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Review article

Drug delivery in intervertebral disc degeneration and osteoarthritis: Selecting the optimal platform for the delivery of disease-modifying agents

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A B S T R A C T

Osteoarthritis (OA) and intervertebral disc degeneration (IVDD) as major cause of chronic low back pain represent the most common degenerative joint pathologies and are leading causes of pain and disability in adults. Articular cartilage (AC) and intervertebral discs are cartilaginous tissues with a similar biochemical composition and pathophysiological aspects of degeneration. Although treatments directed at reversing these conditions are yet to be developed, many promising disease-modifying drug candidates are currently under investigation. Given the localized nature of these chronic diseases, drug delivery systems have the potential to enhance therapeutic outcomes by providing controlled and targeted release of bioactives, minimizing the number of injections needed and increasing drug concentration in the affected areas. This review provides a comprehensive overview of the currently most promising disease-modifying drugs as well as potential drug delivery systems for OA and IVDD therapy.

Abbreviations: AC, Articular cartilage; ACI, Autologous chondrocyte implantation; ACLT, Anterior cruciate ligament transection; ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; AF, Annulus fibrosus; ASOs, Antisense oligonucleotide; BMP, Bone morphogenetic protein; CMC, Critical micelle concentration; COX-2, Cyclooxygenase-2; DMOADs, Disease-modifying drugs for OA; ECM, Extracellular matrix; FGF-18, Fibroblast growth factor 18; GAG, Glycosaminoglycan; GDF-5, Growth and differentiation factor 5; GI, Gastrointestinal; HA, Hyaluronic acid; IGF-1, Insulin-like growth factor 1; IL, Interleukin; IL-1Ra, IL-1 receptor antagonist; iNOS, Inducible nitric oxide synthase; IVD, Intervertebral disc; IVDD, Intervertebral disc degeneration; LBP, Low back pain; MIA, Monoiodoacetate; miRNAs, Micro RNAs; MMPs, Metalloproteinases; mRNA, messenger RNA; MSCs, Mesenchymal stromal cells; NGF, Nerve growth factor; NO, Nitric oxide; NP, Nucleus pulposus; NSAIDs, Non-steroidal anti-inflammatory drugs; OA, Osteoarthritis; PAMAM, Polyamidoamine; PCL, Polycaprolactone; PEA, Polyester amide; PEG, Poly(ethylene-glycol); pHPMAmLacn, Poly(N-(2-hydroxypropyl)methacrylamide-lactate); PLA, poly(lactic) acid; PLGA, Poly(lactic-*co*-glycolic acid); PRP, Platelet-rich plasma; SDF-1α, Stromal cell-derived factor-1 alpha; siRNAs, Small interfering RNAs; TAA, Triamcinolone acetonide; TCP, Tricalcium phosphate; TIMPs, Tissue inhibitors of metalloproteinases; TNF-α, Tumor necrosis factor alpha; WOMAC, The Western Ontario and McMaster Universities Osteoarthritis Index

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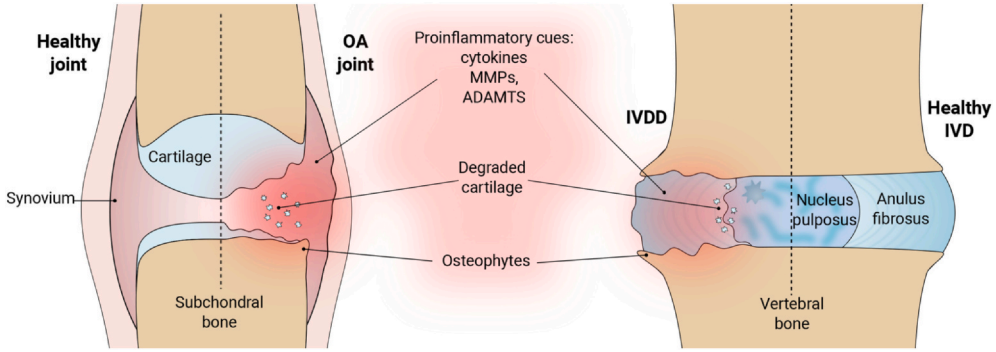

Fig. 1. Schematic representation of healthy joint and IVD and the main pathological and morphological changes in OA and IVDD.

1. Introduction

Musculoskeletal diseases are a major cause of disability and morbidity worldwide [1,2]. Osteoarthritis (OA) and low back pain (LBP) associated with chronic and progressive degeneration of articular cartilage and intervertebral discs (IVDs), respectively, account for more than 50% of patients with musculoskeletal diseases [1]. For early stages, current treatments are generally focused on pain management, while surgical intervention is often needed for late stage disease. Despite the extensive and compelling preclinical evidence on the efficacy of different therapeutic molecules (*i.e.* growth factors and cytokine inhibitors), to date, no disease modifying treatments aiming at tissue repair and regeneration are available [3,4]. The clinical trials carried out so far have shown disappointing results, as the drugs showed only short-lived to no beneficial effects [4]. This is mainly attributed to the heterogenous and multifactorial profile of the diseases, as different tissues and different pathways, from inflammation to degeneration, are involved in their pathophysiology [3–5]. Equally important, the short half-life of the bioactives within the joint and the disc limits the duration of their therapeutic activity, hence decreasing efficacy [4,6,7]. In this regard, drug delivery systems might play a crucial role as part of novel therapeutic strategies due to their capacity to incorporate different types of drugs, tunable release profiles and targeting ability. By concentrating and prolonging the presence of the drugs in the tissues, these systems might contribute to improved drug efficacy and therapeutic effect [6,7].

This review focuses on state-of-the-art bioactives targeting joint degeneration, and current developments on drug delivery systems that can be used to enhance their efficacy.

2. Synovial joints and intervertebral discs: histological, biochemical and physio-pathological features

Synovial joints and IVDs are crucial tissues for body movement and shock absorption due to their unique properties of load distribution, gliding and wear resistance. Articular cartilage is located at the end of long bones in synovial joints, which in turn are together by ligaments and a dense fibrous connective tissue forming the articular capsule [8,9]. Lining the inner part of the synovial capsule is the synovial membrane, which is crucial for maintaining joint homeostasis [10]. The IVDs are composed by two different tissue types: an outer lamellar structure called annulus fibrous (AF) enclosing an inner gelatinous structure called nucleus pulposus (NP) which acts as a shock absorber. At their upper and lower side, IVDs are limited by cartilaginous endplates and the vertebral bodies [11,12]. In the AC, chondrocytes can assume different morphologies whether they are located in the superficial, middle, deep or calcified layer of the cartilage [8,9]. In the IVDs, the fibrocartilaginous AF contains fibroblast-like and spindle-shaped cells, whereas the NP contains rounded chondrocyte-like cells [12].

In the AC, collagen type II represents 90–95% of total collagen fibers and provides resistance to tensile loads. In addition, the collagen network also contains collagen types I, III, IV, V, VI, IX, XI [9,13]. Proteoglycans are also a key component of cartilage matrix due to their highly charged nature, which attracts and retains water within cartilage (65–80% wet weight), enabling resistance against compressive forces and mechanical loading [9,14]. Cartilage matrix also contains a small amount of other non-collagenous proteins such as lubricin and elastin, which are necessary to reduce friction during load bearing articulating activities and provide elasticity to the matrix, respectively [8,9]. In IVDs, the external part of the AF is rich in collagen type I, while collagen type II and proteoglycans are found in the inner NP tissue. Both articular cartilage and IVDs are characterized by an absence of vascular, neural and lymphatic networks, except for the outer AF which contains a limited number of blood vessels and nerves [5,9]. Cartilage nutrition depends on the diffusion of nutrients present in the synovial fluid and basal subchondral bone (bone marrow) and on compression and relaxation cycles of the tissue [9,15], whereas IVDs rely mainly on diffusion through the cartilaginous endplate [16,17]. The lack of vascular networks contributes to the lack of regenerative capacity of these tissues, and additionally makes them less accessible to systemically administered drugs.

