation compared to non-conjugated liposomes, indicating the importance of alendronate moieties for bone targeting applications.[216]

One of the proposed ways to treat osteoclast-dysfunction-induced skeletal diseases is to develop bone-targeted liposomes containing microRNA (miRNA). Cumulating evidence suggested that some of the dysregulated

miRNAs including miR-223, miR-21, miR-155, and miR-148a contribute in multiple physiological and pathological processes of osteoclast differentiation and function. A large number of highly upregulated miRNAs limit bone mass for supporting normal bone, which is probably in response to different physiological cues Figure 6A.[217] In a recent study, Liu *et al*. encapsulated antagomir-148a (a miRNA modulator suppressing the osteoclastogenic miR-148a) in D-Asp_8 decorated liposomes.[218] Biodistribution study revealed that the resultant liposomes were mainly accumulated in the bone, whereas no significant toxicity was observed in liver and kidneys *in* OVX mice (Figure 6B,C).[218] Further studies showed that D-Asp_8 facilitated the enrichment of antagomir-148a, leading to downregulation of miR-148a in osteoclasts *and reduce* bone resorption in osteoporotic mice.[218]

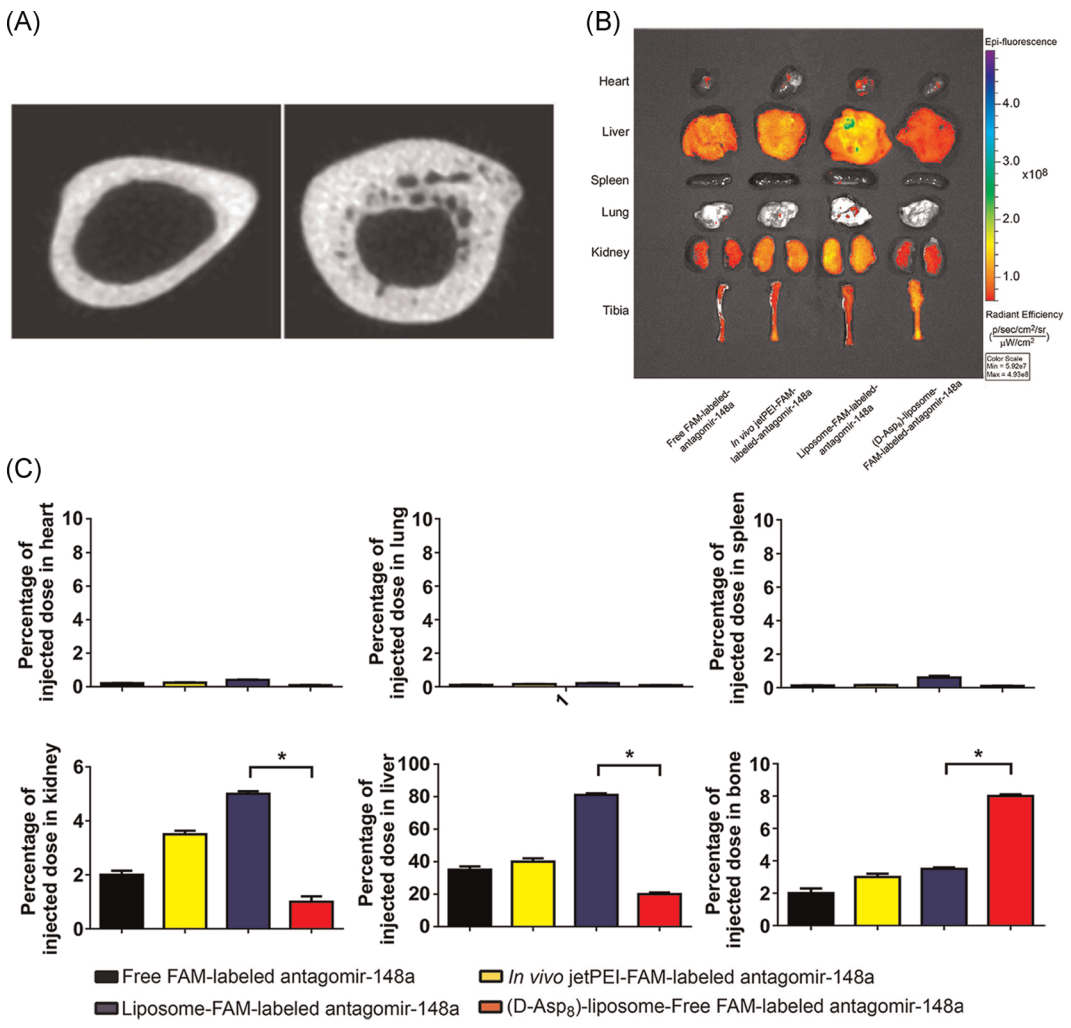

FIGURE 6 Tissue-selective deliveries in vivo. (A) miRNAs for activating Wnt signaling in osteogenesis, left image: The consequence of Dicer deletion in osteoblasts and osteocytes, and right image: driven by osteocalcin.[217] Copyright 2012, Springer Nature. (B) Biophotonic imaging of FAM-antagomir-148a distribution in tissue in OVX mice for free FAM-labeled antagomir-148a, and different nanocarrier-FAM-labeled antagomir-148a, in vivo. (C) Quantification of fluorescence signal density for FAM-labeled antagomir-148a in different organs post injection of both free and nanocarrier-FAM-labeled antagomir-148a, in vivo.[218] Copyright 2015, Elsevier. FAM, carboxyl fluorescein; miRNA, microRNA; OVX, ovariectomized [Color figure can be viewed at wileyonlinelibrary.com]

5.3 | Micelles

Polymeric micelles are the other group of nanocarriers which have been widely explored for targeted therapeutics delivery to the bone tissue. They were first introduced by Kataoka's group in the early 1990s.[219] Polymeric micelles are self-aggregated colloidal particles composed of amphiphilic block copolymers.[200] They have several advantages such as biocompatibility, biodegradability, and high in vitro and in vivo stability. Moreover, they can effectively solubilize various poorly water-soluble drugs, and alter their release profiles.[220] The small size of polymeric micelles enhances their blood circulation time. They can escape from mononuclear phagocytic system in the liver and bypass the filtration of inter-endothelial cells in the spleen.[221] One of the clinically successful formulation of paclitaxel is Genexol PM, which is based on the encapsulation of a drug in polymeric micelles.[222] Genexol PM has lower toxicity compared to free drugs, which allows administration of higher dose of paclitaxel to the patients.[223]

In a recent study, Miller et al. developed PEG forming self-assembled micelles, which were used for co-delivery of two drugs with synergistic interactions at a single administration.[224] Paclitaxel was embedded in the core of the PEG micelles, while alendronate molecules were placed at the outer shell. The in vitro results showed that paclitaxel loaded in alendronate conjugated PEG micelles showed almost the same cytotoxicity as well as anti-angiogenic properties as the free drug.[224] They also evaluated the antitumor effect of paclitaxel loaded in alendronate decorated PEG micelles following intravenous injections on a xenograft mouse model of mCherry-labeled MDA-MB-231 human mammary adenocarcinoma in the tibia. The resultant substance selectively accumulated in tumors and showed higher inhibition of tumor growth (50%) in comparison to control group, which was treated by saline as shown in Figure 7A,B.[224]

