FACULTY OF MEDICINE AND HEALTH SCIENCES

SCOPING REVIEW: SURFACE PROTEINS ON SYNOVIAL FIBROBLASTS AND MACROPHAGES OF PATIENTS WITH OSTEOARTHRITIS

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ABSTRACT (EN)

Background: Osteoarthritis (OA) is the most common joint disease in the world. It is characterised by degradative and reparative processes of the articular cartilage and the subchondral bone. To date, there is no causal treatment available for this disease, partly due to the fact that the pathophysiological mechanisms of OA are still not fully understood. Fibroblasts, which play an important role in the production of extracellular matrix (ECM) and synovial fluid, are the most abundant cell type in the synovium. Furthermore, macrophages are the most prominent immune cells in the synovium. The role of these cells in the pathogenesis of OA seems to be promising and therefore further research is recommended.

Objectives: The aim of this scoping review is to summarise the existing literature concerning surface proteins on synovial fibroblasts and macrophages in patients with osteoarthritis to determine their role in the pathogenesis of OA.

Methods: A systematic search was conducted in three databases (Pubmed, Embase and Web of Science). Broad search terms were selected based on MeSH, Emtree and synonyms obtained from literature. Inclusion criteria entailed: all studies using flow cytometry, mass cytometry, immunohistochemistry or SPECT/CT in combination with radiopharmaceuticals to study surface proteins on synovial fibroblasts or macrophages in patients with OA.

Results: Several surface proteins on synovial fibroblasts and macrophages have been identified that contribute to the development of OA. CD44, CD55, and CD90 were often described on fibroblasts, while on macrophages CD14, CD68 and CD163 were most frequently mentioned. One of the mechanisms through which fibroblasts and macrophages induce damage to the joint, is by stimulating inflammatory processes associated with OA. For macrophages, their direct role has been demonstrated several times in the literature, whereas the role of fibroblasts seems to be more indirect. Both macrophages and fibroblasts have been shown to be involved in the migration of leukocytes from the bloodstream into the synovium. Furthermore, several proteins have been identified whose significance in the pathogenesis of OA requires further investigation.

Conclusion: Synovial fibroblasts and macrophages play a role in the pathogenesis of OA mediated by surface proteins. There are several pathways through which their influence is exerted, inducing inflammation being one of them. Future studies on the pathogenesis of OA should include comparison to healthy tissue samples to further investigate this inflammatory component. In addition, it is recommended to further study joints in the early stages of OA with the aim of developing diagnostic and therapeutic tools for early identification and treatment of patients.

ABSTRACT (NL)

Achtergrond: Osteoartritis (OA) is wereldwijd de meest prevalente gewrichtsaandoening. Kenmerkend zijn de degredatieve en reparatieve processen die plaatsvinden in het kraakbeen en het subchondrale bot. Tot op heden bestaat er geen oorzakelijke behandeling aangezien de pathofysiologische mechanismen van OA onduidelijk blijven. Fibroblasten vormen de meest voorkomende cel soort in het synovium met een belangrijke functie in productie van extracellulaire matrix en synoviaal vocht. Daarnaast zijn macrofagen de best vertegenwoordigde immuuncellen in het synovium. De rol van deze cellen in de pathogenese van OA lijkt veelbelovend, wat maakt dat verder onderzoek aanbevolen is.

Doelstelling: Het doel van deze scoping review bestaat erin om de bestaande literatuur betreffende oppervlakte proteïnes op synoviale fibroblasten en macrofagen in patiënten met osteoartritis weer te geven, om zo hun rol in de pathogenese van OA te identificeren.

Methodologie: Een systematische zoekopdracht werd uitgevoerd in drie databanken (Pubmed, Embase en Web of Science). Brede zoektermen werden geselecteerd gebruikmakend van MeSH, Emtree en synoniemen uit de literatuur. Geïncludeerde studies omvatten: alle studies die op basis van flow cytometrie, massa cytometrie, immunohistochemie of SPECT/CT gecombineerd met radiofarmaceutica, oppervlakte proteïnes bestuderen op synoviale fibroblasten of macrofagen in patiënten met OA.

Resultaten: Oppervlakte proteïnes die bijdragen tot het ontstaan van OA werden geïdentificeerd op synoviale fibroblasten en macrofagen. Op fibroblasten werden voornamelijk CD44, CD55 en CD90 beschreven, terwijl bij macrofagen CD14, CD68 en CD163 frequent benoemd werden. Beide cellen zijn betrokken bij meerdere pathways die invloed hebben op de pathogenese. De directe rol van macrofagen in het ontstekingsproces bij OA werd meermaals aangetoond in de literatuur, terwijl de link voor fibroblasten meer indirect blijkt te zijn. Zowel voor macrofagen als fibroblasten is aangetoond dat ze betrokken zijn bij de migratie van leukocyten uit de bloedbaan naar het synovium. Verder werden proteïnes geïdentificeerd waarvan het belang bij ontstaan van OA verder onderzocht moet worden.

Conclusie: Synoviale fibroblasten en macrofagen spelen een rol in de pathogenese van OA via oppervlakte proteïnes. Verscheidene pathways zijn hierbij betrokken, waaronder een aantal inflammatoire processen. Onderzoek naar de pathogenese van OA moet in de toekomst meer de vergelijking maken met gezonde weefselbiopten om de inflammatoire component verder te onderzoeken. Daarnaast is het raadzaam OA in een vroeg ziektestadium te onderzoeken, met als doel diagnostische en therapeutische middelen te ontwikkelen die patiënten tijdig identificeren en behandelen.

1 INTRODUCTION

1.1 What is osteoarthritis?

Osteoarthritis (OA) is a musculoskeletal condition that is characterised by degradative and reparative processes of the articular cartilage and the subchondral bone (1). Knee, hip and hand joints are most often impacted (2). OA is a global disease that numerous people suffer from. It is the most common cause of pain in peripheral joints in adults of 45 years and over (3). Long et al. (2019) reported in their research that in 2019, 527.81 million people suffered from OA globally, being an increase of 113 percent in prevalence from 1990 to 2019 (4). In Belgium specifically, 44 percent of people aged 75 and older suffer from this disease (5).

Symptoms related to OA are a common reason to visit a general practitioner (6). Patients suffer from mechanical joint pain located in one or multiple joints (2). In addition, patients experience stiffness in the joint after inactivity and a feeling of instability can occur (7, 8). Moreover, mobility is reduced significantly, with decreased quality of life as a consequence (8). Other symptoms that patients may experience are deformity, swelling, crepitus and subsequently, psychological distress (7).

Multiple factors have been identified that enhance the risk of a diagnosis of OA. Personal factors consist of age, gender, obesity, dietary intake and genetics (9). Age and obesity are the strongest predictors of OA (10). Apart from elderly people, women are also more likely to develop OA, especially around menopause (11). Joint-related risk factors include repetitive joint use, injury, muscle strength and malalignment (9).

The diagnosis of OA is generally based on clinical findings (2). The criteria include: patients of 45 years or older, suffering from activity-related joint pain and no morning stiffness for longer than 30 minutes (2). When imaging is used to confirm a structural diagnosis of OA, plain film radiography (RX) is most recommended (12). Radiographic features are narrowing of the joint space, osteophyte formation and the development of sclerosis and cysts (12). For detailed evaluation of structural changes, more sensitive imaging is needed. In this case, Magnetic Resonance Imaging (MRI) forms a better option (13).

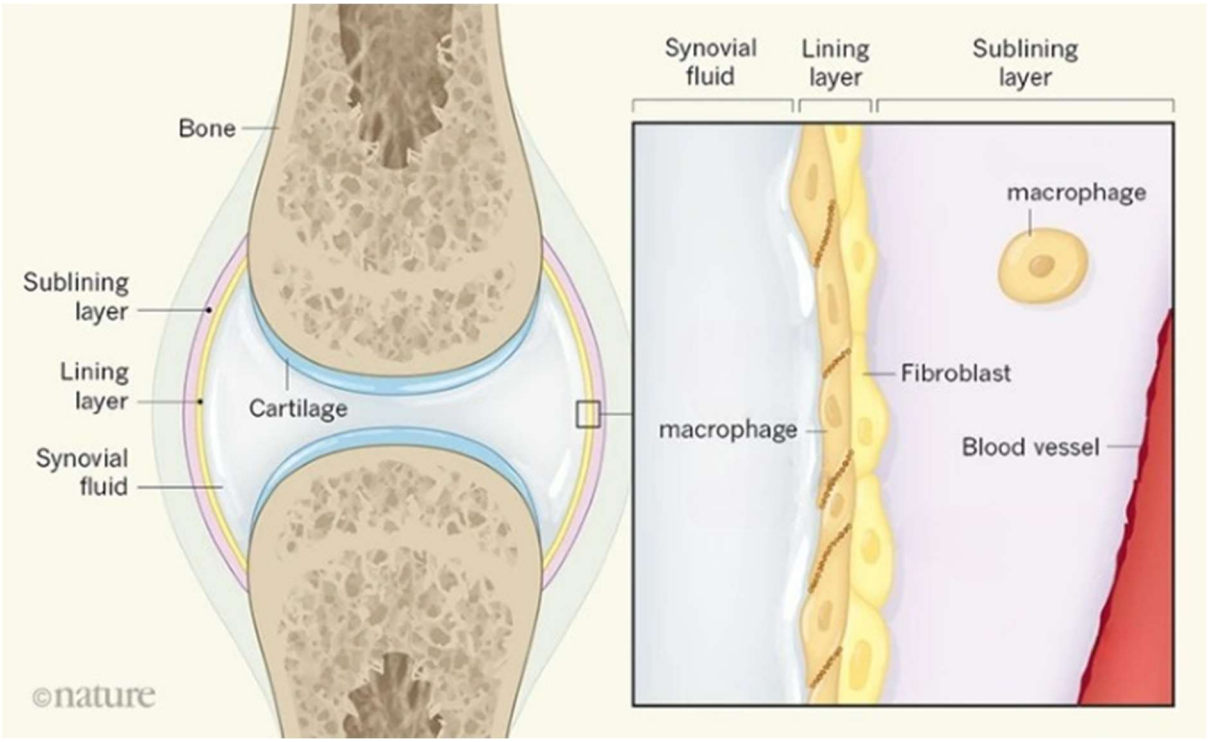
Hitherto, treatment options of OA remain limited. Management aims at reducing symptoms and maximising physical function (14). No treatments acting on disease progression are available to date (14). Treatment options consist of reducing mechanical overload, therapeutic exercise and pharmacological management (14). The latter includes oral or topical nonsteroidal anti-inflammatory drugs (NSAIDs) and intra-articular corticosteroids (14). In severe cases, joint replacement can be undertaken (14).

1.2 Synovial pathogenesis

OA was long seen as a wear-and-tear disease leading to degradation and loss of cartilage (1). In recent years, new insights into the pathogenesis of OA have been gained. Current evidence shows that OA affects the whole joint, including cartilage, subchondral bone, capsule and periarticular structures like ligaments, menisci and synovium (1, 15). Furthermore, modern imaging techniques, such as MRI and ultrasound, showed a high prevalence of inflammation in the joints (16). Even in early stages of the disease, inflammation was observed (1). These findings support the hypothesis that synovitis, albeit low-grade synovitis, plays a significant role in the pathophysiology of OA (16).

