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Review

Microengineering the Synovial Membrane Microenvironment in Rheumatoid Arthritis Research

Running title: Focused on RA synovium-on-a-chip and organoid

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Abstract

Rheumatoid arthritis (RA) is a slow-progressive inflammatory autoimmune disorder characterized by synovial inflammation, hyperplasia, and joint degradation, leading to joint destruction and poor patients' quality of life. Numerous in vitro RA models have been developed to elucidate disease mechanisms and identify therapeutic targets; however, most fail to fully recapitulate the in vivo synovial microenvironment, including the cellular heterogeneity, biomechanical stress, and dynamic cell-matrix interactions, limiting their translational relevance. This translational gap underscores the need for advanced 3D microengineered platforms that integrate patient-specific cells, biomechanical elements, and real-time biosensing to bridge in vitro findings to clinical outcomes. Recent progress in microengineering has enabled the development of systems that closely mimic the physiological and pathological conditions of the RA synovial membrane in vitro. This review highlights recent progress in microengineered synovial models and their applications in elucidating RA pathogenesis and seeking therapeutic interventions. We also introduce persisting technical and biological challenges, and emerging trajectories for innovation within this rapidly advancing discipline.

Keywords: rheumatoid arthritis; synovial membrane microenvironment; microengineering; synovium-on-a-chip; synovial organoid

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1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder characterized by dysregulated immune responses, with the synovial membrane serving as the primary site of pathogenic immune cell activation, autoantibody production (e.g., rheumatoid factor, anti-citrullinated protein antibodies), and early inflammatory cascades that cause synovitis and osteochondral destruction [1–3]. RA affects approximately 1% of the global population and presents a significant burden due to joint pain, stiffness, and progressive disability [4]. Citrullination, a post-translational modification where peptidyl arginine deiminase (PAD) enzymes convert arginine residues to citrulline, is considered a key driver in RA pathogenesis [5]. The citrullination by PADI4(Protein-arginine deiminase type-4) is influenced by multiple factors, including genetic predisposition, smoking, bacterial or viral infections, and autophagy [3,6].

The synovial membrane, or synovium, is the principal site of pathological processes in RA, undergoing hyperplasia of fibroblast-like synoviocytes (FLS), dense infiltration of immune cells, and neovascularization that together form the invasive pannus tissue [7]. In RA, the primary pathological changes in the synovium include synovial hyperplasia, inflammation, increased angiogenesis, and exudation, all of which lead to cartilage degradation and bone erosion. The synovial lining becomes thickened due to the proliferation of synovial cells including fibroblasts and macrophages. The

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immune cell–fibroblast–bone axis constitutes a central regulatory triad in rheumatoid arthritis (RA), wherein dysregulated crosstalk between activated immune cells (e.g., macrophages, T/B lymphocytes), fibroblast-like synoviocytes (FLS), and osteochondral cells (osteoclasts, osteoblasts) drives synovial hyperplasia, chronic inflammation, and osteochondral damage via RANKL/MMP-mediated cartilage degradation and bone erosion [2,7]. Within this altered synovial microenvironment, complex biochemical cues, such as elevated pro-inflammatory cytokines (e.g., TNF-α, IL-6), and biophysical changes, such as increased tissue stiffness, drive the aggressive phenotype of resident stromal and immune cells, perpetuating chronic inflammation [8].

Traditional in vitro culture systems and animal models have provided valuable insights into RA pathogenesis, but they often fail to recapitulate the human-specific, multicellular architecture and dynamic mechanical forces present in the diseased synovium, limiting their predictive power for therapeutic screening [9]. Moreover, these often focus on narrowly defined parameters based on specific study objectives rather than addressing the complex organ-level and tissue or cell interaction dynamics inherent to the human body [9–11]. Consequently, traditional in vitro and animal models are inherently limited in the replication of the microenvironment of synovial tissue. To overcome these limitations, microengineering approaches including microfluidic "synovium-on-a-chip" platforms, tunable hydrogel scaffolds, and 3D-bioprinted synovial organoids have emerged as powerful tools to reconstruct key features of the synovial niche with precise control over cellular composition, mechanical properties, and soluble factor gradients [12].

Recent advances include vascularized synovium-on-a-chip devices that mimic leukocyte extravasation under physiologically relevant shear stresses, droplet-based microfluidic systems for single-cell transcriptomic profiling of patient-derived synovial cells, and stimuli-responsive hydrogels that release therapeutics in response to RA-specific enzymatic activity [13–15]. By integrating microscale engineering with primary human cells and advanced sensing modalities, these platforms enable real-time monitoring of cytokine secretion, barrier integrity, and cell migration, thereby offering unprecedented insight into RA pathophysiology and drug responses in a patient-relevant context [10].

In this review, we outline the pathological hallmarks of the RA synovial microenvironment, then survey traditional models, and finally discuss the latest microengineering strategies to model and manipulate this niche along with emerging opportunities.

2. Microenvironment of RA Synovium

Understanding the in vivo synovial microenvironment, the complex milieu of molecular and mechanical signals, is pivotal in establishing a RA synovial membrane model for unraveling RA pathogenesis and developing targeted therapies.

