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Biomaterial-assisted local and systemic delivery of bioactive agents for bone repair★

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Abstract

Although bone tissues possess an intrinsic capacity for repair, there are cases where bone healing is either impaired or insufficient, such as fracture non-union, osteoporosis, osteomyelitis, and cancers. In these cases, treatments like surgical interventions are used, either alone or in combination with bioactive agents, to promote tissue repair and manage associated clinical complications. Improving the efficacy of bioactive agents often requires carriers, with biomaterials being a pivotal player. In this review, we discuss the role of biomaterials in realizing the local and systemic delivery of biomolecules to the bone tissue. The versatility of biomaterials enables design of carriers with the desired loading efficiency, release profile, and on-demand delivery. Besides local administration, systemic administration of drugs is necessary to combat diseases like osteoporosis, warranting bone-targeting drug delivery systems. Thus, chemical moieties with the affinity towards bone extracellular matrix components like apatite minerals have been widely utilized to create bone-targeting carriers with better biodistribution, which cannot be achieved by the drugs alone. Bone-targeting carriers combined with the desired drugs or bioactive agents have been extensively investigated to enhance bone healing while minimizing off-target effects. Herein, these advancements in the field have been systematically reviewed.

Graphical abstract

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Keywords

Bone; biomaterial; delivery; repair; biomimetic; bone targeting

1. Introduction

Bone injuries are common, with more than 6 million fracture occurrences and over 500,000 bone grafts needed each year in the United States alone [1, 2]. The situation is further plagued as the aged population continues to grow. Large bone defects are frequent outcomes of traumatic events, congenital defects (e.g., Paget's disease), bone deterioration due to altered tissue homeostasis (such as osteoporosis and rheumatoid arthritis), infection/osteomyelitis, and resection of tumors [3]. Even if the problems are addressed with surgical interventions, complications of the procedures, such as infection/sepsis, are reported in over 5% of the total cases, resulting in patient morbidity and mortality [4, 5].

While surgical procedures are key clinical interventions to repair bone defects, the use of pharmaceutical agents is an indispensable part of many treatment regimens. Antibiotics like ciprofloxacin, gentamicin, and vancomycin are used as prophylactics to prevent and treat osteomyelitis and other bone infections [6, 7]. Anticancer chemotherapeutics including Bortezomib and Cisplatin are extensively used to treat tumors [8, 9]. The treatment of osteoporosis entails hormonal therapies such as parathyroid hormone (PTH) and small molecules such as bisphosphonates [10, 11]. Other therapeutics such as ipriflavone [12], cathepsin K inhibitors [13], and adenosine receptor agonists [14] are also currently being experimented to treat various bone conditions. Gene therapies employing functional DNA [15–17] and RNA [18–21] have drawn attention as well. Growth factors, such as transforming growth factors β (TGF- β) super family including bone morphogenetic proteins (BMP) [22], insulin-like growth factor (IGF) [23], and angiogenesis stimulating factors including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) [24], also play an important role in bone repair and regeneration [25–28]. Particularly, BMP-7 is FDA-approved for recalcitrant non-unions and BMP-2 for spinal fusion and tibial fractures [29, 30]. In fact, Infuse™ Bone Graft, a product of recombinant BMP-2 adsorbed in collagen sponge, is currently available in the market. However, concerns have been raised against using BMP-based products, as the supraphysiological level of BMP-2 can cause ectopic bone formation and various other complications such as osteolysis [29, 31].

The pharmaceutical agents and bioactive factors (hereafter, they are collectively referred to as drugs) are often unstable or subject to fast clearance, making them difficult to reach bone tissues, regardless of being supplied locally or systemically. Systemic delivery (oral/intravenous) is heavily utilized in the case of metabolic disorders such as osteoporosis. When administered systemically, the drugs can accumulate in other organs (e.g., liver, spleen, and bone marrow). To prevent unwanted consequences and augment drug efficacy, it is necessary to develop carriers that can help drugs maintain their functions, protect them from decomposition or denaturation, and deliver them to the targeted sites.

The materials used for drug carriers should be biocompatible, nonimmunogenic, and inert to the normal bone healing events. A number of natural and synthetic polymers as well as inorganic minerals have been used to deliver drugs. Natural polymers, such as gelatin [32], collagen [33], fibrin [34], alginate [35], silk [36], hyaluronic acid [37], glycosaminoglycans [38], and chitosan [39], are commonly used as carriers. Additionally, demineralized/decellularized bone matrices, being natural reservoirs of growth factors, are widely utilized [40, 41]. The potential risks of natural polymers include their immunogenicity, processing difficulty, rapid degradation, lack of tunability, and batch-to-batch variations. Approaches including extensive purification, crosslinking, or blending with synthetic polymers have been used to improve the performance of natural polymers [42–48].

Biocompatible synthetic polymers, on the contrary, can be easily modified and processed to achieve the desired properties/functions by varying their molecular weights, functional groups, structures, and/or hydrophobicity. Polyethylene glycol (PEG) [49], poly(lactic-co-glycolic acid) (PLGA) [50], poly(propylene fumarate) (PPF) [51], polycaprolactone (PCL) [52] are a few examples. Another category of carriers consists of bioactive glasses [53], calcium phosphates [54–57], hydroxyapatite [58], and β -tricalcium phosphate (β -TCP) [59], which are inorganic materials that recapitulate some aspects of the native mineral environment.

In this review, we discuss how biomaterials can be designed to deliver drugs to bone tissues. We briefly discuss how physical and chemical interactions of the drugs with the carriers can be used towards loading and their influence on release profile including on-demand delivery. We also explore various approaches used to target the drugs to bone tissue. Cell delivery for bone repair is beyond the scope of this review and has already been covered by others [60–62].

2. Design of carriers for bone repair

2.1. Carrier assembly and drug loading

A drug carrier is assembled through physical interaction or chemical conjugation. It can be processed to achieve various structural forms, such as liposomes and lipid emulsions [15, 63], micelles [64, 65], micro/nanoparticles [47, 66], films [67, 68], injectable hydrogels [69, 70], three-dimensional scaffolds [71, 72], mesoporous materials [57, 73], and composites [74, 75].

