Assignment 2: Development of a (Potentially Xeno-Free) Common Culture Medium for the Joint-on-Chip

Daily supervisor: Laurens R. Spoelstra, MSc
Supervisors: Prof. Marcel Karperien (DBE)
TNW-DBE, 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).

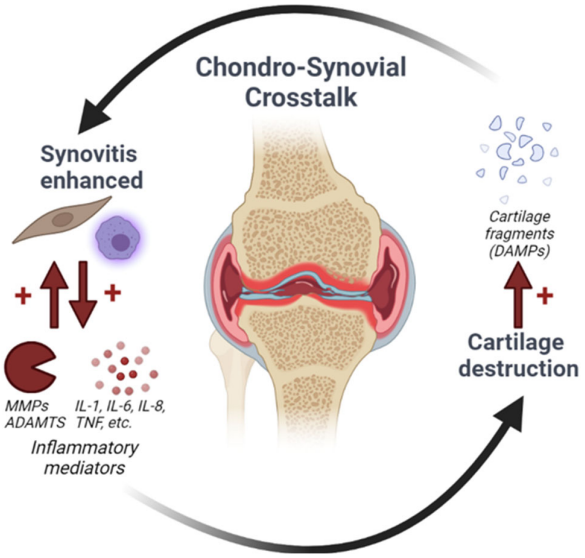
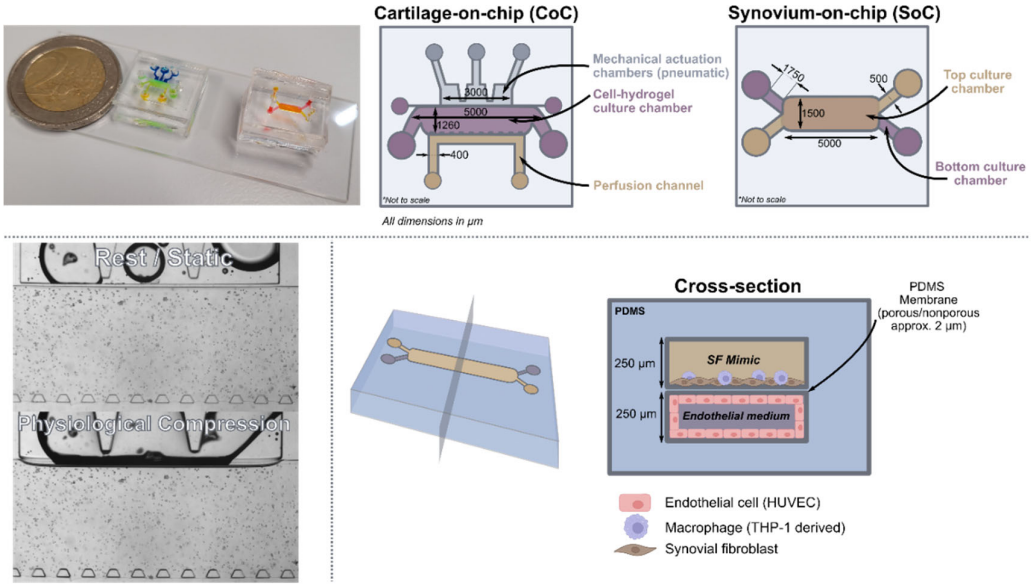


Figure 1: Simplified schematic representation of Chondro-Synovial crosstalk in arthritis.

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).



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In recent years, **the DBE and AMBER groups have developed two OoC models for the key players in Arthritis: the cartilage-on-chip (CoC) and the synovial membrane-on-chip (SoC)**, see Figure above. Currently, **we are working on combining the two devices in one platform: the Joint-on-Chip (JoC).**
One of the key (biological/biochemical) challenges in the development of the JoC, is the development of a common culture medium. Each cell type present in the OoC models used in the JoC (chondrocytes, synovial fibroblasts, THP-1, HUVECs) **requires a different cocktail of nutrients**, growth factors, and other small molecules, f**or optimal maturation, phenotypic stability, and functionality** of these cell types. Unfortunately, additives used for chondrogenic differentiation and maturation (e.g. Dexamethasone, TGF-ß3) are known to have negative effects on the THP-1 and Synovial fibroblasts, for example. Furthermore, THP-1 are known to die when cultured in DMEM-based culture media, which is widely used to culture chondrocytes. Therefore, a common culture medium without such additives needs to be developed. I**n the future, we want to exploit the JoC as a platform that removes the need for animals in drug development, which is why we would like to explore the option of a xeno-free culture medium**, for example by replacing fetal bovine serum (FBS) with human platelet lysate (hPL).

In this assignment, you will explore different culture medium formulations and you will test them on both the Cartilage-on-Chip (CoC) and the Synovium-on-Chip (SoC) to study the effects of the media on the chondrogenic maturation in the CoC, and functionality of the Synovium-on-Chip.

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.