SEMS: RESEARCH PROJECT DESCRIPTION

1. Project Background and Description

Introduction **Chronic diseases (aneurysm, atherosclerosis, myocardial infarction, tumour growth) have been associated with persistent low-grade inflammation. Recently, the composition and stiffness of the immediate 3D micro-environment of cells were shown to be an important, independent modulator of inflammation.**

The emerging role of the cellular micro-environment opens opportunities for therapeutic treatment, but this avenue has not been explored due to a lack of tools to recreate key features of the extracellular matrix (ECM) and study cells in a physiological micro-environment, and to evaluate the molecular mechanism underlying the cellular response in a high throughput manner. Two important innovations at Bioengineering-QMUL overcome previous problems. Firstly, a novel way to modulate the composition and stiffness of the ECM developed by Azevedo's team and secondly, a high throughput CRISPR platform to interrogate all pro-inflammatory pathways in a single experiment developed by Krams' team. We will describe herein, a unique combination of these novel techniques to create the world's-first platform for testing new therapies on micro-environment induced inflammation (Fig. 1).

The ECM, composed of structural, soluble, and signalling molecules, dictates cell behaviour, cell-cell interactions, homeostasis, and regeneration. The spatial presentation of these molecules is crucial in determining the biological outcome. However, methods to enable such molecular presentation within 3D materials do not adequately represent the molecular diversity, chemical composition, and physical features of the natural 3D cellular environment, which significantly limits physiological relevance. Leading approaches using for example peptide-functionalized PEG hydrogels lack whole macromolecules of the ECM that limit their physiological relevance. Another critical feature of the ECM is its stiffness, which dictates cell behaviour. It is known that both the composition and stiffness of the ECM can vary largely in disease and is currently impossible to generate 3D micro-environments where these parameters can be independently tuned to recreate specific physiological scenarios.

Cells react to their (biomechanical) environment with the regulation of ~7,000 genes, in a timely and spatially coordinated manner. The invent 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

Hypothesis. **We hypothesise that spatially controlled 3D hydrogels made from multiple ECM macromolecules integrating specific physical (stiffness) and biological (ECM biomolecules) cues combined with new, high throughput CRISPr-CAS9 genome editing techniques allows to evaluate and modify the role of the ECM on cellular pro-inflammatory pathways**.

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 printable 3D hydrogels that can present specific biomolecular signals and stiffness.
- Combine these gels with mixtures of guiding RNA and DNA templates (for knock in studies).
- Create reproducibly thousands of spots, which contain systematically applied large-scale variations of ECM and spot-specific gRNA.
- Seed a variety of cells on these spots (fibroblasts, macrophages, endothelial cells) and monitor their single cell pro-inflammatory response with fluorescent markers induced by knock in experiments.
- Test a set of antibodies to minimize the effect of integrin activation on inflammation.

3. Desired Skills from the Student

The student is interested in

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

4. Supervisory Team

Professor Rob Krams

Dr. Helena Azevedo

Dr. Thomas Iskratsch