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## REVIEW

# Pathogenic stromal cells as therapeutic targets in joint inflammation

(NRR-17-203V4)

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**Key words:** stroma, inflammation, musculoskeletal, fibroblast, joint, mesenchymal

1 **Abstract**

2

3 Knowledge of how the joint functions as an integrated unit in health and disease  
4 requires an understanding of the stromal cells populating the joint mesenchyme,  
5 including fibroblasts, tissue resident macrophages and endothelial cells.  
6 Physiological and pathological mechanisms in these mesenchymal cells that define  
7 the joint have begun to cast new light on why joint inflammation persists. In this  
8 review, we highlight how the shared embryological origins of fibroblasts and  
9 endothelial cells may shape the behaviour of these cell types in diseased adult  
10 tissues. We review the molecular mechanisms by which cells of mesenchymal origin  
11 sustain inflammation in the synovial membrane and tendons, highlighting the  
12 importance of recently discovered fibroblast subtypes and their associated cross talk  
13 with endothelial cells, tissue resident macrophages and leukocytes. Finally, we  
14 discuss how this knowledge shapes the future therapeutic landscape, emphasising  
15 the requirement for new strategies to address the pathogenic stroma and associated  
16 cross talk of leukocytes with cells of mesenchymal origin.

17

18 **Key points**

19

- 20 • Joint inflammation and tissue damage are mediated by stromal cells ~~derived~~  
21 from of embryonic mesodermal origin
- 22 • Stromal activation and inflammation “memory” of previous inflammatory  
23 insults are shared ~~disease~~ mechanisms exhibited by fibroblasts, tissue  
24 resident macrophages and ~~and~~ endothelial cells
- 25 • Recent advances characterising the phenotype and function of cells of  
26 mesenchymal origin highlight thee distinct fibroblast subtypes mediatinge joint  
27 inflammation and tissue damage
- 28 • Mesenchymal stromal cell niches and their interactions with leukocytes are  
29 implicated in the persistence of joint inflammation
- 30 • To be effective, strategies to treat residual joint disease should target  
31 pathogenic stroma and associated immune cell cross talk

32

33

## 34 **Introduction**

35

36 Chronic inflammatory diseases affecting joint soft tissues include arthritis  
37 (synovium and cartilage), enthesopathy and tendinopathy. Collectively, these  
38 diseases comprise a significant global economic burden<sup>1</sup>. Each are characterized  
39 by inflammation of mesenchymal tissues that form the synovium, tendons,  
40 ligaments and joint capsule and in some cases structural damage to bone and  
41 cartilage. Inflammation of these tissues is broadly characterized by leuckocyte  
42 infiltration, fibroblast accumulation and neovascularization supporting cell  
43 expansion. In this article, we first review the pathophysiological basis of  
44 inflammation and tissue damage with respect to the embryological origins of joint  
45 mesenchymal tissues. We next discuss the stromal cell types populating joint  
46 mesenchymal tissues, including fibroblasts, tissue resident macrophages (TRM)  
47 and endothelial cells (vascular and lymphatic), highlighting their contribution and  
48 roles in chronic synovial inflammation and tissue damage. Finally, we discuss  
49 potential future therapeutic strategies to target inflammation across joint  
50 mesenchymal tissues that address the pathogenic stroma and associated immune  
51 cell cross talk.

52

### 53 **1.0 Embryological origins of the tissues that mediate inflammation** 54 **and damage across the whole joint organ**

55

56 Inflammation and tissue damage are pivotal pathological processes affecting tissues  
57 structures across the whole joint organ. To further understand the mechanisms and  
58 inter-relationships underpinning these fundamental disease processes, it is important  
59 to consider the origins of joint tissues, given that an organ is best defined by its  
60 embryological origin as well as function. This section discusses how the  
61 embryological and anatomical origins of the tissues that comprise the joint might  
62 shape inflammation and tissue damage, highlighting how this knowledge informs  
63 understanding of 'disease patterns' across the joint.

64

65 The anatomical basis of inflammation and tissue damage relative to their  
66 embryological origin is summarized in Figure 1. Although parts of the axial skeleton

67 | ~~derive from neural crest, Embryonic~~ mesoderm is the precursor for mesenchymal  
68 | tissues comprising the ~~axial and~~ appendicular skeleton, synovium, cartilage,  
69 | tendons, ligaments, joint capsule and their associated lymphatics and vasculature.  
70 | These joint soft tissues are predominantly composed of cells of mesenchymal origin,  
71 | including fibroblasts, vascular and lymphatic endothelial cells and TRM. The ~~shared~~  
72 | embryological origins of ~~stromal fibroblasts and endothelial~~ cells may shape the  
73 | behaviour of these cell types in diseased adult tissues. Notably, ~~mesoderm derived~~  
74 | fibroblast and endothelial cell populations both undergo sustained phenotypic  
75 | changes after exposure to inflammatory stimuli, exhibiting stromal activation and ~~a~~  
76 | ~~form of tissue~~ 'memory'<sup>2,3</sup>. ~~However, the distinct molecular markers expressed by~~  
77 | ~~these cell types vary, as we later discuss. TRM also exhibit complex activation~~  
78 | ~~states and "memory"~~<sup>4</sup>. The origins and renewal of TRM have been extensively  
79 | reviewed ~~and will not be repeated here~~<sup>5-8</sup>. The majority of TRM are established  
80 | during embryonic development and persist into adulthood, rather than replacement  
81 | from circulating adult monocytes<sup>7,9-14</sup>. During early gestation, macrophages are first  
82 | observed and expand in the extraembryonic yolk sac during primitive hematopoiesis.  
83 | Yolk sac derived hematopoietic stem cells (HSCs) emerge to form bone marrow  
84 | precursor cells, which subsequently gives rise to all immune cell lineages<sup>7,15</sup> (Figure  
85 | 1). Importantly, yolk sac derived TRM are ~~phenotypically~~ distinct from HSC derived  
86 | progeny<sup>10</sup>. The subspecialized adult tissue niches which TRM occupy dictate  
87 | heterogeneity in the phenotype and functions of these cells in health and disease<sup>16</sup>.  
88 | We next review how these mesenchymal cell populations are implicated in mediating  
89 | inflammation and tissue damage in joint disease.

90

## 91 | **2.0 Cells of mesenchymal origin in the ~~healthy and diseased~~ joint**

92

93 | In this section, we focus exclusively on cells of mesenchymal origin including  
94 | fibroblasts, endothelial cells and TRM rather than on haematopoietically derived cells  
95 | whose role in these processes (in particularly inflammation and damage) has been  
96 | well documented<sup>17-19</sup>. We discuss the roles of these cells in normal joint physiology  
97 | and their impact on inflammation and damage in joint disease. We highlight the  
98 | recently identified mechanisms implicated in sustaining synovial inflammation,

99 discussing the molecular features and pathological phenotypes of fibroblast  
100 subtypes, endothelial cells and TRM.

