An Imaged-Based Methodology to Quantify Ultrasonic Cell Deformation

M.C. Ballard[1a], A. Marek[1] and F. Pierron[1]

[1] Faculty of Engineering and Physical Sciences, University of Southampton,

Southampton, UK

[a]mb2e20@soton.ac.uk

Abstract. We present a new method that enables controllable loading of cells and quantification of their response to ultrasonic vibrations. This involves seeding cells on a substrate resonating at ultrasonic frequencies which transfers the deformation to the cells. The setup is then incorporated into microscopic imaging techniques to obtain high-speed images of ultrasonic cell deformation that can be analysed using digital image correlation (DIC). The images obtained using this method could aid in understanding the effects of ultrasonic stimulation on cells and provide a deeper insight into cell mechanics.

Introduction

The importance of mechanical forces in cell biology has only been recently realised and is a vast area of research [1]. Thus, there is motivation to understand cell deformation and mechanobiological responses [2]. However, the ability to controllably deform cells in the ultrasonic regime and test their response is a noted challenge throughout the literature. Quantifying and eliciting an appropriate stimulus has proven difficult, resulting in methods that are either too aggressive or oversimplified [3]. Here, we present a method based on the Image-based Ultrasonic Shaking (IBUS) test lined out by Seghir and Pierron [4]. It utilises ultra-high-speed imaging to quantify ultrasonic loading and was previously used for determining material properties at high strain rates. Practically, this involves exciting a sample with a sonotrode and caputuring the vibrations with ultra-high-speed and thermal cameras. For our work, this method has been adapted to elicit ultrasonic cell stimulation and obtain high-speed images of the resultant deformation. These images can be analysed using DIC to quantify the cell deformation and combined with data from biological analysis to understand the mechanobiological implications of ultrasonic stimulation.

Methods

The development of this new method can be split into three main components: design of a custom cell containment device, device calibration using the original IBUS test, and the translation of this test to be under microscopic conditions.

First, a custom device consisting of a PMMA coupon and a PDMS well was developed to keep cells submerged in culture media during the test to maintain their health. The length of the PMMA coupon used in this device was specifically tuned to encompass the first mode of longitudinal vibration at 20 kHz ultrasonic excitation, creating a central node of maximum strain. Then, the device was calibrated using the IBUS test to determine the strain and displacement profile of the coupon during excitation at different sonotrode powers, as well as the associated temperature increases. To do this, the device was glued to a 20 kHz sonotrode and its vibrations were imaged with ultra-high-speed and thermal cameras. For the final stage, the device was seeded with cells and glued to the sonotrode, before being placed on a series of translation stages and raised to an appropriate height for imaging. An ultra-high-speed camera was connected to the optical microscope, imaging was realized by means of a phase contrast objective and illumination was provided by a pulsed laser. Fig. 1 Shows images

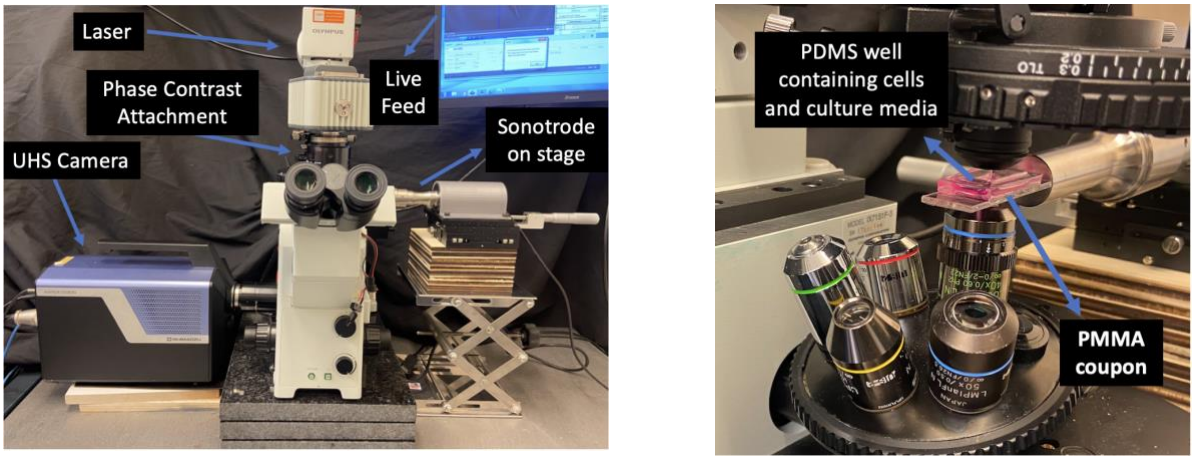

Fig. 1 Left: An image of the experimental setup for capturing images of ultrasonic cell deformation; Right: A close up image of the custom cell containment device under the microscope

of the experimental setup, where high-speed Images of ultrasonic cell deformation were captured at 500,000 frames per second. *In situ* displacement analysis was also undertaken to confirm cell attachment to the substrate through comparison of cell displacement with a substrate scratch.

