Assignment 4: Studying Monocyte Extravasation in the Synovium-on-Chip using Different Inflammatory Stimuli

Daily supervisor: Laurens R. Spoelstra, MSc
Supervisors: Prof. Marcel Karperien (DBE), Prof. Loes Segerink (BIOS), Prof. Séverine Le Gac (AMBER)
TNW-DBE, EEMCS-BIOS, EEMCS-AMBER, University of Twente, The Netherlands

Project background:

Arthritis is a family of diseases affecting millions of patients worldwide, typically characterized by degradation of cartilage, joint inflammation, pain, and joint stiffness. The two most common forms of arthritis are Osteoarthritis (OA) and Rheumatoid Arthritis (RA). Globally, OA affects more than 300 million patients (2017) and RA is estimated to have a global prevalence of up to 1,3%. Furthermore, as obesity and ageing are two major risk factors for OA, and to some extent RA, it is expected that the prevalence of arthritis continues to increase.

Despite the different disease manifestations, there appears to be an overlapping trend in the pathogenesis of both RA and OA: the cartilage destruction resulting from synovitis. Even though synovitis presents differently in both OA and RA, the effects are similar: the release of pro-inflammatory mediators and matrix-degrading enzymes leads to cartilage degradation that can enhance synovitis through the release of matrix products and potentially soluble factors present in the cartilage matrix. Hence, **it can be concluded that both OA and RA involve chondro-synovial crosstalk as part of the disease progression**, which presents as a vicious cycle of cartilage degradation and synovitis, which we call **chondro-synovial crosstalk** (Figure 1).

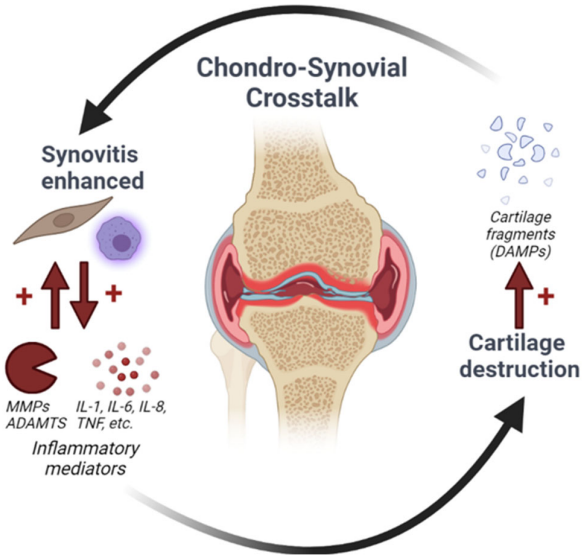
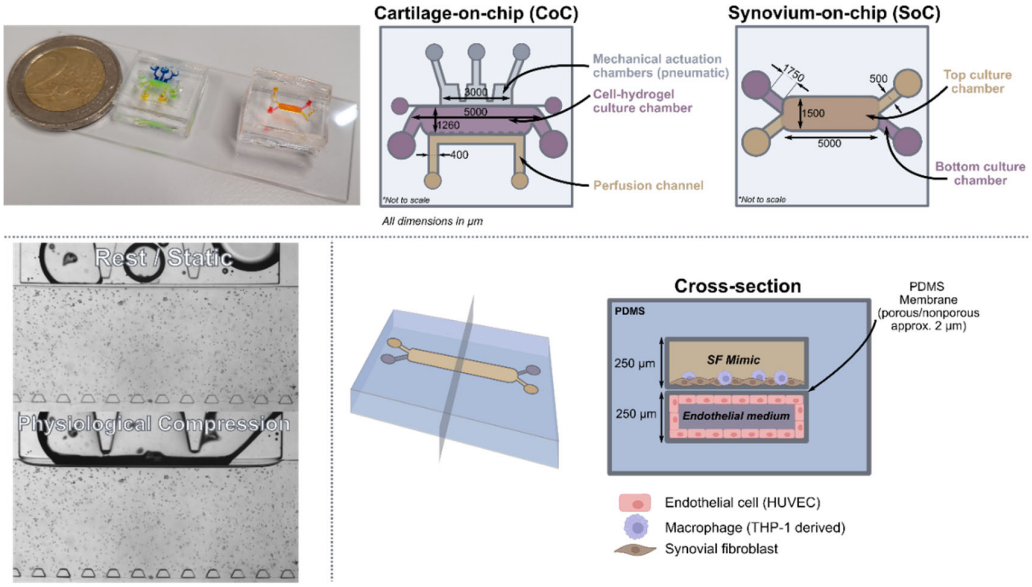