## 101 **2.1 Fibroblasts and the healthy joint**

102

103 The term 'stroma' was originally derived from the Greek word describing "a platform  
104 on which to lie" and is used to describe the supporting substance of tissue. Its  
105 principle role is to maintain the microenvironment required by the parenchyma; the  
106 important functional elements of each body system. The stroma comprises  
107 connective tissue, nerves, vessels and the extracellular matrices (ECM) and fluids  
108 which these cells produce<sup>20</sup>. Joint soft tissues including synovium, capsule, tendon  
109 and enthesis are ~~predominantly composed of mesenchymal stromal cells~~  
110 ~~of cellular and acellular ECM~~. Fibroblasts are the most abundant cell type populating  
111 these joint connective tissues<sup>21</sup> and synthesise the highly organized collagen rich  
112 scaffold necessary for joint structure and movement.

113

114 Fibroblasts are defined by their spindle shaped morphology, the absence of specific  
115 lineage markers of leukocytes, endothelium and epithelium and their ability to adhere  
116 to tissue culture plastic *in vitro*<sup>22</sup>. They are believed to arise from 3 distinct cellular  
117 origins: primary mesenchyme, local epithelial-mesenchymal transition (EMT) or bone  
118 marrow derived precursors (circulating fibrocytes)<sup>23,24</sup>. It is widely accepted that the  
119 majority of fibroblasts originate from primary mesenchymal cells and that fibroblasts  
120 can proliferate to generate new progeny<sup>25,26</sup>. In physiological conditions, fibroblasts  
121 provide mechanical strength to tissues by producing ECM components (type I, III  
122 and V collagen and fibronectin) as well as factors that regulate ECM turnover,  
123 including metalloproteinases (MMPs) and proteins involved in the formation of  
124 basement membranes (type IV collagen and laminin)<sup>27,28</sup>. Fibroblasts synthesise an  
125 array of paracrine factors<sup>29</sup> and exhibit mechanosensitive properties<sup>30</sup> to effect  
126 functional adaptation in normal joint physiology. The intimate relationship between  
127 fibroblasts and mesenchymal stromal cells (MSC) and the clinical use of MSC to  
128 repair damaged tissues has driven a renewed interest in fibroblasts as new  
129 therapeutic targets<sup>21</sup>.

130

## 131 **2.2 Mechanisms sustaining joint inflammation based on pathogenic stroma**

132

## 133 **2.2.1 Fibroblasts and the diseased joint**

134

135 Traditionally, the diversity of stromal cells and in particular fibroblasts and their roles  
136 beyond those of space filling and ECM homeostasis have been underexplored in  
137 inflammation. Mesenchymal tissues in the joint including the synovium, ~~capsule,~~  
138 entheses and tendons undergo phenotypic changes as a consequence of  
139 inflammation<sup>31-33</sup>. These include molecular and structural changes to the ECM,  
140 impacting upon the functional quality of the healed tissue<sup>34</sup>. Whilst it remains  
141 challenging to discern which is the initiating pathogenic cell type, it is clear that  
142 stromal cells populating these tissues provide a niche conducive to sustaining  
143 chronic inflammation<sup>2,35,36</sup>. Recent work shows that fibroblasts vary phenotypically  
144 and functionally at different anatomical sites and contribute significantly to the  
145 identity of individual tissues, providing a so-called 'stromal postcode'<sup>26</sup>. Furthermore,  
146 it is known that, rather than acting as a bystander, fibroblasts are capable of actively  
147 participating and indeed orchestrating inflammation and immunity<sup>36-38</sup>. We next  
148 review how fibroblasts sustain inflammation, highlighting the mechanisms  
149 underpinning their activation, "memory" and phenotypic diversity, with particular  
150 focus on the synovial microenvironment.

151

### 152 ***Fibroblast activation and memory***

153

154 Fibroblast activation is a recognized feature of diseases affecting the joint, whereby  
155 fibroblasts adopt a pro-inflammatory phenotype. This pathological feature has been  
156 identified in cancer<sup>39</sup>, rheumatoid synovium<sup>32,33</sup> and tendon disease<sup>31</sup>. Fibroblast  
157 activation and memory therefore span both innate and adaptive immune responses,  
158 suggesting this is a highly conserved disease mechanism common to tissues of  
159 mesenchymal origin. There is now a growing list of cell surface molecules and  
160 secreted products which collectively provide a fibroblast activation marker "cassette".  
161 These include CD90 (Thy1), CD44, decay accelerating factor (CD55), VCAM-1  
162 (CD106), uridine diphosphoglucose dehydrogenase, and prolyl-4-hydroxylase,  
163 Podoplanin (PDPN/gp38), endosialin (CD248) and Fibroblast Activation Protein  
164 (FAP)<sup>31,36,37,40-42</sup>. Fibroblast activation markers therefore represent important  
165 phenotypic alterations implicated in effecting the switch from resolving to persistent  
166 inflammation<sup>42</sup>.

167  
168 Epigenetic changes are implicated in fibroblast activation and memory. New insights  
169 into the epigenetics of inflammatory rheumatic diseases have been recently  
170 reviewed in detail elsewhere <sup>43</sup>. Prolonged exposure of RA synovial fibroblasts to  
171 TNF $\alpha$  reduce histone H4 levels and promote H4 acetylation <sup>44</sup>. This study showed  
172 that TNF $\alpha$  removed the chromatin barrier from the CXCL10 promoter, permitting  
173 abundant binding of NF- $\kappa$ B family transcription factors and recruitment of  
174 transcriptional machinery <sup>44</sup>. DNA methylation is another important epigenetic  
175 modification identified in RA synovial fibroblasts occurring during the early stage of  
176 disease <sup>45</sup>. Further studies are required to identify the mechanisms underpinning  
177 DNA methylation and there appears to be important prognostic potential for  
178 differentially methylated genes as disease biomarkers <sup>45</sup>. The activated and  
179 aggressive phenotype of RA synovial fibroblasts is associated with global DNA  
180 hypomethylation <sup>46</sup>. Gaur *et al.* investigated if microRNAs moderate the methylation  
181 status of RA synovial fibroblasts, showing L-methionine increased DNA methylation  
182 compared to betaine <sup>47</sup>. Collectively these studies advance our understanding of how  
183 epigenetic changes are implicated in fibroblast activation and memory, informing  
184 future strategies to selectively target pathogenic fibroblasts.

185 ~~Recent work shows that tissue resident fibroblasts help define the pattern of joints~~  
186 ~~involved, not only in arthritis but in other diseases with a prominent stromal~~  
187 ~~component~~ <sup>39</sup>.