Not only the risk factors for OA or intervertebral disc degeneration (IVDD) are similar (*i.e.* trauma, aging, obesity, and abnormal mechanical stress) [5,18], but also the pathological evolution of both diseases share common aspects. During the development of disease, the cells undergo a phenotypic switch which leads to disruption of tissue homeostasis and impaired extracellular matrix (ECM) turnover [18,19], accompanied by low grade inflammation [20,21] (Fig. 1). Cells form clusters [9,18,22], their balance in collagen production switches from collagen II to collagen I, concomitant with the upregulation of matrix-degrading proteinases, such as metalloproteinases (MMPs) and aggrecanases, and inflammatory cytokines. Hypertrophic differentiation is observed at later stages and, ultimately, cells undergo apoptosis [18,22,23]. This abnormal response results in a decreased content of proteoglycans, and collagen type II, increased collagen I, and loss of water. Macroscopically, fibrillation of the cartilage surface and fibrotic changes in the NP are observed. In the AF, the shear stress caused by tissue degeneration stimulates fibrosis and production of nitric oxide by resident cells [24]. The perpetuation of such pathological changes and the progression of degeneration lead to inflammation, stiffness and pain [18]. In IVDD, pain is likely induced by neovascularization and spreading of sensory nerves into the endplate and inner annulus [25–27]. With disease progression, increased tissue calcification occurs. Although in OA vascular penetration into the decalcified layer is seen [9,18], in the degenerating IVD the number of capillary buds in the endplate is reduced [28]. Additionally, cellular senescence has been proposed as a mechanism involved in both OA and IVDD [23,29]. Senescence, which is a stress-response mechanism, is characterized by cell cycle arrest, resistance to apoptosis and a pro-inflammatory phenotype [29,30]. Senescent cells have been found to be more prevalent in OA

cartilage and IVDD than in healthy tissue [31]. Additionally, in the joint, senescent cells can also be found in other tissues such as the synovium, a structure that plays a major role in OA development and progression [29,30].

In sum, OA and IVDDs involve cell phenotype changes that, by promoting inflammation and matrix degradation, drive tissue and joint degeneration. Therefore, there is an urgent need for the development of novel therapeutic strategies aimed at blocking disease progression and/or promoting tissue regeneration.

3. Clinical approaches for treatment of OA and IVDD: the old and the new

First-line therapies for OA and IVDD generally aim at relieving pain and improving function, with a combination of analgesic pharmacological and non-pharmacological treatments. Non-pharmacological approaches include exercise and physiotherapy [32–34]. Pharmacological therapy for OA or IVDD is largely represented by analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), and opioids [33–35]. The administration of oral analgesics, such as paracetamol, represents the first therapeutic solution to control mild pain [35,36]. However, paracetamol is often not effective, leading physicians to prescribe NSAIDs (*e.g.* ibuprofen) at the lowest effective dose and for a limited time, due to the risk of side effects in the gastrointestinal (GI) tract and antiplatelet activity [37–39]. Alternatively, in OA, cyclooxygenase (COX)-2 selective NSAIDs were shown to have the anti-inflammatory and analgesic efficacy of traditional non-selective NSAIDs, with significantly reduced GI-related side effects [40]. However, concerns on the cardiovascular effects of COX-2 inhibitors have been raised [41,42]. In disc degeneration and low back pain, muscle relaxants are sometimes used to control non-specific musculoskeletal pain [43–45]. Opioids are used as an alternative to NSAIDs for both OA and IVDD related back pain [35,36,46], yet this carries a significant risk of side-effects and addiction [37,47]. Local injection of steroids and corticosteroids has also been used in the clinic for pain management of moderate-to-severe pain, although much more frequently in OA [39,48–50]. Also here, adverse reactions such as tissue injury by repeated injections, infections and stimulation of inflammation by crystallized corticosteroids can occur [38].

Intra-articular hyaluronic acid (HA) injections are frequently used for symptom relief in OA in an attempt to restore the viscoelasticity of the synovial fluid [38,51]. The alleged analgesic effect of HA has been shown to be dependent on its molecular weight, with higher molecular weight possibly producing more effective and durable effects [52]. Platelet-rich Plasma (PRP) injection is another intra-articular therapy recently introduced as an experimental treatment for OA [53,54]. PRP is a preparation of concentrated blood plasma with increased platelet concentration, growth factors and other mediators [53]. It has been suggested to have anti-inflammatory effects and to reduce pain [107], and efficiency has been suggested to be higher and more uniform than HA [54,55]. However, the Osteoarthritis Research Society International has recently officially recommended against the use of PRP “because the evidence in support of these treatments is of extremely low quality” and “formulations themselves have not yet been standardized” [56]. The latter is related to the myriad of procedures to prepare PRP [53,56].

When pain management is no longer effective, invasive surgical interventions are necessary, such as end-stage total joint replacement for OA [34] and spinal fusion for disc degeneration [57]. Unfortunately, prostheses have a limited lifetime and revision surgeries have a much higher risk of failure, posing problems for younger patients. Additionally, analogous to the treatment of traumatic cartilage defects [58], autologous chondrocyte implantation (ACI) was tested in patients with knee OA, with a significant clinical improvement after a 5-year evaluation period [59]. However, this technique would be only applicable to patients with small sized and limited number of lesions. Additionally, the population followed in this study was relatively young [59]. Altogether, even though these surgical approaches are effective at reducing pain, their efficacy is often suboptimal in terms of stability and integration, thus failing to restore function [60].

The increasing prevalence of both OA and IVDD as well as the lack of optimal treatment represents a major socio-economic burden worldwide, and therefore strongly calls for more effective therapeutic solutions. In the coming years, the identification of new therapeutic targets followed by the development of disease-modifying drugs for OA (DMOADs) and IVDD aiming at restoring tissue quality and function will be crucial.

The abovementioned treatments focus mainly on symptom treatment rather than on reducing, halting or even reversing disease progression [61,62]. An ideal disease-modifying agent should focus on either inhibiting catabolic pathways or stimulating repair and regeneration [63,64]. However, and despite their enormous potential, to date no disease-modifying agents have been approved for OA or IVDD, owing to their side effects when administered systemically, short half-life in the tissue when injected locally, and ultimately, their lack of efficacy [51,65]. Some of the DMOADs and disease-modifying drugs for IVDD being tested in clinical trials are summarized in Table 1 and Fig. 2 and described in the next sub-chapters.

3.1. Antibodies and cytokine inhibitors

Therapies using monoclonal antibodies and cytokine inhibitors have been the most tested strategies so far. A monoclonal antibody raised against nerve growth factor (NGF), called Tanezumab, was shown to be effective in reducing pain in hip and knee OA [66]. However, serious adverse events were noted, including knee osteonecrosis, rapid progression of OA, and increased incidence of total joint replacement. Although side effects were mainly observed when combined with NSAIDs, some clinical trials have come to a halt [66–68]. Another similar clinical trial administering Tanezumab subcutaneously showed significant function and pain improvements in patients with moderate to severe hip or knee OA, yet more adverse events and total joint replacements were observed [69]. Conflicting results were also obtained with tumor necrosis factor alpha (TNF-α) inhibition. Intra-articular administration of Adalimumab, an antibody against TNF-α, was shown to improve pain and function scores in a trial in knee OA [83], but did not have any therapeutic effect in erosive hand OA [84]. Similarly, use of anti-cytokine therapy for low back pain as been investigated, yet the majority of trials concerned patients with sciatica associated with disc herniation, without any reference to disc degeneration [85]. Also here, the results of the trials are conflicting among each other. Several studies reported improvements in back and leg pain and function upon subcutaneous or epidural injection of Etanercept, a TNF-α selective inhibitor [74,86]. Yet, in two other studies Etanercept did not lead improved primary outcomes, leg pain and disability index, when compared to placebo or steroid-treated groups [87,88]. Whether these treatments are also able to promote disc regeneration remains unclear. Interleukin-1 (IL-1) has also been targeted in joint degeneration. Anakinra, a IL-1 receptor antagonist (IL-1Ra), did not lead to symptomatic improvements compared to placebo in a 3-month follow up clinical study for OA [70]. Likewise, in a study where AMG 108, an antibody against IL-1 receptor type 1, was administered IV or subcutaneously in OA patients, no beneficial clinical effects were observed when compared to the placebo group at a 3-month follow-up [71]. Interestingly, in a pre-clinical study conducted in arthritic mice, combined antibody-mediated inhibition of IL-17 and TNF-α showed improved cartilage protection when compared with single inhibition, showcasing the beneficial therapeutic effect of multi-target blockade [89]. Similarly, combined inhibition of IL-1α and IL-1β showed improved cartilage protection when compared to single inhibition in a mouse model of OA [90]. Nevertheless, in a clinical trial where patients with knee OA were bi-weekly treated subcutaneously with Lutikizumab, a bispecific

