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To cite this article: Ana Cláudia Lima, Helena Ferreira, Rui L. Reis & Nuno M. Neves (2019): Biodegradable polymers: an update on drug delivery in bone and cartilage diseases, Expert Opinion on Drug Delivery, DOI: [10.1080/17425247.2019.1635117](https://doi.org/10.1080/17425247.2019.1635117)

To link to this article: <https://doi.org/10.1080/17425247.2019.1635117>

Accepted author version posted online: 20 Jun 2019.
Published online: 31 Jul 2019.

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REVIEW

Biodegradable polymers: an update on drug delivery in bone and cartilage diseases

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ABSTRACT

Introduction: The unique structure of bone and cartilage makes the systemic delivery of free drugs to those connective tissues very challenging. Consequently, effective and targeted delivery for bone and cartilage is of utmost importance. Engineered biodegradable polymers enable designing carriers for a targeted and temporal controlled release of one or more drugs in concentrations within the therapeutic range. Also, tissue engineering strategies can allow drug delivery to advantageously promote the *in situ* tissue repair.

Areas covered: This review article highlights various drug delivery systems (DDS) based on biodegradable biomaterials to treat bone and/or cartilage diseases. We will review their applications in osteoporosis, inflammatory arthritis (namely osteoarthritis and rheumatoid arthritis), cancer and bone and cartilage tissue engineering.

Expert opinion: The increased knowledge about biomaterials science and of the pathophysiology of diseases, biomarkers, and targets as well as the development of innovative tools has led to the design of high value-added DDS. However, some challenges persist and are mainly related to an appropriate residence time and a controlled and sustained release over a prolonged period of time of the therapeutic agents. Additionally, the poor prediction value of some preclinical animal models hinders the translation of many formulations into the clinical practice.

ARTICLE HISTORY

Received 31 March 2019
Accepted 19 June 2019

KEYWORDS

Bone diseases; cartilage diseases; biodegradable polymers; drug delivery systems; controlled drug release; targeted drug delivery; tissue engineering

1. Introduction

Bone and cartilage-related diseases affect the musculoskeletal system and function, leading to a significant burden over the global public health and economy [1]. The most common disorders involving those connective tissues include osteoporosis, inflammatory arthritis (e.g. osteoarthritis – OA and rheumatoid arthritis – RA), cancer, trauma and defects [2]. Due to the increasing age of the global population, the World Health Organization has predicted a rapid growth of these clinical conditions [3]. The pain and impaired physical condition associated with those diseases generally lead to mental health problems, increased risk of development of comorbidities and, consequently, of mortality. Moreover, impaired musculoskeletal health is responsible for the greatest loss of productive life years in the workforce and for large amounts of money spent in their treatment.

Despite the significant occurrence of bone and cartilages diseases, their effective treatments remain a challenge mainly due to their peculiar and highly organized structures [4]. Bone is a vascularized biomineralized connective tissue, composed of oriented collagen I fibers and nanocrystals of hydroxyapatite, as well as of proteoglycans and glycoproteins [5]. The hierarchical structure ranging from nanoscale to macroscale ensures the high mechanical strength and structural complexity needed to support the force applied to those tissues.

Indeed, it is a multifunctional organ that is reinforced by osteogenic cells, such as osteoblasts and osteoclasts, but also harbors hematopoietic stem cells and mature immune cells, including B cells and macrophages, in the bone marrow. Therefore, bone cells are toughly connected to immune cells, sharing a variety of molecules, including cytokines, hormones, surface receptors and transcription factors [6]. Those interactions are crucial to maintain the physiological processes but can also be responsible to induce pathological conditions. Indeed, despite the ability of self-repair of bone, in elderly patients or in the presence of large defects or congenital abnormalities its regeneration is compromised. Unlike bone, cartilage is an avascular and aneural tissue consisting of collagen type II, proteoglycans (mainly aggrecan), hyaluronic acid and other glycosaminoglycans (GAGs) [7]. The highly organized network of collagen and GAGs fibrils surrounds the cell type characteristic of cartilage, the chondrocytes. Consequently, in response to injury, cartilage has limited intrinsic ability to self-repair. Thus, in order to prevent irreversible or at least to minimize the extent of joint damage, rapid diagnosis and initiation of treatment is required.

In the last years, the increased knowledge of the pathophysiology of cartilage and bone diseases led to a dramatic change in the available treatment modalities. Therefore, the term 'drug' in this review is not limited to the conventional therapeutic agents commonly used (e.g. anti-inflammatory,

Article highlights

- The treatment of bone and cartilage diseases remains an unmet medical need despite the efforts to develop effective and innovative strategies.
- Novel drugs (e.g. chemical substances and biological drugs), innovative tools (e.g. nanotechnology and 3D printing), and smart drug delivery devices (e.g. stimuli-responsive biomaterials) can lead to a revolution in the current available treatments.
- In addition to passive targeting, drug delivery systems can be advanced by their functionalization with targeting moieties specific for bone or cartilage tissues.
- Drug loading and releasing from tissue engineering approaches can modulate and enhance tissue repair.
- Clinical translation of promising treatments has been hindered mainly due to the poor correlation between pre-clinical and clinical results.

This box summarizes the key points contained in the article.

antibiotic and anti-cancer drugs), including also recombinant proteins and genes. Recombinant proteins are used as highly effective medical treatments for a wide range of diseases in which a protein is either lacking or present in an insufficient amount (e.g. transforming growth factor – TGF and bone morphogenetic proteins – BMP) or is abnormally highly expressed (e.g. antibodies to neutralize the excess pro-inflammatory cytokines) [8]. Consequently, therapeutic proteins can include antibodies, hormones, growth factors, anticoagulants, blood factors, enzymes, Fc fusion proteins, interferons, interleukins, and thrombolytic drugs, which are been produced by recombinant DNA technology. If the recombinant proteins are intended to activate or suppress the activity of the immune system, the treatment is referred to as immunotherapy or biological therapy [9]. In inflammatory diseases, such as OA and RA, immunotherapies aim to suppress/reduce the immune system activity (e.g. by neutralizing pro-inflammatory cytokines and by binding and blocking receptors that trigger immune cells activation) or to eliminate and regulate immune cells that contribute to tissue damage (e.g. effector lymphocytes). Conversely, in cancer immunotherapy, the goal is to harness and direct the immune mechanisms to eradicate the tumors (e.g. blockade of cytotoxic T lymphocytes and increasing the expansion and activation of effector T cells). Recently, numerous biological agents have been approved for clinical practice, being even more under development [10]. One of the highest selling classes of biologicals since 2009 is the monoclonal antibodies [11]. Indeed, they gathered significant attention due to their high specificity and potency [12].

Gene therapy is based on the intentional modulation of gene expression in specific cells by introducing exogenous nucleic acids to induce the production of proteins (plasmid deoxyribonucleic acid – pDNA, complementary DNA – cDNA, messenger ribonucleic acid – mRNA- and microRNA – miRNA), or to inhibit the transduction of harmful proteins (small interfering RNA – siRNA- or antisense oligonucleotides) [13,14]. There are two different approaches to deliver nucleic acids to the targeted tissues: direct (using viral or non-viral vectors) or transduced cell-mediated (by *in vitro* genetic manipulation of cells). Even though in recent years, a vast number of

therapeutic gene approaches have demonstrated effectiveness in preclinical models, only a few have moved forward into clinical trials [15].

Despite the significant advancements in the treatment of bone and cartilage diseases, they still present low efficiency and severe side effects. To overcome these limitations, different strategies of drug delivery are currently used in the clinical practice. By definition, drug delivery refers to a method or process of administering a pharmaceutical compound to safely achieve its desired therapeutic effect [16]. These strategies are designed to alter the pharmacokinetic and/or biodistribution of their associated drug, to function as their reservoir, or both. Preclinical and clinical studies are exploring a variety of drug delivery systems (DDS), such as nanoparticles (NPs), microparticles (MPs), micelles, dendrimers, liposomes, hydrogels and scaffolds (Figure 1), which have been slowly translated into the clinical practice.

In this review, we discuss the recent advances in the development of several drug delivery strategies based on biodegradable polymers for treating bone and cartilage diseases. First, different strategies are introduced and systematized. Then, recent advances including new therapeutic drugs, novel targeting approaches, and innovative delivery vehicles are highlighted for each condition. Finally, to enable those systems to reach the clinical practice, an expert opinion of the challenges and future directions is given.

2. Drug delivery by biodegradable polymers networks

Two concepts introduced in the 19th and in the 20th centuries have been revolutionizing the medical field, namely the magic bullet and nanotechnology. The first concept was coined to

Figure 1. Schematic illustration of the various drug delivery systems used in bone and cartilage diseases.