Figure 1: Simplified schematic representation of Chondro-Synovial crosstalk in arthritis.

The need for *in vitro* models for arthritis

To date, no disease-modifying OA drugs (DMOADs) are available and treatment of OA focuses mainly on relieving symptoms and physical therapy. Pharmacological management of OA is mainly based on paracetamol and, alternatively, nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac and naproxen. NSAIDs reduce pain and, to some extent, improve joint functionality but not disease severity. For RA, a few disease-modifying anti RA drugs (DMARDs) are available (e.g. methotrexate (MTX) and sulfasalazine (SSZ)) next to the commonly used NSAIDs that are also used for OA. Unfortunately, most DMARDs result in, amongst others, gastrointestinal and cardiovascular side effects.

There is a need for physiologically relevant arthritis models since the lack of such models limits the development and investigation of DMOADs and DMARDs. Current preclinical models consist mainly of monolayer cultures and animal models, which **do not reflect the biological complexity and whole-joint nature of arthritis; amongst which the chondro-synovial crosstalk**. It has been argued in literature repeatedly that novel physiologically relevant models will lead to an increased understanding of arthritis and aid in the development of new drugs. Furthermore, they could enable personalized medicine to address various phenotypes of OA and RA observed in the clinics. So far, we have developed a Cartilage-on-Chip, Synovium-on-Chip, and Ligament-on-Chip (early phase).



Assignment 4: Studying Monocyte Extravasation in the Synovium-on-Chip using Different Inflammatory Stimuli

A key pathogenic event in many forms of arthritis is the influx of monocyte-derived macrophages and other immune cells from the surrounding tissue and microvasculature, potentially leading to pannus formation and most importantly a disturbed immune balance inside the joint together with the activated synovial fibroblasts. In order to be able to mimic this key pathogenic event in the JoC, it has to be studied on the isolated Synovium-on-Chip first.

In this assignment, you will use the Synovium-on-Chip platform with a thin porous PDMS membrane with 5 µm pores (Zakharova *et al* 2021; doi.org:10.1002/admt.202100138) to study monocyte extravasation of undifferentiated THP-1 monocytes under conditions that mimic RA. You will first create an inflammatory disease model using inflammatory cytokines and you will characterize this disease model using immunofluorescence and qPCR. Next, monocyte extravasation will be studied under various conditions, such as the addition of chemokines (e.g. CCL2). Depending on the outcomes of the experiments, further experiments that study the effects of flow and associated fluid shear stress on monocyte extravasation can be studied as well using a microfluidic setup. T**hese experiments will involve live-cell imaging using our latest organ-on-chip focused automated fluorescence microscope and will combine the cell biological skills and assays with more technical challenges in imaging, data analysis, and microfluidics.**

Key skills/topics the student could learn during this assignment:

- Cell culture of primary cells (Synovial Fibroblasts, HUVECs) and human cell lines (THP-1)
- Immunofluorescence
- Confocal microscopy
- Fluorescence live cell imaging & cell tracking
- Biochemical assays such as ELISA, MMP activity
- qPCR
- Microfabrication of organ-on-chip devices
- Microfluidics: effects of shear stress on monocyte extravasation

Interested? Please contact Laurens Spoelstra (l.r.spoelstra@utwente.nl) for more information.