Synovium in the pathophysiology of osteoarthritis

Osteoarthritis (OA) is a progressive joint disease that worsens with age. It is the most common form of arthritis and a serious clinical concern in an aging population. Several risk factors associated with OA pathophysiology, including trauma-, age-, genetics- and obesity-related triggers, cause a cascade of events resulting in the degradation of joint tissues. OA, previously believed to be a disease of cartilage, is now regarded to be a disease of the whole joint, affecting cartilage, bone and synovium. Recent advances in OA research have demonstrated a critical role of synovium in the pathophysiology of OA. Synovitis is now considered as an active component of OA pathogenesis. This article focuses on the current understanding of the role of synovium in the pathophysiology of OA and its potential as a target for future therapeutic interventions.

Osteoarthritis

Osteoarthritis (OA) is the most common form of arthritis and is among the most prevalent chronic human health disorders in an aging population. It is estimated that over 50% of people aged 65 years and older show radiological signs of OA [1]. This disease mostly affects the hands, hips, knees and spine. The risk factors associated with OA pathophysiology include development-, metabolic-(e.g., obesity), trauma-, age-, sex- and genetic-related triggers, which cause a cascade of events that result in the degradation of joint tissues [2]. The most common characteristics of OA include degradation of articular cartilage, osteophyte formation, meniscal degeneration, subchondral bone sclerosis, bone marrow lesions, synovial inflammation and proliferation. OA, previously believed to be a disease of cartilage, is now considered to be a disease of the whole joint, comprised of cartilage, bone and synovium [3]. While the majority of OA research is still focused on cartilage and its degradative mechanisms, advances in image analysis and molecular pathways involved in OA pathogenesis have revealed the critical role that synovium has in the pathophysiology of OA.

Normal synovium: structure, anatomy & function

The synovium is a well-vascularized and innervated membrane that lines the joint capsule and surrounds all noncartilaginous components within articular joints (Figure 1). The membrane consists of two thin layers of specialized cells termed synoviocytes. The lining layer adjacent to the joint is called the intima while the second layer is called the sub-intima. It is the outer portion of the sub-intima that joins with the fibrous capsule of the joint.

There are two types of synoviocytes within the synovium, type A and B. Type A synoviocytes are macrophage-like cells and type B synoviocytes are synovial fibroblasts. Type A cells are responsible for eliminating excess material and pathogens from the joint, and producing and secreting some enzymes and cytokines/chemokines that contribute to inflammation and cartilage degeneration. Type B cells are presumably responsible for producing hyaluronan and for acting as a barrier that keeps synovial fluid in the joint capsule. Overall, both type A and B cells function as integral players in the maintenance of a healthy environment, which is essential for the proper functioning of all tissues.

The synovial membrane secretes synovial fluid and has two fundamental physiological roles. First, it determines what can and cannot enter the joint space. Second, synovial cells are responsible for producing substances such as hyaluronan and lubricin. These are the main components of synovial fluid as they contribute to its mechanical properties. Synovial fluid is responsible for joint lubrication and the transportation of nutrients and oxygen to cartilage.

Synovium in OA

Synovial cells in OA: their role in cartilage degradation

Osteophyte formation is one of the many features that characterize OA. The exact role of...
osteophytes as contributors to OA symptoms is
not well established; however, it is known that
synovial macrophages play an integral role
in their induction [4,5]. Macrophages are involved
in TGF-β-induced osteophyte formation, as
demonstrated by intra-articular injection or over-
expression of TGF-β in murine knee joints [4].
It is evident that TGF-β has a fundamental role
in osteophyte formation as intra-articular injec-
tions induce their formation [6], while blocking
the growth factor using a soluble receptor pre-
vents its formation [7]. If synovial macrophages
are removed from the synovial compartment via
intra-articular injections of clodronate-containing
liposomes, osteophyte production is largely pre-
vented by viral overexpression or intra-articular
injection of TGF-β. However, this may not be
caused by the decreased level of TGF-β in these
joints, but possibly by other factors instead.
For example, macrophage involvement in osteophyte
formation may be mediated by other anabolic
cytokines such as bone morphogenetic proteins
(BMPs). Immunohistochemical studies exhibited
low expression of BMP-2 and -4 upon macrophage
depletion [8]. In addition, intra-articular injections
of BMPs induced osteophyte formation [6].

Of the three main tissues that comprise OA
joints, the synovium is the inflammatory element.
While OA is associated with signs and symptoms
of inflammation, it is not classically considered an
inflammatory disease. Synovial inflammation has
long been an indicator of rheumatoid arthritis;
however, its participation and role in OA is now
widely accepted.

