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Autophagy-mediated chondrocyte homeostasis: advances in hydrogel carriers for osteoarthritis treatment

Shuya Liu[1,2], Zhewen Mi[1,2], Shijun Liang[1], Zhonghao Jiao[1], Quanxin Du[1], Mingbaicheng Luo[1], Xiaoqing Lu[1], Guangjie Yang[1], Hui Liang[2], Xiaoyang Gao[1], Jing Liu[3], Zhigang Liu[1,*], Daxiang Cui[2,4,*], Qirui Zhao[1,2,*]

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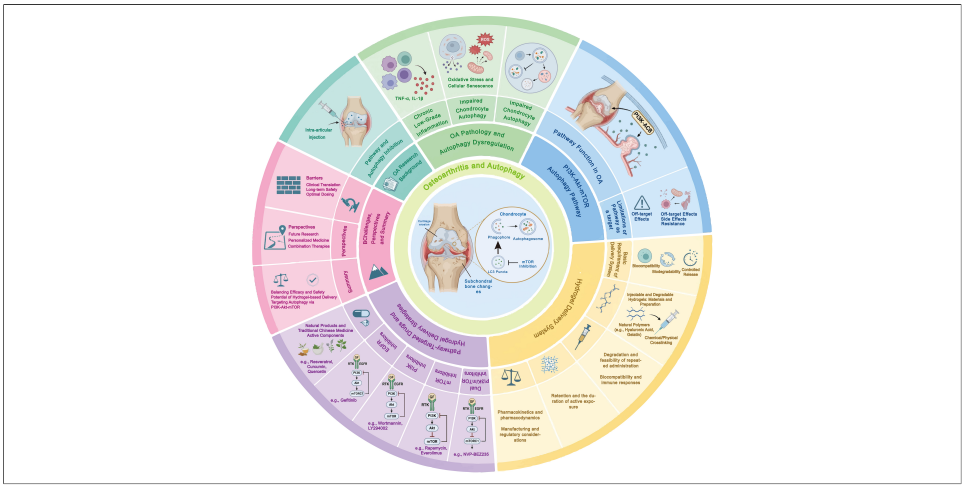

Abstract

Osteoarthritis (OA) is a common chronic degenerative joint disease in older adults, characterized by pain, stiffness, swelling, and functional limitations. Its progression is associated with disruption of the joint microenvironment, inflammation, oxidative stress, cellular senescence, and impaired chondrocyte autophagy. The phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT)-mechanistic target of rapamycin (mTOR) pathway regulates cellular metabolism, survival, and autophagy and may contribute to cartilage matrix degradation and inflammatory responses. This review examines the pathological basis of age-related OA, chondrocyte autophagy dysfunction, pathway regulation, and hydrogel-based local delivery. We discuss hydrogel requirements, material properties, release mechanisms, and limitations, followed by evidence for epidermal growth factor

[1]Department of Orthopedics, The First Affiliated Hospital of Henan University, Henan University, Kaifeng 475004, Henan, China.
[2]Henan Province Engineering Technology Research Center of Intelligent Diagnosis and Treatment, The First Affiliated Hospital, School of Medicine, Henan University, Kaifeng 475004, Henan, China.
[3]Henan Provincial Engineering Center for Tumor Molecular Medicine, School of Basic Medical Sciences, Henan University, Kaifeng 475004, Henan, China.
[4]Institute of Nano Biomedicine and Engineering, School of Sensing Science and Engineering, School of Electronic Information and Electrical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China.

[*]**Correspondence to:** Dr. Qirui Zhao, Dr. Zhigang Liu, Department of Orthopedics, The First Affiliated Hospital of Henan University, Henan University, Kaifeng 475004, Henan, China. E-mail: zhaoqirui@henu.edu.cn; liuzhigang5018@henu.edu.cn; Prof. Daxiang Cui, Henan Province Engineering Technology Research Center of Intelligent Diagnosis and Treatment, The First Affiliated Hospital, School of Medicine, Henan University, Kaifeng 475004, Henan, China. E-mail: dxcui@sjtu.edu.cn

receptor (EGFR) inhibitors, PI3K inhibitors, mTOR inhibitors, dual PI3K/mTOR inhibitors, and natural compounds, including active constituents of traditional Chinese medicine. Hydrogels may prolong intra-articular drug retention, sustain release, and reduce systemic exposure, providing a platform for local delivery of pathway-modulating agents. These delivery advantages do not establish drug efficacy or safety. Most evidence comes from cell experiments, small-animal studies, or non-OA disease models. Autophagic flux assessment, studies in age-appropriate OA models, and long-term evaluation of drug and material safety remain necessary for clinical translation.

INTRODUCTION

Disease burden and therapeutic challenges of age-related osteoarthritis

Osteoarthritis (OA) is a heterogeneous, degenerative joint disease characterized primarily by articular cartilage degeneration, osteophyte formation, and joint dysfunction. It is a leading cause of pain and disability worldwide, with substantial effects on quality of life and socioeconomic costs[1,2]. In China, the prevalence of OA among older adults is a growing concern as the population ages[3]. The hallmark pathological features of OA include articular cartilage degeneration and destruction, osteophyte formation, synovitis, and joint space narrowing. During OA progression, the hyaline cartilage, synovial capsule, and subchondral bone are progressively affected[4-6]. The thinning, fissuring, and eventual erosion of cartilage can cause joint pain, functional limitation, and persistent inflammatory responses. The disease commonly affects weight-bearing or highly mobile joints, including the knee, hip, spine, ankle, and hand joints[7-11].

Current clinical management of OA aims to relieve pain, improve function, and slow disease progression[12,13]. Treatment options include non-steroidal anti-inflammatory drugs (NSAIDs) for analgesia, intra-articular injections (e.g., hyaluronic acid) to enhance joint lubrication, exercise therapy, weight management, and surgical intervention for end-stage cases[14,15]. While oral NSAIDs exert anti-inflammatory and analgesic effects by inhibiting prostaglandin synthesis, their efficacy is largely limited to symptomatic relief, with limited ability to reverse established cartilage degeneration and structural joint damage[16]. Furthermore, exercise therapy and weight management have demonstrated therapeutic benefits for OA. Studies indicate that exercise therapy, regardless of intensity, alleviates symptoms associated with knee OA[17], whereas obesity represents an established, modifiable risk factor for the disease[2]. Nevertheless, these conventional approaches primarily provide symptom control and functional restoration, offering limited disease-modifying effects on underlying pathological processes such as cartilage matrix degradation, synovitis, and dysregulation of the joint microenvironment [Figure 1].

The PI3K-AKT-mTOR pathway and autophagy inhibition

OA pathogenesis involves inflammation, oxidative stress, cellular senescence, and impaired cellular quality control within the joint microenvironment. Autophagy dysfunction can aggravate these processes. The phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT)-mechanistic target of rapamycin (mTOR) pathway regulates metabolism, survival, and autophagy and may link inflammatory signaling to disrupted cartilage matrix metabolism[18,19]. Autophagy enables chondrocytes to remove damaged organelles, abnormal proteins, and metabolic waste. In canonical regulation, mTOR complex 1 (mTORC1) suppresses autophagy initiation. Sustained PI3K-AKT signaling can enhance mTORC1 activity and inhibit unc-51-like autophagy activating kinase 1 (ULK1)-dependent initiation[20,21]. Conversely, mTORC1 inhibition can promote autophagy initiation and may support cellular homeostasis when downstream degradation remains functional. These relationships provide a rationale for investigating local pathway modulation in OA affecting older adults.

Necessity of local delivery therapies

Older patients with OA often have multiple comorbidities, such as cardiovascular diseases, gastrointestinal disorders, reduced renal function, and impaired glucose metabolism, and may receive multiple long-term medications. Therefore, the safety and tolerability of systemic pharmacological treatments are of particular concern in older adults. Although NSAIDs alleviate pain and inflammation by inhibiting the production of inflammatory mediators, they do not specifically target diseased joints. Long-term

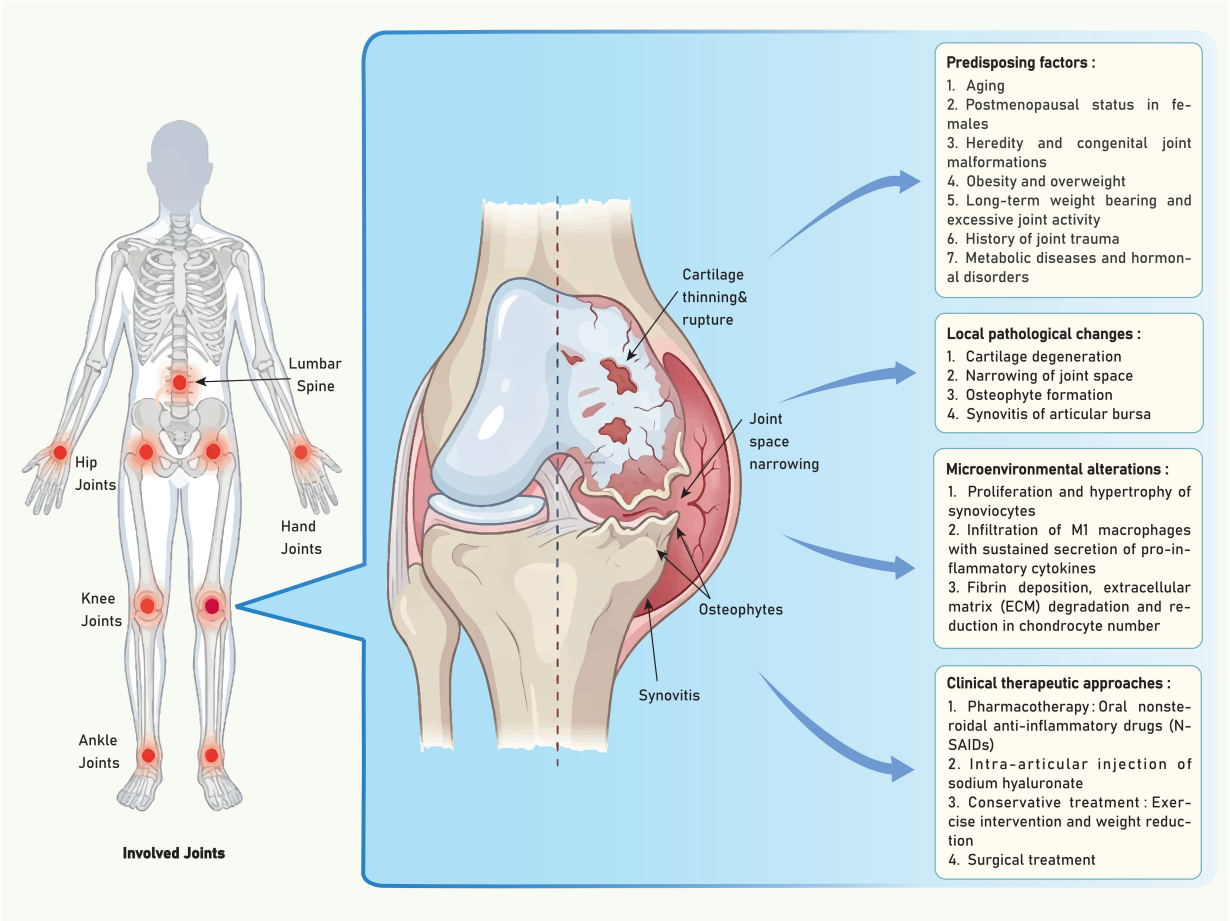

Figure 1. Risk factors, potential influencing factors, and their interaction mechanisms in osteoarthritis. Osteoarthritis mainly involves the hand, hip, knee, ankle joints, and lumbar spine, with characteristic lesions of cartilage thinning and rupture, joint space narrowing, osteophyte formation, and synovitis. Predisposing factors, local pathological changes, microenvironmental alterations, and clinical treatment options are also summarized.

use may increase the risk of gastrointestinal, cardiovascular, and renal adverse reactions, thereby limiting their sustained use in older patients with OA[22]. Furthermore, while intra-articular injections such as sodium hyaluronate can improve joint lubrication and pain symptoms to a certain extent, they predominantly offer short- or medium-term symptomatic relief, with limited disease-modifying effects on pathological processes such as cartilage matrix degradation, synovitis, and subchondral bone remodeling. For patients with end-stage disease, surgery can relieve pain and improve function, but the associated trauma, perioperative risks, and postoperative rehabilitation requirements may reduce its tolerability in some older patients or those with comorbidities.

In this setting, intra-articular (IA) administration provides a rationale for local intervention in older adults with OA. Compared with systemic administration, IA delivery enables therapeutic agents to act more directly on the affected joints, enhancing the bioavailability of drugs at the target site while reducing systemic exposure, systemic side effects, and treatment costs[23]. This approach may be relevant to older patients with OA who require long-term management and face high risks from systemic medication. However, conventional intra-articular injections of free drugs have important limitations: drugs are susceptible to synovial fluid dilution, lymphatic clearance, and joint motion-induced shear forces, leading to a short local retention time, marked fluctuations in drug concentration, and a limited duration of action, potentially necessitating repeated injections to maintain efficacy[24].

Local treatment of OA in older adults therefore requires delivery systems that extend drug retention, control release, and limit the burden of repeated administration. The aim is to maintain an effective

and tolerable exposure profile within the diseased joint, rather than simply to increase intra-articular drug concentration.

