



## Review

# Inflammatory mediators in osteoarthritis: A critical review of the state-of-the-art, current prospects, and future challenges



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## ABSTRACT

Osteoarthritis (OA) has traditionally been defined as a prototypical non-inflammatory arthropathy, but today there is compelling evidence to suggest that it has an inflammatory component. Many recent studies have shown the presence of synovitis in a large number of patients with OA and demonstrated a direct association between joint inflammation and the progression of OA. Pro-inflammatory cytokines, reactive oxygen species (ROS), nitric oxide, matrix degrading enzymes and biomechanical stress are major factors responsible for the progression of OA in synovial joints. The aim of this review is to discuss the significance of a wide range of implicated inflammatory mediators and their contribution to the progression of OA. We also discuss some of the currently available guidelines, practices, and prospects. In addition, this review argues for new innovation in methodologies and instrumentation for the non-invasive detection of inflammation in OA by modern imaging techniques. We propose that identifying early inflammatory events and targeting these alterations will help to ameliorate the major symptoms such as inflammation and pain in OA patients.

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**Abbreviations:** LIF, Factor Leukocyte Inhibitory; PGE2, Prostaglandin E2; IL-1Ra, IL-1 receptor antagonist; TIMPs, Tissue Inhibitor of Matrix metalloproteinases; HA, Hyaluronic Acid; hsCRP, High sensitive C-Reactive Protein; IL-1 $\beta$ , Interleukin-1 $\beta$ ; NO, Nitric Oxide; mPGES-1, Microsomal PGE Synthase-1; sPLA2, Soluble Phospholipase A2; MMPs, Matrix Metalloproteinases; iNOS or NOS2, Isoform of Nitric Oxide Synthase; RA, Rheumatoid Arthritis; TNF- $\alpha$ , Tumor Necrosis Factor-alpha; MRI, Magnetic Resonance Imaging; PP, Pyrophosphate; US, Ultrasound; JSW, Joint Space Width; NSAIDs, Nonsteroidal Anti-Inflammatory Drugs; ACR, American College of Rheumatology; ColX, Type X collagen; C2M, Matrix Metalloproteinase-derived fragments of type II collagen; SF, Synovial Fluid; TKR, Total Knee Replacement; BDNF, Brain-Derived Neurotrophic Factor; TMJ, Temporomandibular Joint; CTX-I and II, Carboxy Terminal Telopeptides types I and II; COMP, Cartilage Oligomeric Matrix Protein; CTGF, Connective Tissue Growth Factor; FLSs, Fibroblast-like Synoviocytes; NF, Nuclear Factor; CCL2, C–C motif Ligand 2; ERK1/2, Extracellular Signal-Related Kinase 1/2; BMI, Body Mass Index; HOA, Hand Osteoarthritis; PTX-3, Pentraxin-3; MCV, Modified Citrullinated Vimentin; NC, Control Subjects; PPAR- $\gamma$ , Peroxisome Proliferator-Activated Receptor- $\gamma$ ; DGAT2, Diacyl Glycerol Acyl Transferase 2; CD36, Cluster of Differentiation; THRSP, Thyroid Hormone Responsive Spot; m-CPPD and t-CPPD, Monoclinic and triclinic Calcium Pyrophosphate Dehydrate; CCL, Cranial Cruciate Ligament; WOMAC, Western Ontario McMaster Universities OA index; SAF-1, Serum Amyloid A-activating Factor 1; CRPM, MMP-mediated breakdown of CRP; C1M, MMP-mediated degradation of type I Collagen; MPO, Myeloperoxidase; HOCl, Hypochlorous acid; Cl<sub>2</sub>, Chlorine gas; ECs, Endothelial Cells; CAT, Chloramphenicol Acetyl Transferase; EMSA, Electrophoretic Mobility Shift Assay; COX-1, Cyclooxygenase-1; COX-2, Cyclooxygenase-2; ECM, Extracellular matrix; MNC, Mononuclear cell; OCT, Optical coherence tomography; WAT, White Adipose Tissue; Gd, Gadolinium; CE-US, Contrast-enhanced ultrasound; PAMPs, Pathogen-associated molecular patterns; PRRs, Pattern recognition receptors; TLRs, Toll-like receptors; MAC, Membrane attack complex; ADAMTS, A Disintegrin And Metalloproteinase with Thrombospondin motifs; NOD, Nucleotide oligomerization domain; NLRs, NOD-like receptors.