Synovial inflammation, or synovitis, seemingly
results from the synthesis and release of various
cytokines and proinflammatory mediators. In
OA, synovial inflammation affects areas adjacent
to damaged cartilage and bone, and, in addition
to being a contributor to inflammation and car-
tilage degradation, synovitis also contributes to
pain. Synovial inflammation is often localized
and can be asymptomatic. Arthroscopic studies
have demonstrated that localized proliferative and
inflammatory changes of the synovium occur in
up to 50% of OA patients, and the activated
synovium can produce both proteases and cyto-
kines, which contribute to disease progression.
Synovitis is evident at the clinical stage of dis-
ease and is a predictor of disease progression [9].
However, advances in the field have recently sug-
gested that it may also be involved in early OA.
Specifically, arthroscopies performed on patients
presenting with pain support this theory. These
studies showed that almost half of patients pre-
senting with pain demonstrated localized synovial
thickening, and repeat arthroscopies performed
1 year later demonstrated that these patients were
more likely to have progression of cartilage deg-
radation [10]. Synovial thickening, which is a sign
of synovial inflammation, has also been evident
through both ultrasound images and MRI in
patients presenting with knee OA [11]. Finally,
histological studies of OA synovium demon-
strate synovial hypertrophy and hyperplasia, an
increased number of lining cells, and infiltration
of the sublining tissue [12].

Cytokines in synovium:
their role in OA
There are three types of cytokines, namely, cata-
bolic, anabolic and regulatory, produced in OA
synovium that are involved in OA pathology.

Catabolic cytokines
IL-1β, produced predominantly by synovial
macrophages and chondrocytes in OA joints,
is the primary catabolic cytokine found in OA

---

**Figure 1. Normal synovium.**
Synovium and it is expressed at high levels, especially in early OA [13]. It plays an integral role in several pathological conditions. First, it suppresses production of the two main cartilage extracellular matrix (ECM) components, type II collagen and proteoglycan, and stimulates synovial cells to release matrix metalloproteases (MMPs). Upon synovium activation, the cytokines produced further enhanced synovium activation, resulting in a cycle of inflammation and cartilage damage (Figure 2). This presents the basis of cytokine inhibition as a potential method of treating OA. The role of synovial inflammation by cytokines in early OA has been established in studies with patients presenting with pre-OA conditions. For example, a study conducted by Cameron et al. demonstrated that patients with acute cruciate ligament injury had IL-1β in their synovial fluid at time of injury, 3 weeks postinjury and 3 weeks postoperatively, suggesting that synovial inflammation begins early in the disease [14].

IL-15 is a catabolic cytokine newly implicated in OA pathogenesis. Recent studies conducted on early knee OA patients demonstrated increased levels of IL-15 in the synovial fluid compared with late-stage disease, thus making this cytokine a potential target for therapeutic strategies. IL-21, another catabolic cytokine, was also identified in the synovial fluid of early knee OA patients [15].

Other inflammatory cytokines expressed in OA joints include TNF-α, and IL-6, -8, -17 and -18. The production of these factors, as well as IL-1β, is increased in the synovial membrane, as demonstrated in both human and experimental OA [16].

Synovitis results in the overexpression of chemokines and MMPs involved in cartilage degeneration and disease progression. It is believed that synovial inflammation is initially a result of cartilage ECM degradation products, which provoke the release of collagenase and other hydrolytic enzymes from synovial cells, thus possibly resulting in vascular hyperplasia in OA synovium. Subsequently, inflammatory processes cause induction of catabolic cytokines, such as IL-1β and TNF-α, thus causing additional inflammation [17]. These degradation products produce components such as wear particles, soluble cartilage-specific neo-antigens, and microcrystals, which are released into the synovial fluid and phagocytosed by synovial lining macrophages, thus causing synovitis through the synthesis of mediators. Next, mediators travel from the synovial fluid to the cartilage causing cartilage degradation and inflammation, thus presenting a vicious cycle of continuous inflammation and cartilage degradation. Synovitis probably contributes to the dysregulation of chondrocyte function, thus causing an imbalance between anabolic and catabolic processes that allow cartilage ECM to maintain its structural integrity in physiological conditions [18]. Breaking this equilibrium causes the degradation process to take precedence, resulting in the loss of articular cartilage. In addition, these cytokines induce chondrocytes and synovial cells to produce inducible nitric oxide synthase and other proinflammatory cytokines, such as IL-6, -8, -17 and -18, leukemia inhibitory factor, prostaglandin E2, and chemokines, which stimulate nitric oxide production [19]. Nitric oxide contributes to inflammation and destruction by enhancing the activation and production of MMPs, inhibiting the synthesis of anabolic macromolecules, such as collagen and proteoglycan, inhibiting the production of the IL-1 receptor antagonist (IL-1Ra), and promoting chondrocyte apoptosis. There is a close relationship between increased levels of prostaglandins and nitric oxide, and levels of IL-1β and TNF-α in OA synovial fluids and joint tissue, which has been well-established. Immunohistological studies conducted on OA synovium for chemokines, stromelysin and collagenase demonstrated that it exhibits active degradative enzymes.