Focus of this review

This review examines the relationships among the OA microenvironment, chondrocyte autophagy, PI3K-AKT-mTOR signaling, and hydrogel-based local delivery, with particular attention to their relevance to older adults. We first discuss OA pathology and autophagy dysfunction, followed by pathway regulation. We then consider hydrogel requirements, material properties, release mechanisms, and delivery limitations. Finally, we evaluate evidence for epidermal growth factor receptor (EGFR) inhibitors, PI3K inhibitors, mTOR inhibitors, dual PI3K/mTOR inhibitors, and natural compounds, including active constituents of traditional Chinese medicine. The discussion distinguishes therapeutic rationale from demonstrated efficacy and identifies limitations relevant to clinical translation[25].

PATHOLOGICAL BASIS AND AUTOPHAGY DYSFUNCTION IN AGE-RELATED OSTEOARTHRITIS

OA is a whole-joint disease involving articular cartilage, synovium, subchondral bone, and surrounding tissues. Its onset and progression are accompanied by multiple pathological changes, including chronic low-grade inflammation, oxidative stress, mitochondrial dysfunction, cellular senescence, and intercellular communication abnormalities, which jointly disrupt intra-articular homeostasis and form a persistent degenerative microenvironment[26,27]. In OA in older adults, the adaptive capacity of chondrocytes to inflammation and oxidative stress declines, and cellular homeostasis maintenance mechanisms such as autophagy are impaired, limiting the timely clearance of damaged organelles and abnormal proteins, which further aggravates cartilage matrix degradation and joint structural destruction. Therefore, understanding the relationship among inflammation, oxidative stress, cellular senescence, and autophagy dysfunction in OA in older adults helps explain the potential relevance of the PI3K-AKT-mTOR pathway [Figure 2].

Chronic low-grade inflammation and cartilage matrix degradation

OA was traditionally considered a "non-inflammatory" degenerative joint disease; however, accumulating evidence indicates that chronic low-grade inflammation contributes to OA onset and progression[28,29]. Within the OA joint microenvironment, synovial cells, chondrocytes, and infiltrating immune cells - particularly macrophages - can be persistently activated by aberrant mechanical stimuli, damage-associated molecular patterns, and matrix degradation products, subsequently releasing pro-inflammatory cytokines and catabolism-related mediators. Elevated levels of pro-inflammatory cytokines such as interleukin-1beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), as well as matrix-degrading enzymes such as matrix metalloproteinase 13 (MMP13) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5), characterize the inflammatory microenvironment and cartilage matrix destruction in OA[30].

Inflammatory cytokines such as IL-1β and TNF-α are frequently overexpressed in the synovial fluid, synovium, cartilage, and subchondral bone tissues of patients with OA[31,32]. These inflammatory cytokines can activate signaling pathways such as nuclear factor kappa B (NF-κB), p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase, promoting the expression of various cytokines, adhesion molecules, inflammatory mediators, and catabolic enzymes, thereby disrupting the metabolic homeostasis between chondrocytes and the extracellular matrix (ECM)[33-36]. Among them, enzymes such as matrix metalloproteinase 1 (MMP1), matrix metalloproteinase 3 (MMP3), MMP13, and ADAMTS5 can degrade cartilage matrix components, including type II collagen and aggrecan, leading to decreased cartilage tissue elasticity, impaired structural integrity, and compromised joint function.

Inflammation can promote matrix degradation and impair chondrocyte autophagy, contributing to OA progression. Sustained stimulation by IL-1β and TNF-α can induce elevated rapamycin-insensitive companion of mTOR (RICTOR) expression and further activate the AKT/mTOR signaling pathway, thereby inhibiting chondrocyte autophagy[37]. Following autophagy suppression, the capacity of chondrocytes to clear damaged organelles, abnormal proteins, and inflammation-associated damage

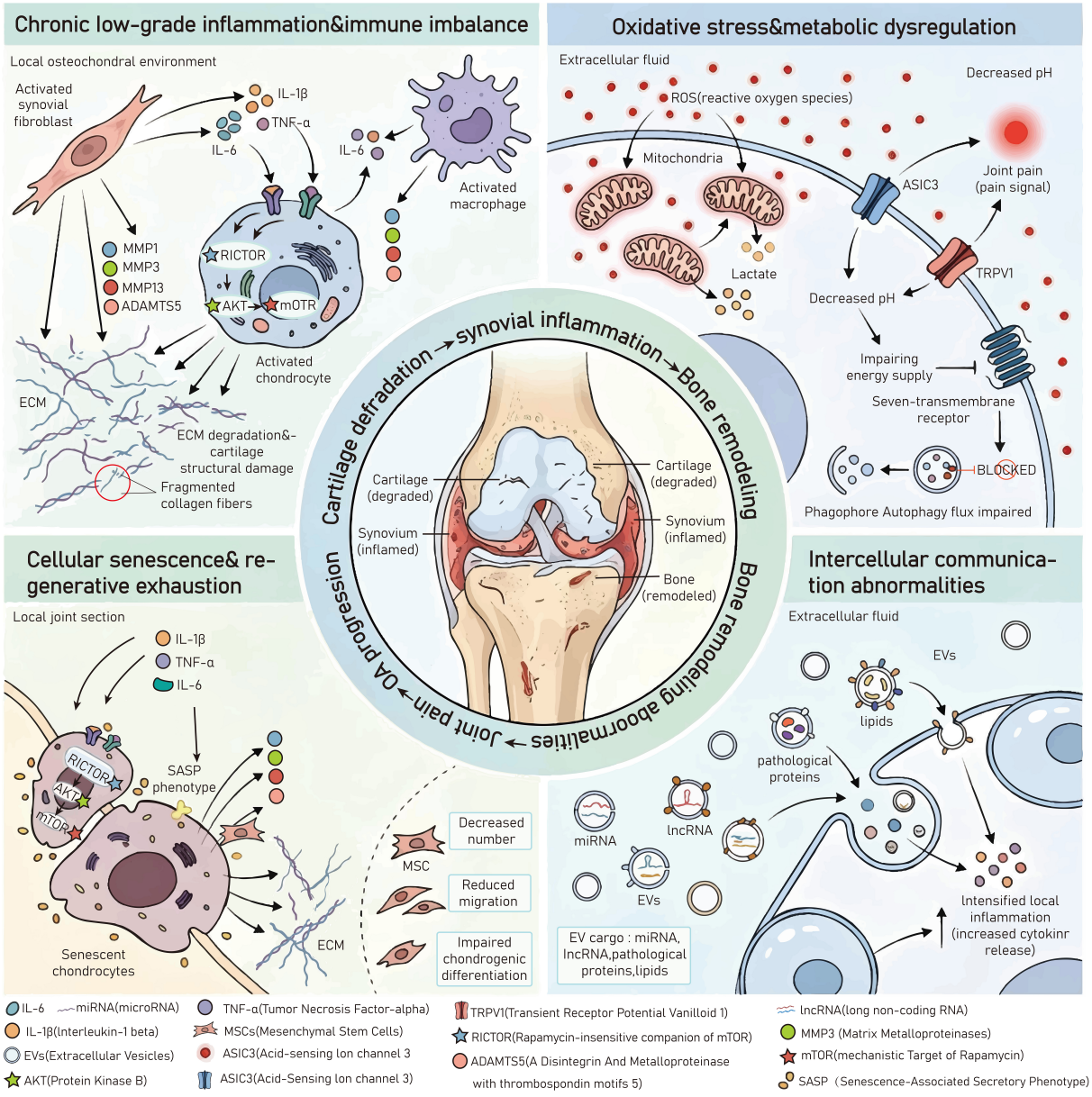

Figure 2. Pathological basis of osteoarthritis regarding inflammation, oxidative stress, cellular senescence, and autophagy dysfunction. The four major pathological dimensions of osteoarthritis include chronic low-grade inflammation and immune imbalance, oxidative stress and metabolic disturbance, cellular senescence and regenerative failure, and aberrant intercellular communication. Chronic low-grade inflammation and immune imbalance: Activated synovial fibroblasts, activated chondrocytes, and infiltrating macrophages secrete pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and matrix-degrading enzymes (MMP1, MMP3, MMP13, ADAMTS5). IL-1β and TNF-α upregulate RICTOR to activate the AKT/mTOR pathway, while MMPs and ADAMTS5 mediate ECM degradation and cartilage structural destruction. Oxidative stress and metabolic disturbance: Excessive ROS damage mitochondria, promote lactate accumulation, and lower the intracellular, extracellular, and intra-articular pH. The acidic environment activates ASIC3 and TRPV1 receptors on chondrocytes to induce joint pain; intracellular acidosis blocks autophagic flux upon activation of seven-transmembrane proteins. Cellular senescence and regenerative failure: Senescent chondrocytes with the SASP senescent phenotype release inflammatory cytokines and matrix-degrading enzymes, which further activate the RICTOR/AKT/mTOR pathway and aggravate ECM destruction. Meanwhile, mesenchymal stem cells (MSCs) exhibit reduced population, impaired migration capacity, and compromised chondrogenic differentiation ability. Aberrant intercellular communication: Extracellular vesicles carrying miRNAs, lncRNAs, pathological proteins, and lipids mediate abnormal cell-to-cell communication and exacerbate local inflammation. MMP: Matrix metalloproteinase; ADAMTS5: a disintegrin and metalloproteinase with thrombospondin motifs 5; RICTOR: rapamycin-insensitive companion of mTOR; AKT: protein kinase B; mTOR: mechanistic target of rapamycin; ROS: reactive oxygen species; ASIC3: acid-sensing ion channel 3; TRPV1: transient receptor potential vanilloid 1; ECM: extracellular matrix; MSC: mesenchymal stem cell; IL-6: interleukin-6; IL-1β: interleukin-1 beta; TNF-α: tumor necrosis factor-α; miRNA: microRNA; lncRNA: long non-coding RNA; EV: extracellular vesicle; SASP: senescence-associated secretory phenotype.

components declines, leading to the further amplification of oxidative stress and inflammatory

responses, creating a feedback loop involving "inflammation-autophagy impairment-matrix degradation". Therefore, chronic low-grade inflammation is not only a major driving factor of OA cartilage degeneration but also a critical pathological link connecting the PI3K-AKT-mTOR pathway, autophagy dysfunction, and cartilage matrix destruction.

Oxidative stress, mitochondrial damage, and cellular senescence

In addition to chronic low-grade inflammation, oxidative stress and cellular metabolic disorders are also important factors driving the progression of age-related OA. Excess reactive oxygen species (ROS) can directly damage chondrocytes and the extracellular matrix and act as signaling molecules that amplify inflammatory responses and disrupt intracellular redox homeostasis[38-40]. In the OA microenvironment, chondrocyte energy metabolism can shift from oxidative phosphorylation toward glycolysis, leading to the accumulation of metabolites such as lactate[41]. The local acidic environment can activate pain-related receptors such as acid-sensing ion channel 3 (ASIC3) and transient receptor potential vanilloid 1 (TRPV1) and aggravate pain, while also inducing chondrocyte stress responses, disrupting autophagic flux, and promoting cellular senescence and apoptosis[42,43].

Mitochondria are central to ROS generation and energy metabolism and are vulnerable to damage in age-related OA. The sustained accumulation of inflammatory cytokines and ROS can lead to decreased mitochondrial membrane potential, reduced adenosine triphosphate (ATP) production, and mitochondrial dysfunction. Concurrently, the diminished autophagy and mitophagy capacities of senescent chondrocytes make it difficult to clear damaged mitochondria in a timely manner, further exacerbating ROS generation and cellular stress responses, thereby forming a positive feedback loop of "mitochondrial damage-oxidative stress-autophagy impairment"[44]. This process not only promotes chondrocyte apoptosis and reduced matrix synthesis but also enhances the expression of pro-inflammatory cytokines and matrix-degrading enzymes, thereby accelerating cartilage degeneration.

Cellular senescence is an important feature of age-related OA and can also arise after injury or other stresses. With increasing age, chondrocytes, synovial cells, and other intra-articular cells can develop a senescent phenotype characterized by cell cycle arrest, decreased stress tolerance, and attenuated repair capacity. Senescent cells in the OA microenvironment frequently present a senescence-associated secretory phenotype (SASP), releasing pro-inflammatory and pro-catabolic factors such as IL-6, IL-1β, TNF-α, chemokines, and matrix-degrading enzymes[29,31]. These SASP factors can not only directly destroy the surrounding cartilage matrix but also further impair the autophagic function of adjacent normal cells, maintaining a local state of chronic inflammation and catabolism[45].

Furthermore, mesenchymal stem cells (MSCs) involved in tissue repair in age-related OA may also exhibit functional decline, including reduced numbers, decreased migration capacity, and impaired chondrogenic differentiation, leading to an insufficient repair response of joint tissues following injury[46]. Therefore, oxidative stress, mitochondrial damage, and cellular senescence interact and contribute to persistent cartilage degeneration in age-related OA. Together, these processes can reduce the ability of chondrocytes to maintain homeostasis and clear damaged components, providing a pathological background for subsequent autophagy dysfunction and aberrant activation of the PI3K-AKT-mTOR pathway.

