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Osteoarthritic synovial fibroblasts drive inflammatory responses and impair chondrocyte function in response to a fibronectin matrikine

Jorge G. Fernandez Davila[a], **Ralph A. Alberto**[a], **Seyoun Byun**[b], **Susan D'Costa**[a], **Jacqueline Shine**[a], **Carolina Alvarez**[a], **Adam B. Yanke**[c], **Christopher W. Olcott**[a,d], **Brian O. Diekman**[a,e], **Douglas H. Phanstiel**[a,b,f], **Richard F. Loeser**[a,g,*]

[a]Thurston Arthritis Research Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA

[b]Curriculum in Bioinformatics and Computational Biology, University of North Carolina, Chapel Hill, NC, USA

[c]Department of Orthopedics, Rush University Medical Center, Chicago, IL, USA

[d]Department of Orthopedics, University of North Carolina School of Medicine, Chapel Hill, NC, USA

[e]Lampe Joint Department of Biomedical Engineering, University of North Carolina and North Carolina State University, Raleigh, NC, USA

[f]Department of Cell Biology and Physiology, University of North Carolina School of Medicine, Chapel Hill, NC, USA

[g]Division of Rheumatology, Allergy and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Abstract

*Corresponding author at: University of North Carolina, 3300 Thurston Building, Campus Box 7280, Chapel Hill, NC 27599-7280, USA. richard_loeser@med.unc.edu (R.F. Loeser).

CRediT authorship contribution statement

Jorge G. Fernandez Davila – contributed to the research design, data acquisition, data interpretation, drafting of the work, and agreed to be accountable for all aspects of the presented work. Ralph A. Alberto – contributed to the research design, data acquisition, and data interpretation. Seyoun Byun – contributed to RNA-seq data processing, analysis, and data submission. Susan D'Costa – contributed to the generation of RNA-seq libraries. Jacqueline Shine – contributed to flow cytometry data acquisition and data interpretation. Carolina Alvarez – contributed to the data analysis and the critical revision of statistics used throughout the manuscript. Adam B. Yanke - contributed to tissue acquisition and data interpretation. Christopher W. Olcott - contributed to tissue acquisition and data interpretation. Brian O. Diekman - contributed to data acquisition and data interpretation. Douglas H. Phanstiel – contributed to research design, data acquisition, and data interpretation of RNA-seq experiments. Richard F. Loeser – contributed to the research design, data acquisition, data interpretation, and drafting of the work. All authors read and approved the submitted version of the manuscript.

Conflict of Interest

No competing interests from any of the authors regarding this work.

Declaration of Generative AI and AI-assisted technologies in the writing process

No AI-assisted technology was used in the writing process.

Objective: To investigate the effects of a matrikine, fibronectin fragment (FN7–10), on human osteoarthritic (OA) synovial fibroblasts and implications for inflammation and cartilage degradation.

Design: Joint tissue was obtained from 50 OA patients undergoing knee arthroplasty. Isolated synovial fibroblasts were treated with 1 μM of FN7–10 or PBS as control. Cytokine protein arrays were used to identify differentially secreted inflammatory mediators in conditioned media (CM). Bulk RNA-seq was used to evaluate transcriptional changes. Monocytic THP-1 cells in transwell assays were used to evaluate chemotactic activity of CM. Macrophage differentiation of THP-1 cells was assessed via qPCR and flow cytometry. Chondrocyte gene expression was evaluated by qPCR and glycosaminoglycan (GAG) production by Alcian blue assays.

Results: FN7–10 stimulation of synovial fibroblasts induced an inflammatory secretome, including production of chemokines. RNA-seq analysis confirmed FN7–10 induced upregulation of chemokines, including *CCL5, CCL7, CCL8, CCL20, CXCL5* and *CXCL10*, which displayed mean transcriptional log-2-fold changes of 3.89 (CI, 2.81 – 4.97). CM from FN7–10-stimulated synovial fibroblasts increased THP-1 chemotaxis by 7.8-fold (CI, 3.26 – 12.35) which was reduced by CCR2 or CXCR2 inhibitors and promoted monocyte to macrophage differentiation characterized by an increase in pro-inflammatory gene expression and CD14. Chondrocytes treated with FN7–10 synovial fibroblast CM showed elevated *IL6* and *MMP1* and decreased *ACAN* and *COL2A1* expression, along with reduced GAG levels.

Conclusions: The matrikine FN7–10 promotes an inflammatory synovial fibroblast phenotype. Proinflammatory mediators, including chemokines, released from activated synovial fibroblasts recruit monocytes that may exacerbate joint inflammation and can also promote increased catabolic activity in chondrocytes.

Keywords

Osteoarthritis; Synovial Fibroblasts; Fibronectin-Fragment; Inflammation; Cell-cell communication

Introduction

Osteoarthritis (OA) is a whole-joint disease, but how the various tissues within the joint interact to promote OA is not fully understood [1]. As OA develops, extracellular matrix (ECM) proteins are enzymatically cleaved by several proteases, including matrix metalloproteinases (MMPs), aggrecanases (ADAMTS-4 and ADAMTS-5), the fibronectinase ADAM-8, and the serine protease HtrA1, resulting in the production of matrix fragments [2,3]. Bioactive ECM-derived fragments, known as matrikines, can act as signaling molecules [4,5] that perpetuate inflammation and drive tissue remodeling within the joint microenvironment. It has been hypothesized that ECM fragments released from cartilage into the synovial fluid could activate cells within the synovium as one of the mechanisms by which synovitis develops; however, few studies have examined this in detail.

Among the bioactive matrikines, fibronectin fragments of various sizes and at micromolar concentrations have been consistently detected in human OA cartilage and synovial fluid and are considered key mediators of cartilage catabolic activity [6–8]. One of these is a

110–140kDa fragment, containing the cell binding domain for the α5β1 integrin, which has been shown to be released after blunt trauma to cartilage [9]. In earlier studies, we treated articular chondrocytes with the 110 kDa fragment and found that it activated catabolic signaling which led to increased production of matrix metalloproteinases (MMPs) and various cytokines and chemokines through activation of MAP kinase signaling leading to activation of NFkB and AP-1 [10–12]. The 110 kDa fragment was generated by proteolytic cleavage of human plasma fibronectin followed by purification steps that led to batch variability in the cellular response. In order to have a consistent source of fibronectin fragment, we adopted a system to produce a recombinant fibronectin fragment, FN7–10 [10,12], that contains both the α5β1 integrin attachment site found in domain 10 of the FN type III module as well as a synergy site in domain 9 [13]. This fragment has been shown to bind and activate α5β1, including on articular chondrocytes [14]. We have shown that stimulating normal human articular chondrocytes with FN7–10 recapitulates the transcriptional features observed in OA chondrocytes [15,16]; however, how other cell types in the joint are affected remains unclear.

Synovial fibroblasts have emerged as active contributors to inflammation and ECM remodeling in both rheumatoid arthritis and OA [17–19]. They produce a range of pro-inflammatory cytokines and matrix-degrading enzymes and are increasingly recognized for their role in altering the joint microenvironment through interactions with other cell types [20,21]. Additionally, individuals with synovitis exhibit more severe cartilage loss compared to those without inflamed synovium [22].

Previous studies have shown that activation of α5β1 integrin on rabbit synovial fibroblasts using an α5β1 antibody [23] or the 120 kDa fibronectin fragment [24] as well as stimulation of human OA synovial fibroblasts with fibronectin fragments generated by HtrA1 [2] can increase the production of matrix-degrading enzymes. Here, we further investigated the phenotypic and functional response of primary human OA synovial fibroblasts using the FN7–10 fragment to determine if activation of α5β1 with a cartilage matrix breakdown product would stimulate production of OA mediators by the synovium. We evaluated the synovial fibroblast transcriptional response and protein secretion and used conditioned media from the FN7–10-stimulated fibroblasts to examine the effects on monocyte migration and chondrocyte function. Collectively, these studies aimed to delineate synovial fibroblast-mediated intercellular communication networks within the OA joint and their contribution to synovial inflammation and cartilage degradation.

