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Review article

A versatile platform based on matrix metalloproteinase-sensitive peptides for novel diagnostic and therapeutic strategies in arthritis

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A B S T R A C T

Matrix metalloproteinases (MMPs), coupled with other proteinases and glycanases, can degrade proteoglycans, collagens, and other extracellular matrix (ECM) components in inflammatory and non-inflammatory arthritis, making them important pathogenic molecules and ideal disease indicators and pharmaceutical intervention triggers. For MMP responsiveness, MMP-sensitive peptides (MSPs) are among the most easily synthesized and cost-effective substrates, with free terminal amine and/or carboxyl groups extensively employed in multiple designs. We hereby provide a comprehensive review over the mechanisms and advances in MSP applications for the management of arthritis. These applications include early and precise diagnosis of MMP activity via fluorescence probe technologies; acting as nanodrug carriers to enable on-demand drug release triggered by pathological microenvironments; and facilitating cartilage engineering through MMP-mediated degradation, which promotes cell migration, matrix synthesis, and tissue integration. Specifically, the ultra-sensitive MSP diagnostic probes could significantly advance the early diagnosis and detection of osteoarthritis (OA), while MSP-based drug carriers for rheumatoid arthritis (RA) can intelligently release anti-inflammatory drugs effectively during flare-ups, or even before symptoms manifest. The continuous progress in MSP development may acceleratedly lead to novel management regimens for arthropathy in the future.

1. Introduction

According to the registers of the Centers for Disease Control, about 22.7 % of the US adult population received a diagnosis of arthritis [1]. The impact of arthritis on quality of life is of particular importance, associated with some of the poorest quality-of-life issues, particularly in terms of bodily pain and physical functioning [2]. The socioeconomic burden of arthritis comprises both the direct costs of medical interventions and indirect costs, such as premature mortality and progressive disability. In addition, the incidence of arthritis increases with age, which means that arthritis, a significant healthcare problem today, will become even more of a burden in the coming years with the increase in the aging subset of the population. Arthritis is an extremely common medical condition, especially in people older than 50, which causes joint pain, stiffness and inflammation. Degenerative osteoarthritis (OA) and autoimmune rheumatoid arthritis (RA) are the most common entities, alongside forms arising owing to trauma, metabolism, and other, less common causes, including septic arthritis and genetic defects. OA is a degenerative joint disease characterized by cartilage degradation, subchondral bone remodeling, synovial inflammation, and osteophyte formation. Its central pathological process involves the enzymatic degradation of articular cartilage and extracellular matrix [3]. RA, on the other hand, is a chronic systemic autoimmune disease primarily marked by synovial hyperplasia, cartilage destruction, and bone erosion, accompanied by systemic inflammatory responses. The pathological mechanisms of RA involve abnormal immune reactions that drive neutrophil infiltration and inflammatory cytokine production within the synovium, ultimately leading to joint deformity and functional loss [4]. Current options for arthritis treatment can only relieve pain and control symptoms, but they can not reverse disease progression. Arthritis has become a major obstacle in orthopedics and sports medicine.

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2. The function of MMPs

In all arthritis, proteolysis of structural molecules of the extracellular matrix (ECM) is an irreversible pathological process [5], which is driven by the action of matrix metalloproteinases (MMPs). MMPs, also referred to matrixins, are zinc-containing endopeptidases that break down ECM [6]. From the first discovery of MMPs in the early 1960s by Gross and Lapière [7], more than 30 MMPs have been discovered, most of which share common functional domains in their structures, including a pro-peptide domain that must be eliminated before activation, an extensively conserved catalytic domain consisting of approximately 170 amino acids that form an oblate sphere and comprise the catalytic Zn^{2+} attached to the three imidazoles of E and H in the motif HEXGHXXGXXH [8], a variable-length hinge area, and a C-terminal region of approximately 200 amino acids with a structure similar to that of hemopexin [9]. A broad spectrum of substrates can be specifically cleaved by corresponding MMPs, such as collagen, gelatin, elastin, aggrecan, and fibrin; therefore, MMPs contribute to tissue homeostasis and remodeling through diverse physiological mechanisms, such as embryonic development, vascularization, and wound healing [10]. MMPs can be categorized into membrane-type MMPs (MT-MMPs) and secretory MMPs [11]. MT-MMPs attach to the cell membrane through covalent bonds, whereas secretory MMPs can adhere to the cell membrane by physically binding to molecules on the membrane surface (such as CD44), performing their main activity in the pericellular space [12]. MMPs are also divided by their specific substrates: 1) Collagenases (MMP1, MMP8, and MMP13), which can degrade interstitial collagens (types I, II, and III); 2) Gelatinases (MMP2 and MMP9), which target type IV collagen in the basement membrane; and 3) Stromelysins (MMP3, MMP10, and MMP11), which degrade noncollagen matrix proteins [13]. The representative roles of MMPs in both physiological and pathological conditions have been comprehensively discussed in previous reviews [14,15]. MMPs have been investigated as potential targets for therapeutic intervention for more than 40 years [16], and several MMP inhibitors have been applied clinically [17]. Nonetheless, MMP inhibitors applied in arthritis treatment have led to severe side effects [18,19], prompting a shift towards exploring more precise arthritis management strategies by targeting MMP activity. The application of designs incorporating MMP enzymatic behavior has been extended to diagnosis, medication delivery, and repair scaffolds in recent years (Fig. 1). In early diagnosis (blue section), MSPs are combined with imaging probes such as fluorophores, aggregation-induced emission (AIEgens), and Förster resonance energy transfer (FRET)-based systems to detect MMP activity. For drug delivery (green section), MSPs are integrated into nanoparticles and hydrogels, or used as responsive modifications to enable targeted and on-demand drug release. In cartilage repair scaffolds (yellow section), MSPs are incorporated into the backbone or crosslinking sites, or function as crosslinkers, facilitating enzyme-responsive degradation and controlled tissue remodeling.

Among the MMP substrates (a table outlining the natural substrates of MMPs can be found in Sternlicht's review [20]), MSPs are among the most easily synthesized and cost-effective ones. The cleavability of MSPs primarily relies on the specific arrangement of the amino acids surrounding the cleavage site. Studies have shown that collagen-like peptide sequences (PLG ~ L) can be cleaved by most MMPs [21]. Peptide pools have been employed to screen out more information on cleavage specificity in distinct MMPs based on the k_{cat} (the catalytic constant) and K_m (the Michaelis constant) [22]. The review by Tu et al. has summarized the amino acid sequences used in the MMP-responsive delivery systems in previous studies [23]. For instance, The substrate preference of MMP3 is characterized by a specificity for serine (S) at the P1 position preceding the cleavage site and a tendency to select methionine (M) or phenylalanine (F) at the P1′ position following the cleavage site. Furthermore, MMP3 exhibits a strong preference for proline (P) at the P3

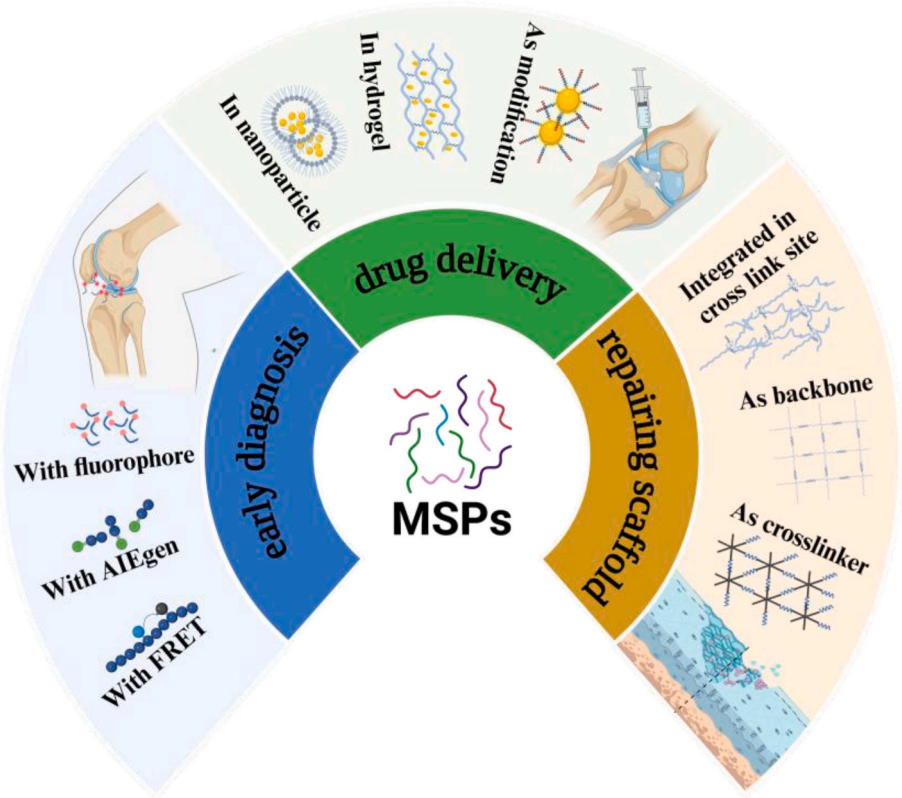

Fig. 1. The Multifaceted Role of MSPs in the Management of Arthritis. The primary applications of MSPs in arthritis can be categorized into three types: accurate early diagnosis, disease-responsive drug delivery, and biomaterial-based repair. Abbreviations: FRET, Förster resonance energy transfer; AIEgen, aggregation-induced emission fluorogen; MSPs, MMP-sensitive peptides.

position and favors hydrophobic or bulky residues, such as methionine (M) and arginine (R), at the P2′ position. This distinct substrate specificity endows MMP3 with high selectivity in degrading matrix proteins, including gelatin and fibronectin. Conversely, MMP13 displays a unique substrate recognition pattern primarily targeting collagen, with a strong predilection for glycine (G) at the P1 position and leucine (L) or methionine (M) at the P1′ position. Similar to MMP3, it exhibits a marked preference for proline (P) at the P3 position, which facilitates efficient cleavage within specific regions of the collagen triple helix. These substrate specificities underscore the pivotal role of MMP13 in the remodeling of cartilage matrix and its involvement in OA [22].

Based on the distinct sources and specificities of various MMPs, many studies have been constantly attempting to investigate the optimal applications of MSPs in arthritis across diverse experimental conditions. The Sankey diagram enumerates the specificity and the MMP sources utilized in such investigations (Fig. 2).

As shown in Fig. 2, OA and RA are the primary focus in MSP research. The large patient populations of these two diseases make the demand for MSPs particularly urgent. Below, we will discuss the role of MMPs in OA and RA separately.