188 ~~Importantly, this concept of epigenetically-driven anatomical diversity of synovial~~  
189 ~~fibroblasts provides an attractive mechanism to explain the clinical observations that~~  
190 ~~different types of arthritis affect distinct types of joints. For example, OA and PsA~~  
191 ~~often involve the distal interphalangeal joints, whereas RA is frequently symmetrical~~  
192 ~~and more commonly affects the MCP joints. In contrast, AS mainly targets spinal~~  
193 ~~ligaments and enthesal tissue~~ <sup>40</sup>. Such studies have prompted improved  
194 ~~characterization of the phenotypes of fibroblast subsets and their different proposed~~  
195 ~~roles. In RA, synovial fibroblasts undergo distinct changes in function, including loss~~  
196 ~~of immunosuppressive response in early disease, followed by later acquisition of an~~  
197 ~~immuno-stimulatory phenotype~~ <sup>44</sup>.

198

199 | Fibroblasts from different joint tissues maintain their phenotype, positional memory  
200 | and topographic differentiation despite culture *ex vivo*. Fibroblasts isolated from RA  
201 | synovium or diseased tendon exhibit stromal 'memory', whereby these cells show an  
202 | enhanced subsequent capacity to respond to an additional inflammatory stimulus  
203 | <sup>2,31,44</sup>. Therefore, sustained expression of activation markers by fibroblasts in the joint  
204 | reflects their 'primed' status after exposure to an inflammatory stimulus. ~~In addition to  
205 | fibroblast activation, this concept of stromal memory also spans innate and adaptive  
206 | immunity, suggestive of a highly conserved disease mechanism across tissues of  
207 | mesenchymal origin.~~ The processes underpinning innate memory have been  
208 | extensively reported for leukocytes <sup>48,49</sup> and are gaining acceptance in tissue  
209 | resident cells of mesenchymal origin. Engagement of TLR4 and downstream  
210 | activation of the NFκB pathway is a prominent pathological feature of fibroblasts  
211 | populating inflamed joint tissues <sup>2,31,44</sup>. These studies suggest that fibroblast memory  
212 | is associated with altered NFκB responsiveness to an inflammatory stimulus <sup>50</sup>.  
213 | Given the longevity of fibroblasts as tissue resident cells and the relatively low rates  
214 | of tissue cell turnover in the joint <sup>51</sup>, the effects of stromal memory in tissues such as  
215 | synovium and tendon are likely to be long lived. In contrast, dermal fibroblasts show  
216 | higher rates of turnover and do not exhibit ~~stromal~~ memory, suggesting this ~~disease~~  
217 | ~~mechanism~~process of stromal memory may vary according to anatomical location  
218 | <sup>2,52,53</sup>. ~~Rheumatic diseases follow a characteristic anatomical pattern of joint and  
219 | organ involvement. Mechanisms regulating the predilection of specific joints for  
220 | developing particular forms of arthritis (for example osteoarthritis (OA) compared to  
221 | rheumatoid arthritis (RA)) have been reviewed in detail <sup>54</sup>. These include site-specific  
222 | local cell types driving disease, systemic triggers affecting local cell types and site-  
223 | specific exogenous factors activating cells locally. Therefore the mechanisms  
224 | underpinning activation of stromal cells depends on the local anatomical tissue  
225 | niche <sup>54</sup>.~~

### 227 **Fibroblast diversity**

228 | ~~Recent work shows that tissue resident fibroblasts help define the pattern of joints  
229 | involved in RA <sup>55,56</sup>. The concept of epigenetically-driven anatomical diversity of  
230 | synovial fibroblasts provides an attractive mechanism to explain the clinical  
231 | observations that different types of arthritis affect distinct types of joints. For~~

232 example, OA and PsA psoriatic arthritis often involve the distal interphalangeal joints,  
233 whereas RA is frequently symmetrical and more commonly affects the MCP joints. In  
234 contrast, AS ankylosing spondylitis (AS) mainly targets spinal ligaments and  
235 enthesal tissue<sup>57</sup>. Such studies have prompted improved characterization of the  
236 phenotypes of fibroblast subsets and their different proposed roles. In RA, synovial  
237 fibroblasts undergo distinct changes in function, including loss of  
238 immunosuppressive response in early disease, followed by later acquisition of an  
239 immuno-stimulatory phenotype<sup>58</sup>. ~~Fibroblasts show considerable variability according~~  
240 ~~to genetic and hormonal factors between individuals~~. Highly conserved homeobox  
241 (HOX) transcription factors specify regional identities of cells and tissues  
242 throughout the body<sup>59,60</sup> and adult fibroblasts retain key features of embryonic  
243 positional HOX gene expression<sup>56</sup>. Fibroblasts also vary according to their  
244 anatomical location in relation to tissue structures at an individual site and the  
245 exogenous stimuli which they receive<sup>54,56,61</sup>. Whether variability can be attributed to  
246 the plasticity of individual fibroblasts necessary for responding to different  
247 environmental cues and whether phenotypic variation can be used to define distinct  
248 subsets of fibroblasts specialized for different niches remains unclear.

249  
250 The synovium is composed of lining and sub-lining layers of fibroblasts which vary in  
251 terms of phenotype and function ~~according to their anatomical sub-location~~. Single  
252 cell RNA sequencing and immunohistochemistry have revealed that RA synovial  
253 fibroblasts can be broadly characterized into 3 subsets, highlighted in Figure 2.  
254 Synovial lining fibroblasts are CD34<sup>-</sup>CD90<sup>-</sup>CD55<sup>+</sup> and Cadherin 11<sup>+</sup>. This lining  
255 subset synthesizes MMP-1 and MMP-3 which mediate tissue damage in the  
256 inflamed joint<sup>62</sup>. Fibroblasts populating the synovial sublining are predominantly  
257 comprised of 2 populations. CD34<sup>+</sup>CD90<sup>-</sup> fibroblasts release CXCL12, CCL2 and IL-  
258 6 and ~~mediate drive fibroblast accumulation cell proliferation~~ and invasion. A second  
259 population of CD34<sup>-</sup>CD90<sup>+</sup> fibroblasts with a pro-inflammatory phenotype highly  
260 express markers of ~~stromal~~ fibroblast activation<sup>62,63</sup>. These ~~'pathogenic'~~ fibroblast  
261 subsets between them degrade articular cartilage, mediate stromal memory, sense  
262 tissue damage via TLR4 activation and have altered responsiveness to signalling  
263 pathways converging on NFκB responsiveness<sup>26,33,50,62</sup> (Figure 2). Having  
264 highlighted the complexity of discrete synovial fibroblast subtypes, we next discuss

Comment [MOU1]: Chris can you suggest a reference for this statement?

265 the phenotypes and functions of other mesenchymal cell types including endothelial  
266 cells and TRM and their respective roles in joint ~~health and~~ disease.

