Modeling Human Joint Health and Disease: A Four-Cell Co-culture Chip Approach Under Varied Fluid Shear Stress

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Fluid shear stress (FSS) above 5 dyn/cm² has a catabolic impact on articular chondrocytes.[1] FSS of 10-20 dyn/cm² is widely known to increase levels of osteogenic gene expression in many different osteoblastic cell types.[2] However, it is not as well understood how FSS levels influence the behavior of synoviocytes, which include fibroblast- and macrophage-like synoviocytes. Nevertheless, 12 dyn/cm^2 FSS induces THP-1-derived human macrophages to secrete inflammatory cytokines (i.e., TNF -α, IL-1β)[3] that are pertinent to the pathogenesis of osteoarthritis (OA). Notably, prior studies were focused almost exclusively on cells in monoculture, whereas here, we examine the effects of FSS under co-culture conditions.

In this study, the multi-tissue nature of the joint as an organ was recapitulated by co-culturing human cell line osteoblasts, primary articular chondrocytes, primary dermal fibroblasts, and THP-1-derived macrophages in Ibidi µ-Slide I Luer chips linked together in series. Different channel heights were utilized for each cell type to allow for exposure to physiologically relevant FSS levels specific for each cell type under a single volumetric flow rate.

To establish a model of healthy joint cell co-culture, cell viability across a 5-day duration using a single shared cell culture media was assessed using multiple assays, including PrestoBlue™ assessment of viability, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assessment of metabolic activity, and lactate dehydrogenase (LDH) cytotoxicity evaluation. To model the OA disease state, macrophages were either separately exposed to lipopolysaccharides (LPS) and interferon-gamma (IFN-γ) prior to co-culture or exposed to inflammatory levels of FSS during co-culture to induce secretion of inflammatory cytokines relevant to OA pathogenesis. Here, secretion of catabolic enzymes commonly associated with OA were then evaluated following up to 5 days of co-culture by utilizing immunoblots of conditioned media to assess levels of MMP-13 and MMP-1 secretion by chondrocytes.

1. Jin, Y., et al., (2021), J Inflamm Res. **14**: p. 6067-6083,DOI:10.2147/jir.S339382.
2. Wittkowske, C. et al., (2016) Front Bioeng Biotechnol. **4**: p. 87, DOI:10.3389/fbioe.2016.00087.
3. Son, H., et al., (2023),Biomedicine & Pharmacotherapy. **161**: p. 114566, DOI: 10.1016/j.biopha.2023.114566.

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