The diagram visually represents the connections between MMPs and the associated roles of corresponding MSPs in osteoarthritis (OA) and rheumatoid arthritis (RA), including diagnosis, theranostic probes, drug delivery, and scaffolds. Specific MMPs such as MMP13, MMP2, MMP7, MMP3, and MMP2/9 and corresponding MSPs are mapped to their respective applications. On the right, models and experimental systems used in research, such as ATDC5 cells, chondrocytes, mesenchymal stem cells (MSC), PDCs, SW1353 cells, mice, rats, and synovial fluid from patients, are linked to MSPs roles. The flow of connections highlights the multifaceted involvement of MSPs in arthritis-related investigations.

3. MMPs in OA

In OA, the presence of inflammatory cytokines triggers the generation of MMPs. These MMPs can break down each element of the ECM present in cartilage [24]. Collagenases (MMP1, MMP8, and MMP13) play primary roles in collagen degradation in OA as they complete the rate-limiting phases. Collagenases catalyze the initial break in the collagen triple helix at the Gly775 and Leu776 positions, resulting in collagen chain unwinding [13]. Subsequently, these molecules that have undergone denaturation become vulnerable to attack and subsequent degradation by other MMPs [13]. Collagenases also exhibit substrate effects: MMP13 in chondrocytes preferentially cleaves type II collagen, the dominant type in joint cartilage [13]. Therefore, MMP13 is the most widely researched MMP in OA.

In addition to collagen, the degradation of noncollagen matrix components, such as proteoglycans and aggrecan, also depends on the activities of MMPs. The MMPs enzymatically break down the aggrecan core protein by cleaving it at the connection between Asn341 and Phe342 in the interglobular domain between G1 and G2 [25].

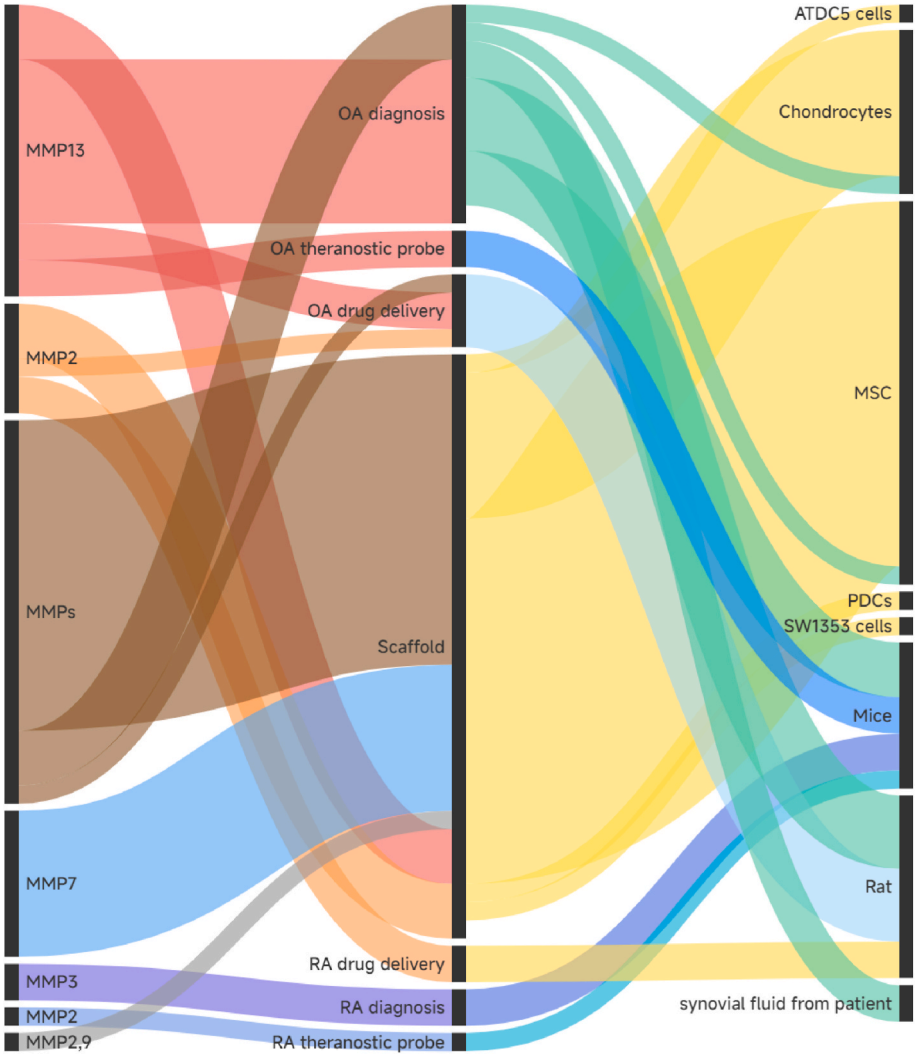

Fig. 2. A Sankey diagram depicting research on MSP in arthritis.

Remarkably, MMP13 can also break down the proteoglycan molecule aggrecan, fulfilling a second function in the matrix degradation in OA [26].

Numerous studies have shown that the expression and protein levels of specific MMPs are elevated in OA. In OA-onset rat models, immunohistochemistry and qRT-PCR of cartilage revealed elevated MMP13 expression, indicating that MMP13 contributes to the early onset of OA. [27,28] Compared with those in non-OA subjects, expression levels of MMP1 [29,30], MMP2 [30], MMP3 [29], MMP9 [30], and MMP13 [29, 31,30] are significantly elevated in OA cartilage. The serum levels of MMP1 [29], MMP3 [29], proMMP3 [32], MMP2 [29], MMP9 [29], and MMP13 [29,33] in patients with OA are markedly elevated. Elevated MMP1 [29], MMP9 [29] and MMP13 [34] expression levels have also been observed in OA synovial fluid.

The prominent participation of MMPs and the elevated expression in OA make them optimal indicators for diagnosis and therapeutic targeting [23].

4. MMPs in RA

The body's reaction to autoantigens or an alien peptide forms the basis of the pathophysiology of RA [35]. The tissue and synovial MMP concentrations have been confirmed with ELISA in previous studies [36, 37]. MMPs derived from pannus tissue and neutrophils in RA breakdown glycoproteins of cartilage into residual epitopes that promote autoimmune processes and consequently contribute to the development of RA [38].

The initiation phase of RA is the arrival and stimulation of neutrophils to release excess MMP8 and MMP9 as inducible pro-inflammatory proteinases [39,40], which are supposed to be balanced by tissue inhibitors of metalloproteinases (TIMPs) brought in by mononuclear leukocytes when they enter the synovial area [41]. Reactive Oxygen Species (ROS) or other triggers activate these MMPs [42] and more than 100 remnant peptides are deprived of type II collagen of cartilage under the combined catalysis of MMP8 and MMP9. Specifically, MMP1, MMP8 and MMP13 cleave the triple-helical collagen into a large (about 3/4) fragment and a small (about 1/4) fragment [43,44]. Subsequently, the two fragments were partially unwound and denatured. These relaxed triple-helical fragments are then split into numerous peptides by inducible MMP9.

A portion of these peptides are shielded against proteolysis by O-linked oligosaccharides and can act as autoantigenic residual epitopes for extended time [43,44]. In addition, MMP9 increases the activity of IL-8 10-fold, further enhancing neutrophil inflow [45]. Finally, in RA, erosion of bone and cartilage also takes place by the invasive pannus tissue, leading to painful and mutilating effects in severe RA. Finally, the invasive pannus tissue contributes to the erosion of bone and cartilage, resulting in painful and deforming damage to the joint.

In clinical practice, MMP3 has been a significant focus, with approximately 2000 registered studies on arthritis investigating its role [5]. A key point of interest in MMP3 is its detectability in both blood and synovial fluid. Comparisons between serum and synovial fluid suggest that the elevated MMP3 levels in the blood of RA patients, which are 10–100 times higher in synovial fluid than in serum, likely reflect leakage from inflamed joints into the bloodstream.

5. The application of MSPs in diagnosis

Traditionally, the diagnosis of arthritis is made by combining clinical symptoms with radiographic findings, such as joint narrowing, osteophytes, and sclerosis [46]. Nevertheless, radiography is limited to depicting skeletal alterations and is insufficient for capturing all the joint tissue involved in arthritis. Thus, patients may have arthritis symptoms in the absence of visible signs on X-rays, which may delay the diagnosis until the disease has progressed to a later stage [46,47]. Magnetic resonance imaging (MRI) techniques have enabled the observation of soft tissue structural damage at an earlier stage. However, it is more expensive, less easily accessible, and still requires noticeable changes in tissue morphology for diagnosis [47,48]. Therefore, currently available imaging techniques cannot capture the early stages of arthritis before the occurrence of any structural damage. It is crucial to develop diagnostic methods that can identify the early stages of the disease. These techniques are essential for preventing secondary complications, slowing the disease's progression, and enhancing the patients' overall well-being [49].

Therefore, there has been a growing interest in utilizing biomolecular methods to address the restrictions of structural imaging techniques and facilitate the development of disease-modifying medicines specifically targeting arthritis. Catabolic proteases are the fundamental components of cartilage breakdown, a hallmark of arthritis. Therefore, these proteases can function as excellent biomolecular targets for innovative diagnostics and therapies for arthritis. Among these catabolic proteases, MMPs are particularly intriguing biomarkers and therapeutic targets because of their crucial function in the development of arthritis.

Several methods of applying MSPs have been developed (Fig. 3) (Table 1). For example, MSP probes for active MMP detection can achieve sensitivity as low as 12.2 fg/mL[50], far below physiological concentrations (for instance, in synovial fluid, the concentration of MMP3 is reported approximately 1.99 nmol/L in healthy individuals, compared to around 20 nmol/L in patients with inflammatory arthritis). Therefore, there is ample room in practical applications to adjust detection conditions and substrate structures to optimally differentiate between physiological and pathological states. This room is particularly important since the reported physiological and pathological variations in MMP levels depend on numerous factors such as the MMP subtype, tissue type, and detection methods. Some detailed data for MMPs concentrations can be found in previous literature [51,52]. The reported sample types for in vitro diagnostics include MMP proteins, chondrocytes, and synovial fluid. The first two approaches primarily focus on validating the properties of probes and lack potential for clinical translation. Synovial fluid diagnosis of OA or RA, on the other hand, demonstrates promising feasibility and safety, representing an important direction for future research. Given the rapid changes in enzymatic activity, synovial fluid should be analyzed immediately after extraction or promptly frozen [53] for transfer to the laboratory. Additionally, due to the high viscosity of synovial fluid, it should be diluted prior to analysis [53,54].

FRET is the most frequently used method for its multiple advantages including simplicity, fast response, high sensitivity, and specificity, which may enable precise quantitative measurements in both laboratory settings (in vitro) and living organisms (in vivo) [55]. FRET is an energy transfer process between a pair of chromophores in close proximity, where the donor molecule that is in an electronically excited state transfers energy to an acceptor molecule [56]. As the distance between the donor and the acceptor increases, the FRET phenomenon disappears, and the original fluorescence emitted by the donor can be detected. For the diagnosis of OA or RA, a pair of chromophores conjugated to the two ends of the MSP releases a fluorescence signal when the MMP cleaves the peptide and separates the chromophore [53,54,57–64].