In another study, Chen et al. developed innovative bone targeting micelles composed of a hydrophobic hyperbranched Boltorn H40 core containing alendronate targeting moiety and PEG as the hydrophilic parts.[226] To incorporate hydrophobic drug, the biodegradable polyester H40 inner core was utilized. However, by using the hydrophilic PEG as the outer shell, the stability of micelles and half-life blood circulation time were improved. Meanwhile, the conjugation of BPs yielded a high affinity to the skeleton mineral HA. Doxorubicin as a hydrophobic anticancer drug model was encapsulated in the micelles, and the high affinity of bone-targeted micelles to bone tissue was evaluated through the HA binding assay.[226] In vitro inhibition of HN-6 cancer cells proliferation showed that micelles loaded by doxorubicin could effectively destroy the cancer cells.[226] Another proposed formulation for targeted delivery of doxorubicin to the bone tissue is self-assembled doxorubicin-PEG-alendronate. The in vivo imaging analysis exhibited a higher accumulation of the drug in the bone metastatic tumor tissue after intravenous administration of bone-targeted doxorubicin-loaded micelles. The new formulation significantly inhibited the growth of tumor, decreased bone loss, and lowered toxicity in tumor-bearing nude of cardiac in mice model as compared to the free drug.[227] In another study, Wang et al. developed self-assembled micelles from a peptide-conjugated diblock copolymer composed of a chimeric peptide CKGHPGGPQAsp$_8$ consisting of an osteotropic anionic Asp8, poly(ethylene glycol) and poly(trimethylene carbonate) (Pep-*b*-PEG-*b*-PTMC).[228] The resultant micelles demonstrated a high affinity to apatite and a negative-to-positive charge conversion during exposure to CTSK, an enzyme over-expressed in bone metastatic microenvironments.[228] These characteristics significantly improved cellular uptake of the micelles over getting to the lesion sites, which enhanced the drug efficacy of loaded doxorubicin as confirmed by both in vitro (to evaluate cytotoxicity) and in vivo (in myeloma-bearing 5TGM1 mouse model) studies.[228] Low et al. also developed a micellar delivery system for doxorubicin using the hydrophilic D-aspartic acid octapeptide as an efficient targeting moiety along with the hydrophilic micelle corona.[229] In vitro HA binding analysis showed that the synthesized micelles had a high affinity to calcified matrix.[229] One of the advantages of their design is locating doxorubicin in the center of the micelles, reducing metabolism by the myocardium and consequently reducing cardiotoxicity.[229]

Another attempt for treating osteomyelitis, an infection of the bone and marrow, was done by Cong et al.[230] They developed self-assembled micelles containing PLGA-block-PEG-alendronate copolymer for bone-targeted