Materials and methods

Study design

This study involved 55 independent human donors: 46 OA synovial donors, 1 OA synovium plus OA cartilage donor, 3 OA cartilage donors, and 5 normal cartilage donors (demographics shown in Supplementary Table 1). Each donor sample was de-identified and assigned a number. Synovial fibroblasts and articular chondrocytes were isolated from fresh tissue, as it was received, using established protocols. The cells obtained from each donor were randomized to a specific experiment and cells from the same donor were used for controls and experimental treatments constituting a paired randomized block experimental

design, with donor number serving as the blocking factor. The OA synovial fibroblasts were treated with FN7–10 or with phosphate-buffered saline (PBS) as a control as detailed below. The initial experiment was to screen for the secretion of pro-inflammatory mediators in the conditioned media (CM) from treated cells using protein arrays. Based on these results, chemokine gene expression in response to FN7–10 was selected as a focus for RNAseq analysis. The chemotactic activity of the CM, and the ability of the CM to promote macrophage differentiation, was evaluated using the THP-1 monocytic cell line. Finally, the CM was applied to cultures of normal articular chondrocytes and expression of OA relevant genes was examined as well as effects of CM on chondrocyte GAG production as a measure of anabolic activity.

Tissue procurement

OA synovial fibroblasts and OA chondrocytes were isolated from synovial tissue and cartilage, respectively, of OA patients undergoing knee arthroplasty at the University of North Carolina Hospital (UNC), Hillsborough, NC. Normal synovial tissue was not available for comparison. Further details of tissue procurement, cell isolation, and culture are provided in the Supplementary Methods.

FN7–10 treatment and conditioned medium collection

Synovial fibroblasts were plated at 20,000 cells/cm^2. The cells were serum-starved in DMEM for 2 h (hrs) before treatment with 1 mL of purified recombinant FN7–10 at a concentration of 1 μM, prepared as previously described [14]. The 1 μM dose was chosen based on the concentration of fibronectin fragments found in OA synovial fluid (1μM or greater) [6] and our previous studies with articular chondrocytes and meniscal cells that used this concentration [14–16,25]. An equal volume of PBS was used as the control condition. For RNA-seq and cytokine array experiments, cell cultures were treated with FN7–10 overnight, followed by RNA or CM collection. To generate fragment-free CM for other experiments, synovial fibroblasts were treated with FN7–10 for 4 hrs. The treatment medium was then removed, cells were washed with PBS to eliminate residual FN7–10, and 1 mL of fresh serum-free medium was added. Cultures were incubated overnight, after which CM was collected, centrifuged at 1800 rpm for 5 min to remove debris, and used immediately or stored at −80 °C.

Cytokine protein array

The C5 human cytokine protein array (RayBiotech, AAH-CYT-5–2) was used to evaluate the secreted proteins found in the CM. The list of 80 array proteins is shown in the array map in Supplementary Table S2. Array membranes were incubated overnight at 4 °C with 1 mL of undiluted serum-free CM from synovial fibroblasts treated with FN7–10 or PBS. Details are described in the Supplementary Methods.

CXCL5 ELISA

CXCL5 levels were measured in CM from FN7–10 and PBS stimulated synovial fibroblasts and normal and OA articular chondrocytes as detailed in the Supplementary Methods.

RNA sequencing (RNA-seq)

RNA sequencing was performed on synovial fibroblasts from five human donors treated with FN7–10 or PBS control. Detailed library preparation, sequencing, and bioinformatic methods are provided in the Supplementary Methods and the complete dataset is available in the database of Genotypes and Phenotypes (dbGaP) under accession #phs003999.v1.p1.

Transwell migration

The transwell migration assay was performed by plating 5×10^5 THP-1 cells resuspended in 100 μL of serum-free RPMI (Gibco, 11875–093) directly into a transwell insert (Thermo, 140627). The transwell was inserted in a well of a 24-well plate containing 600 μL of CM from synovial fibroblasts. Further details are provided in the Supplementary Methods.

RNA isolation and real-time quantitative polymerase chain reaction (qPCR)

RNA was isolated from monolayer adherent cultures (synovial fibroblasts, chondrocytes, and PMA-differentiated THP-1 cells) and from suspension THP-1 cells cells using the RNeasy Mini Kit (Qiagen, 74106) and analyzed using quantitative PCR (qPCR) as detailed in the Supplementary Methods. Primer sequences are listed in Supplementary Table 4.

Flow cytometry

See Supplementary Methods.

Heatmap generation using Morpheus

See Supplementary Methods.

Micromass alcian blue staining

Normal chondrocytes were seeded at a density of 2×10^5 in 10 μL of complete DMEM/F12 in a 48-well dish. Immediately after seeding, the plate was incubated at 37 °C with 5% CO2 for 6–8 hrs to allow the micromass to form. 1 mL of complete DMEM/F12 was then added to each micromass and incubated for 3–4 days before the experiment began. Synovial fibroblast CM from FN7–10 or PBS-treated cultures was spiked with 1% FBS to promote glycosaminoglycan (GAG) production in chondrocytes. 1% FBS DMEM was used as a control. GAG levels were measured using alcian blue as detailed in the Supplementary Methods.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (Version 10.5.0). All synovial fibroblast experiments included at least three independent OA synovial fibroblast donors and sample sizes for each assay are provided in the figure legends. Continuous variables are reported as means with 95% confidence intervals. Cytokine array data and qPCR gene expression analyses in chondrocytes were log2-transformed to address skewness prior to statistical testing and heatmap visualization. For analytic samples with sizes greater than five and meeting assumptions of normality, one sample t tests were used for direct comparisons to a theoretical mean, and paired t tests were used when comparing two correlated, normalized experimental conditions. When normality criteria were not satisfied,

the Wilcoxon signed-rank test was used for correlated samples. Comparisons involving multiple treatment conditions for multiple markers in the flow cytometry experiment were analyzed using a two-way repeated-measures ANOVA with Sidak's multiple comparisons correction. Statistical significance was defined at an alpha level of 0.05. These statistical approaches account for the paired experimental design used in synovial fibroblast, THP-1, and chondrocyte qPCR data, as well as in migration assays and GAG measurements. Corresponding 95% confidence intervals for all reported outcomes in this study are provided in supplementary tables.

Results

Treatment of OA synovial fibroblasts with FN7–10 increases production of proinflammatory mediators

Synovial fibroblasts were treated for 24 h with FN7–10 or a PBS control, and the CM was collected for incubation on C5 cytokine arrays (Fig. 1A). Cytokine arrays incubated with CM from PBS-treated synovial fibroblasts detected several inflammatory cytokines, including IL-6, CXCL8, CCL2, and CXCL1/2/3 (Fig. 1B,C). Exposure to FN7–10 CM increased the levels of every cytokine that was detectable in the controls and also induced the appearance of additional cytokines not observed in the control condition. (Fig. 1B). Comparison of the normalized densitometry of arrays incubated with FN7–10-treated synovial fibroblast CM to those arrays incubated with PBS-treated synovial fibroblast CM revealed increased secretion of inflammatory factors, predominantly chemokines, including CC and CXC ligands. Of the 80 proteins on the arrays 24 were chemokines and 15 of these were increased in the FN7–10 CM condition with the largest mean fold-changes observed for CCL5, CCL7, and CCL20 (Fig. 1D). All means and 95% CIs are shown in Supplementary Table 3. Secretion of CXCL5, the chemokine with the greatest increase across the four synovial fibroblast cultures following FN7–10 treatment, was further validated by ELISA, demonstrating increased detection in the conditioned medium of OA synovial fibroblasts, as well as normal and OA chondrocytes, stimulated with FN7–10 (Supplementary Fig. 1).

Synovial fibroblasts increase chemokine gene expression in response to FN7–10

Based on the protein array results demonstrating a significant increase in chemokines when OA synovial fibroblasts were treated with FN7–10, we examined results from an RNAseq dataset that we had recently generated using OA synovial fibroblasts treated with FN7–10 under similar conditions. A KEGG pathway analysis found the top two enriched pathways in this RNA-seq dataset were TNF signaling and cytokine-cytokine receptor interaction (data being prepared for a separate publication). We examined the genes within the cytokine-cytokine receptor interaction pathway and grouped them into cytokine families, revealing that chemokines were the highest upregulated family in response to FN7–10 stimulus (Fig. 2A). Several chemokines showed strong upregulation (log2FC > 4), including *CCL3, CCL4, CCL5, CCL8, CCL20, CXCL5, CXCL10, and CXCL11*. All mean values and 95% CIs are detailed in Supplementary Table 4. A targeted heatmap of chemokines from the RNA-seq dataset, showed increased expression of 20 chemokine genes (Fig. 2B), including all chemokines identified in our protein arrays.

