Review article

Drug delivery systems functionalized with bone mineral seeking agents for bone targeted therapeutics

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ABSTRACT

The systemic administration of drugs to treat bone diseases is often associated with poor uptake of the drug in the targeted tissue, potential systemic toxicity and suboptimal efficacy. In order to overcome these limitations, many micro- and nano-sized drug carriers have been developed for the treatment of bone pathologies that exhibit specific affinity for bone. Drug carriers can be functionalized with bone mineral seekers (BMS), creating a targeted drug delivery system (DDS) which is able to bind to bone and release therapeutics directly at the site of interest. This class of advanced DDS is of tremendous interest due to their strong affinity to bone, with great expectation to treat life-threatening bone disorders such as osteomyelitis, osteosarcoma or even osteoporosis. In this review, we first explain the mechanisms behind the affinity of several well-known BMS to bone, and then we present several effective approaches allowing the incorporation BMS into advanced DDS. Finally, we report the therapeutic applications of BMS based DDS under development or already established. Understanding the mechanisms behind the biological activity of recently developed BMS and their integration into advanced therapeutic delivery systems are essential prerequisites for further development of bone-targeting therapies with optimal efficacy.

1. Introduction

For any drug to achieve its optimal therapeutic effect, it is important that the compound reaches, and is retained, at the intended site of action (tissue, receptor or molecules) without losing its chemical integrity or biological function. The most frequently applied method to deliver drugs has traditionally been systemic administration. However, this is associated with certain drawbacks, most important being the limited penetration of drugs to their sites of action and the associated systemic side effects of the resulting high dosages. Drug delivery systems (DDS) have emerged to improve drug concentrations in tissues while preventing structural changes of the incorporated drugs. In addition, DDS offer the possibilities to increase the range of applications of hydrophobic compounds (by enhancing their solubility [1]), prolong efficacy of drugs with short biological half-life (by sustained drug release mechanisms [2]), and limit non-specific cellular uptake of drugs (by reducing opsonization by macrophages) [3]. However, conventional DDS that increase biological circulation time are not necessarily designed to actively reach, penetrate and concentrate at the intended site of action. Targeting strategies that can be used by DDS include not

only exploitation of the passive enhanced permeability and retention (EPR) effect, but also active binding to specific tissues when combined with biologically affine moieties [4].

Systemic DDS are usually nanoscale constructs that can be injected intravenously, administered orally or even can be introduced *in vivo* by pulmonary inhalation. Their small size allows them to reach even the smallest capillaries and the limited clearance of such nanoscale constructs from the blood by macrophages gives them stealth-like properties, resulting in longer circulation times.

In the orthopedic field, bone related diseases such as osteoporosis, osteosarcoma and osteomyelitis are regularly treated *via* conventional systemic drug administrations. Nevertheless, inefficient uptake of drugs by bone can limit the utility of these drugs or severely compromise treatment outcome.

For example, bone infections are routinely treated with systemically administered antibiotic agents, for extended periods of time. However, penetration of antibiotics into the affected bone compartment has been reported to be inefficient, with low local drug concentration at the site of infection [5], which can further increase the risk of the development of drug-resistant infections [6]. Additionally, the prolonged antibiotic

regimens required to successfully treat these infections raise healthcare costs and can lead to toxic hepatic side effects and nephrotoxicity [6]. In consequence, systemic antibiotherapies are regularly combined with DDS applied locally in infected bones or bone fractures. The most commonly used local DDS used in these circumstances are antibiotic-loaded bead cements of poly(methyl methacrylate) (PMMA) [7]. Limitations of PMMA implants include a lack of biodegradable properties, the need for invasive implantation and retrieval surgeries and an incomplete release of the loaded antibiotic [8].

In order to establish a high and sustained local concentration of a drug in the proximity of bone, it is desired to have a DDS which can interact intimately with bone tissue on a physical and chemical level. There is a wide array of molecules available that have affinity to bone tissue, called bone mineral seekers (BMS). These compounds can be implemented in a DDS design and would result in DDS exhibiting preferential affinity at bony sites where they can locally release their drug load. This would then lead to high drug concentrations at the therapeutic target site and to a better efficacy of the treatment.

The aim of this review is to provide an overview regarding the features of recent DDS that actively target bone tissue *via* the utilization of bone seekers. Then, in a second part, we report the different fabrication strategies and the bone targeting efficiency of BMS-based DDS. The review concludes with an overview of some of the most promising pre-clinical and clinically applied DDS making successful use of BMS.

2. Composition of bone: possible biological targets for bone-seeking agents

The organic matrix of bone represents roughly 30% of total dry bone mass (see Table 1). This organic matrix includes 90 wt% collagen fibrils in dry weight. The remaining components consist of glycoproteins, proteoglycans and other proteins [9]. The inorganic matrix (65–70% of dry bone mass) consists of calcium-deficient hydroxyapatite (dHAP) nanocrystals which are embedded in the organic matrix [9,10]. Bone cells represent only 1–2% of the total dry bone mass and mostly consist of osteocytes present in the bone matrix. Osteoblast and osteoclast cells regulate bone homeostasis by promoting the synthesis of bone matrix or resorbing bone matrix respectively. Harversian and Volkmann's channels provide space for blood vessels to transport nutrients and oxygen to the organic bone components. These channels of approximately 70 μm of diameter provide accessibility to bone tissue for therapeutic agents [9].

Being the major organic macromolecule present in the bone, collagen could make an attractive target for bone seeker modified DDS. Fibronectin, entactin as well as some glycoproteins have been reported to bind with high affinity to collagen [11]. However, collagen is also the body's most abundant protein [12], highly present in cartilage and connective tissues, meaning that a DDS with specific affinity to collagen will be extremely non-specific to bone tissue. Organic matrix proteins (*i.e.* osteocalcin, osteonectin and osteopontin) could be targeted by a broad range of antibodies [13]. Even though such antibodies would display a high specificity, the relatively low amount of these bone-associated proteins (< 1 wt% of total dry bone mass) could compromise

the effectiveness of such binding strategy. Osteocytes could potentially represent a very specific target for bone tissue. Some osteocyte markers like dentin matrix protein 1 (DMP1), sclerostin and matrix extracellular phosphoglycoprotein (MEPE) are reported in literature and could be used in targeting these cells [14]. Nevertheless, bone cells do not represent attractive targets for DDS as they are embedded in dense inorganic matrix, making them poorly accessible for DDS constructs. The inorganic matrix of bone, consisting nearly entirely of dHAP, is the major component of bone tissue and it offers an excellent target for BMS functionalized DDS due to its exclusive location in bones and developing teeth.

3. Bone affinity of bone mineral seeking agents

There are many different types of BMS, ranging from small molecules (< 1000 Da) to large macromolecular proteins. When considering the use of such molecules or macromolecules for the functionalization of a DDS, several factors are essential for a successful targeted DDS [15,16]. First, the BMS units should have great affinity toward bone mineral and its incorporation in a DDS should not impair its ability to interact with dHAP. Secondly, the BMS-DDS construct should neither trigger any toxic or adverse side effects, nor interfere with healthy bone homeostasis upon administration. Finally, the DDS must not hinder the therapeutic capacities of the delivered drug. Depicted in Table 2 are compounds belonging to the different classes of BMS that are discussed throughout this review, with a summary of their main advantages and disadvantages.

Comparative studies of the mineral affinities of different classes of BMS are rare, and bone affinity is often reported in relation to control groups (usually non-targeting DDS analogues), which hinders any absolute assessment in terms of bone affinity of different classes of BMS. Among the limited literature available, the reports by Ross et al. included comparative studies for bisphosphonate-, ʟ-glutamic acid- and 2-amino-ethylphosphonic acid-functionalized gold nanoparticles (Au-NP), and their interaction to dHAP crystals [17] and bone [18]. Among those Au-NP delivery systems, bisphosphonates showed the highest affinity to dHAP and bone.