267

### 268 **2.2.2 The endolymphatic niche in the ~~healthy and~~ diseased joint**

269

270 Other mesenchymal stromal tissues including the vasculature and lymphatics  
271 contribute to sustaining inflammation across the joint organ. Neo-angiogenesis is a  
272 prominent feature of disease of mesenchymal joint tissues and impacts upon  
273 changes in tissue architecture and pain perception<sup>64</sup>. In health, vascular endothelial  
274 cells regulate blood flow, vessel wall permeability and leukocyte extravasation into  
275 tissues, regulating the inflammatory process<sup>65-68</sup>. In lymph nodes and tertiary  
276 lymphoid tissues, high endothelial vessels (HEVs) provide specialized  
277 microenvironments for efficient entry of lymphocytes into tissues in an L-selectin  
278 dependent process<sup>69</sup>. The phenotypes of endothelial cells change as inflammation  
279 transitions from acute to chronic and also between activation of innate and adaptive  
280 immune systems<sup>67</sup>. Endothelial cell phenotypes are poorly characterized in tendon  
281 and enthesal tissues. However, in RA synovium, these cells have been described  
282 as activated, angiogenic, apoptotic and leaky, a process found in many tumour  
283 microenvironments<sup>70</sup>. During prolonged exposure to inflammatory stimuli endothelial  
284 cells become activated, exhibit memory and express adhesion molecules including  
285 ICAM, VCAM-1 and CD31 (PECAM-1)<sup>3,71-73</sup> (Figure 2). These activated endothelial  
286 cells ~~subsequently also~~ present chemokines and initiate leukocyte migration from  
287 blood to local tissues<sup>70</sup>. Endothelial activation is a cause and consequence of  
288 endothelial dysfunction<sup>74,75</sup>, culminating in increased microvascular permeability,  
289 extravasation of plasma and joint oedema. Release of angiogenic factors including  
290 VEGF triggers angiogenesis, provide necessary nutrients and oxygen to meet the  
291 metabolic demands of the inflamed tissue. Importantly, neo-angiogenesis further  
292 promotes the retention and survival of immune cells at inflamed sites, thereby  
293 sustaining chronic inflammation<sup>38</sup>. These angiogenic processes occur during normal  
294 inflammatory immune responses (i.e vaccination)<sup>76</sup>, however whether angiogenesis  
295 that occurs in joint disease is a cause or effect of pathology remains unclear.

296

297 Stromal lymphatic vessels form a one-way conduit for tissue fluid and leukocytes in  
298 health and disease<sup>77</sup>. During adaptive immune responses, antigen presenting cells

299 travel to lymph nodes via lymphatic vessels, which highly express PDPN, implicated  
300 in ~~stromal~~ fibroblast activation<sup>78</sup>. The permeability of lymphatic vessels is a tightly  
301 regulated dynamic process that alters during health and disease<sup>79</sup>. Lymphatic  
302 vessel growth (lymphangiogenesis) is a primary response during acute inflammation,  
303 which becomes dysregulated in chronically inflamed adult tissues<sup>80</sup>. In experimental  
304 murine models of inflammatory arthritis, lymphatic vessels and nodes draining the  
305 diseased joint undergo an initial expansion phase to expedite lymphatic clearance.  
306 This expansion phase is followed by a collapsed phase, characterized by structural  
307 damage to lymphatic vessels and reduced lymphatic clearance<sup>79,81</sup>. Studies  
308 demonstrate alteration in lymphatic vessel function and lymph node volume also  
309 occur in patients with RA flare<sup>82</sup>. Therapies targeting aberrant lymphatic function  
310 have shown promise in preclinical models of inflammatory arthritis and may prove  
311 efficacious in RA<sup>79</sup>.

312

### 313 **2.2.3 Tissue Resident Macrophages in the ~~healthy and~~ diseased joint**

314

315 TRM mediate a diverse range of biological actions. They are appropriately positioned  
316 and transcriptionally primed to respond to local environmental challenges,  
317 maintaining tissue homeostasis. TRM direct immune surveillance, induce  
318 inflammation and promote subsequent resolution, ~~reviewed in detail elsewhere~~<sup>34,83</sup>.  
319 Given the biological complexity of these roles, TRM are highly heterogeneous and  
320 exhibit diverse phenotypic and functionally distinct subtypes within a single tissue  
321 type<sup>5,84</sup>.

322

323 In inflamed synovium, TRM mediate immune surveillance through expression of a  
324 variety of ~~pattern recognition receptors~~DAMPs, notably Toll-like receptors (TLR)  
325 TLR2 and TLR4 and facilitate the recruitment of infiltrating leukocytes, including  
326 monocyte derived macrophages<sup>85-87</sup>. TRM induce joint inflammation through release  
327 of TNF $\alpha$ , IL-1 $\beta$  IL-6, GM-CSF and PGE<sub>2</sub>, driving fibroblast ~~accumulation~~proliferation,  
328 angiogenesis, leukocyte recruitment and tissue damage via protease secretion  
329 (Figure 2). The essential role of non-classical Ly6C-monocytes has been reported in  
330 murine arthritis models<sup>88</sup>. This study highlights the phenotypic heterogeneity of  
331 synovial TRM, demonstrating how macrophage activation status regulates disease

332 progression and resolution. In support of this, human RA synovial macrophages  
333 exhibit distinct transcriptional profiles associated with disease activity and therapy <sup>89</sup>.  
334 However, distinction between TRM and infiltrating macrophages is currently  
335 hampered by a lack of specific markers that distinguish between these populations in  
336 diseased human tissues.

337

338 | The pro-inflammatory milieu in the inflamed synovium triggers an active process of  
339 lipid mediator class switching and the subsequent release of families of specialized  
340 proresolving mediators (SPM). These include lipoxins, resolvins, protectins and  
341 maresins, that are generated via transcellular biosynthesis and are concerned with  
342 mediating resolution of inflammation<sup>90-94</sup>. These bioactive lipid mediators initiate  
343 programmes which halt neutrophil infiltration, potentiate monocyte recruitment,  
344 moderate vascular permeability and promote phagocytosis and drainage of apoptotic  
345 cells<sup>95</sup>. The mechanisms mediating resolution in inflammatory arthritis have been  
346 reviewed in detail and are not covered here ~~are reviewed in detail elsewhere~~<sup>96</sup>. TRM  
347 are key regulators of repair and fibrosis across all tissue types <sup>34</sup> and are also  
348 implicated in mediating resolution of inflammation. Distinct populations of resolution  
349 phase macrophages have been identified in systemic murine inflammation models  
350 that express Alox15, Timd4 and Tgfb2, which terminate leukocyte recruitment and  
351 promote clearance <sup>97</sup>. However, the precise phenotypes of TRM mediating effecting  
352 resolution in human joint disease requires further investigation.

353

354

355

#### 356 **2.2.4 Cross talk between cells of mesenchymal origin**

357

358 Having highlighted the molecular features and phenotypes of mesenchymal cells  
359 and their roles in mediating joint pathology, we next discuss how cross talk between  
360 these cell populations sustains inflammation. Damage sensing mechanisms,  
361 cytokine and chemokine gradients are pivotal pathological processes involving cross  
362 talk between fibroblast, endothelial cell, TRM and leukocyte populations that sustain  
363 inflammation in the diseased joint <sup>26,98,99</sup>.

