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

To Replace the number of experiments with mice, this project aims to establish a new in vitro platform based on different extracellular matrix (ECM) proteins and mechanical stimulation for human vascular cells research, particularly smooth muscle cells (SMC).

We already have some exciting results.

In this project, we want to compare the effect of 1) extracellular matrix proteins (ECM) and 2) mechanical stretch on human SMC function. First, we used plastic labware to culture the cells with different ECM proteins. We observed that some ECM proteins like collagen I and fibronectin (called here "disease-like" ECM proteins) induce changes in SMCs morphology and change their proliferative and migrative capacities. On the other hand, ECM proteins such as collagen IV and laminin (physiologic-like ECM proteins) keep SMC's elongated shape and reduce their proliferation and migration (Please see some results in fig 1).

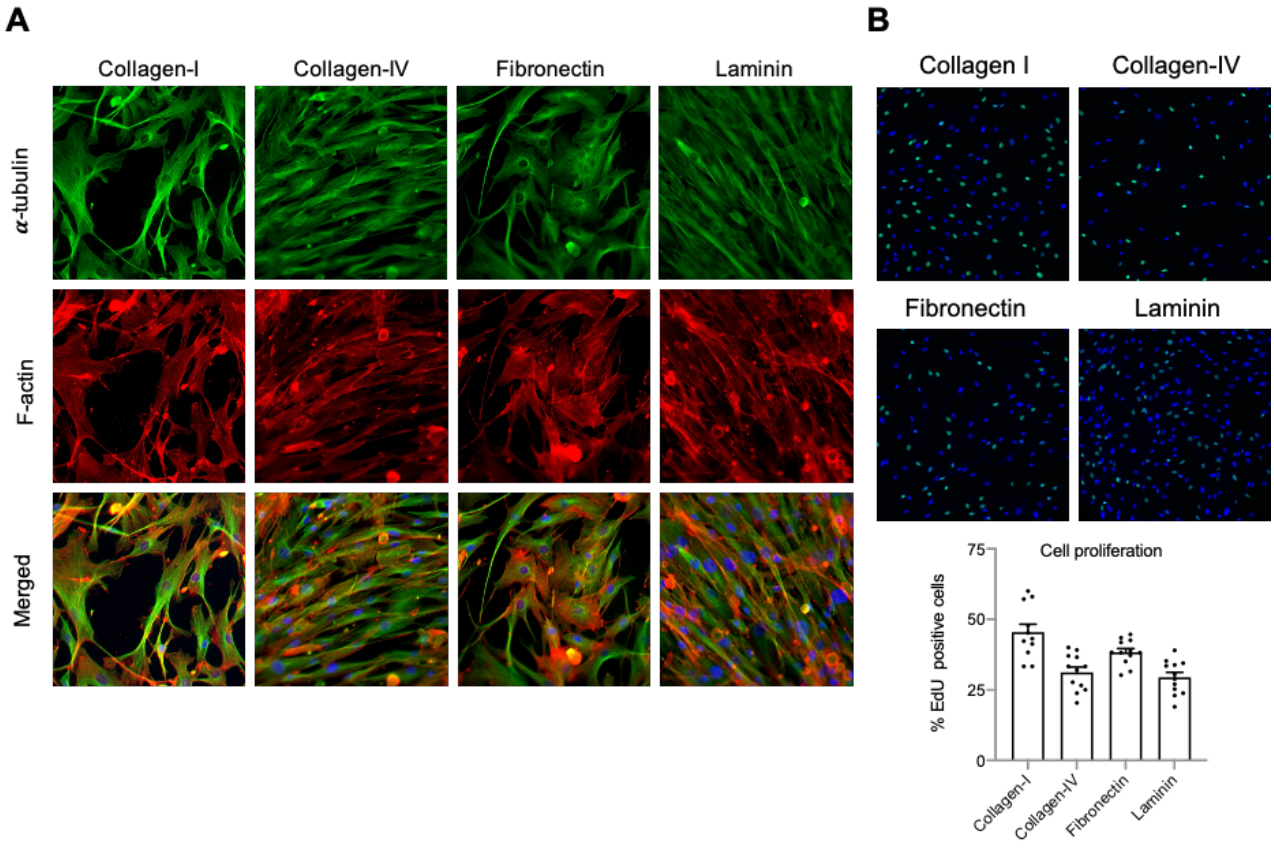

Figure 1. Effect of ECM proteins to human SMCs. **A)** Morphology of human SMCs cultured on plastic plates with different ECM protein coatings. Collagen 1 and Fibronectin induce changes in the normal morphology of the cells. Collagen IV and Laminin keep the SMCs elongated. **B)** Human SMC proliferation after being cultured with different ECM proteins. Proliferative cells are shown in green color (EdU positive).