3.1. Bisphosphonates

Bisphosphonate (BP) molecules have been studied extensively since the 1960's [19] and a multitude of BP-based products are commercially available. BPs contain two phosphonate groups ($PO(O^-)_2$) sharing a common carbon atom, also known as a P–C–P backbone (a generic BP is depicted in Table 2, compound A). BPs are the analogues of naturally occurring pyrophosphate, which is an anhydride and a regulator of bone mineralization, characterized by its P–O–P bond. The nature of this bond makes pyrophosphates prone to fast enzymatic hydrolysis as part of the normal bone physiology and are therefore not suitable as a therapeutic agent or BMS [10]. The P–C–P backbone of BPs is far more stable, while preserving its affinity to bone mineral. Thus, BPs exhibit a prolonged residence in the bone tissue, up to many years [20]. These properties (along with their action on osteoclast inhibition) are the

Table 1
Overview of bone components, their approximate dry weight percentage in healthy bone and examples of potential targeting moieties for DDS.
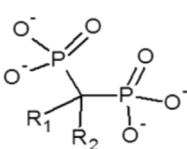
Bone component		Presence in bone (dry weight)	Targeting moieties
Organic matrix	Collagen (type I)	27–32%	Fibronectin, Entactin
	Non-collagenous proteins (*e.g.* Osteocalcin, bone morphogenetic proteins (BMPs) and fibronectin)	< 1%	Multitude of commercially available protein specific antibodies and their fluorophore conjugates
Cellular content	Osteocytes, osteoblasts and osteoclasts	1–2%	DMP1, sclerostin, MEPE
Inorganic matrix	Ca-deficient hydroxyapatite	65–70%	Bisphosphonates, Tetracyclines, Poly (aspartic acid), Poly (glutamic acid)

DMP1: Dentin matrix acidic phosphoprotein 1, MEPE: Matrix extracellular phosphoglycoprotein.
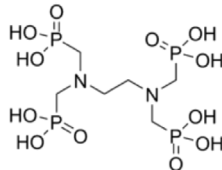
Table 2

Overview of the main classes of BMS and their advantages and disadvantages for utilization in bone seeking DDS. General structure of Bisphosphonates (A), the multi-phosphonate containing molecule EDTMP (B), Tetracycline (C) and the bone seeking peptide poly (α-D-aspartic acid) (D).
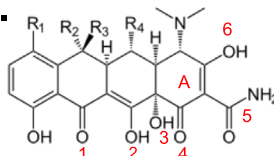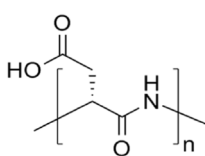

A. *Generic BP structure*

B. *EDTMP*

C. *Tetracycline*

D. *poly (α-D-aspartic acid)*

Group	Advantages	Disadvantages
A. Bisphosphonates	• Strong and rapid affinity to bone mineral • Easily conjugated into DDS by using R_1 and R_2 side chains	• Potency to inhibit osteoclasts and bone homeostasis • Very long presence at bone sites
B. Multi-phosphonate-containing molecules	• Level of bone affinity is scalable with incorporation of more or less phosphonate groups • Many multi-phosphonate-containing molecules can facilitate the transport or radio-pharmaceuticals	• Suboptimal distance between oxygen groups involved in chelation to bone
C. Tetracyclines	• High affinity and specificity to developing bone sites • Intrinsic antibiotic and bone targeting properties	• Low affinity to pathologic bone sites with low bone turn-over • Staining developing teeth and impairment of bone development
D. Bone affine peptides	• Biodegradable properties allow the clearance of the DDS within the therapeutic timeframe • Highly tunable bone affinity due to custom peptide synthesis	• Peptide bonds linking individual amino acids are prone to hydrolysis before target site is reached

EDTMP: ethylenediamine tetra (methylene phosphonic acid).

reason for the utilization of BPs as bone antiresorptive drugs in osteoporotic patients. Mechanistically, BPs exhibit affinity toward bone by chelating with divalent calcium ions (Ca^{2+}) present in dHAP. The deprotonated hydroxyl ($P-O^-$) of the two phosphonates in BPs are separated approximately by 2.9 to 3.1 Å. This is similar to the distance in Ca^{2+}-chelating oxygen atoms naturally present in dHAP crystals, leading to competitive affinity to Ca^{2+} ions [21]. In BPs, the distance between the two deprotonated hydroxyl groups increases when the P–C–P bond is replaced with P–N–P or P–C–C–P bonds, leading to reduced affinity toward dHAP of such compounds [22]. The two remaining groups on the P–C–P carbon atom, R_1 and R_2, can further modulate affinity to dHAP. For instance, the presence of a hydroxyl or an amine group at R_1 leads to additional interaction with the calcium ions and these BPs indeed show a higher affinity toward dHAP compared to other BPs [23,24]. Changing the R_2 group with moieties containing nitrogen atoms leads to a significant change in osteogenic anti-resorption potency, making those BPs not only suitable as bone seekers for DDS but also potent anti-osteoporotic drugs [24,25]. Nitrogen containing BPs inhibit the synthesis of farnesyl pyrophosphate, which controls osteoclast activity [26]. Reduced osteoclast activity shifts the bone homeostasis toward bone formation as osteoblast bone formation remains unaffected. With the affinity of those BPs toward calcium mineral and farnesyl pyrophosphate synthase being highly specific, they preferentially accumulate in bone tissue. Nancollas et al. conducted analysis of 6 different BPs commonly used in clinics [24], and presented BPs ranking as followed on *in vitro* dHAP affinity: Chlodronate ≪ Etidronate < Risedronate < Ibandronate < Alendronate < Zoledronate (Table 3).

The involvement of phosphonate groups in the dHAP binding mechanism was further evaluated by Puljula et al. [27], who investigated the effect of phospho-esters on the ability of the BP to bind to calcium

sufficient dHAP. The BPs with low affinity to bone (*e.g.* Chlodronate) was not able to bind to bone when one of the four chelating oxygen groups was used to form methoxy esters or phenol esters. The more potent BPs with hydroxyl groups on the R_1 side chain were able to chelate with dHAP after the esterification of two oxygen groups, but in significantly reduced quantity compared to their non-modified analogues [27]. This research emphasizes the fact that the hydroxyl R_1 group is involved in dHAP binding and that the amount of BP esterification is negatively correlated to the ability of the BP to bind to dHAP.

Importantly for the bone seeking DSS, the hydroxyl- and amine groups positioned at R_1 and R_2 can be used for chemical conjugation with a drug (to create a prodrug conjugate) [28] or to the surface of a particulate polymer carrier [29] without altering affinity to bone. Interestingly, it is not reported to our knowledge, if using the nitrogen R_2 group for conjugation could decrease the binding affinity of BPs to farnesyl pyrophosphate synthetase and have an impact on its ability to reduce bone mineral resorption. It could be hypothesized that Alendronate tethered at the R_2 position to DDS should exhibit a strong affinity to mineral but with a reduced osteoclast inhibition (so reduced potential side effects), but this has still to be demonstrated.

Nevertheless, it must be emphasized that bisphosphonates are able to display some side effects. A study by Brown et al. listed several potential complications that could be associated with long term (> 5 years) bisphosphonate administration [30]. Bisphosphonate related osteonecrosis of the jaw (BRONJ), atypical sub-trochanteric fractures in the femur and esophageal cancer are some of the reported secondary effects. However, most of these complications are reported in small studies or clinical cases and it remains difficult to establish causative evidence. It is recognized that BP treatment becomes a significant risk factor for the development of BRONJ after invasive dental procedures, like teeth extractions, with incidence up to 27.5% reported after

Table 3
Overview of common BPs with their constitutive side chains. BPs are ranked by potency toward osteoclast inhibition relative to etidronate (due to the presence of nitrogen in the R^2 chain), determined by dHAP crystal growth rates analysis [26]. The kinetic affinity constant (K_L) is an indication of the measure of affinity between dHAP and the different BPs [24].