As shown in *Figure 1*, the synovium, or the synovial membrane, is the soft tissue lining of the inner surface of synovial joint capsules. It consists of two different layers: a continuous surface layer of cells, called the intima or lining layer, and the adjacent tissue, called the subintima or sublining layer (17, 18). The outer layer, the subintima, is a relatively acellular tissue (16). It is up to 5 mm thick and consists of multiple types of connective tissues: fibrous, adipose or loose collagenous (16). This layer is rich in type I collagen and microvascular blood vessels, together with lymphatic vessels and nerve fibres (16). The inner layer, which is the intima, is located directly next to the joint cavity. It is a thin sheet, consisting of 1 to 4 layers of various types of cells, called synoviocytes (16).

Figure 1: Structure of the synovial membrane



From: Buckley CD. Macrophages form a protective cellular barrier in joints. Nature. 2019;572(7771):590-2.

The primary role of synoviocytes is to contribute to the production of synovial fluid by secreting its main components, lubricin and hyaluronic acid (19). By covering the articular surface with lubrification, friction is reduced, thereby protecting and maintaining the integrity of articular cartilage surfaces in diarthrodial joints (19). On top of that, lubricin prevents excessive deposition of proteins at the articular surface (19). As articular cartilage has no intrinsic vasculature or blood supply, it relies heavily on the synovium and synovial fluid for its nutrition (19). This is necessary for maintaining the health of the chondrocyte and articular cartilage (19). The synovium acts as a semipermeable membrane so that nutrients can be supplied, and metabolic waste products can be removed (19). By these means, the normal physiological state of articular cartilage can be preserved (19).

In the intimal layer of the synovium, two types of synoviocytes are distinguished. Type A synoviocytes are identified as macrophage-like cells, which are derived from haematopoietic monocyte lineage (18). In healthy intima tissue, macrophages make up a minority of cells (18). Their primary role is to maintain tissue homeostasis and to protect the host from infection (20). Macrophages are phagocytic cells and form a part of the innate immune system (20).

However, they are capable of bridging and instructing the response of the adaptive immune system via various secretory mediators (20). Type B synoviocytes, the most abundant cell type in the lining layer of healthy synovium, are locally derived fibroblast-like synoviocytes (FLSs) (18). Their main function is to synthesise and secrete major extracellular matrix (ECM) proteins that contribute to the production of the synovial fluid (18). Subintimal regions contain both cells as well (18).

In the pathogenesis of OA, several histological changes are reported in the synovium. The most predominant changes are synovial lining hyperplasia, stromal vascularisation and sublining fibrosis (21). The latter is mainly seen in late stages of OA (16). These changes result from an immunological response to degraded hyaline cartilage (16). Molecules from degraded cartilage are released into the synovial cavity and are likely to initiate synovial inflammation in OA (16). Synovial cells respond by secreting pro-inflammatory mediators, including interleukin-1 bèta (IL-1β) and tumor necrosis factor alfa (TNF-α), which attract immune cells and increase angiogenesis (22). Macrophages are the most abundant immune cell in the OA synovium. In scientific literature, macrophages are frequently divided into pro- and anti-inflammatory, according to their activities. M1 macrophages inhibit cell proliferation and cause tissue damage (23). M2 macrophages have opposite effects, namely promoting cell proliferation and tissue repair (23). In OA, macrophages seem to be the main source of innate immune activation and cytokine production (16, 20). Furthermore, the pro-inflammatory cytokines stimulate the chondrocytes to produce proteases, which cause further degradation and stimulate the inflammation (22, 24). Hence, a vicious cycle sets in (24).

Next to macrophages, another type of leukocytes is involved in synovial infiltration, namely the lymphocytes (25). Lymphocytic infiltration can vary in severity (25). When mild to moderate infiltration occurs, lymphocytes are mainly found in the sublining layer (25). However, heavy infiltration in the synovium can result in closely packed collections of lymphocytes, which are called lymphoid aggregates (25). Furthermore, other immune cells, including B-cells and T-cells, are involved in the inflammatory processes of the synovium (16).

When this chronic synovial inflammation, or synovitis, occurs, various repair mechanisms are installed. Osteoblasts are triggered to build new bone formation to replace the degraded bone (26). The new bone formation causes subchondral sclerosis and the development of osteophytes, characteristic for OA (26). Apart from that, the synovial fibroblasts begin to

proliferate in response to their inflammatory environment (26). In an attempt to repair the mechanical damage incurred, the cells differentiate into myofibroblasts and secrete matrix molecules to rebuild the ECM structure, comparable to a wound healing process (26). The excessive ECM deposition in the synovium leads to fibrosis which eventually results in more inflammation and damage (27). This fibrotic state of the synovium changes the permeability and mechanical properties of the joint (26). Together with the excessive bone formation, this results in pain and stiffness (26).

A prevalent chronic inflammatory joint condition is rheumatoid arthritis (RA) (28). RA is described as a systemic auto-immune disorder that presents as a symmetric polyarthritis associated with swelling and pain in multiple joints, often initially occurring in hands and feet (28). Scientific literature often focuses on the comparison between OA and RA, where OA is considered to be the less inflammatory condition.

1.3 Cell surface proteins and analysis techniques

Research on cell surface markers, or cell surface proteins, can be used to obtain a better overview on different cell subsets with the aim of understanding their role in pathophysiology. On the cell surface of fibroblasts and macrophages, distinct proteins are expressed with each their significance (29). These proteins can reflect either different stages of their lineage-specific differentiation or different states of activation or inactivation (29). Detection of the cell surface molecules is routinely done with monoclonal antibodies (mAbs) (29). On the cell surface of fibroblasts and macrophages, these mAbs react with certain clusters of antigens, known as clusters of differentiation (CDs) (29). Different sub-populations can be distinguished using different combinations of mAbs (29). This allows the immunophenotyping of different cells, including fibroblasts and macrophages (29). The nomenclature of CDs consists of a number with, if necessary, a letter added to indicate different variant (29, 30). These CDs, as well as several other surface proteins, can be studied using the techniques described below.

The most widely used application in tissue and cellular antigen detection is immunohistochemistry (IHC) (31). It is an important tool to examine cell surface markers and thus different cell subsets (31). The technique consists of an antibody used against the antigen of interest to form a complex (31). IHC uses various enzymatic or fluorescent labels linked to antibodies for the visualisation of antigens (31). First, these labelled antibodies are added to

the specimens. Thereafter, incubation with a chromogenic substrate must be completed (32). As a result of the binding of substrate and antibody, the antigen of interest is stained (32). Finally, these complexes can be visualised using a light microscope (32). When fluorescent labels are used, visualisation of the labels can be done without chromogenic substrate, requiring a fluorescence microscope (31). This technique is called immunofluorescence (31).

Flowcytometry, another commonly used technique, measures physical characteristics of a single cell or its components, like cell surface proteins (33). The cells or cell components are buffered in a salt-based solution that direct particles in front of a focused light source (33, 34). To examine surface-bound proteins, dyes or monoclonal antibodies are added to the cell-solution (35). The light excites the labels when passing in front of the laser and fluorescent light of varying wavelengths is emitted (35). Thereafter, an electronic network converts the light scatter signals and fluorescent emissions from the excitation of fluorescent dyes to electronic voltage pulses (35). Finally, the voltage pulses are converted to a digital output and transferred to a computer for manipulation by the operator (35). As a result, targeted proteins are characterised (35). The results are interpreted and can be used for detection or counting of cells or their components (34).

There are a number of cell analysing methods that utilise the principles of flowcytometry and combine them with other techniques. Mass cytometry, for example, is a combination of flow cytometry and time-of-flight mass spectrometry (TOF-MS) (34). Cells are labelled with heavy metal ion-tagged Ab's instead of with fluorescently-tagged Ab's (34). TOF-MS is used to detect these heavy metal ions by measuring the time it takes for the particles to reach the detector (34).

A different application of flowcytometry are cell sorters. As opposed to pure analysers, they have the capacity to separate or sort specific cells from a mixed cell population and divert them from the fluid stream into a collection vessel (35, 36). There are several methods to isolate the targeted cells from the rest. Two of the primary affinity-based techniques used for cell sorting are fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) (37). FACS and MACS both utilise antibodies against specific antigens located on the surface of the cells of interest to distinguish them in a mixed population (37). Using FACS, fluorescent labels are bound to the antibodies and an electric pulse is needed to separate the cells (37). MACS employs magnetic particles that are functionalised with an antibody (37).

When bound to the cells of interest, they are placed in a magnetic field which facilitates the separation from the mixture of cells (37).

Another cell analysis technique that can be used is Single Photon Emission Computed Tomography combined with Computed Tomography (SPECT/CT) in combination with radiopharmaceuticals (38). Radiopharmaceuticals are radioisotopes linked to mAbs that bind to structures in the body, such as surface proteins (38). These tracers emit gamma radiation, which is visualised by SPECT/CT (38). As a result, surface proteins can be observed (38). Other techniques used for analysis of surface markers are beyond the scope of this review.

1.4 Impact of OA and the importance of this scoping review

Considering high age is the most important risk factor and due to the sociodemographic changes, prevalence of OA is increasing more rapidly than any other health condition (10, 39). As stated before, OA may have a substantial impact on quality of life of the affected patients (3, 8). Apart from the individual level, OA creates a major socioeconomic burden (40). For example, data from the US Medicare Expenditure Panel Survey showed the annual expenditures charged to insurers of women with OA was $4833 greater compared to women without OA (40).

A scoping review summarises knowledge using a systematic and iterative approach to identify and integrate the emerging and existing literature on a particular topic (41). The aim of this review is to get a better understanding of the different surface markers found on fibroblasts and macrophages in the synovium of patients with OA. A better apprehension of the synovial cells may bring forward new insights on how they play a role in the origination of this disease. These insights can be used to develop new diagnostic tools and new therapies that intervene with the underlying processes of OA.

2 MATERIALS AND METHODS

A scoping review of the existing literature was performed, based on the framework of Arksey and O'Malley (42). Pubmed, Embase and Web of Science were searched up to November 12th, 2023. To obtain a comprehensive overview of the existing knowledge on different surface proteins of synovial macrophages and fibroblasts in OA, an extensive search with general terms was performed. The search string that was created, was intended to find articles about markers that are located on the surface of fibroblasts and macrophages in the synovium of OA patients. Different combinations of relevant terms have been included in the search. Terms were selected using MeSH and Emtree. Scientific articles were explored in order to identify all possible synonyms. Finally, a filter was added selecting English and Dutch written articles. The search string for each database can be consulted in *Table 1*.