The preparation of carriers should not hinder the efficacy of drugs, specifically during processes that involve thermal treatment or chemical modifications. For instance, heat-sensitive drugs cannot be premixed with carriers that require high temperature sintering such as bioceramics [76]. Likewise, drugs should not affect the assembly and integrity of carriers, putting a limit on the loading capacity. Together, the interplay between drugs and carriers is central to the design of delivery systems and is discussed with examples in the following sections.

2.1.1. Carrier assembly via physical interaction—Drug-bearing carriers built upon physical interactions are prepared by simple mixing of drug and carriers, where the drug is confined through surface adsorption, physical entrapment, ionic complexation, or affinity binding, as depicted in Fig. 1A–C [1, 33, 77]. For instance, Infuse™ Bone Graft is a simple mixture of BMP-2 and collagen, wherein BMP-2 molecules are adsorbed onto the collagen sponge. Physical entrapment, such as encapsulation using emulsion-solvent-extraction methods, is more commonly used, as it yields a better confinement and prolonged release of the drugs [50, 70, 78]. However, this approach often uses organic solvents, and any residual organic solvents could pose potential cytotoxicity. Towards this, many solvent-free methods like mechanical [79], high pressure CO₂ [80, 81], and hot melt [82] mixing have been investigated.

Ionic complexation and affinity binding are further harnessed to strengthen the physical interactions between carriers and drug and to improve drug retention. For example, drugs and carriers with opposite charges are paired up *via* electrostatic interaction, as seen in the delivery systems of cisplatin (+)/hydroxyapatite (–) [9], siRNA (–)/dimethylaminoethyl-dangling copolymer (+) [21], BMP-encoding RNA (–)/polyethylenimine (+) [83], and antimicrobial peptide (+)/calcium phosphate (–) [84]. Many growth factors such as FGF-2, TGF-β, and BMP-2 exhibit affinity towards extracellular matrix (ECM) molecules including heparin, a highly sulfated glycosaminoglycan, through secondary interactions. Hence, heparin peptides and their synthetic analogs have been used to develop carriers for growth factor delivery [85–89].

Drug loading through physical interaction is simple and effective, as it poses minimal effects on the encapsulated drug and its efficacy. The reversible nature of the secondary interactions could also ensure seamless release of the entrapped drug. However, the use of physical interaction for drug loading suffers from various limitations. For instance, drug incorporation through surface adsorption could limit the loading efficiency. Another concern is that physical interactions are generally weak and could be easily interrupted in a complex *in vivo* environment, causing bolus release of drugs. Accumulation of supraphysiological drug at the site and its subsequent diffusion to distant tissues could lead to unwanted ectopic effects [29]. Given these limitations, chemical conjugation has been used to improve drug retention.

2.1.2. Carrier assembly via chemical conjugation—Natural and synthetic materials are rich in functional groups, allowing chemical conjugation of drugs to them (Fig. 1D). One type of conjugation utilizes functional groups like succinimidyl ester, carbonyldiimidazole, or maleimide to covalently bind to the amine/thiol residues commonly

available in the biomolecule drugs [90–95]. For instance, a titanium surface was grafted with carboxymethyl chitosan, followed by immobilization of BMP-2 *via* 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry [96]. Such a coating could enable both antibacterial activity and osseointegration of orthopedic implants. Liu *et al.* noted that covalent conjugation of BMP-2 and other bioactive factors prevents their uptake and entails a more continuous signal transduction [97]. Indeed, Gharat *et al.* reported that a low dose of BMP-2 (100 ng/mL), if tethered, was sufficient to induce osteogenesis of mesenchymal stem cells (MSCs), compared to the milligram level used in physical adsorption [98].

Another conjugation strategy involves generating succinylated, acetylated, or acrylated derivatives of the drugs to bind to the carriers [99]. However, such drug modification and subsequent coupling could introduce conformational changes in the drugs, resulting in inadvertent loss of drug efficacy. To prevent undesirable effects, Tabisz *et al.* have developed BMP-2 variants *via* genetical modification for easy and controlled tethering [100]. Here, the genetically modified sites, either an additional cysteine at the N-terminus or a noncanonical propargyl-L-lysine, were designed to react with the functional groups of the carrier through orthogonal chemistry with high specificity. The authors have showed that only cells being exposed to the BMP-conjugated carriers expressed elevated alkaline phosphatase (ALP), albeit a more thorough examination is needed to verify the efficacy of such variants. Similar work involving genetically modified BMP-2 attached with peptide aptamers, which reversibly binds to orthopedic implants including titanium, has been shown to promote osseointegration [101].

While incorporation of drugs through covalent bonding can be used to prolong drug release, many aspects need to be carefully considered in designing an efficient delivery system. For example, both the drug and the carrier should have the complementary functional groups. The amount of available functional groups in the carrier will determine the drug loading efficiency. It is also important to ensure that the covalent bonds are degradable to assist the release. The linkers formed between the drug and the carrier need to achieve the adequate degradation in the bone environment for a desired release. The degradation kinetics can be tailored *via* the chemistry of the linkers. In addition to the release profile, the chemical nature of the linkers can also influence the drug loading efficiency. The conjugation reaction should be mild to avoid any detrimental effects on the drug [102]. Moreover, covalent coupling is not suitable for loading certain drugs, as a slow and low-level release of the drugs may develop drug resistance [57]. Commonly seen in antibiotic delivery, long exposure of suboptimal dosage often leads to antibiotic resistance.

2.1.3. Bone-mimetic biomaterials as carriers—In addition to coating orthopedic implants, carriers can be generated from bone-mimetic materials that possess mechanical properties resembling native bone tissue and provide more bone specific biochemical cues [16, 72, 103]. Towards this, calcium phosphate-based biomaterials are extensively used [56, 57]. In addition to acting as drug carriers, they can also serve as a reservoir for calcium and phosphate ions, which are necessary for bone homeostasis and mineral formation under physiological conditions, attributing to their osteogenic and osteoinductive properties [104–110]. Furthermore, the intrinsic porosity of these structures not only makes them the ideal

drug carriers, but also facilitates mass transport, cell infiltration, and tissue ingrowth [111–116]. For instance, Levengood *et al.* have used a biphasic calcium scaffold with heterogeneous porous structures, which was further impregnated with gelatin microparticles containing BMP-2 [117]. They showed that the micropores ($<10\text{ }\mu\text{m}$) produced better osseointegration compared to the macropores ($>100\text{ }\mu\text{m}$) in a mandibular defect model, revealing a porosity-dependent effect of the release of BMP-2. In a similar study, dexamethasone-bearing PLGA microparticles were incorporated into the porous hydroxyapatite scaffold, where the PLGA carriers interacted with the hydroxyapatite *via* ionic interactions [118].