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**1. Introduction**

Osteoarthritis (OA) is among the most common joint diseases in the world and a major cause of disability in the aging population [1]. It has been reported that more than 27 million of the US adult population are affected by OA, which is the leading cause of life-years lost to disability in most cases [2]. The disease also affects juveniles, young athletes, many middle-aged people and particularly in older people it can cause severe pain and physical disability [3]. OA is one the major reasons for hip and knee replacement surgeries [4]. Moreover, it most commonly affects the knees, hands, feet, the hips, and the spine. In synovial joints the entire joint is affected, including cartilage, synovial membrane, subchondral bone, ligaments and peri-articular musculature [5,6]. There are a number of major factors affecting the degree of risk for developing OA. These include joint location, obesity, genetic predisposition, joint malalignment, trauma, gender, muscle weakness, physical activity/inactivity, race, bone density, estrogen levels and nutritional status [3,7]. OA is traditionally described as a prototypical non-inflammatory arthropathy but today it is generally accepted that it is an inflammatory disease [8]. Recent studies have provided a much clearer understanding of the role of inflammation in OA, suggesting that inflammation contributes to the symptoms and the progression of OA [9,10]. The most common clinical symptoms are joint pain related to use, pain on movement with a restricted range, cracking of joints (crepitus) and short-lasting inactivity stiffness of joints [9,11]. It has been shown that the inflammatory changes in OA synovium usually take place in the synovial lining with an increased number of inflammatory cells (macrophages) [12,13]. The advanced stages of the disease are visible on plain radiographs, as indicated by narrowing of the joint space (due to cartilage loss), development of osteophytes, and sometimes changes in the subchondral bone [14]. Moreover, a number of ongoing

studies have reported the observation of symptoms responsible for the progression of the risk of this disease by arthroscopy, magnetic resonance imaging (MRI), ultrasound (US) and optical coherence tomography (OCT) [15]. Fig. 1 shows a MRI image of a patient in advanced stages of osteoarthritis, and a detailed schematic on the major inflammatory mediators involving in this disease.

In this review, we systematically summarize the role of major inflammatory mediators in the pathophysiology of OA by focusing mainly on pro-inflammatory cytokines (i.e. IL-1 $\alpha$ , interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-15, IL-17, tumor necrosis factor-alpha (TNF- $\alpha$ )), nitric oxide (NO) and matrix metalloproteinases (MMPs), due to their involvement in this disease. We also discuss the contribution of joint cells, particularly chondrocytes, synoviocytes and inflammatory macrophages to the pathogenesis of OA. The overarching aim of the review is to emphasize the importance of developing new and sensitive methods and diagnostic instruments for the early detection of inflammation in OA by modern imaging techniques. Finally, we put forward a strong argument for developing treatments for decreasing the major symptoms such as inflammation and pain in OA patients.

**2. Synovitis in OA**

During the last few years, the association between OA progression, symptoms of inflammation, and disease activity has been the subject of a large number of basic and clinical studies [14,16]. A variety of studies have recently demonstrated an important link between OA inflammation and progression of structural changes [9]. In his groundbreaking contribution to OA-related public health George Ehrlich emphasized the importance of inflammation as a component of OA [17]. In a paper published in 1975, Ehrlich described a cohort of predominantly menopausal females who presented with a deforming and

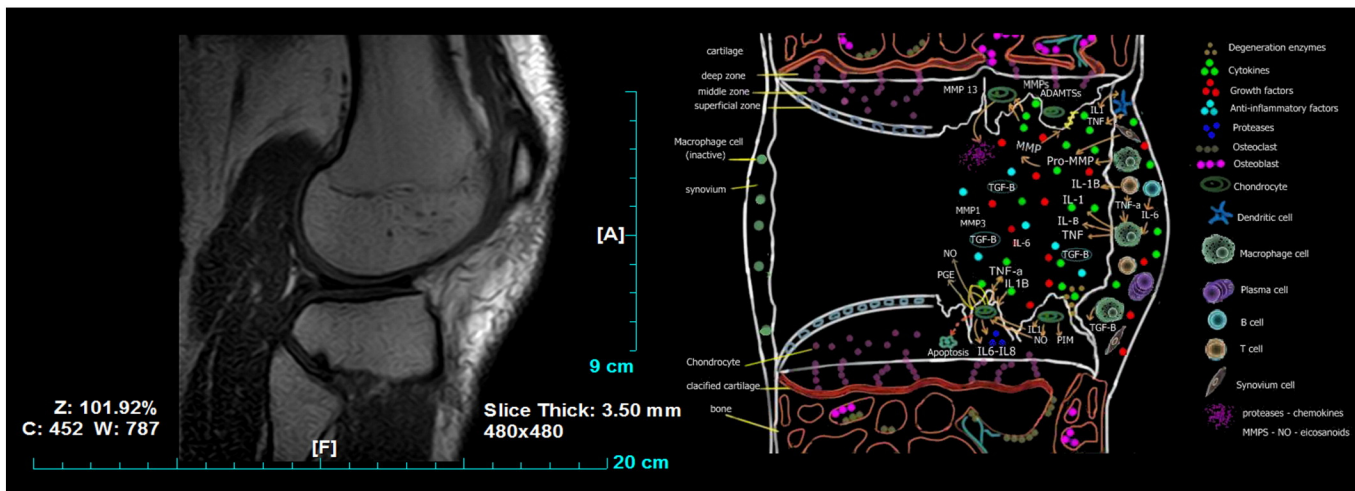


Fig. 1. A MRI image of a patient in advanced stages of osteoarthritis, and a detailed schematic on the major inflammatory mediators involving in this disease.

