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ADVANCED REVIEW

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New insights in osteoarthritis diagnosis and treatment: Nano-strategies for an improved disease management

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Abstract

Osteoarthritis (OA) is a common chronic joint pathology that has become a predominant cause of disability worldwide. Even though the origin and evolution of OA rely on different factors that are not yet elucidated nor understood, the development of novel strategies to treat OA has emerged in the last years. Cartilage degradation is the main hallmark of the pathology though alterations

Abbreviations: ACL, anterior cruciate ligament; ADAMTS-4, a disintegrin and metalloproteinase with thrombospondin motifs 4; ADAMTS-5, a disintegrin and metalloproteinase with thrombospondin motifs 5; ALN, alendronate; AMPK, AMP-activated protein kinase; AuNPs, gold nanoparticles; AuNRs, gold nanorods; BINPs, bismuth oxide nanoparticles; BMPs, bone morphogenetic proteins; COL2, collagen type II; COMP, cartilage oligomeric matrix protein; COX, cyclooxygenase; CMFn, ferritin nanocages; CMFn@HCQ, ferritin nanocages loaded with hydroxychloroquine; CT, computed tomography; CXCL, chemokine (C-X-C motif) ligand; CXCR, α-chemokine receptor; DAF-FM, 4-amino-5-methylamino-2′,7′-difluorofluorescein; DAMP, damage-associated molecular pattern; DDS, drug delivery system; DMM, destabilization of the medial meniscus; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; FDA, Food and Drug Administration; FGFs, fibroblast growth factors; FGFRs, fibroblast growth factors receptors; FOPPR, fiber-optic particle Plasmon resonance; GAGs, glycosaminoglycans; HA, hyaluronic acid, hyaluronan; HCQ, hydroxychloroquine; Hh, Hedgehog; HIF, hypoxia inducible factor; IA, intra-articular; Ihh, Indian Hedgehog; IKK, inhibitory-κB kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; IVIS, in vivo imaging systems; MabCII, monoclonal anti-type II collagen antibodies; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MAPKs, mitogen-activated protein kinases; MEPE, matrix extracellular phosphoglycoprotein; MMP-13, matrix metalloproteinase-13; MMT, medial meniscal transection; MNPs, melanin NPs; mPEG-PDLLA, methoxy poly(ethylene glycol)-b-poly(D,L-lactide); MPs, microparticles; MRI, magnetic resonance imaging; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NIR, near-infrared; NO, nitric oxide; NOS, nitric oxide synthase; NPs, nanoparticles; NSAIDs, nonsteroidal anti-inflammatory drugs; N-S/GQDs, graphene quantum dots co-doped with nitrogen and sulfur; OA, osteoarthritis; OARSI, osteoarthritis Research Society International; PAI, photoacoustic imaging; PCL, polycaprolactone; PEA, polyester amide; PEG, polyethylene glycol; PGA, poly (glycolic acid); Pi, inorganic phosphate; PKCε, protein kinase C ε; PLA, poly (lactic acid); PLGA, poly(lactic-co-glycolic acid); PLL, poly-L-lysine; PLLA, poly (l-lactic acid); PPi, inorganic pyrophosphate; PPIs, proton-pump inhibitors; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; PTOA, posttraumatic in vivo osteoarthritis; RAGE, receptor for advanced glycation end-product; RANKL, receptor activator of nuclear factor κ-B ligand; Rbpj, recombination signal binding protein for Ig κJ; ROS, reactive oxygen species; RUNX, runt-related transcription factor; SIRT1, sirtuin-1; SNPs, single nucleotide polymorphisms; SOD, superoxide dismutase; SOX, SRY-box transcription factor; SPIONs, superparamagnetic iron oxide nanoparticles; SYSADOA, symptomatic slow-acting drugs in osteoarthritis; TFA, trifluoroacetamide; TGF-β, transforming growth factor β; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; TSG-6, TNF-α-stimulated gene-6; VEGF, vascular endothelial growth factor; WHO, World Health Organization.

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in bone and synovial inflammation, among other comorbidities, are also involved during OA progression. From a molecular point of view, a vast amount of signaling pathways are implicated in the progression of the disease, opening up a wide plethora of targets to attenuate or even halt OA. The main purpose of this review is to shed light on the recent strategies published based on nanotechnology for the early diagnosis of the disease as well as the most promising nano-enabling therapeutic approaches validated in preclinical models. To address the clinical issue, the key pathways involved in OA initiation and progression are described as the main potential targets for OA prevention and early treatment. Furthermore, an overview of current therapeutic strategies is depicted. Finally, to solve the drawbacks of current treatments, nanobiomedicine has shown demonstrated benefits when using drug delivery systems compared with the administration of the equivalent doses of the free drugs and the potential of disease-modifying OA drugs when using nanosystems. We anticipate that the development of smart and specific bioresponsive and biocompatible nanosystems will provide a solid and promising basis for effective OA early diagnosis and treatment.

This article is categorized under:
Diagnostic Tools > In Vivo Nanodiagnostics and Imaging
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KEYWORDS
articular cartilage, diagnosis, nanomaterials, osteoarthritis, treatment

1 | INTRODUCTION

Osteoarthritis (OA) is a widely prevalent condition that affects different tissues in the joints. It is no longer considered a condition limited to local cartilage loss as it has been demonstrated that OA affects the whole joint including cartilage, bone, adipose tissue, ligaments, and synovium (Osteoarthritis, 2020). The pathology is commonly identified when a localized loss of cartilage and associated inflammation and remodeling of the adjacent bone are all present. According to WHO estimations, it is predicted that 9.6% of men and 18% of women over 60 years old have symptomatic OA (WHO|Chronic Rheumatic Conditions, 2020). Before age 45, OA is more common in men than in women, but, after that age, it is more common in women, showing ≥3.5-fold higher rates in women aged 50–60 years compared with men (Prieto-Alhambra et al., 2014; Verbrugge, 1995). The current increase in life expectancy and obesity will likely contribute to increase its prevalence. It has been predicted that the prevalence in the US will increase from 47.8 million in 2005 to nearly 67 million by 2030 (25% of the adult population) (Hootman & Helmick, 2006). OA is diagnosed by the physician evaluating as cardinal symptom pain associated with the joint, as well as tenderness, inflammation, redness and flexibility in the affected joint. Medical imaging technologies including X-Rays, computed tomography (CT) and magnetic resonance imaging (MRI) are also used in the diagnosis. By means of these technologies the physician can evaluate the narrowing of the space between the bones in the affected joint and the presence of osteophytes. By using CT, a morphological analysis of the bony structure can be carried out. MRI can also be used to visualize bone and soft tissues, including cartilage without using ionizing radiation. Despite its high resolution, the acquisition times are longer compared with CT (Li, Amano, et al., 2016; Osteoarthritis, 2020). Blood tests may be useful to study physiological and biochemical states that could be responsible for the joint-associated pain and to discriminate it from other pathologies (e.g., rheumatoid arthritis). Joint fluid analysis is also used to confirm or discard the presence of OA or to identify other conditions generally associated with joint inflammation. After OA diagnosis, current management is directed towards reducing joint-associated pain and inflammation while maintaining joint function. Current OA treatments may

alleviate the symptoms of the disease and improve function, though they have not fully demonstrated the reduction or halt of OA to date. Pharmacological interventions include analgesics for pain relief (nonsteroidal anti-inflammatory drugs [NSAIDs], oral or topical), as well as intra-articular (IA) corticosteroids and hyaluronan-based injections (Bannuru et al., 2019; Kolasinski et al., 2020). It is recommended to increase physical activity, physical therapy, weight loss when needed, and to adapt daily activities to avoid bearing excessive loads on the joints. As an example, it has been demonstrated at molecular level that the secretion of pro-inflammatory cytokines by synovial fibroblasts is enhanced in obese OA patients (Pearson et al., 2017). When both pharmacological and non-pharmacological interventions are insufficient to alleviate pain, surgery including osteotomy (i.e., bone realignment to re-equilibrate the load on the weight-bearing joint) or joint replacement with arthroplasty may be applied.

Previous joint injuries, bone misalignment, physically demanding activities, metabolic syndrome and genetics have been identified as potential risk factors for the development of OA (Palazzo et al., 2016). Some studies reveal that OA-associated reduction in physical activity, has been directly related to a significant increase in cardiovascular diseases and all-cause mortality (Hawker et al., 2014). However, several other studies conclude that there are not reliable and confident evidences in the direct association between OA and all-cause mortality (Turkiewicz et al., 2016; Xing et al., 2016). Direct OA costs are associated to the primary healthcare visits, pharmacological treatments, and replacement surgeries. Indirect associated costs reported by patients with disabling hip and/or knee OA have been principally attributable to their need to take unpaid leave from employment and also for their caregivers (Long et al., 2020). According to the Centers for Disease Control and Prevention in the US, OA was the second most costly health condition treated at US hospitals in 2013 accounting for 4.3% of the combined costs for all hospitalizations (Torio & Andrews, 2013). The Global Burden of Disease Study 2017 analyzed the symptomatic OA of the knee and hip in China, stating that 26.1 million people suffered OA in 1990, and this number rose to 61.2 million in 2017, accounting for 1.1% of all years lived with disability of the population (Economic Burden of Disabling Hip and Knee Osteoarthritis (OA) from the Perspective of Individuals Living with This Condition|Rheumatology|Oxford Academic, 2020). Novel approaches not only to relieve the symptoms but also to tackle with the prevention and treatment of early stages of the pathology constitute still an unmet need. This review compiles recent reports on OA management including its diagnosis and treatment being focused on nano-enabling technologies.

2 | OSTEOARTHRITIS: CURRENT LANDSCAPE AND CHALLENGES FOR OSTEOARTHRITIS TREATMENT

OA is a complex disease in which the whole joint is affected. A wide range of cellular and molecular mechanisms are implicated in the progression of OA. The deeper understanding of OA pathogenesis is making possible a change in the treatment strategies which are increasingly focused on prevention and treatment of early OA rather than in pain management and full replacement of the joint at late stages (Carr et al., 2012; Glyn-Jones et al., 2015; Pivec et al., 2012).

2.1 | Cellular and molecular mechanisms involved in osteoarthritis progression

Several studies have demonstrated that cartilage degradation is the main hallmark of OA initiation and progression, followed by alterations in bone formation or subchondral bone remodeling with the subsequent synovial inflammation. Furthermore, fibrosis and damage to ligaments, tendons, menisci and capsules are also involved (Loeser et al., 2012; Meliconi et al., 2013). However, from a cellular and molecular perspective, cartilage degeneration is mainly characterized by the change of the chondrocyte phenotype towards a hypertrophic differentiation, which leads to an elevated production of proteolytic enzymes and therefore, to cartilage damage and loss of joint function. Other events in OA include the expression of chondrocyte hypertrophic markers and matrix mineralization due to chondrocyte calcification (van der Kraan & van den Berg, 2012). The disruption of chondrocyte/cartilage homeostasis can be activated by multiple factors that directly or indirectly initiate a cascade of intracellular changes, in one or multiple pathways, resulting in a chondrocyte hypertrophic/endochondral phenotype (Ripmeester et al., 2018). One of the key events in cartilage catabolism is the destruction of the extracellular matrix (ECM) mediated by the increased expression of Matrix Metalloproteinase 13 (MMP-13 or collagenase 3), A Disintegrin and Metalloproteinase with Thrombospondin motifs-4,5 (ADAMTS-4, ADAMTS-5) and Runt-related transcription factor 2 (RUNX-2) (Tamamura et al., 2005). On the other hand, mechanical or oxidative stress, inflammatory cytokines, or altered amounts or organization of matrix proteins, including degradation

products, have important effects in joint tissue cells. The activation of stress- and inflammation-induced signaling leads to an overexpression of inflammation-related genes, including nitric oxide synthase (NOS)-2, cyclooxygenase (COX)-2, and different MMPs (Goldring et al., 2011). Nitric oxide (NO) is highly expressed in OA chondrocytes and cartilage, and inhibits the synthesis of proteoglycan and collagen, activates MMPs, mediates in chondrocyte apoptosis, and promotes inflammatory responses, all of them resulting in a major catabolic effect. Furthermore, other factors such as inflammation, abnormal mechanical loading, genetics and aging, metabolic and hormonal disorders, vascular and neural invasion, show relevant effects in OA initiation and progression playing a key role in OA cellular and molecular aspects. Consequently, there is an increasing awareness of the systemic dimension of OA, including inflammatory changes in joint tissues and supported by the evidence of mechanisms of feedback and crosstalk among these different joint tissues, such as the impairment of major homeostatic cell responses which ultimately results into the accumulation of oxidative stress, cell dysfunction and senescence (Franceschi et al., 2017; Haigis & Yankner, 2010).

Herein, the key molecular pathways involved in OA initiation and progression are described as the main potential targets in OA prevention and early treatment.

2.1.1 | Wnt/β-catenin signaling pathways

One of the molecular pathways widely studied in chondrocyte differentiation and hypertrophy during OA is the Wnt/β-catenin signaling network, which is extensively recognized as key regulator of bone and cartilage homeostasis and development (Figure 1). It performs important functions in many biological processes, such as the differentiation of

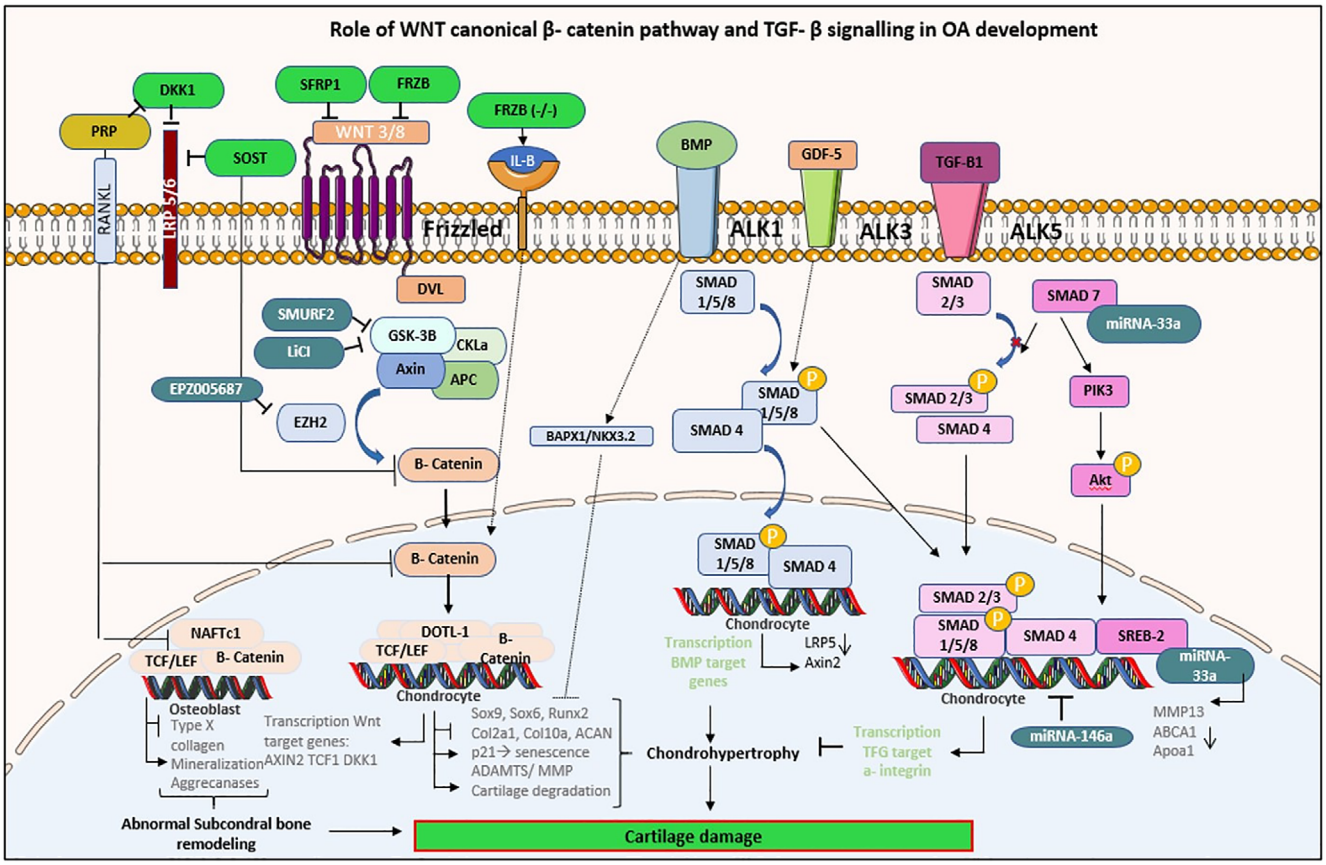

FIGURE 1 Stimulation of the Wnt canonical β-catenin signaling network induces chondrocyte hypertrophy through the transcription of several catabolic markers (RUNX-2, ADAMTS, MMP-13, COL10A1) as well as an abnormal subchondral remodeling, thus promoting degradation of cartilage and subsequent OA progression. Wnt inhibitors that target different components in this pathway may suppress the hypertrophic phenotype in chondrocytes. The activation of the TGF- β signaling through different ALK receptors induces Smad phosphorylation and stimulate the transcription of BMP or TGF target genes resulting in hypertrophic or chondroprotective effects.

mesenchymal cells, the preservation of the mature articular cartilage phenotype, the hypertrophic maturation during endochondral ossification progression, and tissue destruction and renewal (Yuan et al., 2015).