Table 1
Pre-clinical disease modifying drugs for OA and IVDD.

Class	Name/description	Disease	Phase	Main Results	References / clinical trial no
Anti-cytokine therapy	Intravenous administration of Tanezumab (Anti-NGF antibody)	OA	Phase II (completed)	Pain reduction and function improvement	NCT00394563 NCT00399490 [66,67]
	Intravenous and subcutaneous administration of Tanezumab (Anti-NGF antibody)	OA	Phase III (halted)	Study terminated due to adverse side effects such as worsening of OA and bone necrosis	NCT00994890 NCT01089725 [68]
	Subcutaneous administration of Tanezumab (Anti-NGF antibody)	OA	Phase III (completed)	Pain and function improvement in patients with moderate to severe OA, together with a higher prevalence of adverse events	NCT02697773 [69]
	Intra-articular injection of Anakinra (IL-1 receptor antagonist)	OA	Phase II (completed)	Anakinra was well tolerated however no improvements were observed compared to placebo group	NCT00110916 [70]
	Intravenous or subcutaneous administration of AMG 108 (anti-IL-1 receptor antibody)	OA	Phase II (completed)	Safety profile of AMG 108 was comparable to placebo, however no statistically significant improvements were observed	NCT00110942 [71]
	Subcutaneous administration of Lutikizumab (antibody against IL-1a and IL-1β)	OA	Phase II (completed)	No significant changes were observed in pain scores, synovitis and cartilage thickness	NCT02087904 [72]
	Subcutaneous administration of Lutikizumab (antibody against IL-1a and IL-1β)	Hand OA	Phase IIa (completed)	No improvements were observed in pain and imaging outcomes when compared to placebo	NCT02384538 [73]
	Epidural injection of Eternacept (TNF-α inhibitor)	IVDD – herniated disc	Phase II (completed)	Decrease of back and leg pain, and function improvement	NCT00364572 [74]
Growth factors	Intra-articular administration of BMP-7	OA	Phase I (completed)	BMP-7 was shown to have a safe profile, and a trend for symptomatic improvement was observed	NCT00456157 [75]
	Intra-articular injection of FGF-18 (sprifermin)	OA	Phase I (completed)	FGF-18 was shown be safe and non-toxic, but no significant improvement in cartilage thickness was observed. Pain reduction was higher in placebo group.	NCT01033994 [76]
	Single or double intradiscal injection of rhGDF-5	IVDD	Phase I and II (completed)	No results available	NCT01158924 NCT01182337 NCT01124006 NCT00813813
Enzyme inhibitors	Oral administration of doxycycline, broad-spectrum MMP inhibitor	OA	Phase II (completed)	No effect on symptomatic OA; Increased risk of adverse events	NTR1111 [77]
	Oral administration of PG-116800, broad-spectrum MMP inhibitor	OA	Phase II (completed)	No function or pain improvement; Increased musculoskeletal toxicity	NCT00041756 [79]
	M6495, anti-ADAMTS-5 nanobody	OA	Phase I (completed)	No results available	NCT03224702 NCT03583346
	Oral administration of GLPG1972, ADAMTS-5 inhibitor	OA	Phase I (completed) and Phase II (undergoing)	No results available	NCT03311009 NCT03595618
	Oral administration of SD-6010, a selective iNOS inhibitor	OA	Phase II (completed)	The compound was well tolerated, yet no clinical benefit was obtained when compared to placebo	NCT00565812 [79]
	Single local injection of SD-6010, a selective iNOS inhibitor	OA	Phase III (recruiting)	No results available	NCT03928184
Others	Single local injection of SM04690, a Wnt pathway inhibitor	IVDD	Phase I (terminated)	No results available	NCT03246399
	Single local injection of SM04690, a Wnt pathway inhibitor	OA	Phase I (completed)	SM04690 displayed a safe profile, without evidence of systemic exposure	NCT02095548 [80]
	Single local injection of SM04690, a Wnt pathway inhibitor	OA	Phase II (completed)	SM04690 yielded improvement in pain and function measurements	NCT02536833 [81]
	Single local injection of SM04690, a Wnt pathway inhibitor	OA	Phase III (recruiting)	No results available	NCT03928184
	Intra-articular injection of UBX0101, a senolytic drug	OA	Phase I (completed)	No results available	NCT03513016
	Oral administration of calcitonin	OA	Phase III	No beneficial effects observed	NCT00486434 NCT00704847 [82]

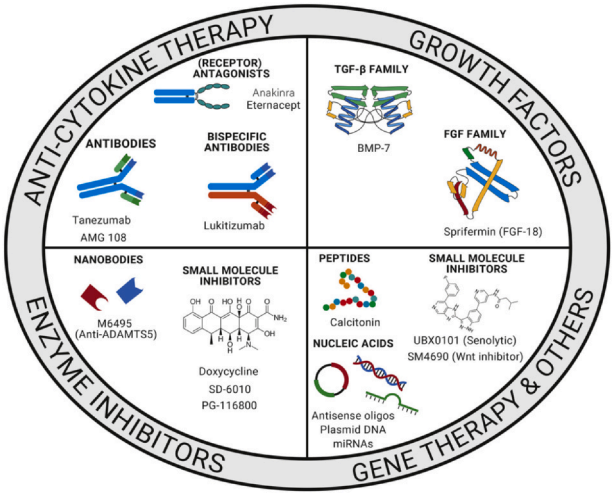

Fig. 2. Pre-clinical drugs that are currently in testing as disease-modifying agents for OA and IVDD therapy, ranging from cytokine/enzyme inhibitors to promoters of anabolic pathways.

antibody for IL-1a and IL-1β, no beneficial effects were observed in pain scores, synovitis or cartilage thickness over a 50-week period [72]. Similar results were observed in a clinical trial testing bi-weekly subcutaneous administration of Lutikizumab for the treatment of erosive hand OA [73]. Despite a decrease in serum levels of inflammatory markers, no significant pain or function improvements were observed when compared to placebo group [73].

3.2. Enzyme inhibitors

Another class of agents that has been proposed as therapeutics for degenerative joint diseases is the enzyme inhibitors, namely MMPs inhibitors [91]. These inhibitors are synthetic agents that mimic the role of endogenous tissue inhibitors of MMPs (TIMPs). Yet, despite promising preclinical data showing chondroprotective effects in OA [91–93], clinical trials have failed to show efficacy of these agents in humans so far and an association with severe side effects [4,78,79,94]. Oral administration of doxycycline, a general MMP inhibitor, did not have any effect on symptom reduction and was associated with more severe side effects in patients with OA [77]. Similar results were observed for oral administration of PG-116800, another broad-range MMP inhibitor [78]. No changes were observed in pain and function scores when compared to placebo group, yet there was an increased occurrence of musculoskeletal toxicity [78]. The side effects are likely associated with the broad spectrum of inhibition of such molecules and the variety of roles MMPs play in several tissues throughout the body, together with the fact that the drugs have been mainly administered orally or systemically for long periods [4].