Paul Ehrlich, in 1900, and is related to a limited effect of the drugs on the cellular target [17]. Therefore, the linking of a targeting moiety to a drug will increase its therapeutic index. Biodegradable polymers are frequently used as carriers in those strategies. Moreover, the assembling of this concept to nanotechnology has provided significant progress in the diagnostic, treatment and prevention of human diseases. The term nanotechnology has been assigned to Richard Feynman, in 1959 [18], but Norio Taniguchi was the first scientist to use that word at 1974 [19]. Nanotechnology embraces 'The design, characterization, production, and application of structures, devices, and systems ... at the nanometre scale' [20], 'with at least one novel/superior characteristic or property' [21]. Although the International Organization for Standardization (ISO/TS 80,004-1:2015) defines nanoscale as the 'length range approximately from 1 nm to 100 nm', there is considerable controversy among the scientific community especially for the upper limit. Indeed, a straight relationship between size and novel effects or functions for different materials does not exist [22]. Therefore, despite the nanoscale definition, in the literature nanostructures frequently include sub-micron particles (1 nm to 1000 nm). The drug delivery field has been advanced and reinforced mainly due to the development of novel and innovative technologies, and the remarkable increase of knowledge about materials science and pathophysiology, biomarkers and targets of the diseases. With an appropriate DDS it is possible to circumvent important drawbacks of the conventional therapies, namely (i) to decrease the dose of drug administered (by avoiding its metabolism/degradation, clearance and distribution in non-target tissues), (ii) to abolish or drastically reduce the systemic side effects (by targeting delivery, which will enhance the pharmacokinetics and pharmacodynamics of the drug, and consequently will increase its therapeutic index) and (iii) to reduce the frequency of administration (by the sustained release of therapeutic concentrations of the drug over time). Therefore, an appropriate delivery system can recover withdrawn drugs from the market by overcoming their side effects in non-target tissues/organs [23,24].

A rational design of a delivery system should consider the nature of the drug to be incorporated (e.g. hydrophobic, hydrophilic or amphipathic), the mechanisms that will control its release (e.g. diffusion, carrier degradation or dissolution, cleavage of chemical bonds, and external, physiological or pathological stimulus) and the disease (e.g. cell/tissue to target or tissue pH and vascularization). Ideally, the drug must be incorporated into the delivery device, being released only in the target cells or tissues in concentrations within the therapeutic range. Moreover, depending on the mechanism of action of the therapeutic agents (e.g. binding to a cell membrane receptor or to an intracellular or nuclear target), the design of delivery carriers should be carefully considered. The selection of the most adequate composition is crucial to obtain DDS with the desirable drug release properties. Additionally, the preparation method as well as the physico-chemical properties of the delivery device (e.g. size and degradation rate in the biological environment) will also influence the release of the drugs [25]. Efforts were also made to achieve a drug release in a pulsatile fashion, triggered by changes in the neighboring milieu (self-regulated delivery

systems using different mechanisms, such as pH-sensitive polymers, enzymes, illness markers and pH-dependent drug solubility) or by an external stimulus (externally triggered systems by a magnetic, thermal, ultrasonic, electric or irradiation stimulus) [26–28]. Among the wide variety of natural, semisynthetic or synthetic materials that can be used to produce DDS, biodegradable polymers (e.g. proteins, polysaccharides, poly(amino acids) and polyesters) [29] have been preferred to produce innovative, effective and specialized release dosage forms, due to their advantages (e.g. avoiding body accumulation and predictable degradation). For instance, the synthetic polymers, poly(lactic-co-glycolic acid) (PLGA) and poly(ϵ -caprolactone) (PCL), and the natural polymers chitosan, hyaluronic acid, alginate and albumin are widely used for the preparation of polymeric NPs (1–1000 nm in size) and MPs (1–1000 μ m) [30,31]. NPs and MPs are collective terms for both nano/microspheres and nano/microcapsules (Figure 1). Nano/microspheres have a compact matrix structure and the drugs can be entrapped, dispersed, dissolved within the polymer matrix or adsorbed at their surfaces [32]. For nano/microcapsules, as they are vesicular systems with a hollow liquid core surrounded by a polymeric membrane, besides the referred locations, the drugs can also be encapsulated in that core [32]. Biodegradable polymers can also be used to produce other DDS, namely micelles and dendrimers (Figure 1). Polymeric micelles are produced from amphiphilic copolymers that self-assemble in nanostructures ($\approx$ 10–200 nm in size) [33,34]. Dendrimers ($\approx$ 2–10 nm in diameter) are highly branched polymeric structures with enhanced functionality, due to the presence of several functional groups at their surface [35,36].

The association of polymers to other biodegradable materials, such as lipids, can be performed to improve their properties. For instance, the binding of polyethylene glycol (PEG) to liposomes (phospholipid bilayers with sizes ranging from 30 nm to several μ m [37,38]) is widely used to increase their residence time in circulation. Moreover, lipid-polymer hybrid NPs were developed to overcome the limitations and combine the advantages of both polymeric NPs and liposomes, leading to more robust DDS in terms of stability and controlled release, for instance [39].

To repair the function and structure of damaged or diseased bone and/or cartilage, the implantation of 3D-engineered structures (e.g. scaffolds or hydrogels) loaded with drugs encapsulated or not in DDS may be more effective. Indeed, due to the limited intrinsic ability to repair of cartilage as well as of bone in elderly patients or in the presence of large defects or congenital abnormalities, tissue engineering approaches may be preferred.

2.1. Targeting strategies

The target delivery of a drug can be either passive or active. Passive targeting is widely investigated mainly in cancer and inflammatory conditions, due to the leaky vasculature or enhanced permeability and retention (EPR) effect [40]. For this and for many other features (e.g. drug release and interaction with cells [30]), the size as well as the surface and shape of the delivery systems are crucial. Active targeting is achieved by attaching to the drug or to the surface of the delivery devices a particular ligand that ideally will bind to a moiety

specifically found in a specific organ, tissue or cell of interest (Figure 2).

2.1.1. Bone and cartilage targeting

The peculiarity of the bone and cartilage structures difficult the attainment of drug concentrations required to elicit the desired biological response at the cell and matrix targets. Therefore, there is a huge interest in designing drug delivery strategies to selectively target bone or cartilage diseased areas delivering the drug where its therapeutic action is required.

In bone, drug-targeting strategies take advantage of its high content of hydroxyapatite and of the existence of specific cells [41,42]. Therefore, the following moieties are usually used for bone targeting:

- (1) Bisphosphonates (BPs) have been widely used for drug delivery into bone, due to their affinity for hydroxyapatite. They are chemically stable derivatives of the naturally occurring inorganic pyrophosphate. Besides being a bone-binding class of molecules (e.g. clodronate, etidronate, alendronate, risedronate, and zoledronate), BPs are also used to treat bone diseases characterized by an imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption,

such as osteoporosis, Paget disease, vascular calcification or bone metastasis [43,44].

- (2) Tetracyclines are broad-spectrum antibiotics used to treat several gram-positive and gram-negative bacterial infections [45] and as bone-targeting moieties due to their ability to bind specifically to hydroxyapatite [46–48]. Indeed, these bone-targeting moieties have been used in bone histomorphometry as new bone formation markers [49,50]. They were the first drugs used as bone-targeting agents, but their use is decreasing mainly due to their poor stability after conjugation. Moreover, as tetracyclines have the ability to inhibit collagenases and other matrix metalloproteinases (MMPs), they can inhibit the degradation of collagen I, the main organic component of connective tissues such as bone. Besides inhibiting bone loss, they can also increase bone formation, because of the pro-anabolic and anti-catabolic properties that these drugs present [49,51].
- (3) Oligopeptides containing acidic amino acids have high affinity toward hydroxyapatite [52–55], being an attractive option due to their lack of adverse effects [42]. Despite the exact mechanism is currently under debate, it is known that the affinity of peptides to hydroxyapatite increases when repeating units of Aspartic acid (Asp) or

Figure 2. Examples of targeting moieties used in drug delivery systems to treat bone and cartilage diseases.

Glutamic acid (Glu) is present in the amino acid sequence [56]. Indeed, natural non-collagenous proteins such as osteocalcin and osteopontin that present high amounts of Asp and Glu amino acids have high affinity to the hydroxyapatite of bone tissue.

Eight repeating sequences of aspartate (Asp₈) strongly bind to highly crystallized hydroxyapatite, which is characteristic of bone-resorption surfaces covered by osteoclasts [57,58]. Conversely, six repetitive sequences of aspartate, serine, and serine (AspSerSer₆) have high selectivity for low-crystallized hydroxyapatite, and, consequently, for bone formation surfaces [52–55,59]. Other peptides, such as the five-amino acid motif Ser-Asp-Ser-Ser-Asp (SDSSD), are used to selectively target osteoblasts via periostin (also called osteoblast-specific factor 2 -OSF-2) [60]. In fact, the conjugation of oligopeptides to drugs (e.g. enzymes or estradiol) was studied for several diseases including osteoporosis and other musculoskeletal disorders [61].

- (4) Aptamers, small single-stranded DNA or RNA sequences, can also be used for drug targeting into bone. An example is the CH6 aptamer to selectively target osteoblasts at the cellular level [62].