Table 1: search strategy

Database	Syntax
Pubmed	(osteoarthritis[MeSH Major Topic] OR osteoarthrit*[Title/Abstract] OR osteoarthrosis [Title/Abstract] OR arthrosis[Title/Abstract] OR "degenerative arthrit*"[Title/Abstract] OR "degenerative joint disease*"[Title/Abstract]) AND (fibroblast[MeSH Major Topic] OR fibroblast*[Title/Abstract] OR "fibroblast like synoviocyte*"[Title/Abstract] OR "fibroblast-like synoviocyte*"[Title/Abstract] OR "fibroblast like synovial cell*"[Title/Abstract] OR "fibroblast-like synovial cell*"[Title/Abstract] OR "fibroblast like cell*"[Title/Abstract] OR "fibroblast-like cell*"[Title/Abstract] OR FLS[Title/Abstract] OR myofibroblast*[Title/Abstract] OR macrophage[MeSH Major Topic] OR macrophage*[tiab] OR "macrophage activation"[MeSH Major Topic] OR "macrophage activation"[tiab]) AND ("joint capsule"[Mesh] OR "joint capsule*"[tiab] OR "joint cavit*"[tiab] OR synovium[tiab] OR "synovial fluid"[tiab] OR "synovial membrane"[Mesh] OR "synovial membrane*"[tiab] OR "synovial tissue*"[tiab] OR synovialis[tiab] OR synovial[tiab] OR "synovial lining"[tiab])

Embase	('osteoarthritis'/exp/mj OR 'osteoarthrit*':ti,ab OR 'osteoarthrosis':ti,ab OR 'arthrosis':ti,ab OR 'degenerative arthrit*':ti,ab OR 'degenerative joint disease*':ti,ab) AND ('fibroblast'/mj OR 'fibroblast*':ti,ab OR 'fibroblast like synoviocyte'/exp OR 'fibroblast like synoviocyte*':ti,ab OR 'fibroblast-like synoviocyte*':ti,ab OR 'fibroblast like synovial cell'/exp OR 'fibroblast like synovial cell*':ti,ab OR 'fibroblast-like synovial cell*':ti,ab OR 'fibroblast like cell'/exp OR 'fibroblast like cell*':ti,ab OR 'fibroblast-like cell*':ti,ab OR 'fls':ti,ab OR 'myofibroblast'/exp OR 'myofibroblast*':ti,ab OR 'macrophage'/exp/mj OR 'macrophage*':ti,ab OR 'macrophage activation'/exp/mj OR 'macrophage activation':ti,ab) AND ('joint capsule'/exp OR 'joint capsule*':ti,ab OR 'joint cavity'/exp OR 'joint cavit*':ti,ab OR 'synovium'/exp OR 'synovium':ti,ab OR 'synovial fluid'/exp OR 'synovial fluid':ti,ab OR 'synovial membrane*':ti,ab OR 'synovial tissue*':ti,ab OR 'synovialis':ti,ab OR 'synovial':ti,ab OR 'synovial lining':ti,ab)
Web of science	TS= ((osteoarthrit* OR osteoarthrosis OR arthrosis OR “degenerative arthrit*” OR “degenerative joint disease*”) AND (fibroblast* OR “fibroblast like synoviocyte*” OR “fibroblast-like synoviocyte*” OR “fibroblast like synovial cell*” OR “fibroblast-like synovial cell*” OR “fibroblast like cell*” OR “fibroblast-like cell*” OR FLS OR myofibroblast* OR macrophage* OR “macrophage activation”) AND (“joint capsule*” OR “joint cavit*” OR synovium OR “synovial fluid” OR “synovial membrane” OR “synovial tissue” OR synovialis OR synovial OR “synovial lining”))

2.1 Article selection

The PRISMA flowchart framework was used as a guide to perform the article selection (43). After applying the search strategy in the different databases, the results were stored in Endnote, where a duplicate removal was completed. Thereafter, the articles were transferred to Rayyan, an AI Powered Tool for Systematic Literature Reviews. Systematic screening of the identified records was performed by two independent reviewers as follows. First, an individual selection was made based on the title. Afterwards, the two reviewers compared their

included articles. When inconsistencies occurred, arguments were shared in order to come to an agreement. This was possible without the need of a third party. This procedure was repeated for the selection based on abstract and full text.

For this scoping review, inclusion criteria were: flow cytometric, mass cytometric, immunohistochemical analysis or SPECT/CT-analysis in combination with radiopharmaceuticals of surface proteins on fibroblasts or macrophages in synovial tissue obtained from patients with diagnosis of OA. The minimum age of the subjects was fixed at 18 years old. No minimum was set on the number of patients included in the study. Non-human studies were excluded, as well as articles studying back, shoulder, temporomandibular and trauma-related OA. Reviews, conference abstracts and other abstracts for which full text could not be found, were excluded. Furthermore, articles not written in Dutch or English were removed. No restrictions were made based on publication date.

2.2 Data extraction

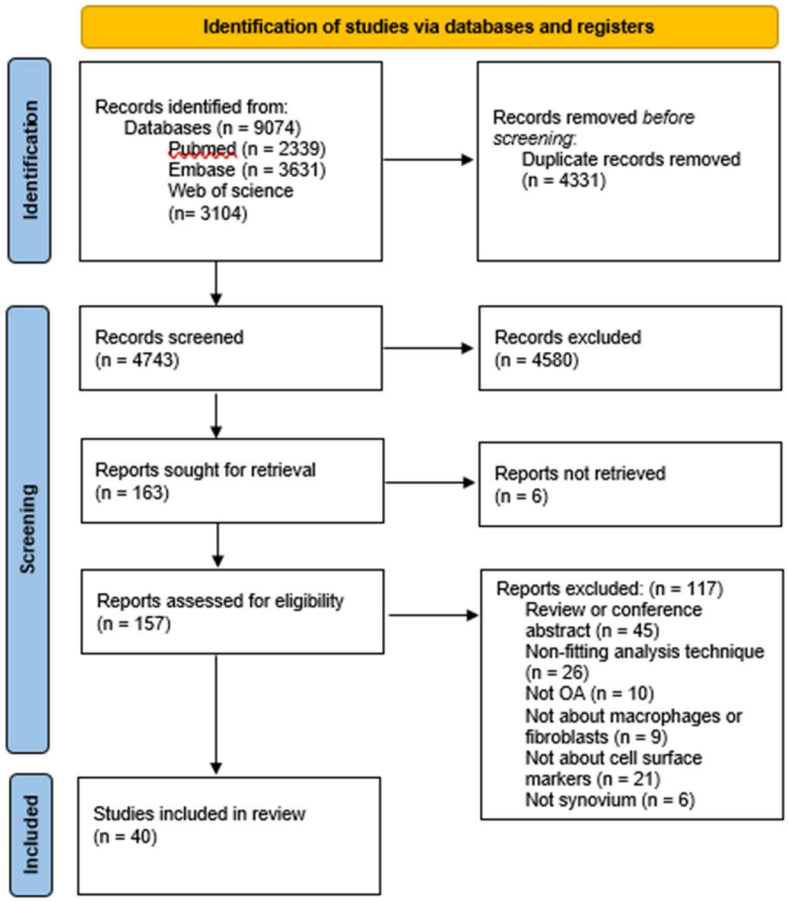
The following data were extracted: demographics, analysis technique, study material collection, cell type and reported surface protein(s). The process of data extraction was separated: one reviewer performed extraction of data on fibroblasts and the other reviewer searched for data on macrophages. Afterwards, the two reviewers looked for resemblances and differences between the two cell types. Lastly, the extracted data were summarised.

3 RESULTS

3.1 Study selection

As seen in *Figure 2*, the extensive literature search yielded 9074 results. Duplicates were removed, coming to a total of 4743 articles to be screened. During the screening based on title 3871 articles were eliminated. During abstract screening, 709 articles were removed, coming to a total of 4580 excluded articles. Full-text version of 163 articles was sought, referred to as reports sought for retrieval in *Figure 2*. Six full-text versions of articles could not be found. Afterwards, 157 full-text articles were assessed for eligibility. Finally, 40 articles met all of the inclusion criteria and were selected. Reasons for exclusion are reported in the PRISMA flowchart *Figure 2*.

Figure 2: Overview of systematic literature selection



3.2 Study characteristics

Of the 40 included articles, 23 articles studied knee OA (44-66), 5 examined hip OA (67-71) and 2 articles observed both knee and hip OA (72, 73). Four articles described knee, hip or hand joints without further differentiation (74-77). Six studies did not mention which joint was examined (25, 74-82). The mean age or range of the study population was not always provided. Of the studies that did provide a mean age (n = 21) or a range (n = 12), minimum and maximum reported were respectively 54.7 and 74.4 years for mean age and 30 and 89 years for range (45, 46, 48, 49, 51, 54-68, 70, 72, 75, 76). Almost all studies (n = 34) obtained their specimens during joint replacement surgery (25, 44-50, 52-60, 62-64, 67-70, 72-80, 82). Concerning the cell type, 18 studies examined fibroblasts (44, 46, 50, 52, 53, 60, 61, 64, 68, 69, 71-74, 77, 78, 81, 82), 14 examined macrophages (25, 48, 49, 51, 54, 63, 66, 67, 76, 79, 80) and 8 articles studied both (45, 55-59, 70, 75). With regard to the techniques used, 16 studies used IHC (25, 45, 47, 48, 59, 60, 62, 65-67, 69, 76-80), 13 studies used FC (44, 49, 52, 53, 57, 61, 70-74, 81, 82) and 10 studies used both (46, 50, 54-56, 58, 63, 64, 68, 75). One study used SPECT/CT in combination with radiopharmaceuticals (51).

3.3 Summary of results

In *Table 2*, the summary of the selected articles is displayed, including demographics, analysis technique, study material collection, cell type and reported surface protein(s).

Table 2: Summary of the results

Author	Title	Study population	Age (in years)	Study material collection	Analysis technique	Cell type	Surface protein(s)
Athanasou et al. (1991)	Immunocytochemical analysis of human synovial lining cells: phenotypic relation to other marrow derived cells	4 HOA	Range: 55 - 70	Joint replacement surgery	IHC	M	CD11a, CD11b, CD11c, CD13, CD14, CD15a, CD16, CD18, CD25, CD31, CD32, CD33, CD34, CD35, CD37, CD39, CD45, CD54, CD64, CD68, CD71, HLA-DR
Bauer et al. (2006)	Fibroblast activation protein is expressed by rheumatoid myofibroblast-like synoviocytes	10 HOA, KOA or HaOA 10 refractory destructive RA	No data available	Joint replacement surgery	IHC	F	$CD44_{v3}$, $CD44_{v7/v8}$, CD90, FAP, MMP-1, MMP-13, SMA
Bröker et al. (1990)	The Prevalence and Distribution of Macrophages Bearing FcyR I, FcyR II, and FcyRIII in Synovium	8 OA, 14 RA, 2 AS, 1 monoarticular chronic synovitis, 3 with sarcoid/reumatic nodules	No data available	Joint replacement surgery	IHC	M	CD14, CD16, CD32, CD64
Brühl et al. (2008)	Functional expression of the chemokine receptor CCR7 on fibroblast-like synoviocytes	10 KOA 10 KRA 6 healthy dermal fibroblasts	No data available	Joint replacement surgery	FC (FACS)	F	CCR7