	Compound	Osteoclast inhibition potency [26]	dHAP/BP K_L ($\times 10^6$) (L·mol^{-1}) [24]	R_1	R_2
Non-nitrogen containing BPs	Etidronate	$1 \times$	1.19	$-OH$	$-CH_3$
	Chlodronate	$10 \times$	0.72	$-Cl$	$-Cl$
Nitrogen containing BPs	Alendronate	$500 \times$	2.94	$-OH$	$-(CH_2)_3-NH_2$
	Ibandronate	$1000 \times$	2.36	$-OH$	$-(CH_2)_2-N(CH_3)-(CH_2)_4-CH_3$
	Risendronate	$2000 \times$	2.19	$-OH$	$-CH_2-(NC_5H_4)$ (ring)
	Zoledronate	$10,000 \times$	3.47	$-OH$	$-CH_2-(N_2C_3H_3)$ (ring)

1 to 4 years of Zoledronate treatment [31]. It is worth pointing out that these risk assessment studies of BPs [30,31] have been carried out to evaluate the side effects of systemic administration of BPs as a standalone therapy over a prolonged duration of administration. When BPs are incorporated in local DDS, the BP associated side effects like BRONJ might be reduced due to the negligible systemic diffusion of the BP and the relatively short duration of the therapy (perhaps even single administration). For comparison, typical dosages of BP therapy for osteoporosis treatment are in the range of 5 to 70 mg per week [32] while a typical BP functionalized DDS would only expose the patient to ± 1 mg of Alendronate for 400 mg of DDS construct [33].

3.2. Other phosphonate-containing molecules

BPs are not the only type of molecules with phosphonate groups that exhibit affinity to dHAP. Ethylenediamine tetra(methylene phosphonic acid) (EDTMP, Table 2 compound B) and tetraazacyclotetradecane-1,4,8,11-tetramethylene phosphonic acid (DOTMP), both with 4 phosphonate groups, are known to chelate to Ca^{2+} ions and have primarily been used to transport radiopharmaceuticals [34] and also proteins to bone [35]. In contrast to BPs, no physiological effects of such phosphonate containing molecules on bone homeostasis have been reported.

To increase the amount of phosphonate groups available to chelate Ca^{2+} ions, multiple BPs can be associated together to form dendritic structure, using the R_2 group of the BP and a spacer (*e.g.* 3,5-di(ethyl-lamino-2,2-bisphosphono)benzoic acid) to create prodrug branched structures [35]. Bansal et al. prepared compounds with incorporated bisphosphonate groups and covalently attached bovine serum albumin or nonspecific bovine immunoglobulin-G as model drugs (Fig. 1A). Mineral affinity was significantly enhanced (compared to non-modified proteins, Fig. 1B), and was proportional to the number of BP moieties (Fig. 1C) [35].

Compared to BP compounds, (multi)-phosphonate-containing molecules are often designed as a targeting group for their conjugated drug load and not as standalone therapeutics, but some of them have been extensively employed for the transportation of radionuclides, which will be discussed in later sections of this review.

3.3. Tetracycline

Tetracycline (TC, Table 2 compound C) is an antibiotic produced by the actinobacterial genus *Streptomyces*, and has been used as a therapeutic agent for decades. In addition to its antimicrobial properties, TC has also affinity to divalent cations such as Ca^{2+} present in dHAP. More specifically, TC accumulates on bone tissues where biological turnover is high, providing a tool to analyze bone propagation fronts as it also emits fluorescence under excitation at 390 nm [36]. The β-diketone system at position 1 and 2, the enol system at position 4 and 6 and the carboxamide group at position 5 are responsible for the chelating behavior of TC (Table 2, compound C) [37].

Research has focused on remodeling the tricarbonylmethane grouping in the A ring of TC [38], which is partly responsible for the molecule's affinity toward dHAP. The resulting 3-amino-2,6-dihydrox-ybenzamide ring structure exhibits a binding affinity increased of up to

50% for dHAP compared to native TC [38]. Besides chelation between TC and dHAP, other interactions might contribute to their association. Van der Waals attractions and hydrogen bonding between the hydroxyl group of dHAP and TC molecules are likely to cause additional surface complexation [39].

As mentioned previously, TC staining is commonly used as a method to image and to quantify new bone formation, as it stains the surface of propagating bone formation front and has fluorescent properties [40,41]. For TC-functionalized DDS, this could result in reduced affinity to pathologic bone sites characterized by low bone turn-over. These factors could make TC a suboptimal candidate as BMS for DDS directed to bone-related diseases like osteomyelitis [42]. In addition, the chelation of TC is permanent, which can result in unwanted side effects such as staining of the teeth. Hence, TC is rarely used anymore for antibacterial purposes and prescribed with care to children still undergoing dental development [43].

3.4. Bone-targeting peptides

Oligopeptides of Aspartic acid (Asp) or Glutamic acid (Glu) have affinity toward dHAP [44], even though the exact mechanism behind is currently under debate [45]. It is known that a peptides affinity to dHAP increases when repeating units of Asp or Glu are present in the amino acid sequence, as it is naturally the case in osteopontin and osteocalcin bone-proteins [46]. The utilization of acidic oligopeptides of Asp or Glu as bone seeking agents is an attractive option due to the fact that they have no apparent adverse effects and a shorter half-life *in vivo* compared to BPs [47].

Ishizakia et al. reported on the application of these acidic oligopeptides to transport various drugs: *i.e.* estradiol, quinolone antibiotics and tissue-non-specific alkaline phosphatase (ALP) [44]. These compounds were conjugated by means of succinate esterification (estradiol and quinolones) or by changing the peptide sequence of ALP at the C-terminus. Interestingly, the authors stated that the measure of affinity between the oligopeptide and dHAP was not influenced by the choice of amino acid (Glu or Asp) or its optical isomer forms (D or L), but that dHAP affinity plateaued at six or more amino acids per oligomer [48]. Due to the non-hydrolysable nature of D-Glu and/or D-Asp rich-oligopeptides, its residence time at bone sites was reported to be longer compared to peptides in L configuration [48]. The structure of Asp can be further classified into α- and β-linkages between the monomers. Nakato et al. have analyzed the difference in chelating properties of polymeric Asp structures including α-L-Asp, α-D-Asp, β-L-Asp and α,β-L-Asp [49]. They discovered that the poly(α-Asp) configuration had the highest chelation properties to Ca^{2+} ions due to the spatial location and configuration of the carboxyl groups on the polymer backbone. It was also confirmed that the chirality of the Asp had no effect on the chelation properties. It can be extrapolated that poly(α-L/D-Asp) must have equal affinity toward dHAP, with desirable degradation properties from the poly(α-D-Asp) configuration (see Table 2, compound D). However, most of the literature does not report in the methodology the nature of the linkage present in the poly(Asp), making a comparison between the studies difficult to conduct.

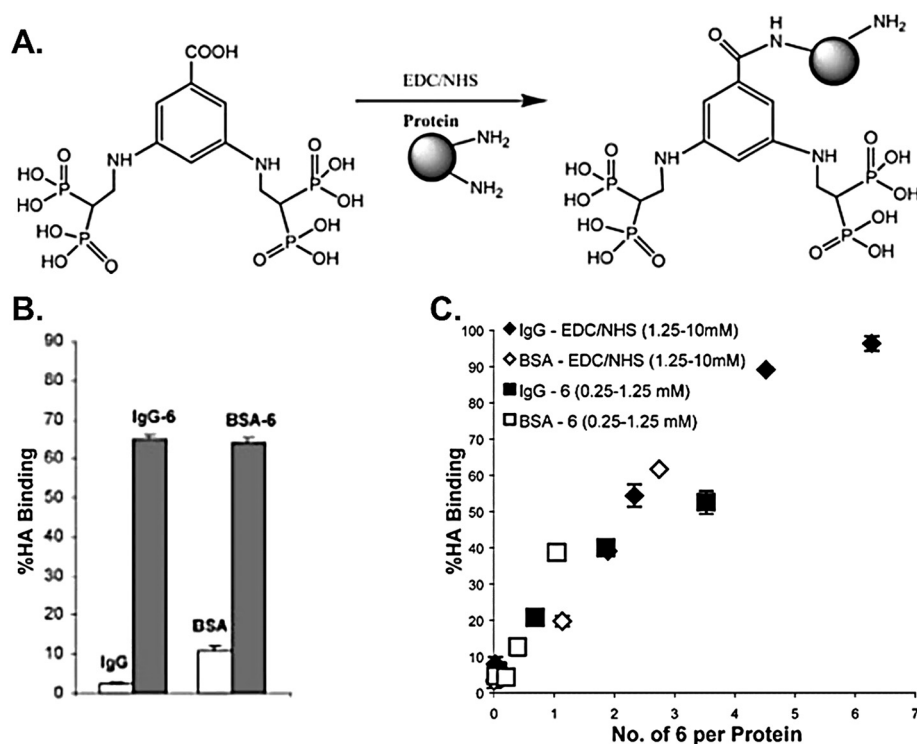Keeping in mind the vast possibilities in peptide combinations,