**Figure 2. Role of synovium in osteoarthritis.**

ADAMTS: A disintegrin and metalloprotease with thrombospondin motif; MMP: Matrix metalloprotease; PGE2: Prostaglandin E2.
Anabolic cytokines
Growth factors are other key mediators involved in OA pathology. Unlike IL-1β and TNF-α, which lead to cartilage degradation, anabolic cytokines, such as TGF-β, which is produced by type B synoviocytes, counteract their effects by promoting cartilage ECM components, and high levels of active TGF-β are found in OA synovial fluids \[20-22\]. Therefore, striking a balance between catabolic and anabolic cytokines is essential for maintaining a normal environment. Depending on which type of cytokine takes precedence, cartilage synthesis will either be promoted or inhibited.

Although these protective mediators promote cartilage ECM components, some display negative effects in other tissues. Specifically, while TGF-β promotes cartilage synthesis, it also causes some adverse side effects. For example, it induces synovial fibrosis, attracts leukocytes to the synovial membrane and induces osteophyte formation.

When anabolic cytokines are administered therapeutically, ECM protein synthesis is promoted, possibly leading to increased cartilage ECM regeneration. Therefore, the growth factors TGF-β and BMP-7 are potential candidates for counteracting IL-1, becoming a possible tool for cartilage repair [23]. However, BMPs contribute to endochondral ossification in addition to upregulating terminal differentiation in chondrocytes. These side effects may be overcome by maintaining the chondrocyte stimulatory capacity of the cartilage, thus allowing this cytokine to be used as a potential therapeutic target.

In addition to the overproduction of catabolic cytokines in the synovium, causing cartilage degradation, insufficient stimulation of anabolic cytokines or cytokines exhibiting insignificant control can result in the same consequences.

Regulatory cytokines
Regulatory cytokines include mediators such as IL-4, -6, -10 and -13. Both IL-10 and -13 have been identified in the human OA synovial membrane [24]. Regulatory cytokines inhibit synovial macrophages from producing the aforementioned catabolic cytokines and upregulate natural inhibitors of catabolic cytokines such as IL-1Ra, and soluble receptors of IL-1β and TNF-α. Specifically, \textit{in vitro} studies have demonstrated that IL-4 inhibits OA synovium from producing IL-1β and TNF-α [25].

- MMPs in synovium
The specific proteases that facilitate cartilage degradation are MMPs, which are metalloenzymes from the metzincin superfamily. In the human genome there exist 25 MMP genes, all of which are zinc- and calcium-dependent endopeptidases. MMPs are first released from cells as inactivated proenzymes. Upon activation, they degrade cartilage ECM proteins, thus contributing to cartilage degradation. Catabolic cytokines found in OA, such as IL-1β and TNF-α, induce or alter MMP expression. Of all the MMPs produced in OA synovium, MMP-13 (a collagenase) is the most highly expressed [26]. Studies using collagen-induced arthritis models have demonstrated its overexpression in the synovium. Compared with cartilage, MMP-13 is more highly upregulated in the synovium. A correlation exists between synovial MMP-2 and -13 and arthroscopically observed cartilage damage, demonstrating that these are the key MMPs in the synovial membrane that contribute to cartilage degradation. Additionally, \textit{in vitro} studies conducted on human OA tissue have shown MMP-10 expression in synovial fibroblasts, OA synovial fluid, and chondrocytes stimulated with catabolic IL-1β and oncostatin M [27].

Rapidly progressing OA possibly represents a more inflammatory process, as levels of both proteases and cytokines are increased. In particular, a specific study of rapidly destructive hip OA observed elevated levels of both MMP-3 and -9 in synovial cells, fluid, plasma and sera [28,29].