Transient FN7–10 synovial fibroblast stimulation reveals significant gene expression changes a day after challenge

To investigate how the FN7–10 induced inflammatory synovial fibroblast phenotype influences other joint-resident cell types, including immune cells and chondrocytes, we established a short (4 hrs) FN7–10 stimulation protocol designed to generate FN7–10–free conditioned medium (CM), thereby eliminating fragment carryover in downstream assays (Fig. 3A). RNA was collected to confirm that pathological gene expression remained elevated for 18 hrs after FN7–10 removal. qPCR gene expression analysis showed that 4 hr FN7–10 treatment induced increased gene expression, maintained at the 24 hr timepoint, of inflammatory cytokines (*IL6, TNFA, and IL1B*) (Fig. 3B) and catabolic mediators (*MMP1 and MMP13*) (Fig. 3C), two potent collagenases found in OA cartilage and synovial fluid, as well as *CRTAC1*, a novel OA biomarker found in plasma of OA patients and involved in ECM degradation. Fibroblast markers *COL1A1* and *PRG4* demonstrated a notable decrease in response to FN7–10 treatment with no change in *TGFB* (Fig. 3D) Proliferation marker *KI67* decreased in response to FN7–10, while *PCNA* showed no change. Gene expression of all chemokine ligands measured demonstrated an increase in response to FN7–10, except for *CXCL12* and chemokine receptor *CXCR3*. All mean values, 95% CIs, and false discovery rates for p-values in this analysis are detailed in Supplementary Table 6.

FN7–10-induced factors secreted by synovial fibroblasts enhance monocyte chemotaxis

To evaluate the functional impact of FN7–10-mediated upregulation of chemokine secretion, a chemotaxis assay was conducted using a transwell system with THP-1 monocytes, as previously established [26]. We used CCL2, a potent monocyte chemoattractant, to confirm the chemotactic response of THP-1 cells. THP-1 migration toward the bottom well increased in a CCL2 dose-dependent manner under our assay conditions (Supplementary Fig. 2). We then utilized synovial fibroblast-conditioned medium as the chemoattractant, as illustrated in Fig. 4A. Representative microscopy images showed a higher number of monocytes in the bottom well in response to FN7–10 CM with a mean THP-1 migration of 2446 cells (95% CI,1762 – 3130) compared to PBS CM with a mean THP-1 migration of 518 cells (95% CI, 364 – 671) (Fig. 4B–C). To determine the involvement of chemokines, THP-1 cells were pre-treated with chemokine receptor inhibitors, as illustrated in Fig. 4D, to assess which receptors potentially mediated the increased chemotactic response.

Chemokine inhibitors RS504393, SB225002, and AMG487 were selected based on their established receptor specificity. RS504393 shows strong selectivity for CCR2, with negligible binding to CXCR1 and CCR3 and minimal binding to CCR1 [27]. CCR2 blockade with RS504393 has been used at slightly higher concentrations (up to 20 μM) to inhibit CCL2-mediated signaling in human chondrocytes *in vitro* [28], further supporting the suitability of the concentrations used in this assay. Concentrations of CXCR2 inhibitor (SB225002) and CXCR3 inhibitor (AMG487) were selected based on previous migration experiments with monocytes and macrophages [29,30]. Cell viability was not affected at 1 or 10 μM with any inhibitor (Supplementary Fig. 3A). Chemotaxis inhibition was greatest with 10 μM of the CCR2 inhibitor and with both 1 μM and 10 μM of the CXCR2 inhibitor. In contrast, the CXCR3 inhibitor showed only a modest effect at 1 μM and no significant effect at 10 μM (Fig. 4E and F). All mean values and 95% CIs in this analysis are detailed

in Supplementary Table 7, and a graph showing the total number of THP-1 cells migrated across the transwell in response to pretreatment with chemokine inhibitors is presented in Supplementary Fig. 3.

FN7–10-stimulated synovial fibroblast conditioned media induces macrophage differentiation in monocytes

We analyzed THP-1 monocyte gene expression in response to synovial fibroblast signals using synovial fibroblast CM (media conditioning step shown in Fig. 3A). THP-1 treatment conditions were serum-free DMEM (control), synovial fibroblast PBS CM, and synovial fibroblast FN7–10 CM (Fig. 5A). We first assessed expression of macrophage differentiation surface markers: *CD14*, *CD86*, *CD163*, and inflammation-related cytokines classically expressed by macrophages including *IL1B*, *IL6*, *TNFA*, *TGFB*, *VEGF*, and *CCL22*, selected based on previous studies. Compared to PBS CM treatment, FN7–10 CM treatment increased gene expression of surface markers *CD14*, *CD86, and CD163* (Fig. 5B), inflammatory-related cytokines *IL1B, VEGF, CCL22*, and chemokine ligand *CXCL5*. Chemokines *CXCL10* and *CXCL11* demonstrated a notable increase in response to FN7–10 CM, while chemokine receptor *CXCR3* showed no change (Fig. 5C–E). Additionally, cell proliferation markers *KI67* and *PCNA* showed a notable decrease(Fig. 5F), consistent with monocyte-to-macrophage differentiation [31]. All mean values and 95% CIs for THP-1 gene expression are detailed in Supplementary Table 8.

Flow cytometry analysis revealed that more than 90% of THP-1 cells stained positively for DAPI after 24-hour incubation with synovial fibroblast CM (Fig. 5G). The FN7–10 CM treatment markedly increased the percentage of $CD14^+$ cells to 95.71%, compared with 52.45% in the PBS CM treatment. The percentage of CD14-high cells was determined across 7 donor samples, which showed a consistent increase in response to the FN7–10 CM (Fig. 5H). FN7–10 CM also increased the percentage of $CD86^+$ cells to 92.47% relative to PBS CM at 58.01% (Fig. 5G). However, due to sample-to-sample variability in this assay, the increase for CD86-high cells was not significant (Fig. 5H). Additionally, FN7–10 CM resulted in a modest 9.91% $CD163^+$ THP-1 cells, compared with 3.83% in response to the PBS CM (Fig. 5G) and the changes to the percentage of CD163-high cells were not significant (Fig. 5H). Compared to controls exposed to media instead of to synovial fibroblast conditioned media, the percentage of THP1 cells with high expression of CD14, CD86, and CD163 was increased by PBS CM treatment and to a greater extent by treatment with FN7–10 CM (Fig. 5H and Supplementary Table 9).

FN7–10-stimulated fibroblast conditioned media increases proinflammatory gene expression in monocyte-derived macrophages

Monocytes can extravasate from blood vessels to tissues and differentiate into macrophages. The tissue environment directs macrophage polarization along an inflammatory to anti-inflammatory spectrum, affecting disease [32]. Our THP-1 gene expression results prompted us to investigate how CM derived from synovial fibroblasts influences monocyte-derived macrophages. Cells were differentiated to macrophages with PMA [33] before incubation with CM from synovial fibroblasts, as illustrated in Fig. 6A. Macrophage differentiation was confirmed by cell adherence in tissue culture (Fig. 6B) and increased expression of *CD14,*

CD86, and *CD163*, and a decrease in *KI67* and *PCNA* (Supplementary Table 10) compared to untreated cells [34,35].

Gene expression of THP-1-derived macrophages in response to CM from synovial fibroblasts was normalized to a serum-free DMEM control. Compared with PBS CM treatment, FN7–10 CM treatment of monocyte-derived macrophages increased gene expression of the surface marker *CD14*, while *CD86* decreased, and *CD163* showed no change (Fig. 6C). Inflammation-related cytokines *TNFA*, *IL1B*, and chemokine ligands *CXCL5*, *CXCL10*, and *CXCL11* demonstrated a notable increase in gene expression in response to FN7–10 CM (Fig. 6D and F). The chemokine receptor *CXCR3* and the anti-inflammatory cytokines *TGFB* and *IL10* showed decreased gene expression in response to FN7–10 CM (Fig. 6E and F). *PCNA* and *KI67* showed no gene expression change in response to CM from PBS or FN7–10 stimulated synovial fibroblast (Fig. 6G). All mean values and 95% CIs are detailed in Supplementary Table 11.