Fig. 1. DDS based on the assembly of multiple BPs.
After activation of the carboxylic acid by carbodiimide chemistry, the di(bisphosphonate) (named here compound 6) can be covalently bound with the amine groups of proteins (represented by the grey sphere, being either bovine serum albumin or IgG) (A). Those di(bisphosphonate)/protein conjugates resulted in strong dHAP affinity compared to pristine proteins (analyzed in vitro, B), which degree of affinity was proportional to the amount of di(bisphosphonate) units bound to the protein (C). Images are republished with permission of Elsevier [35].

other peptide sequences might have enhanced affinity to bone as well. In 2009, using phage display techniques, three peptides with the sequences VTKHLNQISQSY (VTK), STLPIPHEFSRE and APWHLSSQYSRT were identified as having strong and specific affinity toward dHAP and bone like material [50,51]. Additionally, follow-up studies have shown that biomaterials modified with VTK peptides favored osteogenic differentiation of human mesenchymal stem cells (hMSC) and biomineral deposition [52,53]. However, conflicting reports state that VTK peptides also have the ability to inhibit osteoblast mineralization [54], which could be a potential adverse effect. The adsorption mechanism of VTK peptides to dHAP has not been properly described. Surprisingly, the amino acids that are known to have affinity to dHAP (as mentioned above, i.e. Asp (D) and Glu (E)), are not present in this peptide sequence, and the net charge of the peptide sequence is in fact positive. Addison et al. emphasized the importance of the phosphorylation of the serine amino acids in the VTK peptide sequence on their binding energy required to interact to HAP, as phosphorous groups lower the molecular net charge which is beneficial for interactions with calcium [50]. This was further confirmed by computational modeling, which permitted to identify the amino acids responsible for binding to HAP crystals. This approach revealed as well that phosphorylated serine was almost always involved in dHAP binding, and that the hydroxyl side group of tyrosine also interacted with the crystalline surface. To the best of the authors' knowledge, no publications about VTK peptide conjugates to drug delivery systems or direct comparisons with other BMS have been published to date.

4. Drug delivery systems using bone-seeking agents for targeting therapeutics

4.1. Prodrugs with bone affinity

A prodrug is defined as a chemically modified drug that can be metabolized in the body into an active drug. Bone targeting prodrugs based on BMS have been developed to treat bone infection by grafting with antibiotics [55–57], or to treat osteoporosis by grafting with estrogen compounds like estradiol [38,58]. In 2008, Houghton et al.

modified fluoroquinolones with BP groups, by linking the BP with the piperazine group of the fluoroquinolones [59]. The obtained chimeric bisphosphonated drugs are hydrophilic and highly water soluble due to the acidic nature of the BP moiety at physiological pH [60]. An in vivo investigation using a rat bone infection model revealed that bisphosphonated fluoroquinolones have a higher infection prevention rate compared to the systemically administered parent drug control [59]. One limitation of this conjugate system is that not all the prodrugs could dissociate to form the active antibiotic in clinically relevant quantities after its binding to HAP, due to slow hydrolysis of the antibiotic-DDS ester conjugation. While Houghton et al. utilized the piperazine group to link fluoroquinolones to BPs, Tanaka et al. used the carboxylic acid group of moxifloxacin, gatifloxacin and ciprofloxacin to generate their respective prodrug forms with BPs [56]. The same authors report on the production of bisphosphonated glycopetide antibiotic (i.e. vancomycin and oritavancin), with a potential application for osteomyelitis [57]. In vitro experiments showed a high affinity toward bone for all prodrugs (> 96.5% bone binding), but once more, only a small fraction of the prodrug was able to be converted into the active parent drug (< 3.5% in phosphate-buffered saline (PBS) after 24 h) [57], restricting the possibility to reach high local drug release. In rat serum, conversion to active antibiotic was higher (up to 26.4%) due to enzymatic ester cleavage, which was presented as sufficient the for treatment purpose [57].

Bone seeking peptides linked to estradiol, an effective drug to stop or even to reverse osteoporotic phenomena [61,62], have been the focus of extensive researches. Tokogawa et al. linked estradiol with L-Asp hexapeptide via succinate esterification, resulting in estradiol-17b-succinate-(L-aspartate)$_6$ (E$_2$·17D$_6$), for an intranasal administration application [62]. In addition, to enhance nasal uptake, conjugation of E$_2$·17D$_6$ to absorption enhancers (e.g. β-cyclodextrin and hydroxypropyl cellulose) was performed. The results showed that 6 hour post-administration, the amount of estradiol increased in the bone due to the developed E$_2$·17D$_6$ formulation, and that intranasal was a viable and attractive method of administration.

The fabrication of tetracycline-estradiol conjugates was reported by Orme et al. [58]. To link the bone seeker to the drug, a succinic

anhydride linkage was made in presence of 4-dimethylaminopyridine (4-DMAP) catalyst during esterification reaction. The conjugate showed similar bone affinity compared to tetracycline, with over 99% of the compound bound to HAP in 60 min. The authors assume the ester linkage between tetracycline and estradiol being degradable, essential to regenerate the parent drug; nonetheless, no further studies were found to validate parent drug recovery of tetracycline-estradiol conjugates.

4.2. Modified polymer drug carriers

Polymers, either natural or synthetic, are extensively studied materials as carriers to deliver drug to target tissues. With a broad range of biodegradable and biocompatible polymers, the physical, chemical and biological properties of polymer DDS can be highly tunable. In terms of drug release mechanism, polymeric DDS can be separated in four classes: diffusion controlled [63], solvent activated (swelling or osmotic regulated) [64], chemically controlled (degradation regulated) [63] or externally triggered systems (regulated by pH or temperature change) [65]; with some DDS being able to release their drug load in a synergetic manner [63,66]. Unfortunately, most polymers lack the intrinsic ability to target the desired tissue, but are subjectable to chemical functionalization with targeting moieties.

One of the main concerns with polymer/BMS-conjugates is the chemical alteration of the two components, which might alter the bone affinity of the BMS and/or change the desired properties of the polymer. For instance, for BPs to maintain bone affinity, the two phosphonate groups should not be sterically hindered during the binding of the molecule to the surface of polymer particle.

It is important to note that the second-generation BP Alendronate (ALN) is often used as a bone seeker covalently bound to polymer structures, due to its reactivity, sterically free primary amine group on the R_2 side chain which is not involved in chelation processes [67,68]. The drug loaded polymer structures are commonly micro or nano-size particles or micellar structures functionalized with certain BPs.

4.2.1. Solid micro- and nanospheres

During the production of bioactive solid drug delivery particles, the drug is usually incorporated in the polymer matrix by dissolving the polymer and the pharmaceuticals in a common solvent or a co-solvent system before particles fabrication. Nanoprecipitation [69], emulsion [70], solvent displacement [71] or electrospraying techniques [72] are among the conventional methods, which ideally result in particles containing the drug homogeneously distributed throughout the bulk of the particles. The polymers can be functionalized by bioactive molecules (e.g. BP [68], peptides [73] or TC [74]) either before formulation of particles (e.g. chain ends modification [68]) or after the fabrication of particles by surface grafting [75,76].

