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# Synovial inflammation in osteoarthritis progression

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**Abstract** | Osteoarthritis (OA) is a progressive degenerative disease resulting in joint deterioration. Synovial inflammation is present in the OA joint and has been associated with radiographic and pain progression. Several OA risk factors, including ageing, obesity, trauma and mechanical loading, play a role in OA pathogenesis, likely by modifying synovial biology. In addition, other factors, such as mitochondrial dysfunction, damage-associated molecular patterns, cytokines, metabolites and crystals in the synovium, activate synovial cells and mediate synovial inflammation. An understanding of the activated pathways that are involved in OA-related synovial inflammation could form the basis for the stratification of patients and the development of novel therapeutics. This Review focuses on the biology of the OA synovium, how the cells residing in or recruited to the synovium interact with each other, how they become activated, how they contribute to OA progression and their interplay with other joint structures.

Osteoarthritis (OA) is the most common form of arthritis, affecting more than 500 million people worldwide (~7% of the global population), with particularly high prevalence in those of advanced age (>65 years of age)<sup>1</sup>. Epidemiological studies report an increasing incidence of OA in individuals <65 years of age owing to rising obesity, an increasing number of post-traumatic OA (PTOA) cases and diagnosis at an earlier stage<sup>2</sup>. OA is a complex disease characterized by pathological changes across all the joint tissues, including cartilage, subchondral bone, ligaments, menisci, the joint capsule and the synovial membrane<sup>3</sup>. The widely accepted hypothesis of OA pathogenesis implicates an initial injury, frequently biomechanical, of any of these structures, which results in the release of mediators that lead to activation of different inflammatory pathways that damage cartilage. However, increasing evidence indicates that low-grade synovial inflammation (synovitis) contributes to radiographic and pain progression in OA.

Baseline synovitis detected by magnetic resonance imaging (MRI) or ultrasonography is associated with radiographic progression of OA, as defined by worsening of Kallgren and Lawrence (KL) grade or narrowing of joint space<sup>4–11</sup>. Synovitis progression is also associated with more cartilage damage<sup>12</sup>. Radiographic progression and development of erosions in hand OA<sup>13–16</sup> and accelerated knee osteoarthritis (AKOA; defined as a transition from no radiographic knee OA to advanced stage disease within 4 years)<sup>17,18</sup> are also associated with synovitis. More than 2 years before onset, patients with AKOA present with more pain, synovitis-effusion of

larger volumes and signal alterations in the infrapatellar fat pad (IFP) compared with patients who develop typical knee OA<sup>17,18</sup>. MRI and ultrasonography have also been used to evaluate associations between synovitis and pain<sup>5,19–27</sup>, finding that synovitis contributes to pain in OA. Of note, a study found that synovitis partially mediates the association between cartilage damage loss and worsening pain: each 0.1-mm loss of cartilage over 24 months translated to an increase in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale score of 0.32 (95% CI 0.21–0.44)<sup>28</sup>.

However, the results of preclinical studies in animal models and of clinical trials have been contradictory<sup>29</sup>. Although blocking pro-inflammatory mediators secreted by the synovium and cartilage (including IL-6 and IL-1RA) has an analgesic effect and decreases structural progression in several preclinical models of OA<sup>30–33</sup>, not all of these studies confirmed a protective role of cytokine blocking in animal models<sup>34</sup>. In addition, in randomized controlled clinical trials in patients with painful erosive hand OA, whose erosive phenotype was associated with the presence of synovitis<sup>13–16</sup>, inhibition of the inflammatory mediators IL-1 $\beta$ , IL-6 and tumour necrosis factor (TNF) did not improve pain, synovitis or OA progression, as assessed by MRI or ultrasonography<sup>35–39</sup>. Finally, individuals ( $n = 18$ ) with knee OA without inflammation (by ultrasonography) experienced a more prolonged benefit from intra-articular corticosteroid treatment than individuals with ultrasonography-identified inflammation ( $n = 16$ )<sup>40</sup>.

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### Key points

- Imaging studies suggest that synovial inflammation may be present in both early osteoarthritis (OA) and advanced-stage OA and is involved in the development and progression of OA.
- Synovial cells coordinate the production of molecules that initiate and maintain synovial inflammation and contribute to cartilage damage during OA progression.
- Diverse stimuli, including bioactive lipids, prostaglandins, tricarboxylic acid cycle intermediates, cytokines and damage-associated molecular patterns, as well as clinical factors such as obesity, ageing, trauma and excessive mechanical loading, regulate the production of pro-inflammatory and anti-inflammatory mediators by synovial cells.
- There is a need for functional imaging and cellular and molecular studies, together with a more robust histological interpretation at different stages of OA, to better stratify patients with OA and understand the role of synovitis in OA onset and progression.

Taken together, these data raise the question as to whether synovial inflammation is involved in OA pathogenesis, progression or associated joint pain.

In this Review, we describe the current knowledge of synovitis in OA joints, and discuss the pathology (FIG. 1), risk factors (FIG. 2) and cell types associated with synovial inflammation in OA. We focus on the mediators of synovial inflammation (FIG. 3), the crosstalk between synovial cells (FIG. 4) and their clinical relevance (TABLE 1, TABLE 2).

### Synovitis in OA

Synovitis scores based on macroscopic features in the OA joint typically assess the presence and abundance of vascularity, villi, fibrin deposits and hyperplasia assessed by visualization of the synovium during arthroscopy<sup>41,42</sup>, although other scores use features such as hypertrophy, vascularity and global synovitis<sup>43</sup>. Synovitis scores based on microscopic histological features have also been developed, such as the histological score developed by Krenn, which includes assessment of synovial hyperplasia, stromal cell activation and inflammatory infiltrate extent; this score was able to discriminate between degenerative and inflammatory diseases<sup>44–47</sup>. Other synovial OA scores are being developed that include characteristic features of the OA synovium and are based on the predominance of each feature; synovial changes in OA have thus been classified as hyperplastic (villous hyperplasia), fibrotic (capsular fibrosis), detritus-rich (fibrinous exudate and cartilage and bone debris) and inflammatory (diffuse inflammation and aggregates of lymphoplasmacellular infiltrates), despite all of these features usually coexisting<sup>48</sup>. If present, synovial inflammation (synovitis) is characterized by proliferation of fibroblast-like synoviocytes (FLS) and macrophage recruitment, resulting in hyperplasia of the synovial lining. The synovial sub-lining can also be enriched in macrophages, T cells and, to a lesser extent, mast cells, B cells, plasma cells and endothelial cells (as components of blood vessels)<sup>49</sup>.

The two main imaging techniques that are used for synovium assessment, MRI (including contrast-enhanced MRI (CE-MRI) and conventional MRI) and ultrasonography, show good correlation with macroscopic and microscopic histological features of inflammation (BOX 1). Imaging studies revealed that synovitis

in OA has a patchy distribution in different anatomical sites of the synovium, including in suprapatellar, infrapatellar, lateral and medial parapatellar and subpopliteal locations, as well as adjacent to posterior cruciate ligaments, and the extent of synovitis can also be different across these different locations<sup>50</sup>. This distribution may be clinically relevant, as different locations and scores of synovial inflammation determined by CE-MRI correlate differently with pain and radiographic OA severity<sup>51</sup>. Synovitis can be present at any disease stage<sup>49</sup>, and a study reports a correlation between the patterns of patient-reported knee pain and the location of synovitis; specifically, suprapatellar pain was highly associated with suprapatellar synovitis on MRI<sup>52</sup>. Joint effusions and synovitis may be detected by MRI in subjects with OA joint pain and normal or very minimal damage by joint radiography, indicating that synovitis is not restricted to late stages of disease<sup>11</sup>. A post-mortem study reported a prevalence of synovitis of 11% in patients with no OA history or pain, compared with a prevalence of 67% in synovium from end-stage OA joint replacement surgeries<sup>53</sup>. Interestingly, inflammatory infiltrates coexist with fibrotic changes and angiogenesis in OA, which can be more prevalent in the late stages than in the early stages of the disease<sup>48,54</sup> (FIG. 1).

### Cell types in the OA synovium

The inflammatory cell subsets that exist in synovial tissues have been identified by flow cytometry, single-cell transcriptomics and mass cytometry. Evaluation of the synovium from patients with OA undergoing knee replacement showed highly heterogeneous cell populations. Whereas all synovial fibroblasts expressed IL-6, a cytokine independently associated with OA pain and radiographic progression<sup>55,56</sup>, CD34<sup>+</sup>CD90<sup>+</sup> fibroblasts located in the synovial sub-lining express substantially more IL-6 than CD34<sup>−</sup>CD90<sup>−</sup> fibroblasts in the synovial lining<sup>57</sup>. In addition, study participants categorized into clusters based on a high mesenchymal cell content or IL-6 release in the synovial inflammatory response had a history of prior joint surgery<sup>57</sup>. Single-cell RNA sequencing (scRNA-seq) detected 12 different expression profiles in cells of the synovium, including (from most to least abundant) synovial sub-intimal fibroblasts, synovial intimal fibroblasts, *HLA-DRA*<sup>+</sup> cells (immune regulatory macrophages and inflammatory macrophages, dendritic cells, activated pro-inflammatory *HLA-DRA*<sup>+</sup> fibroblasts and B cell clusters), smooth muscle cells, endothelial cells, T cells, mast cells and proliferating immune cells<sup>58</sup>. In addition, OA synovial samples contain more NUPR1<sup>+</sup> monocytes than in leukocyte-rich rheumatoid arthritis (RA) synovial samples ( $P < 0.01$ ), which contain a greater abundance of IL-1 $\beta$ <sup>+</sup> ( $P < 0.001$ ) and IFN-activated monocytes ( $P < 0.01$ ) than OA synovium. NUPR1<sup>+</sup> monocytes express high levels of tissue remodelling factors, such as the receptor tyrosine kinase MERTK and the osteoclast progenitor markers osteostatin and cathepsin K<sup>59</sup>. Together, these studies indicate a considerable heterogeneity in cell subtypes and interaction networks in the OA synovium, which requires further characterization and understanding.

