Assignment 3a/b: Studying Disease Phenotypes and Anti-Inflammatory Drugs in the Synovium- and Cartilage-on-Chip

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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).

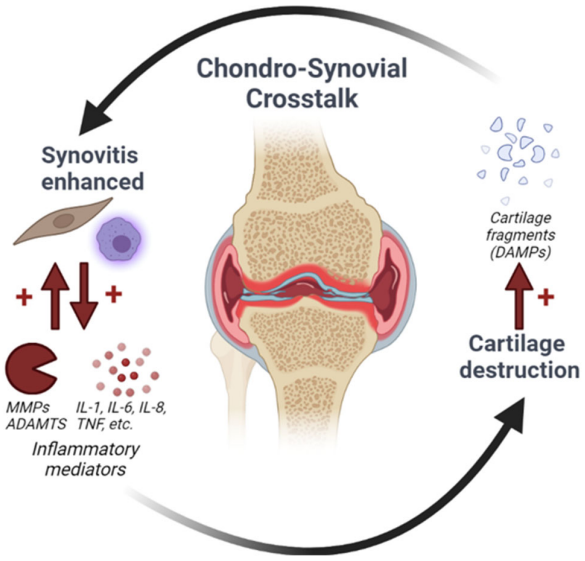
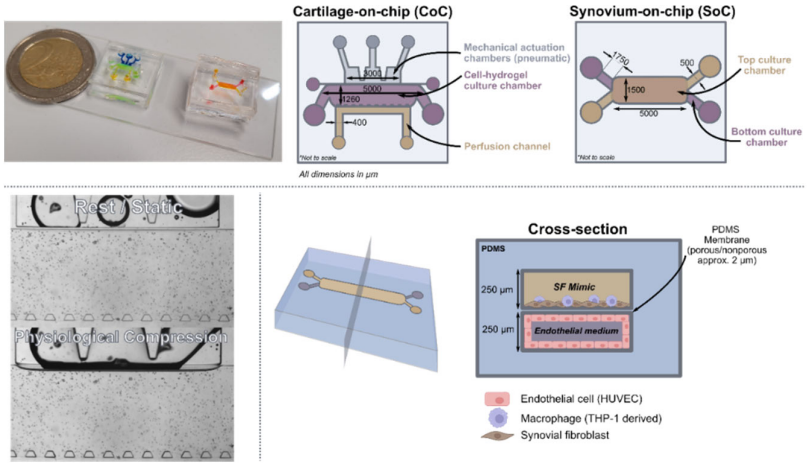


Figure 1: Simplified schematic representation of Chondro-Synovial crosstalk in arthritis.

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).



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The ultimate goal of our JoC platform is to be able to use it for drug screening purposes for any subtype of arthritis, for example OA or RA. Currently, we have generated a RA-like phenotype in the Synovium-on-Chip. The OA phenotype, which is less well-defined but at least has a lower degree of synovitis than RA, is still to be established. In literature, it has been proposed that TGF-ß1 can induce a fibrotic, OA-like, phenotype in synovial micromasses (Broeren, M. G. A. *et al.* 2019. DOI: 10.14573/altex.1804161), which would be the initial approach in this assignment. In this assignment, you will have to define the RA and OA phenotypes, choose readouts (e.g. antibodies, genes of interest) accordingly, and perform experiments to generate the different disease phenotypes. If a disease phenotype has been generated, the platforms can be validated for drug screening (depending on the progress). It would be of interest to test various known anti-rheumatic drugs and/or NSAIDs on our chips. Examples include Sulfasalazine (SSZ), Diclofenac (DIF), Methotrexate (MTX), and Dexamethasone (DEX). The exact experiments will depend on the platform (SoC/CoC) chosen, but could include:

A:Cartilage-on-chip:

For this assignment, you will generate an RA/OA model in the CoC platform and characterise it. For the establishment of a disease model, hyperphysiological compression will be compared to physiological compression levels (Paggi, C. A., Venzac, B., *et al* 2020. DOI: 10.1016/j.snb.2020.127917) with and without the addition of cytokines. After establishment of a disease model, you will make a selection for the drugs to test and you will design experiments to test their effects on key output parameters such as Collagen I/II deposition, protease activity, cytokine release, etc.

B:Synovium-on-chip:

For this assignment, you will generate an RA/OA model in the SoC platform and characterise it. After establishment of a disease model, you will make a selection for the drugs to test and you will design experiments to test their effects on key output parameters such as, cytokine release, gene expression of inflammatory genes, and macrophage polarization.

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

- Cell culture of primary cells (Human chondrocytes, human Synovial Fibroblasts, HUVECs) and human cell lines (THP-1)
- Cell biology: culture medium optimization
- Cell cultures in monolayers & pellets (potentially)
- Immunofluorescence
- Confocal microscopy
- Biochemical assays such as ELISA, SPR, MMP activity
- RT-qPCR
- Microfabrication of organ-on-chip devices
- Cartilage-on-chip mechanical actuation

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