Cartilage is avascular that constitutes an efficient obstacle for drugs as well as for DDS to diffuse and enter in its extracellular matrix (ECM). Therefore, local administration via intraarticular (IA) injection in the joint space has been chosen in detriment of systemic administration to increase the drug bioavailability and to reduce drug dosage, systemic exposure, and adverse events. Unfortunately, drugs injected into the joints are normally cleared very quickly (half-life of 0.1 to 6 h), which is even higher in the presence of inflammatory conditions (e.g. RA and OA). In addition, limited cartilage targeting also limits the therapeutic efficacy of drugs [63]. To penetrate in the cartilage ECM, the design of a DDS should consider its highly anionic charge and dense nature that leads to a 60 nm mesh size provided by the type II collagen [64] and the $\approx$ 3.2 and 4.4 nm of space between GAGs chains along fetal and mature aggrecan, respectively [65]. It was already demonstrated that solutes with a diameter up to 10 nm can penetrate through diffusion or convective transport into the full thickness of an undamaged cartilage [66]. NPs presenting 15 nm of diameter can only access the superficial area of the healthy articular cartilage [66,67]. However, they will be able to penetrate deeper if a damaged ECM is present [66,67]. Indeed, three phenomena influence the penetration of large, positively charged molecules into the avascular negatively charged cartilage: (i) steric hindrance from the dense tissue ECM, (ii) binding to the intra-tissue sites, and (iii) electrostatic interactions. DDS with higher radius can also be useful if they have the ability to specifically bind to the cartilage surface. Therefore, their appropriate functionalization is of extreme importance. As previously referred cartilage presents a high negative charge that can be used to electrostatically bind, hold and accelerate the penetration of positively charged DDS. Indeed, even the smallest DDS able to reach the deep zones of cartilage are usually functionalized to reduce their rapid clearance from the synovium [68]. Consequently, the functionalization of DDS for cartilage target is usually performed with cationic moieties, including: (i) cell-penetrating peptides,

such as the widely used TAT peptide for intracellular delivery [67,69], (ii) amine-terminated PEG [66] and (iii) cationic peptides, such as collagen II α 1 (COL2A1)-binding peptide (WYRGRL) [68], aggrecan-binding peptide (RLDPTSYLRTFW and HDSQLEALIKFM) [70] and heparin-binding peptide (KRKKKGKGLGKKRDPSLRKYK) [71].

2.1.2. Inflammation targeting

The leaky vasculature of the inflamed joints allows using the EPR effect (passive targeting). Nonetheless, active targeting has been taking advantage of the influx of various inflammatory cells including macrophages, fibroblast-like synoviocytes (FLS), and lymphocytes into the synovial space [72]. Many receptors are upregulated on the activated macrophages, such as the folate receptor β and the scavenger receptor. CD44 surface molecules are also overexpressed in macrophages, FLS, and lymphocytes. The activation of epidermal growth factor receptor (EGFR) in FLS induces their proliferation, and consequently RA pathogenesis [73]. In addition, the neovasculature composed by vascular endothelial cells has high expression of intercellular cell-adhesion molecule-1 (ICAM-1), E-selectin and integrins [74]. The upregulated expression of the receptors can be explored by the following moieties:

- (1) Folic acid has high affinity to folate receptor [75], and when conjugated with a DDS can facilitate their internalization in a receptor-specific manner in macrophages.
- (2) Carbohydrates in the form of monosaccharides and polysaccharides interact with different cell receptors. Sialic acid, also known as N-acetylneuraminic acid, is a monosaccharide that specifically binds to E-selectin. The polysaccharide dextran sulfate (DS) selectively binds to scavenger receptor and hyaluronic acid to CD44 molecules.
- (3) Antibodies can be designed to specifically target scavenger receptors (e.g. anti-CD163 antibody [76]), the microvasculature of human arthritic synovium (e.g. single-chain Fv A7 [77]), or EGFR (e.g. monoclonal antibody cetuximab [78]).
- (4) Peptides that have targeting ability to specific molecules were developed mostly by phage display technology [79], including: vasoactive intestinal peptide (VIP) that binds to its G protein-coupled receptors in activated T-lymphocytes, macrophages and FLS [80]; tuftsin that promotes phagocytosis by binding with Fc and neuropilin-1 receptors on macrophages [81]; synovial fibroblast-homing peptide (HAP-1) that facilitate specific internalization in FLS [82]; and GE11, a dodecapeptide with the amino acid sequence YHWYGYTPQNVI, that specifically bind to EGFR [83].

3. Bone and cartilage diseases

Several drug delivery approaches for bone and cartilage diseases (Table 1) will be explored in this section.

Table 1. Examples of DDS for bone and cartilage diseases.

Drug delivery system	Formulation	Drug	Targeting rationale	Property/function	Condition	Ref
Polymer-drug conjugates	PTH-PEG-BP	PTH	Hydroxyapatite via BPs	PTH-PEG-BP conjugates had significant enhanced affinity for bone mineral and improved the trabecular bone volume and structural strength.	Osteoporosis	[90]
	Certolizumab Pegol (Cimzia [®] , in clinical practice)	Certolizumab (Anti-TNF- α antibody)	-	Licensed anti-TNF- α antibody-polymer conjugates that increases the plasma half-life and avoids the complement activation. Patients showed increased physical function and reduced disease signs and symptoms.	RA	[126,127]
NPs	Natural Polymeric NPs	Chitosan-Hyaluronic acid NPs	IA injection	Biofunctionalized NPs with anti-IL-6 antibodies exhibited a prolonged action and stronger efficacy than the free antibody in chondrocytes stimulated towards inflammation.	Arthritis	[129]
	Tufts-in-decorated alginate NPs encapsulating IL-10 plasmid	IL-10 plasmid DNA	Activated macrophages of inflamed joints via tufts	Targeted formulation demonstrated higher transfection efficiency, sustained local expression and reduced systemic and joint tissue pro-inflammatory cytokines, which prevented joint damage and delayed the onset of inflammation.	Arthritis	[132]
Synthetic Polymeric NPs	HPMA copolymer with Asp ₈	siRNA sema4D	Highly crystallized hydroxyapatite surfaces of bone- resorption surfaces via Asp ₈	The inhibition of sema4D by site-specific bone-targeting NPs was able to increase bone mass in healthy animals, prevent bone loss in the early stage of the OVX animal model and gradually recover bone loss in osteopenic animals.	Osteoporosis	[98]
	Pyrophosphate-tri(ethyleneglycol)-cholesterol conjugate (PPI- TEG-Chol)	Icariin	Hydroxyapatite via PPI	Biomimetic-binding liposomes with icariin increased bone density and preserved the trabecular bone microarchitecture.	Osteoporosis	[102]
Liposomes	Cationic liposomes linked to (AspSerSer) ₆	siRNA for Plekho1	Lowly crystallized hydroxyapatite of the bone-formation surfaces via (AspSerSer) ₆	The depletion of Plekho1 markedly promoted bone formation, increased bone mass and enhanced the bone micro-architecture in both healthy and osteoporotic rats.	Osteoporosis	[52]
	PEGylated liposomes conjugate with HAP-1	plasmid containing sema3a	FLS via HAP-1	The expression of Sema3a increased osteoblastic bone formation, and simultaneously suppressing osteoclastic bone resorption in an OVX rat model.	Arthritis	[99]
Lipid-polymer NPs	Post-insertion of alendronate-hyaluronic acid conjugate linked through a bioreducible disulfide linker into liposomes	Doxorubicin	Bone and CD44 using alendronate and hyaluronic acid, respectively	HAP-1 modified liposomes showed a 10 fold increase localization in affected joints compared to unaffected joints and enhanced therapeutic index in an AIA rat model.	Osteosarcoma	[117]
	A clinically tested liposome	Bi-shRNA EWS/FLI1	-	Redox-sensitive liposomes exhibited higher ability for specific intracellular drug delivery and reduced mortality of the animals. The efficacy of the developed system was further improved by coadministration of iRGD.	Osteosarcoma	[138]
Lipid-polymer NPs	PLGA/PVA NPs incorporated into liposomes	All-trans retinoic acid	Osteosarcoma initiating cells via CD133 aptamers	<i>In vitro</i> and <i>in vivo</i> results validated the translation of the developed formulation to clinical trials (NCT02736565).	Ewing's sarcoma	[141]
				The target moiety significantly enhanced the amount of the NPs in CD133+ osteosarcoma initiating cells, demonstrating a higher therapeutic efficacy.	Osteosarcoma	[137]

(Continued)

Table 1. (Continued).

