Transplantable delivery systems for *in situ* controlled release of bisphosphonate in orthopedic therapy

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Importance of the field: Bisphosphonates (BPs), structurally similar to pyrophosphates and functionally superior in restraining osteoclast-induced bone resorption, have been widely used as clinical drugs in the treatment of osteoporosis, bone voids and associated inflammation. However, owing to their high aqueous solubility and the consequently high rate of loss during oral administration, the loading and targeting of BPs pose major challenges in practice. Alternative delivery routes such as nasal, subcutaneous/intramuscular injection have contributed little to improving the bioavailiability and efficacy of BPs. To improve and optimize the delivery efficiency and efficacy of BPs, numerous strategies have been developed and adopted. Studies on controlled release of BPs provide important information on the fabrication of BP delivery systems for *in situ* treatment. As BPs play an important therapeutic role in osteoporosis and similar diseases, it has become essential and vital to survey various reported fabrication methodologies of these systems and the consequential orthopedic treatments so as to keep abreast with advances in their clinical use.

Areas covered in this review: Transplantable delivery systems for controlled release of BP are reviewed from literature published since 2000. The fabrication pathways and the release of BPs from various material systems are discussed in case studies. Recent progress in CaP models based on the strong and specific chelation between BPs and calcium phosphate crystals is highlighted.

What the reader will gain: This review offers an outline of the advances in BP controlled release and delivery systems for orthopedic therapy.

Take home message: Understanding the cutting-edge BP controlled release and delivery systems for *in situ* treatment is key to the successful design of a more promising and perfect delivery system for orthopedic therapy. Moreover, developing such delivery systems incorporating the numerous advantages of BPs and controlled release environment requires substantially more flexible models to control better the fate of BP drugs.

Keywords: bioconjugate, bisphosphonate, controlled release, orthopedics

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1. Introduction

Bisphosphonates (BPs) have been in the spotlight in the field of bone resorption-related treatments for decades. As the only non-hormonal agents, BPs hold promise in effectively reducing the risk of osteoporosis and other similar diseases that pose challenging problems for orthopedists and oncologists. The chemical structure of

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Article highlights.

- Oral administration of bisphosphonates (BPs) is not very efficient and is followed by unfavorable retention of the BPs in bones, and the paper approaches the issue from the point of development of BP controlled delivery systems.
- Silica-based ordered mesoporous materials that confine BPs in their pores prove to be effective for bone defect treatment and lead to better BP adsorption. By adopting this system, BP intake rate can reach 40% compared with 1% when orally administrated.
- Calcium phosphates (CaPs) have been widely used to fabricate various BP delivery systems, which are based on the strong and specific chelation between BPs and CaP crystals. One can achieve fine-tuning over BP controlled release by means of CaP systems, mainly in the forms of hydroxyapatite and CaP bone cement.
- Polymers such as poly(lactic-*co*-glycolic) acid also serve as effective BP carriers. Their combination with inorganic materials can lead to excellent encapsulation efficiencies and better control over the BP release profile.
- Bisphosphonate controlled release and delivery systems drastically improve BP's therapeutic potential, especially in the areas of orthopedic regeneration and bone reconstruction.

This box summarizes key points contained in the article.

BPs is analogous to that of inorganic pyrophosphate (**Figure 1**), which regulates bone mineralization endogenously. These advantages have led to a strong focus on BPs for several therapeutic applications. Based on their three-dimensional structure with two oxygen ions from bilateral phosphonate group acting as chelating sites that can coordinate with two divalent metal ions, BPs have high affinity to bone minerals and the variance of their side chains R_1 or R_2 determines the efficiency of binding. The previous uptake of BPs in bone propels them to contact closely with osteoclasts during bone resorption, which produces an acidic environment for BPs to be conveniently dissociated from bone minerals. Many studies have shown that different BPs have a similar endocytic mechanism by means of which they enter osteoclasts and implement their various biochemical effects [1].

Enzyme farnesyl pyrophosphate synthase (FPPS) is mainly regarded as responsible for bone resorption by many researchers [2]. This enzyme gives birth to isoprenoid lipids in the mevalonate pathway, by which small GTP-binding proteins are modified after their translation. These modified GTP-binding proteins are essential for osteoclast function. Among the major BPs to be administered directly or by means of controlled release (**Figure 2**), alendronate has been widely used in the clinic [3] and shows moderate restriction of FPPS activity. Risedronate, also a clinically relevant BP [4], has a high enzyme binding property that strongly inhibits FPPS. Ibandronate is another FPPS inhibitor, to a degree higher than alendronate but lower than risedronate [5]. Zoledronate achieves stronger inhibition of FPPS activity than ibandronate, and is acknowledged for its extended period of action [6]. Besides, zoledronate can be administered intravenously [7], and has become a standard yearly course of therapy to treat osteoporosis.

Various BPs have been used in the clinic to treat several diseases, including Paget's disease [8], bone oncology [9], osteoporosis [10] and osteogenesis imperfect [11], as well as to enhance bone quality and strength [12] and promote fracture repair [13]. However, BPs are poorly absorbed in the intestines when administrated orally [14,15], and absorbed BPs undergo retention in bones [16]. As BPs have very low bioavailability – < 1% are intestinally absorbed – they have to be used at high dosages, which leads to gastrointestinal disturbances [16,17], chronic renal failure, or osteonecrosis of the jaws [18]. Poor patient compliance is another factor discouraging wider adoption and clinical use of orally administered BPs. A promising solution to these shortcomings is the loading of BPs onto drug delivery systems for *in situ* treatment to increase BP bioavailability, prevent its excess dosage and consequential side effects, and ultimately enhance the quality of life of affected people. Numerous studies have already been targeted at devising BP controlled release systems that act locally, including ceramic supports such as calcium phosphates [19-21], polymeric nano/microspheres for encapsulation [22,23] and hybrid polymeric–inorganic composites [24]. Among the various methodologies available, a strong and specific means of controlled BP delivery by means of chelation between BPs and calcium phosphate crystals has been intensively adopted.