Apart from calcium phosphates, highly ordered metal-organic frameworks and mesoporous silica structures have also been used as carriers [119–125]. The high surface-to-mass ratio (around hundreds of m^2/g) of the mesoporous materials increases the drug loading efficiency. They also provide plentiful docking sites, which again contribute to increasing loading efficiency [73, 124, 125]. Meanwhile, the high porosity lowers the mechanical properties of a carrier and could contribute to burst release, leading to supra-physiological levels of drug [126, 127]. To overcome some of these disadvantages, the mesoporous carriers have been functionalized to load drugs through chemical or secondary interactions, and/or used in conjunction with other reinforcing biomaterials [128, 129].

Emerging 3D printing techniques make it possible to manufacture and prototype versatile architectures and complex geometries for biomimetic carriers [76, 130–135]. Drugs can be printed along with mineralized slurry or paste and processed at mild temperatures without tampering with their efficacy, or they can be coated separately to allow for spatial patterning. Akkineni *et al.* printed a mixture of dextran sulphate/chitosan microparticles and calcium phosphate cement, resulting in a composite structure with the compressive strength comparable to cancellous bone [132]. VEGF or albumin encapsulated in the microparticles presented a near-linear release over 7 days and supported cell proliferation. Vorndran *et al.* devised a multijet 3D printing system, as shown in Fig. 2A, with individual control over the feeding of TCP powders, binding solution, and bioactive components, enabling the spatiotemporal patterning of various bioactive molecules (vancomycin, heparin, and BMP-2) [133]. In this case, TCP ($\text{Ca}_3(\text{PO}_4)_2$) powders were mainly fused into crystalline brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) at room temperature in the presence of a binding solution, where polymers like hydroxypropyl methylcellulose (HPMC) and chitosan were supplemented to enhance binding affinity. Bioactive components, vancomycin, heparin, and BMP-2, were discretely sputtered with polymers and deposited according to a preset pattern. This approach, avoiding the use of organic solvent or high temperature, maintained the bioactivity of the drugs (Fig. 2B). Although 3D printing enables adequate control over the porosity, pore size, pore shape and distribution, as well as functionalization of the carriers, it may give rise to limited resolution and unwanted heterogeneity.

2.2. Methods of drug release

In addition to a stable assembly of carrier with the drug, a successful delivery requires a viable release of the drug without compromising its activity. When delivered, the drug needs to be released to the target bone tissue progressively within a therapeutic time frame and

maintained at an effective dosage to dynamically match the physiological needs [25, 136, 137]. This calls for a detailed understanding of the dissociation mechanisms of the drugs from a carrier as well as their release kinetics.

2.2.1. Diffusion-assisted release—In the case of physical interaction-mediated assembly, the release of drug is mostly diffusion driven. An initial burst or pre-mature release of the drug is often observed in such cases [1, 31]. Adding a polymer coating has been shown to reduce rapid diffusion of drug and burst release [46, 54], where the coating density and dissolution rate can be fine-tuned to achieve the targeted release profile. For instance, a pulsatile local release of PTH was realized by stacking the drug-bearing alginate layers in between polyanhydride protection layers [138]. Surface erosion-induced degradation of the protection layers enabled exposure of a single PTH-laden layer at a given time, allowing a pulsatile delivery of the drug. Boerckel *et al.* have used a PCL nanofiber mesh as a diffusional barrier to the BMP-2 bearing alginate hydrogels, which enabled a sustained release of BMP-2 *in vivo* and promoted bone repair [139]. The authors also demonstrated this carrier to be more efficient than the commercially available collagen sponge in terms of dose response of the BMP-2, thus requiring a lower amount of growth factor to facilitate the repair.

A desirable zero-order (*i.e.* the amount of drug being released per unit time is constant) or pseudo-zero-order release can be further achieved by tailoring the molecular composition, mesh size, crosslinking density, and degradation rate of the carrier [47, 69, 140, 141]. Patel *et al.* described how the release of osteogenic growth factor from gelatin microparticles can be altered by varying the degree of glutaraldehyde-mediated crosslinking and collagenase-mediated degradation [47]. They observed a crosslinking density-dependent release of the drug. The BMP-2 released from the microparticles with low crosslinking exhibited a burst release, whereas the densely crosslinked carrier maintained a linear release kinetics over 28 days, regardless of the presence of enzyme. Moreover, the release was independent of the amount of BMP-2 loaded. Alkhraisat *et al.* altered the surface area of calcium phosphate carriers by strontium doping to improve the loading efficiency of doxycycline and prolong its release *via* Fickian diffusion [142]. It is worth noting that the diffusion was predictable, which followed a simplified Higuchi's law ($M = k\sqrt{t}$) [56, 142], where the cumulative release (M) is proportional to the square root of time (t) and the rate constant (k) is related to the structural properties of scaffold, based on the assumption of uniformly distributed doping and drug adsorption.

2.2.2. On-demand release—The diffusion-assisted release of drugs can be significantly altered when dynamic changes in the physical and chemical interactions between the drug and the carrier take place. These changes can arise from local convection or reactions triggered by environmental fluctuations (*i.e.* hydrolysis, redox, pH, enzymatic activity) or can be achieved with the use of extracorporeal stimuli (*i.e.* light, temperature ultrasound, electromagnetic field) [66, 143–148]. Such carriers susceptible to environmental stimuli are endowed with the capability of on-demand release. This type of release is largely determined by the carrier sensitivity to a trigger and the intensity of the trigger, both of which can be well controlled to achieve targeted release.

The fluctuations in native environment are due to cellular activities, and various cell-secreted factors affecting them are utilized to elicit on-demand release. Zhang *et al.* described a two-stage release strategy to deliver miRNA for augmenting the bone repair capacity of osteoporotic mice [20]. The miRNA strands first formed nanohybrids with the polyethylenimine (PEI)-rich hyperbranched polyester, which were then encapsulated in PLGA microparticles and further incorporated within a porous poly(L-lactic acid) (PLLA) scaffold, as shown in Fig. 3. During the first stage of release, the nanohybrids were engulfed by cells after leaching from the microparticles and scaffold. They were then hydrolyzed after endosomal escape in the second stage, leaving miRNA in the cytosol (Fig. 3B). This strategy greatly improved the transfection efficiency of miRNA, which subsequently promoted osteoblastogenesis of endogenous cells. In another study, copolymers containing oligoethyleneglycol and pyridyldisulfide side chains formed amphiphilic nanoaggregates with pyridyldisulfide groups attracting lipophilic drugs at the core [149]. While the nanoaggregates entrapped the drug by self-crosslinking among the intermolecular thiol pendants, the carriers dissembled in the presence of glutathione (GSH), a cell-secreted redox trigger, thus increasing the drug availability for cellular uptake.