Although OA synovial fluid does not exhibit increased MMP activity, MMPs are expressed at high levels. Specifically, OA synovial fluid demonstrates high levels of both pro-MMP-1 and -3 [30]. Upon activation, they possess cartilage-degrading properties in addition to activating other enzymes. Studies using \textit{ex vivo} synovial cultures from knee OA patients have indicated expression of multiple MMPs, such as MMP-2, -9 and -14, as both mRNA and proteins. Levels of latent and activated forms of MMP-2 and pro-MMP-9 were elevated in synovial lesional cultures compared with paralesional cultures, and MMP-2 and -9 mRNA levels demonstrated increased expression [31]. Pro-MMP-9 is produced by synovial OA macrophages and activated by cartilage-derived MMPs. Once MMP-9 is activated, it activates other MMPs. This depicts the cascade of MMP expression that begins at the cartilage; MMPs are induced by the synovium and its role in cartilage degradation is further enhanced by the MMPs it produces.

- Tissue inhibitors of metalloproteases in synovium
Tissue inhibitors of metalloproteases (TIMPs), the major inhibitors in tissue, are produced by fibroblasts and chondrocytes. In the human
genome, the four members of the TIMP family, TIMP-1, -2, -3 and -4, have some basic similarities but exhibit different expression patterns. Although it is found that TIMP-1, a protective mediator, is highly expressed in human OA synovial fluid, MMP activity is not decreased in OA synovial fluid [34]. The same study demonstrated that in acute joint injury there is a rise in the molar ratio of MMP-3 to MMP-1 and TIMP-1, which results in a catabolic environment. Both TIMP-1 and -2 are found in OA synovial membrane; however, their roles have not yet been established.

**A disintegrin & metalloprotease with thrombospondin motif in synovium**

Aggrecan, one of two main components of cartilage ECM, is cleaved by aggrecanases. Aggrecanases belong to the ADAM (a disintegrin and metalloprotease) family and one form of aggrecanase is ADAMTS, a disintegrin and metalloprotease with thrombospondin motif. Studies have shown that ADAMTS-4 and -5 are of particular interest in OA, and may be important therapeutic targets. For example, mouse models expressing a deficiency in ADAMTS-5 and subjected to surgically induced OA display protection against cartilage degradation and subchondral bone changes [32,33]. In addition, immunohistochemical studies conducted on human arthritic synovium showed ADAMTS-5 in the synovial lining and around blood vessels, both pericellularly and in the matrix. Finally, in vitro studies, in which aggrecanase activity was produced by the synovial membrane, demonstrated high levels of aggrecan depletion. Therefore, ADAMTS-5 from the synovium plays a potential role in aggrecan depletion and, more broadly, cartilage degradation [34]. However, the mechanisms by which ADAMTS-4 and -5 are activated and their exact roles in OA pathophysiology are not yet established.

**Targeting the synovium to treat OA**

Osteoarthritis is a localized disease as it only affects one or two joints. Therefore, gene therapy, a localized treatment, has clear clinical value for reducing systemic side effects and maximizing drug delivery. As the synovial membrane is an active component of OA pathology, it is a key therapeutic target for OA treatment. Gene therapy can be used in one of two ways; by altering the processes in the synovial membrane or by targeting the cartilage with soluble factors. As catabolic cytokines, including IL-1β and TNF-α, are key inflammatory mediators associated with synovial inflammation and cartilage destruction, inhibiting their activity locally is potentially beneficial. Blocking the activity of cytokines locally with intra-articular injection directed against cytokines has yielded mixed results so far. For example, in a dog model of OA, intra-articular injection of anakinra, a recombinant form of IL-1Ra, has been shown to inhibit the development of structural changes associated with OA [35]. Similarly, intra-articular plasmid injections of IL-1Ra in the meniscectomy rabbit model of OA showed a significant reduction in the progression of experimental OA with a significant reduction in the width of osteophytes, and the size and severity of macroscopic lesions. This study also showed that IL-1Ra was detected in the synovial fluid, synovium and cartilage of rabbits that received injections containing the IL-1Ra plasmid [36]. However, in humans, the effectiveness of anakinra in treating OA is still debatable. Despite demonstrating safety in patients with painful OA [37], intra-articular injection of anakinra was not effective at relieving the symptoms of knee OA at 12 weeks post-treatment in a placebo-controlled study that evaluated the efficacy of anakinra in patients with symptomatic knee OA. However, there was a small positive effect of anakinra at day 4, which suggests some short-term control of the disease [38].