Bisphosphonates are multipotent clinical drugs that are used in curing the various illnesses related to bone resorption. By combining the advantages of the therapeutic properties of BPs and their local and controlled release from drug delivery systems, a potent clinical therapeutic tool has been established for the treatment of sites affected by osteoporosis (e.g., proximal femur, vertebral bodies, wrists), sites affected in orthopedic surgeries (e.g., tooth repair, implant fixation), and various other local disorders, diseases and defects of bones. By binding to inorganic carriers, trapped in polymeric systems or conjugated with biomolecular and biopolymeric systems, BPs can be confined in such delivery environments for a long period of time without significant loss, exerting local and long-term therapeutic benefits. This review summarizes the latest advances in realizing BP controlled release and delivery systems and their applications for *in situ* orthopedic medication and treatments.

2. Formulation and conjugation for delivery

As BPs are not easily absorbable by intestine and locate selectively in bone with retention, they have to be used at considerably high doses for oral administration. This results in gastrointestinal and renal complications in patients. Besides oral administration, other administration strategies have been proposed and adopted over the last few years, including delivery by means of the nasal route [25] and injecting the drug

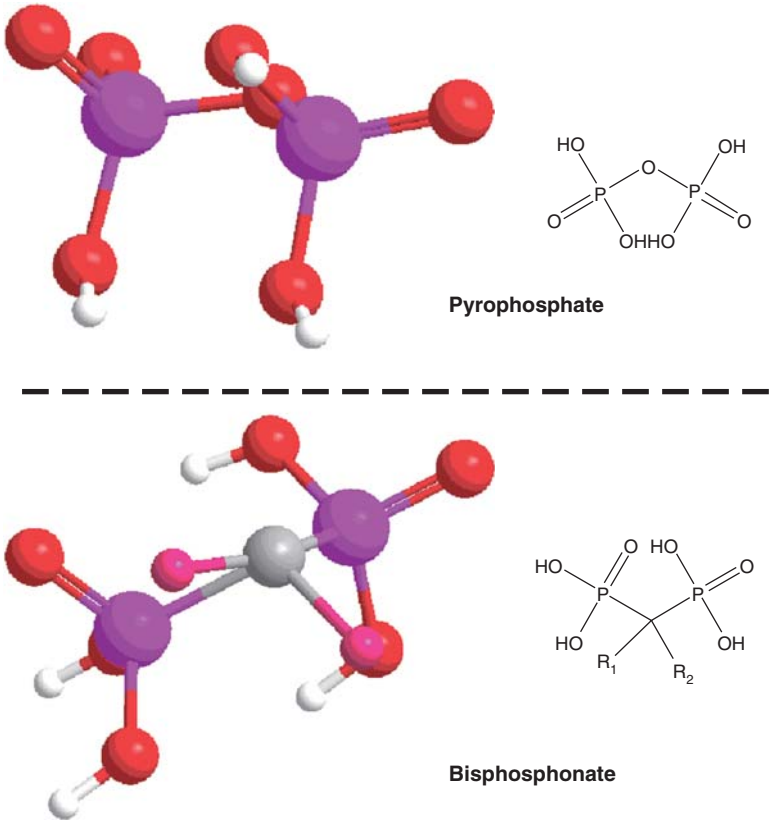

Figure 1. The general structure of a bisphosphonate and pyrophosphate. Bisphosphonate has two phosphonate groups that share a common carbon atom instead of oxygen, which is further attached to two covalently bonded groups (side chains) that determine their clinical applications or relevance.

subcutaneously [26] or intramuscularly [27]. These attempts, however, have not been able to improve markedly BP adsorption and diminish related side effects, and thus have contributed little to improving the bioavailiability and efficacy of BPs [28]. To troubleshoot further these existing problems, a transplantable delivery system that can locally target and control BP release is of immediate significance and has motivated current research in the field of drug delivery. Formulation and conjugation technologies for controlled delivery of BPs have mushroomed over the years (**Figure 3**), and have resulted in improved clinical therapies for orthopedic disorders (**Table 1**).

2.1 Inorganic systems

2.1.1 Mesoporous silica confining bisphosphonate

Mesoporous silica possesses unique properties such as a very high specific surface area and void volume. Structurally highly regular, its pore size distribution is in the range of mesopores, which represents size limits for molecule turnover at the pores. The use of ordered mesoporous silica materials in controlled delivery systems has been reported for a wide range of drugs [29]. Moreover, silica-based ordered mesoporous materials can induce apatite formation in physiological fluids [30]. As an alternative for local and controlled drug delivery, silica-based ordered mesoporous materials that confine BPs in their pores prove to be effective for bone defect treatment and lead to better BP adsorption. Recently, mesoporous silica encapsulating BPs have been reported to inhibit bone resoprtion locally at implantation sites.