Arrighi *et al.* utilized an approach to immobilize a fragment of PTH onto a fibrin scaffold *via* transglutaminase factor XIIIa-mediated crosslinking [150]. They observed that the PTH was functional only when it was in the free form, through the cleavage of a plasmin-sensitive linker, highlighting a release through cell-induced proteolysis. Other protease-sensitive linkers, particularly the ones specific to matrix metalloproteinases (MMPs), have been extensively studied [69, 151–153]. MMPs are upregulated during bone remodeling or metastasis. In a recent work, a BMP-2 carrier was formed by assembling the 4-armed PEG-maleimide units with MMP-cleavable linkers [154]. The carrier allowed a steady release of low doses of BMP-2 *in vivo* as well as the GFOGER ligands. This ECM mimetics derived from type I collagen, specific to $\alpha_2\beta_1$ integrin, not only promoted adhesion of the infiltrated cells but also offered additional benefits relevant to bone healing.

In addition to cellular activities in the milieu, stimulus can be applied remotely to trigger release as needed. For instance, near-infrared light, due to its deep tissue penetration, was used to breakdown the PLGA carriers *via* photothermal effect aiding release of the entrapped drug, strontium [155]. The irradiation, limited to 5 minutes on a weekly basis, was sufficient to promote healing, demonstrating the utility of remote stimulus-mediated drug release in antitumor and repair applications. More carriers, responsive to light, sound, or electromagnetic force, are developed as theranostics (*i.e.* agents with both diagnostic and therapeutic capabilities) [156–159], and the family of carriers equipped with on-demand release is ever-growing [160–163]. On-demand drug release can provide a fine control over the release profile, potentially avoiding premature or off-target release. Since the drug release is sensitive to external stimuli, the release profile can be on/off, resulting in a long-term release of drugs. Furthermore, stimuli-driven on-demand release also offers a precise spatiotemporal control, which can improve the therapeutic efficacy and mitigate the side effects associated with drug over-dosage. However, compared to diffusion-assisted release, the clinical application of an on-demand release system may require painstaking quality control, from the selection of stimuli-responsive material to their consistent performance *in vivo*.

2.2.3. Multivalent release—Given the complexity of the natural healing cascade in bone, from inflammation to cell recruitment to osteoblastogenesis to remodeling, it is conceivable that delivering a single factor and regulating one aspect of healing may not be sufficient to achieve the tissue repair with the necessary functions [1, 164]. This calls for a simultaneous or sequential/coordinated delivery of multiple bioactive factors with spatiotemporal control, as illustrated in Fig. 4 [25, 165–168]. For instance, multiple studies have shown that co-delivery of VEGF and BMP-2 from scaffolds can be used to promote bridging and healing of critical defects [80, 169]. Platelet-rich plasma, containing a myriad of bioactive molecules, has been directly incorporated into scaffolds/carriers to augment bone repair [170–172]. Compartmentalization of different drugs within the carrier creating differential diffusion barrier is another common strategy to release multiple drugs (Fig. 4B) [129, 173].

For better mimicking of natural healing, carriers are created with differential affinity towards the drugs, and a staggered release of multiple drugs is orchestrated to target different phases of the healing process [39, 129, 173–175]. One such design involves varying the binding affinity for each drug (Fig. 4C). In their work, Spiller *et al.* loaded a cytokine, interferon-gamma, onto the decellularized scaffold via physical adsorption, while entrapping another cytokine, biotinylated interleukin-4, via biotin-streptavidin binding [176]. As expected, the interferon-gamma dissociated from the carrier rapidly and induced a pro-inflammatory phenotype (M1 like) of macrophages *in vivo*, whereas the interleukin-4 was slowly released to promote a pro-healing phenotype (M2 like). This approach was able to mirror some of the biological processes, which naturally occur during the transition from angiogenesis to vessel maturation, and subsequently enhanced the integration of bone implant.

In addition, external stimuli can be utilized to achieve an on-demand release of each drug as needed, as discussed in Section 2.2.2. For example, Azagarsamy *et al.* functionalized BMP-2 and BMP-7 with azides that contained different photosensitive linkers, either nitrobenzyl ether (365 nm) or coumarin methylester (405 nm) [177]. These modified growth factors were incorporated into a PEG hydrogel *via* metal-free, strain-promoted click coupling but were then separately severed from the carrier by using different light sources. Along with the flexible incorporation of stimulus-sensitive modalities, more carriers can be envisioned to deliver multiple drugs in a biomimetic manner.

2.3. Bone-targeting carriers for systemic delivery

Bone metabolic disorders such as osteoporosis require systemic administration of drugs. In order to improve the efficiency of such treatments, it is necessary to establish therapeutically relevant concentrations of the drugs in the proximity of the bone tissue. Targeting tissue extracellular matrix (ECM) is an attractive approach to advance the delivery of the drugs [178–182]. Bone ECM has approximately 30% organic and 65–70% mineral components. Given the exclusive mineral component present in the bone, most of the targeting approaches utilize binding to the mineral apatite [160, 182, 183]. A wide variety of bone-targeting agents (BTA) are known to have affinity towards the mineral component of the bone tissue (Fig. 5). These targeting agents can be of small molecular systems (e.g.,

bisphosphonates, tetracycline, etc.) to peptides (e.g., peptides of aspartic or glutamic acids, etc.) (Fig. 5).