Then we use a particular device to induce stretch on the cells and special plates made of soft silicone instead of the hard plastic labware. There is a lot of controversy in the literature regarding the effect of mechanical stretch on SMCs, but few studies have investigated this phenomenon in deep on human SMCs. Therefore, we used this model to induce mechanical stretch on human SMCs cultured under different ECM proteins and test the effect on the cells with three different readouts: 1) gene expression (qPCR and RNA sequencing), 2) cell proliferation (EDU incorporation), and 3) cell migration (scratch assays).

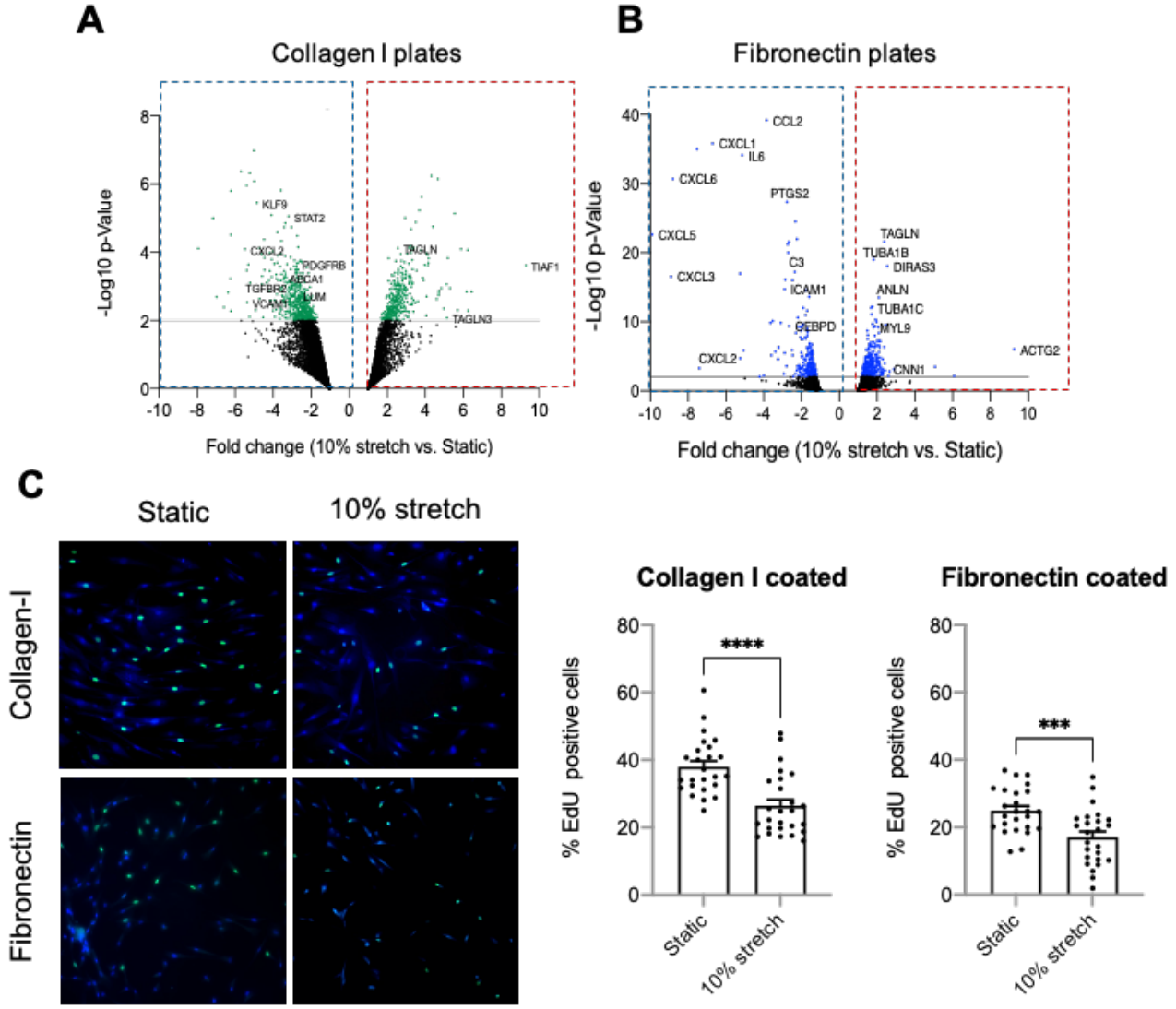

Figure 2. Effects on human SMCs after stretch. **A & B)** Volcano plots of RNA sequencing data of cells exposed to stretch and cultured on collagen I and fibronectin. Green and blue dots indicate genes-of-interest that display both large-magnitude fold-changes as well as high statistical significance. The Blue dashed square indicates downregulated genes, while the red dashed square indicates upregulated genes. The p-value cutoff was < 0.01 and with changes of 2-fold. **C)** Stretch significantly reduces cell proliferation of human smooth muscle cells as indicated by less EdU (green) positive cells.

We have also encountered some challenges:

We aimed to use four different ECM proteins (collagen I, fibronectin, collagen IV, and laminin) buying plates from the company Flexcell to stretch the cells. When culturing the cells, they grew nice and formed a monolayer only with collagen I and fibronectin. However, SMCs cultured on collagen IV and laminin Flexcell plates did not grow as expected (See figure 3).

