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Real-time and non-invasive measurements of cell mechanical behaviour with optical coherence phase microscopy

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ABSTRACT

Cell mechanical behaviour is increasingly recognised as a central biophysical parameter in cancer and stem cell research, and methods of investigating their mechanical behaviour are therefore needed.

We have developed a novel qualitative method based on quantitative phase imaging which is capable of investigating cell mechanical behaviour in real-time at cellular resolution using optical coherence phase microscopy (OCPM), and stimulating the cells non-invasively using hydrostatic pressure. The method was exemplified to distinguish between cells with distinct mechanical properties, and transient change induced by Cytochalasin D.

We showed the potential of quantitative phase imaging to detect nanoscale intracellular displacement induced by varying hydrostatic pressure in microfluidic channels, reflecting cell mechanical behaviour. Further physical modelling is required to yield quantitative mechanical properties.

1. Introduction

There are more than fifteen cancer deaths per minute globally [1], with over 90% of cancer deaths caused by metastasis [2]. Metastasis is known to alter the mechanical behaviour of cells from the nanoscopic to macroscopic scales [3], with metastatic potential increasing as cell stiffness decreases [4–7], and nanoscale features of synthetic surfaces have been shown to influence cell behaviour [8].

Similarly, stem cells are vitally important in regenerative and therapeutic medicine due to their self-renewal and differentiation abilities. Mechanical stimuli have been shown to have a major role in regulating stem cell behaviour, with differentiation controlled by the stiffness of the substrate where stem cells attach, through a mechanosensitive process [9]. Therefore, there is a clear need to investigate the mechanical behaviour of cancer cells and stem cells as well as their response to various mechanical stimuli.

Clinicians have used manual palpation of suspect tissues as a qualitative diagnostic tool for centuries. It is, however, subjective, and carried out on the macroscopic scale. Non-invasive imaging techniques such as ultrasound and Magnetic Resonance Imaging (MRI) elastography have translated to the clinic [10,11], however both lack the spatial resolution to be used on the cellular scale. The measurement of mechanical behaviour on the nano- and microscopic scale has used techniques such as atomic force microscopy (AFM), optical tweezers, and optical coherence elastography (OCE) [12,13]. These do, however, suffer from drawbacks for single cell characterisation in that they use contact loading or are unable to assess cellular mechanics in a 3D microenvironment. Still, AFM is one of the most common techniques currently available to assess cell mechanics [14–16]. It uses a cantilever and tip to determine quantitative cell mechanical properties, achieving high resolution and mechanical sensitivity, but is inherently invasive, and as a surface-based technique it cannot investigate intracellular mechanical properties or when cells are cultured in a 3D environment.

Consequently, optical techniques to investigates cell mechanics are rapidly emerging and are reviewed in [13]. Optical coherence tomography (OCE) maps the mechanical properties of tissue by detecting the depth-resolved deformation produced as a result of compression [13,20–22]. It is an extension of OCT, a low-coherence interferometry based imaging technique which uses the optical scattering properties of a sample in a manner analogous to ultrasound to create either a 2-D or 3-D image which shows structural features at the micrometer scale [17–19]. OCE is comparable to palpation in that a force is applied to the sample under investigation and the resulting displacement tracked [3]. To date,

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OCE systems typically achieve a depth of focus of 0.5–3 mm and A-scan rate greater than 20 kHz [13,22].

Combining OCT with high transverse resolution confocal microscopy results in optical coherence microscopy (OCM), achieving sub-micron resolution imaging with high dynamic range and sensitivity, allowing for 3D cellular imaging. OCM further extends to optical coherence phase microscopy (OCPM), a quantitative phase imaging method, to measure the phase changes and cross-sectional depth information from a sample. As such OCPM is part of a range of label-free optical microscopy techniques known as Quantitative phase imaging (QPI) [23,24] which uses the phase contrast of a sample to improve upon intrinsic contrast imaging. The shift in optical path length (OPL) created by the sample is measured quantitatively at the nanometre scale. It is a powerful label-free tool which has been used to investigate the biophysics of red blood cells [25,26], cell growth [27], and track microbial motility [28].

OCPM is sensitive to sub-micrometer changes in OPL, and achieves high spatial resolution. It is therefore an ideal candidate for monitoring displacements. OCPM has been used to characterise nanoscale cellular dynamics in live cells [29], and has been shown to measure cell viability based on intracellular optical fluctuations [30,31].

In this study, we aimed to propose a method for the contact-less assessment of cell mechanical behaviour in vitro that will allow further longitudinal studies without damaging the cells or compromising cell culture sterility. Therefore, we described a novel method based on a standard commercial OCT that can measure the relative cell mechanical response to hydrostatic pressure non-invasively and in real-time. This method will be easily translatable to any spectral domain OCT and with some modifications to most of the QPI methods.