2.3.1. Bone-targeting agents

2.3.1.1. Bisphosphonates as bone targeting agents: One of the most commonly used bone targeting agents is the family of bisphosphonates (BPs) based compounds. These compounds contain two phosphonate groups (PO_3^{2-}) sharing a common carbon atom in their structures known as P–C–P backbone (Fig. 5A). BPs are the structural analogues of naturally occurring pyrophosphate which is a regulator of bone mineralization. Pyrophosphate is characterized by its P–O–P backbone and is known to have strong affinity to apatite crystals. However, as part of the natural bone physiology and remodeling, pyrophosphates are prone to fast acidic/enzymatic hydrolysis and therefore not suitable as BTA [184]. On the contrary, P–C–P bonds of BPs are far more stable while maintaining their affinity towards the apatite [185–187]. Akin to the calcium (Ca^{2+}) ion chelating oxygen atoms present in natural apatite, deprotonated hydroxyl groups of the two phosphonates in BP molecules (separated by ~ 2.9 to $3.1 \text{ \AA}$) can bind strongly with the Ca^{2+} ions thus endowing BPs with apatite binding affinity [184, 188]. BPs, due to their strong interaction with the apatite, can stay for a prolonged time in the bone, up to many years [189, 190]. The two remaining R_1 and R_2 groups on the carbon atom of the P–C–P backbone can further modulate affinity of the BPs towards apatite (Fig. 5A). For example, the presence of a hydroxyl ($-\text{OH}$) or an amine group ($-\text{NH}_2$) at the R_1 can provide additional interaction with the apatite. This is evident from the fact that BPs with these functional groups at R_1 indeed show higher binding affinity towards bone tissues [191, 192]. Nancollas *et al.* found that among six different BPs which are commonly used in clinics, chlorodronate and etidronate (bisphosphonates without nitrogen) showed significantly less apatite binding affinity compared to risendronate, ibandronate, alendronate and zoledronate (bisphosphonates containing nitrogen moieties) (Fig. 6) [191, 193]. Furthermore, the $-\text{OH}$ or $-\text{NH}_2$ groups positioned at R_1 and/or R_2 of the BPs are available for chemical modifications such as conjugation with a drug (to create a prodrug conjugate) or to a drug carrier (e.g., polymer, nanoparticles, etc.) without compromising their affinity towards bone [194, 195].

Since BPs inhibit bone resorption, their use in targeting bone tissues can potentially interfere with the normal bone remodeling, which is essential to maintain bone health. Long-term and excessive use of BPs has been reported to associate with femoral fractures as well as osteonecrosis of the jaw in rare cases [196, 197]. However, these side effects of BPs may not be a significant concern, due to the low concentration and short-term use of BPs as bone-targeting agents. For example, while 5 to 70 mg of BP are administered per week for osteoporosis treatment, a BP-functionalized delivery system only exposes patient to ~ 1 mg of alendronate in 40 mg of delivery vehicle [198].

2.3.1.2. Peptides as bone targeting agents: Another commonly used bone-targeting agent is polymers of aspartic acid (Asp) or glutamic acid (Glu) (Fig. 5B). Osteopontin and bone sialoproteins, the two major non-collagenous bone proteins, have a repeating sequence of Asp or Glu in their amino acid sequence with the ability to bind to bone apatite [199]. Homo-oligopeptides of Asp or Glu were indeed shown to possess affinity toward bone,

making them promising biomimetic bone-targeting agents [200, 201]. The use of such oligopeptides as bone-targeting agents provides an attractive option, due to lesser side effects and a relatively shorter half-life of the oligopeptides *in vivo* when compared to BPs [202, 203]. Further, binding affinity of the oligopeptides (from either Glu or Asp) towards the bone mineral was not affected by the nature of the amino acids or its enantiomeric forms (L or D). However, apatite binding affinity was found to be plateaued at six or more amino acids per oligomer [204]. In addition, D-Glu or D-Asp rich-oligopeptides were found to be retained at bone sites for longer time (14 days) compared to peptides involving L-amino acids (24 hours) largely due to the non-hydrolysable nature of the former [204]. Nakato *et al.* studied the Ca^{2+} ion chelating abilities of various polymeric Asp structures with α - and/or β -amino Asps (e.g., α -L-Asp, α -D-Asp, β -L-Asp and α,β -D,L-Asp) [203]. Among various bone-targeting Asp peptides, peptide with α -amino acids (poly(α -Asp) configuration) showed the highest Ca^{2+} ion(s) chelation due to the spatial configuration of the $-\text{COOH}$ groups on the peptide backbone. Considering the vast possibilities of amino acid sequences in peptide combinations, other oligopeptides have also been studied as bone-targeting agents [205, 206]. Using phage display techniques, three 12-mer peptides sequences such as VTKHLNQISQSY (VTK), APWHLSSQYSRT and STLPIPHEFSRE were identified and shown to have strong affinity towards bone apatite [206, 207].

2.3.1.3. Tetracycline as bone targeting agents: Tetracycline (TC), an antibiotic produced by the *Streptomyces* bacteria, has been used widely as an antibacterial agent [208]. In addition to its well-known antimicrobial activities, TC is also known to have affinity towards divalent metal ions such as Ca^{2+} ions thus making it a bone-targeting agent [209]. The β -diketone functionality at position 1 and 2, the enol groups at position 4 and 6, and the amide group at position 5 can chelate the Ca^{2+} ions of bone mineral (Fig. 5C) [208]. Besides chelation, other interactions may also contribute to their association - van der Waals attractions and hydrogen bonding between the hydroxyl groups of TC molecules and the apatite are considered to provide additional interactions [210]. Considering the bone binding affinity, TC has been employed to deliver therapeutics to the bone [211, 212]. Since TC is known to accumulate in bone tissues where biological turnover is high, one major concern is the reduced affinity of the TC towards pathological bone sites which are generally characterized by low bone turn-over. In addition, the chelation of TC is permanent, which can result in undesirable side effects, such as tooth staining due to the fluorescence property of TC [213, 214]. This attribute makes TC a suboptimal candidate for bone-targeting agent.