Previous studies have shown that this signaling pathway is intensified in OA in different in vivo models. Yuan et al. (2015) demonstrated in rabbit articular chondrocytes that Wnt/β-catenin signaling enhances catabolic mechanisms leading to excessive remodeling and degradation of cartilage in age-associated joint pathologies. In this regard, the activation of the β-catenin pathway resulted in the earlier differentiation of articular chondrocytes and their transition to an OA-like phenotype in an in vivo murine model (Zhu et al., 2009). As mentioned above, ECM destruction is a critical step in cartilage catabolism (Tamamura et al., 2005), but also the upregulation of Wnt-5a protein is involved in the degradation of collagen type II (COL2a1) in OA rat chondrocytes (Shi et al., 2016).

Chondrocyte hypertrophy has been shown as another relevant event in OA development. Recent studies have demonstrated that the expression levels of Wnt signaling- and β-catenin-inducing factors, as well as downstream Wnt effectors are directly or indirectly linked to OA progression. Cartilage-specific SMURF2 interacts with GSK-3β and induces its ubiquitination and proteasomal degradation resulting in the increased activity of β-catenin signaling (Wu et al., 2009). These hypertrophic changes suggest a direct relationship between early gene expression alterations and OA development.

Wnt/β-catenin signaling promotes osteoblast differentiation and inhibits chondrogenesis (Regard et al., 2012). However, it can also block their differentiation into mature osteoblasts (Eijken et al., 2006; Kahler & Westendorf, 2003). Bone morphogenetic proteins (BMPs) have been suggested as inhibitors of canonical Wnt signaling (Leijten et al., 2013) and as activators of the β-catenin pathway resulting in hypertrophic effects that lead to OA (Papathanasiou et al., 2012).

The receptor activator of nuclear factor κ B ligand (RANKL) is expressed on the surface of osteoblasts and stimulates osteoclast maturation through interactions with its receptor (RANK), expressed on the surface of osteoclast precursors. Osteoprotegerin is a secreted decoy receptor for RANKL that functions to inhibit osteoclastogenesis by disrupting RANKL–RANK interaction. Both RANKL and osteoprotegerin expression can be controlled by Wnt/β-catenin signaling (Regard et al., 2012).

The key role of Wnt in joint homeostasis and OA development has also been demonstrated by the association between single nucleotide polymorphisms (SNPs) and histone modifications in different genes encoding for proteins of the pathway associated with the disease. Both types of epigenetic changes are relevant for cartilage development and homeostasis, and also influence the genetic susceptibility to OA (Cornelis et al., 2019; Monteagudo et al., 2017).

These studies support the pivotal role of the canonical Wnt/β-catenin signaling in maintaining chondrocyte phenotype and articular cartilage homeostasis. The strict control of this pathway is key to avoid OA development as a moderate activity is necessary for chondrocyte proliferation while an increased activity involves chondrocyte hypertrophy.

2.1.2 | Ihh/PTHrP signaling

The Indian Hedgehog (Ihh) and the negative feedback of Ihh/parathyroid hormone-related protein (PTHrP-) are other pivotal pathways in OA being involved in the endochondral cellular phenotypic changes and in cartilage homeostasis (Figure 2). Ihh is a major Hedgehog (Hh) ligand in chondrocytes while PTHrP signals block chondrocyte hypertrophy by maintaining the expression of Nkx3.2/Bapx1, which acts as a transcriptional repressor of genes required for chondrocyte maturation (Provot et al., 2006; Tamamura et al., 2005; Yang et al., 2015).

Several studies have demonstrated a direct relationship between the inhibition of hypertrophic processes and the protection against OA. The administration of pharmacological inhibitors of Ihh attenuated the severity of OA, and the IA injection of PTHrP diminished both glycosaminoglycans (GAGs) loss and COL10A1 levels while increased COL2A1 in the cartilage, as an evidence of protection against OA (Chang et al., 2009; Eswaramoorthy et al., 2012; Lin et al., 2009). The use of conditional Ihh knockout mice and the surgical induction of OA by partial medial meniscectomy showed that the cartilage damage was reduced, compared with wild type mice (control group), accompanied by the decrease of MMP activity and COL10A1 and MMP-13 levels (Zhou et al., 2014). In this regard, the involvement of Ihh expression in chondrocyte hypertrophy and OA progression was highlighted since Ihh expression was observed to be increased in both OA cartilage and synovial fluid of OA patients compared with non-OA patients (Wei et al., 2012). These observations were associated with an enhanced mRNA expression of chondrocyte hypertrophy markers, such as COL10A1 and MMP-13. Conversely, when Ihh was blocked by siRNA and Hh inhibitor cyclopamine, the effects were reversed, pointing to Ihh as a potential therapeutic target in the prevention of OA progression (Wei et al., 2012). This scenario confirms that the Ihh/PTHrP feedback loop is involved in the chondrocyte phenotype determination whose imbalance or disruption could generate early hypertrophic changes, thus subsequently promoting OA disease initiation and progression.

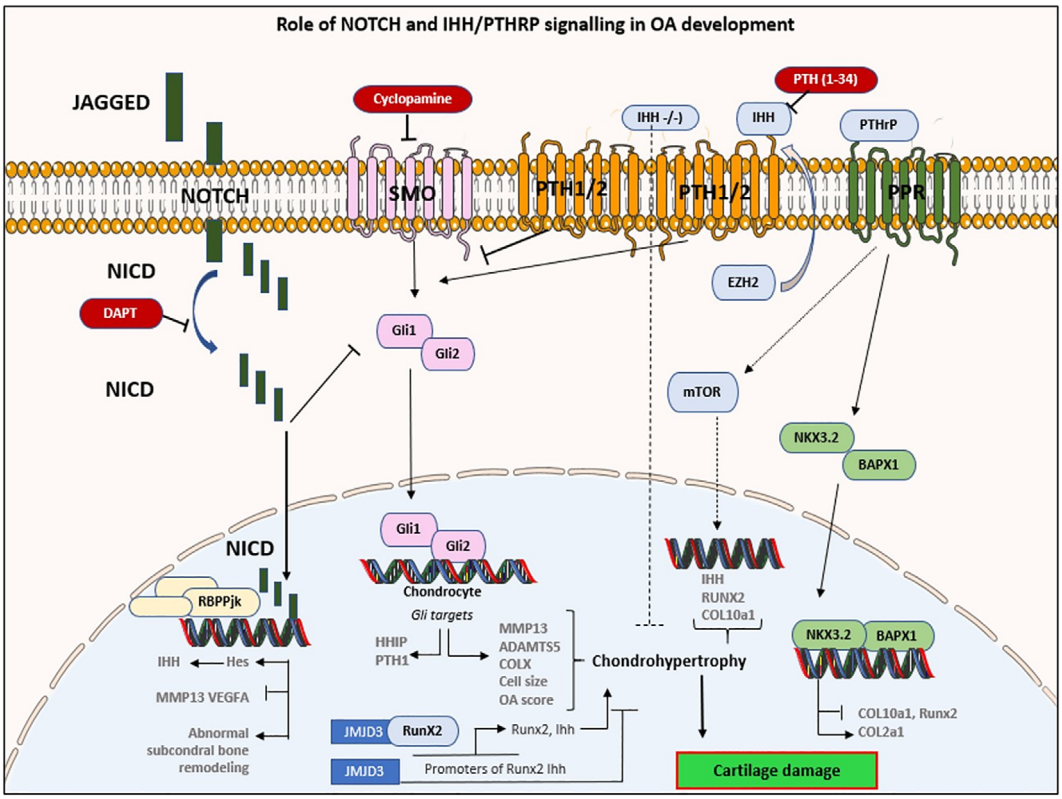

FIGURE 2 IHH/PTHrP and NOTCH signaling pathways in chondrocytes. When Ihh is produced, it binds to PTH and the inhibition of PTH on Smo is attenuated, resulting in the activation of Gli, which enters the nucleus to regulate the expression of target genes (Sox-9, RUNX-2, PTHrP). PTHrP in turn inhibits the hypertrophy of chondrocytes via PPR and Ihh expression is turned off. The activation of NOTCH signaling in OA leads to a cleavage of NOTCH receptor to release the NICD which enters the nucleus and interacts with RBPPjk, to up-regulate the target genes expression, thus contributing to chondrocyte homeostasis.

2.1.3 | Growth factors

Chondrocyte differentiation and maturation during endochondral ossification in OA are strictly regulated by several key growth factors and transcription factors, including members of the transforming growth factor β (TGF-β) superfamily, and fibroblast growth factors (FGFs).

The TGF-β superfamily is a group of multifunctional cytokines generally thought to exert a protective effect in cartilage because of their ability to enhance ECM synthesis. However, TGF-β pathogenic role in OA has been demonstrated through its effects in chondrocyte differentiation and subsequent OA development (Figure 1). TGF-β has demonstrated to signal via activin receptor-like kinase 5 (ALK5)-induced Smad2 and Smad3 phosphorylation (Ten Dijke et al., 1994), but also via ALK1-induced Smad1/5/8 phosphorylation, resulting in chondrocyte hypertrophy and mineralization (Blaney Davidson et al., 2009). In this sense, other members of the TGF-β superfamily may affect the activity and levels of chondrocyte-phenotype downstream transcriptional regulators or target genes, such as α5 integrin, Col10a1, Mmp13, Runx2, and Adamts5, pointing to their key role as downstream target genes related to the TGF-β pathway in OA progression (Caron et al., 2015; Garciadiego-Cázares et al., 2015; Shen et al., 2013). In conclusion, these studies further establish that different TGF-β superfamily members are able to modulate the chondrocyte phenotype and cartilage homeostasis through different downstream factors involved in gene transcription, which ultimately determine chondroprotective or hypertrophic effects.

Fibroblast growth factors (FGFs) and their receptors (FGFRs) control an extensive variety of biological functions, such as endochondral ossification in cartilage response to injury and in the development of OA (Degnin et al., 2010). A wide range of FGFs and FGFRs has been shown to play a pivotal role in cartilage homeostasis, being FGF-2, FGF-18, FGFR-1, and FGFR-3 the most relevant (Figure 3). Even though their effects mainly pointed towards their chondroprotective role (Barr et al., 2014; Chia et al., 2009), other studies have highlighted controversial results indicating a dual role of FGF-2, both anabolic and catabolic, depending on the species (Li et al., 2012). Previous studies have

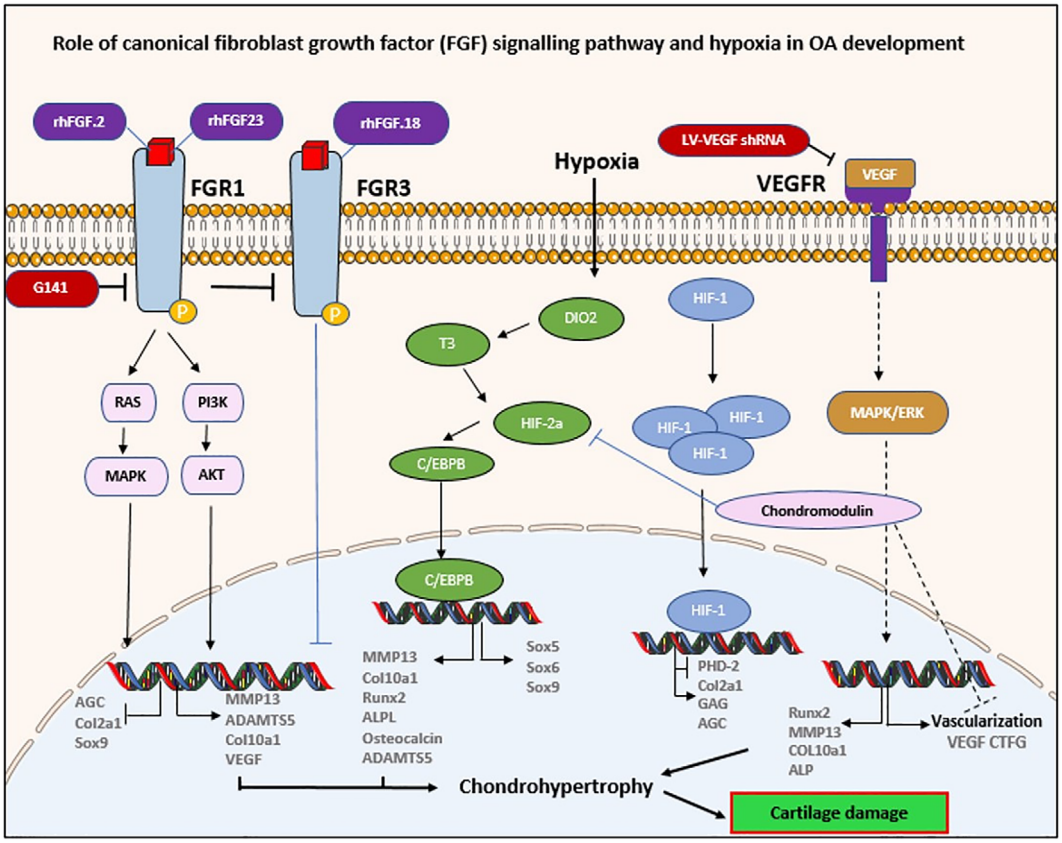

FIGURE 3 FGF signaling and hypoxia pathways in chondrocytes. FGF binds to FGFR to activate the downstream cascade (RAS-MAPK and PI3K-AKT) with a subsequent up-regulation of catabolic markers (VEGF, MMP-13, ADAMTS) thus resulting in chondrocyte hypertrophy. Hypoxia-inducible factors (HIFs) promote the catabolic reprograming of chondrocytes through the up-regulation of hypertrophic and degenerative markers (COL10A1, RUNX-2, ADAMTS, MMP) sustaining both cartilage degradation and OA progression.

examined the effects of FGF-18 on injured cartilage. The combination of the IA injection of recombinant human FGF-18 together with a microfracture procedure enhanced hyaline cartilage production (Power et al., 2014). Moreover, recombinant human FGF-18 also increased proteoglycan synthesis and reduced cell apoptosis (Barr et al., 2014). These results suggest that FGF-18 may improve cartilage repair after mechanical damage. More recent studies have focused on the FGFR-1/FGFR-3 implication in chondrocyte phenotypic alterations in OA cartilage mediated by the deregulation in COL10A1, ADAMTS-5, MMP-13, RUNX-2, and caspase-3 levels (Xu et al., 2016; Zhang et al., 2017; Zhou et al., 2016). Several authors suggest the inhibition of FGFR-1 as a target to reduce or even protect cartilage from OA progression (Xu et al., 2016) whereas FGFR-3 inhibition results in chondrocytes hypertrophy and, therefore, in the initiation of OA (Zhang et al., 2017; Zhou et al., 2016).

