FINAL REPORT

Establishment of an *in vitro* model to investigate extracellular matrix and vascular mechanical interactions in human arterial disease

In this **3R-funded project**, we have established an *in vitro* setup where human smooth muscle cells (SMCs) can be cultured in conditions that accurately simulate healthy or disease arteries. First, cells were cultured on flexible-silicon membranes instead of rigid plastic culture plates. The soft membranes were coated with different extracellular matrix protein substrates that have been associated with a healthier (laminin and collagen IV) or a more pathological state (collagen I and fibronectin) SMCs phenotype. Then, we left the cells under classical static culture conditions or exposed them to different mechanical stretch types, followed by an RNA sequencing analysis. Gene expression of SMCs subjected to static or supraphysiological stretch (15% of elongation), mimicking pathological conditions of the vascular wall, were compared to cells that received physiological or healthy stretch (10% of elongation). Our results showed that cyclic stretch strongly regulates human SMC phenotype through changes in their inflammatory signaling pathways. Static or supraphysiological stretch induces a significant upregulation of several inflammatory molecules. Among those, we found cytokines, chemokines, and adhesion molecules, which may explain the development of arterial diseases. Therefore, this in vitro setup can be used in the future as a platform to **replace** the extensive experiments in genetically modified mice and investigate how mechanical cues control the phenotypic modulation of human SMCs.

In the funded period, we experienced two lockdown periods due to COVID-19. During this time outside the lab and as part of our dissemination plan, I reviewed the literature about mechanical forces in SMCs and submitted an article. The manuscript is entitled “The phenotypic responses of vascular smooth muscle cells exposed to mechanical cues,” and it is published now in the journal Cells (https://doi.org/10.3390/cells10092209). Some results of this project were presented in a poster at the Annual Meeting of the Cardiovascular Research Network, Aarhus University. In this meeting, our 3R work was featured with a poster prize. The final results of this 3R project will be put together and submitted to a peer-review journal.