Capellino et al. (2014)	Increased expression of dopamine receptors in synovial fibroblasts from patients with rheumatoid arthritis: inhibitory effects of dopamine on interleukin-8 and interleukin-6	17 KOA 15 KRA	Mean: 68.8 ± 1.9 Range: 55 - 82	Joint replacement surgery	IHC	F, M	CD163, D1DR, D2DR, D3DR, D4DR, D5DR, dopamine transporter
Chang et al. (2016)	Upregulated expression of CCR3 in osteoarthritis and CCR3 mediated activation of fibroblast-like synoviocytes	15 KOA	Mean: 60.7 ± 4.4	Joint replacement surgery	FC (FACS) IHC	F	CCR3, CD90
Choi et al. (2012)	Effects of the pro-inflammatory milieu on the dedifferentiation of cultured fibroblast-like synoviocytes	? OA ? RA	No data available	Joint replacement surgery	FC (FACS)	F	CD44, CD90, CD106, Stro-1
Dong et al. (2023)	ASIC1a-CMPK2-mediated M1 macrophage polarization exacerbates chondrocyte senescence in osteoarthritis through IL-18	10 KOA 4 healthy	No data available	Joint replacement surgery	IHC	M	ASIC1a, F4/80
Fiorito et al. (2005)	Inflammatory status and cartilage regenerative potential of synovial fibroblasts from patients with osteoarthritis and chondropathy	6 HOA	Range: 59 - 79	Joint replacement surgery	FC IHC	F	CD11a, CD29, CD40, CD44, CD49e, CD54, CD90, CD106, CD166, IL-2Rγ, IL-4Rα, IL-15Rα, SH2, SH3, SH4, Stro-1

Fonseca et al. (2002)	Macrophage subpopulations in rheumatoid synovium: reduced CD163 expression in $CD4^+$ T lymphocyte-rich microenvironments	6 OA 6 RA 3 healthy	No data available	Joint replacement surgery	IHC	M	CD68, CD163
Gu et al. (2023)	MAGL regulates synovial macrophage polarization vis inhibition of mitophagy in osteoarthritic pain	5 KOA 5 anterior cruciate ligament injury	Range: 64 - 74	Joint replacement surgery	IHC	M	CD80, CD206
Honorati et al. (2002)	Contribution of interleukin 17 to human cartilage degradation and synovial inflammation in osteoarthritis	11 KOA 1 HOA	Mean: 68 Range: 51 - 80	Joint replacement surgery	FC	F	IL-17R
Hsueh et al. (2021)	Synergistic Roles of Macrophages and Neutrophils in Osteoarthritis Progression	Sample 1: 39 KOA Sample 2: 18 KOA Sample 3: 60 KOA	Mean: - Sample 1: 69.7 - Sample 2: 62.9 - Sample 3: 61.6 Range: - Sample 1: 56 - 86 - Sample 2: 38 - 80 - Sample 3: 30 - 81	Joint replacement surgery	FC	M	CD11b, CD11c, CD14, CD16, FR, HLA-DR
Huang et al. (2019)	Parallel comparison of fibroblast-like synoviocytes from the surgically removed hyperplastic synovial tissues of rheumatoid arthritis and osteoarthritis patients	23 KOA 23 KRA	No data available	Joint replacement surgery or synovectomy	FC (FACS) IHC	F	CD90, ICAM-1, VCAM-1

Iguchi et al. (1986)	Electron microscopic study of HLA-DR and monocyte/macrophage staining cells in the rheumatoid synovial membrane	3 OA 6 RA 1 healthy	No data available	Joint replacement surgery or arthroscopy	IHC	M	CD14, HLA-DR
Kraus et al. (2013)	Direct in vivo evidence of activated macrophages in human osteoarthritis	25 KOA	Mean: 62.4 ± 15.8 Range: 30 - 89	No specimen	^{99m}Tc-EC20 (SPECT/CT)	M	FRβ (folate)
Kunisch et al. (2004)	Macrophage specificity of three anti-CD68 monoclonal antibodies (KP1, EBM11, and PGM1) widely used for immunohistochemistry and flow cytometry	10 HOA, KOA or HaOA 10 RA	Mean: 67 Range: 46 - 76	Joint replacement surgery or arthroscopic synovectomy	FC (FACS) IHC	F, M	CD14, CD68, CD90
Lin et al. (2012)	The CCL2/CCR2 axis enhances vascular cell adhesion molecule-1 expression in human synovial fibroblasts	33 KOA 15 healthy	No data available	Joint replacement surgery	FC	F	VCAM-1
Liu et al. (2013)	CCN4 induces vascular cell adhesion molecule-1 expression in human synovial fibroblasts and promotes monocyte adhesion	38 KOA 18 healthy	No data available	Joint replacement surgery	FC	F	VCAM-1
Manferdini et al. (2016)	From osteoarthritic synovium to synovial-derived cells characterization:	26 KOA	Mean: 66 ± 11.10	Joint replacement surgery	FC (FACS) IHC	F, M	CD14, CD16, CD55, CD68, CD73, CD80,

	synovial macrophages are key effector cells						CD90, CD105, CD106, CD163
Manferdini et al. (2017)	Adipose stromal cells mediated switching of the pro-inflammatory profile of M1-like macrophages is facilitated by PGE2: in vitro evaluation	12 KOA	Mean: 64 ± 10	Joint replacement surgery	FC (FACS) IHC	M	CD14, CD68, CD80, CD86, CD163, CD206
Manferdini et al. (2020)	Impact of Isolation Procedures on the Development of a Preclinical Synovial Fibroblasts/Macrophages in an In Vitro Model of Osteoarthritis	15 KOA	Mean: 68 ± 6	Joint replacement surgery	FC (FACS) IHC	F, M	CD14, CD55, CD68, CD80, CD86, CD163, CD206
Manni et al. (2003)	Nerve growth factor release by human synovial fibroblasts prior to and following exposure to tumor necrosis factor-alpha, interleukin-1 beta and cholecystokinin-8: the possible role of NGF in the inflammatory response	3 HOA 5 healthy	No data available	Joint replacement surgery	IHC	F	TrkA
Mardanpour et al. (2018)	Is CD163-A marker of progression in osteoarthritis?	20 HOA, KOA or HaOA 10 heatlhy	Mean: 68 ± 8	Joint replacement surgery	IHC	M	CD163
Mimpen et al. (2023)	Cellular characterisation of advanced osteoarthritis knee synovium	10 KOA	Mean: 68.8 Range: 58 - 80	Joint replacement surgery	FC	F, M	CD34, CD40, CD45, CD68, CD90, CD206, FAP, PDPN

Moriya et al. (2020)	Expression and regulation of macrophage-inducible C-type lectin in human synovial macrophages	19 KOA	Mean: 72.2 ± 7.0 Range: 56 - 85	Joint replacement surgery	FC IHC	F, M	CD14, CD45, Mincle
Noda et al. (2021)	Differential inflammation-mediated function of prokineticin 2 in the synovial fibroblasts of patients with rheumatoid arthritis compared with osteoarthritis	79 KOA 67 KRA	Mean: 68.0 ± 8.07	Joint replacement surgery	IHC	F, M	PKR1, PKR2
Ohashi et al. (2022)	Correlation between CD163 expression and resting pain in patients with hip osteoarthritis: Possible contribution of $CD163^{+}$ monocytes/macrophages to pain pathogenesis	8 HOA	Mean: 64.6 ± 10.8	Joint replacement surgery	FC	F, M	CD14, CD80, CD90, CD163, CD206
Pattacini et al. (2007)	Angiotensin II protects fibroblast-like synoviocytes from apoptosis via the AT1-NF-kappaB pathway	? HOA	No data available	No data available	FC	F	AT1
Payet et al. (2023)	Inflammatory mesenchymal stem cells express abundant membrane-bound and soluble forms of C-type lectin-like CD248	? OA ? RA	No data available	Routine surgical intervention	FC (FACS)	F	CD90, CD248

Pörings et al. (2019)	A thyroid hormone network exists in synovial fibroblasts of rheumatoid arthritis and osteoarthritis patients	32 KOA 12 KRA	Mean: 70.3 ± 1.3	Joint replacement surgery	IHC	F	DIO2, DIO3, TRα, TRβ
Ren et al. (2021)	CCL22 induces pro-inflammatory changes in fibroblast-like synoviocytes	13 KOA 10 healthy (cadaver)	Mean: 54.7	Arthroscopic knee biopsy	FC	F	CCR3, CCR5
Saito et al. (2002)	Increased cellular infiltrate in inflammatory synovia of osteoarthritic knees	19 KOA	Mean: 64 Range: 47 - 78	Joint replacement surgery	IHC	M	CD68, HLA-DR
Sampey et al. (2000)	Annexin I surface binding sites and their regulation on human fibroblast-like synoviocytes	6 HOA or KOA 6 HRA or KRA	No data available	Joint replacement surgery	FC	F	Annexin-1 binding sites
Schlaak et al. (1995)	Effects of Th1 and Th2 cytokines on cytokine production and ICAM-1 expression on synovial fibroblasts	? OA	No data available	Joint replacement surgery	FC (FACS)	F	ICAM-1
Tsuneyoshi et al. (2012)	Functional folate receptor beta-expressing macrophages in osteoarthritis synovium and their M1/M2 expression profiles	15 KOA 12 KRA	Mean: 73,5 ± 8,7	Joint replacement surgery	FC (FACS) IHC	M	CD68, CD163, FRβ (folate)
van Nie et al. (2020)	Dopamine induces in vitro migration of synovial fibroblast from patients with rheumatoid arthritis	28 KOA 31 KRA	Mean: 74.4 Range: 58.8 - 88.5	Joint replacement surgery	FC (FACS) IHC	F	D1DR, D2DR, D3DR, D4DR, D5DR

Wäldele et al. (2015)	Deficiency of fibroblast activation protein alpha ameliorates cartilage destruction in inflammatory destructive arthritis	4 HOA, KOA or HaOA 4 RA	No data available	Joint replacement surgery	IHC	F	FAP
Watanabe et al. (2023)	Knee osteotomy decreases joint inflammation based on synovial histology and synovial fluid analysis	21 KOA	Mean: 60.5 ± 1.2	Arthroscopic knee biopsy	IHC	M	CD80, CD163, CX_3CR1, F4/80
Young et al. (2001)	Effects of intraarticular glucocorticoids on macrophage infiltration and mediators of joint damage in osteoarthritis synovial membranes: findings in a double-blind, placebo-controlled study	40 KOA	Mean: 67 ± 12	Arthroscopic knee biopsy	IHC	M	CD68

AS = ankylosing spondylitis, F = fibroblasts, FACS = fluorescence-activated cell sorting, FC = flow cytometry, HaOA = hand osteoarthritis, HOA = hip osteoarthritis, IHC = immunohistochemistry, KOA = knee osteoarthritis, KRA = knee reumatoid arthritis, M = macrophages, OA = osteoarthritis, RA = rheumatoid arthritis, SPECT/CT = combination of single photon emission computed tomography with computed tomography, ^{99m}Tc-EC20 = technetium etarfolatide-labeled peptide

3.3.1 Surface proteins on fibroblasts

The proteins along with studies describing their expression on the cell surface are listed in *Table 3* for fibroblasts.