Secreted factors from FN7–10 stimulated synovial fibroblasts induce an OA phenotype in normal chondrocytes

Considering the ongoing cross-talk between the synovium and cartilage in OA [36,37], we evaluated the effects of CM from FN7–10–activated synovial fibroblasts on the phenotype of normal human chondrocytes, focusing on gene expression and extracellular matrix production. As illustrated in Fig. 7A, chondrocytes were treated under three conditions: serum-free DMEM, PBS CM, or FN7–10 CM. Five independent chondrocyte donors were analyzed using CM from three separate synovial fibroblast donor cultures. A heatmap of log2 fold-changes relative to DMEM control (Fig. 7B) showed consistent down-regulation of *ACAN* and *COL2A1* in response to FN7–10 CM, while *CRTAC1* displayed modest increases. Most remaining genes, including *IL6*, *IL1B*, chemokine ligands, and matrix-degrading enzymes such as *MMP1* and *MMP13*, were upregulated by FN7–10 CM. *IL6* and *MMP1* showed consistent increases across all donors, as highlighted individually in Fig. 7C and 7D. All mean values and 95% CIs are detailed in Supplementary Table 12.

Given the suppression of anabolic matrix genes, *COL2A1* and *ACAN*, we assessed extracellular matrix output using a 72 hr. micromass GAG assay. All CM, including DMEM control, was spiked with 1% FBS to maintain basal deposition. Alcian blue staining revealed a smaller normal chondrocyte micromass in the FN7–10 CM condition (Fig. 7E). Absorbance quantification normalized to 1% FBS DMEM controls showed the averaged micromass in the PBS CM condition had a mean value of 1.05 ± 0.93 – 1.17, while the micromass in the FN7–10 CM condition had a mean value of 0.87 ± 0.75 – 0.99, indicating decreased GAG production in the FN7–10 condition compared with the PBS CM condition (Fig. 7F).

Discussion

Previous studies have increasingly recognized the synovium as a critical contributor to joint pathology [38,39]. Synovitis, a hallmark of OA, has been linked to disease progression and serves as a therapeutic target [40,41]. The present study demonstrated that the matrikine FN7–10 is a useful *in vitro* tool to model inflammatory synovial fibroblasts, a subgroup

of cells increasingly recognized in OA synovium that can contribute to OA pathogenesis [17,41]. We also examined how FN7–10 stimulation of synovial fibroblasts affects two other important cell types in OA: immune cells and chondrocytes (Fig. 8). Our results revealed notable immunomodulatory effects and impairment of chondrocyte activity. Specifically, FN7–10 stimulation turned synovial fibroblasts into active producers of inflammatory mediators, releasing a host of cytokines and chemokines. When chondrocytes isolated from normal cartilage were exposed to CM from FN7–10 treated OA synovial fibroblasts, expression of inflammatory markers were increased while expression of the anabolic genes *ACAN* and *COL2A1* were reduced and this was accompanied by a decrease in production of GAGs. Conditioned media from FN7–10 stimulated synovial fibroblasts increased THP-1 monocyte chemotaxis in transwell migration assays. Furthermore, inhibition of chemokine receptor activity significantly diminished monocyte migration, confirming that chemokine signaling mediates this process.

Of particular interest, CCR2, a receptor for several CC motif ligands, including CCL2/MCP-1, was one of the chemokine receptors mediating THP-1 cell migration in our model. CCR2 and CCL2/MCP-1 signaling are promising therapeutic targets for OA, as they have been shown to induce an OA chondrocyte phenotype [28]. Local, sustained delivery of a CCR2 inhibitor was found to reduce cartilage degradation and synovial hyperplasia in the mouse DMM model [42]. Beyond immune cell migration, we observed signatures of monocyte to macrophage differentiation in THP-1 cells in response to CM from FN7–10 stimulated synovial fibroblasts. This phenotype was characterized by elevated pro-inflammatory mediators, including *IL1B and TNFA*. Notably, *CD14* was upregulated at the transcriptional level in THP-1 cells and in differentiated macrophages in response to FN7–10 CM. Flow cytometry results indicated that CD14 was the only marker with increased cell-surface levels on THP-1 cells in response to FN7–10 CM, compared with PBS CM. Previous studies have found that soluble CD14 levels in synovial fluid are associated with joint space narrowing and increased synovial macrophages and that CD14 deficiency may confer protective effects [43,44].

Notably, CXCL5, was increased in OA synovial fibroblasts treated with FN7–10 as well as in normal and OA chondrocytes. It was also produced by THP-1 cells in response to FN7–10 CM and after THP-1 cells were differentiated into macrophages. While some chemokines have been studied in OA [45,46], the role of CXCL5 remains unclear. We showed that an inhibitor of the CXCL5 receptor, CXCR2, reduced THP-1 cell migration in response to synovial fibroblast CM. These results suggest that further investigation of the role of CXCL5 in OA is warranted.

In summary, FN7–10 stimulation of OA synovial fibroblasts induced a pro-inflammatory phenotype, leading to the production of mediators that recruit immune cells, reduced the anabolic activity of chondrocytes, and promoted a proinflammatory chondrocyte phenotype. While our tissue culture studies utilize only one bioactive matrikine, FN7–10, highlighting a limitation in our approach, it also raises intriguing questions about how other abundant matrikines potentially involved in osteoarthritis may differentially influence joint cells and disease progression. In addition to fibronectin fragments, other matrikines found in joint tissues include fragments of types II and VI collagen, aggrecan, hyaluronan, link protein,

biglycan [4,5]. By understanding how matrikines, generated through cleavage of matrix proteins in the joint, affect synovial cells, we can gain insights into the origins of persistent tissue inflammation in osteoarthritic joints. Such understanding may inform drug discovery and therapeutic approaches aimed not only at alleviating pain and enhancing anabolic activity but also at mitigating the chronic inflammation triggered by damaged cartilage products in osteoarthritis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A.: Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.joca.2026.04.011.

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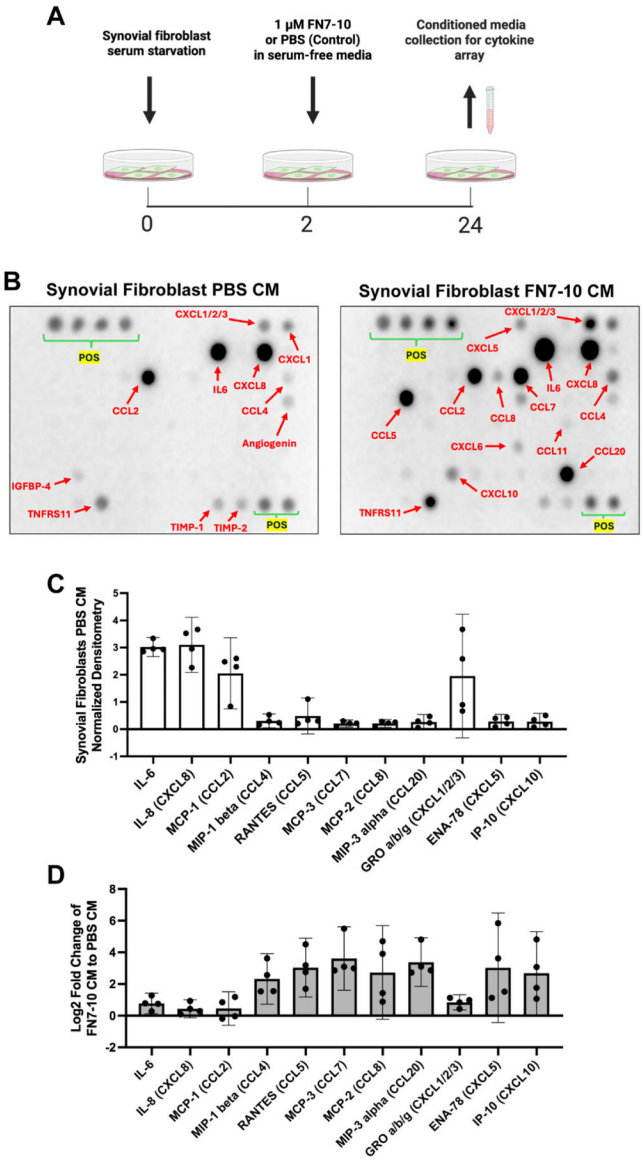

Fig. 1.