The mitogen-activated protein kinases (MAPKs) families are a group of important pathways that regulate, among others, the endochondral ossification and the activity of multiple mediators of cartilage destruction (Figure 4) (Loeser et al., 2008; Stanton et al., 2003). Some authors have shown the involvement of the phosphorylation of some members of one of these pathways, the ERK pathway, in OA by the enhancement of hypertrophic markers (COL10 and RUNX-2) and degenerative cartilage markers (ADAMTS-5 and MMP-13), while the p38 pathway might have a protective role in OA progression (Bianchi et al., 2016; Prasadam et al., 2010, 2013). These data highlight the potential of these signaling pathways as a therapeutic target to attenuate OA progression.

2.1.4 | Inflammatory signaling

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway is involved in OA pathophysiology and regulates the hypertrophic differentiation of chondrocytes during aging and inflammation. Its activation is essential

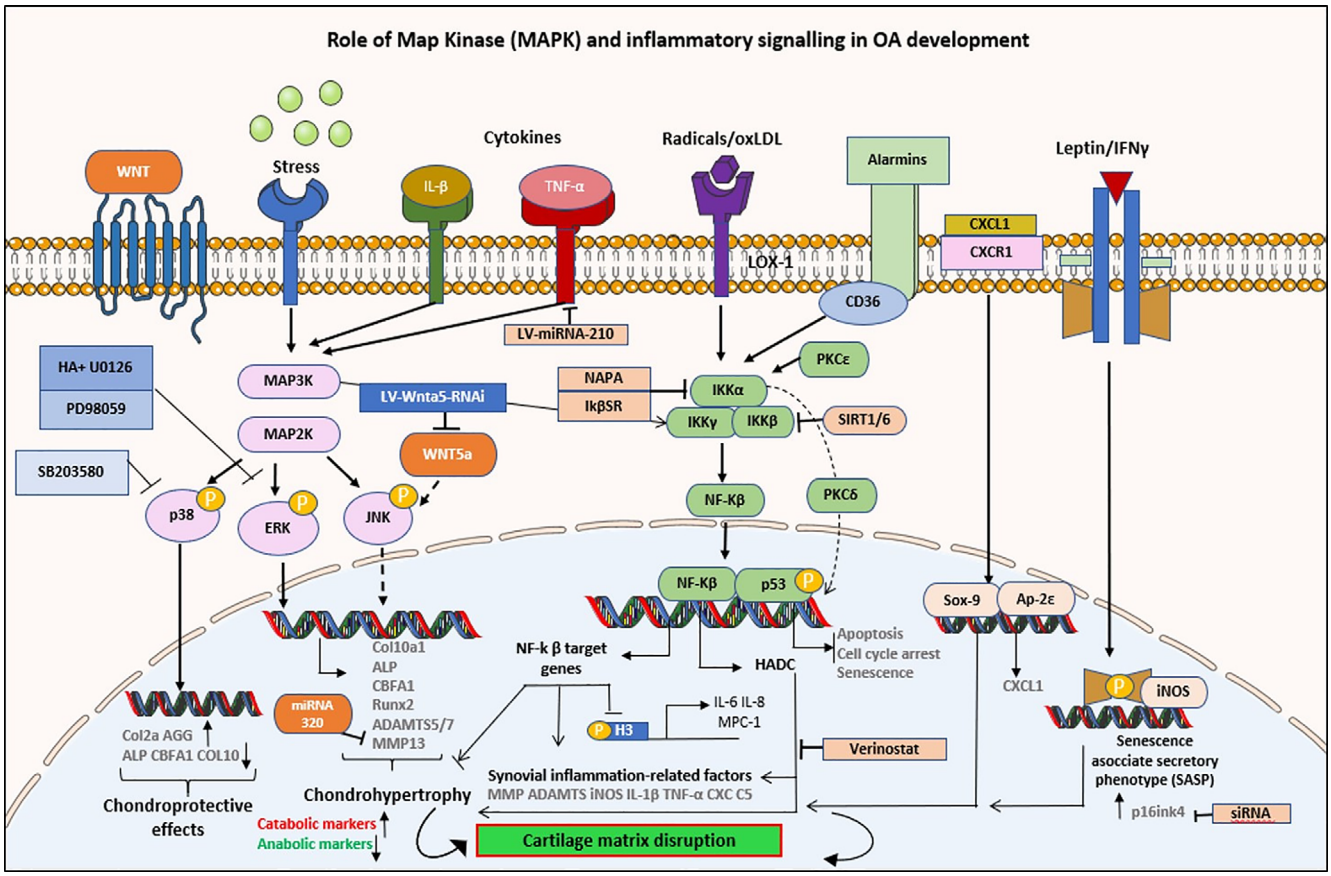

FIGURE 4 MAPK and inflammatory signaling pathways in chondrocytes. Multiple internal and external stimulators (stress, cytokines, alarmins, free radicals, etc.) activate the MAPK and NF-κB cascade which ultimately regulates the activity of transcription factors that reprogram chondrocytes into ECM-catabolizing cells together with proinflammatory and pro-catabolic responses that trigger cartilage matrix disruption.

to induce various inflammation-related factors (MMPs, inducible nitric oxide synthase (iNOS), interleukin (IL)-1β, tumor necrosis factor (TNF)-α) that further activate the signaling cascade (Marcu et al., 2010). Figure 4 depicts the effects of the inflammatory signaling network in OA progression.

In this regard, the silencing of the inhibitory-κB kinases α (IKK-α) and β (IKK-β) in primary human chondrocytes has been shown to increase GAG levels together with SRY-box transcription factor (SOX)-9, RUNX-2 and COL2A1 expression, maintaining chondrocytes in a pre-hypertrophic-like phenotype, pointing to the potential locally targeting of IKKs to diminish NF-κB activity (Olivotto et al., 2008, 2013). However, controversial results were observed in murine articular cartilage where the pharmacological inhibition of IKKs significantly improved its morphology by increasing ECM while reducing the activity of NF-κB and the expression of inflammatory markers such as MMP-10 and -13, and ADAMTS-5, attenuating OA progression (Murahashi et al., 2018; Veronesi et al., 2017). These studies may point out to a different response during IKKs inhibition in OA depending on species, though further studies should be developed to clarify this issue.

The role of chemokines and their receptors in OA pathogenesis has been assessed by evidenced works in patient-based studies and in vivo models of OA (Scanzello, 2017). ERC-CXC chemokines are heparin-binding cytokines that signal through α-chemokine receptor (CXCR)-1 and CXCR-2 receptors and recent evidence demonstrates their role in chondrocytes' hypertrophic changes (Wenke et al., 2011). Normal human cartilage expressed both CXCR2 and its ligand, chemokine (C-X-C motif) ligand (CXCL)-6 and a lower expression of CXCL-6 has been identified in degenerative cartilage. Inhibition of CXCR2 signaling in chondrocytes resulted in a loss of their phenotype and low SOX-9 expression. The development of the destabilization of the medial meniscus (DMM) model in CXCR2(−/−) mice showed more severe OA suggesting the pivotal role of the CXCR2 pathway in chondroprotective responses during OA (Sherwood et al., 2015).

Multiple alarmins (e.g., S100 family proteins or mobility groups proteins), degradation products of ECM proteins and proteoglycans constituents, free fatty acids, and other damage-associated molecular patterns (DAMPs) are increased in OA joints, generally, by interacting with innate immunity. These agents are able to target synovial macrophages (via Toll-like receptor [TLR]-2 and TLR4) and chondrocytes (via TLR2, TLR4, and the receptor for advanced glycation end-products [RAGE]), triggering the production of cytokines and the release of DAMPs, thus sustaining both cartilage degradation and synovial inflammation. The consequent stimulation of synovial cell proliferation and other inflammatory responses can promote procatabolic responses in chondrocytes, contributing to OA worsening (Cecil et al., 2009; Liu-Bryan & Terkeltaub, 2012; Schelbergen et al., 2012).

AMP-activated protein kinase (AMPK) and sirtuin-1 (SIRT1) are the main bioenergy sensors that not only react to changes in energy balance but also have anti-inflammatory effects and control matrix catabolism mediated by chondrocytes. Impairment of AMPK activity in cartilage promotes ECM degradation. However, its activators are able to attenuate procatabolic responses in chondrocytes induced by IL-1β or TNFα, via attenuation of NF-κB activation thus suggesting that changes in AMPK activity supports cartilage homeostasis by protecting cartilage matrix from inflammation-induced degradation (Terkeltaub et al., 2011). Furthermore, AMPK inhibits SIRT1-induced NF-κB activation which is mediated by the deacetylation of p65 subunit of NF-κB and its subsequent proteasomal degradation (Salminen & Kaarniranta, 2012). SIRT1 is a class of deacetylase whose expression is reduced in OA cartilage pointing to its involvement in cartilage homeostasis and OA development (Gabay et al., 2012, 2013; Li, Xiao, et al., 2016; Liu-Bryan & Terkeltaub, 2015).

To sum up, cytokines, chemokines, and other inflammatory signals together with their downstream intracellular pathways exert the regulation of hypertrophic and anti-hypertrophic changes in chondrocytes. Biomechanical injury and oxidative stress compromise the viability of chondrocytes by means of inflammatory mediators, reprogramming them to hypertrophic differentiation together with proinflammatory and pro-catabolic responses, which eventually trigger cartilage matrix disruption. Additionally, inflammation-induced anti-hypertrophic changes have also been described to promote chondroprotective responses.

2.1.5 | Hypoxia associated signaling pathways

In the context of OA, the effects of several stress-related mediators, including inflammatory cytokines or ECM degradation products, can compromise chondrocyte viability, to some extent through the NF-kB signaling. Their effects are transduced by certain "go signals" that transcriptionally reprogram chondrocytes into ECM-catabolizing cells, hence inducing cartilage degeneration (Marcu et al., 2010; Saito et al., 2010; Yang et al., 2015). Hypoxia-inducible factors (HIFs) are autophagy regulators in chondrocytes and their actions compromise the survival and capacity of chondrocyte differentiation in low-oxygen conditions (Maes et al., 2012), which consequently leads to hypertrophic differentiation (Nagase et al., 2013). In addition, vascular endothelial growth factor (VEGF) is a target of the HIF pathway and a powerful angiogenic factor. Thus, both can promote angiogenesis and are also crucial for bone regeneration and endochondral ossification (Hirata et al., 2012). These observations suggest that OA pathophysiology is associated with hypoxic/angiogenic mediators, such as HIFs and VEGF (Bomer et al., 2015; Hirata et al., 2012; Nagase et al., 2013; Wang et al., 2012; Zhang et al., 2016).

Specifically, HIF-2α has been reported as a key factor in OA cartilage as it was highly expressed in OA mice and human cartilage compared with healthy ones (Saito et al., 2010). In addition, it was able to directly enhance the expression of MMP-1, MMP-3, MMP-9, MMP-12 and MMP-13, ADAMTS4, NOS2 in a mouse OA model, confirming its role in the catabolic reprograming of chondrocytes (Yang et al., 2015). Despite the general recognition that HIF expression in cartilage is associated with OA progression, other studies have shown that human articular chondrocytes have developed specific mechanisms to promote tissue function in response to chronic hypoxia. HIF-2α was able to promote both SOX-9-dependent and SOX-9-independent factors relevant for cartilage homeostasis (Lafont et al., 2008). The role of HIF-2α transcriptional signaling pathway in OA remains controversial, given the anabolic effects of HIF-2α in chondrocytes (Xia et al., 2014). It was further demonstrated that cartilage function is regulated by two complementary mechanisms: increasing cartilage matrix production through HIF-2α and decreasing cartilage destruction through HIF-1α (Thoms et al., 2013).

In addition to HIF-2α, VEGF was also involved in OA development. VEGF knockdown has been shown to promote chondrogenesis together with reduced Col10a1 levels (Zhang et al., 2016). Vascularization is necessary for the endochondral ossification process and it has been associated with OA as an increased production of pro-angiogenic factors

(VEGF, CTGF, and MMP-9) was concurrent with vascularization in adjacent hypertrophic chondrocytes during OA. Therefore, hypertrophic chondrocytes may worsen the disruption of the osteochondral junction by the induction of angiogenesis which may drive to OA development (Wang et al., 2012).

It has been demonstrated a link between chondrocyte hypertrophic stimulation via hypoxic and angiogenic pathways and OA progression (Figure 3). Indeed, as it was observed in several studies, the activation of these pathways is a crucial step in OA progression.

2.1.6 | Notch signaling

Notch is a single-pass transmembrane cell surface receptor which regulates a wide variety of cell-fate decisions and cell processes during embryonic development and it is required for the maintenance of tissue homeostasis (D'Souza et al., 2010). Moreover, the activation of Notch signaling in particular chondrocytes is involved in OA progression (Figure 2; Hosaka et al., 2013; Sugita et al., 2015).

NOTCH 1 and NOTCH 2 receptors are strongly expressed in the cell surface of normal articular chondrocytes. However, when the cartilage is degenerated, these domains are translocated into the nucleus in hypertrophic chondrocytes. The role of the transcriptional effector recombination signal binding protein for Ig κJ (Rbpj), one of the major downstream effectors of Notch signaling, was demonstrated in endochondral ossification in an OA murine model (D'Souza et al., 2010). Rbpj knockout chondrocytes were able to suppress OA development as well as decreasing the expression of MMP-13 and VEGFA (D'Souza et al., 2010). Furthermore, the Notch ligands Jag1 and Hes1 were found highly expressed in OA cartilage, indicating that they may be associated with OA pathogenesis (Hosaka et al., 2013; Sugita et al., 2015).