Choi et al. incorporated estrogen in nanospheres made of polylactic-co-glycolic acid (PLGA) and monomethoxy polyethylene glycol (mPEG) copolymers (PLGA-mPEG) and PLGA with ALN grafted on the carboxylic end group. The rationale behind this dual-copolymer strategy was that the hydrophilic surface mPEG can increase the circulation time of the DDS due to the increased hydrodynamic diameter, and that the ALN would increase the site specificity of the particles to bone [68]. The fabrication of such particles required first the covalent grafting of ALN to PLGA using carbodiimide chemistry and secondly the synthesis of PLGA-mPEG. Subsequently, particles were fabricated using both polymers in a dialysis method without the addition of surfactants. The cumulative in vitro estrogen release in PBS over 60 h was 80% of the initial loaded drug. The increase in mPEG chain length did not have a significant effect on the release profile of estrogen, but did result in a lower dHAP affinity. The authors hypothesized that long PEG chains could sterically hinder the ALN moiety to chelate to calcium in dHAP, but further optimization on mPEG chain length to have optimal systemic retention and conservation of strong dHAP binding is still needed

[68].

A study by Chaudarhi et al. used zoledronate as a BMS for targeted delivery of docetaxel loaded PLGA nanoparticles (PLGA-NP) [77]. Solid PLGA docetaxel loaded particles were fabricated using nanoprecipitation after which the surface of the particles was functionalized with PEG chains and zoledronate moieties by NHS-dicyclohexylcarbodiimide (DCC) and N,N′-Carbonyldiimidazole chemistry respectively. Using ^{99m}TC labeling, they determined the blood/liver, bone/blood and tumor containing bone/healthy bone ratio of PLGA-NP accumulation. As expected, the PEGylated particles showed a decrease in liver uptake, while the particles functionalized with Zoledronate had a 7.5-times increase for bone/blood ratio 1 h after intravenous administration. After 24 h, a 504% increase of Zoledronate functionalized particles was detected in bone tumor compared to bare PLGA particles, illustrating the increased retention of the DDS in cancerous bone.

Poly(Asp) can also be used to endow the surface of solid polymer particles with bone affine properties. Jiang et al. used mPEG-PLGA and maleimide-mPEG-PLGA particles in a 9:1 ratio as a potential drug carrier. After particle formation, the maleimide end groups were tagged with Fluorescein isothiocyanate (FITC) labeled oligomer (FITC-Asp_7Cys) by means of an alkylation reaction between the sulfhydryl terminal groups of the peptide and the ring opened maleimide, resulting in thioether bonds [45]. The affinity of these synthesized FITC-Asp_7Cys conjugated nanoparticles (NP) to dHAP was tested by exposing a gel containing dHAP (Fig. 2A) to the particles in dispersion. The resulting diminution of the absorbance intensity of the supernatant (from 100% to 20%) indicated a strong and specific interaction to dHAP (Fig. 2A). In vitro exposure of the FITC-Asp_7Cys conjugated NPs to matrix produced by human mesenchymal stem cells (hMSC) cultivated under osteogenic condition, indicated that the particles had a higher affinity to mineralized matrix, compared to matrix produced by hMSC in normal basic media (Fig. 2B) [45]. The FITC-Asp_7Cys conjugated NPs did not interact with C2C12 (myoblast cell-line) and Sw10 (immortalized Schwann cell cell-line) cell cultures (Fig. 2C), suggesting again specificity of the DDS toward mineralized matrix. This was confirmed by in vivo experiments showing specificity to bone tissue (Fig. 2D).

4.2.2. Micro- and nanocapsules

Micro- and nanocapsules can be defined as vesicular constructs with a typical core/shell structure [78]. The core can contain therapeutics in liquid, solid or dispersed form and the outer (polymer) hard shell provides protection from the biological environment and can provide targeting properties. There are several fabrication methods to produce capsules including nanoprecipitation [79], emulsion diffusion [80,81] and double emulsification [81].

A limited amount of work has been published so far on BMS-nanocapsule conjugates. Khung et al. reported on the fabrication of calcium phosphate (CaP) nanocapsules with sodium dodecyl sulfate (SDS) coating (Fig. 3) [82]. The silane group of silane-PEG-N-hydroxysuccinimide ester was conjugated with the outer SDS layer in a silanisation process. A coupling reaction occurred between an amino terminated BP and the N-hydroxysuccinimide ester terminal group resulting in a CaP core/SDS shell nanocapsule with a silane-PEG-NHS linker that was BP functionalized (Fig. 3A). An in vitro affinity study was performed (by incubating the nanocapsules with dentin discs), revealing an evident adsorption of only the BP functionalized nanocapsules (Fig. 3B).

Very recently, Wang et al. have reported the fabrication and characterization of bone targeting Zeolitic imidazolate framework (ZIF) nanocapsules with a catechol modified gelatin as a wall material [83]. The authors could load the nanocapsules with hydrophobic Simvastatin with high encapsulation efficiency. The catechol groups allow for ALN to be implemented as a BMS after surface conjugation. In vitro experiments showed that the nanocapsules could be internalized by osteoblasts and exhibited affinity to dHAP. Compared to constructs without
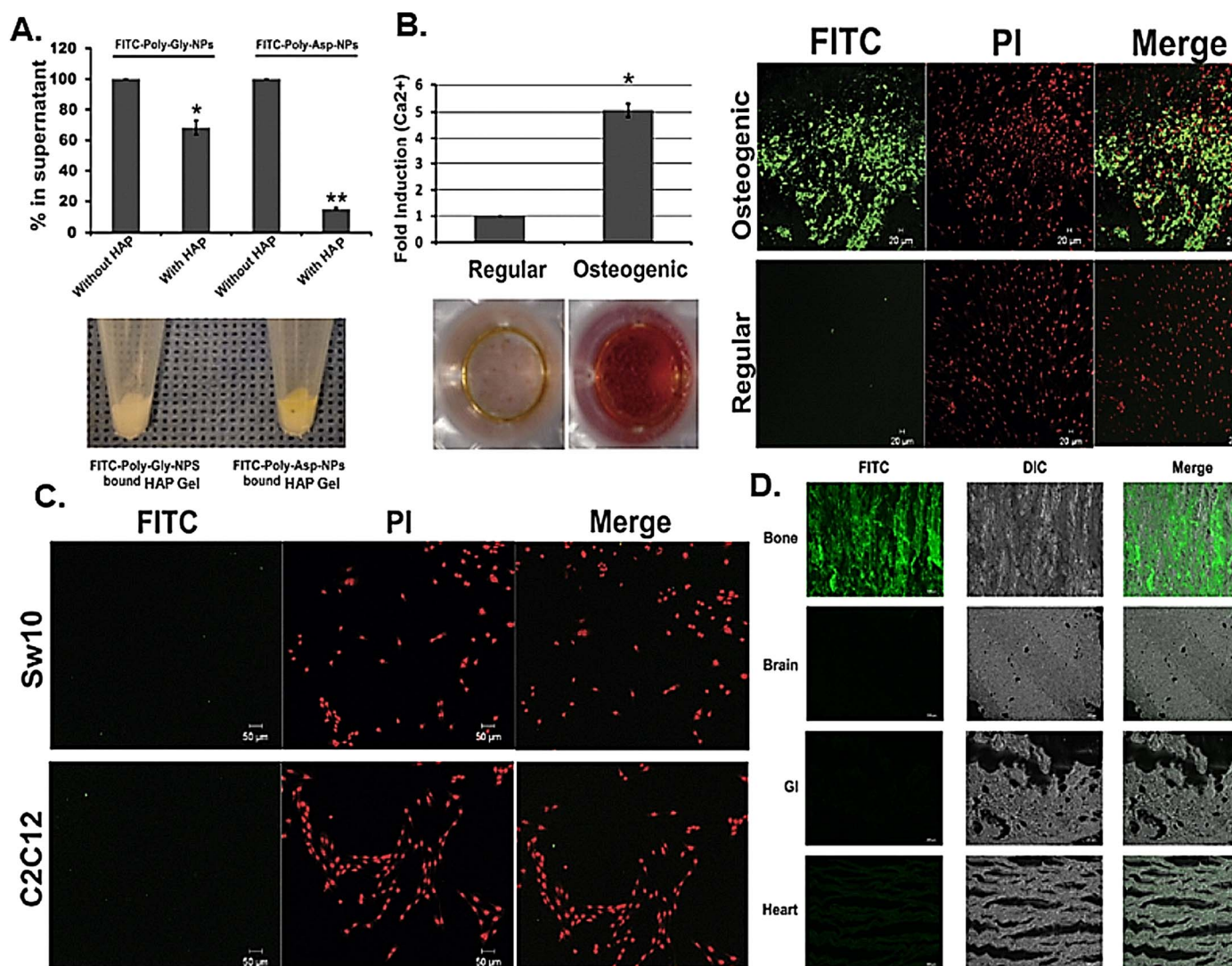
Fig. 2. DDS functionalized with poly(Asp) as therapeutic carrier for specific bone binding