FN7–10-induced changes in synovial fibroblast pro-inflammatory protein secretion. (A) Schematic timeline showing synovial fibroblast serum starvation, stimulation with 1 μM FN7–10, and conditioned media collection for cytokine array analysis (created with BioRender). (B) Representative cytokine arrrays incubated with conditioned media (CM) from synovial fibroblast treated with PBS (left) or FN7–10 (right). Red arrows highlight cytokines detected in the control condition (PBS CM) and increased by at least 1.5-log2fold following FN7–10 stimulation. Positive controls for the array are labeled “POS”. (C) Normalized densitometric quantification of selected cytokines from PBS CM, showing individual donor values with mean ± 95% confidence intervals (n = 4 independent synovial fibroblast donors). (D) Densitometry-based $\log_2$ fold change of selected cytokines detected in the array comparing FN7–10 CM to PBS CM, displayed as individual donor values with mean ± 95% confidence intervals.

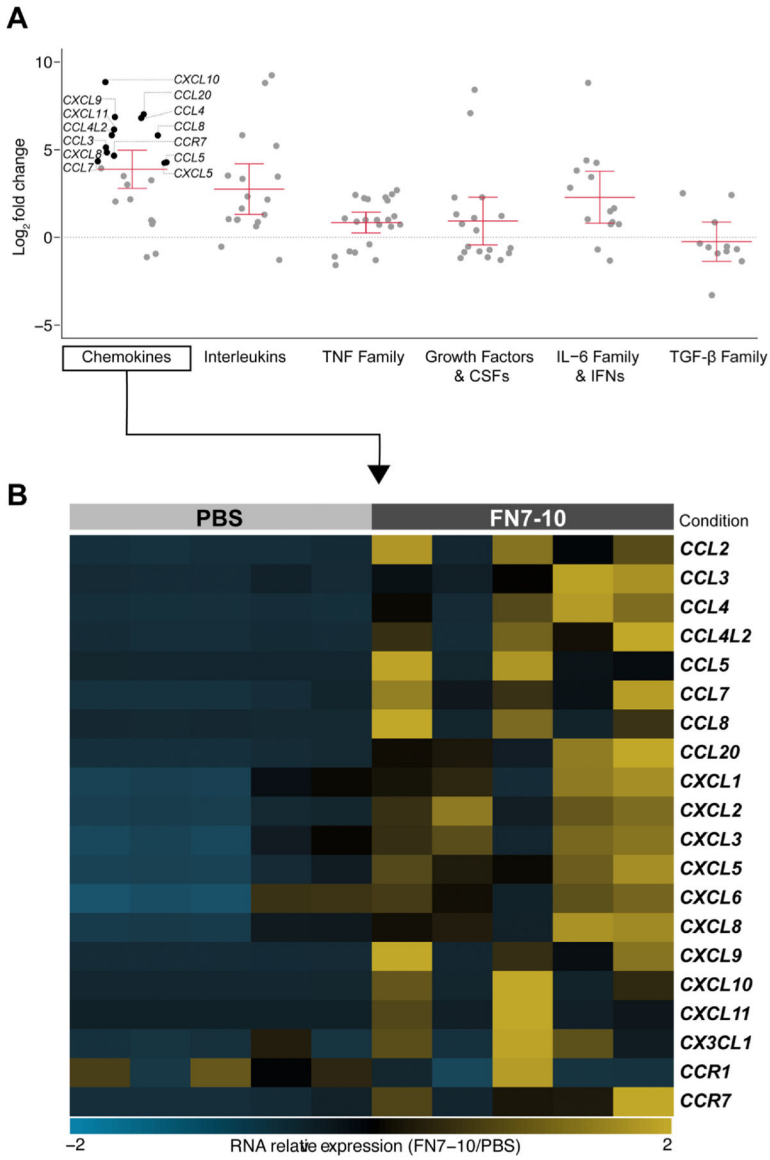

Fig. 2.

FN710–induced changes in synovial fibroblast cytokine and chemokine gene expression. (A) Boxplot showing the $\log_2$ fold change of significantly regulated genes (padj < 0.05) within the KEGG cytokine–cytokine receptor interaction pathway (hsa04060), grouped by major cytokine families. Chemokine datapoints with $|\log_2 FC = > 4$ are bolded and labeled in black, and the horizontal dashed line indicates no change ($\log_2 FC = 0$). (B) Targeted heatmap illustrating expression of chemokine family genes identified in panel A, comparing synovial fibroblasts treated with PBS or FN710 for 18 h (n = 5 independent OA synovial fibroblast donors). Values represent relative RNA expression (FN7–10/PBS).

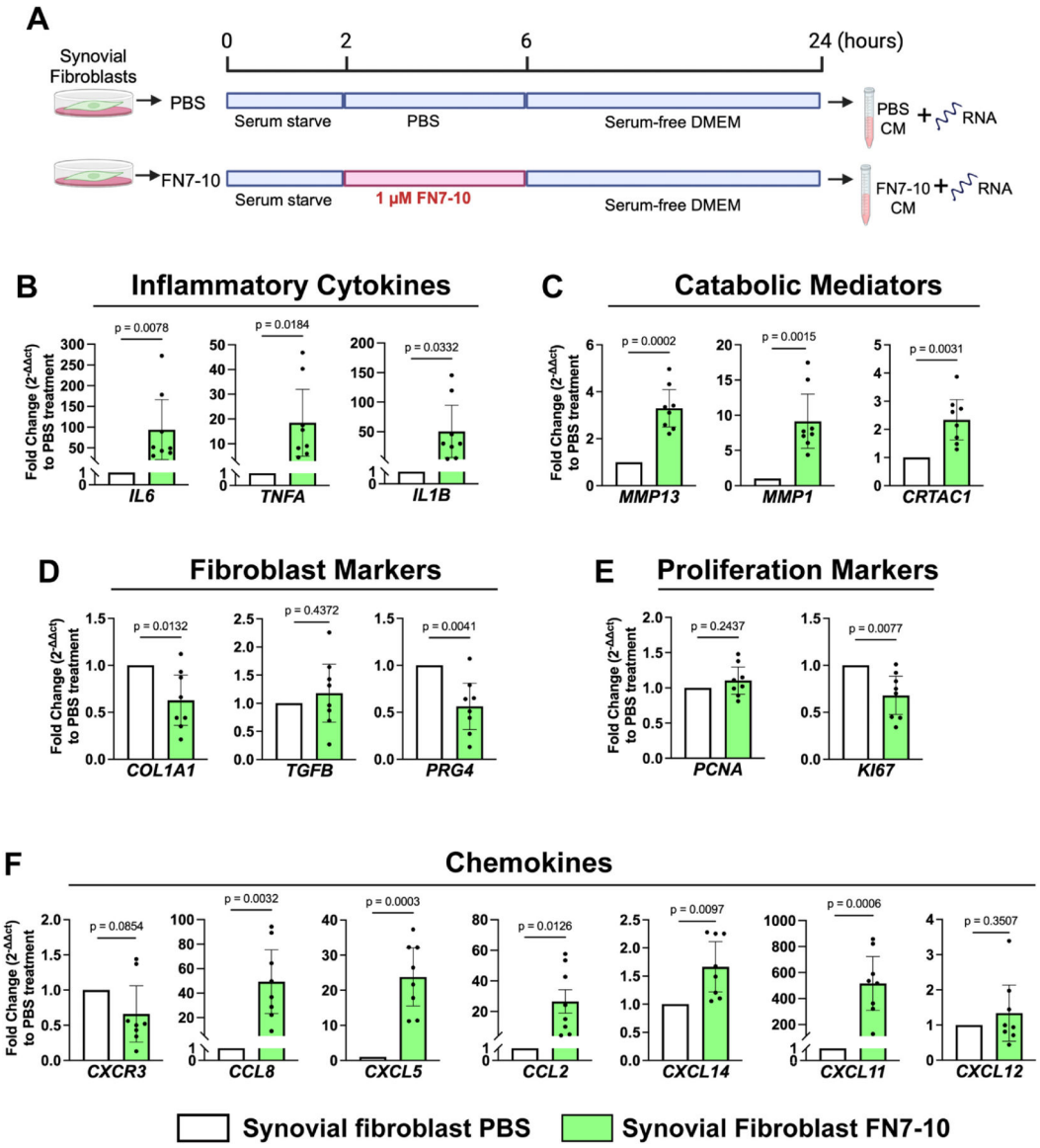

Fig. 3.