However, a recent report demonstrates that the inhibition of Notch signaling accelerates OA progression with age (Liu, Ren, et al., 2016). High mRNA expression of Jagged 1 and its receptor Notch 1 was found in OA human articular cartilage, together with high levels of the target gene HES1. However, the inhibition of NOTCH1 was found to be associated with an increased, HES1 dependent, Ihh signaling activity, thus worsening OA progression (Liu, Ren, et al., 2016). The increase in the observed hypertrophy could be an interaction between the Notch and the hedgehog signaling pathways (Figure 2).

As conclusion, Notch signaling may contribute to chondrocyte homeostasis by maintaining the progenitors while suppressing the regulation of chondrocyte progenitors differentiation. Moreover, its inhibition in differentiated chondrocytes may be associated with hypertrophic changes. The inhibition of the Notch signaling pathway may be a target to avoid OA progression by reducing hypertrophic chondrocytes changes.

2.1.7 | Mineralization

During mineralization, hypertrophic chondrocytes secrete matrix vesicles containing high concentrations of phosphatases, which are able to hydrolyze inorganic pyrophosphate (PPi) into inorganic phosphate (Pi). These ions, in association with calcium, produce mineral crystals which are released into selected areas of the developing matrix (Anderson, 2003). This mineralized matrix is then vascularized leading to osteoblasts and osteoclasts infiltration. Several studies have demonstrated that mineralization is also involved in OA chondrocyte hypertrophy progression (Cavaco et al., 2016; Fukai et al., 2010; Staines et al., 2016; Zhu et al., 2015).

The contribution of Akt1, a serine/threonine kinase, to cartilage calcification has been demonstrated through its mediated increase in the concentration of the calcification inhibitor pyrophosphatase/phosphodiesterase 1. Thus, an increase of PPi mediated by Akt1 inhibition may avoid the crystallization of inorganic phosphate ions during osteophyte formation in OA (Fukai et al., 2010). On the other hand, Rac1 may promote chondrocyte hypertrophy and mineralization in OA, as the overexpression of its inhibitor OCRL1 significantly decreased IL-1β-induced Rac1 activity, chondrocyte hypertrophy, and the up-regulation of ADAMTS5, Col10a1, RUNX-2, MMP-13, and alkaline phosphatase activity (Zhu et al., 2015). Next to the role of mineralization modulators AKT1 and OCRL1, protein kinase C epsilon (PKCε) also modulated calcium deposition affecting ECM mineralization (Queirolo et al., 2016). Furthermore, mineralization is also regulated by the matrix extracellular phosphoglycoprotein (MEPE), a matrix mineralization inhibitor whose expression is directly linked to endochondral growth dynamics (Staines et al., 2016).

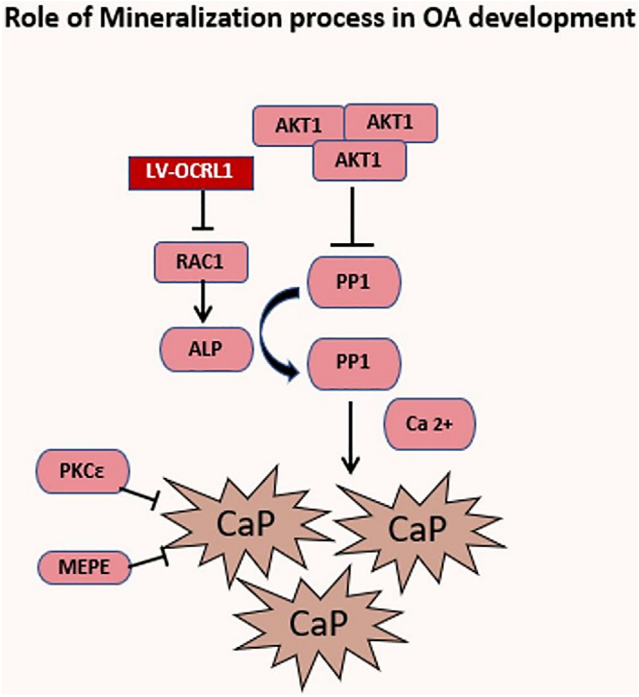

FIGURE 5 Role of several mineralization modulators in the regulation of calcium deposition during ECM mineralization in OA. An increase of PPi and ALP mediated by AKT1 and RAC1 inhibition, respectively, may avoid the crystallization of inorganic phosphate ions during OA. PKCε also modulates calcium deposition affecting ECM mineralization.

In conclusion, ECM mineralization is an important event in OA, and both chondrocyte hypertrophy and mineralization are associated pathologic factors which lead to cartilage degradation (Figure 5).

2.2 | Current diagnosis and treatment strategies

Early diagnosis of OA is a compelling issue. The patients usually experience the first symptoms, including pain, swelling and stiffness, when OA is presumably in an advanced stage and therefore, irreversible (Glyn-Jones et al., 2015). The main challenges in OA treatment are prevention, by finding a key target to halt or even reverse the disease, and the efficient relieve of its associated symptoms to improve patients' quality of life.

The pathological key events in the joint during OA progression are depicted in Figure 6. In early stages, the joint space becomes narrower, osteophytes appear, chondrocytes become hypertrophic and cartilage begins to break until bone ends rub together. Subsequently, synovial fibrosis and inflammation are evident together with cartilage calcification and the loss of joint function. These pathological alterations point to the consideration of the joint as an organ and OA as a joint or organ failure (Loeser et al., 2012).

In fact, the earliest symptom in OA is pain though some patients may be initially asymptomatic. Pain in early OA is reported as intense and deep, worsened by extensive use and physical activity, while it is relieved by rest and analgesia. In later stages of OA, pain becomes more noticeable and unresponsive to drug therapy (Taruc-Uy & Lynch, 2013). In addition, the most widespread symptomatology implicates joint tenderness, increased pain and stiffness, grating or crackling sound or sensation in joints, IA swelling, and impaired movement together with muscle weakening and loss. Radiography, computed tomography (CT) or magnetic resonance imaging (MRI) can help to confirm OA diagnosis. OA progression is typically slow, over the years or even decades, which involves the impairment of patients' mobility and thus, their tendency to develop associated morbidities due to their reduced physical activity such as weight gain or psychological disorders (Lozada & Culpepper Pace, 2018). Furthermore, these comorbidities have been reported to modulate OA progression involving different OA phenotypes and endotypes which hamper an adequate diagnosis and treatment (Boer et al., 2021; Castañeda et al., 2014). The goal to modify the disease is impaired by the limited value of symptomatology in early OA. The symptoms usually change over time and among patients, but also with varied pain

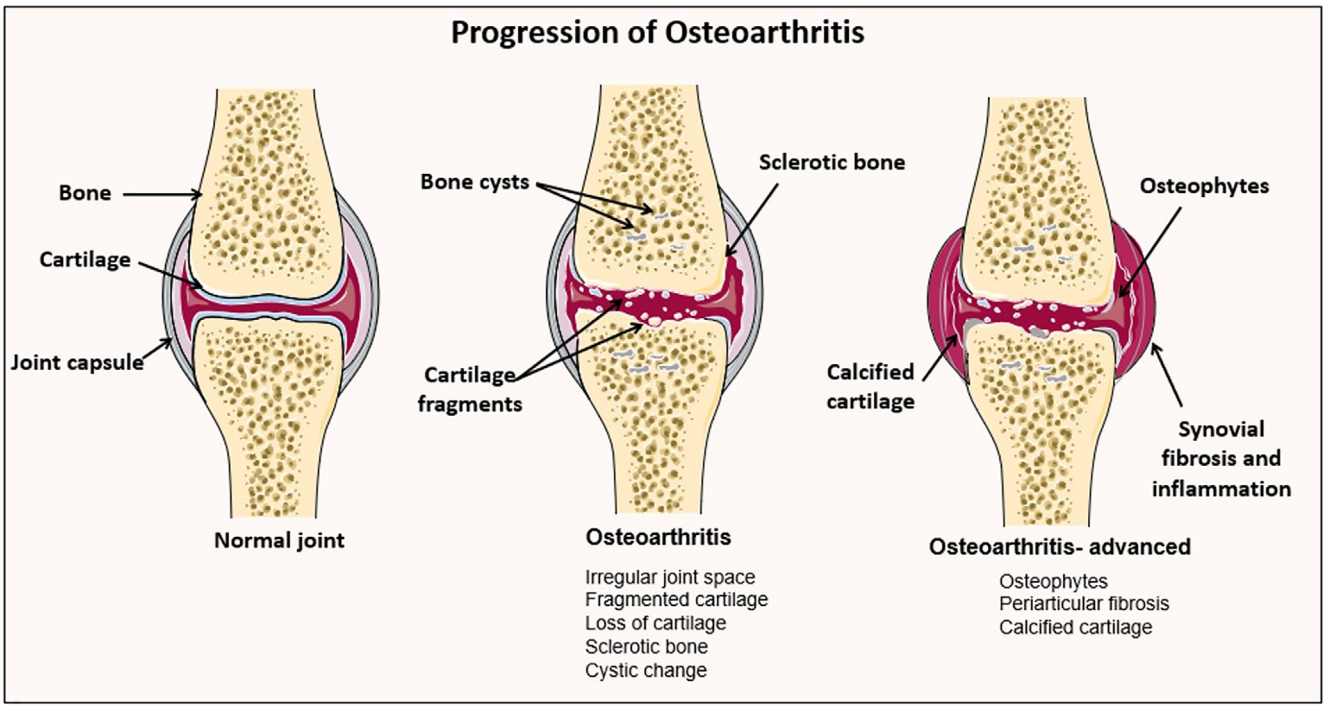

FIGURE 6 Pathologic progression of OA in joints. OA is a progressive disease, which slowly turns from a normal phenotype (left image) to an early OA phenotype (middle image). In early stages (middle image), some chondrocytes undergo a phenotypic change to hypertrophic chondrocytes which leads to a high production of proteolytic enzymes and therefore, to cartilage damage, loss of joint function together with the presence of sclerotic bone lesions. As OA progresses (right image), extensive matrix degradation and cartilage loss occur due to the production of proteases driven by proinflammatory cytokines, which stimulate chondrocytes to produce even more cytokines and proteases. Cartilage loss, as a result of chondrocyte apoptosis and calcification are evident together with osteophyte formation, synovitis, and synovial fibrosis.

perception (Glyn-Jones et al., 2015; Malfait & Schnitzer, 2013). The discovery of an early OA marker would be of paramount importance for its feasible diagnosis and targeted treatment.

Currently, once the disease is diagnosed, the treatments for OA are symptomatic and try to manage pain together with the improvement of joint functionality and mobility. In general, the clinical practice follows the recommendations of different professional groups such as the Osteoarthritis Research Society International (OARSI) (Zhang et al., 2010) or the American College of Rheumatology (Kolasinski et al., 2020). These recommendations are generally divided in two categories: non-pharmacological and pharmacological ones. Among the former, the most prevalent are moderated physical exercise and weight loss. Concerning the pharmacological treatments, NSAIDs (oral or topical), topical capsaicin, IA glucocorticoid and hyaluronan injections, or other analgesics, are commonly used in the clinical practice (Kolasinski et al., 2020). When pharmacological treatments fail, surgical treatments such as osteotomy or total joint replacement may be the next step in OA symptomatic therapy (Hochberg et al., 1995). Herein, the most widespread pharmacological treatments are described in the sequel.

2.2.1 | Anti-inflammatory and analgesics treatments

The mainstay of OA pharmacological treatment is the oral administration of acetaminophen and NSAIDs (some of them with special COX-2 inhibitory potential). However, their use as prolonged treatments may involve side effects including cardiorenal, gastrointestinal and hepatic complications (Adebajo, 2012; Taruc-Uy & Lynch, 2013).

Acetaminophen is a relatively safe compound which alleviates OA pain, but shows a reduced anti-inflammatory effect.

NSAIDs are the mainstay in OA treatment. Their anti-inflammatory mechanism of action is based on the inhibition of COX, which concurrently inhibits prostaglandins necessary for the maintenance of homeostasis in the

gastrointestinal tract. Specifically, COX-1 is constitutive whereas COX-2 is induced by inflammation and plays a pivotal role in inflammation. NSAIDs exert their mechanism of action on COX-2 while their side effects are related to the inhibition of COX-1 (Badri et al., 2016). Prostaglandins are responsible for a superior sensitivity to pain mediated by the sensitization of peripheral nociceptors to painful stimuli (Momin & McNaughton, 2009). The depletion in prostaglandins levels may involve NSAIDs side effects including gastric ulceration, bleeding and kidney dysfunction. Moreover, the prolonged use of NSAIDs may cause cardiovascular events such as myocardial infarction and stroke (Saccomano, 2018).

Other widespread treatments that are not strictly considered as anti-inflammatory nor analgesics treatments are symptomatic slow acting drugs in OA (SYSADOA). They are a therapeutic group of drugs which includes glucosamine, chondroitin sulfate and diacerein. Oral chondroitin sulfate represents an effective symptomatic slow-acting drug (Uebelhart et al., 1998) which also diminishes cartilage volume loss (Wildi et al., 2011) in knee OA. Fidelix et al. (2014) concluded that the symptomatic benefit provided by diacerein in terms of pain reduction is minimal and some adverse effects have been reported. In this regard, glucosamine has been also shown as not as effective as it was previously thought (Towheed et al., 2005).

2.2.2 | Intraarticular administrations

The IA injection of corticosteroids or hyaluronan are two widespread therapies in OA symptoms relief, avoiding the systemic administration of corticosteroids (Hameed & Ihm, 2012; Taruc-Uy & Lynch, 2013). However, there are some drawbacks in IA administration including mild swelling, potential infection and short residence time. The main goals of this technique are the local administration to act directly in the joint tissues, and the achievement of more prolonged retention to reduce the number of required injections (Brown et al., 2019; Evans et al., 2013).

The IA administration of corticosteroids is based on their anti-inflammatory and antinociceptive effects as their effectiveness has been shown for up to 3 weeks. The most prevalent administered are triamcinolone acetonide and methylprednisolone acetate as they are able to persist in the joint in solution avoiding particulate crystalline debris (Ayhan et al., 2014; Saccomano, 2018). Corticosteroids IA administration is not recommended, though not fully confirmed by data, more than 3–4 times per year due to the possible worsening in cartilage damage through repeated administration in weight bearing joints. In fact, most patients whose pathology involves more than those 3–4 IA administrations per year are potential candidates for surgical intervention (Hochberg et al., 1995; Taruc-Uy & Lynch, 2013).

On the other hand, viscosupplementation, the IA administration of hyaluronan or its derivatives, is approved by the Food and Drug Administration (FDA). The administration of hyaluronan is intended not only to restore the mechanical properties of the joint but also to accomplish different biological effects including anti-inflammatory and antioxidant effects, analgesia and increased anabolism of cartilage also mediating the stimulation of endogenous hyaluronan synthesis by synoviocytes (Legré-Boyer, 2015). However, its efficacy and safety is a compelling issue finding evidences about the symptomatic relieve and functional improvement for up to 6 months depending on the studies and the treated joint, whereas other studies claim no clinically relevant improvements in symptomatology, function or structure (Legré-Boyer, 2015; Olivotto et al., 2013; Rutjes et al., 2012; Taruc-Uy & Lynch, 2013). Conversely, the development of novel single-injection and combined forms of hyaluronan derivatives including cross-linked hydrogels is increasing though the election among the wide variety of products on the market is tangled, especially in joints other than the knee (Legré-Boyer, 2015). Thus, the use of viscosupplementation should be customized for each patient as generalizable results have not been yet obtained.