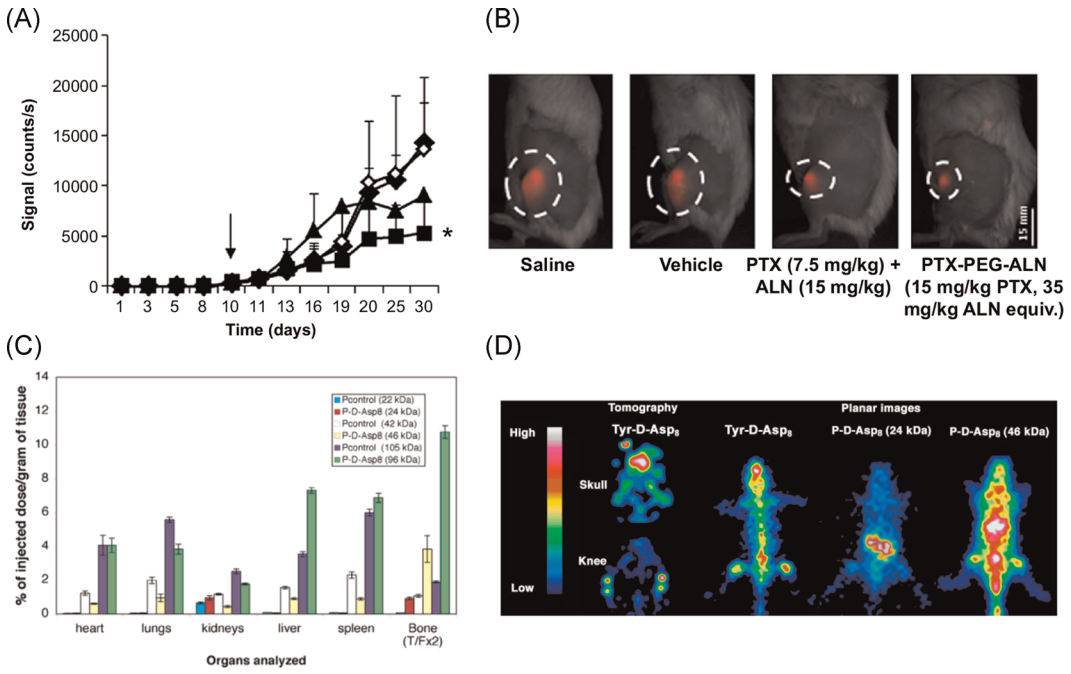

FIGURE 7 Drug-micelles conjugate and copolymer-drug conjugate as bone-targeting structures. (A) Antitumor efficacy of mCherry-MDA-MB-231 tumors in the tibia. (B) Fluorescence images of mCherry-MDA-MB-231 tumors in the tibia treated by different composition of free ALN and PTX (open squares, close triangles, and close squares), as well as saline (close diamonds) or PTX-vehicle (open diamonds) as controls groups.[224] Copyright 2013, Elsevier. (C) The biodistribution of Tyr-labeled HPMA copolymer-D-Asp8 conjugates (P-D-Asp8) and the biodistribution at time intervals 24 h for targeted and nontargeted conjugates for different molecular weights. The targeting strategy showed higher accumulation in the liver and spleen as compared to $P_{control}$. (D) Planar along with tomographic images of BALB/c mice 24 h after iv administration of Tyr-labeled HPMA copolymer-D-Asp8 conjugates and Tyr-D-Asp8.[225] Copyright 2006, American Chemical Society. ALN, alendronate; HPMA, N-(2-hydroxypropyl) methacrylamide; PTX, paclitaxel [Color figure can be viewed at wileyonlinelibrary.com]

delivery of vancomycin. Their in vitro results verified that the conjugation of alendronate did not affect drug loading and release properties of vancomycin from micelles. Moreover, the antibacterial assay against *Staphylococcus aureus* bacteria showed that antibiotic-loaded micelles could efficiently prevent the growth of bacteria.[230]

5.4 | Polymer-Drug conjugates

One well-known way to overcome the limitations of low molecular weight therapeutic substances in treating bone diseases is using polymer-drug conjugates. Polymer–drug conjugates are formed through side-chain grafting of therapeutic agents to polymer chains, and their size is usually below 20 nm.[231] Pioneering work on the conjugation of hydrophobic therapeutic agents to a water-soluble polymer was originally aimed to evade rapid renal clearance and to enhance the aqueous solubility of therapeutics.[232,233] Polymer-drug conjugates also provide an opportunity to modify both pharmacodynamics and pharmacokinetics of therapeutics.[233] They can also improve the blood residence time and decrease adverse side effects of drugs.[168]

One group of hydrophilic polymers used for controlled drug delivery to the bone is derived from *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers.[234,235,236] HPMA copolymers do not show immunogenic effect and can be adapted to a specific target.[237]