Table 3: Surface proteins on fibroblasts

Surface proteins	Synonym	Studies describing expression on the cell surface
Angiotensin II receptors		
• **AT 1**		(71)
Annexin I binding sites		
/		(73)
Chemokine receptors		
• **CCR3**		(46, 61)
• **CCR5**		(61)
• **CCR7**	CD197	(44)
Cluster of differentiation proteins		
• **CD11a**	LFA-1	(68)
• **CD29**	Integrin β1	(68)
• **CD34**		(57)
• **CD40**		(68)
• **CD44**	H-CAM	(68, 74)
• **CD44, v3**		(78)
• **CD44, v7/v8**		(78)
• **CD49e**	Integrin α5	(68)
• **CD54**	ICAM-1	(50, 68, 82)
• **CD55**	DAF	(55, 56)
• **CD68**		(75)
• **CD90**	Thy-1	(46, 50, 55, 57, 68, 70, 74, 75, 78, 81)
• **CD106**	VCAM-1	(50, 52, 53, 55, 68, 74)
• **CD163**		(70)
• **CD166**	ALCAM	(68)
• **CD248**	Endosialin	(81)
Cytokine receptors		
• **IL-2Rγ**	CD132	(68)
• **IL-4Rα**	CD124	(68)
• **IL-15Rα**		(68)
• **IL-17R**		(72)
Dopamine receptors		
• **D1DR-D5DR**		(45, 64)
Dopamine transporters		
/		(45)

Fibroblast activation protein		
• **FAP**		(57, 77, 78)
Matrix metalloproteinases		
• **MMP-1**		(78)
• **MMP-13**		(78)
NGF-receptors		
• **TrkA**		(69)
Podoplanin		
• **PDPN**		(57)
Prokineticin receptors		
• **PKR1-2**		(59)
Smooth muscle actin		
• **SMA**		(78)
Src homology domains		
• **SH2**	CD105	(68)
• **SH3**		(68)
• **SH4**	CD73	(68)
Stro-1		
/		(68, 74)
Thyroid hormone receptors		
• **DIO2-3**		(60)
• **TRα-β**		(60)

3.3.1.1 Common fibroblast typing proteins

CD44

The expression of CD44, a typical FLS-marker, was investigated by three different studies (68, 74, 78). Choi et al. confirmed that CD44 was expressed by more than 90% of OA FLSs (74). This expression was found to be similar to that of FLSs from RA patients, as well as FLSs from healthy patients (68, 74). To evaluate the limitation of an in vitro culture under conditions lacking inflammation, the researchers investigated expression after IL-1β stimulation (74). However, the expression of CD44 on OA FLSs remained unchanged (74). Bauer et al. investigated the presence of specific splice variants of CD44 (78). It was found that, in contrast to the synovial lining layer of RA joints, OA joints show limited staining for v3 and v7/v8 CD44 splice variants (78).

CD55

CD55 is considered to be a commonly found marker on the cell surface of synovial fibroblasts. Two studies analysed the presence of this surface marker (55, 56). Manferdini et al. focused on characterising synovial cells in low- and moderate-grade synovitis and concluded that the majority of all synoviocytes was positive for CD55 in both low- and moderate-grade synovitis (55). In addition, analysis showed that CD55 was positive in both sublining and lining layers (55). Another study of Manferdini et al. compared the number of CD55-positive cells to the number of CD68-positive cells in OA with moderate-grade synovitis (56). A significantly higher percentage of CD55-positive cells was found, compared to CD68-positive cells, confirming a high percentage of synovial fibroblasts in the synovium compared to the percentage of macrophages (56).

CD90

Ten studies analysed CD90-expression on OA FLSs (46, 50, 55, 57, 68, 70, 74, 75, 78, 81). Two studies found that CD90 was expressed on the cell surface of more than 90% of OA FLSs, with similar results for RA and healthy FLSs (68, 74). In contrast to these results, Mimpen et al. discovered that at least 29% of FLSs did not express CD90 on their cell surface (57). Choi et al. analysed the effect of IL-1β on FLSs and found that the expression of CD90 was, similar to CD44, not influenced after stimulation of the cell culture (74).

IHC-analysis provided more information about the localisation of this marker in the synovium (75). In the lining layer of the OA synovial membrane, 1.7% of the cells stained positive for CD90, while the percentage of $CD90^+$ cells in the lining layer of RA synovium was significantly higher (75). Subsequently, two studies have used CD90-presence as a means to distinguish FLSs from other cells (46, 50), while four other studies (70, 75, 78, 81) focused on the co-expression of CD90 with other markers, which will be discussed in the sections due to these specific markers.

3.3.1.2 Non-common fibroblast typing proteins

Other cluster of differentiation proteins

Other cluster of differentiation proteins found on the cell surface of OA FLSs were: CD11a, CD29, CD34, CD40, CD49e, CD166 and CD248 (55, 57, 68, 81). As for CD11a and CD29, no difference in expression was observed after long-term TNFα treatment (68). Mimpen et al. examined the co-expression of CD34 and CD90 on $CD45^{-}PDPN^{+}$ fibroblasts (57). It was described that 23% of these cells were double negative ($CD34^{-}CD90^{-}$), 32% were $CD34^{-}CD90^{+}$ and 45% were $CD34^{+}$ cells (57). With reference to CD163, Ohashi et al. concluded that this protein was sometimes expressed in combination with CD90. However, most FLSs were CD163-negative (70). Lastly, Payet et al. stated that a significant proportion of CD248-expressing cells in OA synovium was $CD90^{+}$, thus confirming that these cells were FLSs (81). There were no differences found in the expression of CD248 on OA FLSs compared to RA FLSs (81).

Adhesion proteins

CD54, known as Intercellular Adhesion Molecule 1 (ICAM-1) has been examined by three different studies (50, 68, 82) while CD106, known as Vascular Cellular Adhesion Molecule 1 (VCAM-1), has been investigated by six (50, 52, 53, 55, 68, 74). ICAM-1 and VCAM-1 expression was compared on FLSs (68, 74). It was found that both proteins were highly expressed on the surface of FLSs found in OA synovium as well as in healthy synovium (55, 68). The expression of these surface markers was influenced by multiple inflammatory factors (68, 74, 82). TNF-α stimulation caused an increase in ICAM-1, as well as VCAM-1 expression on OA and healthy tissue FLSs (68). However, IL-1β only induced an increase of ICAM-1 expression, while VCAM-1 expression remained unchanged (68, 74). Interferon gamma (IFNγ) also induced an increase in ICAM-1 expression on the cell surface, which was antagonised by IL-4 and IL-13 (82). Treatment of OASFs with CCL2 for 24h induced cell surface VCAM-1 expression in a concentration-, as well as in a dose-dependent manner (52). Treatment of OASFs with CCN4 for 24h induced cell surface VCAM-1 expression in a concentration dependant manner (53). A discrepancy between the expression of ICAM-1 and VCAM-1, was that ICAM-1 was found to be expressed in nearly all OA and RA FLSs, while VCAM-1 expression was much higher in OA FLSs than in RA FLSs (50).

Angiotensin II receptors

Pattacini et al. decided to explore the possibility that a component of the renin–angiotensin system, the angiotensin II-receptor AT1, might be expressed on FLSs (71). Their study showed that FLSs indeed showed high expression of AT1 on their cell surface and there was no difference in the percentage of AT1-expressing FLSs from OA and RA patients (71).

Annexin I binding sites

Sampey et al. examined the presence of annexin I binding sites on FLSs obtained from patients with OA compared to FLSs from patients with RA (73). It was demonstrated that annexin I binding site fluorescence was significantly lower on RA FLSs than on OA FLSs at all concentrations of annexin I (73). Additionally, the effect of different proteases on the cells was analysed. Elastase had no significant effect on either OA or RA annexin I binding site numbers, whereas collagenase significantly increased the number of the binding sites on OA FLSs, with a similar trend seen in RA FLSs (73). Addition of the cytokine TNF-α promoted an increase in annexin I binding sites on both OA and RA FLSs, similarly to the effect of IL-1β stimulation (73). Introduction of the glucocorticoid dexamethasone to the FLSs had no significant effect on the number of annexin I binding sites (73).

Chemokine receptors

Ren et al. analysed synovial membrane samples, which tested positive for CCR3-expression, as well as for CCR5-expression (61). Flowcytometry-analysis confirmed that all primary FLSs were indeed positive for CCR3 and CCR5 (61). The percentage of positive CCR3 and CCR5 primary FLSs were quantified, and no significant differences were observed between healthy tissue and OA samples (61). Chang et al. quantified the percentages and concluded that among $CCR3^+$ cells, 58.4% cells were FLSs ($CD90^+CD14^-CD3^-$), indicating that FLSs are major population of $CCR3^+$ cells in the synovial tissue (46).

FACS showed a strong expression of CCR7 on the cell surface of both OA and RA FLSs, whereas only a marginal expression was detected on dermal fibroblasts as a healthy control (44). Furthermore, double staining with antibodies against CCR7 and prolyl-4-hydroxylase, a

typical fibroblast-marker, clearly confirmed CCR7-expression on the FLSs in the synovial tissue of OA and RA patients (44).

Cytokine receptors

Honorati et al. discovered that IL-17R was the only cytokine receptor found to be highly expressed on the surface membrane of synovial fibroblasts and in OA synovium (72). Stimulation by neither IL-17 nor TNF-α modulated the percentage of IL-17R positive cells or fluorescence intensity (72). The constitutive expression of high-affinity receptors for cytokines IL-2 (CD132/Rγc), IL-4 (CD124/Rα) and IL-15, on the contrary, were barely detected on the cell surface of OA or healthy tissue FLSs (68).

Dopamine receptors/transporters

Double staining of synovial tissue revealed that FLSs expressed all dopamine receptor subtypes and dopamine transporter in both patients with RA and patients with OA (45). Furthermore, Capellino et al. discovered that D1-like receptor–positive cells were observed mainly in the actively inflamed area of the synovium and around the blood vessels, while D2-like cells were also present in the sublining layer (45). Van Nie et al. revealed that all DRs were expressed in cultured FLSs, based on analysis of untreated OA FLSs and RA FLSs (64). The density of FLSs positive for all dopamine receptors and dopamine transporter tended to be higher in patients with RA compared with OA (45). In particular, the density of dopamine receptors D3, D4, and D5 and that of dopamine transporter was significantly greater in RA compared with OA (45). However, the overall density of D2-like receptor–positive cells was higher than the density of D1-like receptor–positive cells in both groups (45). IHC-analysis pointed out that D3DR was significantly higher expressed in OA synovial tissue near the cartilage compared to other layers of the synovium (64). Van Nie et al. also investigated if there are any age-related differences to be found in DR-expression (64). No significant age-related differences of DR-expression were observed in OA patients, in contrast to RA patients, where expression of D1DR, D2DR and D4DR was significantly lower in older patients (64).

Fibroblast activation proteins

Bauer et al. concluded that fibroblast activation protein (FAP) was expressed by synovial fibroblasts in patients with OA and RA (78). However, IHC-analysis pointed out that there was a stronger expression of FAP in the inflamed synovium of patients with refractory RA than in the synovium of patients with OA (78). Wäldele et al. confirmed this finding by observing a higher expression of FAP in RASF compared to the expression in OASF, as reflected by significantly higher fluorescence levels (77). During analysis of the location of this marker, it was noticed that there was a high expression of FAP in RA throughout the whole synovial membrane (77). This is in contrast to the synovial tissues of OA patients, where only a marginal expression, predominantly in the lining layer, was detectable (77).