Chondrocyte autophagy dysfunction

Chondrocyte autophagy dysfunction can disturb extracellular matrix homeostasis. Insufficient autophagy reduces the clearance of damaged cellular components and disrupts energy balance, potentially decreasing cell activity and matrix synthesis. It can also sustain inflammatory signaling through NF-κB and MAPK, increasing matrix-degrading enzymes such as MMP13 and ADAMTS5. These changes promote the loss of type II collagen and aggrecan, compromising cartilage structure and mechanical function during OA progression.

Notably, greater autophagy is not uniformly beneficial. At different disease stages and in different cellular states, autophagy may be protective or may reflect a compensatory response following cellular stress or injury. Previous studies suggest a complex relationship between autophagy and chondrocyte

fate. In early OA, moderate enhancement of autophagy can help clear damaged organelles, alleviate inflammation and oxidative stress, thereby reducing chondrocyte apoptosis[47]; however, when autophagy is severely impaired or aberrantly activated, it may also be associated with forms of cell death such as autophagy-dependent ferroptosis[48]. Furthermore, the relationship between autophagy and cellular senescence is not unidirectional, and certain autophagy abnormalities in OA can be related to SASP formation and the chondrocyte senescence phenotype[49,50]. Chondrocyte autophagy is equally important for cartilage development, joint formation, and matrix homeostasis; relevant models show that impaired autophagic flux or aberrant autophagy-related signaling can affect chondrocyte maturation, cartilage matrix formation, and joint homeostasis maintenance[51,52]. Therefore, when evaluating the chondrocyte autophagy state, changes in a single marker, such as microtubule-associated protein 1 light chain 3 (LC3), Beclin-1, autophagy-related protein 5 (ATG5), or sequestosome 1 (p62), need to be interpreted alongside autophagic flux, lysosomal function, cell survival, matrix synthesis, and inflammation. For age-related OA, the therapeutic goal is to restore effective cellular quality control and chondrocyte homeostasis within the diseased joint microenvironment.

In summary, chronic inflammation, oxidative stress, mitochondrial damage, and cellular senescence jointly weaken chondrocyte autophagy function in age-related OA, while reduced autophagy further aggravates amplification of inflammation, cellular damage, and cartilage matrix degradation. As a vital signaling axis regulating cellular metabolism, survival, and autophagy, the PI3K-AKT-mTOR pathway forms an intersection in the pathological network described above. Therefore, elucidating the role of this pathway in chondrocyte autophagy regulation is important for interpreting hydrogel-delivered drug interventions in age-related OA.

REGULATORY ROLE OF THE PI3K-AKT-mTOR PATHWAY IN CHONDROCYTE AUTOPHAGY

The PI3K-AKT-mTOR pathway regulates cell growth, proliferation, metabolism, survival, and autophagy. During OA progression, chondrocytes are chronically stimulated by inflammatory cytokines, oxidative stress, abnormal mechanical loads, and metabolic disorders, resulting in a progressive decline in their capacity to maintain cellular homeostasis. As a critical pathway connecting external stimuli with intracellular metabolic, autophagic, and matrix metabolic responses, the PI3K-AKT-mTOR signaling axis plays an important role in chondrocyte injury, amplification of inflammation, extracellular matrix degradation, and autophagy dysfunction. Therefore, elucidating the role of this pathway in chondrocyte autophagy regulation provides a basis for interpreting drug interventions and hydrogel-based local delivery strategies.

Overview of the PI3K-AKT-mTOR pathway

The PI3K-AKT-mTOR pathway can be activated by growth factors, insulin, receptor tyrosine kinases, and G protein-coupled receptors. Activated PI3K converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), whereas phosphatase and tensin homolog (PTEN) reverses this conversion. PIP3 acts as a membrane-associated second messenger that promotes AKT activation. Activated AKT inhibits tuberous sclerosis complex 2 (TSC2), facilitating Ras homolog enriched in brain (Rheb)-dependent mTORC1 activation. mTORC1 then inhibits the ULK1 complex and suppresses autophagy initiation[20].

Nutrient deprivation, energy insufficiency, and cellular stress can suppress mTORC1 activity, relieving inhibition of the ULK1 complex and favoring autophagy initiation[21]. Under energy stress, liver kinase B1 (LKB1) activates adenosine monophosphate-activated protein kinase (AMPK), which can inhibit mTORC1 and regulate ULK1. The class III PI3K (PI3KC3) complex I supports phagophore nucleation. The complex formed by autophagy-related protein 12 (ATG12), ATG5, and autophagy-related protein 16-like 1 (ATG16L1) acts as an E3-like ubiquitin ligase to promote conversion of cytosolic LC3-I to lipidated LC3-II. The selective autophagy receptor p62 recognizes ubiquitinated cargo and binds membrane-associated LC3-II, directing cargo into autophagic vesicles. WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) supports membrane expansion and autophagosome formation. UV radiation resistance-associated gene protein (UVRAG) and protein associated with UVRAG as autophagy enhancer (Pacer) contribute to autophagosome maturation. Subsequent fusion with

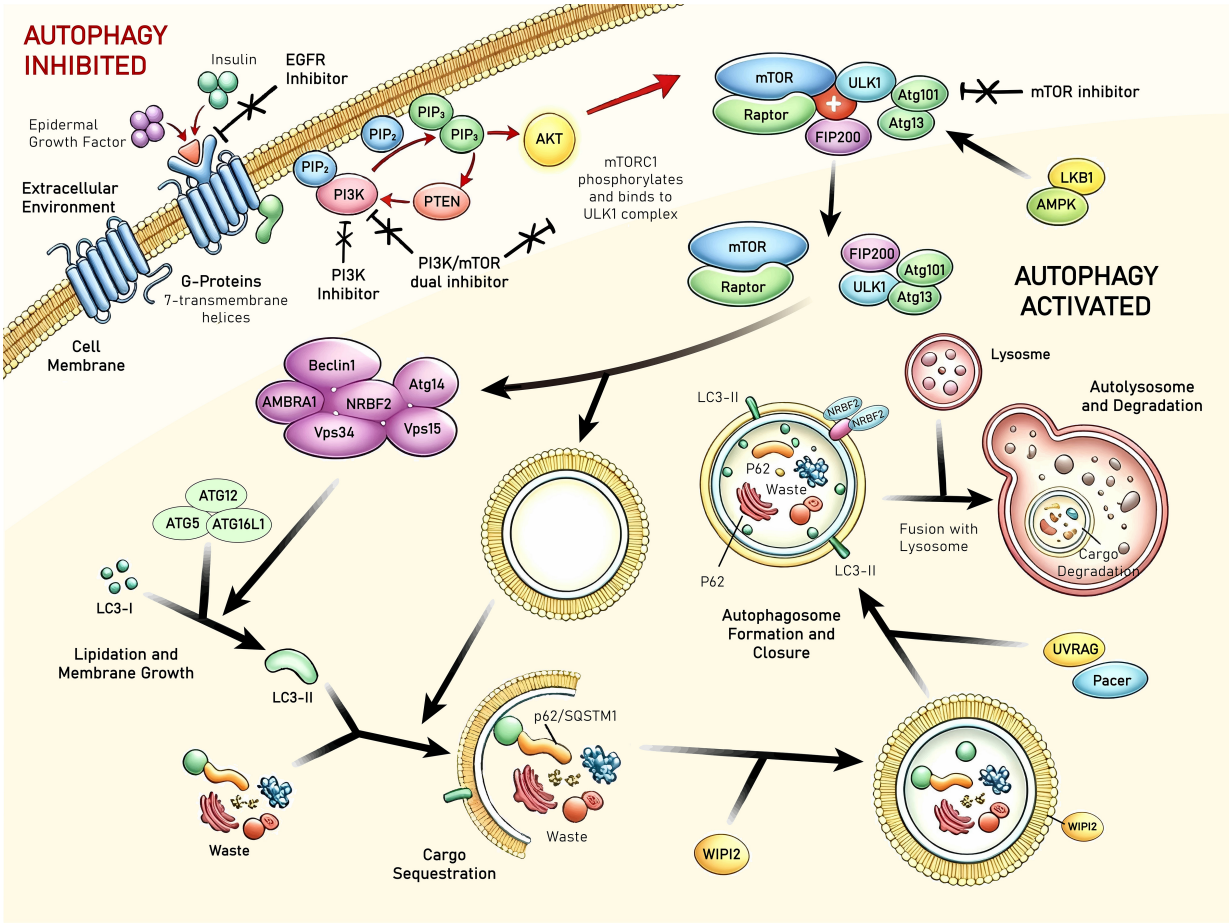

Figure 3. Under physiological conditions, autophagy occurs via intrinsic regulation and contributes to normal cellular processes. During the development of OA, autophagy can be impaired, impeding the timely clearance of intracellular metabolic waste. Therapeutic agents, including EGFR inhibitors, PI3K inhibitors, dual PI3K/mTOR inhibitors, mTOR inhibitors, specific natural compounds, and certain traditional Chinese medicine ingredients, can modulate the PI3K/AKT/mTOR signaling pathway and influence autophagy. EGFR: Epidermal growth factor receptor; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3: phosphatidylinositol 3,4,5-trisphosphate; PI3K: phosphatidylinositol 3-kinase; AMBRA1: autophagy and beclin 1 regulator 1; NRBF2: nuclear receptor binding factor 2; VPS: vacuolar protein sorting protein; ATG: autophagy-related protein; LC3: light chain 3; p62: sequestosome 1; mTOR: mechanistic target of rapamycin; ULK1: unc-51-like autophagy activating kinase 1; FIP200: 200 kDa focal adhesion kinase family-interacting protein; LKB1: liver kinase B1; AMPK: adenosine monophosphate-activated protein kinase; WIPI2: WD repeat domain phosphoinositide-interacting protein 2.

lysosomes produces autolysosomes, in which cargo is degraded. mTOR functions in two complexes: mTORC1 is closely associated with nutrient sensing and autophagy regulation, whereas mTOR complex 2 (mTORC2) regulates AKT activation, cytoskeletal organization, and cell survival [Figure 3].

The role of this pathway in OA

The role of the PI3K-AKT-mTOR pathway in OA extends beyond autophagy regulation and is associated with chondrocyte inflammation, apoptosis, metabolic reprogramming, and ECM metabolism[19]. Persistent exposure to IL-1β, TNF-α, ROS, and abnormal mechanical stimulation within the OA joint microenvironment modulates PI3K-AKT-mTOR signaling through various upstream pathways, thereby altering chondrocyte responses to injurious stimuli. Experimental evidence suggests that G protein-coupled receptor class C group 5 member B (GPRC5B) participates in regulating OA cartilage homeostasis via AKT-mTOR-autophagy signaling; its deletion exacerbates OA induced by destabilization of the medial meniscus (DMM), whereas its overexpression attenuates cartilage degeneration and regulates the balance between matrix synthesis and catabolism[53]. These findings imply that the AKT-mTOR-autophagy axis is not merely a downstream response to cartilage injury but may constitute a mechanistic link for some protective targets. Aberrant activation of this pathway, leading to mTOR-mediated autophagy inhibition, may result in the accumulation of damaged

organelles and abnormal proteins, rendering chondrocytes more susceptible to apoptosis, senescence, and reduced matrix synthesis capacity.

Moreover, the PI3K-AKT-mTOR pathway may modulate the expression of molecules associated with cartilage matrix degradation. Under inflammatory stimuli, chondrocytes and synovial cells produce catabolic factors such as MMP13 and ADAMTS5, which facilitate the degradation of type II collagen and aggrecan. Impaired autophagy leads to the persistent amplification of cellular stress and inflammatory signals, further exacerbating ECM metabolic imbalance. Thus, a mutually reinforcing relationship exists between aberrant PI3K-AKT-mTOR activation, autophagy dysfunction, and cartilage matrix degradation.

However, the PI3K-AKT-mTOR pathway is not a unidirectional "pathogenic switch" in OA. Emerging research indicates that PI3K/AKT signaling may also contribute to cell survival, anti-apoptotic pathways, and repair processes under specific conditions. Consequently, the role of this pathway in OA may be modulated by cell type, disease stage, stimulus intensity, and the timing of therapeutic intervention. For chondrocytes, sustained mTORC1 activation may be detrimental to autophagy maintenance; however, in synovial cells, immune cells, or subchondral bone cells, variations in PI3K-AKT-mTOR signaling may exert distinct or even opposing effects. Therefore, targeting this pathway for OA therapy requires attention to cell type, disease stage, dose, and exposure duration.

Limitations of this pathway as a therapeutic target

Targeting PI3K-AKT-mTOR signaling in OA has important limitations despite its relevance to autophagy and matrix metabolism. First, this pathway regulates fundamental processes, including cell growth, metabolism, immune regulation, and tissue repair; excessive or prolonged inhibition may impair normal cellular functions or interfere with cartilage repair and tissue homeostasis. Thus, pharmacological interventions targeting this pathway require clearly defined exposure windows, treatment durations, and local safety profiles.