### Synovial macrophages in OA

Macrophages are the most abundant immune cells in the synovium, comprising 12–40% of synovial immune cells, depending on the surface markers employed<sup>58,60,61</sup>, and they orchestrate the inflammatory and resolution phases after tissue injury<sup>62</sup>. Macrophages are also the main leukocyte population in synovial fluid in human OA knee joints (median = 36.5% of leukocytes), followed by T cells (25%)<sup>63</sup>. In particular, the CD14<sup>+</sup>CD16<sup>+</sup> macrophage subset (35% of the total macrophage population in synovial fluid) expresses the mature macrophage marker 25F9 (17.3% of the CD14<sup>+</sup>CD16<sup>+</sup> macrophages), indicating activation<sup>63</sup>. Interestingly, linear modelling (adjusted for sex, BMI and age) showed that the ratio of CD14<sup>+</sup> macrophages to total macrophages is a predictor of Knee Injury and Osteoarthritis Outcome Score (KOOS) and WOMAC score, regardless of CD16 expression by this subset of macrophages<sup>63</sup> (TABLE 1). In OA synovia, scRNA-seq of 10,640 synovial cells from 3 patients revealed that ~12.8 % of these cells were HLA-DRA<sup>+</sup>, and this subset includes immunoregulatory and pro-inflammatory macrophages, dendritic cells, pro-inflammatory fibroblasts and B cells<sup>58</sup>. The quantity of activated macrophages in OA knee joints detected by single-photon emission computed tomography (SPECT)–CT with the folate receptor-targeting imaging agent <sup>99m</sup>Tc-EC20 (etarfolatide) correlated with radiographic OA severity and symptoms, including pain and stiffness (self-reported on a scale from 0 to 3)<sup>64</sup>.

Consequently, disruption of pro-inflammatory macrophage infiltration into the synovium has been proposed as a potential therapeutic approach. In a mouse model of OA, inhibition of CC-chemokine receptor type 2 (CCR2; the receptor for the monocyte chemoattractant CCL2) impedes blood monocyte recruitment to injured joints and decreases synovitis and cartilage destruction<sup>65</sup>. In another study, depletion of synovial macrophages by

intra-articular injection of anti-CD14-conjugated magnetic beads or clodronate-loaded liposomes decreased production of IL-1 $\beta$ , TNF and matrix metalloproteinases (MMPs) by synovial fibroblasts and reduced cartilage damage and osteophyte formation<sup>66,67</sup>. By contrast, depletion of joint macrophages in *Csf1r*–GFP<sup>+</sup> macrophage FAS-induced apoptosis transgenic mice resulted in increased synovitis but did not inhibit the development of OA, owing partly to increased infiltration of neutrophils (>eightfold) and CD3<sup>+</sup> T cells (>fivefold) into the synovium of injured joints, which cause additional damage<sup>68</sup>. These results suggest that a better understanding of macrophage subsets and their role in both healthy and injured or inflamed joints is needed. Identification of pathological macrophage subsets might provide a good opportunity to curtail synovitis and tissue damage.

The pro-inflammatory or anti-inflammatory capacity of macrophages is defined based on their effector function, transcription and metabolic programme, and surface marker expression (reviewed elsewhere<sup>69,70</sup>). Considerable effort has been focused on advancing the identification of macrophage subsets in healthy and inflamed joints and understanding how these populations are associated with clinical outcome. In particular, a study in 184 patients with radiographic knee OA from two different cohorts found that the concentration of the macrophage markers CD14 and CD163 in synovial fluid and blood are associated with OA phenotypic outcomes<sup>71</sup>. Levels of both macrophage markers in the synovial fluid were significantly associated with activated macrophages in the joint detected by <sup>99m</sup>Tc-EC20 SPECT–CT, mainly in the capsule ( $P=0.002$  and  $P=0.005$ , respectively) and the synovium ( $P=0.0005$  and  $P=0.002$ , respectively), of patients with knee OA<sup>71</sup>. Interestingly, CD14 and CD163 levels in the synovial fluid were associated with osteophyte severity,

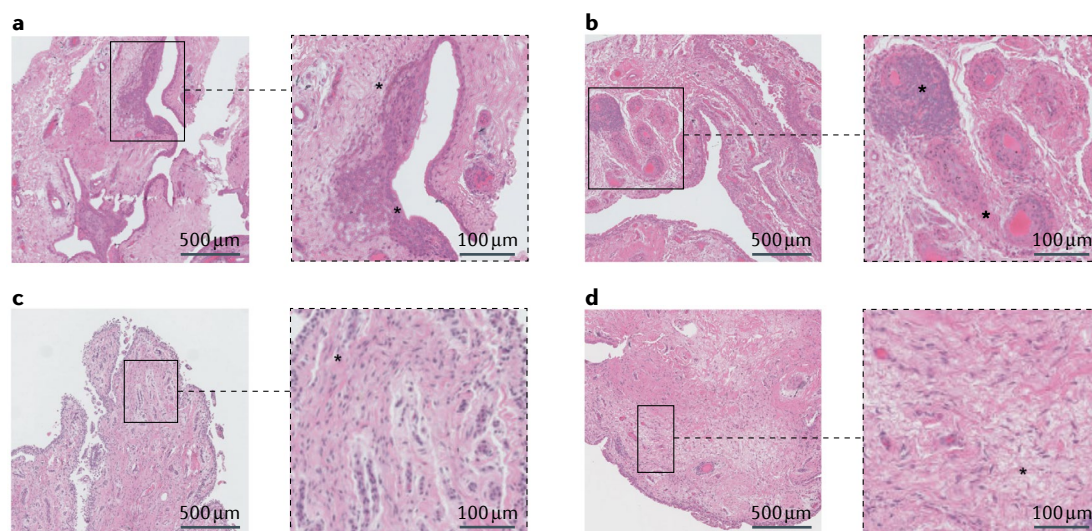


Fig. 1 | **Synovial inflammation and fibrosis in osteoarthritis.** Haematoxylin and eosin staining of synovial tissue from patients who underwent total knee replacement. **a, b** | Features of an inflammatory phenotype are highlighted in the magnified insets, including hyperplasia of the synovial lining (asterisk in part **a**), and cellular infiltrates and vascularization in the sublining layer (asterisk in part **b**). **c, d** | Features of a fibrotic phenotype are highlighted in the magnified insets, including fibrosis in the sublining layer (asterisks in parts **c** and **d**).

whereas synovial fluid CD14 and serum CD163 levels were associated with severity of joint space narrowing. The severity of self-reported knee joint symptoms was associated with CD14 levels in both synovial fluid ( $\beta = 0.773, P = 0.003$ ) and serum ( $\beta = 0.641, P = 0.031$ )<sup>71</sup>. The ratio of CD11c<sup>+</sup> to CD206<sup>+</sup> macrophages or CD86<sup>+</sup> to CD163<sup>+</sup> macrophages in synovial fluid was associated with KL grading and severity of knee OA in patients<sup>72</sup>. Pro-inflammatory macrophages in OA synovium show upregulated production of matrix metalloproteinases (including MMP1, MMP3, MMP13 and MMP9), aggrecanases (including ADAMTS4 and ADAMTS5) and cyclooxygenase 2, leading to articular degeneration<sup>73</sup>. In addition, secretion of the pro-inflammatory cytokines IL-1 $\beta$ , IL-6, and TNF and oncostatin M stimulates destructive processes in chondrocytes and mesenchymal cells, including downregulating synthesis of type II collagen (an indispensable component of healthy articular cartilage) and aggrecan, limiting chondrogenesis<sup>74</sup>. Interestingly, CD68<sup>+</sup> macrophages also contribute to loss of articular type II collagen by engulfing and presenting collagen fragments to CD4<sup>+</sup> T cells<sup>75</sup>.

Anti-inflammatory macrophages also express the mannose receptors MRC1 and MRC2, which bind to collagen and promote its internalization and lysosomal

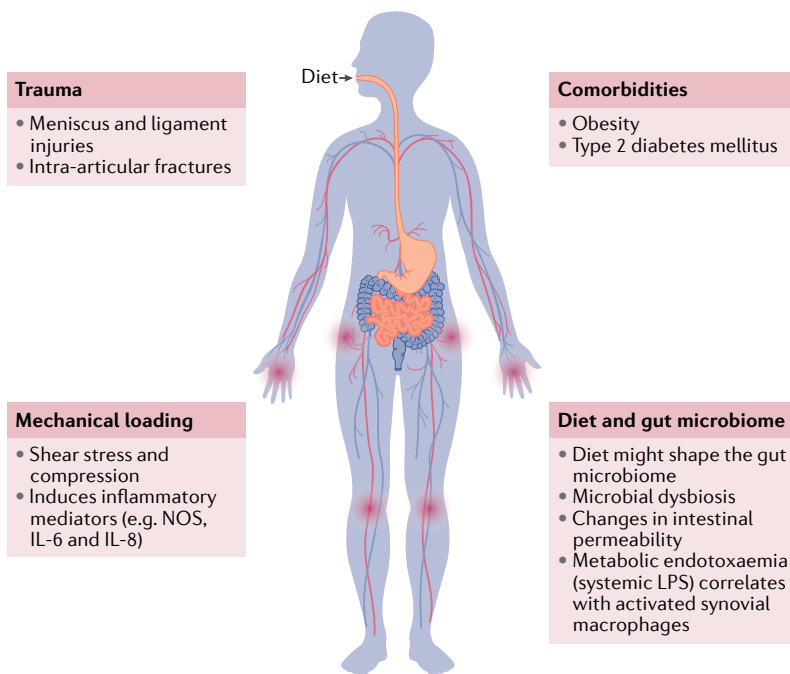
degradation. The resulting improvement in collagen turnover restores ECM homeostasis in the joint and ameliorates cartilage destruction<sup>76</sup>. Importantly, type II collagen helps to maintain expression of anti-inflammatory genes in macrophages as well as pro-chondrogenic cytokines<sup>77</sup>.

Macrophage phenotyping studies have also identified subsets of alternatively activated macrophages (that is, macrophages that are enriched in neither pro-inflammatory nor anti-inflammatory markers), which are more likely to be involved in healing inflammation<sup>78</sup>. Indeed, OA synovial macrophages do not perfectly align with surface marker expression profiles corresponding to classical pro-inflammatory or anti-inflammatory phenotypes, but have been classified as a population that resembles macrophages in RA based on their expression of proliferation genes, and another population is characterized by expression of cartilage-remodelling genes<sup>61</sup>. A scRNA-seq study detected heterogeneous macrophage cell types in the OA synovium, including immunoregulatory (expressing *SEPP1*, *FOLR2*, *STAB1*, *TXNIP* and *CD169*) and pro-inflammatory (expressing *CCL3*, *CCL4*, *IL1B* and *TNF*) macrophage subsets<sup>58</sup>. Interestingly, this immunoregulatory population, which does not align with typical pro-inflammatory or anti-inflammatory phenotypes, exhibits a gene expression profile suggestive of enhanced phagocytic activity and immunosuppressive activity.