2.1. Structural Components

The synovium is a specialized connective tissue that lines the inner surface of synovial joints, bursae, and tendon sheaths [16,17]. It plays a crucial role in joint lubrication, immune regulation, and nutrient exchange. The synovial membrane comprises two distinct layers: the intimal layer (intima, synovial lining) and the subintimal layer (subintima, subsynovial tissue) [16]. The intimal layer with specialized synoviocytes is 1–3 cell layers thick and faces the joint space. Unlike other epithelia, it lacks a basement membrane, allowing for efficient molecular exchange between synovial fluid and the subintima. This layer is responsible for synthesizing hyaluronic acid, lubricin, and cytokines essential for joint lubrication and immune responses. In RA, the intima expands to 10–20 layers, predominantly FLS, forming the aggressive pannus that invades cartilage and bone [18]. The subintimal layer contains blood vessels, lymphatics, and nerves [19–21]. It provides structural support and facilitates immune cell trafficking between the bloodstream and the joint cavity. Nerve endings within this layer contribute to nociception in inflammatory joint diseases and reduced nerve supply is observed in superficial intimal regions of synovial membrane harvested from RA patients [20].

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2.2. Cellular Components

The synovium contains various lineages of resident and infiltrating cells, each playing a key role in joint homeostasis and disease [22]. It harbors two main types of synoviocytes: type A synoviocytes (Resident synovial macrophage, RSMs) and type B synoviocytes (Fibroblast-like synoviocytes, FLSs)(Figure 1).