Results

The custom cell containment device developed for this method was found to be effective in maintaining cell health throughout the test. The calibration showed that strains of up to 1.5 millistrain (mm/m) were achievable using the test and provided an insight into the accompanying temperature increase. Cells were confirmed to be attached to and deforming with the substrate, as shown by in phase displacement of two different cells with a substrate scratch in Fig. 2. High temporal resolution images of ultrasonic cell deformation were achieved,

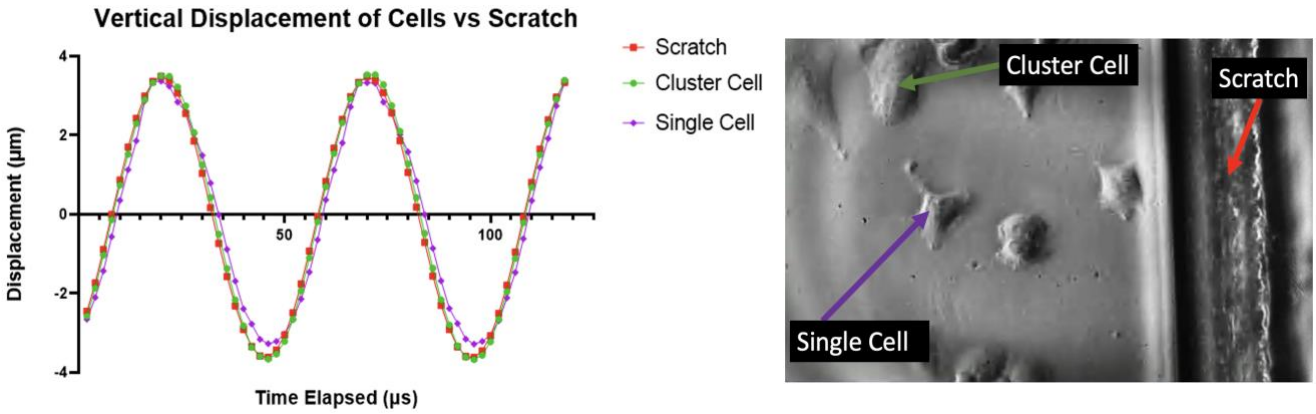

Fig. 2: Displacement of two cells compared with substrate scratch over time to validate cell attachment

examples of which can be seen in Fig. 3. Some internal cell details could also be seen such as nuclei, which also seem to produce a phase lag in the deformation compared with the rest of the cell.

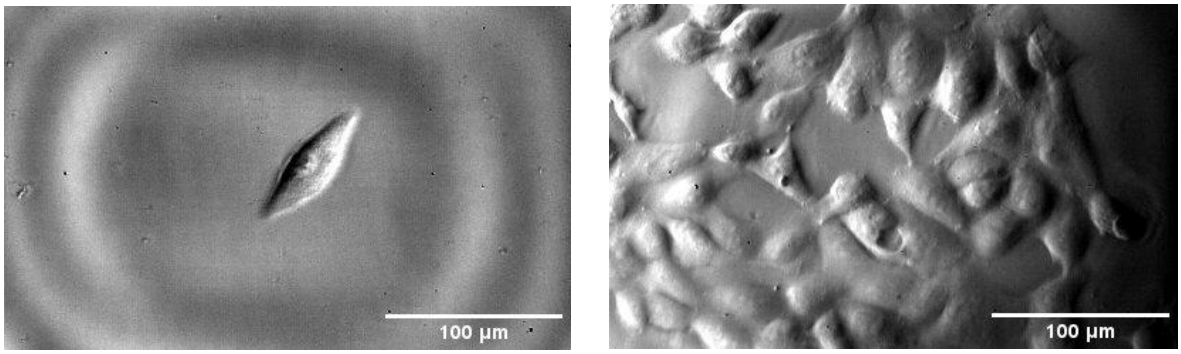

Fig. 3: Static images extracted from videos of cell deformation obtained using the new method

Conclusions

This novel method was found to be effective in capturing high temporal resolution images of ultrasonic cell deformation. Future work involves undertaking DIC analysis to quantify the deformation in both single and groups of cells, and the biological response to this deformation will also be investigated. The images obtained using this method could also be used to inform a mechanical model of cell and aid in filling the gap in knowledge regarding the mechanical properties of tissues at a cellular level.

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