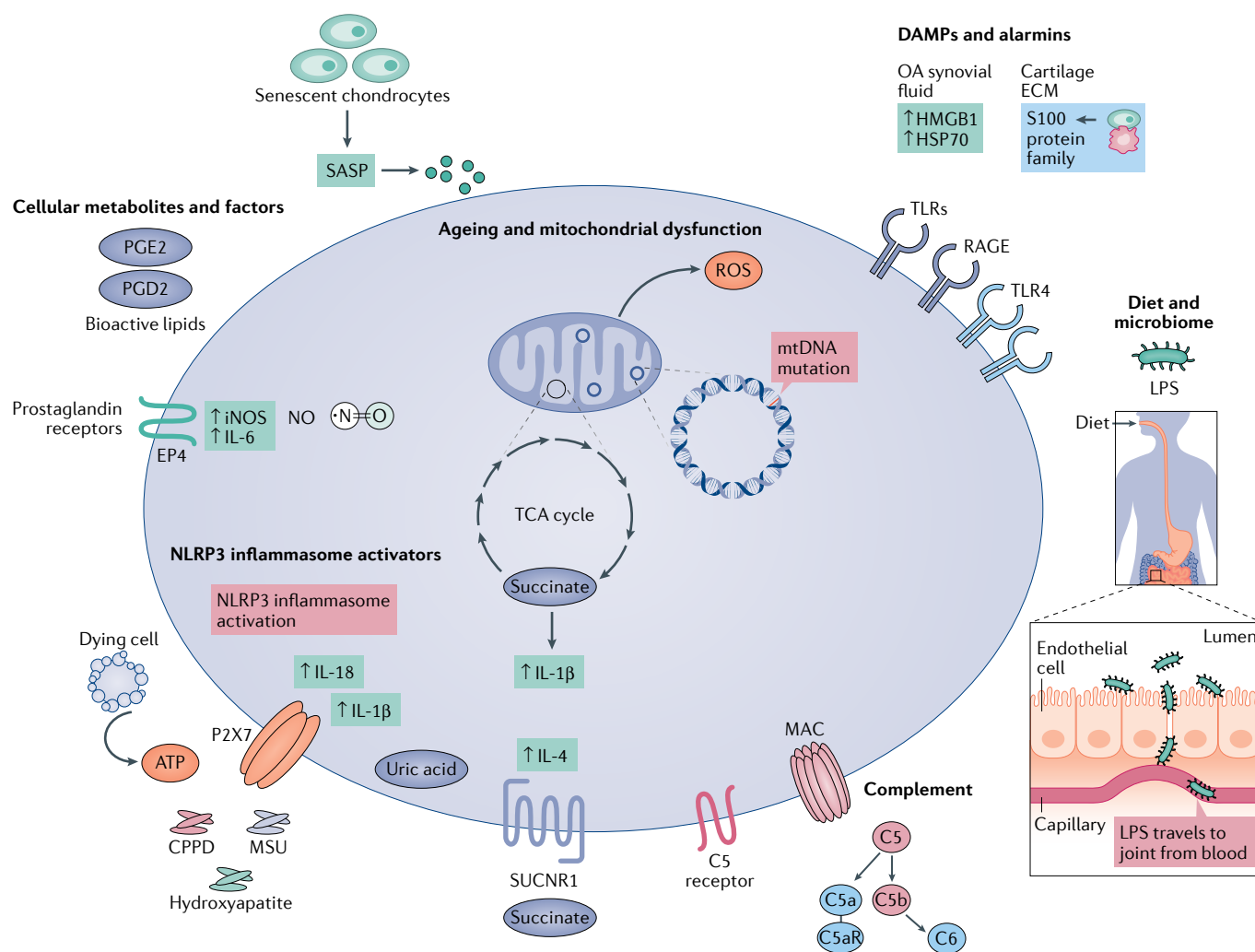
These results suggest that shifting macrophages towards phenotypes that might contribute to restoration of the damaged articular cartilage could represent a potential treatment for OA. Several therapeutic interventions have the ability to modify macrophage phenotypes in OA synovium. For example, glucocorticoids increase the proportion of CD163<sup>+</sup>FR $\beta$ <sup>+</sup> synovial macrophages, and slightly reduce the proportion of CD68<sup>+</sup> macrophages in the synovial lining, in patients with OA, resulting in decreased osteophyte formation<sup>79,80</sup>. Similarly, a cell-mediated gene therapy that is in phase II trials in patients with OA and allows localized delivery of transforming growth factor  $\beta$ 1 (TGF $\beta$ 1) improved the International Knee Documentation Committee, WOMAC and pain (evaluated on a visual analogue scale from 0 to 10) scores, elevated anti-inflammatory markers in the joints and potentiated IL-10 production<sup>81,82</sup>. In addition, functional imaging techniques (besides 99mTc-EC20 SPECT-CT) that allow the identification of macrophage subsets will help in stratifying patients with OA. Novel probes that target other macrophage markers, including CD206 (REF.<sup>83</sup>), formyl peptide receptor 1 (REF.<sup>84</sup>) or somatostatin subtype receptor 2 (REF.<sup>85</sup>), will improve understanding of macrophage phenotypes in OA.

### Synovial FLS in OA

FLS are specialized mesenchymal cells that lubricate the cartilage by producing synovial fluid rich in lubricin and hyaluronic acid (also known as hyaluronan). The concentration of lubricin and hyaluronic acid is decreased in OA synovial fluid, partly owing to changes in synovial membrane permeability, but it is also associated with a change in hyaluronic acid size<sup>86</sup>. Synovial fluid viscosity is decreased in OA and may be related to joint pain, as viscosupplementation therapy with intra-articular



**Fig. 2 | Osteoarthritis risk factors and synovitis.** Among the risk factors associated with osteoarthritis (OA) development and progression, trauma, mechanical loading, comorbidities and diet–microbiome interactions are also related to synovitis. Injury to the meniscus or ligaments and intra-articular fractures lead to the development of synovitis. Aberrant and excessive loading is a known risk factor for developing OA, and subsequent shear stress and compression induce production of inflammatory mediators such as nitric oxide synthase (NOS), IL-6 and IL-8, which contribute to OA pathogenesis. Synovitis has also been related to obesity and type 2 diabetes mellitus. Dietary habit, which has been demonstrated to increase pain prevalence in patients with OA, is one of the factors that influence the composition of the gut microbiome. Microbial dysbiosis (that is, alteration in gut microbiome composition) favours inflammation and metabolic syndrome, as well as changes in intestinal permeability and metabolic endotoxaemia, which correlates with recruitment of activated pro-inflammatory synovial macrophages. LPS, lipopolysaccharide.



**Fig. 3 | Molecular mediators that contribute to synovial inflammation in osteoarthritis.** Ageing and mitochondrial damage increase reactive oxygen species (ROS) production and mitochondrial DNA mutations and can prolong the production of pro-inflammatory cytokines such as IL-1 $\beta$  and IL-6. Senescent cells are associated with age-related pathological conditions such as osteoarthritis (OA), and several senescence-associated secretory phenotype (SASP) factors are inflammatory mediators. Cellular metabolites, such as nitric oxide (NO), succinate and prostaglandins, as well as other bioactive lipids, contribute to inflammation and cartilage damage. NO levels are elevated in chondrocytes and pro-inflammatory macrophages in patients with OA. Succinate accumulates in inflammatory macrophages and supports their pro-inflammatory phenotype. The succinate receptor SUCNR1 is activated by soluble succinate and boosts IL-4 production. Prostaglandin E2 (PGE2) is considered the major contributor to inflammatory pain in the joint and signals through receptors such as EP4, thereby enhancing production of the pro-inflammatory factors NO (by increasing expression of inducible nitric oxide synthase (iNOS)) and IL-6, which also contributes to synovitis and increases hyperalgesia. PGD2 is also enriched in synovial fluid from

patients with OA. Damage-associated molecular patterns (DAMPs) and alarmins, in the context of mechanical stress, interact with Toll-like receptors (TLRs), receptor for advanced glycation end products (RAGE) and other pattern recognition receptors to initiate and propagate inflammation. DAMPs such as high mobility group protein B1 (HMGB1) and heat shock proteins (HSPs) are abundant in OA synovial fluid. S100 family proteins are also upregulated in inflamed synovial tissue. Ectopic deposition of hydroxyapatite crystals, calcium pyrophosphate dihydrate (CPPD) microcrystals and monosodium urate (MSU) crystals, which may signal through P2X7 (depicted) or CD11b, CD16 and CD14 (not shown), as well as ATP released from dying cells, are detected by macrophages and trigger NLRP3 inflammasome activation and IL-1 $\beta$  and IL-18 production. MSU crystals correlate with levels of IL-1 $\beta$  and IL-18 in synovial fluid. Complement factors are highly expressed in OA and play a role in OA pathogenesis. Diet is one of the determining factors of microbiome composition. An altered gut microbial composition is associated with increased intestinal permeability and metabolic endotoxaemia (systemic lipopolysaccharide (LPS)), which is associated with recruitment of pro-inflammatory macrophages in the synovium.

hyaluronic acid decreases pain in patients with OA<sup>87</sup>. The transformation of healthy FLS into activated and pathological cells has been extensively studied in RA and less so in OA. Among the many factors that activate FLS in OA, follistatin-like protein 1 (FSTL1) is overexpressed in the OA synovium, and the levels of this protein correlate with OA severity (assessed by KL and WOMAC scores)<sup>88</sup>.

Activated OA FLS secrete pro-inflammatory cytokines, chemokines and proteolytic enzymes (MMPs and aggrecanases), thereby contributing to the propagation of inflammation and destruction of the cartilage matrix<sup>89</sup>.