Expression of synovial fibroblast genes following FN7–10 stimulation. (A) Schematic showing the experimental set-up. After serum starvation, cells were treated with PBS or FN7–10 for 4hrs then changed to fresh media without PBS or FN7–10 and incubated for an additional 18 hrs (created with BioRender). (B–F) Bar graphs depict fold-change gene expression relative to a theoretical value of 1, and data represent individual donor values (n = 8 independent OA synovial fibroblast donors) with mean ± 95% confidence intervals. Each donor was normalized with its own PBS control. One-sample t-tests were applied using a theoretical mean of 1. Gene groups include: (B) inflammatory cytokines (*IL6*, *TNFA*, *IL1B*), (C) catabolic mediators (*MMP13*, *MMP1*, *CRTAC1*), (D) fibroblast markers (*COL1A1*, *TGFB*, *PRG4*), (E) proliferation markers (*PCNA*, *KI67*), and (F) chemokines (*CXCR3*, *CCL8*, *CXCL5*, *CCL2*, *CXCL14*, *CXCL11*, *CXCL12*).

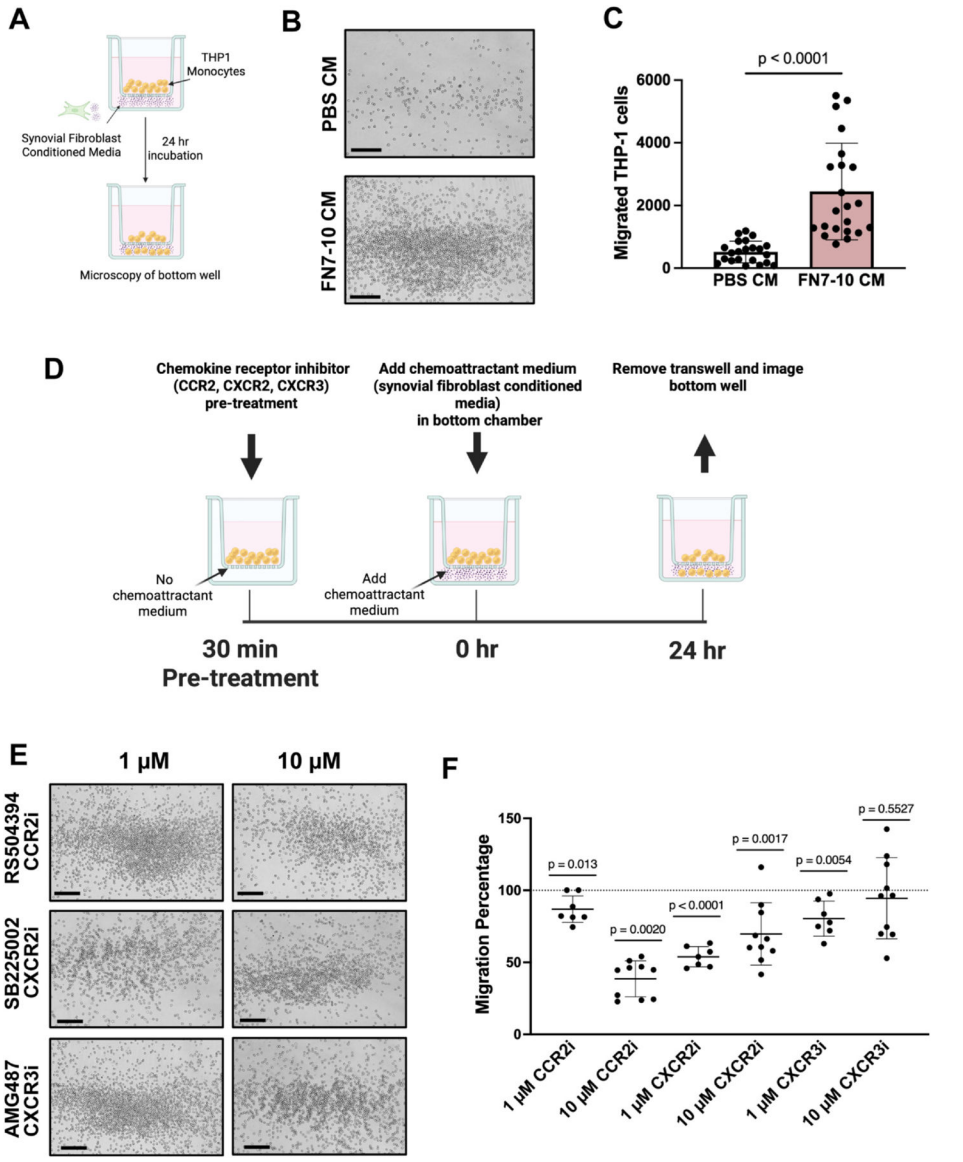

Fig. 4.

Chemokine receptor inhibition decreases monocyte migration induced by synovial fibroblast-conditioned media. (A) Schematic illustration of the THP-1 transwell migration setup using conditioned media from PBS or FN7–10–stimulated synovial fibroblasts (created in BioRender). (B) Representative microscopy images of THP-1 cells that migrated across the transwell membrane to the bottom of the 24 well plate (scale bar = 250 μm). (C) Quantification of migrated THP-1 cells in response to PBS CM versus FN7–10 CM (n = 21 independent OA synovial fibroblast donors), analyzed using a Wilcoxon matched pairs signed rank test. (D) Schematic of the chemokine receptor inhibitor transwell assay (created with BioRender). (E) Representative microscopy images of THP-1 migration after treatment with chemokine receptor inhibitors in the presence of FN7–10 synovial fibroblast conditioned media. (F) Percentage migration of THP-1 cells treated with chemokine receptor inhibitors at 1 μM (n = 7 independent OA synovial fibroblast donors CM) or 10 μM

(n = 10 independent OA synovial fibroblast donors CM). One-sample t-tests were applied using a theoretical migration value of 100%. Data are presented as mean ± 95% confidence intervals, and p values are shown.