The in vivo biodistribution and pharmacokinetic studies of HPMA conjugated with D-Asp_8 [238] or alendronate [239] exhibited a strong binding affinity to the bone after systemic administration. The detailed study on biodistribution and pharmacokinetics of alendronate decorated HPMA copolymer in young healthy BALB/c mice revealed that conjugates with lower alendronate content (1.5 mol%) had similar bone deposition potential as conjugates containing higher alendronate (8.5 mol %). The researchers also studied the influence of molecular weight on the biodistribution of HPMA copolymer−D-Asp8 conjugates. Their results showed that by increasing the copolymer's molecular weight (from 24 to 96 kDa), the total conjugate distribution to bone was increased, owing to the extended half-life circulation time. However, the selectivity of the delivery system was reduced. Figure 7C,D represents the distribution of HPMA copolymer conjugates as well as controls in the body.[225]

Miller et al. designed HPMA copolymer-conjugated with alendronate to target bone metastases selectively with paclitaxel.[240] In vitro results demonstrated that while alendronate facilitated the delivery of paclitaxel to the bones, the conjugation with HPMA copolymer provided targeting to tumor tissue within the bones.[240] HPMA-alendronate-paclitaxel conjugate showed significant antitumor efficacy and antiangiogenic activity in vivo, in comparison to either paclitaxel alone or in combination with alendronate.[240]

In another study, Hruby et al. developed an HPMA carrier containing hydroxyl BP targeting moieties, radiotherapeutic agents,[125] imaging agents,[111] and anticancer doxorubicin drug.[241] As expected the in vitro HA binding assay demonstrated high affinity of the carrier to the mineralized tissue. They also observed that the HA affinity was directly related to the BP content in the copolymers, but was not dependent on pH (in the range of biologically relevant pH: 5–7.4). They also investigated the effect of different bond spacers including stable (amide), enzymatically cleavable (Gly-Phe-Leu-Gly tetrapeptide), and pH cleavable (hydrazone) between the polymer chain and doxorubicin on drug release behavior. Their results showed that the Interaction of the drug carrier with HA slightly decreased the rate of doxorubicin release from the pH-responsive hydrazone bond-containing carrier system. However, the reduction in the rate of doxorubicin release through enzymatic degradation was more noticeable. The pH-dependent release of doxorubicin from hydrazone bonds was slightly dependent on the bond to HA in comparison to the solution. For the enzymatically degradable systems, the rate of release was significantly low after binding to HA.[241] It was also shown that for cleavable spacers, the polymer carrier could release drug following pH or enzymatic changes.[241]