2. Materials and methods

2.1. Cell culture

Breast cancer cells (MCF-7) and mouse fibroblasts (3T3) were used in this study to provide two lineages with distinct mechanical properties. Both were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% foetal bovine serum, 1% L-glutamine and 1% Penicillin-Streptomycin. Cells were incubated at 37 °C and 5% CO_2 and were passaged every 3 days. Cells were dissociated using trypsin-EDTA and transferred to microfluidic channels (microslide IV, Ibidi) 24 h prior to experimentation. We used adherent cells lines that attached to the bottom substrate of the channels.

2.2. Hydrostatic force

In this study, we modulated the hydrostatic pressure in microfluidic channels to induce a hydrostatic force on adherent cells attaching at bottom surface of the channels to produce a non-contact force similar to previous work [32]. In this work, we used controlled cyclic square wave pressure, instead of a pressure column. We generated a change in hydrostatic pressure in the microfluidics channels by altering the air pressure in a fluid container (falcon, 50mL), connected through a Tygon (Saint-Gobain, France) tube to microfluidic channels (microslide IV, Ibidi).

In first approximation, we can consider the cells as half-spheres attached to an incompressible solid substrate (see Fig. 1). Pressure and force are transmitted equally to all directions, and on Fig. 1 we will have equal forces on the right and left side of the cell, with no net horizontal hydrostatic component. Whereas there will be a net vertical force, F_v, at the top of the cell proportional to the projected area. For a cell of radius r, we have:

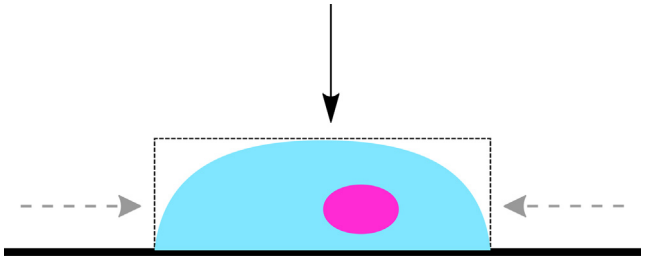

Fig. 1. Resulting hydrostatic force induced on adherent cells above a substrate.

$$F_v = (\Delta P + \rho g h)(\pi r^2) + \rho g V_w \quad (1)$$

where ΔP is the applied pressure change above the atmospheric pressure, ρ the water density (1000 kg m^{-3}), h the height of the water column, and g the gravitational acceleration (9.81 m s^{-2}). V_w is the volume of water on top of the cell starting from the cell top. It can be written as the difference between the cell volume and the volume of an imaginary rectangular box surrounding the cell.

$$V_w = 8r^3 - (4/6)\pi r^2 \quad (2)$$

Typically for a cell of radius 20 μm and ΔP = 1000 N m^{-2} (10 mbar) we calculated a net vertical force of 1.2 μN. We would like to point out that this framework approximated the cross-sectional area as related to cell diameter whereas cells contact the substrate through focal point. This could lead to acting net vertical force an order of magnitude lower, i.e. in the nano-Newton range. Nonetheless, this showed that an actual force is exerted on the cells due to both the hydrostatic pressure and the presence of an underlying substrate.

In this study, MCF-7 and 3T3 cells were exposed to cyclic mechanical stimuli in the form of square wave hydrostatic pressure from a microfluidic pressure pump (AF1, Elveflow, France), inside a microfluidic channel with pressure ranging from 1000 N m^{-2} to 20,000 N m^{-2} with frequencies ranging from 80 to 300 mHz. It was ensured that no air bubbles were present in the sample medium by allowing a flow of media through the microchannel before sealing with a Luer lock plug (Elveflow, France).

2.3. Optical coherence phase microscopy

2.3.1. Experimental setup

The OCPM system was based around a commercial Thorlabs Callisto optical coherence tomography (OCT) system, as shown in Fig. 2. The superluminescent light source was centred at 930 nm with a full width half maximum (FWHM) of 90 nm, with an axial resolution of 5 μm in water. The scanning rate is 1.2 KHz; which was an order of magnitude lower than state of the art OCT used for OCE. The light source was output to a FC/APC fibre, which is the guided with an F280APC-B collimating lens (Thorlabs, NJ, USA). The light path is then directed by galvanometers which control the image acquisition, and finally is coupled into the side port of a Leica DMIRE2 microscope. The system is built in a common path configuration to improve the phase stability [33]. Using a beamsplitter (Thorlabs, NJ, USA), the brightfield image of the sample was collected digitally using a CMOS camera (Thorlabs, NJ, USA). A full list of components can be found in Section 5.

The acquired spectra were then processed as described in Fig. 3. First, the average background was removed, then the signal is resampled in k-space. The modulation of the spectra, collected at a spatial location x_i, y_i, encodes the in-depth location (z_i) of the scattering particles, which are retrieved by zero-padding of the signal and fast Fourier transform. This forms the A-scan at