inflammatory osteoarthritis, some of whom went on to develop changes characteristic of rheumatoid arthritis (RA). Although his original observations were published more than forty years ago, the importance of Ehrlich's findings was not fully appreciated until very recently. The major players in the field of OA research have realized the importance of this and proposed a connection between synovitis and the continuation of structural changes in OA. Although OA is not considered an inflammatory disease in the conventional sense, at the present time this commonly held view is being challenged by clinical studies [18]. In clinical analysis, synovial inflammation obviously features strongly as one of several signs and symptoms of OA. Joint swelling, effusion and pain are typical clinical symptoms of inflammation in OA [9,19]. Investigation of synovial tissues from OA patients obviously demonstrates evidence of inflammation, in spite of the fact that this is not as chronic as the inflammatory arthropathies such as RA [20]. Synovial fluid (SF) in OA inflamed joint features inflammatory cells. Immunohistochemical studies have established that synovial tissue in this disease is characterized by mononuclear cell (MNC) infiltration, production of pro-inflammatory cytokines and other molecular mediators of joint degeneration [21]. Even though the levels of pro-inflammatory cytokines in OA synovitis are lower than in RA, the OA synovitis is itself cytokine-driven, especially involving tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin (IL)-1 [22]. In OA pathogenesis particularly in synovial inflammation, activated chondrocytes and synovial fibroblasts (TNF- $\alpha$  and IL-1) have been identified as vital players [23]. These cytokines stimulate their own production and persuade synovial cells and chondrocytes to produce IL-6, IL-8, leukocyte inhibitory factor (LIF), proteases and prostaglandins [24]. The production of matrix degrading enzymes such as matrix metalloproteinase (MMP)-1 and MMP-3 is also detectable in synovitis [25]. High sensitivity C-reactive protein (hsCRP) is another biomarker that reflects systemic synovitis [26]. In contrast the expression, concentration and viscosity of lubricants such as lubricin and hyaluronic acid (HA) are rather reduced in this disease [27]. Table 1 summarizes the most important studies in which the role of inflammatory factors in OA has been analyzed and discussed.

### 2.1. The role of pro-inflammatory cytokines and other inflammatory mediators in OA

It is believed that the overproduction of cytokines and growth factors by the inflamed synovium and activated chondrocytes is major mediator in the pathophysiology of OA [28]. Many *in vitro* and *in vivo* investigations signify that synoviocytes and chondrocytes can induce the generation of a number of cytokines and chemokines that may also be observed in OA synovial fluid [10]. The major (pro- and anti-inflammatory) cytokines and antagonists that have been considered to play a critical role in the progression of this disease consist of IL-1 $\alpha$ , IL-1 $\beta$ , IL-4, IL-6, IL-8, IL-10, IL-11, IL-13, IL-15, IL-17, leukocyte inhibitory factor (LIF), TNF- $\alpha$  as well as IL-1 receptor antagonist (IL-1Ra) [24,29]. It has frequently been reported that the quantities of inflammatory cytokines such as IL-1, IL-6, IL-15 and TNF $\alpha$  are elevated in the serum of patients with OA [30,31]. In fact, at the time of OA initiation, cytokines and growth factors are thought to be generated by the synovial membrane and subsequently dispersed by synovial fluid into the cartilage. These cytokines stimulate their own expression and activate chondrocytes, inducing them to synthesize MMPs, proteases, chemokines, nitric oxide (NO), and eicosanoids such as prostaglandins and leukotrienes, all of which lead to increased cartilage degradation [32,33]. Furthermore, IL-1 stimulates the expression of MMPs and other catabolic genes, and its interactions with them can damage key extracellular matrix (ECM) macromolecules, creating neo-epitopes from degraded matrix proteins in OA cartilage [32]. Cytokines and other inflammatory mediators that are created by synovium and chondrocytes are detectable in the synovial fluids of OA patients [29]. An increase in the anti-inflammatory

cytokines such as IL-4, IL-10 and IL-13 has been observed in the synovial fluid of OA cases [22]. Through a series of complex mechanisms, these cytokines utilize their anti-inflammatory properties following a reduction in the production of IL-1 $\beta$ , TNF $\alpha$ , MMPs and other inflammatory mediators [22]. Changes in TGF- $\beta$  expression and activity are believed to be a factor in the pathogenesis of OA. It raises the activity of anti-catabolic factors such as ADAMTS-4, tissue inhibitor of matrix metalloproteinases (TIMPs), PAI-1 and other proteins. On the contrary, decreases in the cellular response to pro-inflammatory cytokines consist of MMP-1, MMP-13, IL-1 $\beta$  and TNF- $\alpha$  in chondrocytes [22,34]. Clinical and laboratory investigations have shown that calcium crystals could also act as an important class of mediators in OA joints. Calcium-containing crystals increase inflammation through their interaction with some components of the innate immune system [35]. Excessive levels of calcium pyrophosphate dihydrate (CPPD) crystal formation in OA synovium are also related with the progression of OA [35]. C-reactive protein (CRP) is a biomarker that increases in the acute phase of inflammation. A small increase in the quantity of CRP is potentially of predictive clinical value in the rapid diagnosis of disease progression in early knee OA [11].

