SEMS: RESEARCH PROJECT DESCRIPTION

1. Project Background and Description

Introduction: Chronic diseases (aneurysm, atherosclerosis, myocardial infarction, tumour growth) have been associated with biomechanics induced low-grade inflammation. Biomechanics consists of blood pressure-induced mechanical stress and blood flow induced shear stress. Despite their importance, no specific therapy is available for treating biomechanical related disease.
Cells react to their (biomechanical) environment with the regulation of ~7,000 genes, in a timely and spatially coordinated manner. The invention of CRISPR enables to study this dynamical process either by time or spatially induced fluorescence, barcoding or by induced knock out/ knock-in studies. Performing these studies on a large scale in a parallel way allows one to embrace the above-mentioned complexity. In order to do so, we have created a new high throughput platform which uses reversed transfection to allow eukaryotic cells to uptake guiding RNA in a timely manner. One of the interesting developments is to introduce knock in technology in high throughput platforms. Knock in experiments enable to add fluorescent tags to proteins of interest, add molecular biomarkers, add inducible elements to provide timed expression of genes. In this application we develop "knock in" technology to test new synthetic microRNA for evaluation on signaling pathways.
The general idea is to knock in GFP for components of a signaling pathway and evaluate the effect of microRNA on their expression. A signaling pathway is often represented by 50-100 genes which will be spotted in a single lane. The current HT platform allows to test 10 individual signaling pathways in parallel, but the aim is to expand it to 100 pathways in the current application.
The choice of microRNA is dependent on machine learning software developed in other projects

Hypothesis. We hypothesise that (groups of) microRNA control entire signaling pathways in a spatial and dose dependent way

2. Project Scope

Aims. **Our overall objective is to create a new drug-screening platform capable of monitoring the response of cells to complex and specific 3D environments. Specifically, we aim to:**

- Create a new knock in platform for monitoring of the response of entire signaling pathways.
- Modify the platform so that we can study 100 parallel signaling pathways.
- Inject (combinations of) synthetic microRNA to evaluate their specificity for signaling pathways.

3. Desired Skills from the Student

The student is interested in

- *Working on novel ideas*
- *Molecular interests*
- *Cell culture, gene transfection, plasmid synthesis*

- *Cloning*
- *Microcopic interests.*

4. Supervisory Team

Professor Rob Krams

Dr. Helena Azevedo

Dr. Thomas Iskratsch