Drug delivery system	Formulation	Drug	Targeting rationale	Property/function	Condition	Ref
Micelles	PU nanomicelles	miRNA (Anti-miR-214)	Perostin via an osteoblast-targeting peptide (SDSSD)	Anti-miR-214 delivery to osteoblasts using SDSSD-PU showed increased bone formation and bone mass, and improved bone microarchitecture.	Osteoporosis	[60]
	PEG-Dex	Dex	Bone-resorption surfaces via D-Asp8 Passive effect	DDS deliver anti-miR214 to osteoclasts, and bone microarchitecture and bone mass were improved in OVX osteoporosis mice PEG-Dex micelles, combined with a pH- responsive hydrazone linker, exhibit higher retention in the inflamed joints and enhanced therapeutic efficacy in an AIA rat model	RA	[97]
	Folic acid-Cholesteryl chloroformate – polysialic acid (FA-CC-PSA)	Dex	Folate receptor of macrophages via folic acid	<i>In vitro</i> and <i>in vivo</i> results demonstrated that FA-CC-PSA micelles effectively suppress key pro-inflammatory proteins, improve the drug pharmacokinetics and increased its safety	RA	[112]
	Dextran sulfate-graft-methotrexate conjugate (DS-g-MTX) micelles	MTX	Passive targeting delivery into inflamed joints through the EPR effect	DS-g-MTX micelles showed higher accumulation in the inflamed joints and stronger anti-inflammatory effect, leading to significant alleviation of synovitis and protection of articular cartilage.	RA	[113]
	Sialic acid-dextran-octadecanoic acid (SA-Dex-OA) micelles	MTX	E-selectin receptor of inflammatory vascular endothelial cells via SA	E-selectin-targeting strategy of the SA-Dex-OA/MTX micelles elicited excellent inhibition of inflammatory response and minor adverse effects on liver and kidneys. The synergistic effects between drug and carrier also enhanced bone repair.	RA	[123]
	Hyaluronic acid/Curcumin (HA/Cur) nanomicelles	Curcumin	IA injection	HA/Cur nanomicelles lowered the edema and cartilage degradation in RA rat models, with clear inhibition of the inflammatory response.	RA	[133]
	PEI-Pluronic® L64 copolymers	miRNA-145	-	<i>In vitro</i> studies demonstrated the ability of the micelleplexes to decrease cell proliferation and migration as well as to increase cell death.	Osteosarcoma	[139]
Dendrimers	PEGylated Ethylenediamine-cored and amine-terminated generation 5 (PAMAM) dendrimer with pH-responsive link to the drug	Bortezomib	cRGD	The dendrimers demonstrated efficient reduction of the metastatic bone tumors progression and of the tumor-associated osteolysis in mice.	Bone metastasis	[144]
Others	Hyaluronate/gold nanoparticle/Tocilizumab (HA-AuNP/TCZ) complex	TCZ	-	HA-AuNP/TCZ complex showed the dual targeting activity of the binding to VEGF and IL-6R <i>in vitro</i> . The therapeutic effect on a mouse RA model was verified by ELISA, histological, and Western blot analyses.	RA	[128]
MPs	PLGA MPs	Triamcinolone acetate (TA)	Intra-articular injection	After IA injection, the formulation provides a sustained release of TA, which significantly prolonged analgesia <i>in vivo</i> . The results from clinical trials (phase II and III) indicated the significant clinical pain relief and functional improvement in patients with knee OA.	OA	[118–121]

Abbreviations: Nanoparticles (NPs); Microparticles (MPs); Rheumatoid Arthritis (RA); Osteoarthritis (OA); Intra-articular (IA); Enhanced permeability and retention (EPR); Fibroblast-like synoviocytes (FLS); Polyethylene glycol (PEG); Parathyroid hormone (PTH); Bisphosphonates (BPs); Interleukin (IL); Tumor necrosis factor- α (TNF- α); Dexamethasone (Dex); Methotrexate (MTX); Semaphorin (sema); D-aspartic acid octapeptide (Asp₈); aspartate, serine, serine oligopeptide ((AspSerSer)₆); Synovial fibroblast-homing peptide (HAP-1); Casein kinase-2 interacting protein-1 (Plekho1); Polyurethane (PU); N-(2-hydroxypropyl) methacrylamide (HPMA); Poly(lactic-co-glycolic acid) (PLGA); Polyvinyl alcohol (PVA); Ovariectomized (OVX); Adjuvant-induced arthritis (AIA);

3.1. Osteoporosis

Bone remodeling is a coordinated process in which old bone is reabsorbed by osteoclasts, and new bone is synthesized by osteoblasts. The imbalance of this physiological process frequently leads to osteoporosis. Due to the reduction of bone mass and the deterioration of the bone tissue microarchitectural features, the main clinical consequence of the disease is bone fragility and, consequently, an inherently high risk of fracture [84]. With the increased aging population and life expectancy, osteoporosis incidence is growing, representing a major public health problem. Indeed, it is estimated to affect over 200 million people worldwide [85]. One in three women and one in five men older than 50 years may eventually experience osteoporotic fractures. Consequently, its prevention and effective treatment are crucial.

Although several treatments are currently available to reduce the impact of bone fragility, there is no alternative to restore bone strength with curative effects. Current treatments are limited to anti-resorptive drugs to reduce the bone-resorption rate (e.g. BPs, raloxifene and denosumab), and anabolic agents to increase the bone formation (e.g. recombinant human parathyroid hormone – rPTH- and estrogens). More recently, gene therapies, immunotherapies, and growth factors are in the development stage both in preclinical studies and clinical trials. Due to the lower vascularization of the bone in comparison with soft tissues, biologic agents are usually administered more frequently and at higher doses to have a therapeutic effect. Therefore, one of the most important and persistent problems of osteoporosis treatment is the long-term safety issues of the different current treatments.

BPs are widely used in the treatment and prevention of bone diseases, including osteoporosis and Paget disease, but due to the severe gastrointestinal side effects, its therapeutic use has been narrowed. In order to overcome the adverse effects associated with oral administration, transdermal lipid-based microemulsions were developed as a local alendronate DDS [86]. In ovariectomized (OVX) osteoporotic rats the microemulsions significantly increased alendronate bioavailability in comparison with oral administration, without skin damage. Other strategy was developed using hydroxyapatite-based nano-conjugates of mPEG-PLGA and risendronate as a targeting moiety and a suppressor of osteoclast activity [87,88]. Pharmacokinetic studies confirmed the higher drug transport efficacy after intravenous and oral administration of the NPs, with sixfold and fourfold increase in the relative bioavailability, respectively, as compared to the free drug. Additionally, *in vivo* studies showed a significant enhancement in bone density and micro-architecture after the NPs treatment, confirming the targeted delivery to the bone and the effective synergistic treatment.

PTH is currently the only FDA-approved anabolic drug to treat osteoporosis. As it has a very short half-life (less than 60 min), daily injections are needed to have a positive therapeutic effect. Moreover, PTH treatment cannot exceed 2 years due to the risk of developing osteosarcomas. Therefore, in order to increase bone mineral content, PTH has been conjugated with different carriers. An example involves its adsorption to hydroxyapatite nanorods that possess enhanced affinity to the calcium present in the bone, to specifically release the hormone in the osteoporotic bone [89]. *In vitro* and *in vivo* results confirmed the synergistic effect of PTH and hydroxyapatite, with enhanced osteogenesis and bone

regeneration in an OVX mice model. PTH-PEG-BPs conjugates also significantly enhanced PTH targeting to the bone matrix, which increased bone formation and strength in comparison with systemically administered PTH alone [90]. A novel approach developed 3D biomimetic nanofibrous scaffolds of PLLA to locally deliver PTH in either a pulsatile or continuous release for 21 days using polyanhydride (PA) microspheres [91]. In a mouse model, the local pulsatile delivery of PTH promoted a higher regeneration of a critical-sized bone defect than the standard systemic PTH injection and with insignificant systemic side effects. Other DDS strategies, such as collagen-hydroxyapatite scaffold [92], chitosan/silk fibroin MPs [93], PLGA microspheres [94], and self-dissolving microneedle arrays made of hyaluronic acid for transdermal delivery [95] also incorporated PTH to promote anabolic bone formation with promising results.

Recently, gene therapy has emerged as an innovative strategy to treat osteoporosis [13]. While an increasing number of nucleic acids were identified to participate in osteoclast formation, differentiation, apoptosis and resorption, the challenge is to develop a safe and effective delivery system [96]. Since miR-214 is up-regulated during osteoclastogenesis and inhibits osteoblast function, polyurethane (PU) nanomicelles were modified with an osteoblast-targeting peptide (SDSSD) to selectively deliver anti-miR-214 into osteoblasts [60]. This approach was successful in increasing bone formation and bone mass, with improved bone microarchitecture features in an OVX mouse model. In a similar study, anti-miR-214 was loaded in PU micelles, but Asp₈ was used as the targeting moiety to osteoclasts [97]. The targeting delivery to the bone-resorption surfaces markedly increased bone mass and bone microarchitecture in OVX mice, but in this case, no effects on bone formation were observed. siRNA for Semaphorin4D (sema4D), a major coupling factor expressed on osteoclasts to inhibit osteoblast differentiation, was incorporated in polymeric NPs designed with a site-specific bone-targeting moiety, namely D-Asp₈ [98]. This strategy led to a significant increase in the number of active osteoblasts at the bone surface, which increased the bone volume in OVX animals. Two different cationic liposome formulations were also designed to deliver: (i) siRNA to target casein kinase-2 interacting protein-1 (Plekho1), a negative regulator of osteogenic lineage activity without modulating bone resorption [52], and (ii) plasmids containing sema3A, a protein that potently inhibits osteoclast differentiation [99]. Both strategies ameliorated bone loss and induced bone formation in OVX models. Even though DDS based on gene therapy has been reported as potential strategies to osteoporosis, it is important to mention that the mechanisms of osteoprotective and/or osteoinduction by those genes are not fully understood.