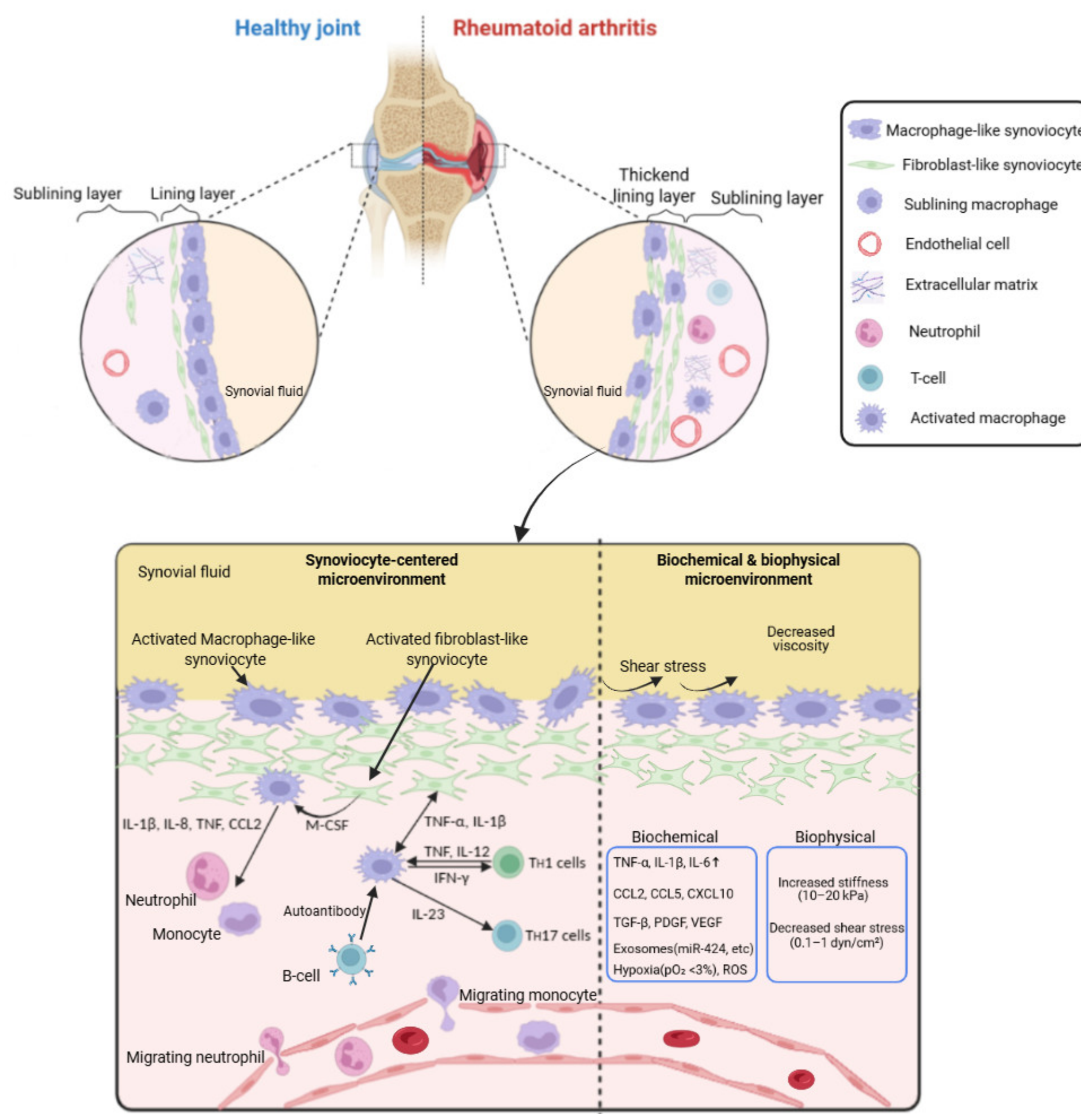

Figure 1. Overview of the rheumatoid arthritis (RA) synovial membrane microenvironment, including cell–cell interactions and biochemical and mechanical components. The figure illustrates the complex microenvironment of the RA synovium, highlighting key resident and infiltrating cell types such as fibroblast-like synoviocytes (FLS), macrophage-like synoviocytes (MLS), T cells, B cells, dendritic cells, neutrophils, and mast cells. Interactions between these cells via cytokines (e.g., TNF-α, IL-6, IL-1β), chemokines, and growth factors contribute to chronic inflammation and joint destruction. The extracellular matrix (ECM) components, along with synovial fluid-derived factors, influence both biochemical and biomechanical signaling. Mechanical forces from joint loading, altered ECM stiffness, and integrin-mediated signaling further modulate cellular behavior and inflammatory responses. This integrated network drives synovial hyperplasia, angiogenesis, cartilage degradation, and bone erosion characteristic of RA pathology.

Synovial tissue contains both tissue-resident macrophages and infiltrating monocyte-derived macrophages. Resident macrophages form a protective barrier at the lining and contribute to homeostasis, while monocyte-derived cells amplify inflammation via TNF-α and IL-1β production.

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[23,24]. RSMs are derived from the monocyte/ macrophage lineage and are highly phagocytic, responsible for clearing debris, apoptotic cells, and immune complexes [24,25]. These cells express Fc-gamma immunoglobin receptor (FcγR) with positive CD68 and CD 163, whereas the expression of major histocompatibility class II molecules (MHC-II) is identified [23]. During the early stages of the immune response, they can absorb and degrade extracellular constituents, cell debris, microorganisms, and many antigens within the synovial membrane. They secrete pro-inflammatory cytokines (e.g., TNF-α, IL-1β) in diseases like RA, contributing to joint inflammation.

FLSs are mesenchymal-derived cells responsible for synovial fluid production and synthesize hyaluronic acid, lubricin, and extracellular matrix components [25]. In RA, FLS become hyperproliferative and produce matrix-degrading enzymes (MMPs, Matrix metalloproteinases), driving cartilage destruction [18]. Recent single-cell RNA sequencing studies identify distinct fibroblast subsets in the synovium which include $CD55^+$ homeostatic FLS with the maintenance of synovial lubrication and ECM integrity and $CD248^+$ pathogenic FLS with the induction of inflammation and cartilage destruction in RA [26]. T and B lymphocytes, plasma cells, and dendritic cells infiltrate the synovium, forming ectopic lymphoid-like structures that sustain autoantibody production and cytokine secretion [27].

2.3. Extracellular Matrix

The extracellular matrix (ECM) of the synovium is a dynamic structure that maintains tissue homeostasis and facilitates cell signaling. It is composed of collagen, proteoglycans (aggrecan, decorin), non-collagen protein, glycoprotein (fibronectin, laminin), and matrix-degrading enzymes, which collectively regulate synovial architecture and function [28–34]. An amorphous or fine fibrillar ultrastructure was found in the synovial inner lining including collagens III, IV, V, and VI with little type I collagen [28]. Altered ECM composition—e.g., fibronectin and type III collagen deposition—changes matrix porosity and ligand presentation, affecting cell adhesion and migration [35]. Once the components and structure of ECM change, it can lead to the occurrence and development of arthritis disease by elevated angiogenesis, accelerated cell differentiation, immune activation, and other adverse immunologic events.

2.4. Biochemical and Mechanical Cues

The RA synovial membrane is a dynamic interface where biochemical and mechanical signals converge to drive disease progression. Biochemical environment factors include proinflammatory cytokines, chemokines, growth factors, extracellular vesicles, hypoxia, and reactive oxygen species. Several proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 are elevated in RA synovial fluid and tissue, driving FLS activation, leukocyte recruitment, and osteoclastogenesis [6,36]. Chemokines orchestrate leukocyte migration into the synovium, monocytes recruiting, and T-cell retention [36]. In RA synovial membrane, VEGF promotes angiogenesis, and TGF-β modulates proliferation and extracellular matrix production of FLS whereas PDGF supports fibroblast survival and migration [37]. Synovial exosomes carry miRNAs and proteins that modulate FLS and immune cell function, propagating inflammation and joint damage [38]. Hypoxia ($pO_2<20$ mm Hg) is characteristic of RA synovium, stabilizing HIF-1α/2α in FLS and macrophages to upregulate angiogenic and glycolytic genes [39,40]. On the other hand, oxidative stress from elevated ROS contributes to DNA damage, NF-κB activation, and MMP induction in FLS [41].

Mechanical cues involve matrix stiffness, shear stress, mechanical strain, and rheological changes of synovial fluid. RA synovial tissue exhibits increased stiffness versus healthy [41]. Stiff matrix enhances FLS activation via integrin-mediated mechanotransduction, promoting invasive behavior and MMP expression [42]. RA synovial fluid flow generates decreased shear stress across the intima due to less viscosity, and FLS become activated and invasive under certain shear on the synovium-on-a-chip model, whereas shear stress modulates FLS calcium signaling and influences cytokine release [43]. During cyclic strain on the synovium in joint movement, FLS senses stretch via cadherin and ADAM15, triggering invasive phenotypes through MAPK pathways [44].