Among the various mesoporous silicas, MCM41 and SBA15 are commonly used materials for BP delivery. Balas *et al.* used hexagonal siliceous ordered MCM41 (D_P = 3.8 nm) and SBA15 (D_P = 9.0 nm) as matrices for alendronate adsorption and release and modified the pore walls' surfaces organically with amine groups [31]. Interestingly, for MCM41-NH_2, BP intake rate could be increased from 1 [15] to 40%, which provides higher local dosage while preventing high systemic dose of BP. Besides, BP loading amount and its delivery rate could be finely tuned through organic modification on the pore surfaces. By covalently grafting amine groups to the silanol groups, BP adsorption was increased almost three times, with following escalation of drug uptake in the target area. Modification of functional groups on silica surface for BP delivery was investigated further by Nieto and co-workers. They found that BP loading could be efficiently regulated by

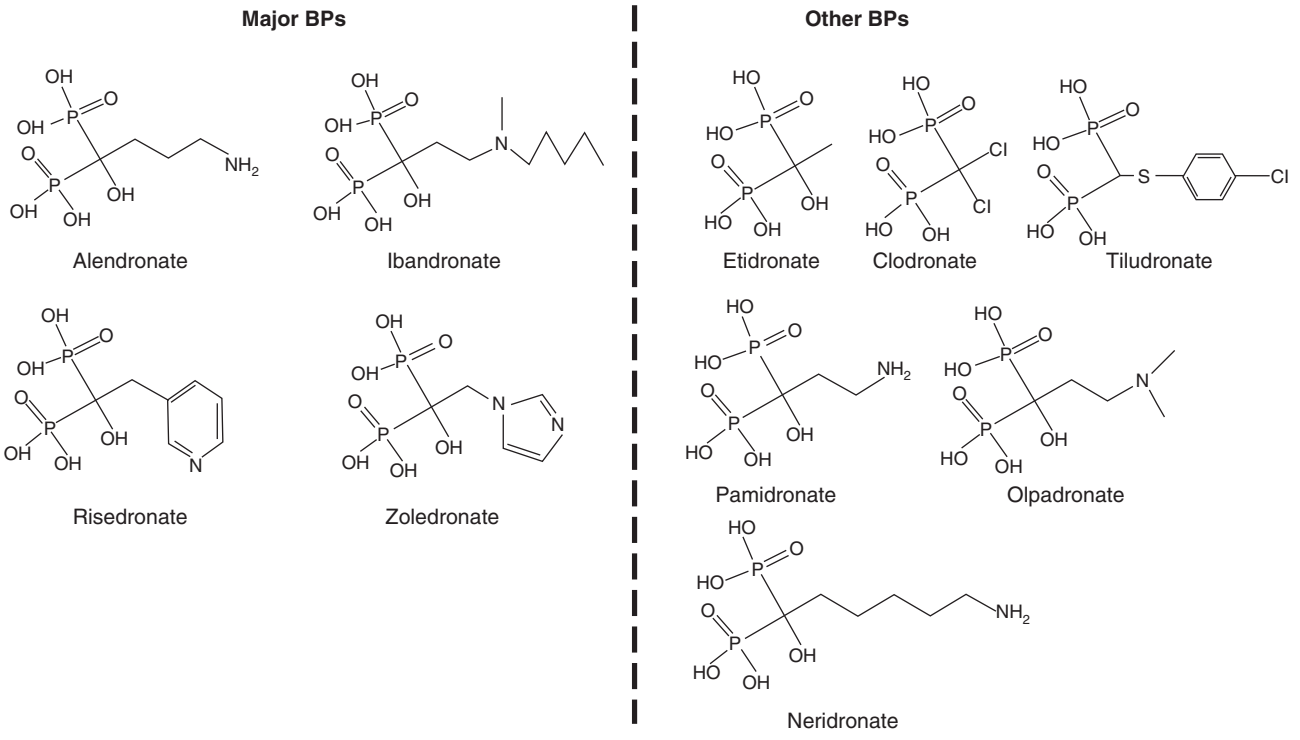

Figure 2. A new classification of bisphosphonates based on their adoption frequency. The four major BPs containing alkyl-amino groups or heterocyclic nitrogen all act by inhibiting the farnesyl pyrophosphate synthase enzyme.
BP: Bisphosphonate.

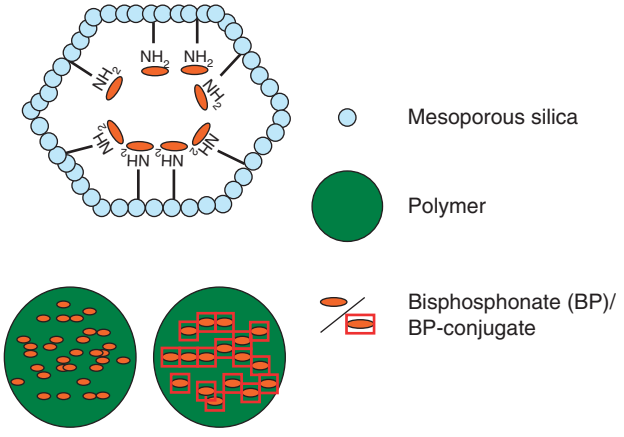

Figure 3. General scheme of two typical BP loading structures. Upper: BP was adsorbed on hexagonally ordered mesoporous silica functionalized with propylamine groups. Lower: BP was hybridized with polymer spheres in micro/nanoscale directly or indirectly via initial conjugation.

a proper change in the amount of amine groups on SBA15 mesopore surfaces [32]. However, the adsorbed alendronate release rate was found to be independent of the functionalization degree. Nieto *et al.* also investigated the effect of surface electrochemistry of SBA15 mesoporous materials on zoledronate adsorption [33]. The release of adsorbed biomolecules was found to be independent of the surface charge but mainly based on diffusion related to the surface area and molecule size. The introduction of other elements into mesoporous silica for BP adsorption and delivery has also been investigated. Colilla *et al.* tested SBA15 mesoporous materials containing phosphorus as BP delivery systems [34]. Compared with pure silica SBA15, silica containing phosphorus showed higher alendronate loading sustained

Table 1. General summary of bisphosphonate formulation pathways and their practical evaluation.