2.3 | Perspectives in osteoarthritis treatment

As described above, OA is a highly prevalent disease which is envisaged to enhance its incidence due to our increasing life expectancy and current lifestyle (obesity, sedentary life, etc.). Furthermore, current treatments for OA are limited to palliative care, and there is currently no pharmacological option that fully impacts disease pathogenesis.

Recently, some products have achieved late stages of development (phase II and III clinical trials) being intended to be more targeted therapies than palliative treatments (Hunter & Bierma-Zeinstra, 2019). Among them, FGF-18 or sprifermin is one of the most promising pharmacological treatments showing increased cartilage thickness (Li, Wang, et al., 2021). Lorecivivint, a small-molecule which modulates Wnt signaling pathway, has demonstrated only limited

effects in a randomized OA phase IIa clinical trial (Li, Wang, et al., 2021). On the other hand, OA phenotyping has emerged in the last few years as a promising approach to classify different OA phenotypes and endotypes exerting clinically close observable features. OA phenotyping aims to define diverse subgroups that may respond to specific treatments. However, further studies are required to corroborate those findings so far and their potential clinical translation (Deveza et al., 2019).

OA progression involves the change in joint tissues, both biochemical and structural, which implies a change also in the clearance of molecules from the joint space and thus, the modification in targeting opportunities. With this scenario in which the microenvironment of the joint changes, the targeting and the efficiency of the treatments are modified progressively with the disease (Brown et al., 2019).

3 | NANO-STRATEGIES FOR OSTEOARTHRITIS MANAGEMENT

Nanomedicine has emerged in the last decades as a vast research field for the diagnosis and treatment of a number of diseases. It is being employed in diverse pathologies, such as cancer, infectious or cardiovascular diseases, among others. The main basis in nanomedicine is the use of nanoparticles (NPs), though other types of nanomaterials including nanostructured scaffolds, nano-based coatings or nanodevices, are being developed for medical purposes. It is estimated that roughly 80% of scientific publications in nanomedicine deal with drug delivery mediated by nanoparticles being their main goal to demonstrate benefits of varied nano-based drug delivery methods compared with the effect of equivalent doses of the same free drugs (James & Highsmith, 2014; Roohani-Esfahani & Zreiqat, 2017; Zazo et al., 2016).

Nanotechnological approaches are good candidates to shed light on OA diagnosis and treatment owing to the attainment of targeted and controlled drug delivery which consequently diminishes side effects and potential drug resistance. Specifically, nanomaterials as drug delivery vectors can solve some of the main limitations of current pharmacological approaches as their characteristics can be customized to fulfill the requirements of each stage of the pathology and prolong the effect of the encapsulated pharmacological cargo by increasing the residence time and the local distribution in the treated joint. Moreover, nanomaterials can penetrate the ECM and cellular barriers enabling intracellular drug delivery (Brown et al., 2019; Zhao et al., 2011).

The thorough design of nanomaterials is very relevant for OA diagnosis and treatment as, in joints, cell and tissue penetration occur concurrently with lymphatic clearance and, due to their reduced size, their potential removal may occur rapidly. The different types of nanomaterials available make possible the delivery of a wide variety of hydrophobic and hydrophilic drugs having tunable release rates, and even environmentally or externally stimulated responsive nanomaterials can be used to trigger drug release on demand (Alam et al., 2017; Brown et al., 2019; Ferrari et al., 2015; Janssen et al., 2014; Kavanaugh et al., 2016). In this regard, the specific targeting within the joint is of paramount importance to localize the designed nanomaterial to the precise target cell, tissue or ECM component. The targeting may be passive or active, depending on the effect of the nanoparticulated carrier given its own characteristics (e.g., size, charge, surface functionalization, chemical composition, targeting ligands, etc.) (Brown et al., 2019). Recent examples of the proposed applications following IA administration and validated in in vivo models or ex vivo in samples from OA patients/animals to date are described in the sequel.

3.1 | Nano-diagnosis of osteoarthritis

3.1.1 | Nanoparticle-based contrast agents for diagnosis

As we mentioned before, one of the challenges for the proper treatment of OA is the identification and characterization of early OA. To be successful in fighting OA, the early diagnosis should be carried out when the articular cartilage damage is reduced, along with a continuous monitoring of the disease progression. Nevertheless, so far, there is still a lack of imaging procedures that can accurately assess cartilage degeneration and changes in the subchondral bone in early stages of the disease. CT and X-rays evaluate indirectly cartilage degeneration by the visualization of joint space narrowing, distinguishable only in the late stage of OA (Mathiessen et al., 2016; Wenham et al., 2014). MRI can identify morphological changes in the degenerated cartilage but submicron defects in the external cartilage layer and disorders in the surface collagen orientation are not detectable using this technique being those morphological defects present in

the early-stage of OA (Liu et al., 2015; Stolz et al., 2009). To solve this issue, the sensitivity of medical imaging methods for the evaluation of early OA could be enhanced by using contrast agents that selectively bind to the damaged areas. Those altered sites show usually disordered collagen, altered cells, fragments of macromolecules or an elevated production of reactive oxygen species (ROS) (Lepetsos & Papavassiliou, 2016; Panula et al., 1998). New contrast agents based on nanotechnology have been proposed for clinical imaging to permit a more accurate visualization of structural tissue variations. For instance, targeted superparamagnetic iron oxide nanoparticles (SPIONs) have been developed as contrast agents for active targeting, as they do not penetrate into healthy cartilage and show specific accumulation in structural cartilage defects, thus labeling areas of therapeutic interest (Soshnikova et al., 2016). Panahifar et al. (Panahifar et al., 2013) decorated SPIONs using alendronate (ALN), a bone targeting moiety containing available phosphonate functional groups that specifically bind to the hydroxyapatite matrix in the bone. Those bone-targeting SPIONs were proposed as non-ionizing contrast agents for bone imaging using MRI, an alternative to radioisotopes commonly used. The in vitro studies showcased selective binding affinity (65%) of ALN-SPIONs for hydroxyapatite, which allows imaging of dynamic bone turnover for diagnosis and monitoring of metabolic bone diseases. Additionally, SPIONs were used to evaluate particle permeation in the context of depletion of ECM components, which typically occurs in early OA (Labens et al., 2017). Results demonstrated an increase in the MRI signal after ECM depletion using SPIONs as IA contrast agents in porcine cartilage ex vivo. SPIONs can penetrate and accumulate at the structural defects, thus indicating the areas of therapeutic interest. Besides, a SPIONs dose dependent inflammatory response was confirmed in vitro using equine articular tissue. Zerrillo et al. (Zerrillo et al., 2021) designed novel fluorinated poly(lactic-co-glycolic acid) (PLGA)-based NPs for multimodal imaging. The copolymer PLGA-PEG-TFA was prepared by integrating the ^{19}F-MRI probe trifluoroacetamide (TFA) into its structure. The resulting PLGA-PEG-TFA NPs prepared by the single emulsion-solvent evaporation method were loaded with a near-infrared (NIR) dye (IR-780 Iodide) for optical imaging purposes. Those dual-modality imaging NPs were tested in vitro in human chondrocytes and also in vivo in an OA mouse model following IA administration. Results showed an optimal visualization of NPs in OA knee joint using fluorescence imaging and ^{19}F-MRI. The multimodal imaging allowed to detect the NPs biodistribution and retention over time, showing that NPs remained in the knee joint for more than 166 h. The main limitation of MRI measurements is the high spatial resolution for small animal models, therefore many contrast agents were only tested in in vitro and ex vivo studies (Herrmann et al., 2012; Leblond et al., 2010).

Photoacoustic imaging (PAI) is a promising alternative to existing techniques that incorporates the benefits of deep tissue penetration ultrasound and high resolution optical imaging (Wang & Hu, 2012). Different NPs have been considered as PAI contrast agents (Pan et al., 2013). PAI has shown the potential to be used for noninvasive in vivo OA studies. Gold nanorods (AuNRs) coated with antibodies were used to target overexpressed TNF-α in the detection of OA-associated inflammation in arthritic mouse knees resulting in an enhancement of PA signal amplitudes (Fournelle et al., 2012). Recently, melanin NPs (MNPs) functionalized with the positively charged polymer poly-L-lysine (PLL) were also assessed as PAI contrast agents (Chen et al., 2018). The ECM components of cartilage are constituted predominantly by anionic GAGs and they exhibit strong electrostatic interaction with PLL-MNPs. As we mentioned before, GAGs depletion from the cartilage is an early marker of cartilage degeneration. PLL-MNPs were administrated via IA injection in OA mouse models. Results showed that healthy joints exhibited twice more PA signal than OA joints due to the reduced GAG content of the latter (Figure 7). Not only the content of GAGs could be determined, but also the content distribution into OA joints could be visualized. Figure 7 depicts a comparison of in vivo diagnostic of early OA using several imaging techniques such as X-ray, MRI and PAI. X-ray images do not demonstrate degenerative lesions or evidences of OA, as the articular cartilage is transparent to X-ray. MR images display symptoms of inflammation in the OA joint (indicated by an arrow), however no definitive evidences of cartilage destruction are observed. In contrast, PAI images of knee joints obtained using PLL-MNPs as contrast agent provide an accurate visualization of cartilage degeneration in vivo, in addition to successfully distinguish early-stage cartilage degeneration from late-stage OA. The biocompatibility, photostability and biodegradability of PLL-MNPs were attributed to their endogenous composition.

Saukko et al. (2017) reported the use of micro-CT in OA diagnosis combined with bismuth oxide nanoparticles (BINPs) mixed with the anionic contrast agent ioxaglate to generate high contrast on the surface of the cartilage due to the lack of penetration of the NPs which remained in the outer layer. Hence, those NPs permitted the detection of the degradation of the cartilage with only a single CT scan, reducing the patient's radiation dose to half compared with the conventional dose used without the nanoparticulated contrast agent. Furthermore, tantalum oxide nanoparticles (Ta_2O_5 NPs) were tested as contrast agents for micro-CT imaging of articular cartilage (Freedman et al., 2014). The role of Ta_2O_5 NPs surface coating was evaluated by their functionalization with ligands having neutral phosphonate, cationic ammonium and anionic carboxylate end groups. Ammonium Ta_2O_5 NPs showed optimal penetration results and

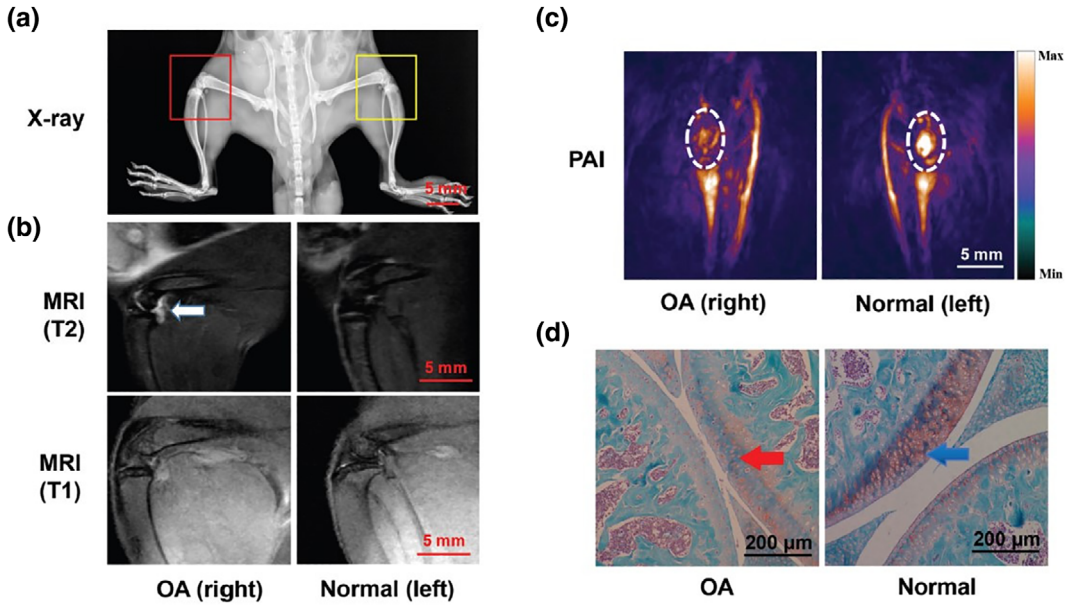

FIGURE 7 In vivo evaluation of early OA using different diagnostic imaging techniques: X-ray, MRI and PAI. (a) X-ray images showed no evidences of cartilage degeneration in the early-stage, being not suitable to detect articular cartilage degeneration. (b) T1 and T2-weighted MRI of healthy and early OA knee joints. T2-weighted MRI exhibited signs of inflammation in the OA joint (white arrow), but images do not allow to confirm the existence of cartilage degeneration. (c) PAI images of knee joints obtained using PLL-MNPs as contrast agent where PLL-MNPs produced higher PA intensity in normal joint compared with early OA joint due to the reduced GAGs levels in the latter, which allow the detection of cartilage degeneration in the early-stage. (d) Safranin-O stained histological sections of the articular cartilage with a decrease of GAGs in the OA joint (red arrow). Reprinted with permission from The Royal Society of Chemistry (Chen et al., 2018). Copyright 2018

preferential accumulation in OA defects in ex vivo murine tibia cartilage and human finger cartilage. The positively charged NPs exhibited a strong affinity for the negatively charged GAGs of the cartilage. The IA knee injection of cationic Ta_2O_5 NPs in an in vivo mouse model resulted in a successful cartilage visualization.