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2.5. Crosstalk Between RA Synoviocytes and Inflammatory Cells

The synovium becomes inflamed, with increased numbers of inflammatory cells including T cells (Th1 and Th17 cells), B cells, dendritic cells, mast cells, and macrophages, and neovascularization—forming the pannus that invades cartilage and bone [45–47]. IFN-γ from Th1 cells activates resident synovial macrophages and promotes inflammation, whereas, IL-17 secreted by Th17 cells enhances neutrophil recruitment and synovial inflammation [46,47]. Activated macrophages release pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and matrix metalloproteinase, which leads to the formation of pannus, an abnormal layer of tissue that invades and damages surrounding cartilage and bone [47–49]. On the other hand, B cells contribute to RA through the production of autoantibodies including the anti-citrullinated protein antibodies and rheumatoid factor which enhances osteoclast activation [3,50].

The autoimmune response selectively directs immunological aggression toward multiple molecular and structural constituents of the synovial membrane, with ECM proteins, and post-translationally modified antigens serving as principal pathogenic foci [46,51]. In some individuals with genetic susceptibility, early self-reactivity against post-translationally modified proteins develops mostly in mucosal areas, with subsequent targeting of synovial tissue by effector T cells [52].

The immune system stimulates fibroblast-like synoviocytes to exert inflammatory and tissue-destructive effects and exacerbate RA pathogenesis [26,48]. FLSs, the resident mesenchymal cells of the synovium, display abnormal behaviors such as hyperproliferation, resistance to cell death, and invasive properties in RA [26]. They can directly interact with T cells through the presentation of antigens and co-stimulatory molecules such as ICAM-1, leading to the activation of autoreactive T cells [52]. Activated T cells, in turn, produce pro-inflammatory cytokines like IL-17 and IFN-γ, which further stimulate FLSs to produce inflammatory mediators and proteases [47,48]. FLSs also release chemokines (e.g., CCL2, CCL5) that recruit macrophages into the synovial tissue. Kuo et al. suggested that HBEGF $^{+}$ inflammatory macrophages are enriched in RA tissues and could promote the fibroblast's invasiveness depending on the response of synovial fibroblast EGFR to EGF ligand expressed by them. This interaction between FLSs and macrophages creates a positive feedback loop that sustains the inflammatory environment within the joint.

2. Traditional Models and Their Limitation

Traditional in vitro models of the rheumatoid arthritis (RA) synovium employ monocultures, co-cultures, and explant-conditioned media to mimic key aspects of the inflamed joint microenvironment.

Primary studies utilized the traditional 2D culture of fibroblast-like synoviocytes derived from synovial fluid to study the inflammatory process in RA for better insights into the pathogenesis of the disease and facilitating the drug discovery processes [53,54]. The monolayer 2D culture could be expanded with the help of coculture models which can be utilized to study complex cell interactions. A previous study suggested that human synovial fibroblasts derived from deidentified synovial tissues of RA patients undergoing total knee arthroplasty remarkably suppressed TNF-mediated induction of IFN-β autocrine loop and downstream expression of IFN-stimulated genes (CXCL9, CXCL10, macrophage activators) without cell contact when coculture macrophages by the trans-well culture mode [54]. Chwastek et al. performed the coculture of human fibroblast-like synoviocytes and peripheral-like neurons differentiated from neuroepithelial stem cells by transwell insert mode to mimic synoviocyte-neuron interaction responsible for the induction of chronic pain in rheumatoid arthritis [55].

Explant-conditioned media models incorporate the full complement of synovial secretome to induce dendritic cell or FLS activation but cannot model cell–cell contact. Tissue explants represent a critical bridge between in vitro cell culture models and in vivo animal studies [56,57]. They consist of ex vivo cultured tissue fragments that retain the three-dimensional structure, cellular diversity, and ECM composition of the original tissue. It maintains native cellular architecture, allowing for a more

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accurate recapitulation of disease pathology compared to traditional 2D or even some 3D culture models. Synovial tissue explants could be obtained from synovial biopsies or surgical synovectomy samples from RA patients and be used to study synovial fibroblast activation, angiogenesis, immune cell infiltration, and testing of targeted therapies, including TNF-α, IL-6, and JAK inhibitors. Wu et al. demonstrated that Kirenol, a diterpenoid extracted from the Chinese herb *Siegesbeckiae* inhibited the migration, invasion, and proinflammatory IL-6 secretion of FLSs by using RA-associated synovial fibroblasts derived from the culture of RA synovial explants [58].

Because they often fail to recapitulate the complex microenvironment of the synovium, more physiologically relevant models should be necessary.

3. Microengineering Strategy to Recapitulate the Microenvironment of RA Synovium

Microengineering models have been developed to overcome the shortcomings of traditional RA synovial models and transformed RA synovial research by providing platforms that recapitulate the complex cellular, molecular, and biophysical characteristics of the inflamed synovium(Figure 2, Table 1).