Profile	Improvement	Cell/animal experiment result	Ref.
Mesoporous silica	Modification of the functionalization degree of the matrix	N/A	[31,32]
	Tuning of the surface electrochemistry of the matrix	N/A	[33]
	Introduction of other elements to the matrix	N/A	[34,35]
Calcium phosphate (CaP)	Incorporation of BPs to CaP/calcium-deficient apatite (CDA) coating	Maintain BP function on bone cells	[42,44,86]
	Hydroxyapatite (HAp) granules to load BPs	Maintain BP function on bone cells	[45]
	BP-containing complexes absorbed onto HAp nanocrystals	Maintain BP function on bone cells	[46-48,50]
	Simulated body fluid-grown (SBF)-HAp layer absorbing BP	Maintain BP function on bone cells	[51]
	Introduction of BPs to calcium phosphate bone cement (CPC)	Maintain BP function on bone cells	[54-57]
Poly (lactic-*co*-gly colic) acid (PLGA)	Conjugate of PLGA nanoparticles with BPs	Maintain BP function on bone cells	[59,82,99]
	Fabrication of PLGA microspheres loaded with BPs	Maintain BP function on bone cells	[60,61]
Poly (D,L-lactide-*co*-glycolide-D-glucose) (PLG-GLU)	PLG-GLU matrix embedding BP-chitosan implant	N/A	[62,63]
Acrylic bone cements	Poly(methyl methacrylate) (PMMA), vitamin E methacrylate and Palacos R, etc. mixed with BP	Maintain BP function on bone cells	[67,69-72]
	Antibiotic simplex: radiopaque with erythromycin and colistin laden with BP	BP systemic delivery performed better	[73]
0rganic–inorganic hybrid	PLGA/mesoporous silica—HAp microspheres loaded with BP	Maintain BP function on bone cells	[64]
	PLGA-hybridizing-HAp microspheres loaded with BP	Maintain BP function on bone cells	[24]
	PLGA/CDA associated with BP	N/A	[65]
Others	The combination of BP with Ga and Gd in nanosuspensions	Maintain BP function on bone cells	[58]
	Synthesis of hyaluronan-BP conjugates having free hydrazide functionality	Maintain BP function on bone cells	[75]
	Osteoprotegenn (OPG) chemically conjugated with BP	Maintain BP function on bone cells	[76]
	Synthesis of alendronate-β-cyclodextrin conjugate	N/A	[77]

release of the drug. The enhanced BP affinity resulted from more interaction sites owing to the incorporation of the PO_4 units. Furthermore, *in vitro* tests showed that an apatite-like layer could develop on the surface of phosphorus-containing SBA15 after 2 weeks. Moreover, Colilla *et al.* described the synthesis of mesoporous silica-zirconia mixed oxides, which used Zr as a component of the mesoporous matrix for the first time [35]. *In vitro* release tests demonstrated that the complexation of phosphonates from BPs with zirconia present in the matrix led to higher retention of BPs and resulted in a more sustained release. These SiO_2-ZrO_2 mixed oxides used as BP delivery systems showed periodic large pore mesostructures with tunable acidity. Moreover, the possibility of adjusting the controlled release of BPs by varying the composition of mesostructured binary oxides was demonstrated. The uptake and release of BPs were found to be finely tunable by adjusting the pH loading and the complexing capacity of the surface. The selection of the material composition allowed controlling of the surface properties of the mesoporous matrix, which could control BP dosage adsorbed into the mesopores.

2.1.2 Calcium phosphate chelating bisphosphonate

Bisphosphonates have high affinity towards calcium owing to the strong cation chelating properties of calcium. Calcium phosphates (CaPs), widely used as direct substitutes for bone rebuilding [36-39], have turned out to be desirable alternatives as drug delivery vehicles. They have been used in various forms, including ceramics, cements and composite coatings. They possess osteoconductive ability, can be resorbed by cells and present a reliable alternative in orthopedic treatment. Calcium phosphates have been examined as vehicles in drug delivery systems [40]. Oliveira *et al.* proposed that sodium clodronate could be introduced in the CaP structure [41]. The dose of the incorporated drug intensively influenced the mechanical and chemical environment for cells in contact with the material, and 0.32 mg/ml was proven to be the ideal concentration for enhancing cell viability and osteoblastic profile. Faucheux *et al.* showed that zoledronate-coated calcium-deficient apatites (CDA) – one kind of CaP – inhibit osteoclastic resorption *in vitro* [42]. They characterized the influence of zoledronate-loaded CDA on osteoclasts and osteoblasts. Their results showed that CDA promoted higher BP adsorption and sustained release of the drug. Local and sustained release of zoledronate from CDA prevented bone resorption by osteoclasts without affecting osteoblast activity.