The bioactive compounds icariin and icaritin present in *Herba Epimedii*, a Traditional Chinese Medicine plant, were reported to prevent primary osteoporosis in clinical trials [100,101], since they promote bone formation. However, those flavonoids have poor water-solubility, first pass metabolism after oral administration and low bioavailability, which limits their clinical applications. Icariin was encapsulated in pyrophosphate-tri(ethyleneglycol)-cholesterol conjugate liposomes [102], taking as target the hydroxyapatite of the bone

owing to the strong affinity of pyrophosphate. The designed formulation significantly increased the therapeutic efficacy of icariin, with improved bone density and preserved trabecular bone microarchitecture. In other work, icaritin was incorporated in Asp₈-targeted liposomes and was able to promote bone formation as well as to suppress bone resorption [103], is, therefore, a potential anabolic candidate for osteoporosis treatment.

3.2. Inflammatory arthritis

Inflammatory arthritis is an umbrella term that encompasses more than 150 different conditions and is characterized by inflammation in one or more joints and/or in the musculoskeletal system [104]. It affects more than 350 million people worldwide, and its incidence and prevalence are increasing. As a result of their chronic, painful and debilitating features, arthritic diseases are one of the leading causes of work disability [105]. Moreover, in 2015 the overall costs of arthritis were estimated in more than \$304 billion in the US.

OA and RA are the most common forms of arthritis, being both diseases associated with persistent arthritic pain, swelling, and stiffness. OA, a local degenerative joint disease, is the leading cause of morbidity and disability in the elderly, affecting 9.6% of men and 18.0% of women aged over 60 years [106]. It is characterized by synovial inflammation and articular cartilage and subchondral bone degradation. The risk factors are genetic predisposition, aging, obesity, trauma, and other systemic diseases. Conversely, RA is a systemic autoimmune disease that usually affects multiple joints [107]. It causes inflammation of joints, synovial hyperplasia, pannus formation, bone erosion, and cartilage destruction. It has a global prevalence of around 1% with the incidence among women being 2–3 times more than in men. Genetic factors, environmental factors, and the adaptive immune response can trigger this chronic inflammatory disease.

Nonetheless, joint damage in OA and RA proceeds via different pathways, they share certain mechanistic similarities [108]. In OA, as a result of cartilage damage and inflammatory process, the phenotype of chondrocytes is altered, becoming degenerated and disturbed. The chondrocytes start to express matrix-degrading enzymes, such as MMPs and aggrecanases (ADAMTS), and due to their increased sensitivity to inflammation, they stimulate a cycle of further cartilage damage. In RA, the immune system activation in combination with the release of ECM products after cartilage damage activates the synovial FLS to a stable, tumor-like phenotype. These activated FLS gradually invade and degrade the cartilage ECM and promote the activation and differentiation of adjacent cells, including the differentiation of monocytes and macrophages into osteoclasts. Therefore, cartilage damage in both diseases is associated with the increment of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukins (IL, particularly IL-1 β and IL-6), which in its turn increase the production of catabolic factors and down-regulates anabolic mediators.

At present, there is no cure for OA and RA [109]. The most commonly used therapeutic strategies include analgesic (such as acetaminophen, propoxyphene, and tramadol), non-steroidal anti-inflammatory drugs (NSAIDs, such as ibuprofen and celecoxib) and glucocorticoids (GCs, such as prednisolone, dexamethasone –

Dex and budesonide). Disease-modified anti-rheumatic drugs (DMARDs, such as methotrexate – MTX) and biological agents are the first line treatment in RA in order to relieve joint damage and control the disease progression, being also in clinical trials for OA. Taking into consideration the mechanisms of initiation and progression of both diseases, in RA the systemic therapy is generally indicated and appropriated, while in OA the local therapy may offer particular advantages over systemic therapy [110,111]. Nowadays, IA injections of hyaluronic acid and glucocorticoids are standard treatment options for the management of OA-related knee pain.

For a long time, NSAIDs and GCs were the first line of treatment, since they reduce pain and inflammation. However, associated with their inability to stop the joint damage, they cause serious side effects. While NSAIDs increase the risk of gastrointestinal bleeding, renal dysfunction, and cardiovascular disease, GCs are associated with immunosuppression, osteoporosis, hyperglycemia and hypertension. Consequently, their long-term administration needs to be carefully considered. Therefore, many DDS were designed to improve drug efficacy and safety. For instance, amphipathic polymer-drug conjugates (PEG-Dex) micelles, combined with a pH-responsive linker, exhibited preferential retention in targeted tissues in a rat model of adjuvant-induced arthritis (AIA) [112]. These micelles had targeted delivery to inflamed sites via the EPR effect, ensuring a higher release in the acidic arthritic joints, and consequently enhanced the therapeutic efficacy of the drug. Cholesteryl chloroformate – polysialic acid (CC-PSA) micelles were modified with folic acid to obtain targeted delivery of Dex in inflamed joints [113]. In an AIA model, the delivery of Dex by folic acid-CC-PSA micelles increased its half-life and bioavailability compared with commercial Dex. Moreover, micelles were retained longer in the joints, leading to reduced paw thickness and clinical arthritis index. A novel twin-drug of diclofenac and Dex was formulated into polylactide (PLA) NPs to improve their solubility and to provide a sustained release system [114]. *In vitro* release studies showed the controlled conversion into its parent drugs by hydrolysis using an esterase enzyme. However, despite the system showing enhanced anti-inflammatory activity *in vivo*, more studies regarding its pharmacokinetics and biodistribution are needed to conclude about their efficacy. Dex was also loaded into GE11-PLGA NPs to be specifically uptaken by EGFR-overexpressed FLS in RA [115]. Even though *in vitro* studies confirmed the active internalization of the NPs in EGFR-overexpressing cells [116], *in vivo* studies are required to validate this hypothesis. A synovium-specific targeted liposomal DDS was produced by conjugating the targeting peptide HAP-1 to the surface of long-circulating PEGylated liposomes encapsulating prednisolone [117]. The DDS displayed 10-fold increased accumulation in affected joints compared to healthy joints, and improved drug therapeutic index in an AIA rat model. Triamcinolone acetonide (TA), a corticosteroid, was encapsulated into biodegradable PLGA MPs (FX006, Flexion Therapeutics) to maintain therapeutic concentrations of the drug in the arthritic joint over a period of months [118]. In an OA rat model, the sustained release provided by FX006 significantly prolonged analgesia and improved histological scores in comparison with the free

drug, and without adverse effects. Phase II and II/III clinical trials showed prolonged and amplified analgesic effect, providing a sustained clinically meaningful pain relief and functional improvement in patients with knee OA, while substantially reducing systemic exposure after IA injection [119–121]. In order to control the TA release in the joints, an arthritis flare-responsive hydrogel platform was also produced by self-assembling the drug with an amphiphilic small-molecule, triglycerol monostearate (TG-18) [122]. The hydrogel disassembly and hence the drug release was controlled by the concentration of enzymes expressed during arthritis flares, such as MMPs and other tissue-degrading enzymes. Even though the TA-loaded TG-18 hydrogel reduced arthritis activity in the injected paw, the injections were performed subcutaneously. Therefore, in order to determine the biocompatibility, efficacy and the residence time in the joints, an IA injection should be performed.

For DMARDs delivery, dextran sulfate-graft-methotrexate conjugate (DS-g-MTX) micelles were developed, with excellent target ability to activated macrophages [123]. DS-g-MTX micelles showed significantly higher accumulation in the inflamed joints and stronger anti-inflammatory effect than the free MTX and the Dextran-g-MTX. In addition, DS-g-MTX efficiently inhibited the expression of pro-inflammatory cytokines, leading to significant relief of synovitis and protection of articular cartilage in collagen-induced arthritis (CIA) mice. Folate-modified dextran-methotrexate conjugate micelles (noted as Dex-g-MTX/FA) were developed for targeting delivery to macrophages [124]. The micelles shown higher cellular uptake mediated by the folate receptor and higher cytotoxicity toward lipopolysaccharide-activated macrophages. Moreover, Dex-g-MTX/FA possessed improved biodistribution at the lesion site and stronger inhibition of pro-inflammatory cytokines, which significantly suppressed the synovitis and effectively protected the articular cartilage. In another study, MTX loaded into sialic acid-dextran-octadecanoic acid (SA-Dex-OA/MTX) micelles considerably improved accumulation and transport to arthritic paws presenting a high expression of E-selectin [125]. In a CIA rat model, the micelles significantly inhibited the inflammatory response, diminished the adverse effects of MTX, and increased the bone mineral density.

