An Ultra-high-speed Imaging Study of Cell Deformation and Mechanobiology from Ultrasonic Stimulation

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Abstract. Here we present a method that enables real time ultra-high-speed imaging of cell deformation during ultrasonic excitation. Cells are cultured on a calibrated Polymethyl Methacrylate (PMMA) substrate attached to a 20 kHz sonotrode and subjected to clinically relevant strains. This is imaged under a phase contrast microscope using a pulsed laser and ultra-high-speed camera to visualise cell deformation. Assays are then undertaken on the cells to determine any mechanobiological implications of ultrasonic excitation. This gives an insight into ultrasound-mediated mechanobiology of cells and may allow for a deeper understanding of how ultrasonic surgical cutting tools affect tissues.

Introduction

Ultrasonic surgical cutting tools are an emerging technology which are becoming increasing popular in the surgical arena and display several benefits over traditional devices. They utilise ultrasonic vibrations to cut biological tissues and have shown to have enhanced precision and improved healing times [1, 2]. However, the response to these tools at a cellular level is largely unexplored. This is likely due to difficulties in studying the incision site and observing probe-tissue interactions, but also challenges around quantifying the strains cells experience during cutting and applying this to them in a controlled and clinically relevant manner. An insight into cell responses could lead to a deeper understanding of the tool-tissue interactions and help inform device design and implementation. Furthermore, ultrasonic vibrations are known to stimulate therapeutic biological effects via mechanobiological pathways in damaged tissues [3]. With further knowledge and understanding of the effects of ultrasound on cells, its regenerative capabilities could be applied to cutting devices to further facilitate tissue healing post-incision.

Method

In this study, we use an Image-Based Ultrasonic Shaking (IBUS) test to excite cells ultrasonically [4]. This involves culturing cells on a rectangular PMMA strip attached to a 20 kHz sonotrode. The shape of the PMMA is specifically tuned to create a standing wave during ultrasonic excitation resulting in a central maximum strain value. The cells are then illuminated under a microscope with a phase contrast objective using a pulsed laser and imaged using an ultra-high-speed camera whilst being ultrasonically vibrated. This allows us to visualise the cells while they are ultrasonically excited.

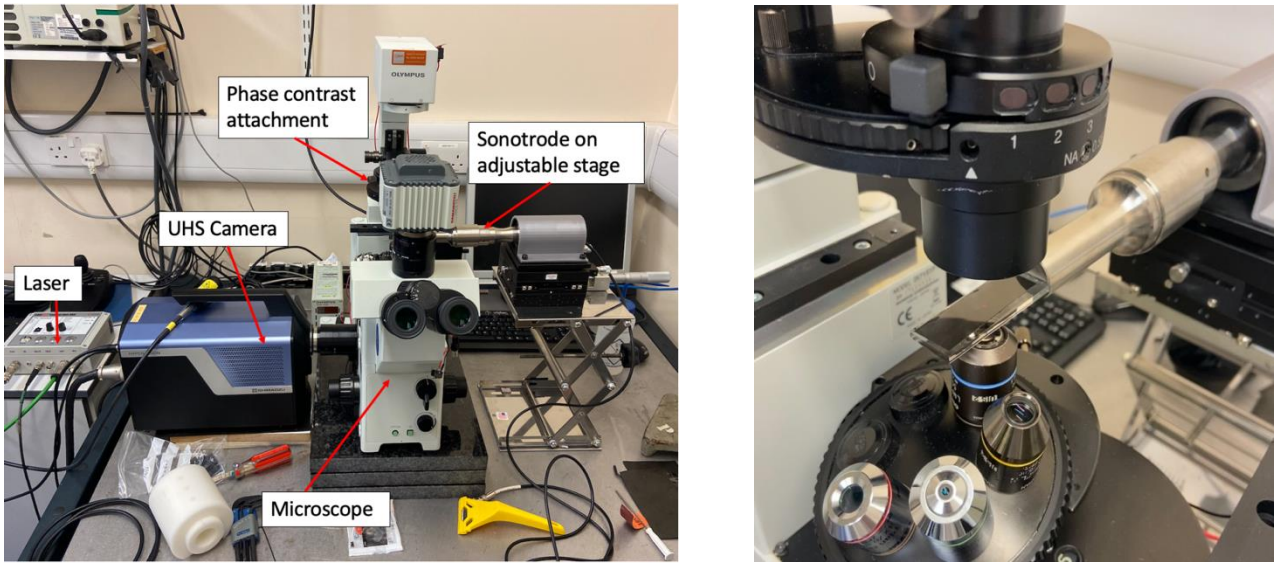

Fig. 1 Left: An image of the experimental setup for high-speed imaging; Right: A close up image of the coupon attached to the sonotrode under the microscope