Of the various forms of CaP, hydroxyapatite (HAp) turns out to be a highly attractive form for BP encapsulation and release. As a calcium-based material, it has a high affinity for BPs and does not hamper BP pharmacological ability with regard to the binding mechanism involved [43]. In addition, HAp's solubility is affected by the pH environment or its crystallinity (lower solubility under higher crystallinity), so that BPs can be released controllably by varying the environment to vary the solubility of HAp [44]. The pH influences the solubility of HAp, so that HAp can release BPs flexibly during bone resorption with pH variation. Seshima and co-workers [45] in their study examined BP controlled release by adopting HAp as a delivery system. They synthesized HAp granules sized between 300 and 500 μm and their composites with BPs. They proved that BP release was regulated by varying the HAp sintering temperature. Lower sintering temperature resulted in lower crystallinity and higher solubility of Hap, which enhanced BP release. This trend was also observed by Iafisco *et al.* when they explored two kinds of BP-containing Pt complex adsorbed and released by HAp. These two complexes differed in some aspects, such as charge and physicochemical property, especially in two kinds of HAp: one is acicular-shaped with low crystallinity, the other is plate-shaped with high crystallinity [46]. Their results showed that the chemical structures of these complexes had an influence on the adsorption and release of BPs, mainly owing to the crystallinity and surface area of the HAp. Boanini *et al.* slightly modified the process of HAp synthesis in aqueous solution, which allowed preparation of composite HAp nanocrystals with up to 7.1 wt% alendronate [47]. The addition of alendronate to calcium solution started soon after completing the addition of phosphate solution, in contrast to synthesis of HAp in aqueous medium in the presence of alendronate. In this way, the undesired formation of amorphous calcium alendronate was excluded to some degree and the crystal structure of HAp related to alendronate loading would be influenced. Based on this modification, Bigi *et al.* investigated the deposition of alendronate containing HAp nanocrystals directly on titanium substrates in order to fabricate desirable coatings combining the bioactivity of HAp with local availability of alendronate [48]. They found that the length of crystalline domain was inversely proportional to alendronate loading content. *In vitro* alendronate controlled release from HAp nanocrystals with a needle shape or plate shape was studied by Palazzo *et al.* [21]. The adsorption and release kinetics of BP drug were found to be dependent on HAp surface area (influencing its solubility [45]), the charge of HAp and loaded alendronate, and their mutual interaction pathway. Ligand exchange between the BP group and two surface phosphate anions promoted strong adsorption [16]. However, some other researchers such as Roussière *et al.* suggested that the two PO_4 groups were not necessarily involved in BP adsorption [49]. Ong *et al.* generated HAp nanoparticles (40 – 200 nm in size) and demonstrated that they could be stably loaded with drugs [50]. Clodronate as a model BP was efficiently loaded onto the particles within 15 min, requiring no further surface modifications of the nanoparticles. McLeod *et al.* showed that pamidronate was adsorbed with higher efficiency when it was applied as a co-precipitated solution of the BP and a simulated body fluid-grown (SBF) as compared with the application of an aqueous solution of BP alone [51]. This approach also led to the formation of a secondary pamidronate-loaded HAp layer on the underlying SBF-HAp layer on the titanium surface. Surface analysis and experiments involving osteoclasts and

osteoblasts showed improved BP adsorption and bioactivity. These results demonstrate that the newly designed co-precipitation approach could be suitable for improving implant localization.

Calcium phosphate bone cements (CPCs), one kind of CaP, possess the properties of biocompatibility and bioactivity, and can easily match the shape of bone cavities and defects. CPCs have been increasingly investigated for medical and dental surgery applications. The cement paste can be hardened by mixing CaP powder with an aqueous solution through a low-temperature dissolution/precipitation reaction [52,53]. As there is no exothermic reaction involved, this process avoids inflammation at the site of administration. These properties make CPCs a desirable alternative for the delivery of drugs such as BPs [54,55]. Panzavolta *et al.* successfully added alendronate and pamidronate to a CPC, which included alpha-tricalcium phosphate (α-TCP), nanocrystalline hydroxyapatite and calcium hydrogen phosphate dihydrate ($CaHPO_4{\cdot}2H_2O$) (90:5:5 in weight percentage, respectively) [56]. The incorporation of BP did not affect the rate of α-TCP transforming into CDA or the microstructure of the cement. Although its compressive strength was reduced, the cement still showed acceptable mechanical properties. $CaHPO_4{\cdot}2H_2O$, or brushite, has excellent biocompatibility and is used in making resorbable biomedical cements. Giocondi *et al.* used scanning probe microscopy to analyze the brushite atomic step motion [57]. Surprisingly, their work demonstrated that BP addition influenced the crystallization kinetic of CPC, which was relevant to BP release profile in return. More recently, Schnitzler *et al.* explored interactions between BPs and the CPC setting process and discovered the role of BP as a retarding agent for CPC setting [55]. The effect was found to be minimized when BP was introduced by means of chemisorption and BP release could be obtained constantly and not abruptly as predicted.

2.1.3 *Other inorganic delivery systems*

Besides the successful fabrication of mesoporous silica and CaP delivery systems for BP, Epstein *et al.* adopted alendronate as a model BP and formulated alendronate-gallium (Ga) and alendronate-gadolinium (Gd) nanosuspensions. Featuring high biocompatibility and biodegradability, these nanosuspensions had high yield with high incorporation efficacy and a charged surface [58]. No additives or excipients were used in this simple formulation. The grouping of alendronate with these two elements in nanosuspensions reduced the early onset of macrophages and at the same time also reduced cytokine production. It was suggested that alendronate-Ga and alendronate-Gd nanosuspensions could be used in disease processes involving monocytes/macrophages and inflammation.

