# Developing Osteoarthritis Models in Mechanically Loaded Cartilage-On-Chips

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Osteoarthritis is the most common musculoskeletal disease worldwide. Its impact is constantly increasing due to the ageing population and the lack of treatment. Osteoarthritis results in the destruction of cartilage made up of a single cell type, the chondrocytes. The initiation and progression of the disease are consequences of the interconnection between the action of proinflammatory cytokines, signaling pathways and mechanical stress. The models widely used in osteoarthritis research do not provide an accurate summary of the disease's pathophysiology. To improve the understanding of the disease and develop treatments, novel cartilage-on-chip microfluidic models, capable of applying mechanical stress, have been developed. This work aims to develop a model of osteoarthritis by applying an inflammatory environment, excessive Wnt signaling and mechanical stimulation to chondrocyte cells, encapsulated in a gelatin methacrylate hydrogel matrix, using a cartilage-on-chip device. The accuracy of the model is assessed by imaging extracellular matrix proteins including aggrecan, collagen II and NF-kB.

Images showing extracellular matrix deposition are obtained using confocal imaging and processed with ImageJ software. To develop the cartilage-on-chip model, an experiment to optimize the seeding density was carried out, followed by an experiment inducing an osteoarthritis phenotype. The experiment to determine the optimal seeding density was carried out under static conditions, without the application of external stimuli, and determined that a seeding density of 20 million cells/mL was optimal for extracellular matrix visualization and for the development of single-cell models. This seeding density was used in the osteoarthritis induction experiment in which chemical and mechanical stimuli were applied. The latter experiment showed conclusive results via analysis of NF-kB protein expression, which showed signs of osteoarthritic phenotype onset. Analysis of aggrecan and collagen II, other proteins that are part of the extracellular matrix, did not show conclusive results. The induction of osteoarthritis by mechanical stimuli could not be evaluated due to a problem with the experimental conditions. However, cartilage-on-chip models are still in their infancy and these results will help to optimize the experimental conditions for future experiments and address the challenges associated with image processing. This work has contributed to the development of the cartilage-on-chip model, which can ultimately prove to be a revolutionary tool for the treatment of osteoarthritis.

**Keywords**: Osteoarthritis - Cartilage-on-chip - Chondrocytes - Gelatin methacrylate - Inflammatory environment - Wnt signaling - Mechanical stress.