Currently, efforts are focused on the development of more specific MMP inhibitors, in particular for MMP-13, which is thought to be the most important MMP in OA cartilage [4,95–98]. Likewise, the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzyme family has an important role in cartilage degradation. M6495, a nanobody against ADAMTS-5 previously shown to be chondroprotective [99,100], is currently being tested in clinical trials for its safety, tolerability and pharmacokinetics upon subcutaneous injection (NCT03224702, NCT03583346). Additionally, the safety and efficiency of the small molecule inhibitor GLPG1972 targeting ADAMTS5 are being currently evaluated in Phase I and II clinical trials (NCT03311009, NCT03595618).

Inducible nitric oxide synthase (iNOS) has been linked to progressive degeneration in OA [101], and its inhibition led to decreased levels of catabolic effectors in a OA dog model [102]. An iNOS inhibitor has also entered a phase II clinical trial for treatment of knee OA, however, no functional benefit or pain decrease were observed when compared to the placebo group upon oral administration [79]. Calcitonin, a small peptide that was shown to have protective effects on cartilage and bone in preclinical studies [103–105], also failed to improve treatment outcomes in two phase III clinical trials carried out in OA patients [82]. In this study, the authors hypothesized the lack of efficacy could be derived, among others, from the low exposure to the compound [82].

3.3. Growth factors

Growth factors have also been proposed for treatment of knee OA. In a randomized, double-blind, placebo-controlled trial, bone morphogenetic protein 7 (BMP-7) was intra-articularly administered to knee OA patients, showing good tolerability and safety profiles [106]. Even though a symptomatic improvement was observed, no follow-up studies have been carried out so far. Based on preclinical studies in rabbit models of IVDD showing the potential of growth and differentiation factor-5 (GDF-5) [107] and BMP-7 [108,109] to restore disc height and structure, phase I and II clinical trials have been carried out testing the safety and efficacy of intradiscal injection of recombinant human GDF-5 and BMP-7. However, these trials have been discontinued for unknown reasons. In a placebo-controlled trial, fibroblast growth factor 18 (FGF-18 or sprifermin) was injected intra-articularly to knee OA patients in single or multiple doses over a 3-week period [76]. FGF-18 has been previously reported to exert anabolic effects on chondrocytes and cartilage [110,111]. After 1 year follow-up, while lower cartilage volume loss and increased joint width in the lateral compartment was reported in patients treated with FGF-18, higher pain relief was observed in the placebo group.

3.4. Gene and oligonucleotide therapy

Gene therapy offers unprecedented tools for the modulation of gene products involved in pathological and repair pathways [112,113]. As the exact causes behind OA remain unknown, gene therapy for OA focuses on either up-regulating therapeutic genes or down-regulating disease-associated genes using plasmid DNA, messenger RNA (mRNA) or short oligonucleotides, such as small interfering RNA (siRNA) and antisense oligonucleotides (ASOs) [113]. Together, these strategies have the common goal to halt degeneration while improving local repair and regeneration [113]. Both strategies can be employed by means of non-viral or viral transfection, and theoretically be performed *ex vivo,* where cells are transfected prior to transplantation into the joint, or *in vivo,* upon direct gene transfer to joint tissues [112,113]. While viral gene delivery strategies for OA have been already tested in clinical trials [114,115], non-viral gene delivery is still in its infancy. The main limitation of non-viral strategies is the low efficiency of gene transfer and subsequently low levels of transgene expression, and the transient effects, as opposed to viral approaches [116]. For IVDD-associated chronic low back pain, clinical trials using either non-viral or viral gene therapy have not been carried out so far [117]. While the preclinical potential of gene therapy to treat degenerative joint diseases is established [118,119], more preclinical and clinical trials need to be carried out to assess the safety and efficacy of such strategies.

The investigation of epigenetic changes during OA development also offers the potential to discover novel therapeutic targets. Recent studies showed that a wide range of microRNAs (miRNAs) plays important roles in the maintenance of cartilage homeostasis, and consequently in the pathological processes preceding or sustaining OA and cartilage degradation [120–127]. MiRNA-140 was shown to exert a crucial role in cartilage development and homeostasis [128,129]. Intra-articular injections of miRNA 140 led to anti-inflammatory effects, cartilage matrix production and slower OA progression in both mice [130] and rat [131] models of OA. Recent preclinical studies showed

the feasibility of antisense oligonucleotide-mediated silencing of miRNA-181a-5p [132], a microRNA found to be increased in OA cartilage [125]. Intra-articular injections of a modified ASO led to attenuation of cartilage degradation and reduction of catabolic molecules in two rodent models of OA [135]. Likewise, evidence is being gathered on the involvement of miRNAs in disc degeneration [133–135]. Modified ASOs may in addition be potential alternatives to small molecules for the inhibition of other OA-associated proteins [136–138]. More recently, other epigenetic regulators such as long noncoding RNAs and circulating miRNAs have been investigated for their roles in the pathophysiology of OA and, therefore, their potential use as biomarkers and therapeutic targets [139–141]. Although there is substantial *in vitro* evidence supporting the potential of these molecules or their inhibitors as therapeutics for both OA or IVDD, a better understanding of their spatiotemporal expression is necessary to avoid cytotoxicity and off-target effects [117,142,143]. Moreover, efficient and targeted delivery of miRNAs or their inhibitors for therapeutic purposes should be carefully evaluated due to potential degradation by RNases and Toll-like receptor-mediated immune system activation [126,135,144].

3.5. Others

The Wnt signaling pathway is known to be not only a key regulator of joint and disc development and function, but also a relevant component involved in joint and disc pathology and hence degeneration [3]. Hence, the Wnt pathway has been a studied therapeutic target for OA and IVDD. Phase I and II clinical trials have been carried out testing the safety and efficacy of SM04690, as treatment for OA [80,81]. In these clinical trials, SM04690 improved pain and function according to the WOMAC score (The Western Ontario and McMaster Universities Osteoarthritis Index) [80,81]. A follow-up phase III clinical trial is currently underway (NCT03928184). The same drug was also proposed as therapeutic for IVDD, following a preclinical study where matrix production and disc height were observed in a rodent-model of disc degeneration [145]. The molecule has since then entered a phase I clinical trial for treatment of disc degeneration, yet the study was halted for business reasons (NCT03246399).

Lately, a new class of drugs targeting senescence mechanisms is emerging as a new therapeutic approach for OA [146]. UBX101, a drug that increases p53 activity and hence inducing apoptosis in senescent cells, entered a phase clinical trial to evaluate safety and tolerability in OA patients [147]. This drug was previously shown to be effective in eliminating senescent cells and in slowing down disease progression in a mouse model of OA [30]. While senescence has been pinned out as a hallmark of IVDD, the development senolytic drugs for disc degeneration is still in its infancy [148].

All in all, most of the novel pharmacological treatments for OA and IVDD show little to no efficiency in cartilage repair in clinical studies, although being promising in pre-clinical research. Most of the drawbacks of the previously tested drugs are related with either prolonged systemic overexposure and subsequent off-target side effects such as for the enzyme inhibitors, but also short bioavailability in the target tissue leading to a lack of efficacy, especially for small molecule drugs are likely to have played a role. Additionally, and specially for IVD, due to the avascularity, presence of endplates and absence of a synovial space, systemically-administered drugs have limited bioavailability within the local tissue [7]. Therefore, improved therapeutic outcomes are likely to be obtained through local injections in drug delivery platforms. These systems can be tailored to provide local and a sustained drug release, as well as targeting of specific cell/tissue types.