364

365 RA synovial fibroblasts act as sentinel cells that can “sense” tissue damage. This  
366 occurs via the binding of damage associated molecular patterns (DAMPs) including  
367 HMGB1, heat shock and S100 proteins<sup>100,101</sup>. [Tenascin-C a matrix protein induced](#)  
368 [upon tissue damage also activates TLR4 mediated sterile inflammation](#)<sup>102</sup>. Binding  
369 of these ligands to TLR4 induces a high alert state, favouring the development of  
370 chronic inflammation<sup>50,103</sup>. Engagement of TLR4 activates Myd88 signalling  
371 pathways, inducing pro-inflammatory cytokine release via NFκB activation<sup>48</sup>.  
372 Consequently, activated synovial fibroblasts are primed to release a broad range of  
373 pro-inflammatory mediators. These localised cytokine and chemokine gradients  
374 promote the migration, retention and survival of leukocytes and TRM,<sup>42,104</sup> creating a  
375 complex functional syncytium conducive to sustaining inflammation, highlighted in  
376 Figure 3. The processes mediating leukocyte trafficking between stromal  
377 compartments in RA are recently reviewed in detail elsewhere<sup>105</sup>.

378

#### 379 *Fibroblast – immune cell cross talk*

380

381 RA synovial fibroblasts promote leukocyte retention via release of cytokines and  
382 chemokines and via contact with other cells of mesenchymal origin. Pro-  
383 inflammatory cytokines released by retained monocytes, T cells and TRM including  
384 IFN $\gamma$ , TNF $\alpha$  and IL-1 $\beta$  induce activated synovial fibroblasts to release high levels of  
385 PGE<sub>2</sub>, GM-CSF, IL-6. These cytokines exert differing effects on leukocyte activation.  
386 PGE<sub>2</sub> moderates chemokine production and promotes Th2, Th17 and Treg  
387 responses<sup>106</sup>. IL-6 drives CD4+ T cells towards Th17 activation<sup>107</sup>, whereas GM-  
388 CSF promotes neutrophil survival and monocyte differentiation in the inflamed  
389 synovium<sup>26,108</sup>. [Nguyen et al. demonstrated that IL-6 and other inflammatory](#)  
390 [cytokines and chemokines are regulated by a positive feedback loop that selectively](#)  
391 [operates in fibroblasts involving leukemia inhibitory factor \(LIF\), LIF receptor and](#)  
392 [STAT4](#)<sup>109</sup>. TGF $\beta$ , also found at high levels in RA synovium induces persistent  
393 expression of CXCR4 on synovial T cells, leading to their active CXCL12 mediated  
394 retention, providing an additional mechanism for immune cell retention<sup>110</sup>. RA  
395 synovial fibroblasts also release a repertoire of chemokines, generating a gradient  
396 consisting of CCL2, CCL4, CCL5, CCL8, CXCL8, CXCL12 and IFN $\beta$ <sup>26,111,112</sup>. This  
397 chemokine gradient actively promotes the recruitment, retention and survival of

398 monocytes and CD4+ T cells at the inflamed synovial site (Figure 3). CXCL12,  
399 VCAM-1 (CD106) and IL-6 therefore constitute part of a 'stromal address code',  
400 critical for leukocyte survival and differentiation <sup>26</sup>.

401

#### 402 *Endothelial cell cross talk*

403

404 Resident stromal cells populating inflamed synovium modulate the ability of  
405 endothelial cells to recruit leukocytes via release of soluble mediators or direct cell-  
406 cell contact. ~~Stromal~~ Fibroblasts isolated from healthy patients are known to  
407 regulate the cytokine-sensitivity of vascular endothelium, while fibroblasts associated  
408 with chronic inflammation adopt a pro-inflammatory phenotype <sup>29,113</sup>. Cytokine and  
409 chemokine gradients mediate and sustain cross talk between endothelial cell,  
410 synovial fibroblast and TRM populations. IL-6, TGF $\beta$ 1 and VEGF released from TRM  
411 provide the necessary cues to promote an angiogenic environment required to  
412 sustain endothelial cell activation and dysfunction (Figure 3). This is supported by  
413 antibody neutralisation of IL-6, which diminished the ability of endothelial cells to bind  
414 lymphocytes in co-cultures with RA fibroblasts <sup>29</sup>.

415

416 The RA synovial fibroblast milieu further sustains an angiogenic environment through  
417 chemokine gradients comprising CXCL1-5 and CXCL8 <sup>26</sup> (Figure 3). RA fibroblasts  
418 regulate expression of endothelial cell adhesion molecules, potentiate leukocyte  
419 extravasation <sup>58</sup> and induce unstimulated HUVEC to bind flowing lymphocytes via a  
420 CXCR4-CXCL12 dependent manner <sup>29</sup>. Consequently, the interactions between cells  
421 of mesenchymal origin create and sustain an inflammatory milieu, whereby synovial  
422 inflammation persists and potentially becomes independent of its inciting cause. We  
423 next consider how persistent inflammation culminates in tissue damage across soft  
424 tissues that comprise the joint.

425

### 426 **2.3 Mesenchymal cells and their role in joint damage**

427

428 In health, early damage repair mechanisms maintain the integrity of joint soft tissues.  
429 In joint disease, sustained inflammation, tissue remodeling and fibrosis ensue,  
430 resulting in irreversible tissue damage. We next discuss how cells of mesenchymal

431 origin mediate fetal scarless healing and highlight the mechanisms by which they  
432 induce damage across adult joint tissues.

433 In contrast to normal adult tissues, early human and murine fetal wounds and  
434 wounds in Nude (FoxN1 deficient) mice heal without scar formation<sup>114</sup>. Fetal wounds  
435 show diminished numbers of immune cells and lower levels of cytokines compared  
436 to adult tissues<sup>115-118</sup>. Differences between embryonic and adult tissue healing are  
437 also attributed to the milieu of pro-fibrotic growth factors released by TRM, including  
438 those of the TGF $\beta$  family. TGF $\beta$ 1 levels are reduced and this growth factor shows  
439 accelerated clearance in embryonic compared to adult tissue repair<sup>119-121</sup>.  
440 Collectively these studies indicate a role for immune cell derived cytokines including  
441 TNF $\alpha$  and TGF $\beta$  in tissue scarring and healing<sup>122</sup>. Other studies highlight differences  
442 between fetal and adult fibroblasts and localized production of MMP-9 and MMP-13  
443 in the scarring process<sup>114</sup>. Fetal fibroblasts show enhanced synthetic function,  
444 increased rate of turnover of collagen, hyaluronic acid, ECM components and  
445 increased migration velocity compared to adult fibroblasts, suggesting rapid healing  
446 may also play a role in scarless tissue repair<sup>123-125</sup>.