3.1. Synovium-on-a-Chip

Synovium-on-a-chip platforms have emerged as a promising tool for modeling synovial membrane structure and function, investigating joint disorders, and evaluating drug responses [59]. These microfluidic devices incorporate synoviocytes, ECM, and biochemical factors to recreate the structural and functional characteristics of the synovial membrane, under physiologically relevant conditions, providing a dynamic platform for disease modeling and drug testing [10]. Furthermore, such a chip system is very cost-effective due to the requirement of fewer cells and microliter levels of reagents compared to traditional 2D and 3D cell culture models.

Vascularization: Because enhanced angiogenesis is known as a major change in RA, the vascularization component of synovium-on-a-chip could not be neglected. Initial vascularized synovium-on-a-chip was designed to be capable of the three-dimensional configuration of synovium and its vasculature as well as simulation of biomechanical stress and inflammatory stimulation, using a commercially available platform, The Chip-S1® (Emulate Inc., Boston, USA) [13]. Their model was established from primary human fibroblast-like synoviocytes for replicating the synovial lining and human umbilical vein endothelial cells (HUVECs) for recreating its associated vasculature, in which the attachment of monocytes to the surface of the endothelial channel occurred in the inflamed condition by IL−1β, thus simulating the cell recruitment in the early inflammatory stages seen in vivo.

Recently, more complicated synovium-on-a-chip models have emerged in order to make a perfect simulation of the synovial microenvironment including vasculature, monocyte, and synovial fluid [60–62]. One of the well-accepted multi-channel OoC systems includes a vascularized synovium compartment with the endothelial channel, synovial fluid compartment, and cartilage compartment. Researchers identified the process of monocyte extravasation into the synovium in the first hours after TNF-α stimulation on this OoC in which fibrin gel was used as a biocompatible matrix to perform the 3D culture of synovial fibroblasts and articular chondrocytes in microfluidic devices.

Mechanical simulation: Fluid shear stress was focused in the synovial research field due to its influences on several synovial components during physiologic and pathologic movement [63]. When co-cultured human dermal fibroblast, macrophage (THP-1 monocyte), primary human osteoblast, and chondrocyte using a μ-Slide I Luer ibiTreat (Ibidi, Germany) system regulated by regulated by a Masterflex® Ismatec® Reglo ICC Peristaltic Pump, it could simulate synovium as well as several components of synovial joint under mechanical stress [61]. In terms of synovium, despite the application of human dermal fibroblast instead of human synovial fibroblast, these results demonstrated that the complex nature of in vivo human joint conditions could be simulated by this microfluidic co-culture system at 24 h and it might serve as a powerful tool for studying the

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pathophysiology of rheumatic diseases and testing potential therapeutics. A research group prepared the microfluidic chip by injection of the suspension of synovial fibroblast at a density of 1×10^7 cells/mL and linkage of a pressure-driven pumping system (Elveflow, OB1 MKII) coupled with a flow rate sensor to perfuse up to 6 cell-loaded microfluidic chips at the same time at a specific level of shear stress [63]. The levels of TNFα released by synovial fibroblasts showed a linear increase with the intensity of the shear, with a 5-fold increase from shear stress of 3 to 8 dyne/cm^2, and a 2-fold increase from shear stress of 8 to 15 dyne/cm^2 in this synovium-on-a-chip model. In this study, the mechanical stimulation downregulated the release of IL-6 and MMPs for a short time exposure, whereas the stimulation for longer than 24 hours upregulated the levels of both IL-6 and MMPs. The other researchers demonstrated that upregulated expression of genes, HAS-1(hyaluronic acid synthase), and HAS-3 were observed when 0%–12% cyclic tensile strain was applied to hFLS for 2 h at 0.2 Hz on this synovium-on-a-chip [13].

Integration of the immune/nervous system: Some authors focused on the importance of the lymphatic system and draining lymph nodes in the pathogenesis of RA inflammation in the synovium, and some authors emphasized that more attention should be given to immune cell and cytokine flow from the lymphatic vasculature in the in vitro model of the RA-afflicted synovium [64,65]. However, there has been no synovium-on-a-chip that combines the synovial membrane with supplying lymphatics, but advanced techniques for microchannel fabrication and controllable fluid dynamics, modeling lymphatic vasculature could allow researchers to find its therapeutic targets and study lymphatic structural and functional change in the pathogenesis of the other diseases.

Some authors attempted to elucidate possible interaction between synoviocytes and neurons in RA [55,66]. Synoviocytes could produce inflammatory mediators that activate and sensitize neurons and neurons, in turn, release neuropeptides like substance P, which further activate synoviocytes and other immune cells, perpetuating inflammation. the combination of synovial inflammation and nerve involvement in RA is a major contributor to the persistent and often debilitating pain that characterizes the disease. However, the synovium-on-a-chip combined with neurons has not been developed.

A research group conducted the study to identify the role of lymphatic dysfunction in RA and first demonstrated altered lymphatic function with near-infrared lymphangiography in RA patients. A researcher established a three-dimensional organoid culture model to simulate the synovial tissue containing synovial fibroblasts, memory CD4+ T cells, and memory B cells [67]. By using fluorescence imaging and image analysis software, they identified the cytokine secretion profiles to recapitulate the interactions between immune cells and synoviocytes in the RA synovium.