Alendronate-monoethyl adipate-(hydrazone)-doxorubicin conjugate (ALN-MA-hyd-doxorubicin) has been also produced for targeted delivery of doxorubicin to the bone tumor tissue. The binding kinetics showed that ALN-MA-hyd-doxorubicin had the potential to bind with HA and bone tissue quickly. The results demonstrated that doxorubicin mostly distributed in tumors of bone after intravenous administration of ALN-MA-hyd-doxorubicin to tumor-bearing nude mice, leading to a remarkable decrease in tumor volume as compared to intravenous administration of free drug.[242]

Pullulan is a linear polysaccharide including α-1, 6-linked maltotriose residues used as polymeric backbones for selectively targeting bone tissues.[243] For example, pullulan-conjugated alendronate showed outstanding capability for treatment of bone neoplasms such as osteosarcoma or breast cancer bone metastases.[244] In vitro studies in murine K7M2, murine 4T1 breast cancer cells, human MDA-MB-231-BM (bone metastases-originated clone), and human SAOS-2 osteosarcoma cells revealed that the modified paclitaxel-conjugated pullulan exhibited more antiproliferative activity than the conjugate without the alendronate.[244]

5.5 | Dendrimers

Dendrimers are a class of synthetic polymers with tree-like hyperbranched structures, which have been extensively studied for gene and drug delivery.[200] Dendrimers have unique feathers such as well-defined architectures, nanoscale size, narrow polydispersity, and good solubility.[245] They also have a high number of terminal groups present in the intermediate and the surface layers, which provide a platform for conjugation of drugs and

targeting moieties.[246] Dendrimer structure can significantly enhance the drug payload compared to linear polymer-drug conjugates.[247] Recently, the capability of dendrimers for targeted drug delivery to bone has been explored. As an example, Pan et al. developed amphiphilic Janus dendrimers, which contained acidic amino acid and naproxen molecules as the peripheral groups.[248] In vitro analysis indicated that all formulation of the dendrimers could bind to HA, with no cytotoxicity against human embryonic kidney (HEK293) cells.[248]

One of the most notable and useful drug delivery systems using polymeric dendrimers is heterobifunctional PEG, which binds two different therapeutic agents together. Clementi et al. synthesized heterobifunctional PEG dendrimers as a drug delivery vehicle for a mixture of paclitaxel and alendronate for cancer bone metastases treatment.[247] The paclitaxel-PEG-alendronate conjugate exhibited a high affinity to the bone tissue, and an enhanced pharmacokinetic profile in comparison to free drugs, mainly because of a significant increase in their half-life.[247] Additionally, they observed that these structures did not decrease the cytotoxicity of the drug against prostate cancer cells as compared to the free paclitaxel/alendronate combination.[247]

5.6 | Other systems used as the delivery systems

Advanced biofunctional materials in tissue engineering are considered as a promising approach for improving impaired bone tissue regeneration and substitution. In this context, bio-functional polymeric materials have been used as supportive extracellular matrices for cell proliferation, adhesion, as well as osteogenic differentiation.[249,250] For instance, a biocompatible OTES-Ti3C2Tz/PLA nanocomposite was developed for the in vitro MC3T3-E1 mouse preosteoblasts proliferation, adhesion, and osteogenic differentiation. They reported a higher ultimate tensile strength of the nanocomposite membranes as compared to the PLA membrane alone (33%).[251] Moreover, it was reported that TiO_2 on the three-dimensional (3D) printed PLGA/TiO_2 construct could improve the ALP activity as well as HA formation of the osteoblast cells cultured on the nanocomposite for bone tissue regeneration. This could be due to the electrostatic interaction between the negatively charged Ti–OH and the Ca^{2+} ion, which could trigger phosphate ions on the positively charged surface.[252]