Another method involves detecting the fluorescence signal from an AIEgen [65]. A water-soluble probe was created by conjugating a hydrophilic MSP with a hydrophobic AIEgen. This probe did not exhibit fluorescence in an aquatic environment. Cleavage of the peptide leads to aggregation of the hydrophobic AIEgen residues, activating a fluorescent signal. The third method is the opposite of the first two strategies: the decrease in fluorescence intensity indicates the activity level of the tested MMPs [66]. MSPs conjugated with the fluorescent group are loaded on the specially treated glass. Cleavage of the peptide will lead to washout of the fluorescent group and a decrease in fluorescence intensity. This method could significantly reduce the cost of using MSPs since the selection of the fluorescent group can be flexible. However, the defect of the third method is that the sensitivity when detecting the

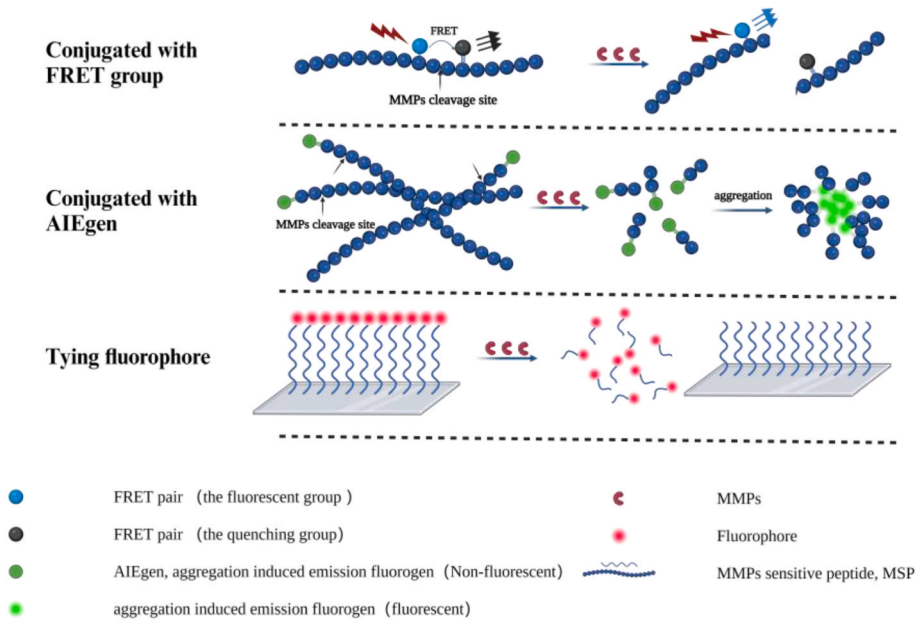

Fig. 3. Strategies for Constructing Diagnostic Probes by Integrating MSPs into Molecules with Different Luminescent Mechanisms. 1. A pair of FRET chromophores conjugated to the two ends of the MSPs releases a fluorescence signal when MMP cleaves the peptide and separates the chromophores. 2. Cleavage of MSPs leads to the aggregation of hydrophobic AIEgen residues, activating a fluorescent signal. 3. Cleavage of MSPs results in the washout of the fluorescent group, leading to a decrease in fluorescence intensity. Abbreviations: FRET, Förster resonance energy transfer; AIEgen, aggregation-induced emission fluorogen; MSPs, MMP-sensitive peptides.

decreased signal is much lower than that when detecting the emerging signal.

In addition to diagnosis, a strategy called theranostic probe was developed to monitor disease conditions during drug delivery. Chen et al. [67] have designed a hydroxychloroquine (HCQ)-loaded ferritin nanocage (CMFn) modified with FRET-MSP that is responsive to MMP13 and can smartly emit light corresponding to severity, facilitating accurate classification. Lan et al. [68] constructed a similar platform, MRC-PPL@PSO, which is specifically cleaved by MMP13 and releases Cy5.5 to emit fluorescence, revealing arthritis conditions. Beside strategies based on fluorescence, Chen et al. [69] reported a more Clinically relevant strategy, in which the probe exhibits a high r1 relaxation rate and X-ray absorption capability, enabling sensitive MR and CT dual-modal imaging. These systems have a main limitation in that the release of the drug is independent of (or scarcely dependent on) the responsiveness of the probes. A smart solution strategy has been proposed in Zhou et al.'s recent research [70]. A micelle comprises both MSP-linked FRET components and MSP-linked polyethylene glycol (PEG) tails, enabling synchronized diagnosis and drug release based on MMP13 activity (Table 2).

Overall, MSPs offer four significant advantages. The first is their exceptional specificity. Since enzymes exhibit a high degree of selectivity and efficiency, enzyme-responsive materials demonstrate superior specificity and fast response rates compared to other systems [71,72]. The exploitation of pathological microenvironmental characteristics, such as pH [73,74] and ROS [75,76], has been extensively studied for arthritis management. However, these features have primarily been applied in drug delivery rather than arthritis diagnosis, primarily due to concerns over their lack of specificity. Even compared to other MMP-responsive materials, MSPs stand out in this regard. For example, PEG [77] and Triglycerol monostearate (TG-18) [78,79] also exhibit MMP responsiveness but fall short of MSPs in terms of specificity. The second advantage is their remarkable sensitivity, derived from the high catalytic efficiency of enzymes. For instance, FRET-based MSPs can detect MMP concentrations at levels as low as fg/mL [50]. The third advantage is the accurate reflection of the activity rather than the quantity of MMPs. MMPs are typically synthesized as inactive zymogens (pro-MMPs) and require activation to become enzymatically active. This activation involves the removal of the pro-domain, which can occur through various mechanisms, including cleavage by other proteases or autocatalytic processes. The regulation of MMP activity is complex and involves multiple levels of control, including activation of the zymogen form and inhibition by TIMPs [80]. In pathological conditions, increased MMP expression is often observed. However, the actual enzymatic activity depends on the activation state of the MMPs and the presence of inhibitors. Therefore, assessing both MMP quantity and activity provides a more comprehensive understanding of their role in disease progression [81]. The fourth advantage is the potential of in vivo administration, given the simplicity and safety of MSPs, which are suitable for the constant and convenient monitoring of therapeutic efficacy in animal models and even in clinical trials. FRET-MSP has been proven effective in monitoring the efficacy of MMP inhibitors in in vivo animal models [61].

6. Application of MSPs in drug delivery

Currently, the main difficulties in medication therapy for arthritis lie in the need to enhance the ability of pharmaceuticals to remain in the joint and be effectively absorbed, as small molecule medicines are efficiently eliminated from the joint via synovial fluid exchange, with half-lives of less than 5 h [82]. Several nano/microdrug delivery methods, including micelles, polymer nanoparticles, liposomes, dendrimers, and microhydrogels, have been studied for their potential use in intravenous and intra-arterial injection therapy for arthritis. Nevertheless, those intra-articular drug delivery systems provide sustained drug release independent of disease activity. This can result in local drug concentrations falling below or exceeding therapeutic levels during periods of high or low disease activity, respectively.

Responsive drug delivery systems, on the other hand, modulate drug release to align with disease activity [83], thereby achieving optimal therapeutic outcomes. Furthermore, these systems minimize unnecessary drug release during periods of low disease activity, prolonging drug retention within the joint and extending the duration of therapeutic effects. Since enzymes possess a high degree of selectivity and efficiency,

Table 1
Studies on arthritis diagnosis via MSP.

Study	Year	Setting	Indicating substrate	Target enzyme	Administration	Detection timing	Fluorescent group	MMP sensitive sequence	Main results
OA diagnosis									
Lee et al. [57]	2008	surgical OA rat models MMPs	MSP conjugated with FRET group	MMP13	IA in vitro	1h after injection 0–40 min after incubation	Cy5.5-BHQ	GPLGMRGLGK	a linear relationship between fluorescent signals and MMP13 concentrations up to 5.5 nmol/L Fluorescence signals of OA knees were 1.3, 3.3, and 7.4 fold of the normal side for 0, 6, and 8 weeks
Ryu et al. [53]	2010	SF from 12 OA patients	MSP conjugated with FRET group	MMP13	in vitro	0–80 min after incubation	Cy5.5-BHQ	GPLGVRGKGG	the fluorescence intensity measured decidedly correlates with the KL grade of OA
Ryu et al. [58]	2011	surgical OA rat models MMPs	MSP conjugated with FRET group	MMP13	IA in vitro	1h after injection 0–80 min after incubation	Cy5.5-BHQ	GPLGVRGKGG/ GVPLSLTMGKGG/ GPLGMRGLGKGG	probe 2 provided an intense fluorescence signal in OA-induced cartilage, whereas weak fluorescence in normal cartilage probe 2 exhibited selective recognition for MMP13 and MMP7, not MMP2 and MMP9 in vitro
Ryu et al. [54]	2012	SF from 33 OA and 5 inflammatory conditions	MSP conjugated with FRET group	MMPs	in vitro	80 min after incubation	Cy5.5-BHQ	GPLGVRGKGG	SF from patients with acute inflammatory conditions presented stronger fluorescent signals than OA.
Lim et al. [59]	2014	surgical OA mice models	MSP conjugated with FRET group	MMP13	IV in vitro	2, 4, 8 h after injection	Cy5.5-QSY21	GGPAG	fluorescent signals of OA knees were 1.5-fold higher against sham surgery at 8 weeks fluorescent signals correlated with histological damage at 6 and 8 weeks
Leahy et al. [60]	2015	surgical OA mice models IL-1β induced human chondrocytes	MSPs conjugated with FRET group	MMPs	IA in vitro	4 or 16 h after incubation 5 min, 2, 6, 24h after injection		MMPSense680(Perkin Elmer)	imaging showed significantly higher fluorescent intensity in OA knees compared to sham knees NIRF imaging results correlated with histological analysis IL-1β-treated human chondrocytes were with enhanced fluorescent intensity
Castano et al. [61]	2018	surgical OA mice models MMPs	MSPs conjugated with FRET group	MMP13	IV in vitro	0–48 h after injection	Cy5.5-QSY21	GPLGMRGL	high catalytic efficiency (kcat/KM = 6.5 × 105 m−1 s−1) and high selectivity for MMP13 among nine MMPs. high catalytic efficiency (kcat/KM = 6.5 × 105 m−1 s−1) and high selectivity for MMP13 among nine MMPs.
Li et al. [65]	2018	surgical OA rat models hMSC in vitro	MSPs conjugated	MMP13	IA in vitro	60 min after injection	AIEgen	PLGVRGKGG	the probe detects early OA in mice before major histological changes this probe can detect increasing MMP13 activity in

(*continued on next page*)

Table 1 (*continued*)