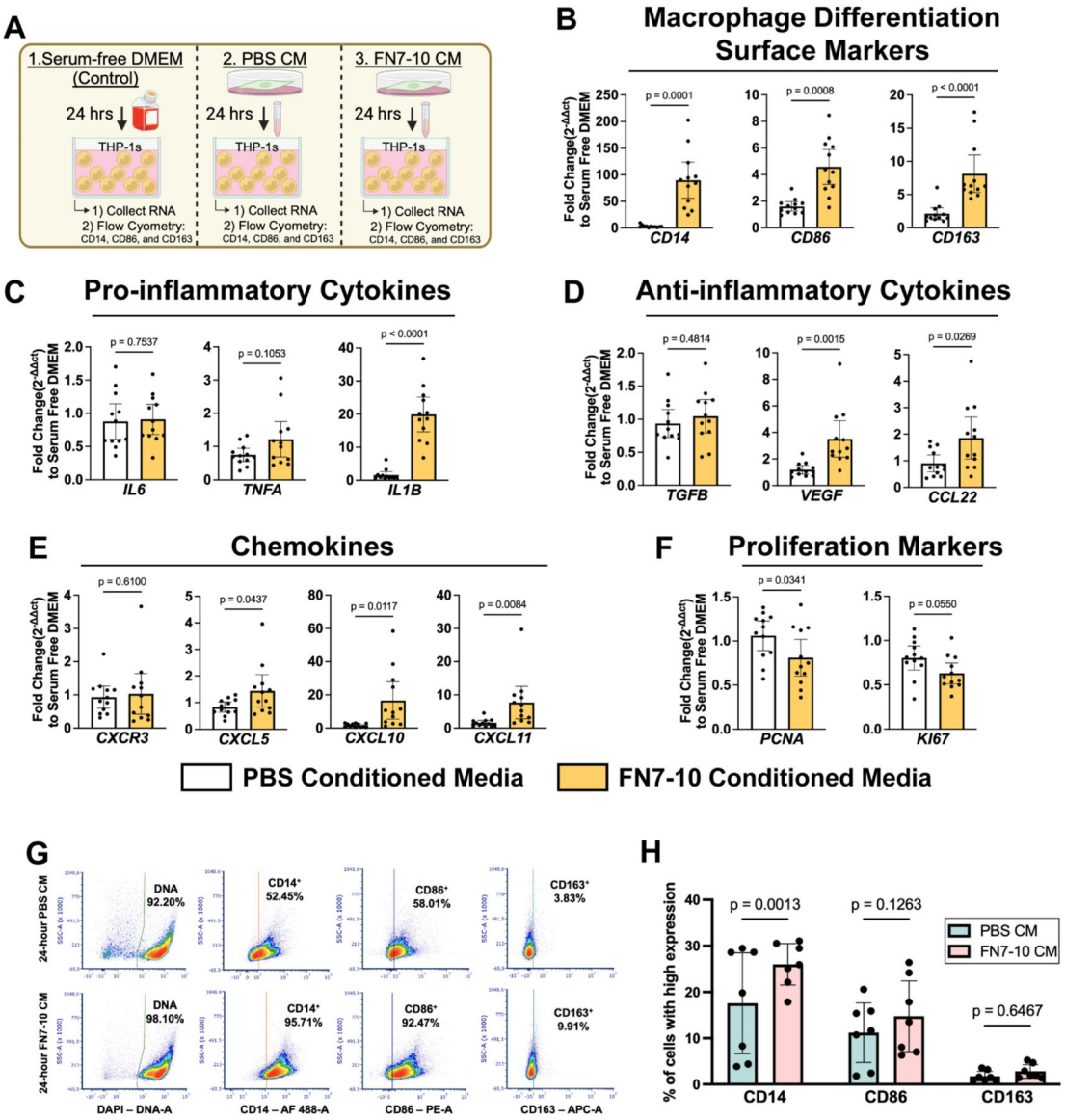

Fig. 5.

Effects of synovial fibroblast conditioned media on THP-1 cell gene expression and surface marker phenotype. (A) Experimental schematic illustrating THP-1 cell treatment with synovial fibroblast conditioned media (CM) (created with BioRender). (B–F) Bar graphs showing fold-change gene expression in THP1 cells treated with synovial fibroblast conditioned media, compared to serum-free DMEM control (n = 12 independent OA synovial fibroblast donors). Data are presented as individual values with mean ± 95% confidence intervals, and paired t tests were used for statistical comparisons. Gene groups include: (B) Macrophage differentiation surface markers (*CD14*, *CD86*, *CD163*); (C) Proinflammatory cytokines (*IL6*, *TNFA*, *IL1B*); (D) Anti-inflammatory cytokines (*TGFB*, *VEGF*, *CCL22*); (E) Chemokines (*CXCR3*, *CXCL5*, *CXCL10*, *CXCL11*); (F) Proliferation markers (*PCNA*, *KI67*). (G) Representative flow cytometry scatter plots illustrating DAPI-positive THP-1 cells and surface marker levels for CD14-AF 488, CD86-PE, and CD163-

APC following a 24-hour treatment with PBS CM or FN7–10 CM. (H) % of THP-1 cells treated with synovial fibroblast CM greater than serum-free DMEM (control) + 2 standard deviations for CD14, CD86, and CD163 from flow cytometry. Data are from cells treated with CM from 6 independent OA synovial fibroblasts donors presented as individual values with mean ± 95% confidence intervals. Two-way ANOVA repeated measures using Sidak's correction was used for statistical analysis.

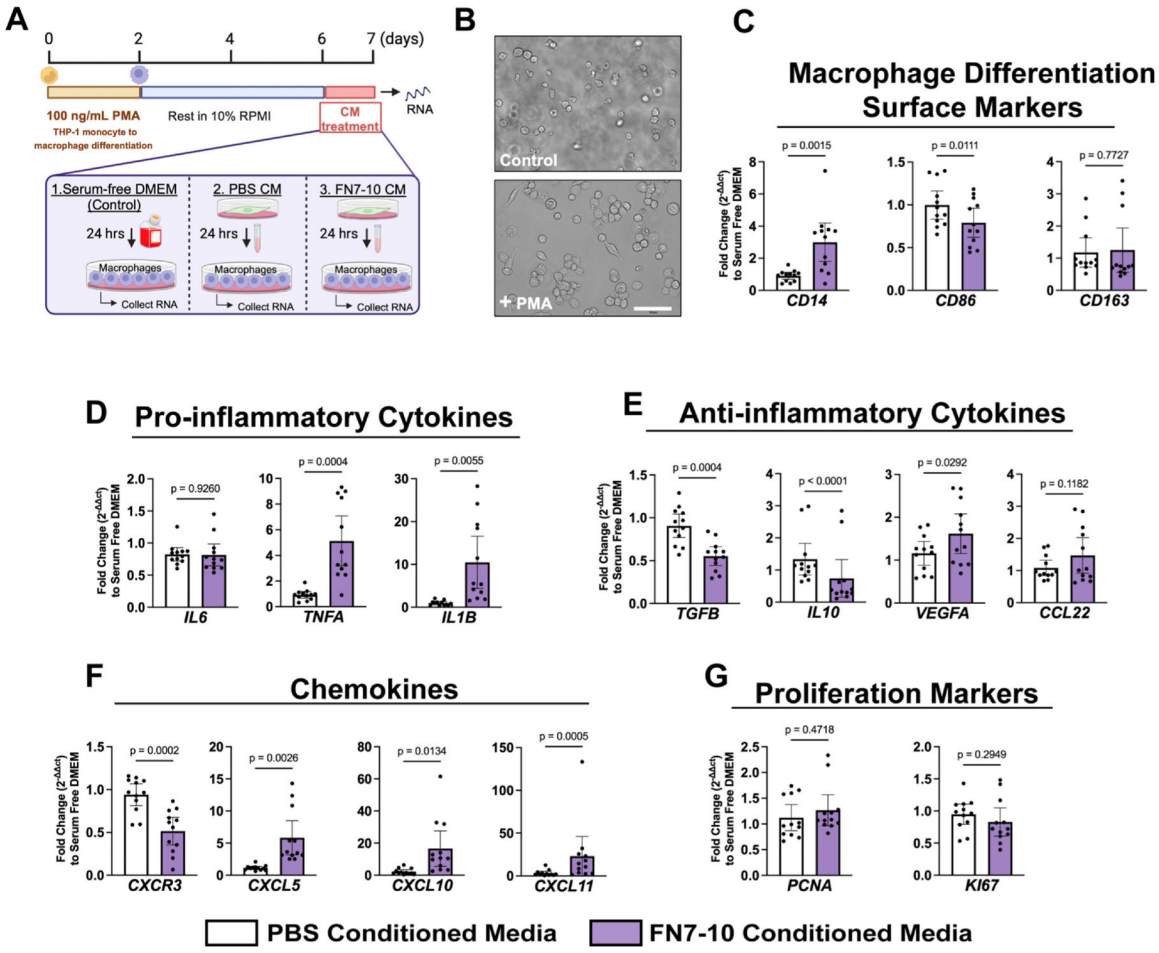

Fig. 6.

Effects of synovial fibroblast conditioned media on macrophage gene expression following PMA-induced THP-1 differentiation. (A) Experimental schematic illustrating THP-1 differentiation with PMA followed by treatment with synovial fibroblast conditioned media (CM) (created with BioRender). (B) Representative microscopy images of THP-1 cells with or without PMA treatment, demonstrating increased adherence following 48-hour PMA stimulation (scale bar = 100 μm). (C–G) Bar graphs showing fold-change gene expression in PMA differentiated THP1 cells treated with synovial fibroblast conditioned media compared to serum-free DMEM control (n = 12 independent OA synovial fibroblast donors). Data are presented as individual values with mean ± 95% confidence intervals. Paired t tests were used for statistical comparisons. Gene groups include: (C) Macrophage differentiation surface markers (*CD14*, *CD86*, *CD163*); (D) Proinflammatory cytokines (*IL6*, *TNFA*, *IL1B*); (E) Anti-inflammatory cytokines (*TGFB*, *IL10*, *VEGF*, *CCL22*); (F) Chemokines (*CXCR3*, *CXCL5*, *CXCL10*, *CXCL11*); (G) Proliferation markers (*PCNA*, *KI67*).