Infliximab, a biological agent that inhibits the action of TNF-α, was shown to be effective in reducing pain and anatomical lesion radiological score with no local or systemic adverse reactions when administered intra-articularly in 12 patients with erosive hand OA. Similarly, 6-month treatment of a 68-year-old male patient with bilateral knee OA with adalimumab, a fully humanized TNF antibody, demonstrated complete relief in nocturnal pain and a decrease in synovial effusion and synovitis with complete abolishment of bone marrow edema [39]. These results are encouraging; however, broader clinical trials with a greater patient size will explore the true efficacy of these drugs when administered locally to treat OA.

Since proinflammatory cytokines produced by synovial cells are involved in induction of MMPs, an alternative therapy would be to inhibit MMP activity and limit joint destruction during OA. A significant effort has been put into developing MMP inhibitors (MMPIs) to prevent or retard cartilage degradation. Although some
have shown effectiveness in animal studies and the early stages of clinical trials, all MMPIs to date have been unsuccessful, possibly owing to specificity issues and consequent adverse side effects [40]. Owing to fact that MMPIs, as well as TIMPs, remain attractive potential therapeutic targets, advancements in the field, and further understanding of the complex structure and nature of MMPs are still underway. Local administration of specific MMPIs will be of great clinical value in the near future.

Future perspective
Recent advances in OA research have identified the synovium as a key player in OA pathophysiology. However, its exact contribution to the disease has not yet been determined. It is expected that, within the next 5–10 years, advances in molecular biology, diagnostic tools and imaging systems will improve the current knowledge of the synovium in OA pathogenesis. We strongly believe that enhancement in drug delivery, bioengineering and gene therapy techniques will help us specifically alter pathological processes initiated in the synovium and help counteract OA progression.

Conclusion
Synovium is now regarded as a key component actively involved in the structural and molecular alterations observed during OA pathogenesis. Targeting the synovium to counteract overproduction of proinflammatory cytokines and to subsequently halt the excess production of destructive MMPs is of great clinical relevance. However, this can only be achieved when the complexity of mediators involved in synovial activation and the complex cytokine network activated by synovial cells are elucidated in the near future.

Acknowledgements
The authors wish to thank S Lamane for all the time and help she gave in the construction of the figures for this article.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

Executive summary

Osteoarthritis
- Osteoarthritis (OA) is a progressive joint disease that gets worse with age. It is the most common form of arthritis and a serious clinical concern in an aging population.
- OA, previously believed to be a disease of cartilage, is now appreciated to be a disease of the whole joint, comprised of cartilage, bone and synovium.
- Synovitis is now considered as an active component of OA pathogenesis.

Normal synovium
- The synovium is a well-vascularized and innervated membrane that lines the joint capsule and surrounds all noncartilaginous components within articular joints.
- There are two types of synoviocytes within the synovium, type A and B. Type A synoviocytes are macrophage-like cells and type B synoviocytes are synovial fibroblasts.

Synovium in OA
- Of the three main tissues (cartilage, bone and synovium) that comprise OA joints, the synovial membrane is the inflammatory element.
- Synovial inflammation has long been an indicator of rheumatoid arthritis; however, its participation and role in OA is now widely accepted.
- Synovial inflammation, or synovitis, seemingly results from the synthesis and release of various cytokines and proinflammatory mediators.
- There are three types of cytokines, catabolic, anabolic and regulatory, produced in OA synovium that are involved in OA pathology.
- Catabolic cytokines secreted by synoviitis in OA induce or alter matrix metalloprotease expression.
- Of all the matrix metalloproteases produced in OA synovium, matrix metalloprotease-13, a collagenase, is the most widely studied and is highly expressed.

Targeting the synovium to treat OA
- Osteoarthritis is a localized disease as it only affects one or two joints. Therefore, gene therapy, a localized treatment, has clear clinical value to reduce systemic side effects and maximize drug delivery.
- Gene therapy can be used to alter processes in the synovial membrane or to target the cartilage with soluble factors that diffuse to the cartilage.

Conclusion
- The complexity of mediators involved in synovial activation and the complex cytokine networks activated by synovial cells still need to be elucidated.
Bibliography

Papers of special note have been highlighted as:
* of interest
** of considerable interest


** A must-read on the role of ADAMTS – a disintegrin and metalloprotease with thrombospondin motif – in OA.


* One of the few case studies to demonstrate successful treatment of OA by blocking TNF.


* Clinical trial describing the musculoskeletal side effects of an matrix metalloproteases inhibitor.