The affinity of the FITC-Asp$_7$Cys conjugated NPs to HAP represented as the decrease in supernatant fluorescence of FITC-tagged particles, and illustration of the high HAP affinity of FITC-Asp$_7$Cys conjugated NPs (yellow color) and low affinity of FITC-Gly$_7$Cys conjugated NPs (whitish color) (A). FITC-Asp$_7$Cys conjugated NPs bind to mineralized matrix deposited by hMSCs cultured in osteogenic medium for 21 days (mineralized matrix was visualized by Alizarin red staining) (B). On monolayer culture, no affinity was shown between FITC-Asp7Cys conjugated NPs and Sw10 or C2C12 cell lines (C). Histological samples of organ tissues from mice after systemic administration of FITC-Asp7Cys conjugated NPs, indicating that the NPs accumulated preferentially in the bone, while its presence was limited in other tissues (D). All figures were modified and adapted with permission from [45].

ALN, a 2.5-fold increase in nanocapsule accumulation in bone was shown after intravenous injections in rats.

4.2.3. Dendrimers

Dendrimers are spherical, branched molecular structures that can act as a carrier for drugs by entrapment of the pharmaceutical molecule in the void internal spaces or by association with the surface groups on the periphery of the dendrimer [84]. Dendrimers are often described in terms of generations (*e.g.* a 2nd generation dendrimer consists of a core with branches whose end-groups also have further branched structures). Ouyang et al. presented the synthesis and *in vitro* bone binding characterization of various 2nd and 3rd generation poly(Asp) functionalized naproxen (anti-inflammatory drug) dendrimers [85]. It was hypothesized that the labile peptide bonds can be readily hydrolyzed, resulting in the release of parent drug at the site of bone infection. In a dHAP binding assay, the dendrimers showed an affinity > 60% within 2 h.

Similarly, Cavero et al. reported on the synthesis of a 2nd generation *aza*-bisphosphonate terminated dendrimers [86], with the prefix *aza*-indicating that the characteristic P−C−P backbone of the BP group was replaced with a P−N−P backbone. To create such multi-branched macromolecular structures, hexachlorophosphazene (HCP) was used as core (and as 1st generation terminus) for further branching. The chlorine on HCP was substituted for the next generation of branched structures, nonetheless no *in vitro* or *in vivo* affinities to bone materials were reported in this study. For optimal BP chelation to calcium, regular BP end-groups are preferred over *aza*-bisphosphonate derivatives, as the distance between the polar oxygen of the BPs might differ significantly and thus reduce the ability of the compound to effectively bind to calcium ions [21].

5. Biomedical uses of bone mineral-seeking agent-modified drug delivery systems

With the development of the previously mentioned drug delivery systems, many varieties of therapeutic agents that are administered systemically can now be accumulated at the region of interest. Several biomedical applications (either for diagnostic or for pathology treatments) have already benefited from such advanced bone-seeking drug delivery systems, and some of the FDA approved therapies, clinical
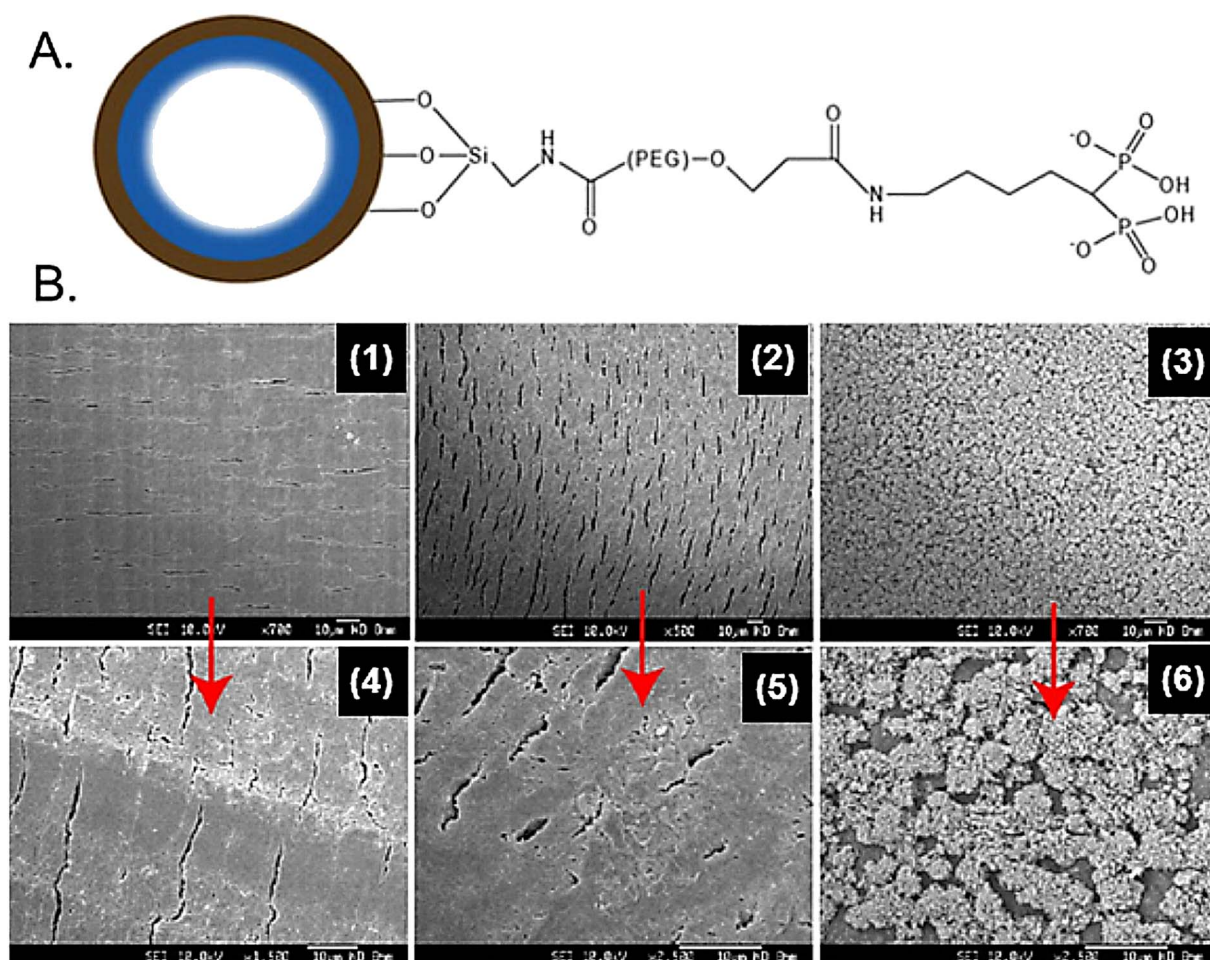
Fig. 3. Calcium phosphate (CaP) based DDS using BP for bone targeting, as described by Khung et al. [82]
CaP - sodium didicyl sulfate (SDS) nanocapsules were functionalized with silane-PEG-NHS by silanisation processing, followed by esterification with an amine terminated BP (A). Field emission scanning electron microscopy images of dentin discs with the addition of: (1) unmodified CaP nanocapsules, (2) PEG functionalized CaP nanocapsules, (3) BP functionalized CaP nanocapsules (B, with the images (4), (5) and (6) representing higher magnifications at 2500 ×). Images published with permission of Springer and [82].

trials under evaluation and the most promising developments still under pre-clinical evaluation will be later reported.