In another study by Farshid et al., the reinforcement of GO and single and multiwalled GO nanoribbons of poly (propylene fumarate) (PPF) nanocomposite was proved. Their results showed that these nanostructures enhanced the mechanical characteristics and also supported the viability of MC3T3 preosteoblast and collagen-I adsorption, which were appropriate for bone tissue regeneration. This effect could be due to the effective interaction between GO and PPF as nanocomposites matrix.[253]

Horch and coworkers also prepared a nanocomposite containing poly (propylene fumarate)/poly (propylene fumarate)-diacrylate (PPF/PPF-DA) and hybrid alumoxane NPs for bone tissue engineering applications. This hybrid nanomaterial dispersed in PPF/PPF-DA showed a three-fold increase in flexural modulus at 1 wt % loading in comparison to the pristine polymer resin. This enhancement could be due to the high level of NPs dispersion within the polymeric matrix as well as covalent bonding between NPs and the polymer chains.[254]

Recently, 3D bioprinting strategies have attracted extensive attention in tissue engineering especially in bone tissue regeneration. This technology allows to produce 3D constructs with interconnected porous structures containing encapsulated living cells and bioactive molecules. It has been reported that 3D-printed structures of bioceramic scaffolds such as GO-modified bioceramic enabled to kill residual tumor cells through photothermal and magnetothermal effects.[255,256] In this case, 3D-printed β-tricalcium phosphate (β-TCP) bioceramic was prepared with Fe_3O_4/GO sandwich-like structure by covering the Fe_3O_4 NPs with GO nanocomposite (β-TCP-Fe-GO) layers, which was used for bone cancer treatment. Interestingly, the synergistic effect of GO and Fe ions by photothermal and magnetothermal effects triggered MG-63 cell death (>75%). Furthermore, OPN, Runx2, and OCN expressions were enhanced in BMSCs using the β-TCP-Fe-GO scaffold in comparison to the pristine β-TCP.[257]

6 | CONCLUSION

The use of nanotechnology in the field of orthopedic studies, diagnostics, and treatment is relative new. While conventional drug delivery systems result in the distribution of therapeutic agents inside the body, ligand-mediated targeted nanomedicines can facilitate their selective accumulation at the site of disease, increasing the effectiveness of treatments. In recent years, several bone-seeking moieties such as BPs, oligopeptides, and tetracyclines, which have a high affinity to the calcified matrix, were utilized in targeted delivery systems to treat a variety of bone diseases. Bone targeted drug delivery aims to increase and prolong pharmacological effects in bone, decrease off-target effects, and enhance compliance due to the less frequent need for medication. Targeted drug delivery to bone by systemic administration is safer, less invasive, and in some bone diseases more effective than local delivery approaches.

In this review, we highlighted targeted drug delivery systems with potential for treatment of bone diseases. Despite the great potential advantages, achieving efficient bone targeting of nanomedicines requires more detailed investigations and understanding of the challenges related to the systemic administration in preclinical and clinical levels. Another aspect of targeted drug delivery to the bone, which should be considered is the accumulation of bone-seeking agents in bone tissue over time, which may cause long-term side effects in the body. Future studies need to evaluate the long-term effects of bone-seeking agents coupled to nanomedicines and their efficacy over the course of treatment. Furthermore, effective collaborations between orthopedic clinicians and nanomaterials and nanotechnology researchers will strengthen research efforts and accelerate the translation of bone-targeted nanomedicines to the clinic in the near future.

Due to advances in nanomedicine, diagnosis of diseases and combining diagnosis with therapy have become available. Nanotechnology plays a significant role in the bone health, which relies on combination of diagnosis, active targeting, multidrug release and bioimaging. In the near future, it is expected that the development of ideal nanomaterials and their translation into the clinic, would allow their enhanced therapeutic outcomes for treatment of bone diseases. For instance, the advancement of high-resolution printing techniques and development of novel bioinks are expected to provide major advancement in bioprinting of hierarchical bone tissues. Additionally, development of new strategies in local and multifunctional drug delivery systems and NPs to target bone tissue will open new avenues for better clinical outcome for these approaches in the near future.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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