SEMS: RESEARCH PROJECT DESCRIPTION

1. Project Background and Description

Investigation of the vascular permeability with combined light-addressable potentiometric sensors (LAPS) and scanning ion conductance microscopy (SICM)

Photoelectrochemical imaging/LAPS and SICM are electrochemical imaging techniques that have been used independently to image living cells [1, 2]. While LAPS shows great promise in measuring ion concentrations, impedance and potentials on the part of the cell membrane facing the substrate on which the cell rests (the basal side), SICM has been used to obtain detailed information about electrochemistry, electrophysiology and topography of the part of membrane facing the extracellular solution (the apical side). This project will make use of a novel instrument that combines the two techniques for the first time for functional electrochemical imaging on both sides of a layer of cells. This instrument is particularly attractive for functional studies of epithelial and endothelial tissues which line the cavities or surfaces of organs such as eyes, lungs, gastrointestinal and urinary tracts, or blood vessels. Impaired function of these tissues is linked to a number of health issues such as blindness, atherosclerosis or diabetes. The polarized apical-basal nature of these tissues is crucial for their function hence there is a great need for tools capable of investigating cellular function at the apical and basal sides simultaneously. In this project, the capabilities of the new combined LAPS and SICM setup will be validated using the vascular endothelium as a model system while gaining new insight in the ion transport mechanisms through the vascular endothelium, which will ultimately help to develop new therapies for diseases associated with increased vascular permeability such as diabetes and atherosclerosis.

[1] Fan Wu et al., Analytical Chemistry 91 (2019) 5896-5903; https://doi.org/10.1021/acs.analchem.9b00304

[2] Yuanshu Zhou et al., Analytical Chemistry 90 (2018) 2891-2895, https://doi.org/10.1021/acs.analchem.7b05112

2. Project Scope

Objective 1. Validation of the LAPS-SICM instrument: Patterns of polyelectrolytes such as PAH and PSS will be stamped onto the LAPS chip surface using microcontact printing (mCP) to obtain areas with different surface charge and thickness to validate simultaneous LAPS and SICM imaging. Dynamic measurements will be simulated by releasing buffer solutions with different pH through the SICM nanopipette and monitoring the local pH changes with LAPS.

Objective 2. Development of ion sensitive LAPS: Silicon-on-sapphire chips modified with organic monolayers have been shown to be suitable for submicron-resolution LAPS imaging. The surfaces of LAPS substrates will be modified with chelators to obtain surfaces with good sensitivity and selectivity for the potentiometric detection of sodium and calcium ions. As sensor surfaces are routinely modified with 1,8-nonadiyne, the chelators will be bound to the surface using a copper catalysed “click” reaction. The effects of the newly designed surfaces will be tested in cell biology experiments to understand the potential effects of these surface modifications on cell viability and phenotype.

Objective 3. The investigation of ion transport across the vascular endothelium: Endothelial cells line all blood vessels including the blood-brain barrier. Dysfunctions in these cells and the negatively charged glycocalyx that covers them have been reported in a number of conditions including atherosclerosis, diabetes and high blood pressure and frequently lead to alterations in the transport of solutes, in particular ions, through the vascular endothelium. The LAPS-SICM instrumentation used in this project offers a unique opportunity to carry out a detailed investigation of the mechanism of ion transport though the endothelium. Endothelial cells will be cultured on a LAPS chip modified with sodium and calcium ionophores. Transcellular transport of sodium ions across a confluent layer of endothelial will be monitored with subcellular resolution and correlated to the morphology of the glycocalyx.

3. Desired Skills from the Student

The student should have a strong background in chemistry or materials science or bioengineering. The student should be interested in gaining skills in photoelectrochemical imaging, sensor fabrication, surface modification, materials characterization techniques such as XRD, AFM, SEM and XPS, cell and tissue culture and the investigation of cell physiological processes.

4. Supervisory Team

Professor Steffi Krause is a Professor of Electroanalytical Systems and an expert in photoelectrochemical imaging and biosensors. https://www.sems.qmul.ac.uk/staff/s.krause

Dr Andrew Shevchuk (Imperial College) is a Lecturer in Nanomedicine. His research focuses on the design and development of SICM and its application to a wide range of studies in cell biology. https://www.imperial.ac.uk/people/a.shevchuk

Professor Wen Wang is a Professor in Biomedical Engineering and Vice Principal for Science and Engineering at QMUL. His research focuses on vascular bioengineering and biomaterial mechanics, including studies on the endothelial glycocalyx, vascular stem cells and progenator cells, transmembrane and transcapillary exchange. https://www.sems.qmul.ac.uk/staff/wen.wang