Study	Year	Setting	Indicating substrate	Target enzyme	Administration	Detection timing	Fluorescent group	MMP sensitive sequence	Main results
			with an AIEgen			0,1, 3, 7 days after incubation			differentiating stem cell the probe could aid in the early diagnosis of OA.
Feng et al. [66]	2019	MMPs	MSPs linked with fluorescein (FITC)	MMP13	in vitro	0,1,2,3,4,5h after incubation	FITC	GRDGPQGIWGQDRC	the peptide-FITC was modified onto the surface of glass by Microcontact printing designed a simple and effective strategy to construct a primary MMP13-sensitive array for the in vitro detection of OA development
Walsh et al. [62]	2023	surgical OA rat models MMPs	MSPs conjugated with FRET group	MMP13	IA in vitro	0h, 6h after injection 0,1,6,24h after incubation	porphyrin-BHQ	GPLG—FRVGK	in OA mouse model, the probe yields strong fluorescence contrast (7-fold higher signal than background) at the diseased joint site.
RA diagnosis									
Ryu et al. [63]		CIA mice models MMPs	MSPs conjugated with FRET group	MMP3	IV in vitro	60 min after injection 0,10,20,30,40,50, 60min after incubation	FPR675-BHQ	CVPLSLTMGKGG	The probe provided a clear early diagnosis of arthritis and visualization of arthritis progression
Lee et al. [64]		CIA mice models, serum and FLSs MMPs	MSPs conjugated with FRET group	MMP3	IA in vitro	60 min after injection 0,10,20,30,40,50, 60min after incubation	Cy5.5-BHQ	GVPLSLTMGKGG	the MMP3 probe was able to selectively detect active MMP3 expression with high sensitivity in FLSs and diluted serum of CIA mice

Abbreviation: OA osteoarthritis; MSPs MMP-sensitive peptides; SF synovial fluid; FRET Förster resonance energy transfer; AIEgen aggregation induced emission fluorogen; IA intraarticular-injection; IV intravenous injection; hMSC Human Stromal Stem Cells; CIA collagen-induced arthritis; FLSs fibroblast-like synoviocytes.

enzyme-responsive materials are highly specific and selective with fast response rates compared to other endogenous stimulus-sensitive systems [71,72]. Given that the previously described advantages of MSPs in diagnostics are almost equally applicable to drug delivery systems, MSPs serve as ideal candidates for MMP-triggered designs (Table 2) (Fig. 4).

Xiang et al. [87] investigated an MMP-responsive micro/nanoscale hydrogel microsphere system to deliver cationic liposomes loaded with celecoxib. Liu et al. [85] designed a diclofenac sodium (DC)-loaded MMP-responsive hydrogel on the upper surface of a repair scaffold. In addition to direct release, researchers have designed hierarchical targeting methods using MSPs. Lu et al. [84] conjugated CuO NPs with integrated peptides with an MSP spacer to accomplish hierarchical targeting: with the guidance of an external peptide, CuO nanoparticles enter the cartilage, and subsequently, inner peptides exposed by MMPs recruit mesenchymal stem cells (MSCs) into the cartilage. Tianyuan et al. [86] also proposed a hierarchical drug release, in which exosomes and KGN-loaded microspheres were encapsulated in MSP-crosslinked hydrogel, thereby delaying the release of KGN. Zhou et al. [88] combined sulfonated azocalixarene (SACA), which has a distinct pattern of release responses under low-oxygen conditions, and an MSP to construct a multi-responsive delivery system for OA. MSP-based drug delivery was also applied in Nguyen et al.'s scaffolds [96,97], which were discussed in the scaffold part.

Unlike OA, RA involves obvious flares, which provide excellent application scenarios for MSP-based drug delivery. Deng's PLGA nanoparticles [89] were modified with PEG2000-MSP to target osteoclasts and macrophages after cleavage by MMPs. Li et al. [90] developed an anti-inflammatory peptide, QAW, conjugated with a penetrating peptide, an MSP, and a guide peptide. These modifications enhanced delivery to the cytoplasm and anti-inflammatory efficacy. Yu et al. [91] designed an amphiphilic polymer, dextran-sulfate-PVGLIG-celastrol (DS-PVGLIG-Cel), to construct MMP2-responsive micelles for delivering celastrol (Cel). Similarly, Guo et al. [92] developed a liposome for delivering triptolide (TP), encapsulated within a biofilm-like bilayer. The liposome's surface featured a hydrated membrane envelope, formed by using MSP as a linker arm to connect DSPE-PEG5000. Overall, MSP-based systems have been extensively developed for on-demand drug delivery in OA and RA, usually via integrating MSPs into advanced materials such as hydrogel microspheres, multi-layered scaffolds, and peptide-conjugated hierarchical nanoparticles.

TG-18 is a small amphiphile molecule with an MMP-cleavable ester linkage. Hydrogels [78,94], lipid nanoparticles [79,95], and nanoparticle–hydrogel composites [78] assembled from TG-18 have been developed to release the loaded drug in response to MMP activity in inflammatory milieus. Although the evidence supporting TG-18 remains limited, it holds significant potential as an alternative to MSPs for drug delivery in arthritis patients.

Overall, by leveraging the pathological overexpression of MMPs in diseased joints, MSP-based delivery systems enable drug release to be activated specifically in response to disease activity, ensuring spatiotemporal precision while minimizing off-target effects. This dynamic, on-demand release adapts to fluctuations in disease activity, optimizing therapeutic outcomes.

Table 2
The application of MSPs and TG-18 in anti-arthritis drug delivery.

Study	Year	Animal model	Delivery system	Material type	Target	Administration	Sequence of MMPs sensitive peptide	Main results
OA application: theranostic probe								
Chen et al. [67]	2019	adjuvant-induced OA mice	HCQ was loaded in ferritin nanocages; the nanocage's surface was conjugated with CTP and MSPs labeled with FRET group	nanoparticle modified with peptide	MMP13	IA	GPLGVRGC	the probe emitted light for OA imaging in response to the level of overexpressed MMP13 in OA microenvironment, corresponding to the degree of OA severity
Lan et al. [68]	2020	adjuvant-induced OA mice	CTP was grafted onto PPL; MSPs labeled with FRET group was grafted onto PPL; Self-assembly micelle of modified PSO	micelle	MMP13	IA	GPLGVRGC	the micelles were activated in OA, produced a fluorescence signal and sustainably released the anti-inflammatory drug molecules
Zhou et al. [70]	2024	Surgical OA mice	micelle modified with MMP13 enzyme-detachable, Cy5 containing PEG, BHQ3, and cRGD ligands and loaded with siRNA silencing MMP13	micelle	MMP13	IA	GPLGVRG	ERMs@siM13 could diagnose the early-stage PTOA, perform timely interventions, ERMs@siM13 could monitor the OA progression level during treatment through a real-time detection of MMP13
OA application: release control								
Lu et al. [84]	2022	Surgical OA rat	Ultrasmall copper oxide NPs were conjugated with CTP and MSC targeting peptide with an MSP as a spacer	nanoparticle modified with peptide	MMP2	IA	PLGLAG	MSPs were cleaved in a time-dependent manner the nanoparticles recruit joint-resident MSCs and induce differentiation into chondrocytes to facilitate repair of articular cartilage
Liu et al. [85]	2021	surgical OA and full-thickness cartilage defect rat	DC-loaded MSP-functioned hydrogel was coated on the top of the scaffold	hydrogel	MMP	implant	GCRDVPMSMRGGDRCG	the scaffold treatments provided functional recovery of the injured joint, effective osteochondral repair and inflammatory management in vivo
Tianyuan et al. [86]	2023	Surgical OA rat	KGN-loaded microspheres and exosomes were encapsulated in MSP-crosslinked hydrogel	hydrogel	MMP13	Implant	GCRRGPLGLSLGKRRCG	the hydrogel exhibits diagnostic logic to identify the pathological cue MMP13 and accordingly determine drug release kinetics satisfactory hyaline cartilage regeneration
Xiang et al. [87]	2024	Surgical OA rat	Celecoxib-loaded cationic liposomes encapsulated within the MSP-crosslinked HAMA microsphere	hydrogel microsphere	MMP13	IA	GCRRGPLGLSLGKRRCG	HAMA/MMP13sp/Lipo@celecoxib exhibited rapid degradation at a physiological concentration of MMP13; effectively decelerating disease progression and promoting articular cartilage repair
Zhou et al. [88]	2024	MIA induced OA rat	Hydroxychloroquine-loaded SAC4A-MA encapsulated within MSP-crosslinked HAMA microsphere	hydrogel microsphere	MMP13	IA	CPLGVRGKGGC	HAM-SA@HCQ can significantly attenuate the oxidative stress, downregulate the expression of hypoxia-induced factor-1α and inflammatory cytokines, and prevent the cartilage from being destroyed.
RA application: theranostic probe								
Chen [69]	2023	adjuvant-induced arthritis mice	PEG-DTIPA-KGPLGVRK-MTX and Pal-GGGGHHHHD-TCZ self-assembled to micelle	micelle	MMP2	IV	KGPLGVRK	the probe exhibits a high r1 relaxation rate and X-ray absorption capability, enabling sensitive MR and CT dual-modal imaging the probe exhibits a strong IL-6R targeting ability toward inflamed joints, and releases drugs in an MMP2-responsive manner
RA application: release control								
Deng et al. [89]	2021	adjuvant-induced arthritis rats	PLGA nanoparticles were functionalized with RGD peptide and PEG2000-MSP	nanoparticle modified with peptide	MMP9	IV	GPLGLAGQC	the nanoparticle had an arthritic joint-specific distribution and efficiently reduced the number of osteoclasts and inflammatory macrophages within these joints.
Li et al. [90]	2019	adjuvant-induced arthritis mice	Peptide: GRGDSPVGLIGRRRQRRKKRGYGGGCQAW	multifunctional peptide	MMP2/9	local injection	PVGLIG	the multifunctional peptide was successfully cleaved by type IV collagenase the designed peptide demonstrates enhanced delivery to the cytoplasm, higher reduction of

(*continued on next page*)

Table 2 (*continued*)