3.1.2 | Nanosensors for the detection of biomarkers

The clinical measurement of biochemical markers would be a promising alternative for the early diagnosis of OA. These biomarkers can be detected in different body fluids such as blood (serum or plasma), urine and synovial fluid (Lotz et al., 2013), though their determination is not widespread in the clinical practice. The most important biomarkers of OA are several proteins of the ECM, inflammatory mediators and metabolic products. The screening of OA biomarkers is usually performed using enzyme-linked immunosorbent assays (ELISA) and western blotting analysis, that are accurate but time-consuming and complex techniques (Jaovisidha et al., 2006). Nanosensors that respond to markers associated with the early phase of OA have been proposed in several studies. Peng et al. (2013) developed a fluorescent probe based on gold NPs (AuNPs) conjugated with a specific peptide for the detection of the ADAMTS-4 enzyme. ADAMTS-4 can cleave aggrecan interglobular domain, whose increased activity can damage aggrecan. The destruction of aggrecan is believed as a critical early event in the degradation of cartilage in OA. The fluorescence of the specific peptide was quenched when it was closely attached to AuNPs but the activation of the sensor occurred when the fluorescence was turned-on by the cleavage of the fluorogenic peptide under the presence of ADAMTS-4. The probe exhibited a fluorescence intensity proportional to the ADAMTS-4 concentration. This fluorescent turn-on probe has also been tested in vivo using a rabbit model of early-stage OA (Liu et al., 2018). In vivo results showed that the fluorescent probe was not clinically superior compared with conventional MRI T2 mapping and ELISA. When NPs were tested ex vivo in synovial fluid samples from rabbits and from patients, the peptide-AuNPs exhibited superior sensitivity than MRI but lacked specificity. The combination of both, peptide-AuNPs probes and MRI, achieved a high sensitivity (82.5%) and specificity (80.0%), outperforming MRI alone. Recently, Chen et al. (2019) designed a new

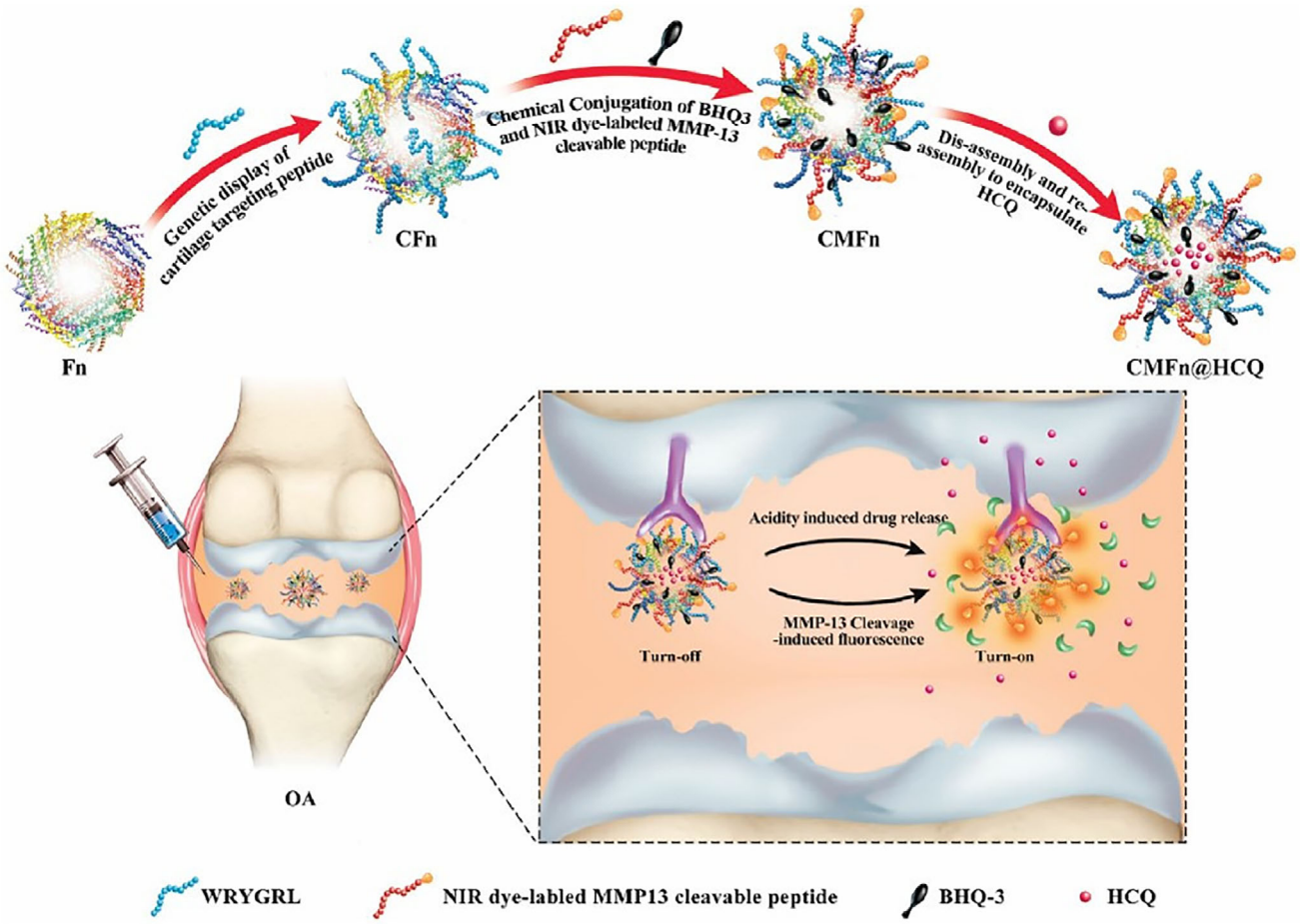

FIGURE 8 Illustration of the cartilage-targeting and MMP-13/pH-responsive CMFn@HCQ probe in which the fluorescence signal was activated under the presence of MMP-13 and drug release was triggered by acidic pH. Reprinted with permission from Elsevier (Chen et al., 2019). Copyright 2019.

nanoparticle-based probe for the detection of MMP-13. The nanoprobe was comprised of ferritin nanocages (CMFn) surface modified with two kinds of peptides, a NIR dye-tagged MMP-13-cleavable peptide and a cartilage-targeting peptide. A quencher of the NIR-dye was also attached to the surface of the CMFn, remaining the probe turned-off in absence of MMP-13 protease expression, and switching on in presence of MMP-13. The anti-inflammatory drug hydroxychloroquine (HCQ) loaded into those pH-responsive nanocages was released specifically in the acidified OA joints. A schematic illustration representing the CMFn loaded with HCQ (CMFn@HCQ) probe and its mode of operation are depicted in Figure 8. The authors found that the in vivo fluorescence signal generated by CMFn@HCQ in an OA mouse model was proportional to the degree of OA severity. This probe with cartilage-targeting capacity can be used to identify the stage of OA disease and to reduce synovial inflammation through the prolonged sustained release of HCQ.

The development of smart nanomicelles based on polycaprolactone (PCL) was carried out in order to obtain a system that specifically targets articular cartilage in the joint for diagnosis and imaging-guide precision therapy (Lan et al., 2020). In particular, poly (2-ethyl-2-oxazoline)-PCL based nanomicelles, functionalized with a specific collagen type II targeting peptide and with a black hole quencher-3 that can quench Cy5.5 fluorescence were prepared to obtain a MMP-13 responsive and pH sensitive polymer, being the resulting nanoparticles loaded with the natural compound psoralidin. This drug delivery system (DDS) was tested in vitro in C57BL6/J chondrocytes stimulated with IL-1β observing that the treatment significantly reverted inflammation and cartilage degradation, even better than the free drug, pointing to the more effective delivery of the hydrophobic drug due to the designed DDS. In vivo fluorescence imaging demonstrated the accumulation of the micelles in the OA joints. Moreover, in vivo experiments in OA mice induced by papain/L-cysteine injection and treated with the loaded nanomicelles displayed a highly significant reduction in OA scores compared with the free drug and to the OA control groups regarding cartilage degradation, ECM integrity, and MMP-13 immunohistochemical staining, highlighting their potential as therapeutic DDS in OA. As an alternative to

proteolytic enzyme-based sensors, COL2 antibodies bound to NPs have been utilized to detect cartilage damage and OA progression (Cho et al., 2015). Liposomes of 100–200 nm in diameter containing a fluorescent NIR dye and conjugated to a monoclonal antibody against COL2 (MabCII) were assessed in vivo. These studies were performed using a mouse model of post-traumatic OA in which knee injury was induced in mice by compressive loading. Fluorescence-imaging (IVIS) showed that the fluorescence intensity attributed to the liposomes correlated with the cartilage damage observed in the histological studies. Further studies (Cho et al., 2018) tested this liposome-based probe in a mouse DMM model that closely mimics early stage OA. The antibody-functionalized liposomes bound to the sites of early lesions on the cartilage and allowed the detection of OA in its early stage and the quantification of cartilage damage.

Jin et al. (Jin et al., 2017) reported the preparation of a NO nanosensor based on PLGA NPs to detect the overproduction of this inflammatory factor. In that work, PLGA NPs were loaded with the NO sensing molecule 4-amino-5-methylamino-2′,7′-difluorofluorescein (DAF-FM). When the DAF-FM molecules react with NO in presence of oxygen, they become fluorescent making their detection possible via fluorescence microscopy. The DAF-FM sensing molecule is highly sensitive to NO; however, DAF-FM can react with other biological molecules generating false signals. Other inconveniences of the DAF-FM probe include its pH sensitivity and its poor retention within tissues and cells because of the fast organism clearance. The encapsulation of DAF-FM molecules into biodegradable PLGA NPs could overcome these disadvantages. In the in vitro experiments performed in IL-1β-stimulated chondrocytes, the nanosensor was able to track the time-dependent NO release presenting a strong correlation between fluorescence signal and NO concentration. In vivo results in rat OA models demonstrated that the NO nanosensor was effective in the quantification of NO levels in the joint. When N^G-monomethyl-L-arginine (a NO inhibitor) and andrographolide (an anti-inflammatory agent) were IA injected, a decrease in the fluorescence intensity was detected which confirms that the sensor permitted to monitor in vivo OA progression. Several authors (Deng et al., 2013; Liu, Xiao, et al., 2016) have reported nanosensors to detect PPi. Graphene quantum dots co-doped with nitrogen and sulfur (N-S/GQDs) are fluorescent nanomaterials that can be quenched by the coordination of Fe^{3+} with N and S atoms (Liu, Xiao, et al., 2016). Upon the presence of PPi, the N-S/GQDs sensor recovers its fluorescence due to the preferential interaction between Fe^{3+} and PPi. The assays using synovial fluid samples from patients corroborated that the sensor fluorescence signal had a linear correlation with the PPi concentration. Deng et al. (2013) developed a sensing method for PPi detection based on AuNPs stabilized with cysteine. The AuNPs dispersion displayed a UV–vis absorbance spectrum centered at 519 nm and a wine red color visible to the naked eye. When Cu^{2+} was added to the cysteine coated AuNPs, the aggregation of the AuNPs produced a change in the color of the solution from wine red-to-blue, and a shift in the absorbance spectrum with a new maximum at 650 nm. The posterior addition of PPi converted the color of the dispersion again from blue-to-wine red, due to the stronger coordination reactivity between Cu^{2+} and PPi than between Cu^{2+} and cysteine in AuNPs. The de-aggregation of the AuNPs produced a measurable UV–vis signal that could be used to quantify the PPi concentration as it was corroborated using synovial fluid of OA patients. Another methodology relying on absorbance spectroscopy was developed by Huang et al. (Huang et al., 2013) in order to detect TNF-α and MMP-13. The method employs a fiber-optic particle plasmon resonance (FOPPR) sensor with antibody-AuNPs in the denude region of optical fiber probes for the accurate detection of TNF-α and MMP-13, achieving a detection limit as low as 8.22 pg ml^{-1} (TNF-α) and 34.3 pg ml^{-1} (MMP-13). Results in TNF-α and MMP-13 quantification in synovial fluid samples showed a good correspondence with those values obtained by ELISA. AuNPs were also used together with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the identification of specific OA biomarkers (López-Cortés et al., 2016). Citrate-AuNPs were used to bind several proteins present in urine samples, such us monocyte chemotactic protein-1 and fibronectin-aggrecan complex, in order to precipitate them by centrifugation. Subsequently, those proteins were digested with trypsin before being introduced in MALDI-TOF MS for direct peptide profiling. The technique achieved a sensitivity of 97% and specificity of 69% in the detection of potential OA biomarkers in urine samples from patients. Ten *m/z* signals were found different between healthy and OA patients. In comparison to common methods of diagnostic such as radiologic and clinical analysis, MALDI-TOF MS presents the highest sensitivity; however, the specificity is lower than those methods although still in the range accepted for an adequate diagnosis.

An immune-chromatographic test based on a lateral-flow immunoassay was designed for the evaluation of cartilage oligomeric matrix protein (COMP) as an OA biomarker (Hong et al., 2012). The method employed AuNPs conjugated with a monoclonal antibody for the identification and quantification of COMP. The assays performed in human synovial fluid revealed that COMP binds successfully to the antibody-functionalized AuNPs. The test strips were designed having a specific antibody that captures the complex COMP-Antibody-AuNPs, so that the color density in the test strip augmented in proportion to the COMP concentration. Assays using lateral-flow immunoassays for COMP quantification presented a

good correlation with those obtained by ELISA. Table 1 summarizes the studies regarding nano-diagnosis of OA that have been gathered in this article.

3.2 | Nano-therapeutics for osteoarthritis treatment

The main challenge in OA treatment is the suitable delivery of therapeutic compounds to the target tissue minimizing side effects and demonstrating efficacy. The direct administration within the joint cavity is the proper route to overcome systemic drawbacks. Articular cartilage is an avascular and aneural tissue difficult to regenerate. The rapid clearance of synovial fluid and the barrier that constitutes the ECM hinder the efficiency of current IA treatments (Jones et al., 2019). Nanotechnology offers enhanced DDS with adequate size to infiltrate into the superficial cartilage and to control drug local pharmacokinetics. Immediately after administration into the joint, NPs interact with cartilage due to their infiltration and penetration within the tissue matrix. The deeper knowledge about OA molecular features and symptomatology facilitates the development of more complex and efficient DDSs for the specific target of different tissues in the joint. Nanomaterials facilitate the incorporation of drugs in the surface or in their matrix to avoid drug degradation, enhance their penetration across the ECM, and allow the modification of drug pharmacokinetics, which involves the reduction in side effects and the increase in drug efficacy (Li, Dai, et al., 2021). Considering these premises, different authors have previously worked on a wide range of materials for OA treatment in in vivo OA models. In fact, some authors have shown the potential of inorganic NPs for OA treatment in vivo (Abdel-Aziz et al., 2021; Chen et al., 2021; He et al., 2021; Li et al., 2019; Sarkar et al., 2019) though as these nanosystems have not fully demonstrated their biodegradability are not considered in this section.

On the other hand, biodegradable synthetic polymers such as PCL, polyester amide (PEA), polyethylene glycol (PEG), poly (glycolic acid) (PGA), poly (lactic acid) (PLA), PLL, poly (l-lactic acid) (PLLA), and PLGA are widely used in OA in vivo studies. These polymers offer a proper control over their physico-chemical properties allowing their customization for different biological approaches. In fact, the negative charge on their surface may be a drawback to penetrate the negative charged cartilage ECM, though cationic modifications on their surface enhance their efficiency. Furthermore, the possibility to encapsulate hydrophobic and hydrophilic drugs is a great advantage for their use as DDS. Another relevant advantage that synthetic polymers show is the possibility to tailor their degradation in order to modify drug release as it can be exemplified with the hydrolytic degradation of PLGA by means of the control of its molecular weight, composition, copolymerization and functionalization, achieving a specific time-dependent drug delivery. However, the main drawback of PLGA degradation is the production of acidic compounds that may enhance ECM degradation and cartilage inflammation (Li, Dai, et al., 2021; Park et al., 2012; te Boekhorst et al., 2012).

PEA microspheres loaded with the COX-2 inhibitor celecoxib, a well-known anti-inflammatory and analgesic drug for OA related pain, were assayed in a rat OA model based on the transection of the anterior cruciate ligament (ACL) (Janssen et al., 2016). In vitro assays demonstrated the efficient drug release from PEA microspheres in Hl-60 lysates pointing to their potential as DDS for the treatment of pathologies with an inflammatory component in which drug release would be reactive to the disease progression. The administered non-loaded microspheres in OA rats and healthy joints showed a differential effect regarding degradation obtaining significantly higher degradation of microspheres in the former group highlighting their profile as an auto regulatory IA DDS. However, the results regarding the joint pathology did not reveal significant differences among control groups and loaded and non-loaded microsphere treated groups, which may be attributed to the chondroneutral effects of celecoxib in vivo.