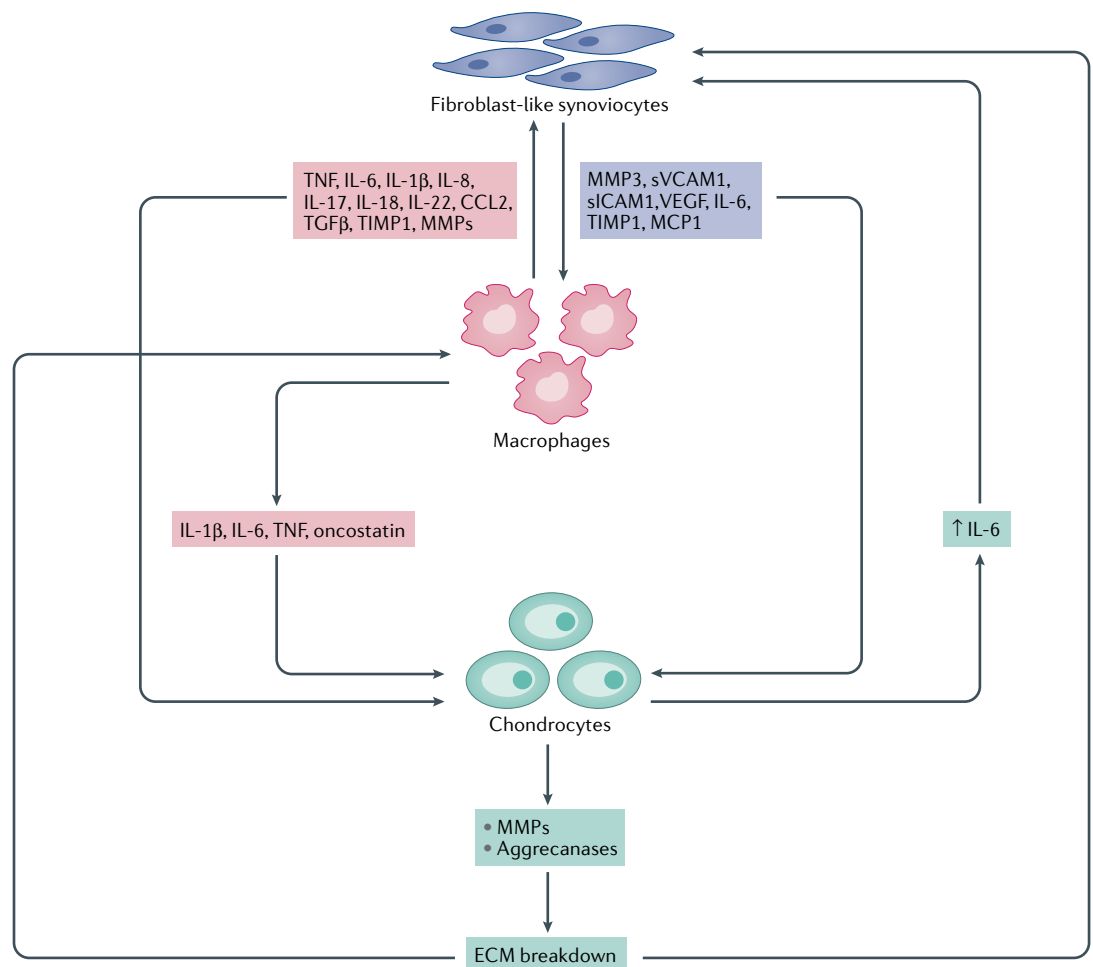
The different FLS phenotypes and their roles in OA pathogenesis have been described in studies in the past few years. A study focusing on RA FLS described

different functional associated phenotypes in FLS isolated from fresh synovial tissue from patients with OA compared with patients with RA who underwent joint replacement<sup>90</sup> (TABLE 1). In a 2021 study, the transcriptomic profiles of synovia and FLS isolated from patients with OA were distinct between patients with early or end-stage OA as well as between patient-reported pain zones and pain-free zones<sup>52</sup>. The transcriptome of synovium from pain zones in patients with early OA was characterized by upregulated expression of pro-fibrotic and pro-inflammatory genes, whereas the transcriptome of both early and end-stage OA showed upregulation of several nociceptive signalling pathways and neuronal growth genes<sup>52</sup>. Interestingly, scRNA-seq analysis of synovial explant FLS revealed that the gene expression profile of an FLS cluster representing the end-stage OA pain zone was associated with eicosanoid signalling, and the most active functions in these cells were “migration of cells” and “cell viability”<sup>52</sup>. Eicosanoid signalling was

also associated with a FLS cluster related to early OA pain zone. The end-stage OA FLS had a transcription profile similar to the leukocyte-rich RA FLS described in a previous study<sup>59</sup>, whereas the early OA FLS resemble the FLS found to be more predominant in OA in this previous study<sup>59</sup>.

**Other synovial cells in OA**

Neutrophils are another innate immune cell type that is found in the OA knee joint and are highly abundant in synovial fluid compared with synovial tissue<sup>91</sup>, although the reason for this distribution is still unknown. The secretion of the key proteolytic enzymes elastase and neutrophil gelatinase-associated lipocalin by activated neutrophils correlates with cartilage damage and radiographic progression<sup>91,92</sup>. Mast cells are also present in the synovium and are associated with inflammation and cartilage destruction in OA<sup>93</sup>. Synovial fluid from individuals with OA is enriched in tryptase (2–25 ng/ml), a mast



**Fig. 4 | Cellular crosstalk in synovitis and OA progression.** Activated fibroblast-like synoviocytes (FLS) in the osteoarthritis (OA) synovium secrete, among other factors, cytokines, growth factors, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), which contribute to macrophage activation and stimulate catabolic pathways in chondrocytes. Similarly, activated macrophages secrete pro-inflammatory mediators that stimulate FLS and chondrocytes, promoting the degradation of extracellular matrix (ECM) components. ECM degradation products further activate both FLS and macrophages, resulting in a repeating cycle of inflammation and cartilage degradation. CCL2, CC-chemokine ligand 2; MCP1, monocyte chemoattractant protein 1; sICAM1, soluble intercellular adhesion molecule 1; sVCAM1, soluble vascular cell adhesion molecule 1; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Table 1 | Clinical relevance of cell types associated with synovitis in OA

Cell type	Markers	Clinical relevance	Refs
Macrophages	Folate receptor detected by <sup>99m</sup> Tc-EC20 SPECT-CT	The quantity of activated macrophages correlated with radiographic OA severity and pain and stiffness	71
	CD14 <sup>+</sup> CD16 <sup>+</sup> macrophages in synovial fluid express mature macrophage marker 25F9 (indicating activation)	CD14 <sup>+</sup> macrophages/total macrophages ratio in synovial fluid is a predictor of KOOS and WOMAC scores, regardless of CD16 expression	63
	CD14 and CD163 in synovial fluid significantly associated with activated macrophages (detected by <sup>99m</sup> Tc-EC20 SPECT-CT), in the capsule ( <i>P</i> =0.002 and <i>P</i> =0.005, respectively) and in the synovium ( <i>P</i> =0.0005 and <i>P</i> =0.002, respectively)	CD14 and CD163 presence in the synovial fluid is associated with osteophyte severity Synovial fluid CD14 and serum CD163 associated with severity of joint space narrowing Severity of self-reported knee joint symptoms associated with both synovial fluid ( $\beta$ =0.773; <i>P</i> =0.003) and serum ( $\beta$ =0.641; <i>P</i> =0.031) CD14 levels	71
	CD11c <sup>+</sup> /CD206 <sup>+</sup> or CD86 <sup>+</sup> /CD163 <sup>+</sup> ratio in synovial fluid	Associated with KL grading and severity of knee OA in patients	72
	Mannose receptors MRC1 and MRC2	MRC1 and MRC2 recognize collagen, promoting its internalization and lysosomal degradation Resulting improvement in collagen turnover restores ECM homeostasis in the joint and ameliorates cartilage destruction Type II collagen helps to maintain expression of anti-inflammatory macrophage-related genes and pro-chondrogenic cytokines	77
	SEPP1, FLOR2, STAB1, TXNIP and CD169	Gene expression profile is indicative of enhanced phagocytic activity and immunosuppressive activity, suggesting an immunoregulatory role	58
	CCR2 <sup>+</sup> macrophages	Present in human synovium Invasive cells that are associated with cartilage erosion in OA	65
	CCL3, CCL4, IL1B and TNF	Pro-inflammatory macrophages	58
FLS	CD34-THY1 <sup>+</sup> FLS	Less abundant in OA synovium than in RA synovium (8% versus 22% of cells, respectively) Perivascular location, proliferative and secrete pro-inflammatory cytokines Proportion of these FLS is correlated with synovitis and synovial hypertrophy assessed by ultrasonography	90
	CD34-THY1 <sup>-</sup> FLS	Located in synovial lining Express the osteoblastic bone formation promoter BMP6 (involved in osteophyte formation) More abundant in OA than in RA synovium	90
	CADM1, COL8A2 and DKK3	Located in synovial lining DKK3 is a strong inhibitor of cytokine-induced collagen loss	59,259
	PTGDS, CXCL3, RSPO3, NRN1, NFKBIA, CXCL2, GEM, VCAM1, LIF, IL6 and INHBA	Associated with painful synovial sites in early OA	52
	HSPA1A, DNAJB1, SLC39A8, HTRA3, ATF3, PTGIS and BNIP3	Associated with painful synovial sites in end-stage OA	52

ECM, extracellular matrix; FLS, fibroblast-like synoviocytes; KL, Kellgren and Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; OA, osteoarthritis; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

cell-specific enzyme that is released during degranulation, compared with control individuals<sup>94</sup>. Deficiency of mast cells reduces cartilage loss, osteophyte formation and synovitis in the destabilization of the medial meniscus (DMM) mouse model of OA<sup>94</sup>. In addition, mast cell-dependent production of prostaglandin D2 in response to elevation in nerve growth factor (NGF) levels leads to an increase in nociceptive signalling in OA joints<sup>95</sup>.