Study	Year	Animal model	Delivery system	Material type	Target	Administration	Sequence of MMPs sensitive peptide	Main results
								pro-inflammatory factors, and better efficacy than peptide QAW.
Yu et al. [91]	2022	adjuvant-induced arthritis rat	DS-PVGLIG-Cel self-assembled to micelle	micelle	MMP2	IV	PVGLIG	DPC@Cel has better anti-RA effects and lower systemic toxicity than free Cel.
Guo et al. [92]	2024	adjuvant-induced arthritis rats	Liposome modified with DSPE-PEG2000-mannose as the targeting molecule and with DSPE-PEG2000-PVGLIG-PEG5000 forming a hydration layer loaded TP	Liposome	MMP2	IV	PVGLIG	liposomes were found to effectively target inflammation sites and enrich activated macrophages.
MMP sensitive TG-18								
Joshi et al. [78]	2018	adjuvant-induced arthritis mice	TG-18 self-assembled to hydrogel and loaded TA	hydrogel	MMP	IA	–	hydrogel loaded with TA releases the drug on demand upon exposure to enzymes or synovial fluid from patients with RA. a single dose of TA-loaded hydrogel but not the equivalent dose of locally injected free TA reduces arthritis activity in the injected paw
He et al. [79]	2020	adjuvant-induced arthritis rats	TG-18 and DSPE-PEG2000 co-assembled the micelle and encapsulated Dex	micelle	MMP	IV	–	the micelle significantly reduces the degree of joint swelling and inhibits the production of TNF-α and IL-1β in joint tissues. The micelle was a smart drug vehicle for the treatment of RA with improved therapeutic efficacy
Wang et al. [93]	2022	adjuvant-induced arthritis mice	TPL and CEL were encapsulated in the PLGA nanoparticles and further hybridized with TG-18	Nanoparticle encapsulated in hydrogel	MMPs	Local injection	–	the release assay indicates that the TPL and CEL can be rapidly released upon exposure to the RA-related enzymes. a single local injection of the nanoparticle/hydrogel composite presents an outstanding therapeutic effect on RA.
Roy et al. [94]	2024	Surgical OA rat	TG-18 self-assembled to hydrogel and loaded BI-4394	Hydrogel	MMPs	Local injection	–	the BI-4394/Hydrogel Reduced Levels Of Inflammation And Bone Erosion
He et al. [95]	2021	adjuvant-induced arthritis rats	PEG−PBA−TGMS self-assembled to micelle and loaded Dex	micelle	MMPs	IV		the release of Dex from the PPT micelles is accelerated in response to acidic pH and overexpressed matrix metalloproteinases.

Abbreviation: PPL Poly (2-ethyl-2-oxazoline)-poly (ε-caprolactone); CTP Collagen-target peptide; CEL deliver celastrol; PSO PPL load psoralidin; TA triamcinolone acetonide; DSPE-PEG2000 1,2-distearoyl-sn-glycero-3-phospho-ethanolamine-poly(ethyleneglycol); Dex dexamethasone; TPL triptolide; TG18 Triglycerol monostearate; IA intraarticular-injection; IV intravenous injection; MIA monoiodoacetate; HAMA methacrylated hyaluronic acid; SAC4A-MA methacrylate-modified sulfonated azocalix[4]arene; DS-PVGLIG-Cel dextran-sulfate-PVGLIG-celastrol; MTX methotrexate; TCZ tocilizumab; Pal palmitic acid; TP triptolide.

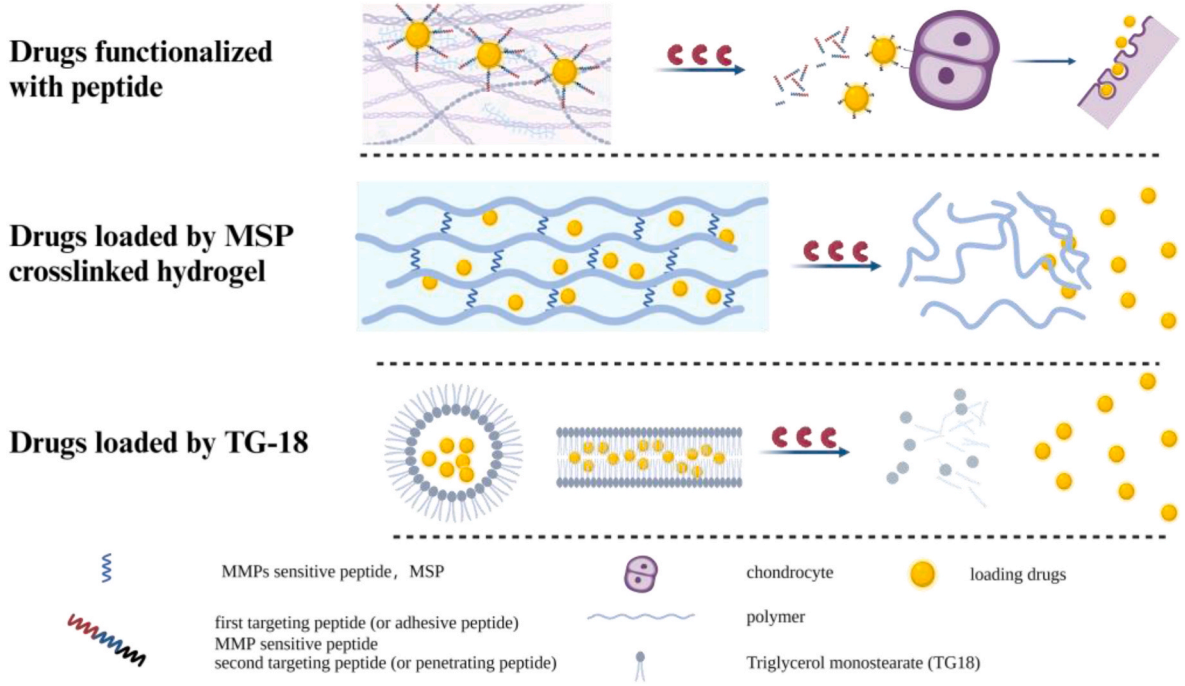

Fig. 4. MMP-Sensitive Drug Delivery in Arthritis. 1. Nano drugs were modified with guiding molecules containing an MSP spacer to achieve hierarchical targeting. 2. Drugs loaded in MSP-crosslinked hydrogels were released upon degradation of the hydrogel by MMPs. 3. Hydrogels, lipid nanoparticles, and nanoparticle-hydrogel composites assembled from TG-18 have the ability to release the loaded drug in response to MMP activity based on the cleavable ester bond of TG-18.

7. The application of MSPs in cartilage engineering

7.1. The demands for cartilage engineering scaffolds

In arthritis, particularly OA, one of the primary pathological features is the degradation and loss of articular cartilage. The regeneration capacity of articular cartilage is limited [98]. Although repair can sometimes occur, the formed fibrocartilage is compositionally different from the articular cartilage and has inferior biomechanics. Defective regeneration is partly attributable to the intrinsic properties of articular cartilage, including low cell density [99] (especially for progenitor cells) [100], low cell mobility [101], and no distribution of vessels or nerves [102]. Traditional therapeutic approaches mainly focus on symptom alleviation and slowing disease progression, falling short of achieving true cartilage repair and functional restoration. Consequently, promoting cartilage regeneration has become a central goal in arthritis treatment research [103]. Facilitating the regeneration and functional recovery of damaged cartilage can effectively suppress the pathological progression of arthritis, providing patients with more durable and comprehensive therapeutic outcomes and laying a solid foundation for the ultimate cure of arthritis.

Since Green et al. [104] reported a tissue engineering scaffold for repairing articular cartilage defects in 1977, scaffold-based tissue engineering strategies have been extensively researched. Numerous approaches have been attempted, including using different cell sources, materials, and construction methods [105]. Although improved repair has been continuously reported, the optimal approach has yet to be determined [106]. The ideal regenerated cartilage should possess adequate mechanical, compositional, and structural properties [107, 108] to address compressive stresses and frictional movement of the joint. During cartilage regeneration, a scaffold could provide (1) restoration of the abnormal load distribution in the joint; (2) a carrier and template for the adherence, proliferation, migration, and differentiation of implanted or inherent cells, and (3) biological or mechanical signaling to promote a chondrocyte phenotype and to guide matrix synthesis and organization [109]. One main challenge for the scaffold is its biodegradation properties. The degradation rate should match the matrix formation rate [110] to balance sufficient space for matrix secretion and well-functioning load bearing. In addition, the degradation of the scaffold can regulate the diffusion of nutrients and waste, cell-scaffold interactions, and the distribution and deposition of ECM proteins. Due to the aforementioned demands, researchers have turned their attention to MSP-based scaffolds, whose specific properties make them particularly well-suited for these applications.

7.2. Characteristics of MSP-based scaffolds

For cartilage regeneration, MMPs are critical components for tissue formation, regardless of whether they are stem cells or chondrocytes [111]. Adding MSPs to the scaffolds mainly has three main purposes: 1. To break down the provisional scaffold to provide a space for seed cell invasion, migration, and new ECM formation in concert with the actual seed cell activities (cell-mediated remodeling); 2. To induce the integration of newly formed cartilage and original tissues via responsiveness for both seed cells and original cells; and 3. To mimick the collagen and provide matrix-derived signals that affect the differentiation of cells [112]. The most common strategy for establishing MMP-sensitive hydrogels is to link one polymer chain to another by MSPs with thiols (usually cysteine) or alkenyls on the two ends of the peptides. The polymer chain could be PEG, HA, proteins, or their derivatives (Fig. 5) (Table 3).

In MSP-based scaffolds, the selection of structural configurations significantly impacts their performance and application. The approach of “MSP to crosslink the side chain of polymers” is suitable for applications emphasizing cellular bioactivity rather than mechanical strength. This strategy is characterized by its simplicity, tunable crosslinking density, and cost-effectiveness, though its lower mechanical properties may limit use in high-load environments. In contrast, embedding “MSP directly into the backbone” could achieve favorable mechanical strength [113]. Although technically more challenging, this configuration is particularly advantageous in scenarios requiring long-term load-bearing functionality, such as knee cartilage repair.

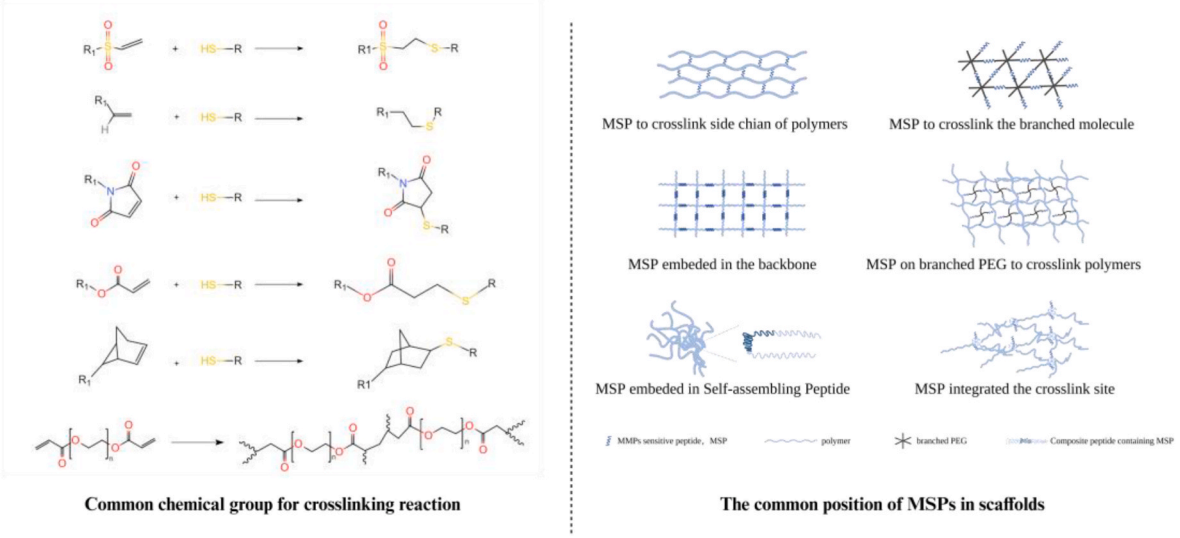

Fig. 5. Construction of Various MSP-Based Scaffolds. The most common crosslinking mechanism employed in MSP-based scaffolds involves the reaction between thiol groups (usually on cysteine) and double bonds (on Vinyl sulfone, Vinyl, Maleimide, Acrylate, or Norbornene), or the reaction between two double bonds. The strategies for achieving MMP sensitivity can be highly flexible, with MSPs acting as crosslinkers, backbones, or both.