4. Drug delivery systems

Several approaches have been taken towards the application of biocompatible and safe drug delivery platforms for the improvement of therapeutic outcomes (Table 2 and Fig. 3). Among these strategies,

Table 2
Main drug delivery systems for OA and IVDD.

Category	Size	Removal mechanism	Modifications	Advantages	Disadvantages
Microparticles	1–1000 μm	Foreign body response. Biodegradation	Different sizes. Tuneable drug loading and release dynamic. Surface coating for controlled drug release. Retention within the joint.	Sustained or controlled drug release. Minimisation of injections needed.	Inflammatory response. Particle solvent might cause toxicity
Nanoparticles (polymeric, liposomes, micelles)	Diameter of 1–1000 nm in at least one dimension and a diameter of 1–100 nm	Foreign body response. Cell internalization. Biodegradation.	Different sizes. Surface modifications for modulation of charge, size and matrix/cell interactions. Antibody or peptide functionalisation for targeted delivery. Matrix penetration/diffusion	Penetration of biological membranes. Targeted drug delivery. Enhancement of solubility of hydrophobic drugs. Enanched drug stability.	Nanoparticles aggregation. Immunogenicity. Particle solvent might cause toxicity
Hydrogels	Mesh size of 5–100 nm	Biodegradation	Modification of mesh size and degradation profile for controlled drug release. Cell homing properties. Local drug delivery.	Sustained or controlled drug release. Tunability. High biocompatibility due to the highwater content.	Hydrophobic drugs are not ideal to be encapsulated. Difficult to handle. Not adherent.

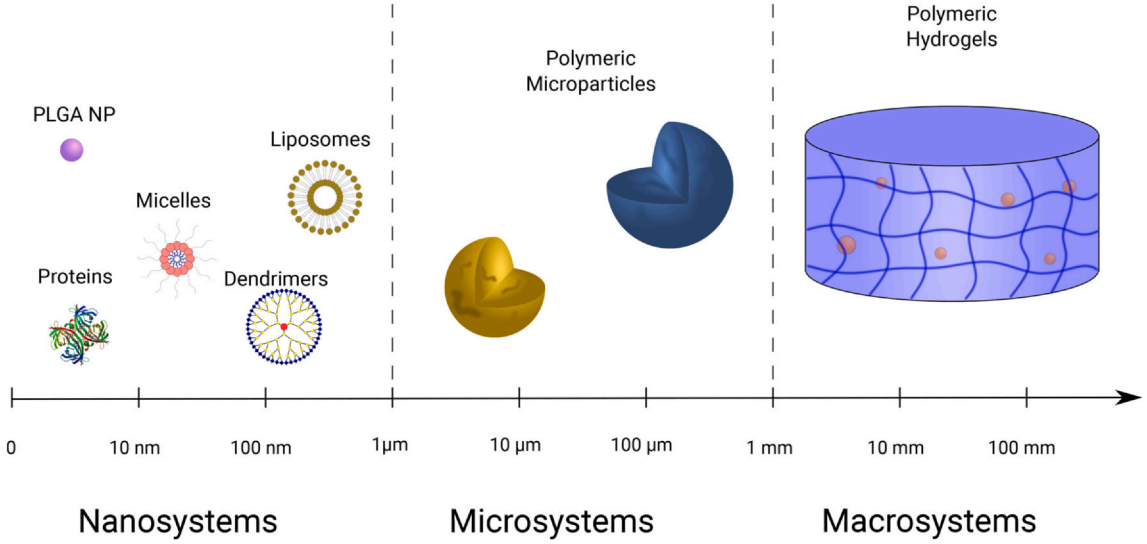

Fig. 3. Schematic representation of the different drug delivery platforms according to their size, from nano to macrosystems.

microparticles and hydrogels are used as drug depot for local extracellular drug release. Continuous drug release over time or upon endogenous (*e.g.* enzymatic activity, temperature, pH) or external stimuli (*e.g.* ultrasound) can be tailored to achieve drug concentrations within the therapeutic window for prolonged periods of time [6,149]. On the other hand, since OA and IVDD involve different tissues with varied roles during pathogenesis, drug delivery using targeting moieties may facilitate and improve drug delivery to specific cells and tissues.

4.1. Microparticles

Microparticles are micron size particulate systems. Due to their size, ranging from one to hundreds of microns, injected microparticles can be well retained within the joint cavity, escaping the main mechanisms of clearance: synovial vasculature and lymphatic systems [6] (Fig. 4). Microparticles can be designed to encapsulate a variety of drug candidates, ranging from small hydrophobic drugs such as corticosteroids and NSAIDs to large macromolecules such as enzymes and antibodies and therefore offering the possibility to encapsulate DMOADs. To tune their degradability and hence their release profile, microparticles can be formulated using different biomaterials.

Up until now, only one microparticle–based drug delivery system has reached the clinic. This product is based on triamcinolone acetonide-loaded poly(lactic-*co*-glycolic acid) (PLGA) microspheres and commercialized under the name of Zilretta® /FX006 [150]. In OA patients, the system showed improved joint retention and prolonged anti-inflammatory effects compared to the injection of bolus triamcinolone acetonide (TAA) (Kenalog 40®) [151]. However, in a phase-3 clinical trial, FX006 failed to outperform the standard of care bolus suspension of microcrystalline TAA in the primary outcome parameter. Nevertheless, the novel formulation did show significantly better results in several secondary outcome parameters [152]. A new clinical trial is ongoing to evaluate the effect of FX006 on synovial inflammation in OA patients [153].

Other microparticle formulations, ranging from synthetic to natural polymers have been used in preclinical studies. Microspheres derived from the synthetic polyester amide (PEA) polymer and loaded with TAA, were tested in several models of joint pathology [154]. In collagenase-induced OA rats, the TAA-PEA microparticles showed a retention time of over two months and reduction of inflammation [154]. In addition, the TAA-PEA formulation was also shown to be superior to TAA-loaded PLGA microparticles in reducing pain, swelling, lameness and synovitis in a rat model of acute arthritis [155]. In a trauma-induced OA model, reduction of prostaglandin E_2 levels, synovial inflammation, osteophyte formation and subchondral bone sclerosis were observed using celecoxib-loaded PEA microspheres, whereas extended

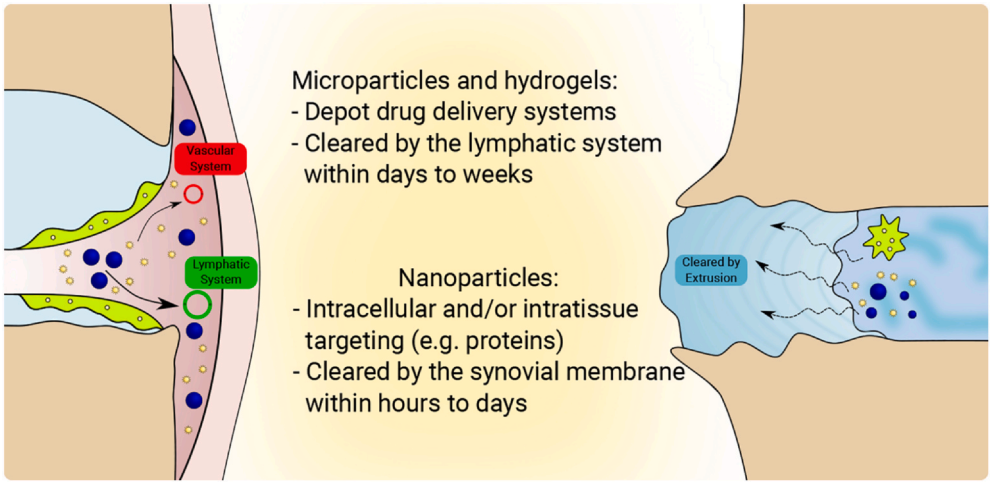

Fig. 4. Schematic representation of the fate and clearance profiles of drug delivery systems in the joint and IVD.