Results

Preliminary results confirm the tunability of this test and its ability to produce clinically relevant strains. Fig 2. (left) displays the calibration curve of PMMA for three different lengths. The strain amplitude was found to be consistent across all sized coupons for different sonotrode powers, meaning that specific amplitudes up to around 2-3 millistrain can be reliably generated during the test.

Initial images have also been obtained of cells during this test, and we have shown that good contrast can be achieved from our experimental setup. Fig. 2 (right) is an image of cells cultured on the substrate taken with the ultra-high-speed camera.

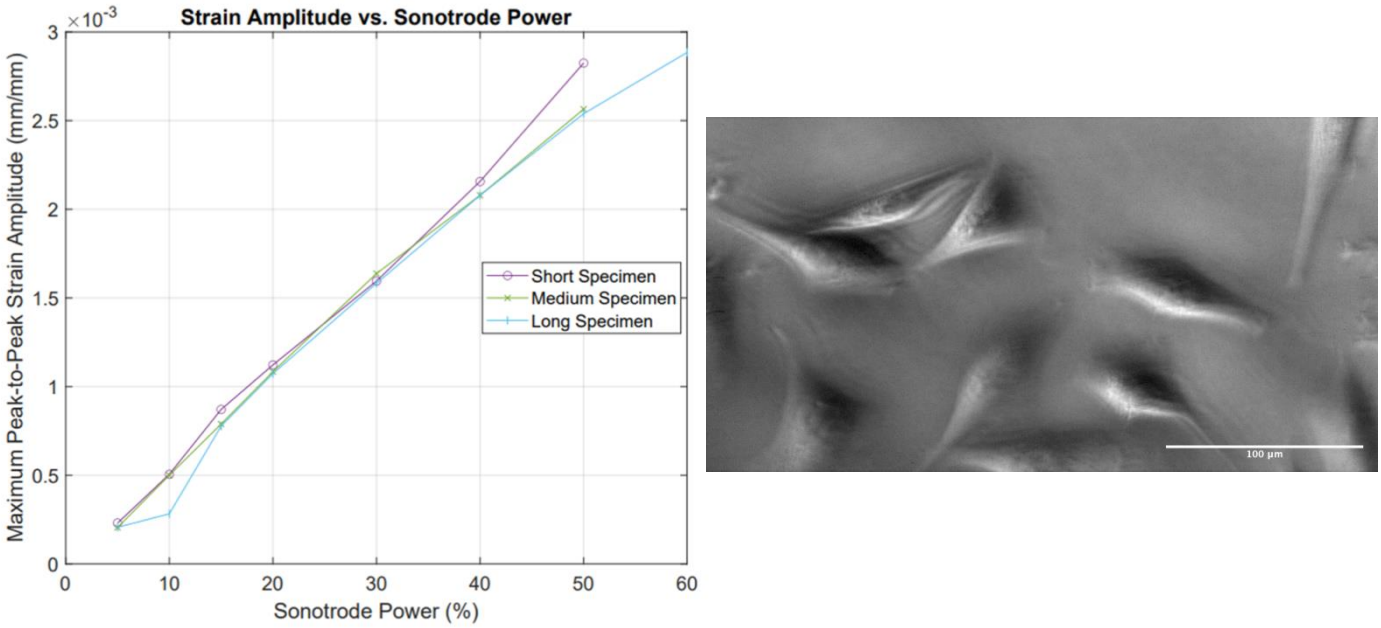

Fig. 2 Left: Graph showing PMMA calibration for different sonotrode powers; Right: Image taken of cells using ultra-high-speed camera at 15 kfps, scale bar represents 100 µm

Conclusion

This test enables the visualisation of cells during ultrasonic excitation and allows us to determine how they deform from clinically relevant strains. Future analysis will involve quantifying cell motion and deformation using digital image correlation (DIC) or other suitable image processing techniques. Results of cell assays can also be combined with this deformation data to relate the ultrasonic stimulation with mechanobiological effects induced to further understand the effect of ultrasonic cutting tools on tissues at a cellular level.

References

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