### 2.2. Chondrocytes as a source of MMPs and inflammatory mediator production

The properties of chondrocytes in the catabolic state are associated with the production of inflammatory mediators by factors in synovial fluid [36]. Chondrocytes generate a number of cytokines and other inflammatory mediators. They produce IL-1 $\beta$ , TNF $\alpha$ , NO, prostaglandins, IL-6, and IL-8 [9]. In addition, IL-1 $\beta$  and TNF- $\alpha$  can stimulate chondrocytes and synovial cells to synthesize IL-8, IL-6, NO, prostaglandin E2 (PGE-2), MMPs, proteases and other inflammatory mediators [9]. These cytokines can elevate the production of PGE-2 by stimulating the gene expression and enzymatic activities of cyclooxygenase-2 (COX-2), soluble phospholipase A2 (sPLA2) and microsomal PGE synthase-1 (mPGES-1) [9,37]. Moreover, chondrocytes control the synthesis of NO with the assistance of inducible isoform nitric oxide synthase (iNOS or NOS2) [38]. In OA, chondrocytes the generation of matrix-degrading enzymes is increased [39]. Metalloproteinases and proteinases play a key role in the progression of OA [9]. Cartilage mainly carries two types of metalloproteinases; ADAMTSs and MMPs. These enzymes play crucial roles in the degradation of cartilage ECM [40]. A number of MMPs have negative impacts on the ECM, and they can serve as significant cofactors in inflammatory reactions in OA [41]. Two principal types of matrix-degrading enzymes are collagenases and aggrecanase (ADAMTS). Collagenase (particularly MMP-1, MMP-3, MMP-8 and MMP-13 by splitting type II collagen and proteoglycans), and aggrecanase groups can potentially invigorate the degeneration of cartilage matrix [9,42]. Among these, MMP-13 has been frequently reported to be the most important player in OA cartilage [9].

### 2.3. Macrophage function in OA synovium

A number of the latest studies have strongly suggested that activated synovial macrophages can have a significant impact on the development of OA [43]. Histologic studies have shown that these cells demonstrate an "activated" constitution via promoting the creation of both pro-inflammatory cytokines and growth factors [24]. In a mouse model of OA, it has been demonstrated that the existence of macrophages in the synovial fluid is important in the production of degenerative mediators in the fluid [44]. Although the amount of macrophages in OA is much lower than in RA, synovial biopsy samples from patients with OA have also exhibited the presence of MNC infiltrates including T cells and macrophages in the synovial membrane of more than fifty percent of patients with OA [45,46]. The production of TNF- $\alpha$  and IL-1 $\beta$  by macrophages greatly depends on the activity of nuclear factor  $\kappa$ B (NF- $\kappa$ B) in synovium RA. However, it has lower influence on the

**Table 1**  
Summary of the key studies in which the role of inflammatory factors in OA has been analyzed and discussed.

Biomarkers	Joint	Tissue	Condition of cases	Number of cases	Controls	Assay	Major outcomes	Ref
C-Col10, C2M, and hsCRP	Knee	Serum	Moderate/severe OA	271	NR	ELISA	An increase in the level of ColX. An increase in the level of hsCRP. No correlation between C2M and hsCRP.	[58]
sTNFR1, and sTNFR2	Knee	Plasma and SF	Primary OA	27	19	ELISA	An increase in the level of sTNFR1. A decrease in the level of sTNFR2. Negative correlation between SF sTNFR1 and sTNFR2 with pain and physical function.	[59]
hsCRP, CRPM, C1M, C2M, and C3M.	Knee	Serum	Symptomatic knee OA and 60 of them underwent total knee replacement (TKR).	342	NR	ELISA	An increase in the level of hsCRP. An increase in the level of CRPM. The levels of MMP-degraded collagens differed between the subgroups.	[60]
BDNF	Knee	Plasma and SF	Primary OA	27	19	ELISA	An increase in the level of BDNF in plasma. A decrease in the level of BDNF in SF. Positive correlation between the level of BDNF and pain in the acute stage of joint inflammatory process.	[61]
COMP, PGE2, and CTX-I and II	TMJ	SF	TMJs OA	30	10	ELISA	No significant differences in the level of CTX-I, CTX-II, COMP, and PGE2. A decrease in the level of CTX-I. An increase in the level of CTX-II.	[62]
CTGF, IL-1 $\beta$ , MMP, and NF-B	Knee	FLSs, and SF	Knee OA	Human knee OA	NR	qPCR, ELISA, and Western blotting	An increase in the level of IL-6, IL-8, CCL2, CCL20, MMP-1, MMP-3, phosphorylated (ERK1/2) and by activation of CTGF and IL-1 $\beta$ .	[63]
Adipokines	Knee	Serum, and SF	Severe knee OA	172	132	ELISA	An increase in the level of adipokines. Positive correlation between adiponectin and leptin with female gender and BMI. No correlation between the level of adipokines and cartilage damage. Positive correlation between the level of adipokines and SF inflammation.	[64]
hsCRP, IL-6, PTX-3, anti-CCP, and anti-MCV.	Hand	Serum	Erosive and non-erosive HOA	99	50	ELISA	No significant differences in the level of hsCRP, IL-6 and PTX3. Positive correlation between the levels of CRP with BMI. No correlation between inflammation markers and disease duration and radiological scores.	[65]
Adipokines	Knee	IFP	Primary and severe knee OA	29 severe and 5 primary OA	34	Lowry	An increase in the level of adipokines. An increase in the level of PPAR- $\gamma$ 1, PPAR- $\gamma$ 2, DGAT2, CD36, and THRSF.	[66]
COMP	Knee	Serum	Primary OA	88	NR	ELISA	A significant increase in the level of COMP.	[67]
NK cells, Chemoattractant, cytokine protein, and RNA	–	Synovial and interfacial	18 patients with OA who were undergoing primary TJR and 22 patients with OA who were undergoing revision TJR.	40	NR	Luminex, qPCR, immunofluorescence and flow cytometry.	An expression the level of CXCR3, CCR5, L-selectin, $\alpha$ 4 integrins, and cutaneous lymphocyte antigen by NK cells.	[68]
CPPD, NO, MMP-13 and PGE2	Knee	SF, and cartilage	Samples were obtained from patients undergoing TKR operations for OA.	5	NR	ELISA, and WST-1	An increase in the level of NO, MMP-13 and PGE2 by m-CPPD and t-CPPD.	[69]
Angiogenic factors, NF-B, TNF $\alpha$ , IL1 $\beta$ , (COX)-1, and COX-2	Knee	FLSs, and SF	Primary and late knee OA	Primary: 10 Late: 15	NR	ELISA	An increase in the levels of CD4+ and CD68+, NF-B1, RelA, COX-2, TNF $\alpha$ and IL1 $\beta$ . No correlation between production of PGE2 and OA.	[21]