Antibodies were also incorporated in DDS to increase their therapeutic efficacy. Certolizumab pegol (Cimzia®) is a licensed anti-TNF- α antibody fragment approved for the treatment of adult patients with moderately to severely active RA [126]. The attachment of the PEG moiety to the Fab fragment increases the plasma half-life to approximately 2 weeks, and the lack of the Fc region avoids the potential complement activation [127]. Therefore, the treatment with the polymer-antibody conjugate significantly increased physical function and reduced RA signs and symptoms, including pain and fatigue. Hyaluronate/gold (Au)NP/Tocilizumab (HA-AuNP/TCZ) complex was developed to synergistically target the vascular endothelial growth factor (VEGF), since AuNPs have angiogenic effects, and the IL-6 receptor (TCZ is a humanized monoclonal antibody against IL-6 receptor) [128]. While *in vitro* results confirmed the simultaneous antiangiogenic and anti-inflammatory effects of the dual targeting, *in vivo* results using a CIA mouse model only showed anti-inflammatory therapeutic efficacy. In another study, the anti-IL-6 antibody was

immobilized at the surface of chitosan-hyaluronic acid NPs, intended for IA administration and allowing the capture and neutralization of the pro-inflammatory cytokine IL-6 in arthritic joints [129]. Although in an *in vitro* inflammatory scenario the DDS clearly exhibited prolonged action and stronger efficacy than the free antibody, *in vivo* studies are needed to confirm the efficacy associated with the long-lasting treatment.

Another strategy to circumvent the side-effects and reduce the dosage is the topical delivery. However, the drug penetration is limited due to the highly effective barrier of the human skin. Moreover, topical delivery of the drug clearly depends on temperature. For topical application, thermoresponsive nanogels (tNG) was developed and successfully encapsulated an anti-TNF α fusion protein, namely etanercept [130]. The application of this DDS to inflammatory skin equivalents or tape striped human skin resulted in an efficient antibody delivery throughout the *stratum corneum* (SC) and into the viable epidermis, which correlated with the high anti-inflammatory effects obtained.

Despite gene therapy having shown promising therapeutic benefits in animal models of arthritis, there is an unmet need to develop DDS that has high encapsulation efficiency and minimum burst release, and even more importantly, that can target inflamed tissues after intravenous administration. A recent study reports the encapsulation of TNF- α -siRNA into solid-lipid NPs composed of biocompatible lipids such as lecithin and cholesterol, and an acid-sensitive stearic acid-PEG hydrazide conjugate (PHC) [131]. In both CIA and collagen antibody-induced arthritis (CAIA), an RA model that do not respond to MTX, the NPs increased the delivery of the siRNA into chronic inflammation sites, reducing paw thickness, bone loss, and histopathological scores. Another approach using tuftsin-decorated alginate NPs encapsulating the anti-inflammatory cytokine, IL-10, plasmid DNA showed enhanced localization in the inflamed paws of arthritic rats upon intraperitoneal administration [132]. Notably, targeted NPs treatment successfully re-polarized macrophages from M1 to M2 sub-type and significantly reduced joint damage.

Natural polyphenols, such as curcumin (Cur), were extensively studied as therapeutic agents for various diseases due to their remarkable anti-inflammatory, antioxidant, antitumor and antimicrobial activities. A novel anti-RA approach composed of hyaluronic acid/Cur micelles was able to overcome the poor bioavailability of Cur, being also able to exert a lubricating action in the joints [133]. When IA injected in a complete Freund's adjuvant (CFA) and Col II RA rat model, the micelles significantly decreased the degree of edema and the expression of pro-inflammatory cytokines (TNF- α and IL-1) and VEGF, which resulted in a marked inhibition of the inflammatory response. Moreover, the friction between the cartilage surfaces of the joints was reduced, protecting the cartilage from further degradation.

3.3. Cancer

Bone cancer has its origin in bone (primary cancer) or in cancer cells that spread to this tissue from their original location (metastatic cancer). Although primary cancers in bone (e.g. osteosarcoma and Ewing's sarcoma) have a low prevalence (less than 0.2% of all cancers [134]), bone metastases are

a common complication of cancers, such as those of breast and prostate [135].

Osteosarcoma, the most common primary bone cancer and frequently diagnosed in young patients is characterized by the presence of malignant mesenchymal stem/stromal cells (MSCs) or MSCs-derived osteogenic progenitors [136]. Therefore, to target osteosarcoma initiating cells, lipid-polymer NPs functionalized with CD133 aptamers and incorporating all-trans retinoic acid were developed [137]. These NPs were more efficient and present a greater specificity in promoting the delivery of all-trans retinoic acid to CD133+ osteosarcoma cells than free all-trans retinoic acid and non-targeted NPs. Moreover, a high therapeutic efficacy in BALB/c nude mice bearing osteosarcoma xenograft was obtained for the functionalized NPs. Therefore, despite CD133 aptamer can target hematopoietic stem cells, which risk is considered by the authors minimal, the *in vitro* and *in vivo* results are promising. Another challenging in the osteosarcoma treatment is the drug resistance, which leads to the need for new drugs and treatment regimens [136]. The co-delivery of gemcitabine and clofazimine (with a recently recognized anti-cancer activity) by a single liposome formulation promoted higher cytotoxicity on bone cancer cells than liposomes encapsulating one of them. The cytotoxicity of clofazimine by apoptotic means and its synergistic activity with gemcitabine demonstrated the potential of this novel combination. A redox-sensitive liposome with bone- and CD44-targeting moieties (alendronate and hyaluronic acid, respectively) was developed for doxorubicin delivery in the intracellular compartment of osteosarcoma cells [138]. The developed formulation presented a higher *in vitro* cytotoxicity and a superior efficacy in an orthotopic human tumor mouse model than the controls (free drug and liposomes functionalized with hyaluronic acid or deprived of redox sensitivity). The dual-targeting liposome was also able to reduce the drug cardiotoxicity and lung metastases (typically associated with this primary bone cancer). Furthermore, the therapeutic effect was enhanced by the co-administration of internalizing-RGD (iRGD). This cyclic peptide facilitated the liposomes penetration into the dense ECM of the tumor, increasing consequently their efficacy. For gene therapy, a micellar nanosystem of Pluronic® L64 chemically conjugated to polyethyleneimine was developed for miRNA-145 delivery into osteosarcoma cells [139]. The non-viral vector allowed an efficient release of the genetic material in the cancer cells cytoplasm, and consequently, an inhibition on cells proliferation, migration, and invasion as well as an enhanced cell death by apoptosis and necrosis was observed.

To treat Ewing's sarcoma, the second most frequent bone cancer of childhood and adolescence [140], a functional plasmid DNA, namely bi-shRNA EWS/FLI1 (to target the most common fusion observed in this bone cancer), was encapsulated into a clinically tested liposome [141]. After *in vitro* confirmation of marked knockdown of Ewing's type 1 fusion protein, Ewing's sarcoma subcutaneous-implanted xenograft (SK-N-MC) mouse model was used. The inhibition of tumor growth, improved survival and safety was observed in a concentration-dependent manner. Reasonable tolerance was also observed in mini-pigs. The promising results led to the testing of the developed formulation in a clinical trial

(NCT02736565). In another study, the loading of irinotecan into a liposome also increased the antitumor activity in pediatric solid tumor xenografts compared with the free drug [142].

To treat bone metastasis, bone-targeting NPs for local MTX controlled release was developed [143]. In this work, PEG 2000 containing alendronate linked to each it ends and bounded to the surface of calcium phosphate NPs was able to bind bone fragments in an *ex vivo* assay. The investigation of the NPs release profile demonstrated that they can promote a faster release of chemotherapeutics in an acidic environment than in a physiological media. Moreover, the amount of MTX loaded into the NPs was able to inhibit cancer cells as when the drug is free. The cytocompatibility and *in vivo* biocompatibility of these NPs also indicate their potential. In another approach, to increase the therapeutic index of bortezomib, a boronate proteasome inhibitor, it was linked to cyclic RGD-targeted and pH-sensitive polyamidoamine dendrimers [144]. After tail vein injection in mice bearing bone tumors, the developed formulation presented a higher tumor accumulation than the non-targeted NPs. Moreover, high efficacy in the inhibition of bone tumor progression and osteolysis was obtained.

Chondrosarcomas are cartilaginous tumors, characterized by the production of hyaline cartilage [145,146]. Unlike Ewing and most osteosarcomas, these cartilage malignancies are typically diagnosed in persons between 40 and 70 years of age [147]. Moreover, despite the advantages of DDS, since 2014 to the best of our knowledge none approach was developed to treat chondrosarcomas. However, drugs have been chemically modified to target the cartilage ECM to increase their therapeutic index [148].