5.1. Cancer: bone metastasis and osteosarcoma

Bone is one of the most frequently affected tissues for cancers to metastasize [87]. Seventy percent of breast cancer metastasis occurs in bone tissue and prostate cancer mainly metastasizes in bone tissue [88,89]. Osteosarcoma (OS) (malignant cancer of the bone), is the most common primary tumor of bone tissue and affects mostly young people between the ages of 10 to 25 [90]. The most evident symptom characterizing OS is the unrestricted production of mineralized bone by tumor cells. The current gold standard for OS and bone metastasis treatment is a combination of surgical removal of the tumor and/or chemotherapy combining doxorubicin, methotrexate with leucovorin, cisplatin and ifosfimide [91]. Despite the effectiveness of these compounds to stop the tumor cell replication, the drugs do not discriminate between tumor cells and healthy cells, resulting in severe systemic side effects. This makes the search for alternative treatments very attractive [92]. Radio-therapeutic treatment is another option to treat metastases in the body and can be done with external beam therapy or radionuclide drugs. Radioisotopes were first used for medical applications in 1940s and are considered one of the greatest medical advances of the 20th century [93] and can be implemented in diagnostics, imaging purposes or radiotherapies. These wide range of applications makes transport of radiopharmaceuticals to bone sites clinically relevant. To

prevent long term systemic damage to tissue surrounding the cancerous area, therapeutic bone targeting radiopharmaceuticals have a relatively short half-life ranging from several hours to multiple days [34]. Still, the systemic damage to healthy tissues is substantial and bone marrow toxicity (myelosuppression) is a general concern associated with radiotherapies [94].

Tomblyn et al. reports on seven radionuclides that are effective and safe for pain palliation in bone metastases, three of which are already approved for general clinical use [95]. The calcimimetic ^{32}P and ^{89}Sr radionuclides do not need to be conjugated to a bone seeker due to intrinsic affinity to bone. Calcimimetic ^{223}Ra is currently commercially available under the tradename Alpharadin™. ^{153}Sm-EDTMP is an approved bone seeking conjugate for clinical applications is commercially available under several tradenames, including Lexidronam™. Some other radio-pharmaceuticals (*e.g.* ^{223}Ra, ^{177}Lu-EDTMP, ^{153}Sm-EDTMP) have also been used for palliative treatment of OS in order to decrease pain caused by bone metastases [96,97]. The mechanism behind the pain relief is currently not fully understood, however Lange et al. hypothesized that it can be attributed to the inhibition and killing of malignant cells [34]. For a comprehensive overview of recent developments in the field of radiopharmaceuticals and their delivery to bone, the authors recommend the review by Lange et al. [34].

When targeting tumors, including OS, one can take advantage of the ERP effect which allows larger molecules or constructs to cross the blood vessel membrane in cancer tissue. ^{99m}TC carrying macromolecules consisting of a polymer backbone with polyphosphonate side
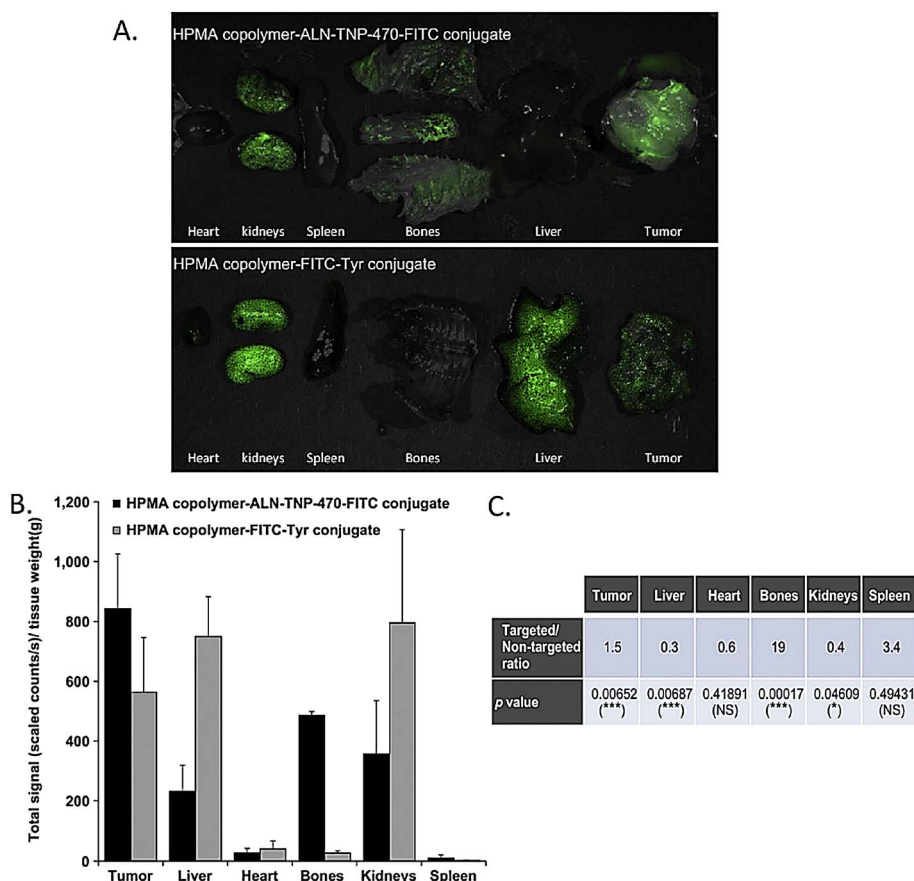
Fig. 4. Bio-distribution of ALN-copolymer-TNP 470 revealed preferential accumulation in bones and tumor in a mice model.

The targeting ability of the FITC-ALN-conjugate was evident by the increased fluorescence in the bone sample compared to the non-targeting copolymers (A). The non-targeting conjugate was cleared by the liver in significantly greater amount than the targeted conjugate, whereas the FITC-ALN-conjugate bound preferentially to bone tissues (B and C). All figures were modified and adapted with permission from [99].

chains were developed and tested in a canine OS model [98]. The difference in DDS uptake of osseous carcinomas and non-osseous carcinomas suggests that the passive EPR effect is not the only factor that plays a role in targeting OS. In fact, phosphonate groups present on the polymer can play an active role in targeting of OS due to their affinity with the calcium in dHAP that is highly present in these bone tumors [98].

Segal et al. reported on ALN and *O*-Chloracetyl-carbamoyl fumagillol (TNP-470) conjugated to *N*-(2-hydroxypropyl)methacrylamide (HPMA) designed for OS drug delivery [99] (Fig. 4). TNP-470 is a potent anti-angiogenic agent aiming to reduce vascularization of OS induced tumors. ALN and TNP-470 were attached to the polymer backbone by cleavable peptide linkers that released the ALN and TNP-470 *in vivo*. It was hypothesized that the anti-angiogenesis properties of the TNP-470 and the tumor regressive properties of ALN could both contribute to treat OS. As a xenogeneic model of human OS, balb/c mice bearing K7M2 murine OS in the tibia were chosen. Bio-distribution study conducted after subcutaneous injection of the targeting conjugate in the mentioned pathogenic mice model indicated that this DDS can effectively target OS sites (Fig. 4A and C). In comparison, kidneys and liver tissue expressed high uptake of non-targeting control conjugates due to blood filtering and the presence of highly permeable sinusoidal blood vessels in the liver (Fig. 4B and C).