Table 1 (continued)

Biomarkers	Joint	Tissue	Condition of cases	Number of cases	Controls	Assay	Major outcomes	Ref
PGE2	Knee	SF	SF after transection of the CCL in dogs	19	NR	Procedure – Force plate measurements, subjective clinical analysis of pain or lameness	An increase in the levels of PGE2. Positive correlation between the levels of PGE2 with the amount of pain or lameness in dogs.	[70]
hsCRP	Knee and hip	Serum	Patients with advanced hip and knee OA	770 patients with hip or knee joint replacement	NR	WOMAC	An increase in the level of CRP. Positive correlation between the levels of CRP with WOMAC (pain).	[71]
MMP-1	Knee	Cartilage	Human patients with knee OA who were undergoing TJR	10	NR	DNase I footprint, EMSA, and CAT	An increase in the level of SAF-1. An increase in the level of MMP-1 promoter.	[72]
IL-17, IL-1 $\beta$ and TNF- $\alpha$	Knee and hip	SFs and chondrocytes	Patients with OA undergoing knee or, in some cases, hip replacement surgery	12 patients (11 knee and one hip) and cartilage samples from 19 patients (16 knee and 3 hip).	NR	ELISA	An increase in the level of IL-17 could contribute to increase in the level of IL-1 $\beta$ and TNF- $\alpha$ .	[73]
MPO, HOCl, and Cl <sub>2</sub>	Knee	SF	Primary and severe OA	In 33 SF samples from 30 patients	4	ELISA	An increase in the level of MPO, HOCl and Cl <sub>2</sub> in patients with early OA. A decrease in the level of MPO, HOCl and Cl <sub>2</sub> in control and advanced OA groups.	[74]
hsCRP, IL-1 $\beta$ , and IL-6	Knee and hip	Plasma, and SF	Patients undergoing total hip or knee arthroplasty or arthroscopy	54	16	ELISA	Positive correlation between the inflammatory infiltrates with progression of OA. An increase in the levels of hsCRP. Positive correlations between hsCRP levels with SF IL-6, degree of synovial inflammatory infiltration and BMI.	[27]
hsCRP	Knee	Serum	318 African-American and 707 Caucasian who are enrollees in a study of musculoskeletal conditions at the mid-life.	1025	NR	Ultrasensitive rate immunonephelometry methodology	An increase in the levels of hsCRP.	[75]
ECs and macrophages	Knee	SF	Undergoing TKR for OA	62	31	Immunofluorescence	Positive correlation between osteochondral vascular density with cartilage severity and clinical disease activity scores. NO correlation between osteochondral vascular density with synovitis. An increase in the levels of ECs, inflammation and macrophage. An increase in the Synovial angiogenesis.	[76]
IL-15	Knee	Serum	Primary knee OA	206	106	ELISA	An increase in the levels of Serum IL-15.	[31]

production of TNF- $\alpha$  and IL-1 $\beta$  in synovium OA [24]. These studies have shown that OA synovial macrophages can perform a major role in the activation of TNF- $\alpha$  and IL-1 $\beta$  in synovial cells.

#### 2.4. Nitric oxide as a major catabolic factor

NO is a predominant mediator in progression of OA by chondrocyte apoptosis [47]. It has been also shown that OA cartilage can increase the level of NO [48]. Farrell et al. [49] have reported a high degree of NO in the serum of arthritis patients in contrast to normal cases. An increase in the level of NO, produced by iNOS, is an important catabolic element in the activation of TNF $\alpha$  and IL-1 $\beta$  [50]. Immunohistochemical analyses have confirmed the existence of iNOS in OA synoviocytes and chondrocytes [51]. NO also induces the production of cartilage matrix macromolecules (such as aggrecans), increases MMP activity and reduces the synthesis of IL-1Ra by chondrocytes [51,52]. In fact, the elevated production of NO is strongly linked with cartilage chondrocyte apoptosis in OA patients. However, the exact mechanism in which

how NO induces apoptosis in OA chondrocytes has not been elucidated yet [53].