447 In adult tissues, fibroblasts and TRM directly contribute to joint destruction, bony  
448 erosions and remodeling through expression of enzymes such as MMPs<sup>126</sup>. MMP-2,  
449 MMP-9 and MMP-13 have been specifically implicated in the pathogenesis of RA and  
450 OA<sup>127</sup>. MMP-9 is also upregulated by CXCL12 (SDF-1) a key chemokine secreted by  
451 synovial fibroblasts<sup>128</sup>. FAP is highly expressed within RA synovium and co-localises  
452 with MMP-13, where it appears to play a role in tissue degradation<sup>129</sup>. Cathepsins, a  
453 major group of proteases involved in joint remodeling are also upregulated in the  
454 diseased joint<sup>130</sup>. Additionally fibroblasts can indirectly contribute through cross talk  
455 with TRM and lymphocytes, further amplifying processes driving tissue damage  
456 (Figure 3), whilst also presenting antigen to tissue infiltrating lymphocytes<sup>131</sup>.

457  
458 Pathological conditions in which cells of mesenchymal origin play a role include  
459 chronic inflammation (e.g. RA, chronic skin wound healing), tissue fibrosis (e.g.  
460 COPD) and cancer (e.g. breast cancer). Interestingly, while these diseases differ  
461 dramatically in aetiology and genetic predispositions, they converge in terms of  
462 phenotype and function of the stromal component. Fibroblasts expand in the RA

463 synovial tissue and in the tumor parenchyma, while fibrosis is characterized by  
464 profound changes in myofibroblast phenotype and function across different organs  
465 such as the lungs and kidneys<sup>132</sup>. Whether these fibroblast properties are intrinsic  
466 phenotypic changes acquired as a consequence of exposure to chronic  
467 inflammation, or are derived from the conditioning of the pathogenic infiltrating cells  
468 is still under investigation and seems to differ in the different conditions<sup>37</sup>. Lafevre *et*  
469 *al* reported epigenetically programmed aggressive cells may “spread” arthritis from  
470 inflamed to uninfamed joints in the early stages of disease,<sup>133</sup>. PDPN expressing  
471 lining synovial fibroblasts are migratory and mediate release of cartilage destructive  
472 MMPs<sup>33,62</sup>. Collectively, these data raise the possibility of distinct mesenchymal cell  
473 subsets implicated in mediating the effects of tissue damage in the diseased joint.  
474 We next discuss how the possibility of selectively targeting pathogenic stromal  
475 subpopulations mediating inflammation and tissue damage informs the development  
476 of future strategies to successfully treat joint disease.

477

### 478 **3.0 Shaping the future landscape: therapeutic targeting of** 479 **mesenchymal cells**

480

481 Cells of mesenchymal origin including fibroblasts, TRM and endothelial cells  
482 constitute the major cell types populating joint soft tissues. We have discussed the  
483 roles and mechanisms by which these cells mediate joint inflammation, highlighting  
484 their ability to act as immune sensing-sentinel cells, their capacity for activation,  
485 positional memory and their altered phenotypes comprising multiple cellular sub-  
486 populations. Multidirectional cross talk between stromal cell populations further fuels  
487 the development of persistent inflammation. Given these important roles and  
488 associated biological complexities, it is likely that residual disease activity in patients  
489 treated with immune therapies may be attributable to stromal mediated inflammatory  
490 responses, which are refractory to current therapies that target immune cell  
491 populations<sup>134</sup>. New therapeutic approaches are therefore required to ‘break the  
492 cycle and reset the system’, particularly in scenarios where inflammation becomes  
493 independent of the inciting stimulus. Given the limited capacity of joint tissues to  
494 regenerate once damaged, there are significant challenges associated with curbing  
495 tissue damage, which might be accomplished through moderating persistent

496 inflammation as a driver of fibrosis. We next discuss the requirement for future  
497 strategies to address the pathobiology concerned with the stromal  
498 microenvironment, targeting cells of mesenchymal origin. We review the drug  
499 classes in current clinical use, those in early phase clinical trials and strategies with  
500 pre-clinical potential to target stromal mediated joint disease. The cellular and  
501 molecular targets and the mechanism of action through which these drug classes  
502 function are summarized in Table 1.

503

#### 504 *Existing licensed therapies*

505

506 Nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide  
507 symptomatic relief for a broad array of conditions targeting inflammation and pain.  
508 Their clinical use in the management of a multitude of diseases affecting the joint is  
509 well established<sup>135-138</sup>. These therapies target fibroblasts, TRM and endothelial cells  
510 via differing biological modes of action. Inhibition of COX activity by NSAIDs  
511 dampens release of prostaglandins, leukotrienes and thromboxane A<sub>2</sub>.  
512 Corticosteroids act via the glucocorticoid receptor to inhibit cPLA<sub>2</sub>, regulate  
513 expression of NFκB / MAPK target genes and dampen release of inflammation  
514 initiating eicosanoids. Whilst NSAIDs and corticosteroids continue to provide  
515 background anti-inflammatory therapy for many rheumatic diseases, they are both  
516 associated with well documented adverse systemic effects. Importantly, COX-2  
517 selective NSAIDs also dampen protective endogenous resolution responses<sup>139,140</sup>,  
518 which may paradoxically impede the capacity of inflamed joint tissues to heal.

519

520 Monoclonal antibodies enable precise molecular targeting of cytokines mediating  
521 joint inflammation. The biological modes of action and efficacy of therapeutic  
522 inhibitors of IL-1, IL-6, TNFα and IL-17 in current clinical use are well reported and  
523 listed in Table 1. One disadvantage associated with selective cytokine inhibition is  
524 the failure of this approach to fully target stromal mediated inflammatory responses  
525 and address the complex multidirectional cross talk between mesenchymal cell  
526 populations. Similarly targeting chemokine gradients is an attractive strategy to  
527 moderate leukocyte retention<sup>141</sup>. However chemokine antagonists including  
528 AMD3100 targeting CXCR4 are associated with adverse systemic effects<sup>142</sup> and the

529 plethora of chemokines mediating stromal inflammatory responses presents a further  
530 therapeutic challenge.

531

### 532 *Therapies in early phase clinical trials*

533

534 GM-CSF, predominantly produced by activated T cells, monocytes and  
535 macrophages is also released by tissue resident cells of mesenchymal origin <sup>143</sup>.

536 Humanised IgG1 monoclonal antibodies to GM-CSF prevent interaction of this  
537 cytokine with its receptor, reducing downstream signalling pathways converging on

538 NF $\kappa$ B. GM-CSF has shown potential as a therapeutic target in autoimmune and  
539 inflammatory disorders, including RA. ~~Early phase clinical trials demonstrated~~

540 ~~disease activity scores reduced in mavrilimumab treated patients with moderate RA.~~

541 Therapies targeting GM-CSF or its receptor have shown encouraging results in more  
542 recent pre-clinical studies and are reviewed in detail elsewhere <sup>143</sup>. Recent phase IIb

543 studies have demonstrated that long term mavrilimumab treatment maintained

544 clinical responses and was well tolerated in RA patients with inadequate response to

545 DMARD's<sup>144</sup>. Further investigation is required to determine the efficacy of GM-CSF

546 targeted therapies to modulate stromal mediated inflammatory responses in the joint.