To test the hypothesis that FAP plays a role in ECM degradation, Bauer et al. examined the co-expression of FAP together with other cell surface proteins associated with matrix degradation (78). To be concrete, they analysed the concomitant involvement of FAP together with metalloproteases MMP-1 and MMP-13, and v3 and v7/v8 splice variants of CD44 in the lining layer of OA and RA synovium contributing to the characteristics of FLSs with myofibroblastic phenotype. It was found that osteoarthritic joints showed limited staining for MMPs and CD44-variants, with only minor expression of MMP-13 and CD44v7/8 (78). This is in contrast to the synovial lining layer of rheumatoid joints, where the expression signature characterised the area of FAP-expressing cells as the centre of high inflammatory activity in the rheumatoid synovium (78). It was also discovered that a distinct FAP-positive fibroblast population in the lining layer of the synovium from both OA and RA patient groups completely lacked CD90-surface expression (78). This population showed MMP1, MMP13 and variants of CD44 on their cell surface as well (78).

The aforementioned finding was supported by Mimpen et al (57). They analysed the co-expression of FAP together with CD90 on the cell surface of a specific fibroblast population defined as $CD45^{-}PDPN^{+}$ fibroblasts (57). It was described that on average, 65% of $CD45^{-}PDPN^{+}$ fibroblasts were $FAP^{+}CD90^{+}$ fibroblasts, while 29% of cells were found to be $FAP^{+}CD90^{-}$ (57). In 9 out of 10 patients, there were more $FAP^{+}CD90^{+}$ than $FAP^{+}CD90^{-}$ fibroblasts located in the synovium (57). Furthermore, Mimpen et al. investigated if there was a correlation between these cell subsets and Body Mass Index (BMI) or global burden of the joint, but no correlation was found (57).

Nerve growth factor receptors

To investigate the effects of nerve growth factor (NGF) in synovial cells obtained from healthy and OA patients, Manni et al. analysed the presence of its receptor, tropomyosin receptor kinase A (TrkA) on synovial fibroblasts (69). Immunoreactivity for TrkA in both unstimulated healthy tissue FLSs and, to a more marked extent, in unstimulated OA FLSs was detected (69). Stimulation of the FLSs with IL-1β, TNF-α or CCK-8 did not induce the expression of TrkA, neither in healthy specimen cells, nor in OA cells (69). On the contrary, TrkA expression was enhanced after exposure of healthy and OA FLSs to NGF (69).

Podoplanin

Mimpen et al. described the presence of podoplanin, known as PDPN, on the surface of FLSs (57). In this study, PDPN was employed as a marker for a specific fibroblast-subset. More specifically, their further analysis of cell surface proteins was conducted on $CD45^{-}PDPN^{+}$ fibroblasts (57).

Prokineticin receptors

To investigate the role of prokineticin 2 (PK2) in the pathogenesis of OA, the presence of its receptors, PKR1 and PKR2, was analysed by Noda et al (59). Immunofluorescent evaluation showed positive expression of PKR1 in OA and RA FLSs, whereas minimal PKR2-expression was detected (59).

SMA

A reliable marker for identifying the subpopulation of synovial fibroblasts with a myofibroblastic phenotype is smooth muscle actin (SMA). Bauer et al. therefore analysed the expression of SMA by FAP-positive FLSs in the intimal lining layer (78). The expression pattern and staining intensity of SMA on the FLS-surface were considerably different between RA and OA synovium (78). In OA synovial tissue, the density of activation markers differed between the

samples from different patients (78). On the contrary, synovial samples from patients with refractory RA showed homogenous expression pattern and staining intensity with regard to the degree of inflammation (78).

Src homology domains

Fiorito et al. studied the expression of Src homology domains in OA synovial fibroblasts compared to healthy synovial fibroblasts (68). It was found that both cells homogeneously expressed SH2, SH3 and SH4 (68).

Thyroid hormone receptors

Pörings et al. investigated the presence of different thyroid hormone receptors (TR) on the cell surface of FLSs (60). In this study, TRα expression was detected in synovial tissue of OA patients, in contrast to TRβ expression, which was not detected under these IHC-conditions. Furthermore, it was found that DIO2, DIO3, TRα and TRβ were present in FLSs under culture conditions, although staining for TRβ was weak (60). Pörings et al. also investigated which effects stimulation with inflammatory cytokines had on their expression. TNF increased protein expression of DIO2, DIO3 and TRα in OA and RA patients, whereas TNF had no influence on TRβ in OA patients (60).

3.3.2 Surface proteins on macrophages

The proteins along with studies describing their expression on the cell surface are listed in *Table 4* for macrophages.

Table 4: Surface proteins on macrophages

Surface proteins	Synonym	Studies describing expression on the cell surface
Chemokine receptors		
• **CX_3CR1**		(65)
Cluster of differentiation proteins		
• **CD11a**	LFA-1	(67)
• **CD11b**	CR3 (α-subunit)	(49, 67)
• **CD11c**	P150,95 (α-subunit)	(49, 67)
• **CD13**		(67)
• **CD14**		(25, 49, 54-56, 58, 67, 70, 75, 80)
• **CD15a**		(67)
• **CD18**	β-subunit	(67)
• **CD25**		(67)
• **CD31**		(67)
• **CD33**		(67)
• **CD34**		(67)
• **CD35**		(67)
• **CD37**		(67)
• **CD39**		(67)
• **CD40**		(57)
• **CD45**	Leukocyte common antigen	(57, 58, 67)
• **CD54**	ICAM-1	(67)
• **CD68**		(54-57, 62, 63, 66, 67, 75, 79)
• **CD71**		(67)
• **CD80**		(48, 54-56, 65, 70)
• **CD86**		(54, 56)
• **CD163**		(45, 54-56, 63, 65, 70, 76, 79)
• **CD206**		(48, 54, 56, 57, 70)
Dopamine receptors		
• **D1DR-D2DR**		(45)
Dopamine transporters		
/		(45)
EGF-TM7 receptors		
• **F4/80**	EMR1	(47, 65)
Fc-receptors		
• **Fc-I**	CD64	(25, 67)
• **Fc-II**	CD32	(25, 67)
• **Fc-III**	CD16	(25, 49, 55, 67)
Folate receptors		
/		(49, 51, 63)

Ion channels		
• **ASIC1a**		(47)
Lectin receptors		
• **Mincle**		(58)
MHC class II receptors		
• **HLA DR**		(49, 62, 67, 80)
Prokineticin receptors		
• **PKR1-2**		(59)

3.3.2.1 Common macrophage typing proteins

CD14

The expression of CD14, a typical macrophage-marker, was investigated by ten different studies (25, 49, 54-56, 58, 67, 70, 75, 80). It was confirmed by Iguchi et al. that $CD14^+$-cells had the appearance of macrophages under a electronic microscope (80). These macrophages can be found in different parts of the synovium. Three studies found expression of this marker in the intima (25, 75, 80), with one article reporting a strong reaction of 83% CD14+-macrophages of all lining layer cells (75). On the cell surface itself, low density was reported on macrophages in the lining layer (25). In the subintima, a high expression of $CD14^+$-macrophages was shown with a high density of the protein on the cell surface (25, 49, 67). Furthermore, a positive reaction was reported in the perivascular zone and the stroma (25, 75). For the lymphoid aggregates, one article described expression of the protein on macrophages (75), while one article described no expression (25). In addition, $CD14^+$-cells produced more TNF-α than $CD14^-$-cells (58). Bröker et al. concluded that large numbers of $CD14^+$-macrophages were found to be a constant feature of synovial inflammation (25). On CD14-positive cells, expression of other proteins, namely CD68, CD86 and CD206 were found (56). None of these proteins were expressed in CD14-negative cells (56).

CD68

CD68 has been examined in ten different studies (54-57, 62, 63, 66, 67, 75, 79). In the lining layer, three studies reported a strong antigen reaction of CD68 (54, 55, 62). These $CD68^+$-lining cells contained cartilaginous fragments inside the cell body (62). While Athanasou et al. showed a strong presence of $CD68^+$-macrophages in the subintima of the synovium (67), three studies only reported a small number of $CD68^+$-macrophages (54, 55, 62). Furthermore, one study reported a strong reaction of $CD68^+$-macrophages around lymphoid aggregates and a moderate reaction within aggregates (79).

Young et al. studied the effect of methylprednisolone acetate on $CD68^+$-macrophages in the synovial lining and sublining layer (66). A small reduction (30%) in CD68-expression was detected in the synovial lining post-treatment. This reduction was not observed in the placebo group. The researchers reported no effect on mediators of cartilage destruction together with the decline of $CD68^+$-macrophages. Furthermore, no decrease of $CD68^+$-macrophages due to methylprednisolone acetate was seen in the synovial sublining (66).

Apart from differences in localisation, one study described differences in the CD68-reaction depending on the intensity of the synovitis (55). Manferdini et al. showed a stronger reaction to CD68 in moderate-grade synovitis than in low-grade synovitis (55). In joints showing moderate-grade synovitis, they observed expression of CD68 together with S100A8 and CCL3/MIP1α, respectively a degradative factor and an inflammatory factor, on macrophages in synovial tissue (55). Co-expression of CD68 with CD40 was found in 12% of the macrophages (57).

CD163

Nine studies discussed the expression of surface protein CD163 on macrophages (45, 54-56, 63, 65, 70, 76, 79). Ohashi et al. confirmed CD163 as a macrophage marker by comparing expression of CD14 (70). A significantly higher amount of CD14 expression was found in the $CD163^+$ fraction compared to the $CD163^-$ fraction (70). Fonseca et al. studied CD163-positivity on macrophages in RA and OA specimens (79). It was found that CD163 was better in distinguishing mononuclear phagocytes, in comparison to CD14 and CD68. Mature macrophages showed the highest expression of CD163 (79).

A positive reaction for CD163 was found in the intima and subintima (76, 79), with one study reporting a higher expression in the intima than in the subintima (63). Staining was scarce in the periphery of lymphoid aggregates and absent from aggregates centres (79). The same results were found for RA (63). Compared to healthy tissue, Mardanpour et al. showed an increase of CD163 in OA tissue, with enhanced phagocytosis and migratory activities was confirmed within the OA synovium (76). Apart from that, Tsuneyoshi et al. found that more M1-markers, including TNF-α and iNOS and M2-markers, including IL10 and transforming growth factor bèta (TGF-β) were expressed with $CD163^+$-macrophages in the lining layer than in the sublining layer (63).

3.3.2.2 Non- common macrophage typing proteins

Adhesion receptors

The adhesion receptor CD11 is a heterodimer with three different antigens, namely CD11a, CD11b and CD11c (83). Two studies discussed CD11 (49, 67). Athanasou et al. found a strong membrane reaction against CD11a and CD11c in the subintima (67). CD11a- and CD11c-antibodies stained more than 50% of the $CD14^+$- or $CD68^+$-cells (67).