3.2. Hydrogel-Based 3D Scaffolds

Hydrogel-based 3D scaffolds offer a biomimetic approach to engineering the RA synovial microenvironment by providing customizable mechanical properties, biochemical cues, and spatial organization that recapitulate key aspects of synovial physiology and pathology in vitro. Hydrogels are hydrophilic polymer networks capable of absorbing large amounts of water, closely mimicking the hydrated nature of native ECM and providing a supportive 3D milieu for cell encapsulation and migration [68,69]. Key tunable properties include crosslinking density (controlling pore size and stiffness), degradation rate (via hydrolytic or enzymatic mechanisms), and presentation of bioactive ligands (e.g., RGD peptides) to direct cell adhesion and signaling [69,70]. Mechanical properties can be tailored to match the RA synovial stiffness range (5–20 kPa) by adjusting polymer concentration, crosslinker type (e.g., UV-initiated methacrylation, Schiff base chemistry), and incorporation of reinforcing nanomaterials (e.g., graphene oxide, nanoclay) [71].

3.2.1. Natural Hydrogel Scaffolds

Collagen type I and type II hydrogels replicate the primary protein constituents of synovial ECM, supporting FLS attachment, proliferation, and MMP-mediated remodeling [72]. Collagen hydrogels crosslinked with genipin or riboflavin exhibit tunable stiffness (1–10 kPa) and degradation rates,

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enabling studies of mechanotransduction in FLS activation and invasion [73]. HA-based hydrogels could support chondrocyte and FLS viability while providing a promising platform for designing immunomodulatory biomaterials toward the treatment of RA [74]. Methacrylated HA (HAMA) crosslinked under UV light yields hydrogels with tunable stiffness (2–15 kPa) and degradation, enabling studies of FLS migration under variable mechanical settings [75]. GelMA hydrogels combine gelatin's cell-adhesive motifs with photo-crosslinkable methacrylate groups, allowing precise control over crosslink density and mechanical properties [76]. GelMA scaffolds with stiffness tuned to 5–20 kPa have been used to culture RA FLS and MSCs, reproducing hyperplastic lining formation and inflammatory cytokine profiles comparable to patient synovium [15]. Alginate hydrogels crosslinked with calcium ions provide a simple, rapid gelation system for encapsulating synovial cells, though lack inherent cell-adhesive motifs [77]. Composite alginate-collagen or alginate-gelatin hydrogels combine alginate's mechanical tunability with biological cues from proteins, supporting co-culture of FLS, macrophages, and endothelial cells to model pannus tissue [72,77]. Chitosan–Matrigel composites (70:30 v/v) provide a low-cost, biocompatible 3D matrix in which both RA and non-RA FLS form dense networks, with compressive moduli comparable to soft tissues (~1–10 kPa) and high cell viability over 7 days [78].

3.2.2. Synthetic Hydrogel Scaffolds

PEG hydrogels offer low protein adsorption, batch consistency, and facile functionalization with cell-adhesive peptides (e.g., RGD) and protease-sensitive linkers [79]. PEG-diacrylate (PEGDA) hydrogels with stiffness gradients from 2 to 20 kPa have been employed to investigate FLS durotaxis and mechanosensitive gene expression (e.g., PRG4, MMPs). Short amphiphilic peptides (e.g., RADA16-I) spontaneously form nanofiber networks mimicking ECM fibrils, supporting 3D culture of FLS and immune cells without exogenous crosslinkers [80]. These hydrogels enable injectable scaffold formats that gel in situ within the synovial cavity, providing minimally invasive platforms for drug delivery and cell therapy [71].

Encapsulation of FLS and macrophages within hydrogels enables measurement of cell invasion, ECM degradation, and cytokine secretion under controlled mechanics [14]. Dual-responsive hydrogels release therapeutics in response to MMP activity or pH changes in the inflamed synovium [15]. Hydrogel scaffolds serve as platforms for testing anti-fibrotic and anti-inflammatory agents, revealing stiffness-dependent drug efficacy and FLS apoptosis profiles [81].

3.3. Spheroids and Organoids

The 3D tissue engineering approach(spheroid) is a promising strategy that can recapitulate 3D physiological structures based on the cell-cell and cell-extracellular matrix interactions [43,82]. They might stimulate the synovial structural features and RA pathogenesis with various cellular components including synovial fibroblast, endothelial cells, macrophages, neurons, and immune cells. Furthermore, its application is expanding through advanced 3D in vitro tissue engineering approaches, including scaffold-free strategies (e.g., cell sheets and self-organization) and scaffold-based systems utilizing synthetic or natural polymers [82–84]. Kiener et al. demonstrated that FLSs formed a compacted lining architecture through 3-week cultivation in spherical extracellular-matrix micromasses [85]. The histological evaluation of FLSs micromass architecture showed rearranged FLSs around the extracellular matrix resembling the synovial lining, and the survival and compaction of monocytes/macrophages were observed in the new lining structure. Philippon et al. developed a 3D spheroid model of synovial tissue having the umbilical vein endothelial cells, FLSs, and macrophages derived from monocytes with a collagen-based 3D scaffold [86]. In the presence of fibroblast growth factor 2, vascular endothelial growth factor, and RA synovial fluid, spheroids with RA fibroblast-like-synoviocytes showed outgrowth of macrophages within the spheroids. A 3D co-culture platform, a novel synovial organoid system developed by Chinese researchers contains THP-1-derived M1 macrophages, human umbilical vein endothelial cells, rheumatoid arthritis fibroblast-like synoviocytes and bacterial cellulose as a scaffold, which can mimic inflammatory and vascular