Intermediate designs, such as "MSP to crosslink the branched molecule" and "MSP on branched PEG to crosslink polymers," offer a balance between simplicity and mechanical strength. Additionally, "MSP embedded in self-assembling peptides" leverages the peptides' inherent self-assembly properties to achieve superior biocompatibility.

The degradation rate of MSP-based scaffolds is another pivotal factor influencing their functionality. Generally, the rate at which the scaffold breaks down is primarily determined by the quantity of MSPs and the degradation characteristics of MSPs. However, some results are contradictory. He et al. [114] reported a faster degradation rate with increasing MSPs concentrations, while Tsanaktsidou et al. [115] demonstrated a slower rate. This discrepancy may be attributed to the difference in crosslinking methods. Tsanaktsidou et al. have applied both MSPs and BISAM crosslinkers, and the lack of sensitivity of BISAM to the MMP compensated for the reduction in MSPs; therefore, the degradation rate increased. These two strategies may be flexibly used in cartilage engineering according to practical demands. Regarding the optimal degradation rates of MSP scaffolds, Kudva et al.[116 117] reported that hydrogels incorporating MSPs with a normal degradation rate had greater cellular viability and proliferation, GAG production, and chondrogenic gene expression compared with those using fast-degrading MSPs. However, the exact distinction between these two MSP degradation rates has not been quantitively elucidated.

The responsiveness of the MSP-based scaffold has been well validated, whether in response to MMPs [118–120], chondrocytes [121], or MSCs [122]. Seed cells cultured in MSP scaffolds show a spherical morphology [123] and an arrangement of isogenous groups [124], consistent with native cartilage. Seed cells express more chondrogenic genes (SOX9, ACAN, and COL2A1) [125–127] and accumulate more ECM (deposition of GAGs and COLII) [123,127–129] in MSP scaffolds. The compressive modulus of repaired cartilage has been confirmed to increase in the presence of MSP scaffolds [113,123]. Although most of the evidence has been obtained from in vitro assays, pilot results from *ex vivo* [124] and in vivo [130] studies [131] also supported that MSP scaffolds can facilitate the formation of native-like articular cartilage.

MSPs play an essential and pivotal role in cartilage engineering. To be specific,the key contributions of MSPs can be summarized as follows: 1. Facilitation of cell proliferation and differentiation: MSP-based scaffolds provide a favorable microenvironment for chondrocyte proliferation and differentiation. This includes promoting a spread cell morphology [126,132], supporting the formation of larger chondrocyte clusters [125], and upregulating the expression of key chondrogenic markers such as COL II and aggrecan [125]. Additionally, these scaffolds improve cell viability significantly compared to non-degradable or non-responsive materials [114]. 2. Enhanced ECM accumulation: MSPs contribute to increased ECM deposition while maintaining a native-like cartilage phenotype. They facilitate less calcification [126], produce a more diffuse cell-derived matrix [125], and enhance mechanical properties, such as the dynamic compressive modulus. 3. Improved cartilage integration: The enzymatic responsiveness of MSPs enables better integration between newly formed cartilage and surrounding tissues. This is reflected in the indistinct boundary between the cell-derived matrix and the hydrogel scaffold [125].

7.3. *Innovation in MSP-based scaffolds*

With the clear safety and efficacy of MSPs established through the aforementioned extensive research, it is only natural that an increasing number of studies are now attempting to integrate MSPs with other novel designs. Based on the versatility and ease of constructing MSP scaffolds, multifunctional MSP scaffolds have been extensively investigated. Studies exploring additives, bioactive matrices, crosslinking techniques, and fabrication methods have greatly enhanced the capabilities of MSP-based scaffolds (Fig. 6).

Functional peptides that promote adherence of chondrocytes, collagen, HA, heparin, integrins, and similar components have been extensively researched in MSP-based scaffolds to enhance matrix deposition. Additionally, growth factors such as TGF-β1, TGF-β3, IGF-1, and chondroitin sulfate have also been studied in MSP-based scaffolds for their potential to promote chondrocyte proliferation. Although these additives are common in cartilage repair scaffolds, the inclusion of MSPs introduces the capability of "revealing as needed." For instance, Salinas et al. [128] incorporated an MSP into the arginylglycylaspartic acid (RGD) peptide sequence to release RGD via a native differentiation-mimicking timeline. This strategy increased the glycosaminoglycan produced by hMSCs 10 times compared with that produced by a nondegraded scaffold after 21 days of culture.

To achieve optimal biocompatibility, natural and biological materials are highly anticipated. Collagen, which makes up approximately 60 % of the dry weight of hyaline cartilage, is the primary protein in the cartilage ECM [141,142]. Therefore, collagen or collagen mimetics are promising choices. Since MSPs and collagen peptides are commonly

Table 3
Studies on the MMP-sensitive scaffold in cartilage repairment.

Study	Year	Loading cell	Hydrogel/scaffold	Reactive groups for crosslinking	Target enzyme	Sensitive sequence	results
Park et al. [121]	2002	Primary bovine chondrocytes	Branched PEG vinyl sulfone linked by MSP	Vinyl sulfone-thiol reaction	MMP	GCRDGPQGIWGQDRCG	chondrocytes were able to degrade the MMP-sensitive hydrogels and expand the size of the pericellular domain
Park et al. [125]	2004	Primary bovine chondrocytes	Branched PEG vinyl sulfone linked by MSP	Vinyl sulfone-thiol reaction	MMP	GCRDGPQGIWGQDRCG	MSP hydrogel demonstrated larger clusters and more diffuse, less cell surface-constrained cell-derived matrix in the chondron the gene expression of COL II and aggrecan was increased in the MMP-sensitive hydrogels the border between cell-derived matrix and hydrogel was indistinct
He et al. [114]	2007	rats MSC	PLEOF was crosslinked with Acrylated MSPs	Alkenyl-Acryl reaction	MMP13	QPQGLAK	cell viability was significantly higher in the MSP hydrogel the degradation rate of the hydrogel depended on the ratio of the peptide to the BISAM crosslinker
Chau et al. [118]	2008	None	A centrically positioned MSP flanked with three RADA units on each side	Beta-sheet assembling	MMP2	PVGLIG	exposure of the hydrogel to MMP2 resulted in peptide cleavage, and a decrease in surface hardness
Kim et al. [132]	2008	hMSC	Acrylated HA was crosslinked with MSPs, and mixed with cell adhesion peptides (RGD)	Acryl-thiol reaction	MMP	GCRDGPQGIWGQDRCG	as the concentration of collagenase increased, the degradation rate of peptide hydrogels increased cells in MSP hydrogels spread by degrading surrounding hydrogels with MMPs
Salinas et al. [128]	2008	hMSCs	RGD with MSP sequence covalently bound to PEGDA gel	Thiol-acrylate polymerization	MMP13	PENFF	an MMP13 cleavage site was incorporated into the peptide sequence to release RGD mimicking the native differentiation timeline by 21 days of culture, hMSCs encapsulated in RGD-releasing gels produced 10 times as much glycosaminoglycan as cells with uncleavable RGD gels, and 75 % of the cells stain positive for COL II deposition where RGD was cleavable, as compared to 19 % for control.
Bahney et al. [113]	2011	hMSC	MSPs were embedded within a PEGDA backbone	Acryl-acryl reaction	MMP7	PLELRA or VPLSLTMG	hMSCs photoencapsulated in MMP7-sensitive scaffolds produced neocartilage constructs with more extensive collagenous matrices. Furthermore, these changes translated into an increased dynamic compressive modulus.
Giano et al. [119]	2011	SW1353 cells	Self-assembling hydrogels by β-hairpin peptide containing MSP sequence	Beta-sheet assembling	MMP13	PTGXKV(X could be F/L/I/A)	by 14 days, the overall degradation of the gels varies from 5 % to 70 % and the rates differ according to MSPs. cells were capable of transversing the different MSP gels.
Nguyen et al. [96,97]	2011	mice MSC	Superficial zone: PEGDA, acrylated MSPs, and methacrylated CS were crosslinked. Transitional zone: PEG: CS hydrogels Deep zone: PEG: HA hydrogels	Acryl-acryl reaction multi-layer	MMPs	QPQGLAK	the addition of MSPs or CS lowered the compressive modulus of the hydrogel while adding HA raised the compressive modulus. the combined addition of CS and MSPs in PEG hydrogels significantly increased collagen II expression as compared to PEG-only hydrogels

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Table 3 (*continued*)