Aini et al. (2016) carried out the in situ mRNA delivery of a therapeutic cartilage-anabolic transcription factor (RUNX-1) through polyplexed nanomicelles which comprised PEG-polyamino acid block copolymer showing a PEG outer layer and a mRNA-containing core. This DDS successfully delivered mRNA into mice joint cavity as IVIS demonstrated by testing labeled nanomicelles with luciferase and with enhanced GFP production. Moreover, the fabricated DDS demonstrated in vivo, in a DMM mouse model, its potential as therapeutic system by the arrest of OA progression together with the increase in anabolic cartilage markers. This may be attributed to the gene therapeutic effect mediated by RUNX-1 though the comparison with the non-loaded mRNA was not shown in that study. Kang et al. (2020) synthesized acid-activatable curcumin polymer micelles based on the synthetic polymer PEG (Figure 9). This DDS was tested in an in vitro model of LPS stimulated RAW 264.7 macrophages demonstrating a significantly superior anti-inflammatory effect compared with the addition of free curcumin to the cultures. In addition, in vivo studies in a

TABLE 1 Overview of nanoparticle-based strategies used for OA diagnosis

Nanomaterial	Detection method	Cell line and/or patients' samples /animal model	Targeting/activation method	Outcomes	References
ALN-SPIONs (17 nm)	MRI	Hydroxyapatite microparticles (diameter of 20 μm)	Hydroxyapatite	Nonionizing dynamic contrast agent, in vitro selective binding affinity (65%) for hydroxyapatite	Panahifar et al. (2013)
SPIONs (12 nm)	MRI	Porcine cartilage and equine articular tissue	Depletion of extracellular matrix components of cartilage	Increased MRI signal, SPIONs dose dependent inflammatory response	Labens et al. (2017)
PLGA-PEG-TFA NPs loaded with IR-780 Iodide (203 ± 2 nm)	^{19}F-MRI and fluorescence	C28/I2 human chondrocytes; Mouse model	Cellular uptake/Dye release	NPs for multimodal non-invasive imaging, successful in vivo visualization of OA knee joint	Zerrillo et al. (2021)
Antibody-coated AuNRs (6–12 nm width)	PAI	Mouse model	TNF-α	Enhancement of optoacoustic signal amplitudes, detection of high expression level of TNF-α	Fournelle et al. (2012)
PLL-MNPs (39 ± 2.3 nm)	PAI	Cartilage of rat knee joints; Mouse model	GAGs	Enhanced PAI signal, discerned early-stage from late-stage OA	Chen et al. (2018)
BINPs mixed with ioxaglate (260 ± 80 nm)	Micro-CT	Bovine osteochondral plugs	Surface of cartilage	Dual contrast method, detection of mechanical and enzymatic damage of cartilage, reduction in patient radiation dose to half	Saukko et al. (2017)
Ta_2O_5 NPs (5–10 nm)	Micro-CT	Murine tibia and human index finger cartilage; Mouse model	GAGs	Optimal penetration of cationic NPs and preferential accumulation in OA defects	Freedman et al. (2014)
AuNPs-peptide (9.7 ± 0.5 nm)	Fluorescence	Synovial fluid samples from patients and rabbits; Rabbit model	ADAMTS-4 enzyme	Fluorescence intensity proportional to the ADAMTS-4 concentration, combined AU-probe/MRI testing outperformed MRI alone	Zhenlong Liu et al. (2018); Peng et al. (2013)
CMFn@HCQ (22 nm)	Fluorescence	Chondrocytes; Mouse model	MMP-13	Fluorescence intensity proportional to the level of overexpressed MMP-13, cartilage-targeting probe and	Haimin Chen et al. (2019)

TABLE 1 (Continued)

Nanomaterial	Detection method	Cell line and/or patients' samples /animal model	Targeting/activation method	Outcomes	References
				release of drug under specific conditions	
PCL-based nanomicelles loaded with psoralidin (120.3 ± 22.8 nm)	Fluorescence	C57BL6/J primary chondrocytes; Papain/L-cysteine injection in mouse	MMP-13 and Type II collagen	In vitro reversion of inflammation and cartilage degradation. In vivo significant reduction in OA scores compared with free drug and OA control groups	Lan et al. (2020)
MabCII-coated nanosomes with a NIR-dye (200 nm)	Fluorescence/IVIS	Porcine cartilage explants; Post-traumatic OA mouse model and destabilization of the medial meniscus model	Type II collagen	Specific binding to the cartilage according to the severity of damage, quantitative diagnostic	Cho et al. (2015, 2018)
PLGA NPs loaded with DAF-FM probe (1000 nm)	Fluorescence	IL-1β-stimulated chondrocytes; Rat model	NO	Nanosensor permitted the in vivo quantification of NO levels in joint fluid	Jin et al. (2017)
N-S/GQDs (3 nm)	Fluorescence	Synovial fluid samples from patients	Pyrophosphate ion	Detection of pyrophosphate ion concentration in synovial fluid of OA patients	Liu, Xiao, et al. (2016)
Cysteine-coated AuNPs (13 nm)	UV–vis spectroscopy	Synovial fluid samples from patients	Pyrophosphate ion	Highly sensitive and selective method with low technical and instrumental demands	Deng et al. (2013)
Antibody-coated AuNPs (8.4 ± 2.8 nm)	FOPPR sensor	Synovial fluid samples from patients	TNF-α and MMP-13	Label-free, real-time, and high sensitivity detection platform, good correlation with ELISA	Huang et al. (2013)
Citrate-AuNPs	MALDI-TOF MS	Urine samples from OA patients	Urine proteins	Fast, non-expensive and robust test, higher sensitivity but lower specificity than common methods	López-Cortés et al. (2016)
Antibody-coated AuNPs (30 nm)	Lateral-flow immunoassay	Synovial fluid samples from patients	COMP	Quantitative detection of COMP in a broad detection range, good correlation with ELISA	Hong et al. (2012)

monoiodoacetate OA mouse model confirmed the in vitro results displaying the higher suppression of the upregulated inflammatory and cartilage degradation markers compared with the administration of the free drug, together with an excellent safety profile, pointing to the suitability of this developed DDS in OA management.

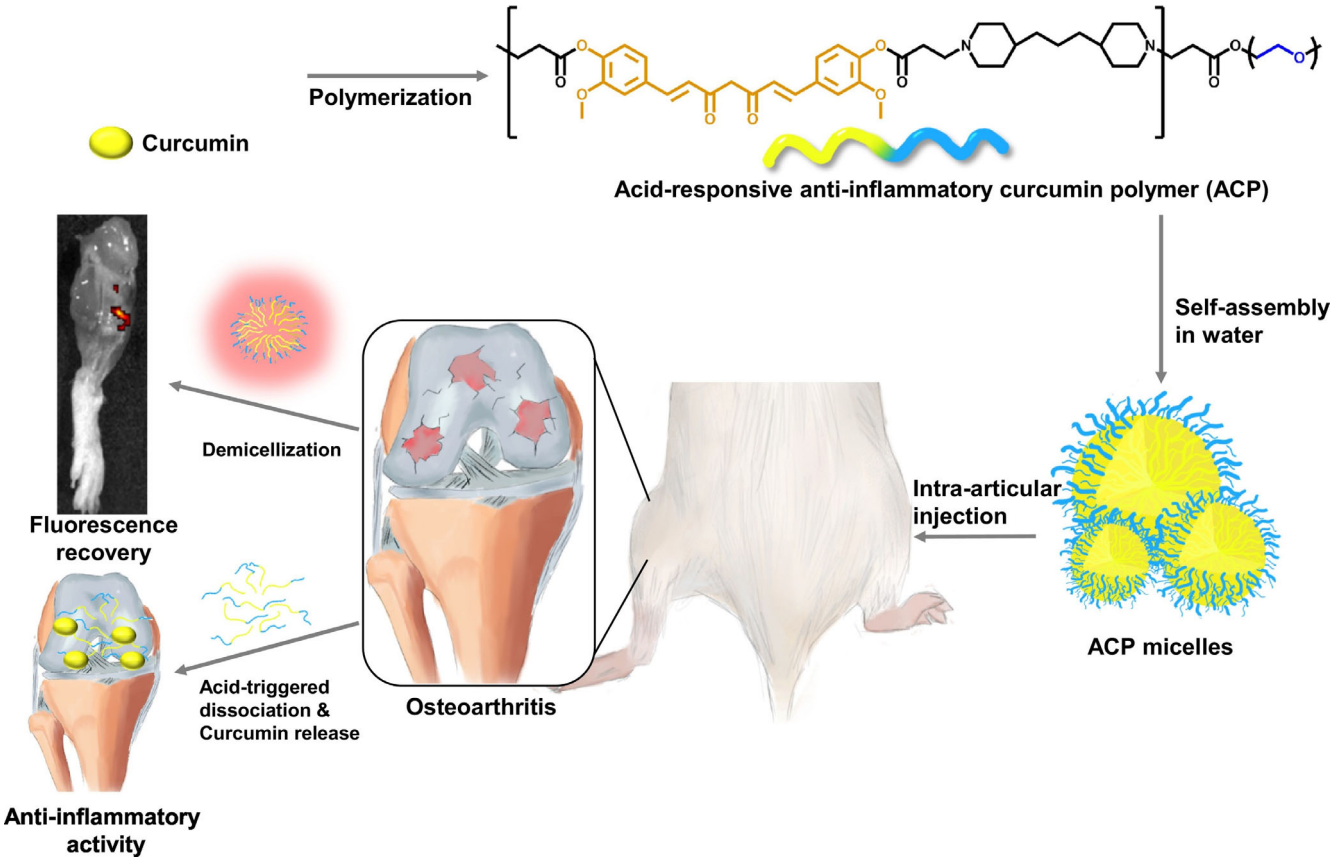

FIGURE 9 Scheme of the pH-responsive polymeric prodrug of curcumin developed as a therapeutic drug delivery system for osteoarthritis. Reprinted with permission from Elsevier (Kang et al., 2020). Copyright 2020.

PLA has been also explored as suitable polymer for the fabrication of microparticles with very high drug loadings and prolonged drug delivery ability (Maudens et al., 2018). In particular, these authors synthesized kartogenin nanocrystals by wet milling which then were loaded into PLA microparticles covalently labeled with Cy7. This DDS exerted an extended drug delivery of ~62% up to 92 days and good viability percentages in human OA fibroblast-like synoviocytes cultures. Moreover, the treatment of OA joints in a model of DMM developed in mice highlighted the therapeutic potential of these loaded microparticles by the improvement of OA scores though inflammatory scores were not alleviated probably due to the mechanism of action of kartogenin. In addition, the efficacy of the loaded microparticles were significantly higher than that of the free kartogenin group, pointing to the benefits of the crystallization and encapsulation of the drug in therapeutic applications. Finally, the persistence of this developed DDS in the tissues was revealed up to 2 months. Liu et al. (2019) investigated the use of click chemistry for the development of six targeted polymeric (PEG-b-PLA) NPs for their use as adenosine receptor agonists by binding adenosine at different positions with an acetylene group. RAW264.7 macrophages were used to test the stimulation of adenosine receptors showing the binding and activation of functionalized NPs to adenosine and to its receptors, being more efficient those in which the copolymer was bound to the adenosine on the 3′,4′-hydroxyl groups. Furthermore, a posttraumatic in vivo OA (PTOA) model inducing anterior cruciate ligament rupture in rat knees confirmed the therapeutic potential of this DDS through the amelioration of OA markers, specifically knee swelling, fibrillation of cartilage surface and proteoglycan loss.

PLL-based NPs have been recently reported as an effective DDS in OA treatment by favoring macrophage polarization towards the anti-inflammatory phenotype M2 (Kou et al., 2022). These authors developed a bilirubin-PLL conjugated polymer in which berberine, a well-known anti-inflammatory compound, was loaded. Then, IgG was added to obtained opsonized NPs in order to achieve the specific recognition and uptake of these NPs by macrophages. Their efficiency was tested in vitro in RAW 264.7 macrophages and in rat cartilage cells whereas in vivo experiments were carried out in a rat ACL transection model. Both in vitro and in vivo assays revealed the successful alleviation of inflammatory markers in chondrocytes and joint synovium as well as the regulation of macrophages polarization to M2 phenotype to stimulate cartilage regeneration. Furthermore, the developed DDS exerted the selective phagocytosis by

M1 macrophages (pro-inflammatory phenotype) leading to M2 polarization restraining inflammation in surrounding chondrocytes and therefore, boosting cartilage repair to alleviate OA.

PLGA based DDSs have been widely explored for IA administration in the treatment of OA as this polymer has been approved as safe biomaterial in many DDSs and medical devices by the FDA. In fact, one of the IA formulations currently approved for the management of OA, Zilretta®, is based on PLGA microspheres loaded with the corticosteroid triamcinolone acetonide (Paik et al., 2019; ZILRETTA [Triamcinolone Acetonide Extended-Release Injectable Suspension], 2021). PLGA enables the slow release of the drug achieving a significant relief of pain and stiffness though the efficacy and safety of repeated administration have not been yet demonstrated (ZILRETTA [Triamcinolone Acetonide Extended-Release Injectable Suspension], 2021). The encapsulation of the parathyroid hormone (PTH) (1–34) into PLGA microspheres was developed in order to test its suitability as a therapeutic platform in papain-induced OA rats (Eswaramoorthy et al., 2012). The results revealed no significant differences between PTH/PLGA microspheres and free PTH (1–34) administration effects in cartilage, being both strategies successful in the effective suppression of OA progression. PLGA has been also used to encapsulate statins such as fluvastatin (Goto et al., 2017). In vitro assays in human OA chondrocytes revealed the potential of fluvastatin in ameliorating OA catabolic markers and promoting cartilage anabolic markers versus other statins. Then, fluvastatin was encapsulated in PLGA and tested in a surgical OA model (ACL transection) in rabbits which not revealed significant differences in morphological changes among control (treated with non-loaded microparticles) and experimental groups though the OARSI scores and biochemical changes pointed to the remission of OA in treated animals.