Endothelial cells are present in joint structures and angiogenesis is implicated in OA pathogenesis<sup>96</sup>. Histological analysis of established OA synovium detected pericytes in all blood vessels, suggesting that these vessels are fully mature and stable, which might explain the persistent inflammation in OA; by contrast, the synovial vasculature in inflammatory arthritis is

characterized by a mixture of mature and immature vessels<sup>97</sup>. This study also found that blood vessels were distributed throughout the depth of the synovial membrane in OA, without preferential distribution in synovial lining cells<sup>97</sup>. Endothelial-cell-derived vascular endothelial growth factor (VEGF) seems to play an important part in OA pathogenesis, as the serum and synovial fluid concentration of VEGF correlates positively with WOMAC, radiographic severity (KL score) and the presence of osteophytes and a power Doppler ultrasonography signal of synovitis<sup>98</sup>. Although VEGF is crucial for cartilage formation, its expression seems to be upregulated in the joint of patients with OA and in surgically induced knee OA in mice; increased VEGF expression is associated with catabolic processes in



Table 2 | Risk factors and activators of synovial inflammation in OA

Risk factor or activator	Clinical relevance	Ref.
Obesity	Patients with obesity have a higher prevalence and severity of synovial inflammation assessed by conventional MRI	25
T2DM	Higher rates of ultrasonography-detected synovitis and effusion in patients with T2DM with end-stage knee OA who underwent arthroplasty compared with patients without T2DM, independent of patient BMI	139
Metabolic endotoxaemia	The presence of LPS in both plasma and synovial fluid from patients with OA correlates with the presence of activated macrophages in the joint capsule and synovium, radiographic severity (by <sup>99m</sup> Tc-EC20 SPECT-CT), and total WOMAC score	158
Microbiome — increased intestinal permeability and endotoxaemia	Pro-inflammatory <i>Streptococcus</i> species are associated with higher effusion on MRI and WOMAC knee pain, independent of BMI	161
Senescent cells	Positive correlation of the percentage of p16 <sup>INK4A</sup> -expressing synoviocytes and IL-6 concentration in the synovial fluid with the degree of synovitis at the site of biopsy	168
Bioactive lipids	11,12-DHET and 14,15-DHET levels are higher in OA knees versus unaffected knees of people with unilateral disease ( $P < 0.014$ and $P < 0.003$ , respectively) and are associated with radiographic progression over 3.3 years	180
HMGB1	HMGB1 levels in the synovial fluid higher in patients with KL 4 than in those with KL 2 ( $P < 0.01$ ) and KL 3 ( $P < 0.05$ )	184
	Synovial fluid HMGB1 levels associated with the severity of synovitis and pain	185
HSP70	HSP70 levels higher in both serum and synovial fluid of individuals with knee OA than in healthy controls and both correlate with radiographic severity	189
MSU crystals	MSU crystals in the joint are associated with increased synovial fluid concentrations of IL-1 $\beta$ ( $r^2 = 0.34$ , $P < 0.0001$ ) and IL-18 ( $r^2 = 0.41$ , $P < 0.0001$ ), OA severity and radiographic progression, and osteophyte formation ( $P = 0.001$ and $P < 0.0001$ , respectively)	204

KL, Kellgren and Lawrence; LPS, lipopolysaccharide; MSU, monosodium urate; OA, osteoarthritis; SPECT, single-photon emission computed tomography; T2DM, type 2 diabetes mellitus; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

chondrocytes and synovial cells (related to cartilage destruction)<sup>99</sup>. Furthermore, conditional knockdown of *Vegf* attenuated injury-induced OA in mice and intra-articular anti-VEGF antibodies suppressed OA progression and blocked VEGF signalling, as revealed by reduced levels of phosphorylated VEGFR receptors in articular chondrocytes, synovial cells and dorsal root ganglia<sup>99</sup>. Indeed, oral administration of the VEGFR2 kinase inhibitor vandetanib attenuated OA progression<sup>99</sup>.

Studies investigating the composition of the synovial membrane also reported the presence of T cells, including T helper (T<sub>H</sub>) 1 cells, T<sub>H</sub>2 cells, T<sub>H</sub>9 cells, T<sub>H</sub>17 cells, T<sub>H</sub>22 cells, T regulatory (T<sub>reg</sub>) cells and cytotoxic T cells<sup>100</sup>, even in the earliest stages of disease<sup>101</sup>. Although a change in the profile of T cell subtypes was described to correlate with disease activity and pain<sup>102</sup>, the role of T cells in the development and progression of OA has yet to be determined<sup>100</sup>.

The presence of the varied cellular players in the synovial tissue might complicate histological evaluation of the OA synovium. As alteration in the equilibrium and interaction between these cell types shape OA progression and symptomatology, understanding of the mediators of this intricate network is therefore crucial.

### OA risk factors and synovitis

#### Trauma

PTOA (FIG. 2) represents ~12% of all cases of symptomatic OA<sup>103</sup>. A study found that patients with a 3–10-year history of sport-related intra-articular knee injury developed OA<sup>104</sup>. Both animal and human studies have

demonstrated that joint injuries (to menisci and ligaments, as well as intra-articular fractures) lead to the development of synovitis<sup>105–107</sup>. For example, data from the Osteoarthritis Initiative showed that injury was associated with accelerated OA development, as assessed by KL grade<sup>108,109</sup>, whereas other studies found a higher incidence of OA in patients with joint injuries than in those without injuries<sup>110</sup>. As a surrogate of the presence of joint inflammation, pro-inflammatory cytokines, including IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-12, IFN $\gamma$  and TNF, as well as the cartilage-degrading markers MMP1, MMP3 and MMP9, are substantially elevated immediately after injury in the synovial fluid of patients with joint injuries<sup>111–114</sup>, and the elevated cytokine levels persist after bone healing<sup>115</sup>. As pro-inflammatory factors induce the production of cartilage-degrading enzymes, an association between synovial inflammation and PTOA is a prevalent hypothesis. However, in a study of 113 patients with acute anterior cruciate ligament injury, the levels of inflammatory mediators in the synovial fluid or the presence of moderate-to-severe Hoffa synovitis or of effusion synovitis at 2 years after anterior cruciate ligament injury did not predict structural knee OA at the 5-year follow-up<sup>116</sup>. More long-term longitudinal studies are needed to evaluate the contribution of synovial inflammation to the initiation and progression of PTOA.

#### Mechanical loading

Mechanical loading is essential for healthy joint maintenance. Nonetheless, aberrant excessive loading is a known OA risk factor<sup>117</sup> and is thought to act through

several molecular pathways, including IL-1 $\beta$ , TNF, NF- $\kappa$ B, WNT, microRNA and oxidative stress signalling pathways, which lead to chondrocyte apoptosis and ECM degradation<sup>118</sup>. However, excessive loading might also affect the synovium. For example, in vitro studies on FLS showed that mechanical loading induced the expression of several mediators involved in OA pathogenesis, such as prolyl-4-hydroxylase- $\alpha$ 1 (P4HA1), collagen  $\alpha$ 2 (I) chain (COL1A2), cyclooxygenase 2 (COX2) and IL-6 (REF.<sup>119</sup>). In addition, similar studies in human monocytes revealed that mechanical loading, shear stress and compression induce expression of nitric oxide synthase 2 (NOS2), IL-12B, IL-6 and IL-8 (REF.<sup>120</sup>). Despite all this evidence suggesting that abnormal mechanical loading can facilitate the accumulation of inflammatory mediators in the synovium, the exact mechanism by which aberrant or excessive mechanical loading induces synovitis is still unknown and might involve multiple cellular factors. Of interest, moderate physical activity has been proposed to modulate the immune response by priming circulating monocytes towards an anti-inflammatory macrophage-like differentiation, mediated potentially by peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) signalling that implicates increased expression of CD36 ( $1.9 \pm 1.5$ -fold) and liver X receptor- $\alpha$  (LXR $\alpha$ ) ( $5.0 \pm 4.7$ -fold) compared with sedentary individuals<sup>121</sup>. In a randomized controlled trial in women with knee OA, physical activity increased total intra-articular and perisynovial concentration of the anti-inflammatory cytokine IL-10 (REF.<sup>122</sup>),

which is mainly produced by anti-inflammatory macrophages<sup>123</sup> and has a chondroprotective role<sup>122</sup>.

### Obesity and T2DM

Obesity is a well-known risk factor for both OA incidence and progression<sup>124</sup>, and its role in OA development is different according to sex and also depends on the affected joint<sup>125</sup>. Incidence of knee, hip and hand OA is higher in women<sup>126–128</sup> than in men, and the prevalence of symptomatic OA is higher in women with obesity than in men with obesity<sup>2</sup>. The contribution of obesity to OA occurs not only through so-called mechanoinflammation but also through systemic low-grade inflammation or meta-inflammation, as obesity is linked to OA in weight-bearing joints such as the knees and in non-weight-bearing joints such as the hands<sup>129</sup>. The synovium of individuals with obesity displays marked fibrosis, increased macrophage infiltration and elevated expression of the Toll-like receptor 4 (TLR4) gene, but reduced levels of adiponectin and PPAR $\gamma$ <sup>130</sup>. In addition, abundance of CD14<sup>+</sup> and CD206<sup>+</sup> macrophages is increased in the synovial tissue of obese individuals<sup>130</sup>. Furthermore, the synovial fluid levels of the pro-inflammatory adipokine leptin are significantly higher in individuals with obesity than in those without obesity and correlate positively with BMI<sup>131</sup>. Finally, levels of mast-cell-produced  $\beta$ -tryptase in synovial fluid are also higher in individuals with obesity than in those without obesity<sup>132</sup>. Studies performed in rat<sup>133</sup> and rabbit<sup>134</sup> models with diet-induced obesity and surgically induced OA also showed an increase in pro-inflammatory macrophages<sup>133</sup> and the pro-inflammatory mediators IL-1 $\beta$ , IL-6 and TNF<sup>134</sup> in the synovium, which promote OA. Some studies also report more pain in patients with obesity with OA<sup>135,136</sup>. FLS isolated from patients with obesity with hip OA who underwent joint replacement surgery secrete higher amounts of IL-6 than FLS from lean patients, which was enhanced by crosstalk with chondrocytes via leptin<sup>137</sup>. Although patients with obesity have a higher prevalence and severity of synovial inflammation, as assessed by conventional MRI<sup>25</sup>, improvement in knee pain in patients with obesity with >20% weight loss at 1 year after dietary intervention or bariatric surgery was not mediated by a decrease in synovitis or bone marrow lesions (BMLs), as evaluated by MRI, but was partially explained by improvement in pressure pain threshold (at the patella and wrist) and depression score (CES-D)<sup>138</sup>. Furthermore, there was no noticeable improvement in BMLs (number and volume on MRI) or synovitis score after weight loss, which is in agreement with results of previous studies<sup>139,140</sup>. In fact, weight loss had no effect on synovial inflammation, evaluated by both static conventional MRI and dynamic CE-MRI, or on pain, evaluated by KOOS in a Danish study<sup>141</sup>. The fundamental reasons why obesity seems to facilitate synovitis but weight loss does not reverse this process are still unknown, although it is possible that obesity causes irreversible or long-lasting changes, such as epigenetic modifications or tissue structure alterations, which support OA progression even when individuals lose weight.