### 3. The role of innate immunity system activation in osteoarthritis

In recent years, some studies have shown that the innate immune system has not only a potential role in the host defense against microbial invasion but also in various forms of tissue injuries such as OA [35]. This host-defense system is initiated by contact with pathogen-associated molecular patterns (PAMPs) that are molecules associated with groups of pathogens, and unsafe signals released by damaged or dying cells or tissues [54]. Toll-like receptors (TLRs), type I transmembrane glycoproteins, which are members of the largest pattern recognition receptors (PRRs), are the central part of this system. It has been observed that by activation of TLRs, the amount of transcription factors with NF- $\kappa$ B and in turn, the pro-inflammatory mediators has increased [54]. It has been suggested that cartilage matrix degradation products may cause the response of innate immune system through TLRs. In

addition, nucleotide-binding, leucine-rich repeat containing proteins [nucleotide oligomerization domain (NOD)-like receptors (NLRs)] that activate inflammatory mediators especially IL-1 $\beta$  through their proteins, are another significant receptors in innate immunity [40]. Some studies have demonstrated that TLRs could activate MMPs such as MMP1 and MMP13, which increase the inflammatory response by producing cartilage matrix fragments that bind to TLRs [55]. The complement system is another important constituent of this system, which includes more than 30 plasma and membrane-bound proteins. The complement system can be activated through the turnover of C<sub>3</sub> and the formation of membrane attack complex (MAC, C<sub>5</sub>b-9) [56]. Wang et al. [57] have investigated the role of the complement components in the pathogenesis of OA. The results exhibited that complement proteins such as C<sub>3</sub>a and C<sub>5</sub>b-9 were overexpressed in the SF from early stage OA patients, compared with SF from healthy donors; suggesting that complement activation occurs early in the joint during OA development [57].

#### 4. Biomechanical stress and the progression of inflammation in OA

A variety of mechanical risk factors including overweight/obesity, muscle weakness, joint instability and overload can potentially contribute to the progression of OA [77]. Studies have emphasized that chronic mechanical stresses cause the destruction of chondrocytes and collagen network by inducing chondrocytes to deliver degenerative enzymes [78]. Via mechanoreceptors (ion channels, integrins) and cartilage-degenerating proteinases, these stresses are capable of being translated into intracellular signals in joint cells. When these signals reach a certain threshold, they can raise the levels of MMP-3, MMP-13, PGE-2 and other inflammatory mediators [79,80]. By regulating or increasing the production of inflammatory cytokines chondrocytes can respond to mechanical risk factors. They may respond to these forces by stimulating the expression or activities of cytokines even in the presence of low level inflammation [81]. Furthermore, it is believed that subchondral bone is a source of inflammatory mediators and the source of OA pain and further degeneration of articular cartilage [82].

The mechanical stress applied to the load-bearing joints (e.g., spine, hip, knee and ankle) is an important factor in OA [83]. Epidemiological studies have indicated that, among the most common mechanical forces, obesity is the most harmful risk factor linked to the development of knee OA [84]. Unfortunately, while obesity has a key role in the management of OA, the prevalence of obesity is growing fast throughout the world [85]. In the gait sequence, a force of 3 to 6 times higher than the body weight usually applies across the knee and hip joints [86]. These stresses are elevated in the time of high-impact activities (the relation of obesity with hip OA is weaker than its relation with knee OA). As a consequence, when an obese person walks, any excess weight can contribute additional mechanical stress across the load-bearing joints [86]. In addition, some studies suggest that obesity is a key player in chronic inflammation of cartilage through the administration of these forces on the joints and also the reduction of joint space [87]. In an important commentary published by Francis Berenbaum's group ten years ago it was suggested that elevated levels of systemic metabolic factors, especially adipokines produced by white adipose tissue are responsible for the high prevalence of OA among obese people [88]. In fact, producing these pro-inflammatory cytokines is thoroughly connected with obesity and is a key player in cartilage and bone homeostasis, which associate obesity and adiposity with inflammation and OA [89]. Additionally overweighting alters gait, posture and physical activity levels [90]. Some recent studies clearly showed that overweight can elevate the synthesis process of mechanoreceptors (stretch-activated channels,  $\alpha$ -5B1 integrin, CD44 on chondrocytes), and as a result, increase the levels of cytokines, growth factors, MMPs, and other mediators in the cartilage [91,92].