Chemokine receptors

Watanabe et al. studied the effect of around-knee osteotomy (AKO) on inflammation in the OA joint (65). In their research, CX_3CR1, a chemokine receptor, was observed in the superficial layers of the intima. Whereas a disorganised arrangement of these macrophages was found prior to surgery, well-aligned macrophages were seen post-operatively (65). In the deeper layers of the intima, CX_3CR1-negative macrophages were found, with a decrease in number after surgery (65).

Dopamine receptors/transporters

Capellino et al. studied the expression of dopamine receptor 1 to 5 (D1DR –D5DR) and the dopamine transporter on synovial macrophages (45). A positive reaction of D1DR and D2DR was reported (45). D1DR-positivity showed high expression in the actively inflamed area of the synovium and around blood vessels (45). D2DR-positivity was seen in the D1DR-positive area and additionally in the sublining layer (45). For dopamine receptors D3, D4, D5 and the dopamine transporter, no expression was found on macrophages in the synovial tissue (45).

EGF-TM7 receptors

Dong et al. showed the expression of F4/80 protein on the cell surface of macrophages in OA tissue (47). F4/80, a specific macrophage surface marker, can distinguish synovial macrophages from synovial fibroblasts and is part of the epithelial growth factor- like seven transmembrane motif (EGF-TM7) receptor family (47). A significant decrease of $F4/80^+$-macrophages was seen after AKO compared to before (47).

Fc-receptors

Fc-receptors for IgG (FcyR) are reported by four studies (25, 49, 55, 67). The receptors described are FcyRI (CD64), FcyRII (CD32) and FcyRIII (CD16) (25, 49, 55, 67). All three receptors were represented on macrophages in synovial tissue of OA patients (25). FcyRI (CD64) was weakly expressed in the lining layer (25). In the sublining layer, macrophages showed weak to intermediate expression (25, 67). Furthermore, intermediate expression was also reported in the stroma, while strong expression of this receptor was detected in the perivascular region (25). In lymphoid aggregates, no expression was found (25). Bröker et al. reported similar expression patterns between FcyRI and CD14 (25). FcyRII (CD32) had a homogeneous distribution in the tissue (25). Athanasou et al. focused on the subintimal macrophages and found a weak expression for FcyRII in this area (67). In the lining layer, high levels of FcyRIII (CD16)- macrophages were expressed, while low levels were reported in the subintima and stroma (25, 67). In the perivascular area and in lymphoid aggregates, expression was negative (25). Compared to RA, no significant differences in $FcyR^+$-cells were observed (25).

Folate receptors

Three studies reported expression of folate receptors on macrophages in OA (49, 51, 63). Tsuneyoshi et al. examined in synovial tissue of OA and RA patients the expression of FR-β on mononuclear cells, including macrophages (63). In inflamed synovial tissue of OA patients, they reported expression of FR-β predominantly in the lining layer, while FR-$β^+$-macrophages in RA are most expressed in in the sublining layer (63). Apart from that, it was found that more M1 markers, including TNF-α and iNOS and more M2 markers, such as IL-10 and TGF-β, were expressed on FR-β-macrophages in the lining layer than in the sublining layer (63). CD163 showed similar expression (63). $CD163^+$FR-$β^+$-cells and $CD163^-$FR-$β^+$-cells were identified as macrophages using CD68, a pan-macrophage marker, and a folate uptake assay (63). The functional specificity of FR on macrophages was confirmed by Hseuh et al. (49). A significant reduction in fluorescent folic acid uptake was observed with the addition of non-labelled folic acid (49). On cell surface level, Tsuneyoschi et al. found that FR-β-expression levels were higher in $CD163^+$FR-$β^+$-macrophages than in $CD163^-$FR-$β^+$-macrophages (63). This result was found in both OA and RA (63). Furthermore, in the $CD163^+$ FR-$β^+$-population, expression of FR-β on the cell surface was scarcer in OA than in RA (63).

One study examined the folate receptor by using Technetium Etarfolatide (^{99m}Tc-EC20), a folate receptor-specific molecular imaging agent (51). Subsequently, SPECT/CT analysis was performed. Using this technique, the presence of FR$^+$-macrophages in the synovial membranes of OA patients was confirmed (51). Apart from that, Kraus et al. analysed the correlation between typical OA features and folate uptake by the folate receptor (51). They found a positive correlation with joint symptoms, severity of joint space narrowing, indicative of cartilage loss and/or meniscal extrusion and osteophytes, indicative of the anabolic joint response to the disease (51).

Ion channels

Dong et al. studied the acid-sensing ion channel 1a (ASICI1a) in KOA specimens (47). By using IHC, high expression of this protein was shown on the cell surface of macrophages (47).

Lectin receptors

Moriya et al. studied the expression of Macrophage inducible Ca2+-dependent lectin receptor (Mincle) (58). It was found that CD45$^+$CD14$^+$-macrophages tested positively for Mincle in the synovial lining layer (58).

MHC class II receptors

The expression of HLA-DR, an MHC class II receptor, was investigated by four different studies (49, 62, 67, 80). Iguchi et al. confirmed that HLA-DR$^+$ cells had the appearance of macrophages by using an electronic microscope (80). In patients with OA, abundant expression of these HLA-DR$^+$-macrophages was found, predominantly in the lining layer (62, 80). In the lining and sublining layer, a high density of HLA-DR was found on the cell surface of macrophages (67). The distribution of HLA-DR was similar to distribution of CD68 (62).

M1 and M2 macrophage markers

CD80 and CD86, known as M1 macrophage markers, were found primarily in the lining layer of OA tissue (54, 56). When compared to patients with an anterior cruciate ligament injury, higher density of CD80+-macrophages was shown in OA patients (48). CD206, a M2 macrophage marker, was found in the lining and sublining of the tissue (54, 56). Of the CD68$^+$-

macrophages, proximately half of the cells had CD206-positivity and 9.4% expressed both CD40 and CD206 (57). Low levels were found in OA patients, with no difference to those with an anterior cruciate ligament injury (57). Watanabe et al. compared M1/M2 ratio before and after AKO, using respectively CD80 and CD163 as a marker (65). In OA tissue, they showed a significant decrease postoperatively in the M1/M2 ratio (65).

Ohashi et al. analysed the intensity of the expression of CD14 on $CD163^{+}$-cells and its relation to CD80 and CD206 (70). It was found that the $CD163^{+}$-cell group could be divided into a $CD163^{+}CD14^{high}$-population and a $CD163^{+}CD14^{low}$-population (70). Whereas $CD80^{+}$-cells were detected in the $CD163^{+}CD14^{high}$-population, this was not the case for the $CD163^{+}CD14^{low}$-population (70). CD206 was found in both populations (70).

Prokineticin receptors

Noda et al. studied the expression of prokineticin receptor 1 and 2 on mononuclear cells, including synovial fibroblasts, synovial macrophages and inflammatory cells (59). It was observed that most mononuclear cells in OA and RA tissues showed a positive reaction to PKR1 and PKR2 (59). Compared to RA, PKR1-expression in OA synovium was significantly higher in the lining and sublining layers (59). This difference between OA and RA was not found for PKR2 (59).

4 DISCUSSION

This scoping review provides an extensive overview of existing literature concerning cell surface markers on fibroblasts and macrophages in the synovium of OA joints. The main purpose of this study was to gain a better understanding of the different surface markers and to provide new insights into how they play a role in the development of this disease. Several contributing surface proteins on synovial fibroblasts and macrophages have been identified. CD44, CD55 and CD90 were often described on fibroblasts, while on macrophages, CD14, CD68 and CD163 have been frequently mentioned. One of the mechanisms by which fibroblasts and macrophages induce joint damage is by, directly or indirectly, stimulating the inflammatory process associated with OA. In this scoping review, the findings are supported by the existing literature on fibroblasts and macrophages in general, including surface protein functions and pathways involved in inflammatory processes.

For fibroblasts, different surface proteins have been identified in OA synovium. Three markers that are frequently found on the cell surface of healthy fibroblasts, as well as on osteoarthritic fibroblasts, are CD44, CD55, CD90 (55, 68, 74). These proteins are seen as typical fibroblast-markers and are often used to distinguish fibroblasts from other cells (55). Their functions are wide-ranging. CD55, for example, also known as decay accelerating factor, is a well-known factor involved in complement activation and regulation (85). CD44 stimulates hyaluronate degradation, while CD90 contributes to synthesis and release of ECM components (86, 87). Additionally, these surface markers share functions in cell-adhesion and release of cytokines (86, 88). The features listed above are mainly associated with the mechanisms of action of healthy tissue fibroblasts (18). The finding that these proteins are, for the most part, homogeneously expressed by synovial fibroblasts and that expression is not influenced by pro-inflammatory factors, like IL-1β, in the environment, supports the hypothesis that these proteins are not involved in the pathogenesis of OA (55, 74).

However, two studies discovered certain subpopulations of FLSs that did not express CD90 on their cell surface (57, 78). These findings imply that CD90 could be a less reliable fibroblast-marker than first assumed. When this surface protein is used as a means to identify FLSs, it is possible that subpopulations are overlooked. This hypothesis is supported by the study of Bradley et al., where absence of CD90 on synovial fibroblasts indicates a more fibrotic myofibroblast phenotype (84). Since it is observed that synovial fibroblasts in OA differentiate into myofibroblasts in response to inflammatory factors in the environment, it can imply that CD90-absence assumes a more pathological state of synovial fibroblasts (26). Therefore, when this marker is used as an identifier, the role of fibroblasts in the pathogenesis of OA might be overlooked.

There are some indications to assume a pro-inflammatory character. An example of a link between a surface protein on synovial fibroblasts and synovial inflammation is the presence of CCR3. Studies show that CCR3 is found on the cell membrane of synovial fibroblasts in OA joints (46, 61). One of the known chemokines that bind to CCR3 is eotaxin-1 (CCL11). It was revealed that a greater amount of eotaxin-1 was present in the synovial fluid of patients with OA (46). Moreover, eotaxin-1 was found to upregulate MMP9 production via binding to CCR3 (46). In a recent study, MMP9 was linked to fibroblast survival, proliferation, migration and invasion and to the production of pro-inflammatory cytokines (89). Accordingly, a possible relationship between CCR3-expression and inflammatory responses in the synovium has been identified (46). Contradicting the importance of this chemokine receptor was the observation that there was no difference between the percentage of OA fibroblasts expressing CCR3 and the percentage of healthy tissue fibroblasts expressing CCR3 (46). Since it is

eotaxin-1 that is upregulated, and not the receptor, screening for this receptor for diagnostic purposes seems useless.

Other examples of proteins involved in pro-inflammatory activity are CD40, a protein with known function in producing pro-inflammatory cytokines, CD166, which is responsible for lymphocyte migration and T-cell activation, and FAP (68, 78, 93, 94). The latter contributes to the inflammatory processes correlated with OA through its stimulating effect on cell migration and invasion (78, 90, 91). Moreover, FAP has recently been demonstrated to promote cartilage breakdown in a collagen-induced arthritic mouse model (92). However, this proteolytic activity of FAP is still controversial and needs to be explored more thoroughly. Although significant expression of FAP was shown on fibroblasts in OA synovium, its expression in RA synovium is found to be more prominent. This suggests that the inflammatory component is less important in OA pathogenesis than in RA (77).