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microenvironment as well as synovial pathology in RA synovial tissues [87]. In 2021, Rothbauer et al. presented a novel organoid-on-a-chip platform that simulates the interactions between synovium and cartilage in human joints [88]. They established a chip-based three-dimensional tissue coculture model that recapitulates the reciprocal cross-talk between individual synovial and chondral organoids. Synovial organoids were made from Matrigel suspension containing FLSs harvested from RA patients undergoing synovectomy and chondral organoids from commercial human chondrocytes. In RA-FLS organoids, a considerably higher secretion performance was observed in MMP-13 and VEGF with an increase for later passages, which again equalized over three weeks of cell culture. Their results indicated that when co-cultivated with synovial organoids, chondral organoids induced a higher degree of cartilage physiology and architecture and showed different cytokine responses compared to their respective monocultures, highlighting the importance of reciprocal tissue-level cross-talk in the modeling of arthritic diseases.

3.4. 3D bioprinting

Bioprinting technologies—extrusion, stereolithography—pattern FLS, macrophages, and endothelial cells within GelMA or fibrin bioinks to fabricate vascularized synovial tissue analogs [89]. High resolution printing (<50 μm) permits microvascular network formation and precise spatial organization of lining and sublining layers. GelMA-based bioinks have been successfully 3D-printed into microarchitectures that guide FLS alignment and vascular channel formation, facilitating studies of angiogenesis and cell invasion [90]. Low-cost, 3D-printed droplet microfluidic instruments enable single-cell RNA-seq of disaggregated RA synovial tissue, identifying pathogenic fibroblast and immune subpopulations [14,91]. Such platforms facilitate routine clinical profiling of synovial biopsies and discovery of novel therapeutic targets.

3.5. Embedded Sensors and Real-Time Readouts

Embedding sensors into microengineered RA synovial membrane platforms can transform static models into dynamic, data-rich systems. Real-time readouts of pH, O_2, metabolites, cytokines, and mechanics yield unprecedented insight into RA pathophysiology and therapeutic responses [92,93]. The first three-dimensional synovium-on-a-chip platform integrated with non-invasive light scattering biosensing technology was developed to track the initiation and progression of pro-inflammatory synovial tissue responses, facilitating real-time, longitudinal analysis of dynamic tissue-level remodeling during pathological processes such as RA [94]. This synovium-on-a-chip platform employs a microengineered hydrogel matrix combined with a dynamic particle suspension system, encapsulating synovial cells and functionalized polystyrene nanoparticles. In this study, researchers only used purified primary FLSs harvested from RA patients through at least five passages from immune cells like lymphocytes and resident synovial macrophages inside microfluidic devices. This microfluidic 3D light-scattering technology could discriminate the diseased phenotype as early as post-seeding day 2-3 instead of approximately 14 to 21 days post-seeding for conventional microtiter-based 3D synovial models with proliferation assays and cytokine assays such as ELISA. This innovative platform successfully recapitulated synovial inflammation, fibroblast activation, and extracellular matrix remodeling, offering a powerful tool for studying arthritis pathophysiology and drug responses under physiologically relevant conditions.

Table 1. Comparative Evaluation of Microengineering Strategies to Recapitulate the RA Synovial Membrane.

Strategy	Key Features	Advantages	Limitations	Ref
Synovium-on-a-C hip	Two-chamber PDMS device with FLS/macrophage layer and perfusable endothelial chamber	Precise fluid control; Real-time imaging; Replication of synovial shear stress and	PDMS's absorption; moderate throughput; Bubble formation;	[10,60,95,96]

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		gradients; Immune cell-endothelial-FLS crosstalk	material adsorption; Limited long-term culture, Complex fabrication; specialized imaging	
Hydrogel-Based Micropatterned Scaffolds	PEG/collagen hydrogels patterned with micro-wells for FLS + endothelial co-culture; static or low-flow conditions	Accessible fabrication; tunable mechanics; supports basic co-culture assays	Lacks dynamic shear; limited remodeling; incomplete ECM complexity	[15,69,71,79,81]
Spheroid Microtissues	Self-assembled RAFLS/macrophage spheroids in non-adherent microwells; can integrate with perfusion	Mimics cellular condensation; easy high-throughput; relevant cell–cell contacts	Lacks perfusion; limited diffusion; no mechanical cues	[86]
Synovial Organoid	3D encapsulation of Fibroblasts/HUVEC /Macrophages in a 3D fibrin-GelMA hydrogel system to investigate inflammation-mediated angiogenesis	Captures 3D architecture; High-throughput imaging: Real-time visualization of angiogenesis through fluorescence imaging	Limited mechanical loading; organoid heterogeneity; standardization challenges	[96–99]
3D Bioprinted Synovial Constructs	Bioinks of decellularized ECM + FLS printed into defined geometries; optional perfusable channels	Customizable geometry, tunable stiffness, patient-specific potential	Avascular constructs; microvasculature printing limits; bioink optimization challenges	[72,77,90,96]
Biosensor-Integrated Platforms	Electrochemical/optical sensors embedded in microfluidic chips to monitor pH, O_2, cytokines in real-time	Real-time biochemical or optical monitoring; non-invasive; multiplex capability	Sensor drift; integration complexity; potential interference	[94,100]