exposure to TAA enhanced degeneration [156,157]. In a canine model of IVDD, celecoxib-loaded PEA microspheres inhibited osteophyte formation and sclerosis similarly to the rat OA model, and even prevented further degeneration [158]. However, TAA delivery in the same model only reduced the expression of a pain marker [158,159]. In contrast, in a rat model of IVD trauma, prevention of IVD degeneration was observed after delivery of corticosterone using tricalcium phosphate (TCP) microcapsules [160]. A new drug formulation, combining synthetic poly(lactic) acid (PLA) nano- and microparticles, encapsulating drug nanocrystals of a few hundred nanometers, allowed an extended release profile of the encapsulated drugs, specifically kartogenin and p38α/β MAPK inhibitor [161,162]. Using these drug formulations, a protective effect on cartilage integrity and reduction of disease markers were observed in an OA mouse model [161,162]. Microparticles of silk fibroin, a natural polymer, also increased the retention of a fluorescent dye within the joint cavity after intra-articular injection [163]. By exploiting their biodegradability and biocompatibility, PRP-containing gelatin hydrogel microspheres were shown to significantly delay OA and IVDD progression in an anterior cruciate ligament transection (ACLT) and degenerated IVD rabbit models, respectively [164–166].

Another approach towards controlling drug release dynamics and avoid the typical drug burst release of microparticles was reported in a study where ibuprofen was chemically functionalized to the backbone of a polymer chain instead of loaded into the particles [168]. Here, a methacrylic derivative of ibuprofen was *co*-polymerized with an oligo (ethylene-glycol) methacrylate and poly(PLGA-PEG) dimethacrylate, and used to form microparticles of 40–100 μm. The release of regenerated ibuprofen was obtained by hydrolysis of the ester bond, allowing a gradual release of 13% over a three-month period. [167]

Sustained delivery of IL-1Ra-loaded PLGA microspheres in IVDD led to attenuation of NP degeneration in *in vitro* human IVD tissue culture [168]. Upon intradiscal injection in a rat model of IVDD, the same system showed protection against GAG degradation [169]. Regenerative factors have been formulated in microparticle drug delivery systems to a much lesser extent. Only in one study PLGA microparticles loaded with dexamethasone and FGF-2-embedded heparin/poly(L-lysine) nanoparticles were shown to promote rat mesenchymal stem cell (MSC) proliferation and differentiation into NP cells *in vitro* [170] and induce partial tissue regeneration in a rat model of disc degeneration [171].

Microparticles have longer retention times than smaller delivery systems mainly owing to their bigger size and limited clearance *via* small capillary networks [172] (Fig. 4). *In vivo* studies have reported a joint residence time of several months for different microparticle-based delivery systems [154,156]. Additionally, PLA microparticles of above 3 μm in diameter exhibited slower joint clearance than nanoparticles of 300 nm in diameter [173]. Remarkably, the inflammatory state of the joint was also shown to affect clearance times, with 3 μm microparticles having faster clearance in diseased joints as opposed to healthy joints [173]. The authors hypothesized that the increase in clearance rate was associated with the permeability of the synovial capillary network. Additionally, bigger particles are known to be entrapped in the synovial membrane and subsequently phagocytized by macrophages [174,175]. Although synovial entrapment and subsequent phagocytosis is often regarded as a limitation for drug delivery platforms, phagocytosis by macrophages has also been exploited as a strategy for increased joint retention and as a mechanism for triggering drug release [175–177]. Fewer studies have reported retention and degradation kinetics of microparticulate systems upon intradiscal delivery. PLGA microspheres were shown to be retained in the disc up to 28 days after injection and were localized both in the NP and AF [169].

Importantly, special attention should be given to the cytotoxic profile and biocompatibility of drug delivery systems. Biocompatibility is not only related to the particle itself, but also to its degradation products. For instance, PLGA is known for its biocompatibility but acidic breakdown products can lower the surrounding pH, and potentially cause inflammation [178]. Microparticles can also stimulate a host cell response, and this can be influenced by the particle size. In example, PCL, PLA and PLGA microspheres have shown to provoke an adverse inflammatory reaction in rabbit joints when their size was around 1–20 μm but not 35–105 μm [179]. Particle shape can also influence the inflammatory response as observed when joints were injected with irregularly shaped chitosan microspheres despite the widely reported biocompatibility of such polymer [179]. In sum, many factors, from physicochemical properties to biodegradability and degradation products, need to be considered in order to design biocompatible particles with minimized host response.

4.2. Nanoparticles

Nanoparticles can be either exploited to increase drug retention time or to target specific areas within the joint, as their smaller size allows for efficient use of targeting moieties and facilitates penetration and diffusion within the dense cartilage matrix. Using different nanoparticle formulations and production methods, size and surface properties can be modulated, allowing loading of a large range of drugs. However, the resulting drug release profile depends on several factors such as pH, temperature, nanoparticle degradation, drug diffusion and loss of binding interactions. Different types of nanoparticles have been widely used for OA and IVDD treatment in preclinical animal models, including polymeric nanoparticle, micelles and liposomes.

In general, although microparticles allow for longer retention time in the joint space and drug delivery to the synovium, they do not guarantee sufficient drug concentrations in the cartilage matrix as their cargo is diluted in the synovial fluid and thereby dispersed throughout the joint (Fig. 4). In this regard, nanoparticles present an attractive vehicle for drug delivery into the cartilage matrix. Due to their tunable properties and smaller size, nanoparticles can be tailored to preferentially bind cartilage surface and promote full-thickness matrix penetration. HA nanogels were shown to remain in the joint for up to two months upon a single intra-articular injection in mice [180]. Additionally, they were also shown to locate in the synovial lining. Nanoparticles below 90 nm were shown to penetrate full-thickness cartilage explants, while bigger nanoparticles tend to accumulate in the superficial layers [181–184]. Finally, interaction with the synovial fluid must be taken into account when designing drug delivery systems, as Synovial fluid has been shown to induce aggregation of nanoparticles limiting cartilage uptake [182].

As for microparticles, nanoparticle properties are also crucial due to their influence on biocompatibility. Positively charged particles are known to be more toxic, as they can potentially lead to cell membrane lysis and mitochondrial/lysosomal damage [185]. Hydrophobicity can also favor particle interactions with proteins of the complement immune system, promoting inflammation and particle removal by phagocytosis [186]. On top of that, since nanoparticles can also be taken up by immune cells, their immunostimulatory properties should also be evaluated [187]. However, nanoparticle tunability and manipulation could be exploited to obtain optimal drug delivery properties as well as minimal or no adverse effects.

4.2.1. Polymeric nanoparticles

Polymeric nanoparticles can be derived from several biocompatible and biodegradable polymers, allowing tuning of their drug loading and release properties. In addition, due to their small size and flexibility, polymeric nanoparticles can be functionalized to allow targeted delivery to specific tissues or cells by the addition of extracellular matrix- or cell-binding ligands. For instance, using the collagen II α1-binding peptide (WYRGRL) nanoparticles retention was increased 72-fold within the articular cartilage of murine knee joints, exploiting the tissue as a drug reservoir [181]. Targeting can also be achieved by making use of electrostatic interaction. Avidin is a positively charged protein with an isoelectric point of ~8 in physiological conditions and with a

hydrodynamic radius of 7 nm which makes it small enough to diffuse through the dense cartilage matrix [189]. Due to its positive charge, avidin has a strong affinity to the negatively charged cartilage matrix, and this facilitates a faster penetration and longer retention of the protein carrier within cartilage [188–190]. Drugs like dexamethasone and insulin-like growth factor 1 (IGF-1) were conjugated to avidin nanoparticles through a biotin linker allowing the loading of the carrier with potentially four different molecules [188–190]. Avidin nanoparticles showed full thickness penetration into the articular cartilage of rat knee joints, a half-life of 29 h and retention time within the joint of 7 days [189]. This study shows how the engineering of nanoparticles can improve the delivery of therapeutics to specific cell types or areas within the joiit. Amine terminal polyamidoamine (PAMAM) dendrimers were functionalized with poly(ethylene glycol) (PEG) and utilized to deliver IGF-1 for the regeneration of articular cartilage in a rat model of OA [183]. The electrostatic interaction between the positively charged dendrimer and the negatively charged cartilage matrix allowed a 10-times higher nanoparticle retention up to thirty days. The subsequent *in vivo* study in an OA rat model demonstrated improved cartilage repair when using IGF-1 conjugated to dendrimers as opposed to injection of free IGF-1 [183]. Altogether, these studies highlight the beneficial effect of using positively charged nanoparticles and their interaction with the negatively charged matrix for OA treatment.