In contrast, markers that suggest an anti-inflammatory potential of synovial fibroblasts have been retrieved on the cell surface. CD73, for example, is known for its ability to shift ATP-driven pro-inflammatory immune cell activity toward an anti-inflammatory state mediated by adenosine (95, 96). In addition, more annexin I binding sites are expressed on the surface of OA FLS compared to RA FLS (73). Although its exact operating mechanisms remain unclear, a study has revealed that annexin I reduces production of prostaglandin E2 and TNF-α and is necessary for the anti-inflammatory effect of dexamethasone (97, 98).

Other surface proteins were detected on the cell membrane of fibroblasts that may indicate other mechanisms of action in OA synovium. CD29 and CD49e, together forming the VLA-5 complex, CD11a, CD34 and CD248 are examples of proteins with a possible function in cell-matrix adhesion that have been discovered on the surface of fibroblasts (57, 68, 81, 99, 100). These adhesion proteins may play a role in the process of fibroblast invasion into the synovium, which is a hallmark of OA pathogenesis. Furthermore, synovial fibroblasts are reported to play a role in ECM production, resulting in fibrosis of the synovium (101). For example, CD105 is a surface marker found on the cell membrane of OA FLS (68) responsible for ECM production and thus contributing to synovial fibrosis (102). In addition, angiotensin II receptors have been identified on the membrane of synovial fibroblasts. A previous study located these receptors on the membrane of cardiac fibroblasts, where they were linked to a role in ECM synthesis, ECM activity and consequently, to fibrosis (103). The difficulty in these observations lies in the fact that the expression of these markers was only examined on fibroblasts in OA-joints and not on healthy tissue fibroblasts. Therefore, it is not possible to

make a comparison to verify whether there is a difference in expression which could imply a difference in functional effect.

CD14 is the most commonly reported marker on macrophages in this review. High numbers were observed on macrophages in all parts of the synovium (25, 49, 67, 75). In terms of function, this protein is a receptor for lipopolysaccharide (LPS), associated with the monocyte/macrophage cell lineage (75, 104). CD14 contributes to the inflammatory process by providing a signal for the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α (104). It has been confirmed that the introduction of TNF-α into a cell culture of fibroblasts increases the expression of VCAM-1 and ICAM-1 on the cell surface (68). By enhancing these proteins, fibroblasts promote the migration of leukocytes from blood vessels (105, 106). Among these leukocytes are macrophages, on which the ligand for ICAM-1, LFA-1 (CD11a), has been found (67). This suggests that macrophages sustain their presence during the inflammatory process and that fibroblasts contribute indirectly through promoting migration of leukocytes.

The macrophage marker CD68 was observed in synovitis associated with OA (55, 62). Strong expression of CD68 was found in the lining layer (54, 55, 62). Here, $CD68^{+}$-cells contained cartilage debris inside the cell body (56). Macrophages are known to incorporate foreign fragments via lysosomes containing CD68 (56). After processing, the antigens reappear on the cell surface of macrophages using HLA-DR (62, 80, 107). HLA-DR belongs to the major histocompatibility complexes expressing class II antigens (MHC class II) and contributes to the inflammatory process by presenting antigens to $CD4^{+}$-T cells (108). This results in T-cell activation (108). HLA-DR was shown multiple times on macrophages in this review (49, 62, 67, 80, 108). Apart from that, results showed that the distribution of CD68 in the synovium was followed by the distribution of HLA-DR (107, 109). Upregulation of CD68 on macrophages indicated increased phagocytic activity and was accompanied by co-expression of degradative and inflammatory proteins (55, 110). Young et al. reported a reduction in $CD68^{+}$-macrophages with intra-articular glucocorticoid treatment (66). Contrary to what was expected, this reduction was not followed by a reduction in inflammatory and degradative mediators, like MCP-1, MIP-1α, MMPs and TIMPs (66). In explanation of this, it was hypothesised that the majority of these factors are produced by synovial fibroblasts, which may be more resistant to intra-articular glucocorticoids (111-113).

Another example of how macrophages play a role during inflammation is through CD163. This protein, which promotes phagocytosis and migration, has been correlated with resting pain in OA (70). They postulated the increase in TNF-α in the $CD163^+$ group as a possible explanation, since it was not observed in the $CD163^-$ group. TNF-α is considered to be a pro-inflammatory cytokine that contributes to pain sensitisation (70). Mardanpour et al. hypothesised that the excessive phagocytosis, occurring in OA, sensitises these $CD163^+$ -macrophages to cell death (76). This may lead to a release of pro-inflammatory cytokines, possibly TNF-α, which causes pain (76).

This aforementioned pro-inflammatory role of CD163 is questionable. For a long time, macrophages have been divided into two subsets, called M1 and M2 macrophages. M1 was characterised by the production of pro-inflammatory cytokines, while M2 by the production of anti-inflammatory cytokines (63). CD163 is considered to be an M2 macrophage marker, suggesting an anti-inflammatory character (63). Tsuneyoshi et al. confirmed that the classification of macrophages into pro-inflammatory and anti-inflammatory was not as straightforward (63). It was found that both M1 and M2 markers were abundant on $CD163^+$ macrophages (63). Because of these conflicting results, a more broad-based view of macrophages in OA needs to be encouraged. Macrophages may be difficult to categorise into two groups. Rather, they should be viewed in terms of a continuum of activation states based on local tissue and cytokine environment (51). This is in line with the findings of this review.

Furthermore, CX_3CR1^+-macrophages and CX_3CR1^--macrophages were both found in OA synovium (65). Based on this finding, two hypotheses can be put forward. First, not all macrophages may be uniformly derived from the blood. Distinct subsets of these cells have been reported in organs during early development that were able to maintain their population independently of blood supply (114-116). In arthritic mice, Culemann et al. demonstrated that the CX_3CR1^+-macrophages self-sustain their presence via a pool of locally proliferating CX_3CR1^--mononuclear cells in the synovium (117). In this review, macrophages expressing CX_3CR1 were found in the superficial layer of the synovium, whereas CX_3CR1^--cells were retrieved in deeper layers (65). This was consistent with findings in this review, suggesting a subset of synovial macrophages in OA that are not blood derived. Secondly, it is hypothesised that these resident CX_3CR1^+-macrophages restrict the inflammatory reaction. Watanabe et al. found that CX_3CR1^+-cells were disorganised before AKO, whereas a well-aligned organisation was found postoperatively (65). This is consistent with results found by Culemann et al. (117). Within synovial macrophages, CX_3CR1 expression was found on a specific subset of

membrane-forming macrophages (117). These CX_3CR1^+-cells form an immunological barrier between synovium and joint cavity and physically seclude the joint through tight junctions, hereby restricting the inflammatory reaction by providing a shield for intra-articular structures (117). This is in contradiction to recruited blood-derived macrophages, which actively contribute to joint inflammation (55, 62, 117). These findings suggest a state of reduced inflammation achieved through the forming of a barrier of self-sustained macrophages.

To address the need for further in vivo studies, macrophages in OA tissues have been investigated by using their folate receptors and SPECT/CT. In RA, there is evidence that folate uptake correlates with inflammatory parameters (51). Several studies have reported that this folate uptake is mediated by macrophages in OA. This makes SPECT/CT with Etarfolatide a non-invasive diagnostic technique linking activated macrophages to inflammation. This technique was used in a study by Kraus et al. who showed strong correlations between macrophages and inflammation on the one hand, and radiographic OA and joint pain on the other (51). It is postulated that macrophage-targeted therapies may be anti-arthritic. Studies of folate-targeted therapy in adjuvant-induced arthritis in rats support this claim (118). For example, Yi et al. showed that different folate therapies could alleviate the main symptoms of adjuvant-induced arthritis (118). Although these results sound very promising, they should be interpreted with some caution. In this review, FR-β expression was shown to be lower on the surface of OA macrophages than RA macrophages (63). This may indicate a different distribution of these proteins and consequently a different impact on the disease process in comparison to RA (63).

In this review, a number of other proteins were found on the surface of fibroblasts that may play a role in the pathogenesis of OA, namely chemokine receptors CCR5 and CCR7, cytokine receptor IL-17R, NGF receptor TrkA, glycoprotein PDPN and thyroid hormone receptors TRα and TRβ (44, 57, 60, 61, 69, 72). Other proteins found on the surface of synovial macrophages are EGF-TM7 receptors, ion channels, Mincle and Fc receptor (25, 49, 54, 58, 67). Finally, D1DR-D5DR, dopamine transporter and PKR1 and PKR2 proteins were present on both synovial fibroblasts and macrophages (45, 59). A link with inflammation is suggested for these surface markers, however more research needs to be carried out.

There were several limitations to this scoping review. Firstly, six articles gave no indication of which joint was examined. Therefore, it cannot be guaranteed that the back, shoulder and temporomandibular joint have been entirely excluded. Furthermore, the comparison of results

does not take into account the joint from which the biopsy was taken. For example, cells obtained from osteoarthritic knee joints are compared with cells from osteoarthritic hip joints. Secondly, this review does not include techniques that investigate gene expression, such as PCR. Therefore, information about the relationship between gene and protein expression is not provided. A third limitation is that there is no standardised method of isolating and culturing cells throughout the studies included. When cells are extracted from the body and put into culture, the influence of many environmental factors is removed. This hampers to study the influence of the inflammatory environment on the expression of cell membrane proteins. As a consequence, the quality of the results might be reduced to a sub-optimal level.

In the future, research on inflammation in OA should be undertaken in comparison to healthy tissue. In current studies, OA is frequently compared to RA, where OA serves as a non-inflammatory counterpart. As a result, the contribution of inflammation in the pathogenesis of OA may be systematically underestimated. Another area of concern was the fact that almost all synovial tissue had been obtained at the time of prosthesis placement, indicating, in a late stage of disease. However, it is of great importance to also conduct studies on specimens obtained from joints of patients in earlier stages of OA. This research could allow the development of diagnostic tools with the aim of treating the disease at an early stage by interfering with pathological processes occurring in the joint. In this way, the placement of joint prostheses could be avoided, as well as the associated costs and risks. This as opposed to now, where patients can only be treated symptomatically while the joint continues to wear down until it must be replaced.

In conclusion, this review provides insight into the surface proteins on synovial fibroblasts and macrophages and their involvement in the pathogenesis of OA. From the current literature, it can be concluded that both macrophages and fibroblasts contribute to the pathogenesis via their surface proteins. Multiple different pathways are involved in how they exert their influence, of which many remain to be explored. Their role in the inflammatory process is one of the mechanisms involved. Furthermore, new directions for future research and clinical practice are identified in this review. For research purposes, more studies on the comparison between OA tissue and healthy tissue should be conducted to fully comprehend the low-grade inflammatory component. These studies should be carried out at all stages of the disease, with particular attention paid to early stages. In the future, it is important to gain a better understanding of these surface markers in order to diagnose and treat the disease in an early symptomatic stage.

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