2.2 Polymeric and polymer-inorganic material composite systems

Like inorganic systems, BP delivery systems that make use of polymers should also be biocompatible, biodegradable, have high BP incorporation efficiency and be of suitable size so that they can be inserted into and match bone defects as precisely as possible. Poly(lactic-*co*-glycolic) acid (PLGA) has been commonly used as a drug delivery vehicle in the previous two decades. PLGA has a faster degradation rate compared with PLA, and it can achieve release of the entire amount of encapsulated drug in 2 – 4 weeks. Hence, PLGA is often suitable for formulations of previously daily administered drugs, and trials have been conducted to enhance BP loading into PLGA.

2.2.1 *Polymeric nano/microspheres encapsulating bisphosphonate*

Fabrication of PLGA particles in nanometer scale has always been of high interest to improve drug loading. Cohen-Sela *et al.* described a new nanoparticle (NP) preparation technique – double-emulsion solvent diffusion (DES-D) – in comparison with the double-emulsion solvent evaporation (DES-E) technique [59]. They used alendronate as a model BP and found that its entrapment was better when the DES-D technique was adopted, possibly owing to the creation of an extra temporary phase. Besides, the formulated NPs with BPs showed inhibition of monocyte/macrophage activity as well as the inhibition of restenosis in rat models.

PLGA microspheres (MSs) have also attracted increasing attention for BP delivery; however, the hydrophilicity of BPs acts as a barrier in their formulation for achieving high loading efficiency. Many studies have been aimed at increasing BP loading into MSs. For example, NaCl was included in the water/oil/water (W/O/W) multiple emulsion, acting as a physical barrier by creating high osmotic pressure during the second emulsification step and thus permitting 49 – 75% clodronate loading in PLGA MS [60]. By adopting various emulsification approaches, the fabricated PLGA MS could show high pamidronate encapsulation efficiency ranging from 61 to 87% [61]. However, alendronate sodium, unlike the above two BPs, could hardly be fabricated in the same system to reach comparable loading efficiencies. Preparation of alendronate sodium, chitosan and PLGA MSs resulted in poor loading efficiencies, 7.7% at highest, in a recent study [61]. This created a need to develop an improved delivery system in the form of MSs with well-rounded BP pharmaceutical activity. Nafea *et al.* [23] studied alendronate-containing PLGA MSs and successfully attained high encapsulation efficiency. Unlike previous emulsion techniques, the W/O1/O2 double-emulsion technique was found to develop MSs with desirable properties as a carrier system for the hydrophilic BPs. The MSs prepared by this technique showed maximized BP incorporation efficiency. Bisphosphonate controlled delivery was shown to be sustained for up to 13 days. By regulating fabrication and the relevant parameters involved, BP release could be finely controlled.

Besides PLGA MSs, the preparation of other microparticles (MPs) to load efficiently water-soluble BPs has also been

investigated. Weidenauer *et al*. investigated a terpolymer with star branches, poly(DL-lactide-*co*-glycolide-D-glucose) (PLGGLU), to microencapsulate BP [62]. W/O/W and solid/oil/water (S/O/W) microencapsulation techniques were studied, and another method excluding aqueous phase that suspended BP in organic solvent (SOO) was applied. It was quite hard to develop MPs with high drug loading efficiency using the aqueous microencapsulation techniques. A modified SOO technique increased the MP loading efficiency to 28% at highest. Bisphosphonate prepared in micronized form together with an organic solvent system containing the same amounts of acetonitrile (ACN) and dichloromethane (DCM) were the key components involved in the SOO-based microencapsulation. In addition, Weidenauer *et al*. investigated pamidronate-containing implants prepared from spray-dried MPs using a laboratory ram extruder [63]. A pamidronate-chitosan implant was embedded in PLGGLU, as compared with the micronized pamidronate particles they milled before [62]. The pamidronate-chitosan matrix system showed a prolonged delivery of BPs from hydrophobic polymer MPs with high drug loading (31.92%).

2.2.2 *Polymer-inorganic material composite systems containing bisphosphonate*

In terms of the BP delivery advantages of PLGA mentioned above and inorganic materials described earlier, Shi *et al*. devised PLGA/mesoporous silica-HAp (hybridized with alendronate) composite MSs to form PLGA composite MSs (PLGA/MSH-AL) [64]. This system was built with triphasic structures. After hybridization with alendronate, the HAp-AL composites were then self-assembled into the mesoporous silicas *in situ*. Finally, the HAp-AL-laden mesoporous hybrids (MSH-AL) were incorporated into the bulk of PLGA MSs. Compared with other single-component constructs, this multicomponent system showed higher encapsulation efficiency of the model BP–alendronate, with more control over the alendronate release profile. Besides, Shi *et al*. also fabricated new PLGA-hybridizing-HAp MSs loaded with alendronate, which were developed by an S/O/W or W/O/W approach [24]. The single-emulsion MSs showed better BP encapsulation efficiency (~ 90%) than double-emulsion MSs. All groups of MSs showed sustainable controlled release profile of BPs. Similarly, Billon-Chabaud *et al*. developed a controlled release device to deliver BPs locally and sustainably for a long duration [65]. First, they investigated the microencapsulation of methylene bisphosphonic acid as a model BP into PLGA 50/50 MSs. The optimized process of double-emulsion technique was used [66]. Next, they prepared implants binding BPs with CDA by mixing or compression. Hydroxypropylmethylcellulose (HPMC) was also added into the formulation to evaluate its binding capacity. The latter association of CDA and BPs resulted in a longer period of BP release, owing to the addition of HPMC. Therefore, encapsulation in PLGA appears to be a good way to allow MS fabrication with high yields and lower drug release rates.