Study	Year	Loading cell	Hydrogel/scaffold	Reactive groups for crosslinking	Target enzyme	Sensitive sequence	results
Feng et al. [126]	2014	hMSCs	HA macromers were modified with maleimide groups and crosslinked with MSPs.	Maleimide-thiol reaction	MMPs	GCRDVPMSQMRGGDRCG	it was found that hMSCs encapsulated in the MSP gels switched to a more spread morphology, and expressed a higher level of chondrogenic marker genes but a lower level of hypertrophic genes more cartilage specific matrix molecules but less calcification was observed in the MSP gels
Gao et al. [133]	2015	hMSCs	PEGDMA, acrylated RGD and acrylated MSPs were crosslinked	Acryl-acryl reaction	MMPs	GCRDGPQGIWGQDRCG	the gel has excellent biocompatibility with embedded hMSC and significantly enhanced bone and cartilage differentiation
Parmar et al. [122]	2015	hMSCs	Scl 2 was modified with HA- or CS-binding peptides and then crosslinked with MSP	Acryl-thiol groups	MMP7	CGGGPLELRAGGGC	hMSCs encapsulated in MSP-based hydrogels functioned with glycosaminoglycan-binding peptides exhibited improved viability and significantly enhanced chondrogenic differentiation
Sridhar et al. [123]	2015	porcine Chondrocytes and hMSCs	4-arm PEG norbornene was functionalized with thiolated TGF-β1 and crosslinked with MSP	Norbornene-thiol reaction	MMPs	KCGPQGIWGQCK	both encapsulated cell types maintained a high viability and a spherical morphology in the gels, and the generated ECM resembles articular cartilage with respect to collagen typing (high type II collagen: type I collagen ratio). there was a significant increase ($p < 0.001$) between day 1 and 14 of the compressive modulus in degradable scaffolds while the values between day 1 and 14 were not statistically different in non-degradable scaffolds
Amer et al. [120]	2016	mice MSCs	8-arm PEG was functionalized with norbornene, and crosslinked with MSPs	Norbornene-thiol reaction	MMP2,9	CVPLSLYSGC	these hydrogels rapidly degraded upon exposure to exogenous MMP2 and MMP9. in vivo, these hydrogels remained intact after 4 weeks and exhibited a classic FBR with inflammatory cells at the hydrogel surface and a fibrous capsule.
Broguiere et al. [134]	2016	hCCs	HA conjugated with MSPs containing an active Lys, and HA conjugated with an active Gln were crosslinked via the transglutaminase activity of FXIIIa	Lys-Gln reaction under transglutaminase	MMPs	GPQGIWGQ	these gels have an ideal set of properties for treating cartilage lesions: they are injectable, very fast gelling, adhesive to cartilage tissue, retain their shape, and have excellent biocompatibility. human chondroprogenitors encapsulated in the gels showed tunable proliferation and good cartilage matrix deposition, transforming the gels into cartilage like tissue within 3 weeks.
Parmar et al. [135]	2016	hMSCs	Site-directed mutagenic Scl2 was crosslinked by MSPs and aggrecanase (ADAMTS4) cleavable peptides	Acryl-thiol reaction	MMP7	PLELRA	hMSCs encapsulated within the hydrogels crosselinked with both degradable peptides exhibited enhanced chondrogenic characteristics as demonstrated by gene expression and extracellular matrix deposition compared to the hydrogels crosselinked with a single peptide
Kudva et al. [116]	2017	human chondrocytes	4-arm PEG was crosslinked with MSP	Vinyl sulfone cysteine	MMPs	regular degrading crosslinker GCREGPQGIWGQERCG	the hydrogels based on regular degrading MSPs had higher GAGs production and chondrogenic gene expression

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Table 3 (*continued*)

Study	Year	Loading cell	Hydrogel/scaffold	Reactive groups for crosslinking	Target enzyme	Sensitive sequence	results
						the fast degrading: GCRDVPMSMRGGDRCG	than those based on fast degrading MSPs.
Parmar et al. [129]	2017	hMSCs	Glycosaminoglycan-binding peptide-functionalized Scl2 proteins and RGDs were crosslinked with 4-arm acrylate-functionalized MSP	Acryl-thiol reaction	MMP7	PLELRA	the cleavable hydrogel had greater extracellular matrix accumulation
Aisenbrey et al. [136]	2018	hMSC	CS and RGD were linked into branched PEG, which was crosslinked with MSP	Norbornene-thiol reaction	MMP7	CRDPLELRADRC	there was a substantial deposition of aggrecan and collagen II, which correlated with degradation of the hydrogel a linear relationship was observed between PEG intensity per nuclei and collagen II intensity per nuclei from weeks 1–6.
Hesse et al. [137]	2018	human MSC and porcine chondrocytes	Maleimide functionalized heparin was mixed with adhesion-binding (RGD) and collagen-binding (CKLER/CWYRGRL) peptides, and then was crosslinked with branched PEG carrying MSPs in each arm	Maleimide-thiol reaction	MMPs	GCGGPQGIWGQGGCG	MSP-based hydrogel enhanced cell viability and proliferation correlated with MSP ratio.
Kudva et al. [117]	2018	hPDCs and ATDC5 cells	4-arm PEG was functionalized with RGD, and then was crosslinked with MSP	Vinyl sulfone-thiol reaction	MMPs	VPMSMRGG GPQGIWGQ	PEG-VS macromer and cross-linked with the protease sensitive peptide cross-linkers can support both the proliferation and chondrogenic differentiation of these two cell types
Pascual-Garrido et al. [131]	2019	rabbit MSCs	8 arm PEG norbornene was mixed with thiolated CS, RGD, and then crosslinked with MSP	Norbornene-thiol reaction	MMP2	CVPLSLYSGC	chondrogenesis and the degradable behavior of the hydrogel by MSCs were confirmed the hydrogel group had the highest scores on the modified O'Driscoll scoring system and showed higher safranin-O staining, although significance was not detected for either parameter. the hydrogel remained in the defects, did not result in inflammation, and showed good cartilage healing capacity
Schneider et al. [130]	2019	Porcine chondrocytes	Multi-arm PEG norbornene monomers, bis-cysteine MSPs, and thiolated-TGF-b3 were crosslinked.	Norbornene-thiol reaction	MMPs	GCVPLSLYSGCG	the total amount of ECM deposited in the hydrogel constructs was similar in vitro and in vivo. the in vivo environment led to more elaborate ECM, which correlated with higher MMP activity, and an overall higher quality of engineered tissue that was rich in aggrecan, decorin, biglycan and COL II with minimal collagen type I.
Tsanaktsidou et al. [115]	2019	hMSCs	MeHA was functionalized with CS binding peptide, and then was crosslinked with MSP	Alkenyl-thiol reaction	MMP7	CGGGPLELRAGGGC	the concentration of the peptide crosslinker increased, the gelation onset time decreased as well as the degradation rate of the synthesized hydrogels while their storage modulus, G′, increased.
Ren et al. [138]	2020	Rabbit BMSC	Maleimide-modified HA was functionalized with collagen mimetic peptide, (GPO)8-CG-RGDS, and then was crosslinked with MSP	Maleimide-thiol reaction	MMP2	GCRDGPQGIWGQDRCG	combining these collagen mimetic peptides with an MMP-sensitive peptide may have the potential to induce the differentiation of BMSCs into cartilage and inhibit the

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Table 3 (*continued*)

Study	Year	Loading cell	Hydrogel/scaffold	Reactive groups for crosslinking	Target enzyme	Sensitive sequence	results
							hypertrophic phenotype during differentiation.
Tsanaktsidou et al. [124]	2020	hMSCs and Porcine Chondrocytes	CS-biofunctionalized MeHA were crosslinked with MSP	Acryl-thiol reaction	MMP7	CGGGPLELRAGGGC	the developed hydrogels were found to create a proper environment for the growth and proliferation of hMSCs and to promote their differentiation towards a chondrogenic phenotype chondrocyte-laden MeHA hydrogels. cultured on an *ex vivo* osteochondral platform revealed the deposition of GAGs and the arrangement of chondrocyte clusters in isogenous groups, which was characteristic of hyaline cartilage morphology.
Maples et al. [139]	2023	Bovine chondrocytes	8-arm PEG-norbornene tethered with TGF-β3 was crosslinked with MSP	Norbornene-thiol reaction	MMPs	GCVPLSLYSGCG	age-dependent variations of donor chondrocytes induced different levels of MMPs and TIMPs, which influenced the timing of the gel-to-tissue transition in MMP-sensitive hydrogels.
Stefani et al. [140]	2023	Rat bone-marrow derived MSCs	8-arm PEG-norbornene tethered with TGF-b3, IGF-1, RGDs, and CS was crosslinked with MSP	Norbornene-thiol reaction	MMPs	GCVPLS-LYSGC	the cartilage-mimetic hydrogel supports the incorporation of bioactive growth factors and chondrogenesis of encapsulated MSCs leading to hyaline cartilage matrix production.

Abbreviation: COLII collagen type II; HA hyaluronic acid; PEGDA poly(ethylene glycol) dimethacrylate; PEGDMA poly(ethylene glycol) dimethacrylate; Scl 2 Streptococcal collagen-like 2 protein; hCCs human chondroprogenitor cells; MeHA methacrylated hyaluronic acid; CS chondroitin sulfate; PEG poly (ethylene glycol); TGF transforming growth factor; PLEOF Poly (lactide-co-ethylene oxide-co-fumarate); 4aPEG-OPA o-phthalaldehyde-grafted four-arm poly(ethyleneglycol); RADA arginine-alanine-aspartate-alanine; hPDCs Human periosteum-derived cells; ECM extracellular matrix; GAG sglycosaminoglycans; TIMPs tissue inhibitors of MMPs; IGF Insulin-like growth factor; MSCs mesenchymal stem cells.

used bioactive materials with similar basic structures, constructing a hydrogel with a collagen backbone and MSP linker is simple and feasible. Additionally, directly synthesizing collagen peptides with MSP sequences is also promising. In another direction, bacterial collagen has emerged as an important alternative. Unlike mammalian collagens, Streptococcal collagen-like protein 2 (Scl2) proteins are non-immunogenic, non-cytotoxic, and can be recombinantly produced in high yields with minimal batch-to-batch variation [143]. Parmar [144,145] modified the structure of Scl2 helices to include MSP sequence through tethering or site-directed mutagenesis.

The methods for crosslinking MSP-based scaffolds have evolved to enhance their biocompatibility and safety. One such method has involved beta-sheet assembly, where segments of a polypeptide chain align to form a sheet-like structure stabilized by hydrogen bonds [146]. Chau et al. [118] and Giano et al. [119] have engineered MSP-containing β-hairpin peptides that can undergo folding and self-assembly in response to environmental triggers under physiological conditions. Another biological strategy has involved crosslinking hyaluronan modified with MSPs containing active lysine residues, and hyaluronan with sequences containing active glutamine residues, mediated by the transglutaminase activity of FXIIIa [134]. These biological crosslinking methods have conferred significant advantages in biocompatibility and safety by reducing or avoiding synthetic chemical catalysts and harmful side reaction products.

With rapid technological advancements in biomedical engineering, MSP-based scaffolds have been fabricated with increased precision and enhanced functionality. Parmar et al. [135] have developed a bimodal enzymatically degradable scaffold by crosslinking the main chain with a combination of MSPs and aggrecanase (ADAMTS4) cleavable peptides. This highly adaptable and finely tunable strategy could better mimic native cellular temporal processes. Compared with scaffolds incorporating only one sensitive peptide, hMSCs in multiple enzymatically degradable scaffolds have exhibited enhanced chondrogenic characteristics. Nguyen et al. [96,97] constructed a multilayered scaffold with PEG, acrylated MSPs, and acrylated chitosan (CS) as the superficial layer, PEG and acrylated CS as the central layer, and PEG and acrylated HA as the bottom layer. The superficial layer generated elevated amounts of collagen II and reduced levels of proteoglycans. The middle layer produced moderate amounts of collagen II and proteoglycans. The bottom layer resulted in elevated proteoglycan levels and decreased collagen II levels. These three layers have provided a growth gradient for the compressive modulus. Gao et al. [133] developed Inkjet-bioprinted hydrogels consisting of acrylated MSPs, PEG, and hMSCs, which combined the advantages of 3D printing and MMP sensitivity. The bioprinted scaffold achieved excellent cartilage matrix deposition and mechanical properties and inhibited hMSC hypertrophy. Considering that the native cartilage is zonally organized and functional, spatially varying biomaterial compositions are attractive.