#### 5. Imaging techniques for diagnosis of inflammation in OA

There are a variety of imaging techniques (such as X-ray radiography, MRI, US, OCT and arthroscopy) available for monitoring the progression of cartilage loss, synovial inflammation and subchondral bone changes in OA [12]. Radiographic techniques can confirm the diagnosis of OA, but are generally poor for monitoring the progression of OA. However, X-ray radiography does not allow us to see evidence of inflammation and more powerful imaging techniques are needed. Currently MRI is one of the most powerful imaging techniques although it is not recommended by the regulatory authorities for use in clinical trials — only X-ray radiography is accepted by regulatory authorities, despite its poor ability for monitoring responses to pharmacological and non-pharmacological treatments. Since finding early changes in arthritis by conventional X-ray methods are usually difficult [15], modern imaging methods such as MRI and US are being increasingly used for investigating early signs of arthritis. These modern imaging techniques are highly sensitive for the detection of joint damage and the signs of inflammation when conventional X-ray radiographs cannot give satisfactory information [15,93]. These evolving techniques play a key role in the observation of small effusions, discovering specific areas of cartilage damage and distinguishing inflammatory from non-inflammatory joint diseases [93,94]. Joint space narrowing (JSW), cyst and osteophyte formation, and subchondral bone sclerosis, are classic radiographic findings in OA joints [95]. It has been accepted that Doppler techniques such as US are among the best choices for an indirect evaluation of progression of synovitis with the assessment of vascularity. This can be potentially used for detecting synovial inflammation and progression of OA [96]. In the knee, the suprapatellar pouch and the medial and lateral recesses are two common imaged sites for detecting synovial hypertrophy by US [94]. Keen et al. have reported that modern US technologies can provide images with extensive fields of view by means of high resolution at frequencies of up to 20 MHz [96], which is useful in the detection of synovial pathologies (including hypertrophy, vascularity, and synovitis) [96]. As a novel technique, Song et al. have offered contrast-enhanced ultrasound (CE-US) for measuring synovial vascularization in OA knee [94]. They reported that CE-US could exhibit greater sensitivity (95%) in detecting synovitis than CE-MRI (82%), power Doppler US (64%) or grayscale US (58%) [94]. MRI is another modern technique for detecting synovitis and progression of OA. It has been shown that MRI can provide excellent images of synovium deep within joints (like hip and shoulder) without being covered by bone structures. Non-CE-MRI and gadolinium (Gd)-based CE-MRI are two common techniques for detecting inflammation in OA. Fernandez-Madrid et al. have shown that synovitis was first associated with hypointense signal variations in Hoffa's fat pad on sagittal, non-CE T1-weighted spin-echo images [97]. Moreover, it has been reported that hyperintense signal alterations in Hoffa's fat pad on fat-suppressed PD or T2-weighted spin-echo sequences are useful signs for synovitis in OA [98]. Non-CE MRI is a more common technique for imaging of synovitis, while CE-MRI can clearly distinguish inflamed synovium from joint effusion. In fact, in non-CE MRI, both synovium and effusion are frequently shown as signal hyperintensity, while in CE-MRI, effusion remains hypointense but synovium with inflammatory activity is usually elevated. In addition, Loeuille et al. have demonstrated that since CE-MRI detected inflammation of synovium is associated with histology, it is more sensitive and more accurate compared with non-CE MRI [14,99]. In OA patients, when the clinical signs of pain and inflammation increase, the association of these with radiographic observations is crucial. Among these criteria, the measurement of JSW has the most significant role for the detection of changes over time, detectable by using a number of different techniques [100]. Many studies have reported a weak correlation between pain and radiographic findings, in which some patients with advanced radiographic findings have shown no pain symptom and some patients with pain demonstrated no radiographic signs of OA [101].

## 6. Therapeutic implications for inflammation in OA

OA is an important cause of chronic disability for the elderly and retired population and also for middle-aged people of working age [3]. Unfortunately, as of now no pharmaceutical or drug has been developed with the capacity for structure modification. Therefore, the challenge of developing and commercializing useful and efficacious drugs for this public-health problem remains [102]. As a result, there have been several attempts to come up with preventive approaches to address this problem. Prevention of general disability and halting the progression of the structural changes are important for the management of OA. In OA, increased joint inflammation is associated with articular cartilage damage. Furthermore, the main treatments developed to date are focused on the reduction of inflammation and pain in these patients [103,104]. The treatment of OA can be divided into three interventions namely nonpharmacological, pharmacological, and finally surgical options [90].

### 6.1. Nonpharmacological interventions

Improvement in self-management and lifestyle changes are capable of reducing pain in OA patients. This is particularly important for obese patients and those with sarcopenia (muscle loss) and muscle weakness [105]. Moderate exercise and regular physical activity can significantly reduce the general signs of OA in these patients [106]. This improvement can be achieved with weight loss, enhancement of muscle strength to improve joint function and reduce inflammation [107]. Aerobic exercise can effectively enhance the function of joints, reduce pain and also improve joint function in patients with the risk of OA [108]. Dias et al. [109] have reported that dynamic and moderate exercise can positively influence physical activity. Orthotics (ranging from insoles to braces) are other treatment ways that significantly decrease the pain. A number of studies have emphasized that these interventions are able to improve the physical activity and reduce the pain in these cases [110]. Additionally, Jones et al. [111] have highlighted the benefits of using a cane for knee OA. On the other hand, superficial joint application of heat or cold may be an effective method to reduce these symptoms [105]. If these approaches fail, drugs and surgical intervention are currently the only remaining options [110].