4. Bone and cartilage tissue regeneration

Despite the advances in surgical and pharmacological interventions over the last years, the repair of bone and cartilage tissues, and function is still a major challenge in the field of orthopedic medicine. Surgical interventions to restore tissue function include microfracture [149,150], osteochondral autografts and allografts [151–153] and arthroplasty [154–156]. However, all present limitations, such as formation of fibrocartilage [157], donor site morbidity [158], durability of the implant, residual pain, stiffness and/or recurrent swelling [154,155]. Over the past decades, tissue engineering approaches have also been developed to regenerate bone and cartilage tissues, for instance, autologous chondrocyte implantation (ACI) [159,160] and matrix-associated chondrocyte implantation (MACI) [161–164]. Those techniques also present limitations, such as graft hypertrophy and fibrosis, need of two surgical interventions and high costs. Therefore, efforts have been made to implement single-step surgeries by implanting cell-free biomaterials or enriched with stem cells and/or progenitor cells with positive results [165–168]. For instance, a scaffold of chitosan (BST-CarGel®) led to better results in terms of quantity and quality of cartilage repair than microfracture [169]. Among the different types of stem cells, MSCs gained particular interest in tissue engineering approaches, due to their differentiation capacity towards bone and cartilage (besides adipose) tissues in the presence of appropriate physical and/or chemical cues. Moreover, they

possess well-recognized immunomodulatory properties that can assist tissue repair/regeneration and are easily collected from the human body.

Bone grafts are usually based on biodegradable polymer composites [e.g. silk fibroin [170], PCL [171], gellan-gum [172], chitosan [173] and/or hyaluronic acid] with calcium phosphate materials (e.g. hydroxyapatite and tricalcium phosphate). The same polymers are also widely used to produce cartilage scaffolds (Figure 3). Moreover, for osteochondral applications, a gradient osteoconductive phase can be obtained by decreasing the level of porosity and calcium phosphate content from the base (bone side) to the superficial (cartilage side) surface as found *in vivo* [172]. Advances in tissue engineering were also made by the rational design of 3D structures with adequate structural cues (e.g. size, shape, and topography [174–178]) to allow recapitulating the damaged tissue developmental patterns. However, to push the forefront of tissue engineering it can be necessary to chemically modify the surface of the engineered grafts or to enrich them with different substances, such as drugs (e.g. antibiotics) or cell signaling molecules (e.g. cytokines and growth factors), to influence the endogenous or exogenous stem or non-stem cells (e.g. endothelial cells and immune cells) function and fate. Indeed, the enrichment of the engineered structures with bioactive molecules can avoid the use of cells and consequently their inherent disadvantages (e.g. high costs and stringent regulatory processes). Drugs can be included or linked as free entities or after their incorporation in delivery systems. The porosity, swelling, erosion, and biodegradability of the 3D structures, which should be coordinated with the rate of tissue growth, and/or the facility to enzymatically or hydrolytically break the bonds can allow a higher or lower release of the incorporated drug loaded or not into delivery devices. Moreover, different drugs can be included and

released concomitantly or sequentially, for example, to govern stem cell differentiation (e.g. TGF- β 3, BMP-6, and Dex) and to increase vascularization and angiogenesis (e.g. VEGF). This enhancement of the scaffolds or hydrogels functionality will allow a local drug release of adequate concentrations of the bioactive molecules during a desired period of time to support, accelerate and assist the recovery of damaged tissues in a much more effective way. Overall, the combined strategies allow modulating the cell proliferation and differentiation as well as the immune response, avoiding immunorejection and implant failure. An example of a delivery matrix for large weight-bearing bone defect repair proposes a hydroxyapatite and PCL scaffold produced by 3D printing, being its pores loaded with a thermosensitive PLGA-PEG-PLGA hydrogel containing two cytokines, namely VEGF-165 and BMP-2 [179]. The sustained release over time of the two bioactive factors as well as their synergistic effects were demonstrated. Moreover, the produced biomimetic bone was able to repair the defect in rabbits with an efficacy similar to autogenous bone graft. Another cell-free approach to regenerate critical-sized bone defects consisted of nanofibrous polymeric scaffolds functionalized with microspheres incorporating polyplexes carrying miRNA-26a [180]. Besides the spatial and temporal control, this approach also enabled a two-stage delivery of miRNA (first from the polymeric microspheres and then from the polyplexes). The developed approach efficiently targeted the glycogen synthase kinase-3 β (Gsk-3 β), enabling the prolonged expression of several osteogenic genes at therapeutic levels. Therefore, the repair of critical-sized calvarial defect in osteoporotic mice was observed.

Osteomyelitis is a common complication of the implantation of 3D structures, thus efforts have been made to develop engineered structures loading antibiotics to be released at the site of infection [181–183]. For instance, vancomycin, an antibiotic used in the treatment of methicillin-resistant *Staphylococcus aureus*, was loaded in silk fibroin NPs and afterwards in silk fibroin scaffolds [184]. After 6 weeks, the proposed strategy promoted a higher reduction of the infection at the defect site in a severe osteomyelitis rat model than the untreated control or when scaffolds and NPs were used alone. Instead of vancomycin, gentamicin was loaded into PLGA-PEG MPs that were further incorporated in a bone graft [185]. *In vitro* studies showed the potential of the developed 3D structure to counteract the infection. Local delivery and increased therapeutic efficacy of gentamicin were also advanced by its loading in an injectable thermosetting composite scaffold of chitosan and bovine bone substitutes using beta-glycerophosphate as cross-linker [186]. *In vitro* studies demonstrated a synergistic activity of chitosan and antibiotic and a bactericidal effect for 24 h. Gentamicin was also included in a thermo-responsive hydrogel of hyaluronic acid-poly (N-isopropylacrylamide) [187]. The efficacy of this strategy was evaluated and well demonstrated in a rabbit model of osteosynthesis presenting a *Staphylococcus aureus* infection.

Regarding cartilage, severe defects are challenging to self-repair due to the avascular, aneural and a lymphatic nature of this tissue. Therefore, several approaches were developed to facilitate cartilage repair. Nel-like molecule-1 (Nell-1) growth factor loaded in chitosan NPs was incorporated into

Figure 3. Diagram representing the flexible tailoring of novel tissue-engineered strategies aiming drug delivery.

electrospinning nanofibers organized in oriented and large-sized scaffolds [188]. The incorporation of the Nell-1 into chitosan NPs extended the bioactivity of the growth factor than its mere incorporation into the electrospinning nanofibers. Moreover, *in vitro* studies shown that hBMSCs chondrogenic differentiation and ECM production is enhanced in the presence of Nell-1, demonstrating the potential of this strategy for cartilage tissue engineering. A PCL shell of coaxial electrospun fiber scaffold was also developed to co-deliver a bone marrow-derived (B)MSC-affinity peptide (E7) and the rhTGF- β 1 [189]. The functionalized scaffold was able to enhance BMSCs adhesion and proliferation and to promote their chondrogenic differentiation, presenting the characteristics required for cartilage engineering. Afterwards, E7 peptide was used to functionalize a biphasic scaffold platform of demineralized bone matrix prefunded with a chitosan hydrogel to assist microfracture procedure *in vivo* [190]. It was able to enhance quantitatively and qualitatively the cartilage repair comparatively to controls. An enzymatically degradable (using an MMP-degradable peptide sequence) and TGF- β 1-functionalized PEG hydrogel were used to co-encapsulate chondrocytes and MSCs in a ratio of 8:1 [191]. These cellularly- and locally degraded materials promoted higher production of ECM as well as of constructs with improved mechanical properties for cartilage tissue engineering applications than their counterpart, but non-degradable constructs. In another study, the combination of BMSCs with insulin-like growth factor-1 (IGF-1) and TGF- β 1 into laminin gel scaffolds demonstrated a higher efficacy in the formation of hyaline cartilage in an osteochondral defect in a rabbit model than the cells alone or in combination with one growth factor [192]. The efficacy of Y27632 [(1R,4r)-4-((R)-1-aminoethyl)-N-(pyridin-4-yl) cyclohexanecarboxamide] was compared with the TGF- β 3 after their incorporation in water-based 3D printing scaffolds of polyurethane elastic NPs and hyaluronan [193]. The timely release of the bioactive factors allowed the chondrogenesis of MSCs organized in self-clusters within the 3D-printed scaffolds. Moreover, the transplantation of the MSCs-seeded scaffold containing Y27632 was effective in regenerating rabbit cartilage defect. Therefore, this work demonstrated the potential of this strategy in customizing tissue engineering avoiding the use of growth factors and their inherent disadvantages (e.g. to be expensive and can cause hypertrophy). To improve cartilage/bone tissue regeneration, radially oriented collagen scaffolds incorporating stromal cell-derived factor-1 (SDF-1) to enhance cell homing were developed [194]. The efficacy of these scaffolds in promoting cartilage repair in osteochondral defects in rabbits was higher than the chemokine-free scaffold and random scaffolds with or without SDF-1.