8. Challenges and future directions

Several main limitations have been encountered in such studies. The behaviour of MMPs is complex in arthritis pathology, and the dynamics of specific MMPs at various stages are conflicting. With respect to gene expression detected locally, previous studies have yielded conflict results in OA. Some have reported that the expression of MMP1 [147], MMP3, [147,148] MMP2 [149], and MMP13 [147,150] is lower in damaged regions of human osteoarthritic cartilage compared with intact regions, whereas others reported that the expression of MMP1 [151] and MMP13 [151,152] is greater in damaged regions. Moreover, MMP1

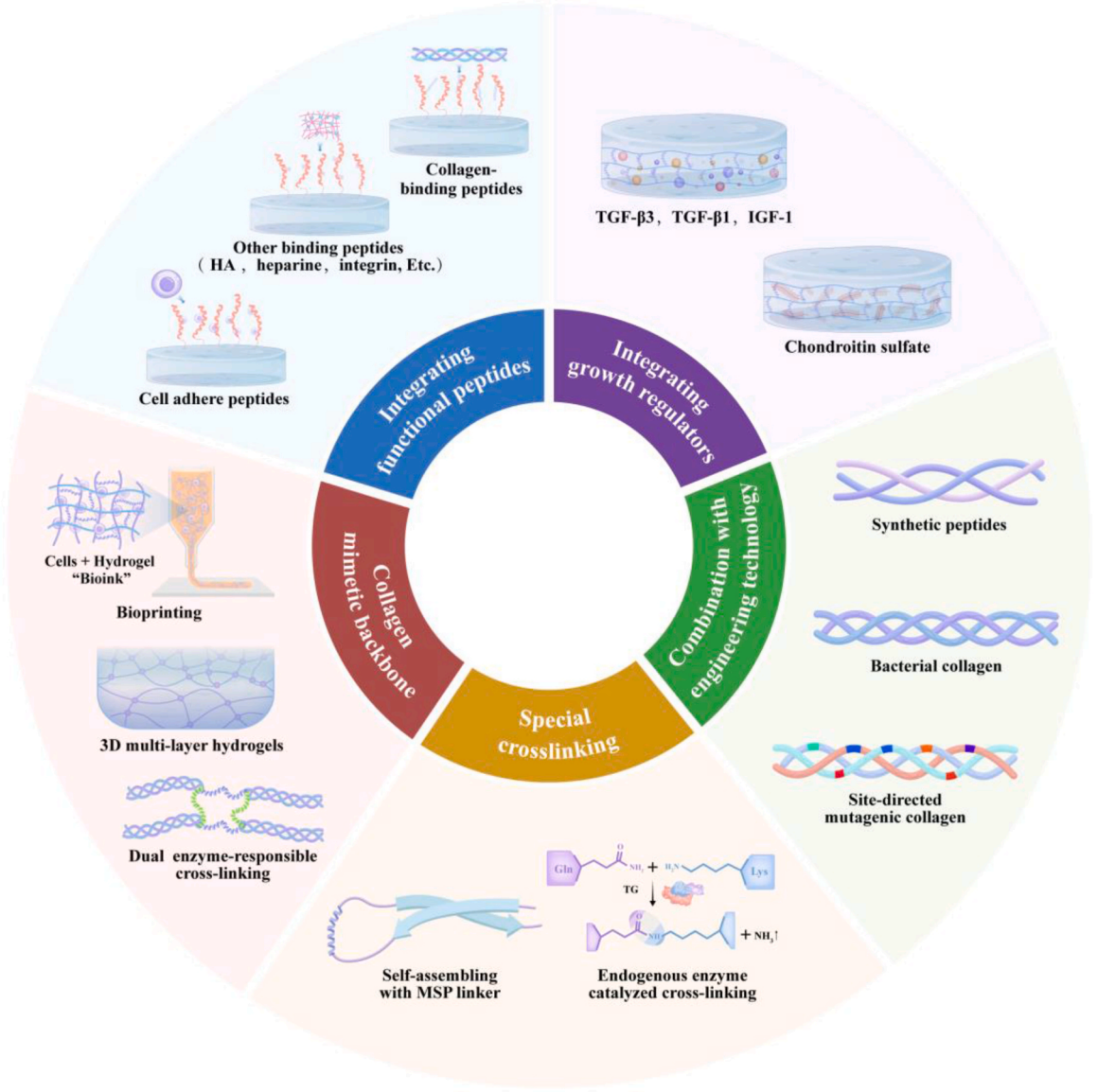

Fig. 6. Novel MSP-based Scaffolds with Multiple Functions. Multifunctional MSP scaffolds have been thoroughly investigated, encompassing the integration of additives, bioactive matrices, advancements in crosslinking methodologies, and the incorporation of novel fabrication techniques. HA, hyaluronic Acid; TG, transglutaminase.

[148] and MMP13 [148] were not differentially expressed between damaged and intact sites in some studies. Similarly, the expression of MMPs in different disease stages was also unclear. A decrease in MMP13 has been reported in advanced compared with minimal lesions [150], whereas opposite results have also been published [153]. Some researchers have shown that the increased production of MMPs in intact cartilage neighboring a lesion may indicate an active effort to rebuild undamaged cartilage in response to shifts in the cellular environment [147]. Other explanations included that the contradiction in the expression of MMPs observed in the above studies may be associated with the fluctuation in the proportion of distinct zones [150] since MMPs are expressed in a zone-dependent manner [154,155]. The cross-reactivity of MSPs to various kinds of enzymes remains troublesome. Some basic studies on the chemical structure of MMPs have raised the possibility of developing new highly selective MSPs that are only labile for targeting the MMP. MacColl et al. demonstrated that the interaction between the MMP and its substrate is determined by distinct subsites or pockets (S) within the MMP that interact with the corresponding substituents (P) in the substrate [156]. The S1′ pocket exhibits the highest degree of variability in terms of the composition of amino acids and the depth of the pocket [157]. The S1′ pocket can vary in depth, ranging from shallow (e.g., MMP1 and MMP7) to intermediate (e.g., MMP2, MMP8, and MMP9) to deep (e.g., MMP3, MMP11, MMP12, MMP13, and MMP14) [158,159,160]. Exploring the characteristics of each MMP therefore may lead to the development of new more selective MSPs. In the design of drug delivery and diagnostic systems utilizing MSPs, the selection of peptide chain length must balance substrate specificity, enzymatic cleavage efficiency, and system stability. Short peptide sequences may reduce MMP-specific recognition, while long sequences could negatively impact the distribution and pharmacokinetics of the whole system [161]. Therefore, design considerations should prioritize efficiency and specificity based on the intended application. For instance, diagnostic probes emphasize specificity, whereas therapeutic delivery systems may prioritize stability. Additionally, short peptides, lacking complex tertiary structures, can only partially replicate MMP cleavage characteristics for natural substrates [162]. In contrast, longer peptides better reflect MMP biological activity but present challenges in experimental control and synthesis

complexity. This underscores the importance to align length choices with research objectives, for example, focusing on biological mechanisms or translational potential.

MSPs are expected to achieve rapid clinical translation by being integrated into arthritis-related products currently under clinical trials, such as hyaluronic acid (HA) hydrogels, microparticles, and liposomes [163]. MSPs are easily-synthesized, cost-effective and biocompatible. The free terminal amine and carboxyl groups of the peptide are highly suitable and commonly employed for conjugating the peptide with other molecules. It has been reported that various small drugs, [164,165] proteins [166], polymers [167], or inorganic nanoparticles [168] are MMP-responsive when covalently conjugating or physically incorporating MSPs.

In the further future, MSPs possess significant translational potential in the management of arthritis, owing to their multifunctional capabilities and adaptable nature. By leveraging the central role of MMPs in arthritis pathophysiology and tissue remodeling, MSPs can be developed into precise diagnostic indicators, responsive drug delivery triggers, and regulators for cartilage remodeling. Their ability to specifically respond to MMP activity enables spatiotemporal precision, making them ideal for early disease detection and targeted therapy. Moreover, the excellent usability and stability of MSPs allow for seamless integration into diverse biomaterial designs. These features collectively position MSPs as a versatile platform with immense potential for clinical translation. For example, a multi-responsive system can be achieved through hierarchical design, where the outer layer comprises one type of responsive molecule, the intermediate layer consists of MSP-linked drug carriers, and the inner layer encapsulates the drug, enabling multistage release. Such a design fully exploits the synergistic effects of different environmentally responsive components and overcomes multiple physiological barriers encountered during therapeutic delivery, which is critical for achieving efficient targeted drug delivery. Alternatively, combining MSPs with mechanically robust materials can impart scaffolds with both mechanical support and enzyme-responsive degradation properties. Novel scaffolds with excellent mechanical properties have been developed, exhibiting properties almost comparable to native cartilage. Lin et al. [169] reported a liposome-incorporating HA-based hydrogel microsphere, which improved joint lubrication by forming self-renewable hydration layers. Fu et al. [170] developed a method to significantly enhance the stiffness and toughness of protein-based hydrogels by introducing chain entanglements into the network of folded elastomeric proteins. This innovation enables the engineering of materials that combine high stiffness, high toughness, fast recovery, and ultrahigh compressive strength, closely resembling the mechanical properties of cartilage. Similarly, Guo et al. [171] have explored the translation of natural hierarchical structures and toughening mechanisms into biomimetic hydrogels, achieving strong and tough materials through a freeze-casting-assisted solution substitution method. In the early stages, the scaffold provides sufficient mechanical strength to meet cartilage load-bearing requirements. As the MSPs gradually degrade, the resulting space promotes cell migration and extracellular matrix production, ultimately enabling functional cartilage regeneration.

9. Conclusion

MMPs play essential roles in arthritis pathophysiology and cartilage engineering, thus, MSPs are potential diagnostic indicators, drug-delivering triggers, and cartilage remodeling regulators. Translational potentials include early and precise diagnosis of MMP activity via fluorescence probe technologies; acting as nanodrug carriers to enable on-demand drug release triggered by pathological microenvironments; and facilitating cartilage engineering through MMP-mediated degradation, which promotes cell migration, matrix synthesis, and tissue integration. Considering the excellent usability and stability of peptides, using MSPs could lead to major breakthroughs in arthritis management through designs integrating various peptides.

CRediT authorship contribution statement

Mingyang Li: Investigation, Formal analysis, Data curation, Conceptualization. **Tao Deng:** Writing – review & editing, Formal analysis, Conceptualization. **Quan Chen:** Writing – review & editing, Validation. **Shenghu Jiang:** Writing – review & editing. **Hang Li:** Writing – review & editing. **Jiayi Li:** Writing – review & editing. **Shenglan You:** Writing – review & editing. **Hui-qi Xie:** Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Bin Shen:** Validation, Supervision, Resources, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

Not applicable for this review.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationship which could have appeared to influence the work reported in this paper.

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