547 Kinase inhibitors targeting JAK and SYK signalling pathways have been investigated  
548 for their therapeutic utility to reduce cytokine release through JAK STAT <sup>145,146</sup> or

549 MAPK / PKC <sup>147,148</sup> blockade respectively (Table 1). Baricitinib, an oral reversible

550 inhibitor of JAK1 and JAK2 has shown therapeutic value in RA patients. This  
551 treatment was associated with significant clinical improvements in patients with an

552 inadequate response to methotrexate compared with placebo and adalimumab  
553 treated groups <sup>149</sup>. Protein kinase inhibitors target a broad range of cells types with

554 reported off target effects, highlighting the importance of understanding the  
555 pharmacology of these drugs beyond the kinome <sup>150</sup>.

556

### 557 *Potential future strategies to target pathogenic stroma*

558

559 Developments in cancer medicine targeting cancer associated fibroblasts populating  
560 tumour stroma have informed potential future strategies to target pathogenic stroma

561 in rheumatic disease <sup>151,152</sup>. Targeting pathogenic stroma presents a considerable

562 therapeutic challenge due to the biological complexity underpinning activation,  
563 memory and phenotypic diversity exhibited by these mesenchymal cell populations.  
564 Potential future strategies to treat residual rheumatic disease might include targeting  
565 activated fibroblast subtypes, use of epigenetic modifiers or resolution agonists to  
566 target stromal mediated inflammation. Pre-clinical evidence supporting these  
567 approaches are discussed below.

568  
569 Selective targeting of distinct fibroblast subtypes mediating joint inflammation and  
570 tissue damage is a potential therapeutic strategy to target pathogenic stroma.  
571 Cadherin-11 is known to regulate synovial fibroblast inflammation, synergizing with  
572 IL-1 $\beta$  and TNF $\alpha$  to regulate IL-6 release <sup>153</sup>. This study showed that cad-11 deficient  
573 mice or anti-cad-11 mAb therapies reduced inflammation in arthritic mice,  
574 suggesting that cadherin expression regulates the inflammatory capacity of synovial  
575 fibroblasts. Cyclin dependent kinases regulate cell proliferation and survival via  
576 specific inhibitors (CDKi) and are potential therapies to target fibroblast  
577 **accumulationproliferation** in RA synovium (Table 1). CDK pathways become  
578 dysregulated in cancer, leading to the development of anti-cancer drugs including  
579 the CDKi Roscovitine <sup>154</sup>. In synovial fibroblasts, IL-6 and MMP-1 are known to be  
580 regulated by CDKi p21 <sup>155</sup>. Given that CD34<sup>+</sup>CD90<sup>-</sup> 'immunoregulatory' fibroblasts  
581 are highly proliferative, invasive and produce IL-6<sup>62</sup>, CDKi therapies are a potential  
582 strategy to target this fibroblast subset mediating joint disease.

583  
584 We previously discussed how epigenetic changes are implicated in mediating  
585 **stromal** fibroblast activation and memory. Epigenetic alterations in RA synovial  
586 fibroblasts are listed in Table 1, identifying DNA methylation, histone modification  
587 and miRNA as potential processes to therapeutically target <sup>43,45-47,156</sup>. Moderating the  
588 epigenetic landscape is likely to have broad ranging effects on a variety of cell types,  
589 with off target effects. Hence improved understanding of the pharmacology of these  
590 drugs beyond the epigenome is essential before we can appreciate their potential  
591 utility to treat joint disease.

592  
593 The roles of proresolving mediators in joint health and disease are increasingly  
594 understood, identifying resolution agonists as potential therapies to moderate joint

595 inflammation and promote tissue repair <sup>96</sup>. The biological modes of action of  
596 proresolving mediators or ‘immunoresolvents’ are well established from *in vitro* and  
597 *in vivo* studies and include limiting PMN infiltration, stimulating efferocytosis and  
598 activation of endogenous tissue protective mechanisms <sup>90-93,157,158</sup>. Whilst  
599 immunoresolvents target leukocytes, their biological actions are not associated with  
600 immunosuppression <sup>83,159</sup>. Importantly, proresolving mediators also target fibroblasts,  
601 TRM and endothelial cells types <sup>160-162</sup> and therefore possess the capacity to  
602 modulate stromal mediated inflammatory responses across joint tissues. Approaches  
603 to potentiate resolution processes include dietary supplementation with proresolving  
604 precursors, blocking catabolism of proresolving mediators or local delivery of stable  
605 analogues binding proresolving receptors <sup>96</sup>. The pro-resolving mediator RvD3 was  
606 found to limit leukocyte infiltration and paw joint eicosanoid levels in murine  
607 inflammatory arthritis <sup>163</sup>. The stable epimer 17R-RvD1 significantly attenuated  
608 arthritis severity, cachexia, paw oedema, leukocyte infiltration and shortened the  
609 remission interval, showing cartilage protective actions in murine models of acute  
610 inflammatory arthritis<sup>164</sup>. *In vitro* studies also highlight the capacity of 15-epi-LXA<sub>4</sub>  
611 and MaR1 stable epimers to regulate PDPN, STAT-1 and IL-6 in IL-1 $\beta$  stimulated  
612 diseased human tendon stromal cells <sup>35,165</sup>. Collectively these studies suggest  
613 resolution pharmacology may be an important future therapeutic tool to address  
614 stromal pathobiology in the joint.

615

## 616 **Conclusions**

617

618 Stromal cells of mesenchymal origin including fibroblasts, tissue resident  
619 macrophages and endothelial cells are pivotal populations regulating health and  
620 disease in musculoskeletal tissues. New insights are beginning to reveal the  
621 mechanisms underpinning the activation and dysfunction of mesenchymal stromal  
622 cells and their contribution to sustaining chronic joint inflammation. The discovery  
623 that distinct synovial fibroblast subsets mediate joint inflammation and damage will  
624 inform precision therapeutic targeting of pathogenic stromal cell populations. These  
625 discoveries shape the future therapeutic landscape, presenting exciting new  
626 approaches to address the pathogenic stromal microenvironment. Harnessing the  
627 capacity to modulate cross talk between leukocyte and pathogenic stromal cell

628 populations is a critical barrier to overcome in our quest to advance therapeutic  
629 strategies for patients with refractory joint disease.

630

631 **Glossary of terms**

632

633 **Mesoderm:** Middle embryonic primary germ layer residing between ectoderm and  
634 endoderm

635

636 **Mesenchymal:** Embryonic connective tissue derived from the mesoderm

637

638 **Mesenchymal tissue:** Tissue of the musculoskeletal, circulatory and lymphatic  
639 systems

640

641 **Stromal cell:** Non-haematopoietic, tissue resident cells.

642

643 **Stromal cell activation:** Process whereby stromal cells including fibroblasts, tissue  
644 resident macrophages and endothelial cells adopt a pro-inflammatory phenotype and  
645 express distinct molecular markers after exposure to an inflammatory stimulus.