2.3.1.4. Other bone-targeting agents: Besides the afore-discussed, a few other candidates such as molecules with multiple phosphonate groups, poly(ethylene sodium phosphate), aptamer, etc. have been developed as bone-targeting agents [18, 215–217]. Some examples of molecules with multiple phosphate groups include ethylenediamine tetra(methylene phosphonic acid) (EDTMP, Fig. 5D) and tetraazacyclotetradecane-1,4,8,11-tetramethylene phosphonic acid (DOTMP). These molecules, containing four phosphate groups that can bind to Ca^{2+} ions, are used to deliver radiopharmaceuticals and proteins to the bone tissue [215, 218]. In sharp contrast to the BPs, no physiological/biological effects were observed on bone homeostasis. In order to increase the chelation with Ca^{2+} ions, dendritic structures with multiple BPs have been developed [216]. Using 3,5-di(ethylamino)-2,2-

bisphosphono)benzoic acid as spacer, a branched phosphonate structure was created which increased the amount of available phosphate groups for Ca^{2+} binding. In a recent report, poly(ethylene sodium phosphate) (PEP·Na) that showed excellent cytocompatibility was found to have *in vivo* bone affinity in a mice model. A cyanine 5 dye conjugated PEP·Na, *i.e.* Cy5-PEP·Na, was found to be in spine for more than three days after its intravenous injection [217].

Though rarely studied, bone-targeting agents focusing on bone cells such as osteoblast-specific molecules have also been developed by using cell-specific aptamers [18]. Using a cell-SELEX method, Liang *et al.* found that aptamers with TCTATGGCCTGTAGTCCGCCATCCGGCGTAGCTTTGCAAGTGG (CH2 aptamer), CTTGATTGGACGAAGCATCGTGCGCATCCGGCGAAATCCGTGG (CH5 aptamer) and AGTCTGTTGGACCGAATCCCGTGGACGCACCCTTTGGACG (CH6 aptamer) had a significant binding affinity towards osteoblasts with an equilibrium dissociation constant in the 10^{-9} to 10^{-12} molar range. The stability of these aptamers against nuclease degradation can be improved *via* 2'-O-methyl-nucleotide substitutions without affecting their affinity. Among these aptamers, the presence of shorter nucleotide sequences and satisfactory secondary structure makes CH6 aptamer an interesting bone-targeting candidate compared to CH2 and CH5 [18].

2.3.2. Assembly of bone-targeting carriers—A direct assembly of bone-targeting carriers can be achieved by combining both BTAs and therapeutics into a common carrier such as polymeric or dendritic macromolecules [205, 219–235]. The two common methods to load drugs involve either covalent immobilization of drugs to carriers or noncovalent encapsulation of drugs in carriers, as discussed in Section 2.1. Similarly, BTAs can be introduced either before the formulation of carriers *via* macromolecular chain/backbone modification or after the carrier fabrication *via* surface grafting. The use of carriers containing multivalent BTAs allows for better targeting and delivery efficiency *via* improved pharmacokinetics and biodistribution [178, 236]. One key factor in developing carriers for systemic delivery is the size of the carriers. Nanoparticles with diameters smaller than 10 nm and larger than 200 nm are known to get eliminated rapidly from the body through extravasation and renal clearance or uptake by the macrophages [237, 238]. Though the particles in the size range from 10 to 70 nm are known to penetrate very small capillaries including the lacuna of the bone tissue, 70 to 100 nm particles are generally used to target bone because of their extended blood circulation time [225, 239]. In addition, a degradable carrier can improve the release kinetics of therapeutics and prevent the carrier accumulation through controlled degradation of their backbone [240–250].

One of the major concerns with bone-targeting carriers is the potential steric hindrance from the carrier structures, affecting the binding of BTAs to the apatite minerals [219]. Hence, alendronate (ALN), a second-generation BP, is often used in macromolecular carriers, due to its sterically free primary amine group on the R_2 side chain. It is commonly used with *N*-(2-hydroxypropyl) methacrylamide (HPMA), which is biocompatible and non-immunogenic [251, 252]. For instance, Miller *et al.* have shown the delivery of an anticancer drug paclitaxel (PTX) selectively into bone upon conjugation of ALN and PTX with HPMA copolymer [243, 253]. ALN and PTX were conjugated with HPMA copolymer *via* Gly-Phe-

Leu-Gly (GFLG) and GFLG-Phe-Lys-p-aminobenzyl carbonate (GFLG-FK-PABC) spacers, respectively (Fig. 7). The cleavage of the FK dipeptide in GFLG-FK-PABC spacer by cathepsin B (an enzyme overexpressed by the epithelial and endothelial cells associated with tumor) released an amine intermediate (PABC-PTX), which then dissociated spontaneously (through 1,6-elimination and decarboxylation) to release active PTX (Fig. 7). The ALN-HPMA-PTX-conjugate was shown to inhibit the proliferation of human umbilical vein endothelial cells (HUVECs) *in vitro*. Furthermore, it was observed in a follow-up study that an anti-angiogenic agent, TNP-470, conjugated ALN-HPMA (ALN-HPMA-TNP 470 conjugate) carrier inhibited tumor growth by 60% in mice bearing adenocarcinoma in the tibia, whereas the use of a physical mixture of ALN and TNP 470 showed a reduced effect: a tumor growth inhibition by 37% [245].

In addition to steric hindrance, the molecular weight of a carrier also affects the bone-targeting behavior. A recent study used HPMA copolymers of varying molecular weights (24, 46 and 96 kDa) to conjugate with D-Asp8 (HPMA-D-Asp8) and suggested a strong influence of the molecular weight of the polymers on the bone binding affinity [247]. Though all HPMA-D-Asp8 were found to accumulate within the tibia and femur within just 15 minutes post-injection, HPMA-D-Asp8 with lower molecular weight (HPMA of 24 kDa) showed higher levels of accumulation compared to HPMA-D-Asp8 involving high molecular weight HPMA. However, with time (after ~24 hours), HPMA-D-Asp8 with high molecular weight HPMA was found to accumulate more compared to the low molecular weight products.

Another approach to create bone-targeting carriers is modifying drugs with BTAs as prodrugs. Prodrugs are chemically modified drugs that can be metabolized into active compounds *in vivo*. In general, they offer several advantages such as improved pharmacokinetics, decreased toxicity, and targeted delivery to specific cells or tissues [254–256]. Bone-targeting prodrugs *via* direct conjugation of the therapeutics with various BTAs have been extensively studied. Some examples include grafting BTA with estrogen compounds (e.g., estradiol which is known to stop or even reverse osteoporosis), parathyroid hormone, cell receptor agonist, growth factors such as BMPs, etc. [257–260]. Similarly, BTA-conjugated antibiotics have been developed for treating osteomyelitis [261–265]. Other drugs such as anticancer drugs, miRNA, *etc.* have also been successfully delivered to the bone tissue for treating bone disorders [266–269].