### Box 1 | Imaging techniques for synovitis assessment

#### Magnetic resonance imaging

- Two techniques: conventional MRI and contrast-enhanced MRI (CE-MRI).
- Signs of synovial inflammation in MRI scans include:
  - an increase in synovial membrane thickness and/or volume
  - enhanced signal intensity after intravenous gadolinium injection
  - the presence of effusion
  - alterations in the infrapatellar fat pad<sup>26,258</sup>
- Conventional MRI is still the most frequently used technique in OA, despite being unable to distinguish between synovial hypertrophy and joint effusion
- Synovial hypertrophy and joint effusion were correlated in a study that identified definite synovitis (synovial thickness  $\geq 2$  mm by CE-MRI) in 96.3% of knees with an effusion<sup>259</sup>
- A meta-analysis of 8 studies found that both CE-MRI (6 studies) and conventional MRI (2 studies) findings of synovitis correlated with macroscopic (vascularity, hyperplasia and villi) and microscopic (inflammatory infiltrates, synovial lining cells number, oedema and fibrosis) histological features of inflammation<sup>260</sup>

#### Ultrasonography<sup>261,262</sup>

- Ultrasonography can assess and distinguish between synovial hypertrophy and joint effusion
- Synovitis appears as thickening of the synovial membrane in grey scale (usually scored on a scale of 0–3)
- Power Doppler ultrasonography can detect active synovial inflammation in OA (also scored on a scale of 0–3)
- Power Doppler signal correlates with histologically confirmed inflammatory cell infiltrates, increased synovial lining layer thickness and increased vascularity
- Power Doppler signal also correlates with MRI findings of synovitis (joint effusion: CI = 0.61;  $P < 0.001$ ; synovial thickening: CI = 0.45;  $P = 0.01$ )<sup>263</sup>

These techniques could possibly be used to stratify patients with synovial inflammation who could benefit from specific anti-inflammatory treatments.

Epidemiological studies show a higher prevalence of OA (radiographic and symptomatic) in patients with type 2 diabetes mellitus (T2DM) and a higher rate of arthroplasty<sup>142,143</sup>, with a meta-analysis reporting a higher risk of OA development in patients with T2DM than in those without T2DM (OR 1.46; 95% CI 1.08–1.96;  $P=0.01$ )<sup>144</sup>. Although some studies neither confirmed these findings after adjustment for BMI<sup>145,146</sup> nor detected an association between T2DM and prevalence or incidence of OA<sup>147,148</sup>, some of the studies included in the meta-analysis reported the same increased risk after BMI adjustment, suggesting that T2DM is an independent risk factor for OA development<sup>143</sup>. For example, ultrasonography-detected synovitis and effusion were higher in patients with T2DM and end-stage knee OA who underwent arthroplasty than in those without T2DM, independent of patient BMI<sup>143</sup>. Several reports have described the effect of hyperglycaemia on synovial inflammation. For example, synovial levels of the pro-inflammatory cytokine TNF were higher in obese patients with OA and T2DM than in those without T2DM<sup>149</sup>. FLS in patients with diabetes and obesity with OA are also insulin resistant, implying a diminished ability of insulin to decrease production of pro-inflammatory and catabolic mediators that contribute to OA development<sup>150</sup>. High glucose levels induce VEGF secretion and reactive oxygen species (ROS) production in FLS in OA, increasing angiogenesis, tissue damage and inflammation<sup>151</sup>. Finally, both diabetes and ageing are associated with the accumulation of advanced glycosylated end-products, which induce an increase in proMMP1 secretion by FLS and in transcription of bone morphogenetic protein (BMP) genes that are involved in osteophyte formation<sup>152</sup>.

#### **Diet and the gut microbiome**

Although obesity is one of the most important modifiable risk factors to improve outcomes in OA, diet might have a role beyond weight control. A higher dietary inflammatory index score is associated with a higher prevalence of radiographic, symptomatic KOA, independent of patient weight (OR 1.40; 95% CI 1.14–1.72;  $P=0.002$ )<sup>153</sup>. Interestingly, a randomized controlled trial of vitamin D supplementation slowed the progression of effusion-synovitis volume increase<sup>154</sup>, supporting the premise that micronutrients might have an effect on chronic pain by modulation of intra-articular inflammation. A subsequent randomized controlled trial showed no effect of vitamin D supplementation on BML volume and synovitis<sup>155</sup>. In a randomized, controlled trial, *Curcuma longa* extract, a proposed anti-inflammatory natural product, was superior to placebo in controlling pain but had no effect on knee effusion-synovitis or cartilage damage<sup>156</sup>. The Mediterranean diet is also believed to have positive effects in patients with OA<sup>157</sup>, and epidemiological studies from the Osteoarthritis Initiative found that a western diet was associated with progression of OA (higher KL and WOMAC score)<sup>158</sup>, although no data on its effect on synovitis were provided.

Diet is one of the modifiable factors that influence the composition of the gut microbiome. As germ-free mice have reduced susceptibility to OA from DMM<sup>159</sup>,

and microbial DNA signatures have been detected in the cartilage and synovial tissue of patients with OA<sup>160,161</sup>, interest in the role of the microbiome in OA development and progression has increased. Western diets lack prebiotic-rich foods, in the form of dietary fibre, other complex carbohydrates and sugar alcohols present in fruits, which might be beneficial in supporting a healthy microbiome. Microbial dysbiosis — adverse alterations of the gut microbiota composition — may favour metabolic syndrome and inflammation. Indeed, obesity is associated with a loss of beneficial *Bifidobacterium* species and an increased abundance of pro-inflammatory bacterial species, which might increase macrophage recruitment from the gut to the synovium and accelerate knee OA<sup>162</sup>.

The bacterial endotoxin lipopolysaccharide (LPS) is a known activator of synovial inflammation through TLR4 (REF.<sup>163</sup>). Metabolic endotoxaemia (that is, the presence of bacterial products such as LPS in the blood) has been linked to changes in intestinal permeability induced by diet<sup>164</sup> and has been described in patients with obesity and metabolic syndrome<sup>165</sup>. Interestingly, the presence of LPS in both plasma and synovial fluid from patients with OA correlates with the presence of activated macrophages in the joint capsule and synovium, radiographic severity (by <sup>99m</sup>Tc-EC20 SPECT-CT), and total WOMAC score<sup>163</sup>. Another study in individuals with knee OA found an association of the pro-inflammatory *Streptococcus* species with higher effusion (on MRI) and WOMAC knee pain, independent of BMI<sup>166</sup>. Whether changes in the gut microbiota that support inflammation are present in early stages of OA and are a contributing factor in OA radiographic or clinical progression or a consequence, possibly influenced by obesity, needs to be further examined.

#### **Molecular mediators of synovitis in OA**

##### **Ageing and mitochondrial damage**

Mitochondrial dysfunction (FIG. 3) is characterized by reduced mitochondrial integrity, including decreased mass, number and mitochondrial DNA (mtDNA) content, and impaired mitochondrial respiration, which increases ROS production<sup>167</sup>. Ageing is postulated to play a role in the mitochondrial dysfunction observed in OA. For example, mice that aged prematurely from accumulation of mtDNA mutations exhibited osteopenia, changes in epiphyseal trabecular bone and the subchondral cortical plate, and elevated numbers of hypertrophic chondrocytes in articular calcified cartilage<sup>168</sup>. Evaluation of mtDNA single nucleotide polymorphisms has defined mtDNA haplogroups as potential biomarkers for diagnosis or prognosis of OA<sup>169</sup>. In OA synoviocytes, the frequency of mtDNA mutations is substantially lower than in RA synoviocytes<sup>170</sup>, but complete characterization and larger population studies are needed to define the involvement of mtDNA mutations in synoviocytes in OA development. Ageing has also been associated with chronic low-grade inflammation, which could promote OA development, although the exact mechanism of the ageing–inflammation link is still unknown<sup>3</sup>. Mitochondrial dysfunction and deficient ROS scavenging prolong the production of

pro-inflammatory cytokines (such as IL-1 $\beta$  and IL-6) and prevent the repolarization of macrophages from a pro-inflammatory to an anti-inflammatory phenotype in other tissues<sup>171</sup>, all of which could influence the development and progression of OA.

Senescent cells are a feature of various age-related pathological conditions, including OA. In particular, senescent chondrocytes in both PTOA and age-related OA accumulate mostly in the articular cartilage and synovium, and their elimination attenuates the development of OA, reduces pain and increases cartilage formation<sup>172</sup>. Several senescent-associated secretory phenotype factors are also inflammatory mediators, supporting the hypothesis that senescent cells in OA synovium could play a role in initiating or maintaining synovial inflammation. Studies in ex vivo human OA knee specimens and in a surgically induced OA mouse model have detected senescent synoviocytes in the OA synovium and demonstrated a positive correlation between the degree of synovitis at the biopsy site and the percentage of p16<sup>INK4A</sup>-expressing synoviocytes and levels of IL-6 in synovial fluid<sup>173</sup>. Together, these results suggest that 'aged synovium', indicated by the presence of senescent cells, is associated with synovitis. Early-stage clinical studies with senolytic agents are now underway, with results forthcoming (NCT04210986, NCT04770064 and NCT04815902).

#### **Metabolites affecting the synovium**

The OA synovium is a rich environment containing a wide variety of metabolites and soluble factors that contribute to both inflammation and cartilage damage<sup>174</sup>.

**Nitric oxide.** Synovial fluid from patients with OA contains elevated levels of nitrite and the enzyme responsible for nitric oxide (NO) production, inducible nitric oxide synthase (iNOS; encoded by *NOS2*)<sup>175</sup>. NO, which is mainly produced by chondrocytes in the OA joint<sup>176</sup>, mediates inflammatory mediator production, angiogenesis and cartilage destruction<sup>177</sup>. Pro-inflammatory macrophages are also an important source of NO, through the metabolic rewiring of arginine metabolism towards NO and L-citrulline production<sup>70</sup>. Inhibition of iNOS dramatically reduces the production of catabolic and pro-inflammatory factors and prevents OA development in dogs<sup>178</sup>. Although animal studies support the investigation of iNOS inhibitors as a potential disease-modifying intervention for OA, no successful clinical trials of these agents have been reported<sup>179</sup>.

**Succinate.** Pro-inflammatory macrophages exhibit a dysfunctional Krebs cycle that results in accumulation of succinate, which is shuttled from mitochondria to the cytosol to prevent hydroxylation and degradation of hypoxia inducible factor 1 $\alpha$  (HIF1 $\alpha$ ), a key transcription factor involved in IL-1 $\beta$  production<sup>180</sup>. Although intracellular succinate supports the pro-inflammatory phenotype of macrophages, activation of succinate receptor 1 (SUCNR1; also known as GPR91) by soluble succinate boosts IL-4 production<sup>181</sup>, a cytokine that induces macrophage polarization towards an anti-inflammatory phenotype. However, succinate signalling through SUCNR1 in FLS links inflammation with fibrosis and angiogenesis and, indeed,

exacerbates RA<sup>182</sup>. Despite these animal and in vitro data in RA, there are no studies investigating the relationship between succinate levels in the synovium and radiographic progression or clinical symptoms in OA. Indeed, the role of succinate and other intermediate metabolites in glycolysis and the Krebs cycle in FLS in OA is still unknown, and additional metabolic and functional studies are needed to understand the phenotype of FLS in OA.