Second, OA is a whole-joint organ disease involving chondrocytes, synovial cells, subchondral bone cells, and immune cells, all of which contribute to disease progression. The functional role of the PI3K-AKT-mTOR pathway varies among these cell types. For example, *in vitro* studies suggest that the PI3K inhibitor LY294002 attenuates TNF-α-induced overexpression of cadherin-11 in OA fibroblast-like synoviocytes and reduces their invasive capacity. However, in collagenase-induced OA mouse models, systemic administration of LY294002, while reducing cadherin-11 expression in the synovium, failed to significantly ameliorate synovitis or cartilage injury[54]. This result suggests that protective phenotypes observed via *in vitro* pathway inhibition do not necessarily translate into improved joint outcomes *in vivo*.

Third, autophagy regulation itself depends on disease stage and cellular context. In early OA, moderate restoration of chondrocyte autophagy may facilitate the clearance of damaged organelles, mitigate oxidative stress, and maintain matrix metabolism. However, in late-stage disease, characterized by severe cartilage structural destruction, subchondral bone remodeling, and persistent synovitis, autophagy modulation alone may be insufficient to reverse complex joint structural damage. Moreover, studies often infer autophagy from changes in markers such as microtubule-associated protein 1 light chain 3 beta (LC3B), ATG5, Beclin-1, or p62, which do not fully represent the actual restoration of autophagic flux. Therefore, future studies should integrate autophagic flux assays, indicators of cartilage matrix synthesis and degradation, histological scoring, and joint function evaluations to assess the therapeutic effects of PI3K-AKT-mTOR targeted interventions.

Finally, systemic inhibition of the PI3K-AKT-mTOR pathway may trigger off-target effects and systemic adverse reactions, contributing to interest in local delivery strategies. Intra-articular delivery platforms, such as hydrogels, may support local administration of pathway-modulating drugs by prolonging drug retention, minimizing systemic exposure, and enabling sustained release. Nevertheless, hydrogel-based delivery does not inherently resolve the safety and efficacy concerns of the drugs themselves; further investigations into drug release kinetics, intra-articular distribution, chondrotoxicity, and long-term therapeutic outcomes remain essential.

Table 1. Primary categories and characteristics of hydrogels for intra-articular OA delivery and cartilage functional replacement

Category	Natural biodegradable hydrogels	Composite injectable hydrogels	Nanoparticle-hydrogel composite hydrogels	Non-degradable or slowly degradable hydrogels
Representative materials	HA; gelatin/GelMA; collagen; chitosan; alginate	PEG; PLGA-PEG-PLGA; PNIPAAm; Pluronic F127; mPEG-polypeptide	Liposomes, micelles, and polymer nanoparticles or microspheres in a hydrogel	PVA-based hydrogels; double-network hydrogels; cartilage-like hydrogels
Gelation characteristics	Physical/chemical crosslinking, ionic crosslinking, photocrosslinking	Thermosensitive gelation, *in situ* drug depot, tunable release	Hierarchical release, mitigated burst release, enhanced drug stability	Long-term mechanical support, lubrication, and cartilage functional replacement
Main cargos or applications	Anti-inflammatory drugs, natural compounds, rapamycin, *etc.*	Glucocorticoids, quercetin, etanercept, *etc.*	Rapamycin, siRNA, metabolic regulatory agents, *etc.*	Functional replacement for end-stage OA or exploration of delaying surgical intervention
Evidence boundaries and limitations	Biocompatible, ECM-like matrices; mechanical strength, batch variability, and degradation require optimization[56]	Adjustable gelation and release; burst release, drug stability, and long-term local safety require evaluation[62,66-68]	Can reduce burst release and improve stability; manufacturing and batch consistency remain challenging[56,57,69,70]	Potential mechanical support and lubrication; fixation, wear debris, tissue compatibility, foreign-body responses, and long-term safety require evaluation[61,71,72]

HA: Hyaluronic acid; GelMA: gelatin methacryloyl; PEG: polyethylene glycol; PLGA: poly(lactic-co-glycolic acid); PNIPAAm: poly(N-isopropylacrylamide); PF127: Pluronic F127; mPEG: methoxy polyethylene glycol; PVA: polyvinyl alcohol; siRNA: small interfering ribonucleic acid. PLGA-PEG-PLGA denotes a triblock copolymer, and mPEG-polypeptide denotes a copolymer containing methoxy polyethylene glycol and polypeptide blocks.

HYDROGELS AS PLATFORMS FOR INTRA-ARTICULAR DELIVERY AND CARTILAGE FUNCTIONAL REPLACEMENT

Intra-articular administration can increase drug exposure within diseased joints. However, synovial fluid dilution, lymphatic clearance, the cartilage ECM barrier, and joint motion can limit free-drug retention and produce fluctuating local concentrations, necessitating repeated administration[24,55]. Delivery platforms are therefore being developed to prolong retention, control release, improve lubrication, and reduce systemic exposure. Their high water content, ECM-like structure, injectability, and tunable release properties make hydrogels suitable candidates for local OA delivery[56]. Hydrogels can act as reservoirs for PI3K-AKT-mTOR-modulating drugs, although their effects depend on the specific formulation [Table 1]. The following sections examine retention, degradation, repeated injection, biocompatibility, immunogenicity, pharmacokinetics, and regulation of combination products.

Fundamental requirements for intra-articular delivery systems

The OA joint microenvironment imposes multiple requirements on delivery systems. First, the system needs suitable injectability and *in situ* gelation to allow minimally invasive administration and adaptation to irregular joint spaces. Second, the material must maintain structural stability under synovial fluid flow and joint motion-induced shear forces to prevent rapid drug clearance. Third, the delivery system should provide release kinetics suited to the drug, thereby mitigating initial burst release and local toxicity while maintaining a sufficiently long therapeutic window. Furthermore, the dense, negatively charged ECM of cartilage limits drug penetration and retention; thus, parameters such as particle size, surface charge, cartilage adhesion, and tissue penetration capabilities are critical determinants of therapeutic efficacy[57,58].

Ideal hydrogels for intra-articular use should also balance biocompatibility, low immunogenicity, predictable degradation profiles, and reproducible fabrication techniques. In older patients with OA, the joint microenvironment is often characterized by chronic inflammation, oxidative stress, chondrocyte senescence, and reduced repair capacity; the material itself should not exacerbate synovitis or induce chronic foreign body responses. Therefore, the evaluation of hydrogels includes gelation and release behavior as well as intra-articular retention time, drug release kinetics, cartilage/synovial safety, histological outcomes, and measures of pain and gait [Figure 4].

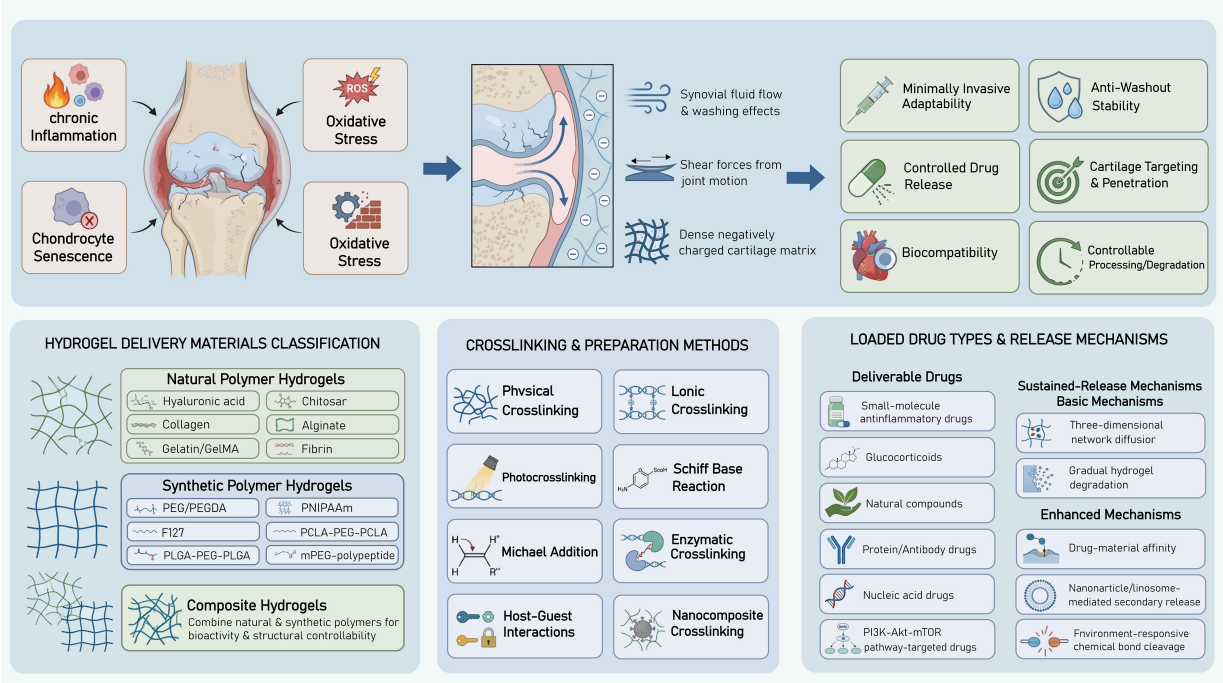

Figure 4. Overview of design requirements, material systems, and drug release mechanisms of intra-articular injectable hydrogel delivery systems for age-related OA. The left panel illustrates the pathological triggers in OA joints, including chronic inflammation, oxidative stress, and chondrocyte senescence. The central panel depicts the joint microenvironment and the advantages of hydrogel platforms, such as resistance to synovial fluid washout, shear force adaptation, and dense negatively charged matrices for sustained retention. The right panel summarizes the favorable properties of hydrogels: minimally invasive adaptability, controlled drug release, cartilage targeting and penetration, biocompatibility, and controllable processing/degradation. The lower sections further classify hydrogel delivery materials (natural polymer, synthetic polymer, and composite hydrogels), outline common crosslinking and preparation methods, and summarize loaded drug categories and their sustained-release mechanisms. PCLA-PEG-PCLA: Poly(ε-caprolactone-co-lactide)-block-poly(ethylene glycol)-block-poly(ε-caprolactone-co-lactide); PEG: poly(ethylene glycol); PEGDA: poly(ethylene glycol) diacrylate; PLGA-PEG-PLGA: poly(lactide-co-glycolide)-block-poly(ethylene glycol)-block-poly(lactide-co-glycolide); PNIPAAm: poly(N-isopropylacrylamide); mPEG: methoxy polyethylene glycol.

Hydrogel materials and release mechanisms

Injectable hydrogels for intra-articular OA delivery can be broadly classified as natural, synthetic, or composite polymer systems. They can be formed through physical or ionic crosslinking, thermal gelation, photocrosslinking, Schiff base reactions, Michael addition, enzymatic crosslinking, host-guest interactions, or incorporation of nanoparticles and microspheres. Their cargos include small-molecule anti-inflammatory drugs, glucocorticoids, natural compounds, proteins, antibodies, nucleic acids, and candidate pathway-modulating agents. Release can involve diffusion through the three-dimensional network, material degradation, drug-matrix affinity, secondary release from nanoparticles or liposomes, and cleavage of stimulus-responsive bonds[59,60]. Targeting, lubrication, and stimulus responsiveness provide additional functions; the rapamycin microspheres discussed below, for example, combine drug delivery with lubrication.

Superelastic hydrogels with large reversible biaxial strain have expanded the possibilities for mechanical support and cartilage-like materials. Non-degradable systems primarily provide sustained support, lubrication, friction reduction, or functional replacement rather than degradation-controlled drug release. High elasticity, strength, and reversible deformation are relevant to artificial cartilage design[61]. Here, these materials are considered briefly in relation to the delivery platforms central to this review.

Retention and the duration of active exposure

Intra-articular residence is often cited as an advantage of hydrogels, but different measurements address different components. Persistence of a fluorescent material signal indicates label retention; it does not necessarily establish intact material, retained active drug, or sustained target engagement. *In vitro* release duration, *in vivo* drug residence, pharmacodynamic activity, and persistence of a ther-

apeutic effect are distinct endpoints[23,24]. The rapamycin gelatin study illustrates this distinction: protection at a later histological endpoint cannot be equated with release throughout the entire follow-up period.

Joint motion, synovial turnover, depot location, inflammation, and cartilage distribution can alter exposure relative to an *in vitro* release assay. No single retention duration is established as optimal for all pathway-modulating cargos. For drugs with important physiological targets, prolonged exposure is advantageous only within a tolerable pharmacological window.

Degradation and feasibility of repeated administration

Material degradation and drug release need not proceed at the same rate. Rapid degradation can terminate a depot prematurely, whereas persistent material or poorly cleared degradation products can prolong synovial contact. Network fragments, incorporated particles, and residual crosslinking components may have different clearance pathways[56,62]. Their biological effects cannot be inferred from the properties of the starting polymer alone.

Repeat-injection feasibility is consequently more than successful needle passage. It depends on the remaining depot burden, cumulative drug exposure, inflammatory responses, effects on joint motion, and the practical reproducibility of injection into a diseased joint. A formulation compatible with one administration may behave differently after repeated dosing. Current short-term or single-administration findings leave these issues incompletely resolved.

Biocompatibility and immune responses

Low acute cytotoxicity is useful but does not establish long-term biocompatibility. Cartilage, synovium, subchondral bone, and local immune cells can respond differently to the drug, intact hydrogel, carriers, and degradation products. Natural polymers can vary in purity and source, while synthetic formulations can contain residual reactive components; neither origin guarantees low immunogenicity[56,62].