One of the first reports of delivering prodrugs selectively into the bone tissue both *in vitro* and *in vivo* was by Uldag *et al.* using BP conjugated albumin, where albumin was used as a model protein [268–270]. Here, albumin was chemically modified to introduce maleimide functionality and then reacted with a thiolated amino-BP via Michael addition reaction [268]. While majority of the BP-conjugated albumin ($91.8 \pm 0.8\%$) was bound to apatite, minimal binding of the unmodified albumin was observed ($<3\%$). The positive correlation ($r^2 = 0.967$) between the extent of conjugation (i.e., number of BP molecules per albumin) and apatite binding indicated that BP is directly responsible for the binding of albumin to the apatite. Furthermore, it was observed that the BP conjugation enhanced delivery of the protein to the bone after subcutaneous (s.c.) and intravenous (i.v.) injections; with the i.v. injection to be more effective. This conclusion was based on the calculated targeting

efficiency (TE, obtained by dividing mean delivery of BP-conjugated protein to bone with that of the unmodified protein) for i.v.-injected compared to s.c.-injected albumins (3.7 for i.v. and 1.9 for s.c. after 24 h) [269]. The BP-conjugated albumin was also effective in targeting osteoporotic bones (e.g., tibia and femur, etc. of osteoporotic rats). Moreover, TE of BP-conjugated albumin increased with time; TE for the tibia was found to be 2.2 after the first day and 7.5 after the fourth day in the osteoporotic rats [270]. Houghton *et al.* developed BP-fluoroquinolone prodrug by conjugating BP with the free amino (piperazine) group of the fluoroquinolones for osteoporosis [261]. A β -aminoketone linkage was established between the BP and fluoroquinolone whereby the antibiotic was released by elimination reaction. The BP-fluoroquinolone conjugates showed excellent (80–90%) bone mineral binding capacity in just one hour *in vitro*. *In vivo* investigation revealed that bisphosphonate-conjugated fluoroquinolones have a higher rate of infection prevention compared to the systemically administered parent antibiotic in a rat bone infection model [261]. Chemotherapeutic drugs such as cisplatin (which shows severe systemic toxicity) were used to effectively treat osteosarcoma by using a bone-targeting approach [267]. These drugs showed reduced acute toxicity upon conjugation with BP while maintaining their anticancer properties. Specifically, the acute toxicity of drug-BP conjugate, cis-[PtL(NH₃)₂Cl]NO₃ {L = tetraethyl [2-(pyridine-2-yl)ethane-1,1-diyl]bisphosphonate}, in mice was found to be seven-fold lower than that of the unbound cisplatin. The relative low systemic toxicity may result from the steric hindrance of the ligand, which blocks the conjugate approaching the bases of DNA [267]. However, there is one key limitation of the afore-mentioned systems: not all the prodrugs could release the active molecules in therapeutically relevant quantities after their binding to bone mineral, due to slow dissociation of the antibiotic-BP conjugates.

It is worth noting that bone-targeting carriers with on-demand release of drugs have also been developed to mitigate the inefficient release of drugs [240, 242, 243, 245, 248, 257–260, 263–265, 271, 272]. As discussed in Section 2.2.2, such carriers can release drugs in response to external stimuli, further improving the drug efficacy. For instance, a photo-active dendritic platinum-copper alloy nanoparticle upon conjugation with Asp8 not only displayed higher affinity towards bone tissue, but also efficiently reduced the tumor growth and bone resorption after the photo-responsive therapy [273].

Because bone tissues under pathological conditions (e.g., osteoporosis, osteomyelitis or osteosarcoma) experience significantly lower pH, pH-sensitive linkers (e.g., ester, carbonate or carbamate, etc.) have been used in the on-demand delivery systems [236, 259–261, 263, 274]. In a recent study, Yewle *et al.* demonstrated that parathyroid (PTH, 1–34) hormone conjugated with single molecule of bisphosphonate *via* acid-labile hydrazone bond had 60–80% higher HA binding affinity over the corresponding unmodified PTH. The bioactivity of the PTH (1–34) was found to be retained following its chemical modification [260]. Doxorubicin, an anticancer drug, was conjugated with bone-targeting D-Asp8 *via* an acid-sensitive hydrazone linkage. D-Asp8-drug conjugates were shown to have as high as 91 ± 3.1% binding efficacy towards bone mineral and a sigmoidal release kinetics of the drug at pH 4.5–5.5 [274].

Studies have also leveraged the overexpression of protease cathepsin in bone tissues to support on-demand and tissue-specific delivery of drugs by using cathepsin-cleavable peptides as linkers [236, 257–259, 262, 263]. For example, a bone-targeting dual-action prodrug has been developed by Xie *et al.* for osteoporosis (Fig. 8A). The BP-drug conjugates consisted of an anabolic agonist of the prostaglandin EP4 receptor and alendronic acid, chemically linked *via* a cathepsin K cleavable peptide *N*-4-carboxymethylphenyl-methyloxycarbonylleucinyl-argininyl-para aminophenylmethyl alcohol (Leu-Arg-PABA) (Fig. 8B). The BP-agonist conjugates were shown to bind rapidly and almost completely (>95%) to bone mineral within 30 minutes both in PBS and in rat plasma. Furthermore, the conjugates showed considerable degree of hydrolysis (approximately 55% over 24 hours) in rat plasma *in vitro*. *In vivo* studies indicated that the agonist was liberated *via* degradation of cleavable linker Leu-Arg-PABA (half-life of 4.4 days) with ~2.2% bone uptake [257].