646

647 **Stromal cell memory:** A change in the capacity of stromal cells to respond to  
648 inflammatory stimuli

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**Table 1: Drugs to target the pathogenic stroma and associated immune cell cross talk in joint disease**

Drug Class	Target Mesenchymal Cell	Molecular Target	Mechanism of Action	References
<b>NSAIDs</b>	Fibroblast (F) Tissue Resident Macrophage (TRM) Endothelial Cell (EC)	COX-1 COX-2	Selective / non-selective inhibition of COX to reduce release of prostaglandins, leukotrienes, thromboxane	135,136
<b>Corticosteroids</b>	F, TRM, EC	glucocorticoid receptor	cPLA2 inhibition regulate NFκB / MAPK target genes reduce prostaglandins, leukotrienes, thromboxane	137,138
<b>Monoclonal Ab</b>				
IL-1	TRM (F)	IL-1R	Reduce effects of inflammasome and caspase activation	166,167
IL-6	TRM, F	IL-6R	Reduce STAT-3 signalling	168-172
TNF	TRM (F)	TNFR 1/2	Reduce NFκB / MAPK signalling	173-178
GM-CSF	TRM, F, EC	GM-CSFR	Reduce JAK STAT, PI3K, MAPK and NFκB signalling	179,180
<u>IL-17</u>	<u>TRM</u>	<u>IL-17R family</u>	<u>Reduce TRAF6, MAPK, TAK1 &amp; NFκB signalling</u>	<u>181-183</u>
<b>Kinase Inhibitors</b>				
JAK inhibitors	F, TRM	JAK1 JAK2 JAK3 TYK2	Blockade of cytokine signalling via JAK STAT	145,146,149
SYK inhibitors	F, TRM	Fcγ receptor	Reduce IL-6 via MAPK / PKC	147,148
<b>Fibroblast activation</b>				
Cadherin-11 mAb	F	Cadherin-11	Reduce MAPK, NFκB, IL-6	153
Cyclin dependent kinase inhibitors (CDKi)	F	CDK1,2,4,6	Inhibit cell proliferation & survival, induce apoptosis	142,154,155
<b>Epigenetic Modifier</b>				
	F	DNA methylation Histone modification  miRNA	Hypomethylation <u>LBH enhancer region</u>  Increase H4 acetylation CXCL10 promoter Increase H4 acetylation IL-6 promoter Reduce miR-22 Reduce miR-20a Reduce miR203	<u>154,159</u> 44 184 185 186 187
<b>Pro-resolving</b>				
17-R RvD1		ALX, DRV1	Chondroprotective	164
Annexin A1	F, TRM, EC	ALX	Chondroprotective, increased TGFβ, prevent apoptosis	188
RvD3		ALX	Reduce leukocyte infiltration, prostaglandins, leukotrienes and thromboxane	163
15-epi-LXA <sub>4</sub>		ALX	Reduced STAT-1, IL-6,	

**Comment [MOU2]:** New references supporting therapies targeting IL-17 for the treatment of PsA

**Comment [MOU3]:** New references supporting therapies targeting DNA methylation

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**Figures:****Figure 1. Embryological origins of mesenchymal tissues in the whole joint organ.**

To further understand the mechanisms and inter-relationships underpinning inflammation and tissue damage across the joint, it is important to consider the embryonic origins of joint tissues, which may shape the behaviour of these cell types in diseased adult tissues. Embryonic Mesoderm is the precursor for mesenchymal tissues comprising the ~~axial and~~ appendicular skeleton, synovium, cartilage, tendons, ligaments, joint capsule and their associated lymphatics and vasculature. Adult joint soft tissues are predominantly composed of cells of mesenchymal origin, including fibroblasts, endothelial cells and tissue resident macrophages (TRM). ~~The shared embryological origins of fibroblasts and endothelial cells shape the behavior of these cell types in diseased adult tissues in terms of their ability to exhibit activation and memory after exposure to inflammatory stimuli.~~ Yolk sac derived TRM are phenotypically genetically distinct from HSC derived lineages. TRM occupy subspecialized niches which dictate their heterogeneity and phenotype in adult tissues.

**Figure 2. Molecular features of cells of mesenchymal origin in Rheumatoid pathological synovium.**

Inset shows topographical location of cell types comprising RA synovium, consisting of lining and sublining layers. Synovial lining fibroblasts (blue) are CD34<sup>-</sup>CD90<sup>-</sup>, express PDPN, CD55 and release MMP-1 and MMP-13 implicated in tissue destruction. Fibroblast subsets concerned with proliferation, accumulation and inflammation ~~proliferation and inflammation~~ occupy the synovial sublining. ~~Proliferative~~ immunoregulatory fibroblasts (green) promote fibroblast accumulation and invasion. ~~These cells~~ express CD34 and release chemokines and cytokines generating gradients that promote leukocyte retention. Pathogenic fibroblasts (red) are a CD34<sup>-</sup>CD90<sup>+</sup> subpopulation that highly express markers of ~~stromal~~ fibroblast activation and exhibit inflammation stromal memory. Pathogenic fibroblasts express TLR4 which mediates the damage sensing properties of these cells and downstream activation of the NFκB pathway via MAPK, JNK and JAK-STAT signalling pathways.

These phenotypic features sustain the pro-inflammatory pathogenic phenotype of this fibroblast subset. Fibroblasts in the synovial sublining are in close proximity to activated endothelial cells, expressing CD31, VCAM-1 and ICAM-1 and CD68<sup>+</sup> tissue resident macrophages (TRM) which release pro-inflammatory mediators and proteases.

**Figure 3: Mechanisms sustaining synovial inflammation, highlighting cross talk between cells of mesenchymal origin and leukocytes.**

Cells of mesenchymal origin including fibroblast subsets, endothelial cells and tissue resident macrophages (TRM) are engaged in multidirectional cross talk, which sustains synovial inflammation. RA synovial fibroblasts promote leukocyte retention via release of cytokines and chemokine gradients and via contact with other cells of mesenchymal origin. Pro-inflammatory cytokines released by retained monocytes, T cells and TRM including IFN $\gamma$ , TNF $\alpha$  and IL-1 $\beta$  induce activated synovial fibroblasts to release high levels of PGE<sub>2</sub>, GM-CSF and IL-6. TGF $\beta$  released by TRM induces persistent expression of CXCR4 on synovial T cells, leading to their active CXCL12 mediated retention. RA synovial fibroblasts also release chemokines including CCL2, CCL4, CCL5, CCL8, CXCL8, CXCL12 and IFN $\beta$  that promotes the recruitment, retention and survival of monocytes and CD4<sup>+</sup> T cells. IL-6, TGF $\beta$ 1 and VEGF released from TRM provide the necessary cues to promote an angiogenic environment required to sustain endothelial cell activation and dysfunction. The RA synovial fibroblast milieu further sustains an angiogenic environment through chemokine gradients comprising CXCL1-5 and CXCL8.