Relevant safety outcomes include persistent synovitis, macrophage or foreign-body responses, tissue toxicity, and systemic effects from released drug. For rapamycin and other pathway inhibitors, suppression of physiological survival, repair, or immune functions is a separate concern from material compatibility. Local administration may reduce systemic exposure, but that reduction requires demonstration rather than assumption. Older patients may have additional vulnerabilities that are not represented by otherwise healthy experimental animals.

Pharmacokinetics and pharmacodynamics

The drug exposure profile includes release from the depot, distribution within synovial fluid and cartilage, uptake into target cells, metabolism, and clearance from the joint. Plasma exposure and exposure outside the injected joint remain relevant. Pharmacokinetic interpretation therefore distinguishes drug measurements from tracking of the polymer or nanocarrier[23,24,63].

Pharmacodynamic interpretation likewise distinguishes mTORC1-related signaling from mTORC2/AKT effects and from changes in complete autophagic turnover. A depot that extends exposure can shift the intensity or duration of target inhibition, including effects not seen after a brief free-drug dose[64]. Joint protection, local toxicity, and systemic exposure together determine whether an altered release profile improves the benefit-risk balance. However, the ocular or intramuscular pharmacokinetics described subsequently cannot substitute for intra-articular measurements.

Manufacturing and regulatory considerations

Translation depends on reproducible composition, drug content, particle or network properties, gelation, release, degradation, and storage stability. Sterilization can alter the polymer network or drug, and complex carrier-in-hydrogel products may introduce additional sources of batch variability. A biologically favorable laboratory formulation is therefore not equivalent to a reproducibly manufactured clinical product[57,62].

A drug-loaded hydrogel may fall within a drug, device, or combination-product framework, depending on its components, intended function, and the applicable jurisdiction. In the United States, assignment of a combination product to a lead regulatory center is based on its primary mode of action; chemical drug action and physical functions such as lubrication may both be relevant[65]. Classification is

product-specific rather than universal for all hydrogels. Evidence concerns the combined finished product, including interactions between constituents, and is not supplied merely by previous use or approval of its separate components. Existing clinical use of a polymer or systemic drug does not establish approval or suitability of a new intra-articular formulation.

THERAPEUTIC AGENTS TARGETING THE PI3K-AKT-mTOR PATHWAY AND HYDROGEL-BASED DELIVERY STRATEGIES

Given the regulatory role of the PI3K-AKT-mTOR pathway in chondrocyte autophagy, inflammatory responses, and matrix metabolism, pharmacological intervention targeting various nodes of this pathway has attracted interest in OA research[19]. Theoretically, EGFR inhibitors, PI3K inhibitors, mTOR inhibitors, dual PI3K/mTOR inhibitors, as well as select natural compounds and active ingredients from traditional Chinese medicine, may regulate chondrocyte autophagy and ECM metabolism by modulating this signaling axis. Concurrently, hydrogel-based delivery systems offer a platform for local delivery of these agents by prolonging intra-articular retention, achieving sustained release, and minimizing systemic exposure.

The strength of evidence differs across these agents. Relatively direct formulation evidence is concentrated in mTOR inhibitors and selected hydrogel systems. Evidence for EGFR inhibitors, PI3K inhibitors, and dual PI3K/mTOR inhibitors includes model-dependent responses, differences between *in vitro* and *in vivo* findings, and extrapolation from other diseases. The following sections therefore examine each drug class in terms of its mechanism, experimental setting, and relevance to hydrogel-based OA delivery [Table 2].

EGFR inhibitors

EGFR belongs to the erythroblastic leukemia viral oncogene homolog (ErbB) family of receptor tyrosine kinases[73-75]. Its activation initiates the downstream PI3K-AKT-mTOR pathway and may modulate autophagic processes via mTOR-dependent signaling. Gefitinib, a small-molecule EGFR inhibitor, has been investigated in models of intervertebral disc degeneration (IVDD). These studies demonstrate that gefitinib can attenuate the degenerative phenotype of chondrocyte-like cells *in vitro* and promote ECM synthesis. When integrated with a thermosensitive injectable hydrogel delivery system, the formulation provides sustained local release in puncture-induced rat IVDD models, and by persistently blocking EGFR activity, it enhances matrix production, activates autophagy, and suppresses matrix degradation[76] [Figure 5].

These findings suggest that the combination of EGFR inhibitors and injectable hydrogels may modulate matrix metabolism and autophagic processes via local delivery. However, it is important to recognize that this evidence is primarily derived from intervertebral disc degeneration models rather than articular OA models. Given the differences between intervertebral disc tissue and articular cartilage regarding mechanical environments, cellular composition, nutritional conditions, and pathological progression, these results cannot be directly extrapolated to the treatment of age-related OA. For OA, intra-articular injection of gefitinib-encapsulated hydrogels remains a candidate strategy; its efficacy requires further validation in OA-related chondrocytes, synoviocytes, subchondral bone microenvironments, and aged animal models.

The potential value of a gefitinib hydrogel lies in maintaining local drug exposure while limiting systemic exposure. Its intra-articular retention, release profile, effects on chondrocytes and synoviocytes, autophagic flux, and long-term cartilage safety remain unresolved. EGFR inhibition is therefore a candidate approach to upstream pathway modulation rather than an established OA treatment.

PI3K inhibitors

The PI3K/AKT signaling pathway has a role in cell survival, metabolic regulation, inflammatory responses, and autophagy, serving as a critical midstream node within the PI3K-AKT-mTOR axis. LY294002 is a commonly used PI3K inhibitor that has been extensively investigated in both *in vitro* and animal models (including non-OA models).*In vitro* studies have demonstrated that LY294002 attenuates TNF-α-induced cadherin-11 overexpression and invasive capacity in OA fibroblast-like synoviocytes[54], however, evidence also shows that it attenuates the pro-survival, anti-apoptotic, and matrix-promoting effects of certain protective factors on chondrocytes[77].
The *in vivo* effects of PI3K inhibitors do not fully

Table 2. Representative agents targeting the PI3K-AKT-mTOR pathway, autophagy-related findings, and evidence limitations

Drug and target	Research findings	References
Gefitinib (EGFR Inhibitor)	As an EGFR inhibitor, gefitinib effectively reverses the degenerative phenotype of chondrocytes *in vitro* and promotes matrix synthesis. When combined with advanced thermosensitive injectable hydrogel delivery systems, it enables localized sustained release and long-term therapeutic effects in animal models, effectively delaying intervertebral disc degeneration	[76]
LY294002 (PI3K Inhibitor)	LY294002 attenuates TNF-α-induced overexpression of cadherin-11 in OA fibroblast-like synoviocytes and reduces their invasive capacity, though it shows limited efficacy in ameliorating synovitis and cartilage injury *in vivo*	[54]
Rapamycin (mTOR Inhibitor)	By inhibiting mTORC1, rapamycin lifts the suppression of autophagy initiation. Hydrogel-based local delivery increases autophagy-related markers and improves histological outcomes and matrix metabolism in experimental OA models, although assessments of autophagic flux and long-term safety remain necessary	[69,70,81]
BEZ235 (Dual PI3K/mTOR Inhibitor)	As a dual PI3K/mTOR inhibitor, preclinical studies demonstrated its radiosensitizing efficacy in colorectal cancer; however, Phase Ib clinical trials in advanced renal cell carcinoma revealed significant toxicity and a lack of objective therapeutic benefit, leading to early termination of the trial	[84,85]
GNE-947 (Dual PI3K/mTOR Inhibitor)	GNE-947 acts as a dual PI3K/mTOR inhibitor with documented anti-angiogenic activity in laser-induced rat choroidal neovascularization models, characterized by an extended half-life	[86]
Neolymphostin A (Dual PI3K/mTOR Inhibitor)	Neolymphostin A exhibits high selectivity for PI3K and mTOR, alongside favorable cell permeability and functional activity, blocking AKT phosphorylation with nanomolar half-maximal inhibitory concentration (IC50) values in living cells; however, its synthesis remains challenging	[87,88]
Eupatorin (Natural Compound)	Eupatorin targets key molecules such as MMP9, EGFR, and PTGS2. It modulates the PI3K/AKT and estrogen-related signaling pathways, thereby inhibiting inflammation and ECM degradation while facilitating the restoration of autophagy	[94]
Puerarin (Natural Compound)	Puerarin exhibits multi-target regulatory characteristics, manifesting anti-inflammatory, anti-apoptotic, and ECM-promoting effects, potentially mediated by the activation of the PI3K-AKT pathway	[95]
Digoxin (Natural Compound)	Digoxin stimulates chondrocyte differentiation and anabolic metabolism to counteract cartilage degradation; it suppresses M1 polarization of synovial macrophages to alleviate synovitis, and downregulates miR-146b-5p in M1-derived exosomes to modulate the inflammatory microenvironment via USP3 and SOX5	[97,98]
Quercetin (Natural Compound/TCM)	While quercetin possesses anti-inflammatory and antioxidant activities (with evidence of PI3K/AKT/mTOR-mediated autophagy regulation primarily from diabetes, oncology, or fibrosis models), its application in OA - via mPEG-polypeptide thermosensitive hydrogels - showed a trend toward improvement in gait-related parameters and Osteoarthritis Research Society International (OARSI) scores. Its pathway-specific mechanism and localized delivery efficacy in OA require independent evaluation	[68,101-104,106-108]
Andrographolide (Natural Compound/TCM)	A four-arm PEG/micelle hydrogel incorporating PF enables approximately 24-day sustained release of andrographolide. This agent may enhance chondrocyte autophagy via PRKCA/EGFR-related mechanisms, ameliorating inflammation, matrix degradation, and apoptosis. The direct causal linkage to the PI3K-AKT-mTOR axis requires further validation	[109]

EGFR: Epidermal growth factor receptor; PI3K: phosphatidylinositol 3-kinase; mTOR: mechanistic target of rapamycin; TNF-α: tumor necrosis factor-α; OA: osteoarthritis; mTORC1: mTOR complex 1; PRKCA: protein kinase C alpha; MMP: matrix metalloproteinase; ECM: extracellular matrix; AKT: protein kinase B; mPEG: methoxy polyethylene glycol; USP3: ubiquitin-specific peptidase 3; SOX5: SRY-box transcription factor 5; PTGS2: prostaglandin-endoperoxide synthase 2; TCM: traditional Chinese medicine.

correspond to *in vitro* experimental results. In collagenase-induced OA (CIOA) mouse models, intraperitoneal injection of LY294002 reduces cadherin-11 protein expression in the synovium but fails to significantly ameliorate synovitis or cartilage injury. In contrast, intra-articular intervention targeting specific downstream molecules - such as cadherin-11 antibodies - in the same model has shown greater effects on synovitis and cartilage damage[54]. These findings imply that systemic inhibition of the PI3K/AKT pathway may not adequately replicate the protective effects observed *in vitro*, as therapeutic outcomes may be modulated by cell type, administration route, drug distribution, off-target effects, and systemic feedback regulation.

PI3K inhibitors remain candidates for modulating OA inflammation and autophagy rather than established treatments. Their incorporation into hydrogels would require evaluation of local dosing, release kinetics, chondrocyte and synoviocyte toxicity, autophagic flux, and long-term joint outcomes. Pro-

