Contractile response of alveolar epithelial cells to biochemical or mechanical stimulation probed by traction microscopy

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A dissertation by
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in partial fulfilment of the requirements for
the degree of Doctor of Philosophy

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Chapter 2. Aims of the thesis

General aim

The general aim of this thesis was to study the generation of contractile force by human alveolar epithelial cells in culture in response to biochemical or mechanical stimuli using traction microscopy.

Specific aims

1. To implement a traction microscopy setup to measure the contractile force generated by human alveolar epithelial cells in culture.

    1.1. To implement and validate a software to determine the deformation field induced by adhered cells on the elastic substrate, following previously described algorithms.
    1.2. To implement and validate a software to determine the traction field induced by adhered cells and other contractility parameters, following previously described algorithms.
    1.3. To implement a software to determine the contour of an adhered cell from a brightfield or phase contrast image of the cell.

2. To study the contractile response of human alveolar epithelial cells in response to thrombin.

    2.1. To determine the gel substrate conditions and gel fabrication procedure which enable suitable cell culture and optimal detection of traction forces exerted by human alveolar epithelial cells. These gel conditions include: concentration of

polyacrilamide gel components to provide optimal gels stiffness; concentration of fluorescent beads to optimally compute gel deformation; and suitable gel coating to enable cell attachment.

2.2. To determine the gel elastic properties (Young's modulus) by atomic force microscopy.

2.3. To measure the time-course of the contractile response to thrombin challenge.

2.4. To study the distribution of contractile forces exerted by adhered cells before and after thrombin stimulation.

2.5. To measure actin polymerization and reorganization induced by thrombin challenge.

2.6. To study the role of the actin cytoskeleton in the contractile response to thrombin by pre-treatments with cytochalasin D.

2.7. To study the role of pathways signalling MLC phosphorilation in the contractile response to thrombin by pre-treatments with ML7 and Y-27632.

3. To study the contractile response of human alveolar epithelial cells subjected to stretch.

3.1. To determine a suitable gel substrate that firmly attaches to a flexible membrane, allowing biaxial stretch application (max ~15%) and cell culture.

3.2. To determine the gel elastic properties (Young's modulus) of the gel at different strain levels by atomic force microscopy.

3.3. To implement and validate a stretching device to apply controlled biaxial and uniform strains to cultured cells and simultaneously measure contractile forces by deforming the gel substrate to which they are adhered.

3.4. To adapt the existing traction microscopy algorithms and software to allow computation of large bead displacements (~20 μm) and corresponding stretch fields.

3.5. To measure contractile forces exerted by human alveolar epithelial cells before, during and after being subjected to a stepwise deformation of up to 11.5% linear strain.

3.6. To assess the role of actin polymerization in the contractile response to stretch.

3.7. To assess the role of actomyosin crossbridges attachment or detachment in the contractile response to stretch.

3.8. To assess temporal changes in cell contractility after stretch release.