2.2.3 *Other polymeric composite systems*

Other than CPCs, which are inorganic bone cements, mentioned above, BP encapsulation in organic bone cements has also been carried out [67-69]. The combination of etidronate in poly(methyl methacrylate) (PMMA) resulted in inhibition of bone resorption [70]. Rodríguez-Lorenzo *et al*. designed acrylic systems with injectability and self-curing ability that contained amino-BPs for the treatment of osteoporosis and related diseases [71]. They incorporated alendronate in these systems based on two kinds of methacrylate. The synthesized BP release was inferior to that of alendronic acid monosodium trihydrated salt, which is, however, compensated by the higher potency of the synthesized BPs. Studies showed improved cell behavior with the synthesized BP-incorporated systems, proving them to be more biocompatible. Zenios *et al*. studied the mixture of a commercial bone cement named Palacos R and pamidronate as a model BP for drug delivery [72]. The BP and the cement were both mixed in liquid form. The resulting acrylic bone cement showed reduced mechanical performance. Regarding conflicting investigations on the influence of BP encapsulation on the mechanical properties of such cements [67,69,72], Yu *et al*. [73] tested the influence of zoledronic acid and pamidronate loading on commonly available organic bone cement *in vitro* and *in vivo*. Different concentrations and diluent volumes were chosen, and they found that reduction in mechanical properties was related to dilution of liquid monomer but not to BP *per se*.

2.3 Other biomolecular and biopolymeric systems

Varghese *et al*. synthesized a new high-molecular-mass hyaluronan-BP (HA-BP) conjugate having free hydrazide functionality that can be used as an antiosteoclastic and antineoplastic drug in injectable hydrogel formulations [74]. The potency of the prodrug was triggered by a ubiquitous enzyme, Hase, which cleaved HA-BP to suitable sizes so as to be internalized by CD44-positive cells by receptor-mediated endocytosis. Being a hydrogel, it would avoid systemic drug exposure and allow its controlled release at the site of implantation. The hydrazide group of the HA-BP conjugate, primarily used for crosslinking, could also be used to explore hydrazone linking of other drug molecules such as doxorubicin, and so on, which could be integrated into the hydrogel matrix.

The delivery of osteoprotegerin (OPG), a model therapeutic protein, together with a BP to bone sites in an osteoarthritic animal model was investigated by Doschak *et al*. [75]. OPG was conjugated to a thiol-BP by means of a disulfide linkage. The OPG-thiol-BP conjugates displayed higher HAp affinity *in vitro* compared with unmodified OPG. This conjugate had high retention rates after 72 h. After 24 h of administration to osteoarthritic rats, there was > 4 times more presentation of OPG-thiol-BP conjugate at the bone site than that of control OPG. These results suggest that conjugation of cytokines with a thiol-BP for treatment of osteoporosis and similar diseases of bone is more efficacious than administering the cytokines alone.

An alendronate-β-cyclodextrin conjugate (ALN-β-CD) was developed as a new system for BP controlled release. 'Click' chemistry was adopted for this conjugation, as reported by Liu and co-workers [76]. The delivery system showed very high affinity to HAp and could gradually release dextran from ALN-β-CD/dextran complex bound to HA. The BP delivery system has promising potential in the treatment of oral disorders.

3. Applications

Systemic application of BPs results in gastrointestinal complications and has a poor gastrointestinal adsorption [15]. These limitations led to the investigation and development of local BP delivery systems for orthopedic applications. Local BP delivery makes it possible to administer a higher dose of BP to the target region in the affected bones [77]. Radiolabeled alendronate when applied locally was seen to have high *in situ* presentation and low absorption into surrounding tissues [78]. For example, Tengvall *et al.* [79] showed that locally immobilized BPs accelerate screw fixation in bone surgeries, by means of their effect on inhibiting bone resorption.

3.1 Orthopedic regeneration

Bisphosphonate delivery systems have been observed to treat ischemic osteonecrosis of the femoral head (IOFH) effectively as they prevent bone resorption by osteoclasts. As IOFH is a localized condition, local intraosseous administration of BP would be advantageous and desired. Aya-Ay *et al.* investigated the effects of intraosseous administration of ibandronate for IOFH treatment [80]. This was a pioneering study in local administration of ibandronate to prevent the deterioration of femoral head in IFOH. Nitrogen-containing BPs have been adopted in clinic to inhibit bone resorption by osteoclasts [6]. Greiner *et al.* investigated the effect of zoledronate (containing nitrogen, seen in **Figure 2**) on bone resorption by osteoclast-like cells (OLCs) *in vitro* [81]. ZOL was loaded in a poly(DL-lactide) (PDLLA) coating at different concentrations. The results showed that the activity of OLCs was significantly reduced with higher ZOL dosage, comparable to when ZOL was used alone at similar dosages. These effects on osteoclasts might be clinically beneficial in preventing the loosening of the orthopedic implant and to promote healing of the fracture.

3.2 Bone and dental reconstruction

It is difficult to obtain sufficient fixation of an orthopedic implant in weak osteoporotic bones. Poor fixation leads to micro movements at the bone–implant interface activating osteoclasts, which further leads to loosening of the implant. Recent data [42,45,64,69,72,74,75,82] suggest local BP delivery at the implant surface could be a promising solution. Local BP delivery improves implant fixation, as demonstrated in a study on rats [83]. The administration of alendronate around HAp-coated implants improved formation of bone around the implant and also improved implant fixation [84]. Using drug delivery systems, BPs can be applied to avascular regions of necrotic bone, which cannot be achieved via application of BPs systematically [77]. Local delivery of zoledronate improved bone density around implants in sheep, as demonstrated in a study by Stadelmann *et al.* [85]. Zoledronate application resulted in 50% higher bone formation at the implant surface. These results were similar to those seen in osteoporotic rat models. Hence, it can be said that local zoledronate treatment improves implant fixation in different species. Recently, Verron *et al.* reported a BP-loaded CDA for bone augmentation in osteopenic rats and sheep [86]. Zoledronate-loaded CDA was used *in vivo* as a CaP matrix to improve bone micro-architecture and increase relative bone content. New bone was found to form, and existing trabeculae were strengthened in the osteoporotic site.