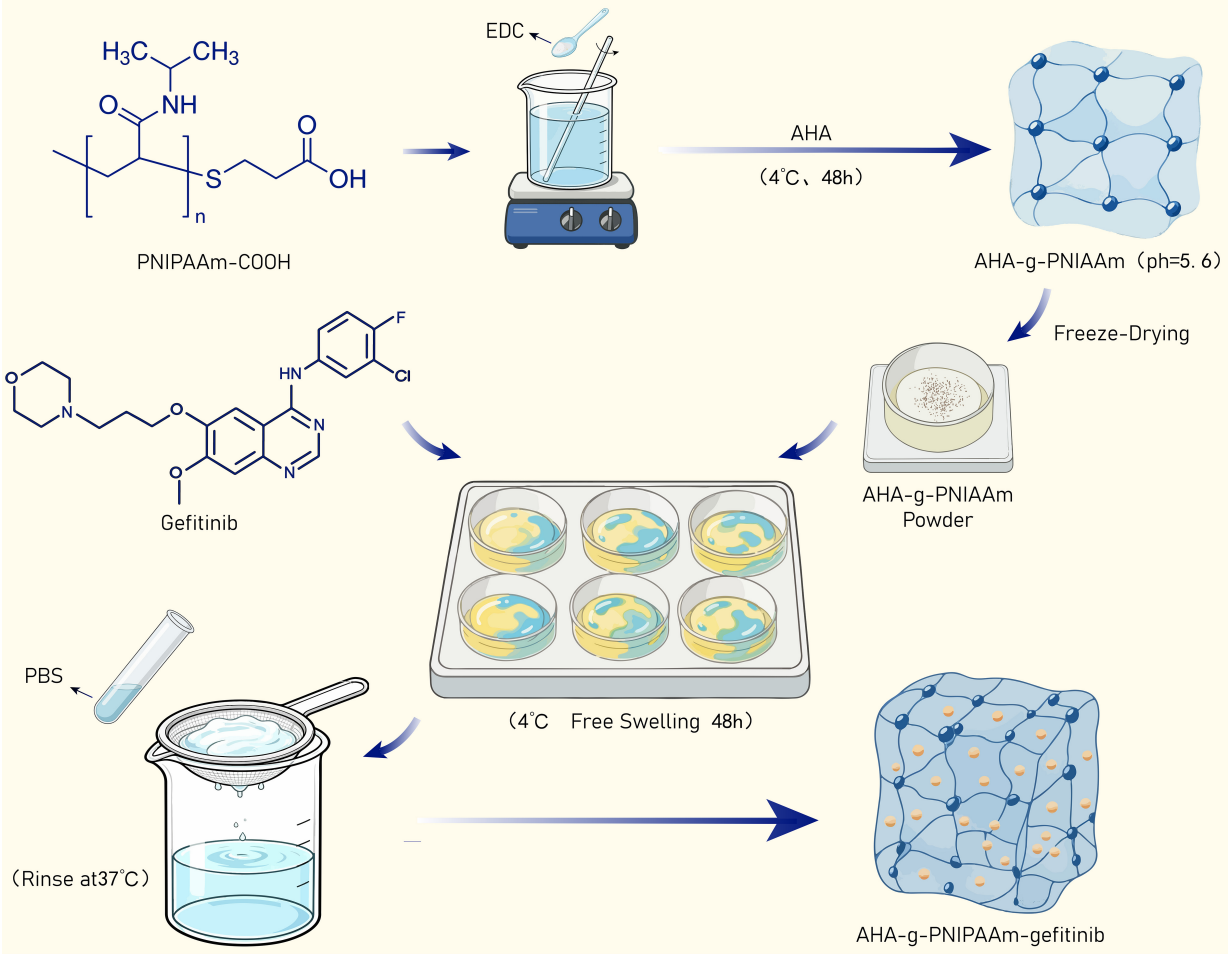

Figure 5. Fabrication workflow of gefitinib-loaded hydrogels. The synthesis starts from PNIPAAm-COOH, followed by 1-ethyl-(3-dimethylaminopropyl) carbodiimide (EDC)-mediated reaction with aminated hyaluronic acid (AHA) at 4 °C for 48 h to obtain the AHA-g-PNIPAAm hydrogel. After freeze-drying, the powder is loaded with gefitinib and swollen in PBS at 4 °C for 48 h. The product is rinsed at 37 °C to yield the final gefitinib-loaded AHA-g-PNIPAAm hydrogel. EDC: 1-ethyl-(3-dimethylaminopropyl) carbodiimide; PNIPAAm: poly(N-isopropylacrylamide); AHA: aminated hyaluronic acid; PBS: phosphate-buffered saline; AHA-g-PNIPAAm: aminated hyaluronic acid-g-poly (N-isopropylacrylamide).

longed retention and reduced systemic exposure alone would not resolve uncertainty about their *in vivo* efficacy.

mTOR inhibitors

mTOR is the central regulatory node within the PI3K-AKT-mTOR pathway; as a serine/threonine protein kinase, it regulates cell growth, metabolism, survival, and autophagy via two distinct complexes, mTORC1 and mTORC2[78,79]. Among these, mTORC1 is most closely associated with the initiation of autophagy and is a canonical negative regulator of the autophagic process. Rapamycin is a well-characterized mTOR inhibitor, primarily suppressing mTORC1 activity through binding to FK506-binding protein 12 (FKBP12), though its inhibitory effect on mTORC2 remains relatively weak[78,80].

Among current candidate drugs targeting the PI3K-AKT-mTOR pathway, mTOR inhibitors have a relatively direct pharmacological link to chondrocyte autophagy modulation. *In vitro* studies indicate that rapamycin-mediated mTORC1 inhibition reduces sulfated glycosaminoglycan (sGAG) release from chondrocytes under inflammatory conditions, enhances the expression of chondrogenic markers such as SRY-box transcription factor 9 (SOX9), collagen type II alpha 1 chain (COL2A1), and aggrecan (ACAN), and mitigates cell death and inflammatory processes, suggesting that mTORC1 inhibition may contribute to maintaining chondrocyte phenotype and ECM homeostasis[81]. At the delivery level, gelatin hydrogels loaded with rapamycin-micelles enable controlled intra-articular release; in murine OA models, this system increases the number of

autophagy-marker-positive chondrocytes, improves histological scores, and downregulates OA-associated mediators[70].

Subsequent research on hydrogel microspheres further integrated rapamycin-loaded liposomes, hyaluronic acid hydrogel microspheres, and self-renewing hydration-based lubrication layers. This system demonstrated elevated expression of autophagy-related proteins such as LC3B and ATG5, alongside decreased MMP13 expression. Furthermore, it achieved local enrichment via electrostatic interactions between cationic liposomes and the negatively charged cartilage matrix, while simultaneously improving joint interface lubrication[69]. Compared with free rapamycin, the advantage of rapamycin-loaded hydrogels lies in the simultaneous improvement of local retention, lubrication, and sustained drug release, providing a more stable intra-articular microenvironment for mTORC1-mediated autophagic regulation. However, these findings must be interpreted with caution. Upregulation of LC3B and ATG5 indicates changes in autophagy-related protein expression but cannot be equated to restored autophagic flux; reduced MMP13 is consistent with reduced matrix catabolism, necessitating further validation through autophagic flux assays, histological scores, long-term joint function assessments, and aged OA models. Clinical translation also depends on the local rapamycin dosage window, release kinetics, long-term chondrotoxicity, synovial responses, and risks associated with repeated administration [Figure 6].

Other mTOR inhibitors include the second-generation agent PP242 and the third-generation agent RapaLink-1. Second-generation inhibitors can suppress both mTORC1 and mTORC2, whereas third-generation inhibitors have increased potency and can overcome certain resistance mutations[78]. These agents have mainly been studied in oncology. Their efficacy, safety, and suitability for intra-articular hydrogel delivery in OA remain uncertain.

Dual PI3K/mTOR inhibitors

Dual PI3K/mTOR inhibitors simultaneously target PI3K and mTOR; mechanistically, they may more comprehensively block the PI3K-AKT-mTOR signaling axis and, to some extent, circumvent feedback activation resulting from single-target inhibition[82]. Pharmacological inhibition of the PI3K/AKT/mTOR axis has been extensively investigated in oncology, providing a mechanistic and drug-development framework for these inhibitors[83]. However, direct evidence for these agents in OA autophagy regulation and intra-articular hydrogel delivery remains limited, with existing inferences primarily derived from oncology, ophthalmology, or other disease models. Accordingly, this section positions dual PI3K/mTOR inhibitors as prospective candidates rather than validated therapeutic strategies for OA.

BEZ235 is among the most extensively studied dual PI3K/mTOR inhibitors; preclinical research demonstrated its potential to enhance radiosensitivity in colorectal cancer[84]. However, in a phase Ib clinical trial for advanced renal cell carcinoma, the drug caused substantial toxicity, including fatigue, rash, nausea, vomiting, diarrhea, and mucositis, alongside a lack of objective clinical benefit, leading to early termination of the trial[85]. These findings imply that while dual inhibition of PI3K and mTOR offers theoretical advantages in pathway blockade, the associated systemic toxicity and the narrow therapeutic window are critical concerns. Given the higher safety standards required for chronic non-lethal conditions such as OA, potent pathway inhibitors established in oncology cannot be simply extrapolated to intra-articular applications.

GNE-947, a dual PI3K/mTOR inhibitor, has demonstrated anti-angiogenic activity in laser-induced rat choroidal neovascularization models, and when administered via intravitreal injection as a suspension, it exhibits an extended half-life in rabbit eyes, offering a pharmacokinetic reference for long-acting local exposure[86]. However, important differences exist between intraocular and intra-articular environments; the latter involves unique factors such as synovial fluid dilution, mechanical shear forces, the cartilaginous barrier, and synovial reactions. Consequently, the long-acting exposure characteristics of GNE-947 suggest possible relevance in local sustained-release designs, but do not establish its suitability for intra-articular delivery in OA.

Neolymphostin A, a covalent dual inhibitor derived from the bacterium Salinispora arenicola, binds covalently to the Lys802 residue of PI3Kα via a vinylogous ester moiety, exhibiting potent PI3K/mTOR inhibitory activity in living cells[87]. However, the

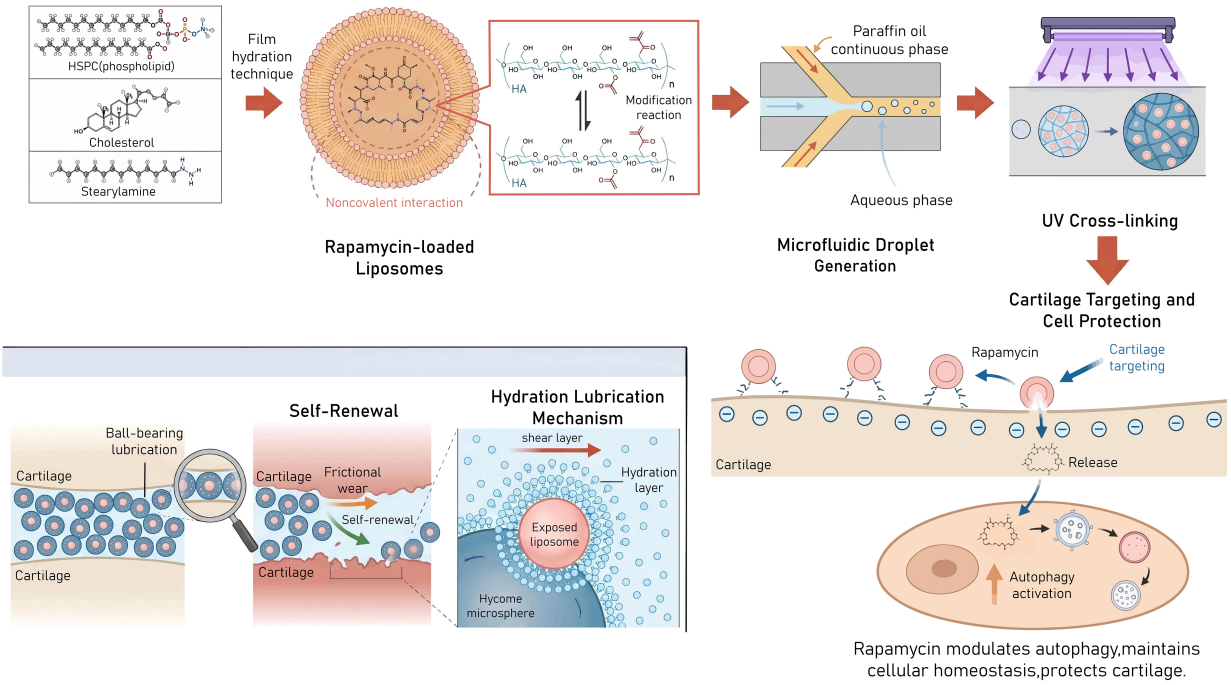

Figure 6. Preparation process and proposed therapeutic mechanism of rapamycin-loaded hydrogels. Liposomes are coated on the surface of hydrogel microspheres (HMS) to introduce a hydration layer, which can effectively reduce friction. To endow HMS with the ability to form a self-renewable hydration layer and maintain intracellular homeostasis, rapamycin-liposome-hyaluronic acid HMS (RapA@Lipo@HMS) was fabricated using microfluidic technology combined with photopolymerization. UV: Ultraviolet.

chemical synthesis of this agent is complex, and evidence regarding its utility in OA models or local intra-articular administration is currently lacking[88]. Other novel inhibitors - such as VDC597, PKI-179, imidazo[1,2-a]pyridine-based compounds, SHR8443, and ZDQ-0620 - have likewise mainly been examined in osteosarcoma, colorectal cancer, or other oncological investigations[89-92]. Therefore, these agents are more appropriately positioned as candidate molecules for OA-focused evaluation; their intra-articular adaptability, chondrotoxicity, release characteristics, and therapeutic window for chronic disease management warrant dedicated investigation before their clinical relevance to OA can be assessed.

Natural compounds and active ingredients of traditional Chinese medicine

Natural compounds and active ingredients derived from traditional Chinese medicine have attracted interest in OA intervention research due to their multi-target and multi-pathway regulatory characteristics[93]. Compared with single-target small-molecule inhibitors, these agents may simultaneously modulate inflammatory responses, oxidative stress, apoptosis, ECM metabolism, and autophagic processes. However, their complex composition, multiple molecular targets, pharmacokinetic instability, and the challenges in elucidating their precise mechanisms require careful evaluation of the available evidence. For the theme of this review, the mechanistic links between natural compounds and active components of traditional Chinese medicine (TCM) and the PI3K-AKT-mTOR autophagy pathway remain incompletely elucidated. Therefore, the discussion focuses on compounds relevant to OA, PI3K-AKT-mTOR signaling, autophagy, or ECM metabolism.

Eupatorin and puerarin illustrate the evidence available for natural compounds. Eupatorin potentially acts on key targets such as matrix metalloproteinase 9 (MMP9), EGFR, and prostaglandin-endoperoxide synthase 2 (PTGS2), inhibiting inflammation and ECM degradation while modulating autophagic processes via the PI3K/AKT and estrogen-related signaling pathways[94]. Puerarin exhibits multi-target regulatory properties, exerting anti-inflammatory, anti-apoptotic, and matrix-promoting effects, which may partially be mediated by the PI3K-AKT pathway[95].