An example of nanoparticles used for IVDD therapy are albumin/heparin nanoparticles which were used for the release of stromal cell-derived factor-1α (SDF-1α) and the recruitment of bone marrow resident MSCs [191]. Using this strategy, disc regeneration was observed as opposed to SDF-1α without delivery system.

4.2.2. Micelles

Micelles are supramolecular self-assembled nanoparticles that spontaneously form upon hydration of amphiphiles. Amphiphiles are molecules which contain a hydrophilic and a hydrophobic part. When hydrated in aqueous solution, amphiphiles reorganize themselves to form nanoparticles containing a hydrophobic core and a hydrophilic external shell facing the water media. These vesicles are formed in aqueous solution when the amphiphilic molecule reaches a certain concentration threshold known as critical micelle concentration (CMC). These particles allow the encapsulation of poorly soluble hydrophobic drugs within the hydrophobic core [192]. Expectably, incorporation of hydrophilic molecules is rather difficult and dependent on covalent conjugation to the external hydrophilic shell of the micelles [193]. The vesicle diameter usually ranges between 10 and 100 nm, depending on the ratio between the hydrophilic and hydrophobic part of the amphiphilic molecule. Size can also vary depending of the nature of the encapsulated drug. Advances in polymer chemistry, and the use of block copolymers with lower CMC for the preparation of polymeric micelles has been yielding vesicles with higher *in vivo* stability, which makes them suitable candidates for drug delivery [192]. Several micelle formulations have been investigated for OA therapy. Rapamycin-loaded micelles delivered intra-articularly in a gelatin hydrogel were shown to delay OA progression in arthritic mice [194]. In a similar approach, PEGylated kartogenin-based micelles delivered in a HA hydrogel were used to prevent OA progression in an ACLT rat model [195]. PEGylation is a common strategy to provide particles with stealth properties and allow longer circulation time and retention *in vivo*. Several other micelle formulations have been tested for the treatment of inflammatory arthritis by delivering dexamethasone [196–199], cyclosporin-A [200], and indomethacin [201]. The covalent entrapment of dexamethasone in core-crosslinked polymeric micelles composed by PEG and poly(N-(2-hydroxypropyl)methacrylamide-lactate) rendered particles with controllable and tunable release kinetics. Once injected in two animal models of rheumatoid arthritis, the micelles induced improvements in arthritic scores [196]. Also folic acid-functionalized polysialic acid/cholesterol micelles loaded with dexamethasone demonstrated improved arthritic scores when compared to non-targeted micelles and dexamethasone alone [202].

Micelle polyplexes composed of PEG-polyamino acid block copolymers and loaded with an anabolic mRNA were also used for IVDD therapy in a rat model of disc degeneration [203]. The strategy led to maintenance of disc integrity and prevention of inflammation induced by administration of naked mRNA. MiR-29a-loaded micelle polyplexes encapsulated into hydrogels were also used for IVDD treatment in animal models [204]. The polymers of both hydrogel and particles were MMP-responsive, causing the release of micelles from the gel. Subsequent removal of the PEG shell from particles, enhanced their cellular uptake and endosomal escape. The strategy led to reduced MMP-2 levels and attenuation of IVD fibrosis *in vivo* [204].

4.2.3. Liposomes

Liposomes, which were the first nanoparticles to be translated to clinical applications, are synthetic vesicles made of phospholipid bilayer(s) and structurally arranged as a cellular membrane [205–207]. Phospholipids are natural amphiphiles which contain a hydrophobic apolar tail and a hydrophilic polar head. The size of the liposomes can range from 50 nm to 5 μm, depending on the number of bilayers constituting the liposome. As opposed to micelles, liposomes contain a hydrophilic core and a hydrophobic lipid outer bilayer which allows the encapsulation of hydrophilic and hydrophobic drugs, respectively. [205,208]. Among the different attractive properties of liposomes, particular attention is given to their elevated degree of biocompatibility, the possibility of encapsulating drugs of different nature, and their wide range of functionalization possibilities [205]. Similarly, to micelles, liposomes have highly versatile physical and chemical properties that can be tailored according to the intended use. Different components can be added to the lipid bilayer to achieve longer circulation times, targeting of specific tissues and controlled release profiles.

The use of liposomes for treatment of joint diseases has been explored in the last decades. For instance, a study demonstrated the feasibility of targeted liposomal-based delivery of plasmid DNA to chondrocytes [209]. Efficient *in vivo* gene transfer and expression in chondrocytes was observed upon intra-articular injection in rats. Expression of the exogenous gene was limited to chondrocytes located in both superficial and middle layer. The limited penetration and transfection of the 200 nm vesicles again draws attention to the importance of the particle size for efficient full depth diffusion, and therefore efficacy. Multilamellar liposomes were used for intra-articular controlled delivery of dexamethasone and diclofenac [210]. The liposomes were composed of soybean phosphatidylcholine and dipalmitoyl phosphatidylethanolamine, and further functionalized with HA and collagen to increase their bioadhesive properties and affinity for extracellular matrix [210,211]. Single or combined delivery of dexamethasone and diclofenac reduced knee-joint inflammation in a monoiodoacetate (MIA)-induced rat model of OA over a time span of 17 days [210]. Furthermore, HA-coated liposomes were shown to have a greater effect when compared to the collagen-coated counterpart, which was attributed to higher cartilage? binding affinity [210,211]. Similarly, liposomes formulated from soybean phosphatidylcholine and cholesterol for encapsulation and delivery of celecoxib and embedded within a HA gel protected cartilage from degeneration as compared to the free celecoxib in a rat model of OA [212]. However, as the effect of the liposome alone was not evaluated, the beneficial effect cannot be attributed with certainty to the controlled and sustained release of celecoxib, especially as liposomes are known to increase joint lubrication [213,214]. Nevertheless, this dual treatment modality of liposomes does increase their attractivity as therapeutic strategy in degenerative joint disease.

The use of liposomes for IVDD treatment has been minimally investigated so far, but a study optimized lipofectamine for the transfection of a human telomerase reverse transcriptase construct in cultured NP cells [215]. Liposomal siRNA could also downregulate Caspase 3 and ADAMT5 levels in a rabbit model of disc degeneration, showing beneficial effects compared to saline control [216].

In the past years, also drug delivery systems derived from cell membranes have been a subject of research as, due to their autologous nature, they can reduce adverse effects observed with exogenous liposomes. One example of cell-derived particles are nanoghosts, MSC-derived nanoparticles obtained after removal of the cell content and subsequent extrusion [217]. Nanoghosts have been recently applied for drug delivery in mouse models of cancer, leading to significant results in terms of tumor regression and mouse survival [217]. In addition, because of the possibility to produce these nanoparticles in larger scale, they also offer a significant advantage in comparison with exosomes, which lack satisfactory scalable production methods [218].