### 6.2. Pharmacological interventions

Although the American College of Rheumatology (ACR) recommended acetaminophen as the first step for therapy of OA, several studies have proposed that nonsteroidal anti-inflammatory drugs (NSAIDs) have more efficacy for pain relief in this disease [112,113]. It is known that acetaminophen has no anti-inflammatory properties at the prescribed doses [114]. In general NSAIDs are the most commonly recommended medications for the reduction of pain caused by inflammation [113]. Some clinical studies have reported that NSAIDs and paracetamol can reduce pain complaints in the patients suffering OA [110]. In addition, some studies have reported significant reduction of inflammation in OA after application of gels or transdermal patches containing lidocaine [115]. However, gastrointestinal, renal, and cardiovascular side-effects are the most challenging disadvantages of NSAIDs, and for this reason, alternative treatments are receiving more attention [114]. Local intra-articular injections of corticosteroids and HA are also useful for the management of OA [114,116]. Corticosteroids can slow down the synthesis of inflammatory mediators such as IL-1 $\beta$ , TNF $\alpha$  and COX-2 in the synovial fluid [11]. An enhancement in the level of glucosamine and chondroitin sulfate through dietary consumption of supplements is the other method to decrease symptoms of OA but the clinical evidence for their efficacy is generally weak [16,117]. Recent studies have demonstrated that glucosamine is only as efficacious as placebo [118].

Experimental studies have shown that some anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 are elevated in the serum of OA patients. These cytokines can have anti-inflammatory properties against TNF- $\alpha$ , IL-1 $\beta$ , and MMPs. These studies have reported that using anti-IL-1 $\beta$  antibody, anti-TNF- $\alpha$  antibody and a selective iNOS inhibitor is associated with the reduction of inflammatory mediators in OA cartilage [119,120]. Antibiotics particularly tetracycline, are other important therapeutic indications having inhibitory effects on MMP activity and NO production [121,122]. Some studies have explored the effect of diclofenac on the development of cartilage damage, and exhibited that diclofenac could reduce pain in OA cases. In addition, this has fewer reactions compared with naproxen, ibuprofen and piroxicam [123]. Moreover, because ADAMTS-5 has been considered the main protease responsible for aggrecan degradation in OA, these days extensive work has been put on the improvement of aggrecanase inhibitors such as arylsulfonamido-based hydroxamates that are able to decelerate or stop the progression of OA [124]. Finally, recent studies have reported on the vast advantages of anti-inflammatory compounds of natural origin due to the lack of gastrointestinal side effects (such as saponins, bromelain, flavonoids, curcumin and parthenolide) [125,126].

### 6.3. Surgical interventions

Surgical intervention is widely considered to be the last treatment option for OA. Joint replacement is considered as major surgery, so it is normally only recommended if other treatments, such as non-steroidal anti-inflammatory drugs, opioids, physiotherapy or steroid injections, have not reduced pain or improved mobility. It is only recommended for patients who have not responded to any other form of treatment and in cases where joint destruction has been extensive and previously mentioned treatments have not been successful [110]. Osteotomy and joint replacement surgery are two interventions that surgeons use for the treatment of severe OA [127]. Osteotomy patients usually also require joint arthroplasty subsequently. These two surgical methods can reduce pain and also increase the ability of patients to have a better daily physical activity [110]. Due to the enormous economic and personal burden of OA, we need new combinations of surgical, pharmacological and biological therapies to treat this disease.

## 7. Conclusions and future perspectives

This review is a rich source of information supporting the notion that inflammatory mediators play a key role in the initiation and propagation of pathogenic OA processes. It has been shown that most important inflammatory mediators produced by chondrocytes and synoviocytes are cytokines, NO, ROS and matrix degrading enzymes. In addition, it has been demonstrated that biomechanical stress and joint over-load can significantly increase the synthesis of inflammatory mediators. Overexpression of these mediators causes the gradual deterioration of cartilage tissue, synovial membrane and subchondral bone. More mechanistic research needs to be conducted to fully understand the processes involved in the initiation and propagation of pathogenic OA processes. Therefore, future clinical studies should systematically assess the effects of several inflammatory and anti-inflammatory mediators in OA. For instance, there is a critical need for further investigations on the relative impact of each cytokine produced by inflamed synovial tissue, in order to determine the exact role of the various cell types in this tissue. For the future, understanding of the communication pathways between cartilage, subchondral bone and synovial tissue would greatly help to overcome the difficulties in the treatment of this disease. In addition, interdisciplinary research and effective collaboration between the public and private sectors can potentially overcome the major issues related to OA. Finally, basic scientists, clinicians and radiologists need to acknowledge the shortcomings of existing imaging modalities. We need new, sensitive and non-invasive methods and novel diagnostic instrumentation for imaging synovitis in real-time and with these innovations

in hand we should be in a much stronger position to formulate new clinical trials and preventive strategies for OA.

### Conflict of interest statement

The authors wrote this paper within the scope of their academic and affiliated research positions. The authors declare no conflict of interests. The authors do not have any commercial relationships that could be construed as biased or inappropriate.

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