These studies suggest that natural compounds may participate in OA intervention by influencing PI3K-AKT-mTOR signaling; however, most evidence is from cellular or animal studies, with limited evaluation in hydrogel-based delivery systems.

Evidence regarding digoxigenin/digoxin-related compounds must be interpreted with caution. In hepatocellular carcinoma cells, digoxigenin has been reported to activate autophagy through regulation of the PI3K/AKT/mTOR pathway[96]. By contrast, OA-specific studies on digoxin indicate that it targets low-density lipoprotein receptor-related protein 4 (LRP4) and exerts chondroprotective effects[97], while additional work suggests that digoxin can ameliorate the joint inflammatory microenvironment by suppressing M1-like synovial macrophage polarization and the exosomal microRNA-146b-5p (miR-146b-5p)/ubiquitin-specific peptidase 3 (USP3)/SRY-box transcription factor 5 (SOX5) axis[98]. These findings arise from different compounds and disease contexts and should not be merged into a single mechanistic claim. Accordingly, their precise relevance to PI3K-AKT-mTOR-directed intra-articular therapy in age-related OA remains to be established.

Traditional Chinese medicine and its active components have established a preliminary experimental foundation in OA research, with some formulations or TCM-based interventions demonstrating potential in alleviating pain, inflammation, and cartilage injury in OA animal models[99,100]. Regarding specific active components, quercetin and andrographolide are common representatives in this research domain. Notably, quercetin is a widely distributed polyphenolic flavonoid in plants and should not be oversimplified as a "proprietary Chinese medicine". Studies have indicated that the diverse biological effects of quercetin are linked to the regulation of the PI3K/AKT/mTOR pathway[101-104]. Quercetin has also been reported to regulate autophagy through the PI3K/AKT/mTOR pathway in a non-OA viral infection model[105]. In diabetic Goto-Kakizaki (GK) rat models, quercetin promotes protective autophagy by inhibiting the PI3K/AKT/mTOR pathway, thereby mitigating hepatic, splenic, and renal injury[106]; in Burkitt lymphoma, it suppresses the PI3K/AKT/mTOR pathway, induces complete autophagic flux, and facilitates the degradation of mutant MYC proto-oncogene protein (c-Myc)[107]; in epidural fibrosis models, it modulates fibroblast proliferation via PI3K/AKT/mTOR-dependent autophagy regulation[108]. However, these findings are primarily drawn from diabetes, oncology, or fibrosis models and cannot be directly extrapolated to age-related OA.

Andrographolide is a bioactive plant-derived component extracted from Andrographis paniculata. A study developed a four-arm PEG/micelle hydrogel incorporating Pluronic F127 (PF127) for the sustained release of andrographolide (approximately 24 days). In OA models, a single injection ameliorated joint degeneration, improved gait, and altered chondrocyte autophagy-related markers, while concurrently reducing inflammation, matrix degradation, and apoptosis[109]. Mechanistically, the effects of andrographolide on chondrocyte autophagy may involve the regulation of protein kinase C alpha (PRKCA)/EGFR signaling, with related evidence from prior studies of PRKCA-mediated EGFR suppression[59]. However, further clarification regarding the long-term safety, release kinetics, and the direct causal connection with the PI3K-AKT-mTOR pathway is needed for this strategy [Figure 7].

Natural compounds and active TCM constituents are therefore best regarded as candidates whose mechanisms require further validation. Their incorporation into hydrogels raises questions about chemical stability, encapsulation, release, local safety, autophagic flux, and matrix metabolism. Network pharmacology and molecular docking can generate hypotheses, but they cannot replace experimental validation in relevant cells and disease models.

CURRENT CHALLENGES AND FUTURE DIRECTIONS

Combining PI3K-AKT-mTOR modulation with hydrogel-based intra-articular delivery offers a potential approach to OA treatment, but substantial limitations remain. Most evidence comes from cell experiments, small-animal studies, or non-OA disease models. Few studies establish pathway modulation, restored autophagy, cartilage protection, and formulation efficacy within the same experimental system. Mechanistic interpretation, assay standardization, material safety, and clinical applicability therefore require careful evaluation.

Insufficient mechanistic evidence

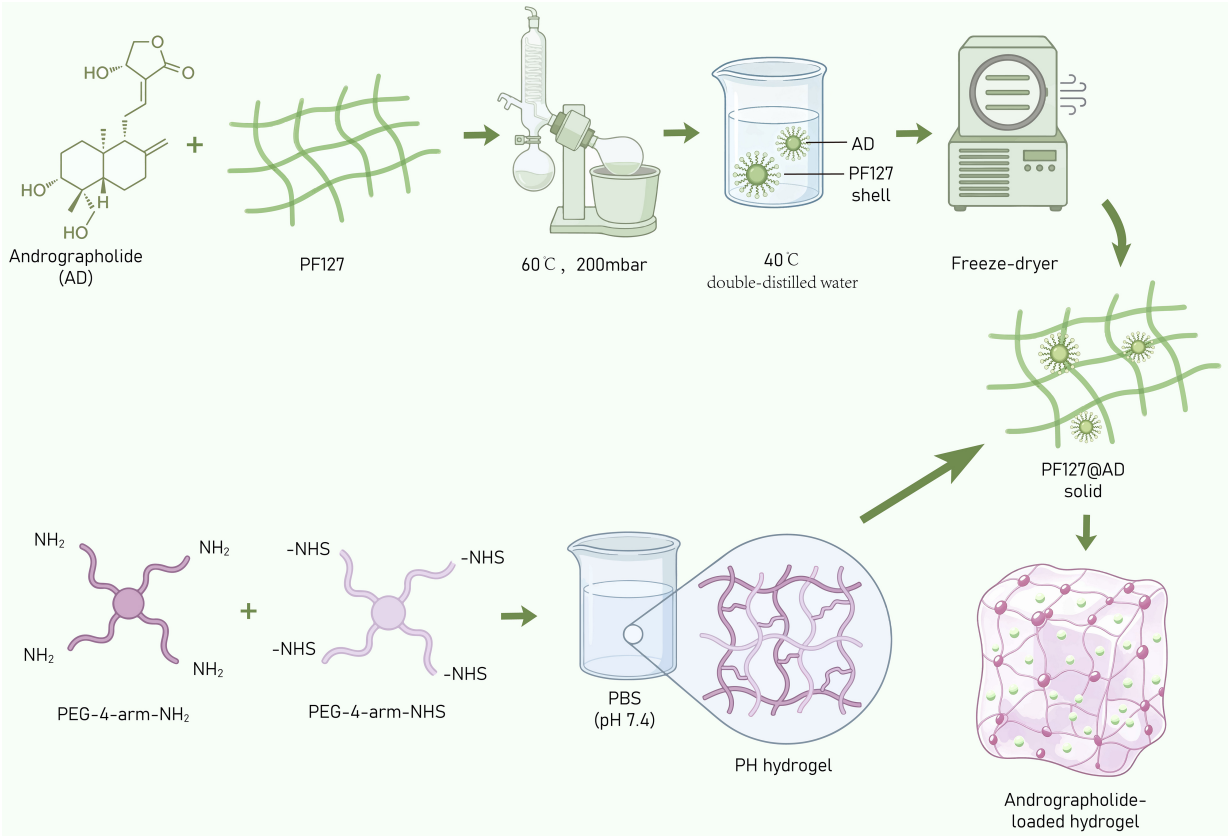

Figure 7. Fabrication workflow of andrographolide-loaded hydrogels. Andrographolide is encapsulated within the hydrophilic PF127 network to fabricate PF127@AD nanoparticles, which are then embedded in the PH hydrogel prepared by dissolving PEG-4-arm-NH_2 and PEG-4-arm-NHS at a 1:1 ratio in PBS for sustained drug release. PF127: Pluronic F127; AD: andrographolide; PEG: polyethylene glycol; PBS: phosphate-buffered saline.

While studies on PI3K-AKT-mTOR pathway-mediated OA progression suggest its association with chondrocyte autophagy, inflammation, apoptosis, and ECM metabolism, the precise underlying mechanisms remain incompletely understood. First, the PI3K-AKT-mTOR pathway is not uniformly pathogenic. It is broadly involved in cell growth, metabolism, survival, and immune regulation, potentially exerting divergent effects depending on cell type and disease stage. In chondrocytes, sustained mTORC1 activation may suppress protective autophagy and exacerbate cellular damage, whereas in synovial cells, immune cells, and subchondral bone cells, alterations in this pathway may elicit distinct or even opposing biological effects. Consequently, "inhibiting the PI3K-AKT-mTOR pathway" cannot be equated with "treating OA".

Second, the sources of evidence for many candidate drugs do not fully align with the research context of OA. Primary evidence for the EGFR inhibitor gefitinib is derived from models of intervertebral disc degeneration, while the bulk of data concerning dual PI3K/mTOR inhibitors stems from oncological or ophthalmic models; furthermore, evidence for certain natural compounds and active ingredients of traditional Chinese medicine relies heavily on network pharmacology, molecular docking, or non-OA disease models. While such studies may provide insights for OA drug screening, they do not establish therapeutic efficacy for age-related OA. Future research should prioritize the use of OA-relevant cellular models, cartilage-synovium co-culture systems, aged animal models, and large animal models to evaluate the effects of diverse agents on chondrocyte autophagy, synovitis, subchondral bone remodeling, and joint function.

Many studies focus on individual agents or pathway nodes, although age-related OA involves interacting inflammatory, oxidative, mitochondrial, senescence-related, mechanical, and metabolic processes.

PI3K-AKT-mTOR signaling is one component of this network. Its interactions with NF-κB, MAPK, AMPK, sirtuin 1 (SIRT1), and nuclear factor erythroid 2-related factor 2 (Nrf2) are relevant to interpreting treatment effects and avoiding an overly simple account of OA pathogenesis.

Lack of standardized autophagy detection

Autophagy is dynamic, and changes in LC3B, Beclin-1, ATG5, or p62 abundance alone cannot establish autophagic flux. Increased LC3B or ATG5 may accompany increased initiation, autophagosome accumulation, or impaired lysosomal degradation. Such observations therefore do not independently demonstrate restoration of complete autophagic turnover.

This distinction is particularly relevant to OA, in which inflammation, oxidative stress, mitochondrial damage, and lysosomal dysfunction can alter both autophagosome formation and degradation. Static protein measurements cannot determine whether chondrocytes have regained effective clearance capacity. Flux assays distinguish increased initiation from autophagosome accumulation and completed degradation[63]. Table 3 applies this distinction to selected cited studies.

Autophagy measurements also require interpretation alongside functional outcomes. Relevant OA endpoints include chondrocyte homeostasis, MMP13 and ADAMTS5 expression, COL2A1 and aggrecan expression, cartilage histology, pain, and joint function. Integrating flux measurements with matrix metabolism and functional assessments provides a firmer basis for interpreting therapeutic effects than marker abundance alone.

Barriers to clinical translation of hydrogels

As intra-articular delivery platforms, hydrogels can extend drug retention, enable sustained release, and reduce systemic exposure; however, their clinical translation faces several challenges. First, the physicochemical properties of hydrogels must be highly compatible with the intra-articular environment. The OA joint cavity is characterized by synovial fluid flow, mechanical shear, repetitive loading, and fluctuating inflammatory microenvironments. Excessive degradation rates can prevent maintenance of therapeutic drug concentrations, while slow degradation may increase risks of foreign body responses, synovial irritation, or restricted joint mobility. Therefore, parameters such as gelation time, mechanical properties, degradation rates, release kinetics, and intra-articular retention time require systematic optimization[24].

Second, the long-term safety of hydrogel delivery systems remains uncertain. The PI3K-AKT-mTOR pathway regulates fundamental processes, including cell growth, metabolism, and immune regulation; thus, even with minimized systemic exposure via local delivery, the long-term effects of its inhibitors on chondrocytes, synoviocytes, subchondral bone cells, and immune cells must be rigorously evaluated. Particularly in older patients with OA, whose joint microenvironment is already marked by cellular senescence and diminished repair capacity, over-inhibition of the PI3K-AKT-mTOR pathway may compromise tissue homeostasis and repair responses. Consequently, hydrogel-based delivery does not replace drug safety validation.

Third, the standardization of material fabrication and clinical application remains a critical challenge. Batch-to-batch consistency, sterilization methods, drug encapsulation efficiency, release reproducibility, storage stability, and the controllability of injection procedures all significantly influence clinical translation. For stimuli-responsive hydrogels, it is essential to demonstrate that triggering conditions correspond to those in OA joints. For example, the stability, predictability, and reproducibility of drug release from ROS-, pH-, or matrix metalloproteinase (MMP)-responsive systems in the joint microenvironment require further confirmation via animal studies and preclinical research.