Bisphosphonates have also been used at dental sites, delivered in gelatin sponges [87,88] or as surgical pellets [61,89]. Bone resorption after dental surgical procedures was significantly reduced with local application of alendronate [90], which also enhanced tooth repair after dental replantation in a dog model [91]. As oral BP administration may lead to jaw necrosis, careful investigations need to be done regarding pathological effects of local BP controlled release systems applied at dental/oral sites.

3.3 Anticancer

Bisphosphonates are potent antiangiogenesis drugs [92-94]; however, their adverse side effects when administrated orally, such as gastrointestinal disturbances, osteonecrosis of the jaw and ocular inflammation, also necessitate local delivery of BPs [95]. Moreover, the retention of BPs in bones and their slow release over the years may hamper skeletal maturation in children. Promisingly, efforts have been made to design new local BP delivery systems for antiangiogenesis [96]. Biocompatible and absorbable CaP ceramics, widely used in clinics for bone reconstruction [97], have been developed as BP drug delivery vehicles for cancer treatment in the clinic [42]. Zoledronate is loaded onto CaP drug carrier when the two are reacted in an aqueous solution. By adjusting the properties of these carriers, the timing and rate of drug release can be controlled accurately [19]. Local delivery of BPs with CaP particles has been proposed as a potential alternative for treating hemangiomas. Slow and sustained release of desired amounts of BPs for antiangiogenesis can be obtained by controlling the structure of CaP, leading to effective treatment of hemangiomas [98]. Furthermore, Cenni and co-workers fabricated NPs made of a conjugate of PLGA and alendronate using an emulsion/solvent evaporation technique [82,99]. Alendronate was found to be homogeneously distributed in the fabricated NP networks, which was beneficial for NP bone-seeking ability. Interestingly, a higher dilution of these NPs brought about increased prothrombin activity and an anticancer drug could be loaded inside them.

4. Conclusion

As various drug carriers/conjugates have been shown to have a benign admission of BPs, the synergetic combination of the therapeutic properties of BPs and controlled release functions of drug delivery systems have had a huge impact on engineered regenerative medicine treatments for orthopedic therapy. By exploiting this knowledge, many delivery platforms have been set up to load BPs for the suppression of osteoclasts and enhancement of osteoblasts at the target site. In this review, the various BP-incorporated systems that have been designed to attain a local and controlled release of this bone antiresorptive agent so as to influence bone cells in implants and substitute tissues *in situ* have been discussed. A variety of structural modifications have also been made to manipulate and control the loading efficiency of BPs or their conjugates, yielding higher carrying capacity and consequential higher release amount and prolonged release duration for bone tissue regeneration. In conclusion, given the inadequate understanding of the interactions between released BP and various bone minerals, challenges remain in the development of practical designs for BP delivery-related *in vivo* therapeutic platforms. Nonetheless, the pilot works discussed in this review have laid the foundation for future BP delivery-related engineered regenerative medicine; therefore, it may not be long before this drug-bone substitute is widely used in the clinical setting.

5. Expert opinion

Bisphosphonates have emerged as important inhibitors of bone resorption for orthopedic therapy and their application in bone medicine has widened drastically over the years. However, owing to their poor intestinal absorption and highly selective bone location and retention, high doses of BPs have been orally administered, which results in jaw osteonecrosis, abdominal discomfort and even hypocalcemia [16,100]. This has led researchers to focus on the utilization of drug delivery systems to load BPs and then unload them locally.

The bottleneck for current BP delivery system design lies in the comparatively low loading efficiency of the drug. Drug loading density relies on scaffolds, microcarriers, or other transplantable materials, the role of which has evolved from a mere structure to provide mechanical support and encapsulation for drugs to a dual-functional accommodating 'niche' capable of releasing drugs such as small growth factor molecules, big protein molecules and other therapeutic agents with a wide range of molecular masses, and so on, in a controlled manner to improve the microenvironment for drug residence. By doing so, the required drug amount for orthopedic therapy can be optimized. By improving drug loading efficiency, the higher local dose of loaded BPs can better repress osteoclasts and other unwelcome cells such as blood monocytes and macrophage-like cells without affecting non-hostile cells such as osteoblasts.

Mere incorporation of BP in solution form into bone substitutes, however, increases the complexity during fabrication, and its short lifespan and susceptibility to surrounding enzymatic degradation lower its efficacy. For this reason, inorganic/organic BP delivery systems on the basis of simple interactions between BP and the material solutions may not be a suitable choice for attaining long-term sustained release. A possible key is to surface modify these substitutes or tie them into inorganic and organic materials; whereby chemically modified drug vehicles secrete BPs locally and continuously, maintaining a stable BP gradient for suppressing osteoclastic activity in the long run.

Current BP delivery designs and platforms still hold much room for improvement. However, regardless of the different techniques of BP incorporation, they prove to be effective in local and controlled release of the therapeutic drug. The pursuit of BP controlled release and delivery-related regenerative medicine has already begun. It is thus conceivable that its use in clinical settings will be a reality in the near future.

Declaration of interest

The authors declare no conflict of interest and have received no payment in preparation of this manuscript.

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