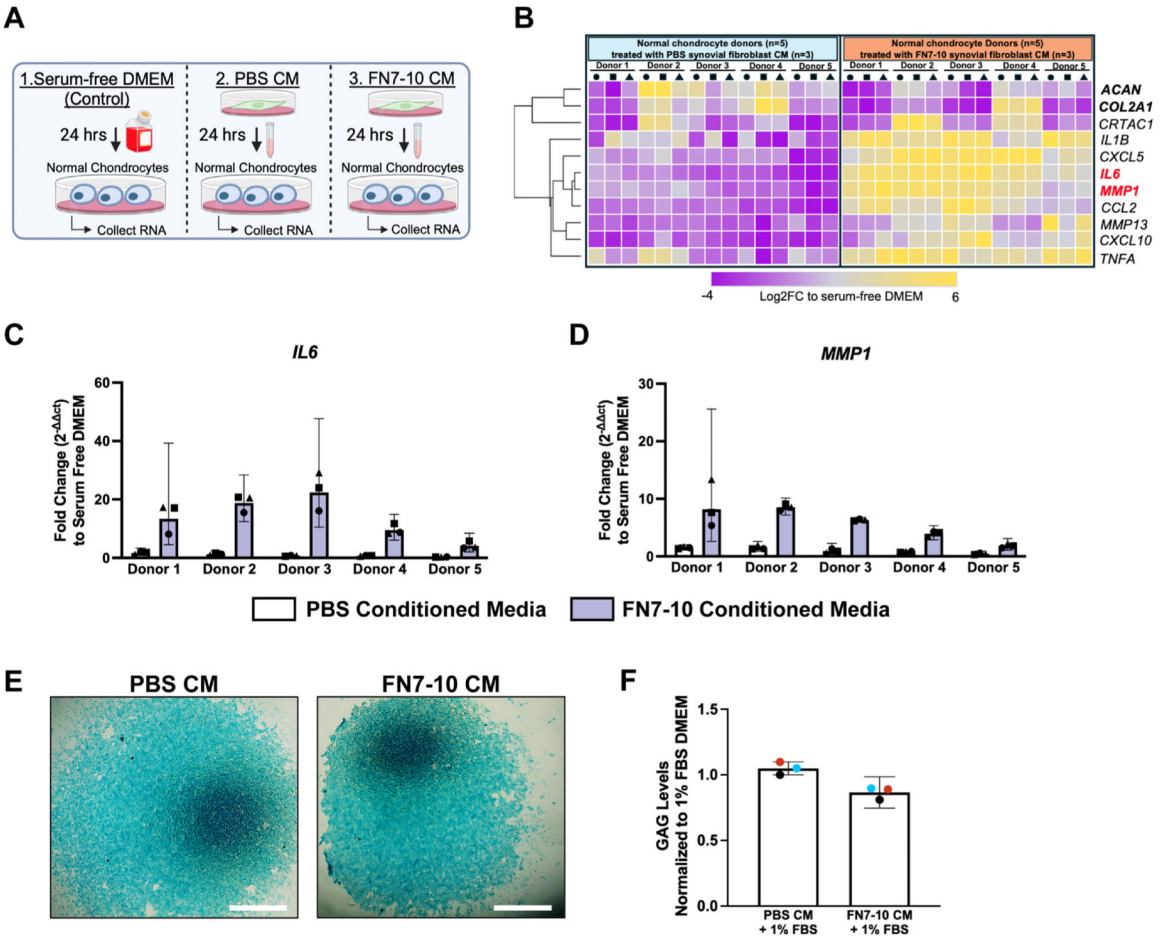

Fig. 7.

Effects of synovial fibroblast conditioned media on chondrocyte gene expression and GAG production. (A) Experimental schematic illustrating chondrocyte treatment with conditioned media (CM) from treated synovial fibroblasts (created with BioRender). (B) Heatmap of $\log_2$ fold-change gene expression in normal chondrocytes treated with synovial fibroblast conditioned media relative to serum-free DMEM controls. Data represent n = 5 independent chondrocyte donors treated with conditioned media from n=3 independent OA synovial fibroblast donors. Hierarchical clustering of genes was performed using a 1 − Spearman rank correlation distance metric. (C–D) Bar graphs of *IL6* (C) and *MMP1* (D) gene expression in normal chondrocytes from five independent donors following treatment with PBS CM or FN7–10 CM from three synovial fibroblast donors. Data are presented as individual donor values with geometric mean ± geometric 95% confidence intervals. (E) Representative brightfield images of chondrocyte micromasses stained with Alcian blue after 72 h exposure to PBS CM or FN7–10 CM spiked with 1% FBS (scale bar = 1000 μm). (F) Quantification of GAG content in chondrocyte micromasses normalized to the 1% FBS DMEM control. Independent normal chondrocyte donors (n=3) were each treated with synovial fibroblast PBS CM or FN7–10 CM (n=3 independent donors) spiked with 1% FBS, and the results were averaged.

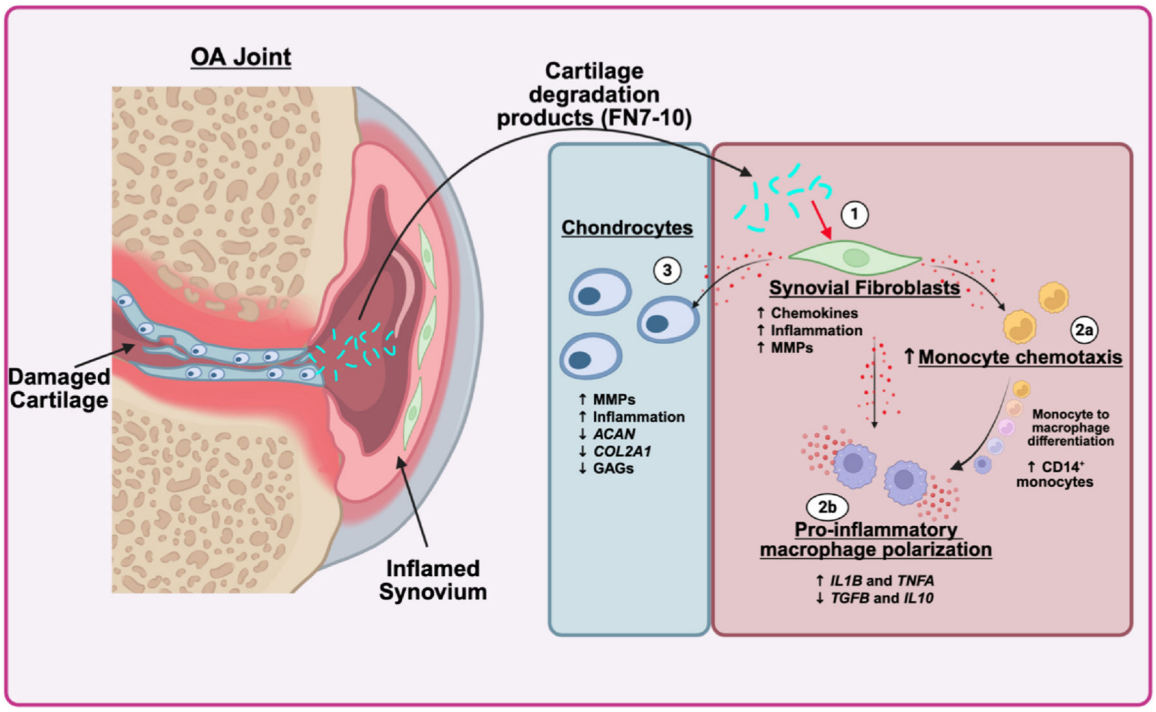

Fig. 8.

Proposed model of FN7–10-mediated synovial fibroblast signaling to immune cells and chondrocytes. The matrikine FN7–10 promotes synovial fibroblast activation characterized by increased production of pro-inflammatory mediators including chemokines, cytokines, and matrix metalloproteinases (MMPs). These mediators promote monocyte chemotaxis and differentiation to pro-inflammatory macrophages. Factors released from activated synovial fibroblasts also stimulate chondrocytes to produce MMPs and pro-inflammatory factors as well as inhibit chondrocyte anabolic activity characterized by decreased *ACAN* and *COL2A1* expression and glycosaminoglycan (GAG) production. (Created with BioRender).