Since we have found out that mechanical stretch on human aortic SMCs reduces the inflammatory and synthetic gene expression and reduces cell proliferation on cells cultured under the "disease-like" matrices. It is essential for the project to evaluate if stretch on cells cultured under "physiological-like matrices further reduce disease characteristics in human SMCs. One possibility to overcome this problem is to order uncoated silicon plates from the Flexcell company and perform the coating with collagen IV and laminin proteins ourselves (we have already ordered these plates and the ECM matrices for coating).

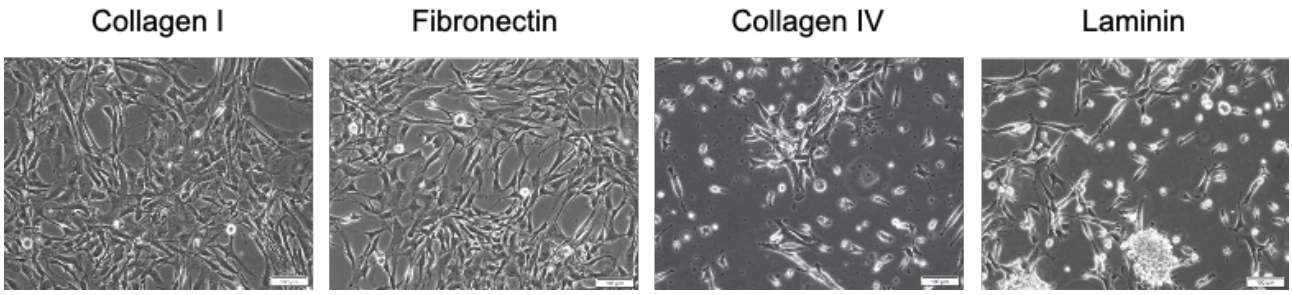

Figure 3: Cells grew nicely on the Flexcell company coated plates with the ECM collagen I and fibronectin but not in plates coated with collagen IV and laminin. Therefore, we ordered new plates "uncoated" from the same company that we would like to coat with collagen IV and laminin ourselves.

To validate our RNA sequencing results, we have performed stretch experiments again. We want to perform real-time qPCR to evaluate gene expression of the most significantly regulated genes obtained in the RNA sequencing. We have isolated RNA that we would like to use to prepare cDNA and run the gene expression analysis, but unfortunately, our nanodrop is broken. We have tried to get it to repair, but the results we get after it came back from the workshop are not consistent, and therefore, I would like to buy a new device to further continue with my research project.

To highlight the importance of our findings in human arterial disease, we plan to perform two final experiments. 1) To evaluate our in vitro model on a diseased human SMCs line (originated from a diabetic and hypertensive human donor), we would like to buy it as soon as possible. Moreover, 2) use antibodies and fluorescence microscopy to demonstrate the top three regulated genes' relevance in our RNA sequencing on human samples with atherosclerosis (we have the human samples). We only need to buy the selected antibodies.

During the lockdown, we have been preparing a review article about the importance of this in vitro model for investigating arterial disease and how this could reduce the number of mice used for research.