The work of Qiao et al. describes the development of mesoporous silica covered Gadolinium(III) up-conversion nanoparticles (UCZP) which combine both diagnostic and therapeutic applications for early (breast cancer) metastases into bone [100]. The surface of these UCZP are functionalized with the BP Zolendronate and poly(acrylic acid), a pH-responsive polymer. The diagnostic properties of UCZP were tested in an *in vivo* mouse model where malignant cells were deposited in the heart of the mice, mimicking bone metastasis of breast cancer. Two weeks after intracardial injection, the sites of metastasis could be

identified by non-invasive *in vivo* bioluminescence imaging and magnetic resonance imaging of the UCZP. With X-ray imaging bone metastasis could identified significantly later, after three weeks. The therapeutic capabilities of the UCZP in the form of a DDS for Plumbagin were showed by high encapsulation efficiencies of the drug and by increased Plumbagin release in acidic environments, like the site of bone metastases. Plumbagin acts as a suppressor of osteocyte-related osteoclastogenesis by inhibiting expression of nuclear κB ligand (RANKL) and Sclerostin. A reduced recruiting of Osteoclasts was observed, suggesting the effectiveness of the reported UCZP DDS to diminish the processes that enable breast cancer related bone metastases [100].

5.2. Osteomyelitis

Osteomyelitis (OM), infection of bone and bone marrow, can be categorized in chronic- and acute OM. Despite the nomenclature, the defining characteristic of OM classification does not include the duration of the infection, but rather the histopathologic features [42]. Chronic OM includes the presence of necrotic bone and is generally a consequence of an open fracture, bacteremia or contiguous soft tissue infection that has not been treated or treated unsuccessfully [42]. The incidence of chronic OM is increasing due to higher prevalence of predisposing factors like diabetes mellitus and peripheral vascular diseases [42]. The gold standard of treatment of chronic OM is a surgical debridement of necrotic bone and surrounding infected tissue, followed by several weeks of systemic antibiotic treatment [42,101–103]. It has been stated repeatedly that there are no convincing clinical studies showing that long-term duration of systemic antibiotic treatment is more effective than shorter therapies, however 4 to 6 weeks of therapy is used based on empirical findings [102,104].

As was introduced earlier, a local DDS that releases antibiotics over

time might be preferable over systemic therapies. For such purposes, PMMA bone cements have been used in clinics for decades to keep prostheses in place and to prevent or treat infections on a local level [7,105]. Unfortunately, PMMA is non-biodegradable, can only be loaded with a limited number of heat stable antibiotics due to its exothermic polymerization properties and has an incomplete release of its loaded drug content [7,105].

As previously mentioned, prodrugs based on bisphosphonate modified antibiotics have been developed with the goal to reduce the systemic dosage and increase the presence of the parent antibiotic at bony sites [28,55,59]. Moxifloxacin, gatifloxacin, and ciprofloxacin have a free amino position in which tethering with a BP group is a possible approach. Indeed, *in vivo* studies showed that a single dose of BP-gatifloxacin conjugates could prevent OM in a prophylactic OM rat model due to a longer presence of the antibiotic at the bone site [59].

Acidic oligopeptides have also been linked to quinolone antibiotics to treat osteomyelitis [106]. For instance, levofloxacin (LVFX) was linked to oligopeptide (L-Asp$_6$) resulting in LVFX-D$_6$ conjugates. A second antibiotic, Norfloxacin (NFLX), was conjugated in similar fashion (NFLX-D$_6$), which showed increased *in vitro* affinity toward dHAP compared to the free quinolones, however LVFX-D$_6$ did not show similar properties [106]. The authors stated that the 3-carboxylate and 4-carbonyl groups in quinolones are also responsible for the chelating properties to divalent metals in bone, such as calcium. This explains their main findings as during the conjugation of LVFX-D$_6$ these groups are compromised while they remain unaltered during the formation of NFLX-D$_6$. In a mouse with OM, colony forming unit (CFU) count decreased of approximately 3-fold compared to untreated control mice and the LVFX-D$_6$ possessed antimicrobial activity up for 6 days. In contrast, the unconjugated LVFX group could not slow down bacteria proliferation and an increase of CFU after only 4 days was observed. Although LVFX-D$_6$ induced a significant CFU decrease, it might hardly be sufficient as a monotherapy agent and future applications as cotherapy would most likely be envisioned.

To summarize, prodrugs with bone-affinity are still the main focus of newly marketed DDS to treat OM. Advances in the field of polymer based DDS with bone-targeting moieties, other than prodrugs, could bring clear benefits, but to the best of our knowledge, such advanced therapeutics have neither been FDA-approved, nor reported as being in advanced stage in the literature.

5.3. Osteoporosis

Osteoporosis (OP) is a bone pathology with a prevalence of 10 million people in the US alone. Over 2 million of bone fractures per year are a direct cause of the low bone density of patients affected by OP, and associated medical costs are expected to be as high as $25 billion US dollar annually in 2025 [107,108]. General strategies to treat OP are either to decrease osteoclast activity, to stimulate the formation of new bone, or a combination of the both. The BP bone seeker class (*e.g.* ALN) is often used as a treatment to inhibit the dissolution of dHAP crystals by osteoclasts during the disturbed bone homeostasis in osteoporosis patients [21,30]. Currently, the only FDA approved anabolic drug for new bone formation is parathyroid hormone (PTH), commercially available under the tradename Teriparatide™ [109]. Nonetheless, BP therapy is still the norm when treating OP and only a limited amount of advanced DDS combined to BMS have been developed.

As a treatment for Pagets disease and a second line therapy for postmenopausal OP, salmon calcitonin (sCT) is used to inhibit osteoclast bone resorbtion. With a 40 fold increase in osteoclast inhibition potency and a longer biological half-life compared to human calcitonin, sCT is a short-chain peptide with promising properties [110,111]. Due to low oral bioavailability, intravenous injections have been the prevered method of administration [111]. To increase the biological half-life of sCT, PEGylation of the protein can be performed [112], and to specifically improve the bone targeting property, Bhandari

et al.conjugated such sCT with a BP moiety, which resulted in an prolonged presence (on an *in vitro* HAP binding assay) [113].

As novel OP treatment, Wang et al. functionalized PLGA NP nanoparticles with TC [74]. TC-PLGA NP had a significantly higher affinity toward dHAP compared to PLGA NP, which revealed that grafting TC on macromolecules does not inhibit TC chelating activity. As a proosteogenic drug model, simvastatin (SIM) was loaded in the TC-PLGA. Following incubation with osteoblast precursor cell line (MC3T3-E1) those particles underwent lower cellular compared to free-TC controls NP, which had a direct influence on the degree of osteogenic differentiation of the cell line. The authors argued that the increasing hydrophilicity of the TC grafted NP resulted in a decrease of cellular internalization of the NP. Around 80% of the loaded SIM was released in 72 h, leading to high concentrations of drug in the bone. SIM loaded TC-PLGA NP efficiently deliver SIM to the bone where it partially restores the bone mineral density (BMD) of ovariectomized rats. Of interest, they observed that femur and femoral head had a different response to the delivered SIM, which was explained by the fact that the cancellous bone in the femoral head is more susceptible to the drug's influence on osteoblast differentiation due to its greater surface area, compared to the denser cortical bone more prevalent in the femur [74].

6. Conclusions

Bone mineral seekers (BMS) are powerful (macro)-molecular tools to improve the delivery of therapeutic compounds to hydroxyapatite in bones. Among the described BMS, bisphosphonates, tetracycline and the oligopeptides poly(Asp) and poly(Glu) are the most frequently reported. The latter providing a promising alternative to un-reversible BP chelation. Drug-BMS conjugates show significant increased accumulation and retention at bony sites but need to effectively release the parent drug in an appropriate time frame. For this reason, a significant amount of research has been dedicated to drug delivery systems implementing BMS, which have the benefits to protect the encapsulated therapeutic compounds and to guide the conjugate to bone for an efficient local drug release. Bone pathologies like osteosarcoma, osteomyelitis and osteoporosis can benefit from the mentioned functionalized delivery vehicles to optimize therapies. Although many studies have shown promising *in vivo* results, few BMS modified DDS have successfully made translation to the clinics yet. Nevertheless, with the great amount of promising pre-clinical studies, with the main ones challenged in this review, we can envision an increase in BMS modified DDS to enter the clinics in the following years to alleviate current limitations of bone therapies and to relieve affected patients.

Declaration of interests

The authors have no interests to declare that would influence the content of this work.

Acknowledgements

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