Other widely used compounds are natural polymers, mostly GAGs analogues, and also ECM components such as hyaluronic acid (HA), chondroitin sulfate and collagen. The former show good biocompatibility and easy surface modification though its low water solubility and poor targeting efficiency may impair its successful application. Chitosan-based NPs were successfully tested in a monoiodoacetate OA in vivo model (Gao et al., 2020). In particular, IA administration of cationic functionalized chitosan chemically conjugated with superoxide dismutase (SOD) produced a decrease in articular cartilage damage and mechanical allodynia as well as anti-inflammatory effects. These effects were attributed to the enhanced targeted intracellular ROS clearance activity and pharmacokinetic profiles mediated by the NPs. In this sense, chitosan modified MoS_2 nanosheets as NIR photo-responsive DDSs loaded with the anti-inflammatory drug dexamethasone were tested in a murine model of OA induced by IA papain injection (Zhao et al., 2019). RAW 264.7 macrophages were treated with the developed DDSs and then irradiated to study the NP uptake and the inflammatory status of cells after induction of inflammation with LPS. These in vitro studies demonstrated a maximum NP uptake after 6 h with a time dependent trend being increased cell viability and uptake efficiency when the modification of chitosan was included in the DDSs versus the plain MoS_2 nanosheets. Moreover, NIR irradiation increased drug release efficiency up to 42% pointing to the prolonged residence time of the drug, together with a significant downregulation of synovial inflammatory factors. In vivo, these results were confirmed demonstrating improved retention in the joint space, better anti-inflammatory effects compared with the free drug and enhanced chondroprotective ability. Other authors have also shown the suitability of chitosan based nanocarriers loaded with anti-inflammatory drugs following IA administration. Chitosan/tripolyphosphate microspheres loaded with lornoxicam were IA administered in a rat OA model mediated by monoiodoacetate injection. At the end of the experiments, the results showed a significant inhibition of joint swelling of the loaded microspheres together with the inhibition of IL-6 release and diminished cartilage damage compared with control and free lornoxicam treated groups (Abd-Allah et al., 2016). Desulphated heparin based microparticles (MPs) were explored as suitable carriers for TNF-α-stimulated gene-6 (TSG-6) (Tellier et al., 2018). IA administration of the loaded MPs in an in vivo model of OA mediated by the medial meniscal transection (MMT) demonstrated the relieve of cartilage damage. The treatment did not show increase of cartilage volume, thickness and attenuation compared with the untreated group and even more interestingly, soluble TSG-6 treated group at higher concentrations, pointing to an enhanced TSG-6 activity when encapsulated into desulphated heparin MPs (Figure 10). The delivery of curcumin from gelatin/silk fibroin microspheres was studied in an OA model induced in rats by monoiodoacetate administration (Ratanavaraporn et al., 2017). IL-6 levels in serum were significantly decreased after treatment with the developed DDS compared with the control group (non-treated OA animals) as well as radiographic and histologic data revealed the significant attenuation of OA signs in animals treated with the fabricated microspheres. The authors point to the high encapsulation efficiency (55–59%) and the slow degradation rate as the reasons behind the enhanced therapeutic potential of the developed DDS.

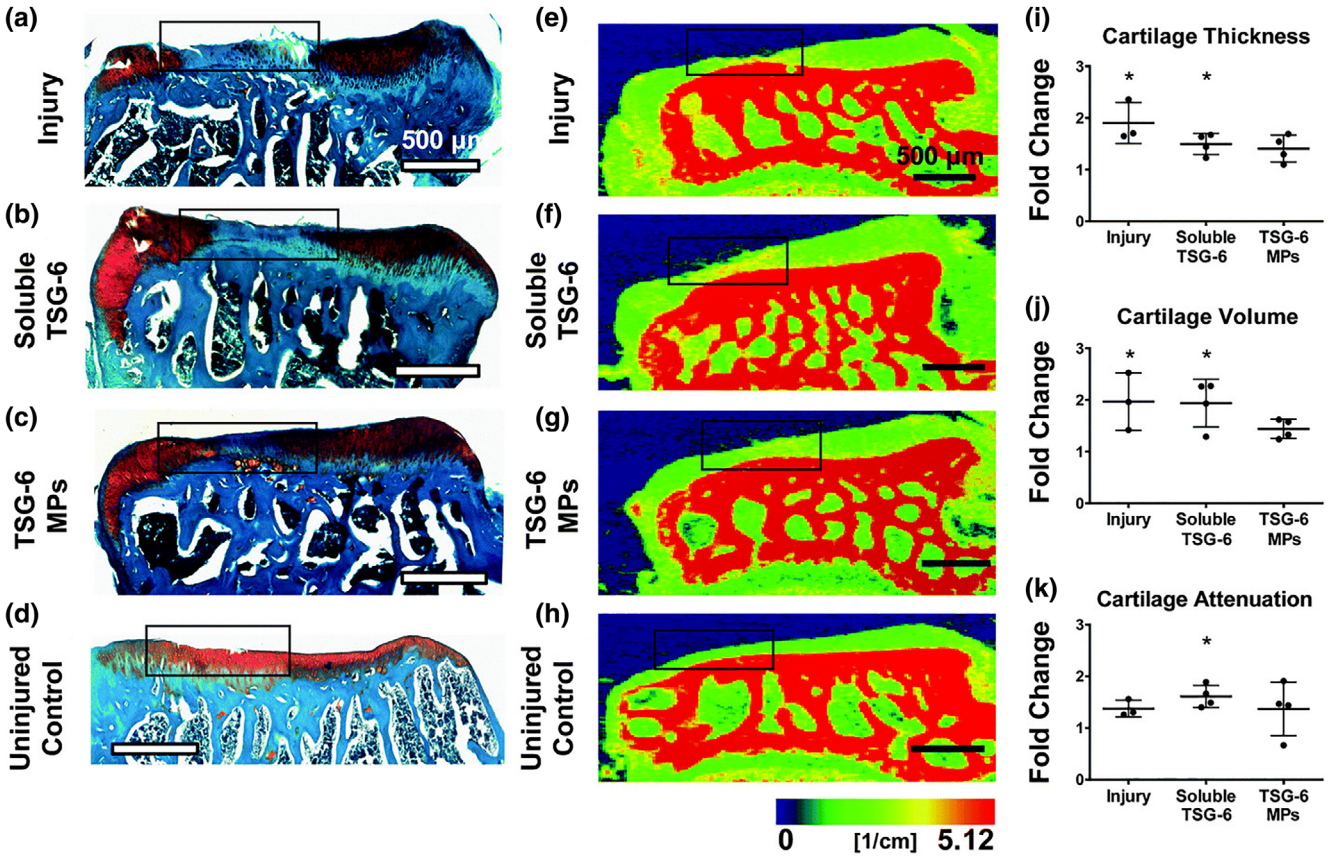

FIGURE 10 TSG-6 loaded MPs reduce cartilage damage 3 weeks following MMT injury. (a–d) Safranin-O stained coronal sections of tibiae 3 weeks following injury and treatment indicated that GAG loss was observed in the (a) injured and (b) soluble TSG-6, but not in the (c) TSG-6 loaded MP group. (e–h) contrast-enhanced μCT imaging of the same samples indicated that cartilage fibrillation was present in the (e) injured and (f) soluble TSG-6 groups but was not present in the (G) TSG-6 loaded MP group. (i–k) quantified evaluation of articular cartilage indicated that (i) cartilage thickness and (j) volume were significantly increased compared with uninjured controls after injury and soluble TSG-6 treatment, and (k) cartilage attenuation was significantly increased after soluble TSG-6 treatment. In contrast, no significant increase in cartilage thickness, volume, or attenuation was observed in the TSG-6 loaded MP group compared with uninjured controls. *Significantly different than contralateral control ($p \leq 0.05$). Reprinted with permission from The Royal Society of Chemistry (Tellier et al., 2018). Copyright 2018.

ECM components are very appealing as building blocks in the synthesis of NPs as they show anti-inflammatory, antioxidant and analgesic effects and they produce an improvement in the physical properties of the joint by providing viscoelastic properties, compression resistance and enhancing the joint biomechanics (Li, Dai, et al., 2021). In this sense, self-assembled HA-NPs were obtained by chemical conjugation of a low molecular weight hyaluronic acid backbone (MW = 10 kDa) with hydrophobic 5β-cholanic acid (Kang et al., 2021). In vitro studies in primary articular murine chondrocytes and in samples obtained from human OA donors subjected to total knee arthroplasty, together with an in vivo DMM OA model, highlighted the chondroprotective ability of HA-NPs through the inhibition of the catabolic gene axis CD44-NF-κB. These results point out to the inhibition of cartilage degeneration and OA progression even more effectively than current treatments with high molecular weight HA. Phosphatidylcholine lipid was the basis for the fabrication of superlubricated nanospheres (poly [2-methacryloyloxyethyl phosphorylcholine]-grafted mesoporous silica nanospheres) through the photopolymerization and loading of sodium diclofenac (Hao Chen et al., 2020). This work demonstrated the improved lubrication and sustained release of the developed DDS. Moreover, the in vitro and in vivo assays showed the inhibition of OA progression upregulating cartilage anabolic components and downregulating catabolic proteases and pain-related genes although in vitro no significant differences were found between the administration of the free drug versus the loaded DDS (Table 2).

TABLE 2 Overview of nanomaterials-based strategies for IA treatment in in vivo OA models

Nanomaterial	Cell line and/or patients' samples/animal model	Outcomes	References
Celecoxib-loaded PEA microspheres (10–100 μm)	Hl-60 lysates, synovial fluid and synovium from patients; Transection of ACL OA model in rat	In vitro, drug release reactive to inflammation and auto regulatory degradation. Chondroneutral effects in vivo	Janssen et al. (2016)
mRNA (RUNX-1) loaded into PEG-polyamino acid block copolymer (50 nm)	DMM in mouse	Suppression of OA progression, improved expression of anabolic cartilage markers	Aini et al. (2016)
Activatable curcumin PEG micelles (∼170 nm)	RAW 264.7 macrophages; Monoiodoacetate OA model in mouse	Anti-inflammatory and chondroprotective effects, no in vivo toxicity	Kang et al. (2020)
PLA microparticles loaded with kartogenin nanocrystals (13.8 μm)	Human OA fibroblast-like synoviocytes; DMM in mouse	Improvement of OA scores though inflammatory scores were not alleviated. Persistence in tissues up to 2 months	Maudens et al. (2018)
Adenosine bound to PEG-b-PLA NPs (129–141 nm)	RAW 264.7 macrophages; PTOA model inducing anterior cruciate ligament rupture in rat	In vitro binding and activation of functionalized NPs to adenosine and receptors. In vivo amelioration of knee swelling, fibrillation of cartilage surface and proteoglycan loss	Liu et al. (2019)
Bilirubin PLL NPs loaded with berberine and opsonized with IgG (212 nm)	RAW 264.7 macrophages and rat chondrocytes; Transection of ACL OA model in rat	In vitro selective targeting of M1 macrophages and attenuation of inflammation and OA markers. In vivo macrophage polarization and OA attenuation	Kou et al. (2022)
PTH (1–34) loaded PLGA microspheres (69 ± 25 μm)	MC3T3-E1 cells; Papain IA injection in rat	No significant differences between loaded microspheres and free PTH (1–34) groups in the in vivo OA treatment	Eswaramoorthy et al. (2012)
PLGA loaded with fluvastatin (15–40 μm)	Human OA primary chondrocytes; Transection of ACL OA model in rabbit	No significant differences in morphological changes among groups, OARSI scores and biochemical changes pointed to the remission of OA	Goto et al. (2017)
Chitosan based NPs functionalized with SOD (236.7 nm)	Primary rat chondrocytes; Monoiodoacetate OA model in rat	Inhibition of cartilage degeneration. Reduction of mechanical allodynia and inflammation	Gao et al. (2020)
Chitosan modified MoS_2 nanosheets loaded with dexamethasone (78 ± 18 nm)	RAW 264.7 macrophages; Papain IA injection in mouse	In vitro prolonged residence time of the drug and improved anti-inflammatory effect. In vivo, improved retention in the joint space and better anti-inflammatory and chondroprotective effects	Zhao et al. (2019)
Chitosan/tripolyphosphate microspheres loaded with lornoxicam (3.57–6.12 μm)	Monoiodoacetate OA rat model	Significant inhibition of joint swelling, IL-6 levels and cartilage damage	Abd-Allah et al. (2016)
Heparin desulphated MPs loaded with TSG-6 (80 ± 60 μm)	MMT OA model in rat	Cartilage thickness, volume and attenuation were not increased after loaded MPs treatment in contrast to soluble TSG-6 group	Tellier et al. (2018)

(Continues)

TABLE 2 (Continued)

Nanomaterial	Cell line and/or patients' samples/animal model	Outcomes	References
Gelatin/silk fibroin microspheres loaded with curcumin (100–300 μm)	Monoiodoacetate OA model in rat	Significant attenuation of OA radiographic and histologic signs in OA treated animals	Ratanavaraporn et al. (2017)
HA-NPs (221 ± 1 nm)	Articular chondrocytes (mouse and human explants); DMM in mouse	Chondroprotective effect through the inhibition of the catabolic gene axis CD44-NF-κB. Inhibition of cartilage degeneration and OA progression	Kang et al. (2021)
Phosphatidylcholine lipid based superlubricated nanospheres loading sodium diclofenac (260 nm)	Primary rat chondrocytes; DMM in rat	Inhibition of OA progression by the upregulation of cartilage anabolic components and downregulation of catabolic proteases and pain-related genes	Hao Chen et al. (2020)

4 | CONCLUSION

OA is a complex pathology showing various phenotypes. The identification and specific targeting of these phenotypes seems to be the key strategy in the development of novel and more efficient diagnostic and therapeutic strategies. Until then, the careful management of symptoms in early and moderate OA together with surgical procedures in advanced OA remain as the basis of OA therapies. Nanomaterials have offered novel approaches and platforms for OA diagnosis and treatment. Nano-diagnosis has been addressed mainly through two strategies: nanoparticles as contrast agents in medical imaging and in the development of nanoprobes for the sensitive detection of OA-associated biomarkers. Novel approaches in which NPs monitor OA disease progression in real time have already been described in this review. Despite their potential, further research should be conducted in order to validate in vivo their diagnostic capabilities, specially detecting the early stages of OA. Future work should therefore include the development of targeted nanomaterials highly sensitive and selective to biomarkers in the synovial cavity or in the articular cartilage, that would enable the early diagnosis and improve the tracking of OA progression. The tracking of the disease evolution, at the same time that therapeutic drugs are being delivered, would allow a rapid patient stratification according to the severity of the disease and the design of personalized treatments and also fast treatment modifications when needed. Another essential requirement is the biocompatibility and degradability of the systems used for diagnosis and therapy. Vectors should be designed taking into account that the material should be degraded once the diagnostic process has ended, at the same time that they should be stable enough to report accurate and reliable data during the monitoring process.

Summing up, we foresee that future research in the field of nano-diagnosis will be addressed towards the use of targeted NPs with specific and selective binding to OA markers, which would allow the imaging and treatment while reporting the progression of the disease in real time. Nanoenabled DDSs should demonstrate not moderate but exponential benefits compared with the administration of the free drugs which would justify their use; otherwise, we will not witness their rapid translation to the clinic. The development of smart DDSs that fulfill the adequate drug release at the target site, considering that the disease changes over time and including highly sensitive nanoprobes for the accurate diagnosis of OA progression, appears to be a very promising approach for OA treatment. The advancement in the design of internal stimuli-responsive nanomaterials will reveal a wide range of novel and potentially successful diagnostic tools and therapies for OA, as their basis is the triggering depending on objective pathological changes. Future studies in the development of smart nanomaterials for OA diagnosis and treatment based on advanced materials and the unraveling of OA molecular mechanisms are of paramount relevance for the progress of nanomedicine in the early diagnosis and treatment of the disease.

AUTHOR CONTRIBUTIONS

Monica Paesa: Formal analysis (equal); investigation (equal); software (equal); writing – original draft (equal); writing – review and editing (equal). **Teresa Alejo:** Formal analysis (equal); investigation (equal); software (equal); writing – original draft (equal); writing – review and editing (equal). **Felicito Garcia-Alvarez:** Formal analysis (equal);

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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