**Prostaglandins and other bioactive lipids.** Both IL-1 $\beta$  and IL-18 substantially increase the production of prostaglandin E2 (PGE2) in the synovium after articular cartilage damage<sup>183</sup>. In synovial fluid from patients with knee OA, the levels of IL-18 and PGE2 correlate greatly<sup>184</sup>. PGE2 is considered the major contributor to inflammatory pain in the OA joint. PGE2 signals through multiple receptors that are expressed differentially in both peripheral sensory neurons and the spinal cord. Through the EP4 receptor, PGE2 has been proposed to participate in enhancing the production of aggrecanases and MMP13 induced by pro-inflammatory cytokines<sup>185</sup>. PGE2 also induces the expression of iNOS and IL-6, which further contributes to maintaining synovitis and increasing hyperalgesia. PGD2 is also enriched in synovial fluid from patients with OA<sup>186</sup> and has been suggested to potentiate nociception<sup>95</sup>. In a study that compared synovial fluid samples from 112 knees of 102 individuals (of whom 58 had knee OA and 44 were healthy controls), including both affected and unaffected knees in those with unilateral OA, increased levels of PGD2, 11,12-dihydroxyeicosatrienoic acid (11,12-DHET) and 14,15-dihydroxyeicosatrienoic acid (14,15-DHET) were associated with the presence of OA<sup>186</sup>. The levels of 11,12-DHET and 14,15-DHET were higher in affected than in unaffected knees of people with unilateral OA ( $P < 0.014$  and  $P < 0.003$ , respectively) and were associated with radiographic progression over 3.3 years of follow-up<sup>186</sup>.

#### **DAMPs and alarmins**

Excess mechanical stress or injury leads to the release of damage-associated molecular patterns (DAMPs), which interact with pattern recognition receptors (PRRs), including TLRs, receptor for advanced glycation end products (RAGE) and others to initiate the innate immune response and propagate inflammation. DAMPs implicated in OA are very heterogeneous and include cartilage fragments, ECM proteins, secreted intracellular proteins, plasma proteins and crystals (extensively reviewed elsewhere<sup>187,188</sup>).

**High mobility group protein B1.** HMGB1 is a non-histone nuclear protein that facilitates transcription factor and nucleosome stability. However, HMGB1 acts as an endogenous danger signal and is released from cytokine-activated cells and damaged or dying cells supporting the inflammatory response<sup>189</sup>. In OA joints, HMGB1 is secreted by damaged and necrotic chondrocytes<sup>189</sup>. HMGB1 levels in synovium and synovial fluid are higher in patients with OA than in healthy controls<sup>190,191</sup>, and they are also higher in the synovial fluid of patients with KL 4 than in those with

KL 2 ( $P < 0.01$ ) or KL 3 ( $P < 0.05$ )<sup>190</sup>. HMGB1 levels in synovial fluid have also been associated with the severity of synovitis, pain and daily activity reduction<sup>191</sup>. The therapeutic potential of HMGB1 neutralizing antibodies has been evaluated in the DMM mouse model, in which they showed cartilage protective effects<sup>192</sup>.

**Heat shock proteins.** Heat-shock proteins (HSPs) are induced by cellular stress to protect and maintain cellular integrity and function<sup>193</sup>. In OA synovium, HSP60, HSP70 and HSP90 are the most abundant members of the HSP family<sup>194</sup>. HSP70 levels in serum and synovial fluid are higher in individuals with knee OA than in healthy controls and correlate with radiographic disease severity<sup>195</sup>. In the DMM mouse model, overexpression of the HSP70 family member HSPA1A abrogated cartilage erosion while having no effect on DMM-induced osteophyte formation or subchondral bone plate thickening<sup>196</sup>.

**S100 protein family members.** S100 proteins are intracellular calcium-binding proteins that participate in regulating the cytoskeleton and cell migration<sup>188</sup>. S100A9 is strongly upregulated in inflamed synovial tissue<sup>197</sup> and is involved in cartilage matrix degradation and osteophyte formation<sup>198</sup>. A study in 141 individuals with clinical knee OA showed that serum levels of S100A8 or S100A9 correlated with total WOMAC scores ( $P = 0.021$ ), weight-bearing pain ( $P = 0.043$ ) and physical dysfunction ( $P = 0.010$ )<sup>199</sup>. Similar results were obtained in 294 patients with hand OA<sup>200</sup>.

#### Other NLRP3 inflammasome activators

Evidence of the participation of the NLRP3 inflammasome, a protein complex involved in processing and maturation of IL-1 $\beta$  and IL-18, in OA onset and progression has led to this complex being proposed as a potential biomarker for OA diagnosis and patient classification<sup>201–203</sup>. Maturation of IL-1 $\beta$  and IL-18 is a two-step process. First, activation of NF- $\kappa$ B-dependent transcription of *NLRP3* and *IL1B*<sup>204</sup>, *p62* (also known as *SQSTM1*)<sup>205</sup> and *SLC44A1* (REF.<sup>206</sup>) among other genes, and de novo synthesis of mtDNA<sup>207</sup>. Second, the assembly of the NLRP3 inflammasome, activation of caspase 1 and processing of pro-IL-1 $\beta$  and pro-IL-18 to mature cytokines<sup>204</sup>. In the OA synovium, ectopic deposition of hydroxyapatite crystals, calcium pyrophosphate dihydrate microcrystals and monosodium urate crystals, and ATP released from dying cells are detected by macrophages and trigger NLRP3 inflammasome activation and IL-1 $\beta$  and IL-18 production<sup>208,209</sup>. Of note, monosodium urate crystals in the joint are associated with increased synovial fluid levels of IL-1 $\beta$  ( $r^2 = 0.34$ ,  $P < 0.0001$ ) and IL-18 ( $r^2 = 0.41$ ,  $P < 0.0001$ ), OA severity and radiographic progression ( $P = 0.001$ ), and osteophyte formation ( $P < 0.0001$ )<sup>210</sup>. IL-1 $\beta$  induced chondrocyte catabolism, increased MMP and ADAMTS5 activity and suppressed proteoglycan synthesis<sup>183</sup>. The pathogenetic role of IL-1 $\beta$  in OA synovium might also be due in part to the lack of production of the natural IL-1 $\beta$  antagonist IL-1 receptor antagonist (IL-1Ra) by OA chondrocytes<sup>58</sup>. Preclinical studies using recombinant IL-1Ra (anakinra) demonstrated a strong protection in OA animal models by improving

lubricin expression, preserving cartilage integrity and reducing synovial hyperplasia and inflammatory cell infiltration<sup>211</sup>. Furthermore, in an exploratory analysis of a randomized controlled trial for the prevention of cardiovascular events, canakinumab (anti-IL-1 $\beta$ ) treatment was associated with a lower incidence of hip and knee replacement than placebo<sup>212,213</sup>, although in a randomized controlled trial, intra-articular injection of anakinra did not improve OA symptoms compared with placebo<sup>214</sup>. These disparate outcomes have resulted in a lack of consensus regarding the use of IL-1 signalling therapy for OA, concluding that inhibiting the actions of IL-1 $\beta$  alone is not enough to block OA pathogenesis. In this sense, NLRP3 inflammasome inhibitors, which block not only the production of IL-1 $\beta$  but also that of IL-18 and active caspase 1, might be a more potent intervention. In the OA synovium, IL-18 promotes chondrocyte proliferation and expression of COX2, iNOS and MMPs, and boosts IL-6 production, further supporting a pro-catabolic environment<sup>215</sup>. Importantly, active caspase 1 also cleaves gasdermin D, releasing the active amino terminal portion that has pyroptotic activity (by pore formation), which could contribute to maintaining persistent inflammation within the synovium<sup>216</sup>.

#### Complement

Complement factors are highly expressed in the main tissues involved in OA, including cartilage, bone and synovium, in patients with OA compared with in healthy controls<sup>217,218</sup>. Furthermore, expression of complement effectors is higher and that of complement inhibitors is lower in the synovium of patients with OA than in healthy donors<sup>217</sup>. In addition, C5 and C6 deficiency are protective against the development of synovitis and cartilage damage in animal models of OA, and complement activation is associated with increased production of ECM-degrading proteins and inflammatory mediators<sup>217</sup>. These data suggest a role for complement activation not only in synovitis but also in the development and progression of OA, and it could therefore represent a potential therapeutic target.

#### Cellular interplay in OA synovium

##### Interactions between synoviocytes, chondrocytes and osteocytes

In a healthy joint, chondrocytes balance the synthesis and breakdown of the cartilaginous matrix to ensure correct distribution of load across the joint, thereby reducing friction. Both non-mechanical and mechanical factors contribute to OA development, which involves a shift in chondrocyte metabolism to increased proteolytic activity and inflammation and cartilage degradation. Direct and indirect communication between chondrocytes and synoviocytes is thought to contribute to maintaining anabolic and catabolic responses of each cell type<sup>219</sup> (FIG. 3). In the inflamed joint, chondrocytes and synoviocytes mutually induce alterations in their transcription programme to favour the production of MMPs<sup>220</sup>. Cartilage fragments, aggrecan, fibronectin and other DAMPs are sensed by synoviocytes to shift their transcriptomic profile towards chronic inflammatory responses, including cytokine, NLRP3 inflammasome,

hypoxia, scavenger receptor and TLR, and integrin pathways<sup>221</sup>. Importantly, the array of upregulated genes related to maintenance of an inflammatory phenotype are under control of transcription factors that support synovitis, including ATF2, STAT3 and NFκB1 (REF.<sup>221</sup>). Cartilage fragments can also induce inflammatory responses in synoviocytes by reorganization of the actin cytoskeleton, enhanced production of NO and PGE2 and increased deposition of collagen<sup>222</sup> (FIG. 4).

Synovitis can coexist with BML and both can precede the development of radiographic OA<sup>223,224</sup>. Although it is thought that BMLs result from excessive mechanical loading, it is not known whether synovitis contributes to subchondral bone pathology<sup>225</sup>, other than by invasion of subchondral bone by pannus-like tissue in the medial compartment<sup>226</sup> without producing the marginal erosions typically seen in RA<sup>227</sup>. The temporal relationship between the synovitis and BMLs is still not known. In a study in patients with end-stage OA before joint replacement, histologically assessed synovitis correlated moderately with the presence of subchondral cysts by MRI ( $r = 0.350$ ;  $P = 0.03$ )<sup>228</sup>. However, a subsequent analysis of the contribution of different OA pathological processes to pain found no association between subchondral pathology and synovitis, suggesting that subchondral pathology is associated with knee pain independently of cartilage and synovial pathology<sup>229</sup>.