4.3. Hydrogels

Similarly to microparticles, hydrogel can be used as drug delivery systems for small and big molecules, acting as a localized drug depot. Hydrogels are 3D water-containing structures formed by crosslinked natural or synthetic polymers. Importantly, their physical properties such as density and porosity can be tuned by adjusting polymer composition and concentration, therefore allowing for optimal drug incorporation and release [219,220]. While the high water content gives the hydrogel a biocompatible profile, the polymer mesh provides adjustable mechanical properties [219,220]. Moreover, drug release profiles can be further modified by adjusting polymer crosslinking and degradability. Hence, many hydrogel formulations have been explored for intra-articular and intra-discal drug delivery, ranging from natural-derived (alginate, chitosan, collagen, gellan gum, hyaluronic acid) to synthetic materials (PEG, poly-N-isopropylacrylamide, polyvinyl alcohol, polyvinylpyrrolidone). Natural polymers which are exploited to mimic the original ECM have the advantage of not inducing inflammatory reactions at appropriate ratio of purity and dosage [221]. On the other hand, synthetic hydrogels might be less biocompatible but with the advantage of being more tailorable, which may be desired if they need to meet certain biomechanical demands. For hydrogels, the degradation mechanism seems to be the main factor influencing biocompatibility, as the degradation products can potentially trigger inflammation, leading to a exacerbated biomaterial response and, ultimately, compromising the therapeutic goals [222].

For OA treatment, HA-hydrogels represent the most frequently used formulation because of the improvement of joint function through slow release of HA and the possible loading of several drugs. For instance, a HA-doxycycline hydrogel was demonstrated to have improved pharmacokinetic and therapeutic profiles over HA or doxycycline alone [223]. Upon partial meniscectomy and unilateral fibular ligament transection in rabbits, the proposed system displayed decreased occurrence of cartilage fibrilation and osteophyte formation. Importantly, lower degrees of pain were observed for rabbits treated with the HA-doxycycline formulation. A HA-based hydrogel was also shown to improve SDF-1α delivery and recruitment of MSCs in an *ex vivo* model of nucleotomy [224].

Stimuli-responsive hydrogels have also attracted great interest due to their ability to release the drug after chemical or physical stimuli [225,226]. A thermoresponsive hydrogel composed by polycaprolactone (PCL)-PEG-PCL triblock copolymer loaded with celecoxib was demonstrated to have a sustained drug release of about 4–8 weeks upon intra-articular injection in rats [227,228]. The same system was then used for intra-articular injection in a horse model, and celecoxib release was observed for up to 28 days [229].

In addition, hydrogel systems in which the release of therapeutics is triggered by the overexpression of tissue remodeling enzymes have been developed [226,230]. Using this strategy, TAA-loaded hydrogel disassembly and drug release were demonstrated to be specifically triggered by synovial MMP levels *in vitro,* and dependent on arthritis flares *in vivo* [230]. In a canine model of IVD degeneration, a thermoresponsive poly-N-isopropylacrylamide MgFe-layered double hydroxide hydrogel was used for celecoxib delivery [231]. Despite the excellent *in vivo* biocompatibility, the strategy only led to a limited reduction of prostaglandin levels in a canine model of mild IVD degeneration, requiring further investigation in models with a more severe phenotype [231]. In a large animal model of disc degeneration, BMP-2 and BMP-2/7 heterodimers were conjugated to a HA hydrogel and intradiscally injected [232]. Even though conjugation was shown to be effective, no improved disc regeneration was observed, which the authors hypothesized to be related to low dosage and low release from the hydrogel.

Finally, hydrogels represent a very versatile drug delivery systems which has also been applied for gene therapy such as modulation of disease-causing genes or anti-chondrogenic factors through the delivery of siRNA, antisense oligonucleotides and anti-miRNA drugs [137,138,233,234].

As for other drug delivery systems, the release rate of hydrogels is highly dependent on their degradation profiles and physicochemical characteristics. A thermoresponsive PCLA–PEG–PCLA hydrogel was shown to promote celecoxib release for 4 and 8 weeks in a horse and mice model, respectively [227–229]. Regarding intradiscal and intra-articular injection of hydrogels, the main limitation is the hydrogel extrusion or fragmentation due to mechanical loading and high pressure [228,235,236] (Fig. 4). Hence, a rapidly solidifying hydrogel would in theory have a lower probability of extrusion [237]. Additionally, fast integration of the hydrogel with the surrounding tissue can limit extrusion or fragmentation [238].

Importantly, an advantage of hydrogels over other delivery systems is the fact that, besides providing prolonged release of bioactives, they can act as scaffolds for endogenous tissue repair in both AC and IVD [138,239–242]. This can be further enhanced by functionalizing hydrogel matrices with bioactive components that will promote endogenous cell migration, differentiation and matrix production.

5. Conclusions and future perspectives

The articular cartilage and intervertebral disc are tissues with similar structural and biochemical properties. There is an unmet clinical need for new therapeutic molecules that can act on the effector pathways that lead to degeneration, aiming at repair and regeneration. Different disease modifying drugs have been proposed and extensively tested *in vitro* and in pre-clinical models. However, the undesired side effects and limited efficacy observed in clinical trials have delayed their clinical approval. One of the factors greatly affecting therapeutic efficacy lies on the short-term retention of many drugs within joints and limited targeting to specific tissues. Thus, the combination of an effective disease-modifying drug with a safe delivery strategy is a crucial step forward towards regeneration.

In terms of drug delivery strategies applied in OA and IVDD, microparticles and hydrogels are currently being used as site-specific drug depots. Both systems can derive from different synthetic or natural polymers and, depending on the production methods, degradation and drug release profiles can be tuned according with the application and target tissue. Besides the enhanced bioavailability and prolonged therapeutic periods, these systems reduce the need for multiple injections and systemic drug exposure. In addition, hydrogels can also be applied for cell delivery or recruitment and differentiation of endogenous MSCs. On the other hand, the selective targeting of different cell/tissue types in different disease stages using functionalized nanoparticles could allow a more selective and patient-specific therapeutic strategy. Positive outcomes have been obtained in preclinical models using either polymeric nanoparticles, liposomes or micelles. For cartilage targeting, small size and positive charge are ideal requirements for efficient drug delivery within the cartilage matrix. Nanoparticle functionalized with cartilage-binding peptides and liposomes are also attractive platforms due to their ability to favor drug accumulation in specific areas and intrinsic lubricative properties, respectively. IVDD therapy have been less explored so far. Microparticles demonstrated

how enhanced therapeutic outcomes can be obtained through sustained delivery of off-the-shelf drugs. Hydrogels have still only been largely explored for cell homing or delivery properties, yet more drug delivery applications in preclinical models are needed. Since the ideal strategy should adapt the drug delivery to the actual disease stage, enzyme-responsive hydrogels are an exciting option which was proven effective. Furthermore, since the NP is more prone to degeneration, more attention has been paid to the regeneration of this tissue. However, future therapies should aim at restoration of both NP and AF.

Importantly, also the choice of drug is a crucial step towards the design of an efficient therapy. Current disease modifying disease drugs for OA and IVDD allow wide intervention on many aspects of the diseases, ranging from blockage of matrix-degradative enzymes, inflammation and bone resorption to stimulation of new ECM synthesis. Selecting the best drug and intervention time goes in parallel with the necessity of the identification of novel disease biomarkers. It is also conceivable that multiple drugs or different drugs at different disease stages might be more effective, favoring the parallel development of multiscale-drug delivery platforms.

A final consideration for the development of any drug delivery approach regards their biocompatibility and cytotoxicity. In the future, a larger focus on studies of specific methods to identify local and systemic off-target effects might facilitate the screening and translation of safe and effective drug delivery systems into clinical practice.

To sum up, ongoing studies on the pathophysiology of OA and IVDD, the development of therapeutic aimed at blocking disease progression or inducing a regenerative response, and the optimization of drug delivery strategies have the potential to meet the current necessities for curative therapies.

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