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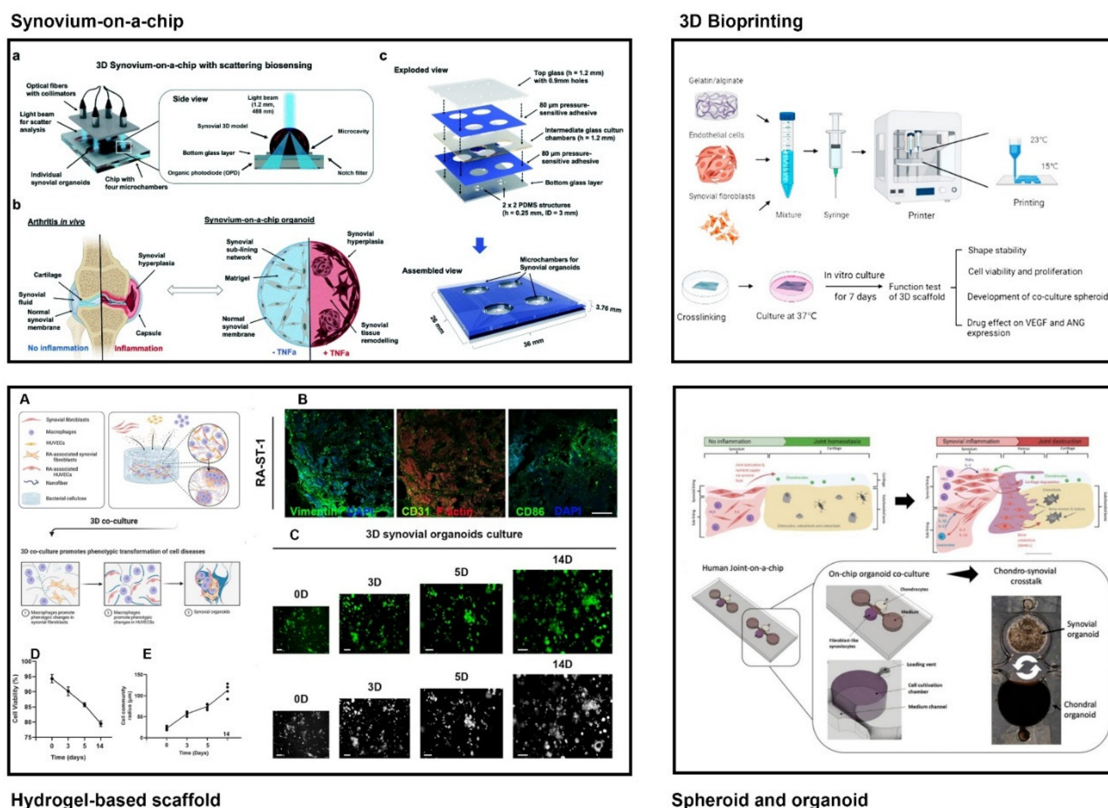

Figure 2. Microengineering of rheumatoid arthritis synovial membrane models. Reproduced from [13,87,88,94]. Licensed under Creative Commons Attribution 4.0 (CC BY 4.0).

4. Conclusion and Future Perspective

In this review, we have described recent progress in the development of microengineering RA synovial membrane models for better recapitulation of its microenvironment. Microengineering has yielded a suite of sophisticated in vitro models that capture the multifactorial nature of the RA synovial microenvironment. By integrating cellular heterogeneity, hydrogel-based scaffolds, biochemical gradients, mechanical forces, 3D bioprinting, organoids, and real-time biosensing, these platforms might overcome the limitations of traditional culture and animal models, offering unprecedented opportunities for mechanistic insight, drug discovery, and personalized therapy.

Despite substantial progress, numerous challenges still require scientific solutions. Due to variability in hydrogel composition, chip fabrication, and primary cell sources, we should establish consensus protocols and quality-control metrics. Incorporation of immune cells and tertiary lymphoid structures remains limited in the existing synovium-on-a-chip.

Therefore, future models should integrate adaptive immune components and lymphatics to make a perfect recapitulation of RA synovial microenvironment, by combining organoid and organ-on-a-chip technique. In addition, long-term culture (> weeks) under dynamic conditions to simulate chronic inflammation and tissue remodeling should be realized. In the near future, AI-based analysis of imaging and sensor data should be developed to identify predictive biomarkers and optimize device parameters on microengineering models.

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Conflicts of Interest: None

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