The lesions of cartilage are usually extended into the subchondral bone and consequently, efforts have been made to develop engineered structures to regenerate both tissues simultaneously. An example of a drug release scaffold to treat osteochondral defects is composed by alginate, chitosan, β -tricalcium phosphate and Dex sodium phosphate [195]. After implantation of the biomimetic monolithic three-layered scaffold into the defects formed in the trochlea of Sprague–Dawley rats, the biocompatibility and higher efficacy in osteochondral healing than the one obtained in the

control group (defect filled with MaioRegen®) were demonstrated. Cartilage/bone tissue regeneration was also promoted by the preparation of bilayered oligo(poly(ethylene glycol) fumarate) (OPF) composites incorporating gelatin MPs loaded with IGF-1 into the chondral layer and with BMP-2 into the subchondral layer [196]. Besides this 3D structure, other two scaffolds were tested, one containing only IGF-1 in the chondral layer and the other presenting solely BMP-2 in the subchondral layer. The implantation of these three scaffolds with spatially controlled distribution of growth factors in the medial femoral condyle osteochondral defects in rabbits demonstrated minimal differences for cartilage repair at 12 weeks post-implantation. However, for bone regeneration, the spatial controlled delivery of the two growth factors synergistically enhanced the degree of subchondral bone formation, demonstrating the potential of that scaffold for osteochondral tissue repair. An osteochondral tissue engineering strategy to produce functional cartilage and subchondral bone tissue comprised the development of a gene-activated matrix (plasmid DNA encoding BMP2 and TGF- β 3 were included in the cartilage and bone layers, respectively) to promote the transfection of hMSCs [197]. The bilayer scaffold was composed by type I collagen and hydroxyapatite for the subchondral bone layer, and type II collagen for the cartilage layer. After enrichment of these layers with NPs presenting a calcium phosphate core and DNA/calcium phosphate shells conjugated with polyethyleneimine, they were crosslinked with transglutaminase. The bilayer scaffold was able to promote prolonged transgene expression and enhanced hMSCs osteogenic and chondrogenic differentiation. Despite its potential, *in vivo* studies are needed to clearly demonstrate the benefits of the developed enzyme-crosslinked gene-activated matrix in osteochondral healing. Instead of plasmid DNA encoding growth factors, DNA encoding transcription factors, namely Runt-related transcription factor 2 (RUNX2, to induce osteogenic differentiation) and SRY (sex-determining region Y)-box 5, 6, and 9 (the SOX trio, to induce chondrogenic differentiation) were used to induce osteoarticular tissue regeneration [198]. After their complexation with the branched poly(ethylenimine)-hyaluronic acid, they were loaded into the osteogenic or chondrogenic layers, respectively, of a porous oligo[poly(ethylene glycol) fumarate] hydrogel scaffold crosslinked with carboxymethyl cellulose particles. The implantation of the bilayered scaffolds with a spatial controlled distribution of both plasmids DNA in a rat osteochondral defect was more efficacious to improve the quantity and quality of the generated tissues than empty hydrogels or either transcription factor alone. An anti-inflammatory cell-free scaffold was also developed to repair osteochondral defects [199]. Polyacrylic acid was used to graft resveratrol and then scaffolds were produced by its inclusion into atelocollagen hydrogels presenting a compressive strength comparable to normal cartilage. These scaffolds were able to promote the proliferation, maintain the phenotype and protect against reactive oxygen species of chondrocytes and BMSCs. The filling of a rabbit osteochondral defect with the anti-inflammatory scaffold down-regulated inflammatory-related genes (IL-1, MMP13, and COX-2) and up-regulated bone

and cartilage-related genes (SOX-9, aggrecan, Coll II and Coll I). Moreover, the repair of the osteochondral defect in rabbit joint was observed after 12 weeks of implantation, demonstrating its potential in this field of tissue engineering.

5. Conclusion

Drug delivery strategies enhance the therapeutic index of the drugs and, consequently, can significantly improve the effectiveness of the therapeutic agents to treat bone and cartilage diseases. However, despite the huge advances obtained in early preclinical studies, the clinical successes are still limited.

Biodegradable polymers play a crucial role in the development of innovative structures to act either as carriers or tissue-engineered structures. These polymers have the advantage of being susceptible to enzymatic or hydrolytic degradation *in vivo* into non-toxic products that can be cleared by the normal excretion routes of the body. The functionalization of the polymeric structures to target specific moieties of bone or cartilage is essential to increase their specificity and affinity for those tissues. Moreover, DDS, such as MPs, NPs, micelles, liposomes, and tissue-engineered structures should allow for a local and controlled drug release over time in relevant therapeutic concentrations. Despite the promising results in preclinical small animals, such as mice and rats, further investigation in large animal models and clinical trials are required to increase the translation of those strategies into clinical practice.

6. Expert opinion

Drug delivery strategies based on biodegradable polymers hold the promise of contributing to more effective treatments for bone and cartilage diseases. Indeed, the multidisciplinary expertise in nanotechnology, materials science, medicine, cell biology, and tissue engineering fields open new avenues and are revolutionizing the drug delivery field.

Ideally, DDS should improve the drugs therapeutic index by simultaneously (i) promoting targeted delivery to the diseased/injured tissues, (ii) providing an optimal control and sustained release over the needed period of time, and (iii) reducing undesirable side effects and/or toxicity of the therapeutic agents. To accomplish a precise target delivery, ligands such as BPs, oligopeptides, and aptamers were designed to increase the affinity of the DDS toward bone and/or cartilage tissues. On-demand drug release can be achieved by using stimuli-responsive polymers (e.g. pH, temperature, enzymes). Moreover, 3D biodegradable structures enriched with bioactive molecules enable designing advanced and effective bone and/or cartilage tissue engineering applications.

Notwithstanding all the progress in the field, most of the existing delivery vehicles present short-term release caused by the kinetics of the drug release mechanisms, namely diffusion or hydrolysis and/or have limited loading capacity. Moreover, the residence time in circulation after systemic administration is limited by opsonization (protein adsorption at DDS surfaces). This problem is widely prevented by PEGylation, but

this can have a negative impact on DDS internalization by the target cells (interference with particle–cell interactions). Additionally, the protective effect is lost after repeated administrations. Consequently, alternatives to PEGylation should be developed to overcome these limitations.

The incorporation of bone-targeting moieties into DDS increased the drug accumulation and retention at the site of action in many preclinical studies. Bone vascularization allows using systemic administration of targeted DDS to reach specifically this tissue. Conversely, the avascular, highly dense anionic ECM and small pore size of the cartilage make drug delivery and diffusion very difficult. In recent years, local administration of DDS has attracted great interest to improve drug retention in the synovial cavity. However, due to the rapid turnover of the synovial fluid, it still needs improvements to become a long-lasting therapeutic strategy for cartilage diseases. Therefore, future direction involves the design of DDS with higher capacity to attach and/or permeate through cartilaginous tissues. Albeit the advances in drug delivery for several skeletal diseases, there are many others that still need to be addressed in this field. Indeed, only few drug delivery strategies can be found in the literature for, e.g. inherited systemic skeletal dysplasia, intervertebral disc calcification, heterotopic ossification, and Paget's disease. Therefore, researchers should take into consideration recent discoveries regarding the targets and molecular mechanisms behind those diseases and design DDS capable of increasing the efficacy of therapeutic agents and minimizing off-targeted delivery.

Advanced *in vitro* studies, including bioreactors and co-cultures of different and several cells, are needed to be more representative and predictive of the performance of the DDS *in vivo*. Despite the importance of rodents, due to the genotypic similarity, relatively inexpensive, easy to handle and an essential preliminary assessment, confirmation of efficacy in large animal models are frequently required. Indeed, many clinical trials were suspended due to safety and/or efficacy issues. Therefore, animals presenting a high similarity to humans, such as humanized animal models or animals presenting biomechanics and anatomy similar to human are needed to provide a better extrapolation to the human scenario and a high chance of success in clinical trials. When designing DDS researchers should make a compromise between innovative concepts (to achieve smart-responsive, controlled and highly efficient delivery) and simple and easy of use systems to have higher possibilities of clinical application.

In summary, considering the great amount of promising DDS in preclinical studies, with the main ones discussed in this review, it is foreseeable that in future years an increased number of DDS will enter the clinics in order to alleviate current treatment limitations and, more importantly, to radically improve the safety and efficacy of drugs when administrated in patients.

Acknowledgments

Authors acknowledge the financial support from FCT (Portuguese Foundation for Science and Technology) for the project PTDC/CTM-BIO/4388/2014 – SPARTAN, the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER) (NORTE-01-0145-FEDER-000023 FRONThERA) and the NORTE 2020 Structured Project, cofunded by Norte2020 and Horizon 2020 by the contract number H2020-NMBP-PILOTS-2016 721062 Project Flexpol.

Authors would also like to acknowledge FCT/MCTES (Ministry of Science, Technology and Higher Education) and the FSE/POCH (European Social Fund through the Operational Program of Human Capital), for the PhD scholarship PD/BD/11384/2015 of A. C. Lima (PD/59/2013).

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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