Combining optical tweezing and confocal microscopy for the study of cell mechanics

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Abstract We have developed a system combining an optical tweezers and a confocal microscope for the study of cell mechanics. The system enables us to measure how mechanical forces are distributed within cells. This provides an important insight into the mechanisms by which how cells sense and respond to mechanical forces.

It is well established that cells alter their biological function in response to mechanical loads. In fact, mechanical loads regulate the health of tissues such as cartilage, muscle and bone [1], and have been shown to influence the development of cancerous tumors [2] and the differentiation of stem cells [3]. Understanding how cells sense and transduce mechanical forces requires a full description of their mechanical properties.

Rhe viscoelastic properties of cells are primarily controlled by the cytoskeleton [6] that is an intricate network of protein filaments spanning the cytoplasm. It is well established that the cytoskeleton is a key component of mechanotransduction pathways. However, a full description of its role requires a thorough understanding of the relationship between cytoskeletal structures and the mechanical properties of cells.

A variety of tools have been used to measure the mechanical properties of cells. These tools include micropipette aspiration, compression chambers, magnetic cytometers and optical tweezers. Generally, the deformation of the entire cell in response to a known load is measured, and the bulk viscoelastic properties of the cell are derived. The limitation of measuring the bulk deformation of a cell is that it does not resolve the mechanical behavior on a subcellular level. Therefore it cannot provide the detailed information about how loads are distributed within cells, and how this distribution might relate to the underlying cellular structures.

In recent years, two techniques that can resolve mechanical properties on a subcellular level have emerged. These techniques can be divided into two categories (1) passive microrheology and (2) active microrheology. In passive microrheology, tracer particles are embedded into the cell, and their Brownian motion is tracked using either a laser beam and a quadrant detector, or fluorescence video microscopy and a multiple particle tracking algorithm. Because the Brownian motion of a bead reflects

the viscoealastic properties of its immediate surroundings, this technique can be used to resolve cytoplasmic mechanics on a highly localized scale. In active microrheology, organelles such as mitochonria are fluorescently labeled, and their displacements in response to mechanical loads are measured using a pattern-matching algorithm [4]. This information is then used to calculate intracellular strains, and estimate how mechanical forces are distributed throughout the cytoplasm.

We have recently developed a system combining a fluorescent microscope with optical tweezers. The system provides an extremely flexible platform for the study of cells as it enables both active and passive microrheology measurements. In particular this system can be used to measure the deformation of specific cytoskeletal networks (eg. actin) in response to well-defined loads. The optical tweezers are used to pull on microspheres attached to cells expressing YFP-tagged proteins, and the resulting deformation is imaged with the confocal microscope. Digital image correlation is then used to map the displacements and strains that occur within the tagged protein network, thereby providing new information on the connection between cytoskeletal structures and the mechanical properties of the cell. In the future, the confocal nature of the microscope will also be utilized to perform fluorescence correlation spectroscopy (FCS) [5], and test how protein mobility is altered in response to mechanical loads. This system will provide an new insight into how cells respond to mechanical loads, and contribute to a better understanding of pathologies such as osteoarthritis, osteoporosis and cancer.

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4. References

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