Future research directions

Appropriate disease models remain essential to evaluating relevance to older adults. The principal hydrogel studies cited here did not use naturally aged OA models. Rapamycin-loaded microspheres were tested after surgery in 12-week-old male rats; the andrographolide study used a DMM model in 8-week-old C57BL/6 mice; and quercetin thermogel was administered 24 weeks after anterior cruciate ligament transection (ACLT) performed in rats at 12 weeks of age. Young animals and surgically induced disease do not fully reproduce the senescence, chronic inflammation, mitochondrial dysfunction, and reduced repair capacity associated with aging. Natu-

Table 3. Reassessment of autophagy measurements in selected cited studies

Drug	Study and experimental setting	Autophagy measurements	Conclusion
GPRC5B[53]	Human cartilage, cells, and DMM mice	LC3, Beclin-1 (BECN1) and sequestosome 1 (SQSTM1), bafilomycin A1 challenge, chloroquine modification of matrix effects	Inhibitor-supported cellular involvement; *in vivo* staining does not measure joint flux
Rapamycin HA microspheres[69]	Cells and surgical rat OA	LC3B and ATG5 transcripts, LC3B immunofluorescence, matrix markers	Static marker changes with lubrication and joint protection; completed turnover and mediation remain unestablished
Andrographolide hydrogel[109]	Cells and DMM model	LC3-I/II, Beclin-1, p62, PRKCA overexpression, static cartilage measurements	Autophagy-associated and PRKCA/EGFR-related findings, not independent flux quantification
Digoxin[97,98]	OA experiments and cohort	LRP4/anabolism or macrophage and exosomal mechanisms; available primary reports do not establish a turnover mechanism	OA mechanisms and an observational association
Quercetin diabetes study[106]	GK rat organs	LC3, Beclin-1, p62, PTEN-induced kinase 1 (PINK1) and Parkin abundance, no late-blockade turnover comparison in the reported methods	Static organ-marker changes, not independently measured completed flux
Quercetin fibrosis study[108]	Fibroblasts and epidural fibrosis	LC3-I/II, Beclin-1, ATG5, p62, LC3 staining and 3-methyladenine (3-MA)	Autophagy-associated effects were observed, but static markers and 3-MA do not establish completed turnover

DMM: Destabilization of the medial meniscus; LC3: light chain 3; HA: hyaluronic acid; OA: osteoarthritis; ATG5: autophagy-related protein 5; PRKCA: protein kinase C alpha; p62: LRP4: lipoprotein receptor-related protein 4; GK: Goto-Kakizaki; PTEN: phosphatase and tensin homolog; EGFR: epidermal growth factor receptor.

rally aged animals, surgically induced OA in aged animals, and models incorporating metabolic abnormalities address different aspects of this translational gap.

The timing and cellular context of pathway modulation also require consideration. PI3K-AKT-mTOR signaling can have different roles across disease stages and joint cell populations, so stronger or longer inhibition does not necessarily improve outcomes. Release profiles and exposure windows need to reflect these differences. Suppression of inflammation and maintenance of cellular quality control may be relevant in early disease, whereas advanced OA also involves substantial cartilage loss, subchondral bone remodeling, synovitis, and pain.

Stimuli-responsive and multifunctional hydrogels may combine controlled release with injectability, appropriate degradation, cartilage adhesion, and lubrication. Potential triggers include inflammatory mediators, ROS, matrix metalloproteinases (MMPs), and local pH. For pathway-modulating drugs, encapsulation, release kinetics, and local tolerability determine whether such designs improve exposure without causing toxicity from burst release or prolonged inhibition.

Finally, future research necessitates the establishment of evaluation frameworks relevant to clinical translation. Beyond monitoring molecular markers such as LC3B, ATG5, p62, and MMP13, evaluation should incorporate assessments of autophagic flux, histological scores, cartilage thickness, subchondral bone alterations, synovitis, pain-related behaviors, gait analysis, and long-term safety profiles. For hydrogel-based systems exhibiting translational potential, further investigations into large animal models, repeat-dose studies, immunotoxicity assessments, and pharmacokinetic analyses are needed. Only after systematic validation of mechanistic evidence, material performance, and preclinical safety can hydrogel-based strategies targeting the PI3K-AKT-mTOR pathway progress toward clinical translation for age-related OA. Furthermore, while exosome delivery, three-dimensional (3D)-printed osteochondral scaffolds, and piezoelectric/piezoresistive materials represent potential long-term extensions of the hydrogel platform[110-122], these fields entail independent challenges regarding source quality control, structural reconstruction, or electrical stimulation. Given the requirement for deeper mechanistic integration with the core theme of PI3K-AKT-mTOR/autophagy-based intra-articular delivery, these are not presented as primary strategies herein

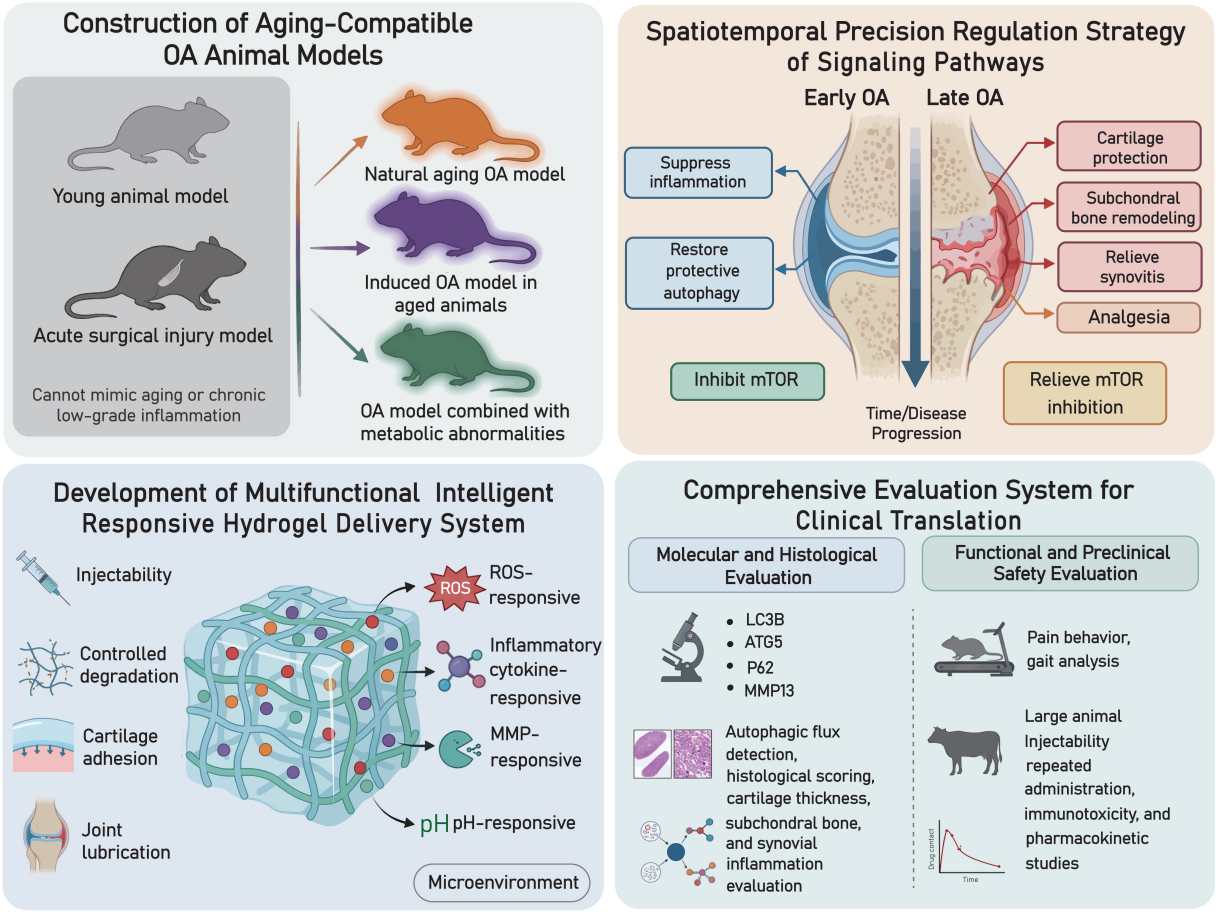

Figure 8. Considerations for hydrogel development for articular joints. The upper-left panel illustrates the construction of suitable OA animal models, comparing traditional models (young animal model, acute surgical injury model) which fail to recapitulate aging and chronic low-grade inflammation, with optimal candidate models including the natural aging OA model, an induced OA model in aged animals, and an OA model combined with metabolic abnormalities. The upper-right panel presents the spatiotemporal precision regulation strategy of signaling pathways: for early OA, inflammation suppression and restoration of protective autophagy are achieved via mTOR inhibition; for late OA, cartilage protection, subchondral bone remodeling, synovitis relief, and analgesia are realized by relieving mTOR inhibition. The lower-left panel shows the development of a multifunctional intelligent responsive hydrogel delivery system, featuring injectability, controlled degradation, cartilage adhesion and joint lubrication, with multi-stimuli responsiveness to ROS, inflammatory cytokines, MMPs and pH in the joint microenvironment. The lower-right panel depicts the comprehensive evaluation system for clinical translation, divided into molecular and histological assessment (markers LC3B, ATG5, p62, MMP13; autophagic flux detection, histological scoring, cartilage thickness, subchondral bone and synovial inflammation evaluation) and functional/preclinical safety evaluation (pain behavior, gait analysis, large animal studies, repeated administration, immunotoxicity and pharmacokinetic studies). OA: Osteoarthritis; mTOR: mechanistic target of rapamycin; ROS: reactive oxygen species; LC3B: light chain 3 beta; ATG5: autophagy-related protein 5; p62: sequestosome 1; MMP13: matrix metalloproteinase 13.

[Figure 8].

CONCLUSION AND OUTLOOK

Age-related OA is a degenerative joint disease associated with interacting processes, including chronic low-grade inflammation, oxidative stress, mitochondrial dysfunction, cellular senescence, and imbalanced extracellular matrix metabolism. Diminished chondrocyte autophagy is a critical consequence of these intersecting pathological factors, further impairing the cellular capacity to clear damaged organelles and aberrant proteins, thereby amplifying inflammation, cellular senescence, and extracellular matrix degradation. Consequently, targeting chondrocyte autophagy provides a rationale for investigating disease-modifying therapeutic strategies for age-related OA.

As a central signaling axis regulating cellular metabolism, survival, and autophagy, the PI3K-AKT-mTOR pathway has an important role in chondrocyte injury, inflammatory responses, and matrix degradation during OA progression. Current evidence suggests that EGFR inhibitors, PI3K inhibitors, mTOR inhibitors, dual PI3K/mTOR

inhibitors, as well as certain natural compounds and active traditional Chinese medicine ingredients, may modulate the PI3K-AKT-mTOR pathway and downstream autophagic processes via distinct nodes. Among these, the mechanistic linkage between mTOR inhibitors and autophagy regulation is relatively direct, and strategies such as hydrogel-mediated rapamycin delivery provide experimental examples for local modulation of the OA microenvironment. However, the strength of evidence varies significantly across these agents, and some candidates primarily derived from non-OA models, *in vitro* assays, or mechanistic predictions cannot be directly extrapolated to established treatments for age-related OA.

As platforms for intra-articular delivery and cartilage functional replacement, hydrogels provide a platform for PI3K-AKT-mTOR-targeted therapeutics by extending drug retention, enabling sustained release, minimizing systemic exposure, enhancing local lubrication, and facilitating cartilage-specific targeting. Injectable biodegradable hydrogels are particularly suited for establishing drug reservoirs and controlled release, whereas nanoparticle-hydrogel composite systems, lubricating hydrogel microspheres, and stimuli-responsive hydrogels further enhance local residency and on-demand release capabilities; conversely, non-degradable or slowly degradable hydrogels predominantly represent exploratory avenues for functional replacement or load-bearing support in end-stage OA. Particularly for older patients with OA, local delivery strategies may reduce systemic exposure and stabilize local drug concentrations within the diseased joint. However, hydrogels cannot replace rigorous validation of drug efficacy and safety; their composition, degradation behavior, release kinetics, intra-articular retention time, immunogenicity, and long-term safety profile require systematic evaluation.

Hydrogel-based local delivery of PI3K-AKT-mTOR-modulating agents remains a promising preclinical approach to OA. Its interpretation depends on cell- and stage-specific pathway effects, reliable autophagic flux measurements, appropriate disease models, and long-term evaluation of drug and material safety. Mechanistic evidence, formulation performance, and functional outcomes must be considered together when assessing its potential relevance to older adults.

DECLARATIONS

Authors' contributions

Writing - original draft, Methodology: Liu S, Mi Z, Liang S, Jiao Z

Formal analysis, Data curation: Liang H, Gao X

Writing - review & editing, Visualization, Supervision: Lu X

Writing - review & editing, Validation: Liu J, Du Q, Luo M

Writing - review & editing, Visualization, Investigation, Conceptualization: Yang G

Writing - review & editing, Supervision, Conceptualization: Liu Z, Cui D, Zhao Q

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

Artificial intelligence (AI)-assisted tools were used during preparation of this review. ChatGPT (version 6.1 Sol, released 2026-09-29) supported literature checking, reference ordering, translation, and English-language editing. Citexs (no official version number; first released in 2024) was used to generate preliminary sketches for literature-related Figure 1-8 and Graphical Abstract. All authors revised and finalized the figures and reviewed, revised, and verified the outputs of these tools. The authors independently developed the research perspectives, structure, arguments, and conclusions. No original scientific findings were generated by AI. All authors take responsibility for the accuracy and integrity of the manuscript.

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Conflicts of interest

All authors declare that they have no conflicts of

interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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