Treatments of various bone disorders such as osteosarcoma, osteoporosis, osteomyelitis, *etc.* have benefited from the bone-targeting drug delivery systems. By using such delivery systems, different types of therapeutics were administered systemically and shown to accumulate at the target bone tissue with improved therapeutic outcome [240–250]. For instance, bone myeloma was significantly prevented/delayed in female Nod/SCID beige mice with bone-targeting alendronate (ALN) conjugated PLGA nanoparticles (NPs) containing an anticancer drug bortezomib compared to the nanoparticles without any drug. This was evident by the fact that bioluminescent imaging (BLI) flux from mice obtained after injecting Luc⁺GFP⁺ MM1S cells was found to be significantly lower in bortezomib containing ALN-PLGA NPs compared with that of ALN-PLGA NPs without drug or free drug groups at different time interval (Fig. 9A). Survival rate was found to increase in the group treated with drug containing ALNPLGA NPs (median survival of 41 days) compared to the other groups (median survival of < 36 days) (Fig. 9B) [222]. In another study, bone-seeking oligopeptide (Asp8) conjugated liposome, encapsulated with an osteogenic molecule icaritin (Asp8-ICT-Lip), was shown to enhance bone tissue formation in osteoporotic mice compared to the liposome containing icaritin but lacking the bone targeting Asp8 moiety (ICT-Lip). Mice treated with Asp8-ICTLip had significantly higher bone mineral density (BMD) and bone volume ratio (BV/TV) compared to the untreated mice or mice treated with the ICT-Lip. A better architecture and connectivity of the trabecular bone was also observed in the Asp8-ICT-Lip group compared to the ICT-Lip group [234]. These studies demonstrated that bone-targeting delivery systems are not only efficient in carrying drugs/bioactive agents to the target site but also effective in enhancing its therapeutic efficacy *in vivo*.

3. Conclusions and future perspectives

The delivery of bone therapeutics has benefited greatly from biomaterial-based carriers. These carriers are widely used locally and systemically to deliver the desired bioactive agents to the bone tissue. A number of biomaterial-assisted approaches ranging from prodrugs to bone-targeting agents have been extensively devised and developed. For instance, therapeutic efficacy of various drugs can be increased by connecting the drug to a bone-targeting moiety via a degradable linker or a multi-valent polymer. Such strategies have shown increased bone binding and retention while delivering the drug upon cleavage of

the linker. Further, bone-targeting drug delivery systems have been shown to provide the protection to the encapsulated drug with effective release at the bone tissue, as evident from an array of *in vitro* and *in vivo* studies. Yet many of the approaches have yielded different levels of success clinically, suggesting that significant technological challenges still persist. This is largely because bone regeneration requires a complex and orchestrated cascade of signaling, which is regulated by various signaling molecules such as growth factors. Thus, moving forward, carriers that can store and release multiple bioactive agents in a spatiotemporal manner are desired to activate various phases involved in bone healing. They may not only enhance bone regeneration but will also yield neo-bone tissues with the adequate biological and mechanical functions.

Systemic administration of drug-bearing carriers functionalized with bone-targeting moieties provides a non-invasive and targeted therapy for treating various bone defects. Endowing such systems with stimuli-responsive groups can be used to achieve on-demand delivery of drugs. It is possible that the resulting smart, remote-controlled systems can be administered at any time, even before a fracture occurs, especially for individuals suffering from osteoporosis or other systemic bone diseases, so that the drug can be activated and released immediately after the diagnosis with fractures. Furthermore, these systems can be used as long-lasting drug depots to treat systemic diseases like osteoporosis. Alternatively, these delivery devices loaded with antibiotics and pain-relieving drugs can be implanted to the targeted site following surgery to manage pain and potential infections, if any. Such systems with on-demand drug delivery can also be useful in combating opioid addiction, as the release of drugs and their duration can be controlled remotely.

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Statement of significance

Drug delivery is imperative when surgical interventions are not sufficient to address various bone diseases/defects. Biomaterial-assisted delivery systems have been designed to provide drugs with the desired loading efficiency, sustained release, and on-demand delivery to enhance bone healing. By surveying recent advances in the field, this review outlines the design of biomaterials as carriers for the local and systemic delivery of bioactive agents to the bone tissue. Particularly, biomaterials that bear chemical moieties with affinity to bone are attractive, as they can present the desired bioactive agents to the bone tissue efficiently and thus enhance the drug efficacy for bone repair.

Figure 1.

Schematic showing drug loading *via* different forms of interaction. (A) Simple adsorption of drug molecules (triangles) by the carrier. (B) Entrapment/encapsulation of drug molecules within biomaterials (shaded circles), which are further loaded in the carrier. (C) Drug molecules (hexagons) designed to interact with the carrier *via* affinity binding (dashed lines). (D) Confinement of drug molecules (stars) within the carrier *via* covalent bonding (spiral lines).

Figure 2.

A multijet 3D powder printing system. (A) The components and layout of the printer. (B) Bioactive molecules were patterned in 3D along with the bioceramics, showing minimal impact of the printing process on their bioactivity. Reprinted with permission from [133].

Figure 3.

Delivery of miRNA with a two-stage release from a carrier [20]. (A) Self-assembly of the nanohybrids with miRNA-26a entrapped the microRNA *via* electrostatic interaction. (B) The nanohybrids were encapsulated within PLGA microparticles, which were embedded in a PLLA scaffold. The nanohybrids were released via diffusion and engulfed into the cytosol, where miRNA-26a was freed to activate osteogenesis.

Figure 4. Delivery of multiple drugs (triangles, hexagons, and stars) mimicking the natural healing process. (A) Drugs are homogenously distributed in the carrier with similar release profiles. (B) Drugs are compartmentalized in the carrier with diffusion barriers (shaded areas), leading to a staggered release. (C) Drugs are loaded in the carrier exhibiting varied physical/chemical interactions (presented as different shades), which contribute to their sustained release. The conceptual release profiles are presented correspondingly.

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Figure 6.

Structures of clinically used various bisphosphonates (BPs) and their bone binding affinity represented by the kinetic affinity constant (K_L). K_L is the measure of affinity between apatite and the BPs.

Figure 7.

Schematic representation showing mechanism for the cleavage of the ALN-HPMAPTX conjugate (HPMA copolymer-PTX-FK-ALN), by cathepsin B. Adapted from [243].

Figure 8.

(A) Schematic representation of bisphosphonate-EP4 agonist prodrug targeting bone and enzymatic release of active molecules; (B) Differential enzymatic release of active EP4 agonist from the prodrug conjugate *in situ* by local hydrolytic enzymes. Adapted from [257].

Figure 9.

Alendronate-bortezomib nanoparticles (Ald-Bort-NPs) pretreatment inhibits myeloma growth more effectively than the nanoparticles without drug (Ald-NPs) or the free drug alone. (A-B) Female Nod/SCID beige mice were subjected to pretreatment for 3 weeks with Ald-NPs, bortezomib (Free Drug), or Ald-Bort-NPs. (A) Bioluminescent imaging (BLI) flux from mice obtained after injecting Luc⁺GFP⁺ MM1S cells *via* tail-vein at different time

interval. (B) % of Survival of mice for different groups at different time intervals. Adapted from [222].

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