Synovitis is also associated with osteophyte formation. Hoffa synovitis is significantly associated with osteophyte development in both anterior ( $P = 0.013$ , adjusted OR 1.12, 95% CI 1.03–1.23) and medial ( $P = 0.000$ , adjusted OR 1.21, 95% CI 1.11–1.31) lesions of the tibia<sup>230</sup>. In support of the association between Hoffa synovitis and osteophyte development, depletion of macrophages in the collagenase-induced OA model strongly reduced the formation of osteophytes and fibrosis<sup>231,232</sup>. Macrophages, which together with FLS are the main source of TGFβ in the synovium, contribute to the stimulation of bone formation and production of

proteoglycan and type II collagen, and enhance chondrogenesis. TGFβ and the related proteins BMP2 and BMP4 are essential growth factors implicated in osteophyte formation<sup>233</sup> and pathological type I collagen deposition during fibrosis<sup>234</sup>.

Although the relationship between synovitis and OA structural progression is better defined, the role of synovial inflammation in OA pain is not completely understood (BOX 2). Treatment with antibodies against granulocyte–macrophage colony-stimulating factor (GM-CSF), which signals in both the immune and nervous systems, has an analgesic effect in OA without affecting synovitis scores<sup>235</sup>. Indeed, the number of GM-CSF- and GM-CSFRα-expressing cells per mm<sup>2</sup> synovial sub-lining correlated negatively with knee pain<sup>235</sup>, reinforcing the idea that synovitis and pain are not always associated. Research in OA-related pain has also focused on nerve growth factor (NGF)<sup>236</sup>, which is a neurotrophin that activates nociceptive neurons to transmit pain signals from the periphery to the central nervous system. NGF is expressed by FLS and macrophages in the synovium<sup>237</sup> and by subchondral mononuclear cells, osteoclasts and chondrocytes in the cartilage from patients with knee OA<sup>229</sup>. Other studies suggest that symptomatic OA is associated with upregulation of MMP1 in the synovium and downregulation of IL-1R1 and VEGF compared with the levels of these molecules in individuals with asymptomatic chondropathy with similar macroscopic joint surface appearances who did not seek total knee replacement<sup>18</sup>. IL-1β signals through its receptor IL-1R1 and induces the expression and release of MMP1 by FLS. After IL-1–IL-1R1 engagement, IL-1R1 is downregulated, which may explain why therapy with an IL-1R1 antagonist failed in clinical trials<sup>238</sup>. Angiogenesis in the subchondral bone has been postulated as the initial event in OA pain, as the blood vessels supply nutrients for axonal growth and neo-innervation of the osteochondral junction, likely driven by NGF released from basal articular chondrocytes<sup>239</sup>. The synovium then contributes to pain by secreting pro-inflammatory factors, such as TRKB (the receptor for brain-derived growth factor), CCL14 and ADAMTS15, angiotensinogen, angiotensin-converting enzyme, netrin 1, CCL2 and CCL8 (REFS<sup>18,237,240</sup>), either independently or amplifying the process already initiated by NGF.

### **Interactions between synovial tissue and the infrapatellar fat pad**

Fibrosis may contribute to joint stiffness and pain, which are the main symptoms in OA, but most of the clinical studies relate to postoperative synovial fibrosis. Intra-articular fibrosis can be detected by using MRI scans with advanced metal suppression and with gadolinium contrast<sup>241</sup> in patients with stiff and painful arthroplasty. Patients with diagnosed fibrosis<sup>242</sup> exhibit thicker tissue (4.4 mm ± 0.2 mm) than patients with a non-fibrotic phenotype (2.5 mm ± 0.4 mm) after total knee arthroplasty (1.9 mm ± 0.2 mm;  $P < 0.05$ )<sup>241</sup>. A promising fibroblast radiotracer for PET, <sup>18</sup>F-labelled glycosylated FAPI, demonstrated highly specific uptake in bone structures and joints<sup>243</sup> and could aid in

## **Box 2 | Gaps in and proposed agenda for synovitis research in OA**

### **Research gap**

To date, the fundamental mechanisms underlying the crosstalk between synovitis and clinical symptoms of osteoarthritis (OA) are not completely identified. Current studies describe histological and molecular characteristics at end-stage OA using imaging but do not capture change over time.

### **Proposed research agenda**

Longitudinal studies that combine cellular and molecular evaluation in combination with histology and imaging at different stages of OA to establish mechanistic links with clinical OA progression.

### **Research gap**

Current imaging techniques cannot capture all histological features of the OA synovium.

### **Proposed research agenda**

Optimization of imaging, including MRI, functional imaging and positron emission tomography to capture the different histological patterns and phenotype subsets in OA.

### **Research gap**

Defining phenotypes that capture the heterogeneous features of OA synovitis.

### **Proposed research agenda**

Personalized medicine for patients with OA by defining phenotypes of OA that capture the inflammatory subtypes through advanced imaging.

improving understanding of the role of fibroblasts and fibrosis in OA and whether or not fibrosis in the synovial tissue contributes to joint pain.

The exact mechanism by which fibrosis occurs in OA synovium is still not entirely clear. It is generally accepted that there is more inflammation, with an increased number of macrophages and T cells in the lining layer, in early OA than in advanced OA, in which inflammation and fibrosis coexist<sup>48,244</sup>. These observations suggest that the progression of OA is accompanied by a transition from an inflammatory phase to a fibrotic stage and that factors that initiate fibrosis might be induced during synovitis. Several factors that are increased in inflamed OA joints are associated with fibrosis, including TGF $\beta$ , procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2), tissue inhibitor of metalloproteinase 1 (TIMP1), connective tissue growth factor (CTGF), disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) and prostaglandin F2a. For example, ADAM12 mRNA and protein levels in synovium correlate with the severity of histological synovitis<sup>244</sup>. Both PLOD2 and TIMP1 have been directly implicated in synovial fibrosis and are elevated in the synovium of patients with end-stage OA and mice with experimental OA<sup>245</sup>.

Another compartment that is involved in the development of synovial fibrosis is the IFP, which is located below the kneecap between the joint capsule and the synovial membrane, protects the knee from mechanical stress and provides a vascular supply. However, during OA development and progression the IFP also undergoes structural changes characterized by increased fibrosis and neovascularization, lymphocyte infiltration in the interlobular septum and smaller fat lobules<sup>246–248</sup>. Of note, individuals who develop AKOA are more likely to have altered signal intensity in the IFP (30%) than those with no knee OA at 2 years prior to the index visit (OR 2.07, 95% CI 1.14–3.78), and these odds increase twofold at 1 year prior to disease onset and for the next 3 years<sup>249</sup>. The infiltration of immune cells and increased fibrosis cause the disappearance of adipocytes in the parenchymal region of the IFP<sup>250</sup>. Part of the contribution of the IFP to synovial fibrosis is mediated by the activation of FLS: PLOD2 expression and collagen production by FLS increases sixfold and 1.8-fold respectively when FLS are co-cultured with fat-conditioned medium from the IFP of patients with OA<sup>247</sup>. Furthermore, collagen production by FLS correlated with increased levels of prostaglandin F2a in the fat-conditioned medium, whereas no correlation with TGF $\beta$  amounts was observed<sup>247</sup>. In addition, IFP tissue in obese individuals shows increased expression of genes associated with fibrosis and ECM production, but no change in adipocyte size, inflammatory cell infiltration, macrophage polarization, formation of crown-like structures, or expression of genes encoding inflammatory cytokines and chemokines<sup>251</sup>.

In another study, IFP volume was not associated with BMI<sup>252</sup>. However, macrophages in the IFP from patients with obesity with knee OA were positive for the surface markers CD206 and CD163 (~80% and 40% of all CD14<sup>+</sup> macrophages, respectively), and these macrophages produce IL-6 and TNF but not much IL-10; of note, none of these features correlated with BMI<sup>252</sup>. Animal models of OA also showed an association between synovitis, changes in macrophage polarization (including enrichment of pro-inflammatory macrophage phenotype or increased crown-like structures), and fibrosis in both synovial tissue and the IFP, and treatment that decreased inflammation was associated with changes in macrophage phenotypes and attenuation of fibrosis<sup>251–253</sup>. Although all these studies showed some contribution of IFP dysfunction to joint inflammation and fibrosis, the mediators of these interactions remain unknown.

Of interest, TGF $\beta$  has been proposed as a nexus between fibrosis and pain in OA. In a study of patients with radiographic KL grade 3–4 after total knee arthroplasty, NGF expression in synovial tissue correlated positively with TGF $\beta$  expression ( $P < 0.001$ ) while showing no association with levels of the pro-inflammatory cytokines IL-1 $\beta$  and TNF ( $P = 0.576$  and  $P = 0.616$ , respectively). Both TGF $\beta$  and NGF colocalized in the lining layer, mainly in the CD45<sup>-</sup>CD90<sup>+</sup> fibroblast population (86.3% of analysed cells in the synovial tissue)<sup>254</sup>. Similar to findings in articular cartilage<sup>255</sup>, TGF $\beta$ –ALK5 signalling mediates NGF production through the TAK1–p38 pathway in the synovium of patients with knee OA<sup>254</sup>.

## Conclusions

OA is a complex disease in which symptoms and joint function are often dissociated from structural damage. In an effort to identify pathobiological mechanisms in OA, the OA community is intensely investigating synovial inflammation. Consequently, we now know more about the cellular and molecular players in synovitis, although more in-depth studies are needed to evaluate the association of these factors with radiographic progression and contribution to OA symptoms at both early and late stages of disease. Hurdles to be overcome might include the heterogeneous nature of the OA synovium and the complex network of interactions that are involved in synovial inflammation, fibrosis and cartilage damage, processes that often cannot be completely dissociated and evaluated using current imaging techniques. Research advances in phenotype-specific treatment options have provided several novel therapies that could target the inflammatory component of OA<sup>256,257</sup>. However, further research is needed to determine whether synovial inflammation is relevant for diagnosis, risk stratification or identification of therapeutic targets.

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## Author contributions

E.S.-L., R.C., N.E.L. and M.G. researched data for the article and contributed substantially to discussion of the content. All authors wrote the article. All authors reviewed and/or edited the manuscript before submission.

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