Lab on a Chip

Supporting Information

A Lab-on-chip Ultrasonic Platform for Real-time and Nondestructive Assessment of Extracellular Matrix Stiffness

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Assessment of bubble disturbances on stiffness measurements

In order to effectively quantify the effect of air bubble formation and its size on the measurements, a control set of experiments were conducted by intentionally introducing air bubbles into the central surface area of the hydrogel inside the culture platform (between the piezoelectric transmitter and receiver transducers). In this test, a hydrogel with the stiffness set to 236.5 kPa (1.5 %w/v agarose gel) was placed inside the platform and different sizes of bubble defects were punched into the surface of the hydrogel, and the relative change in output signal at the receiver was recorded. **Figure S1a** shows a schematic of the test setup utilized for this experiment. The recorded relative change in electrical signal from the piezoelectric receiver showed an overall decrease as the bubble size increases, **Figure S1b**.

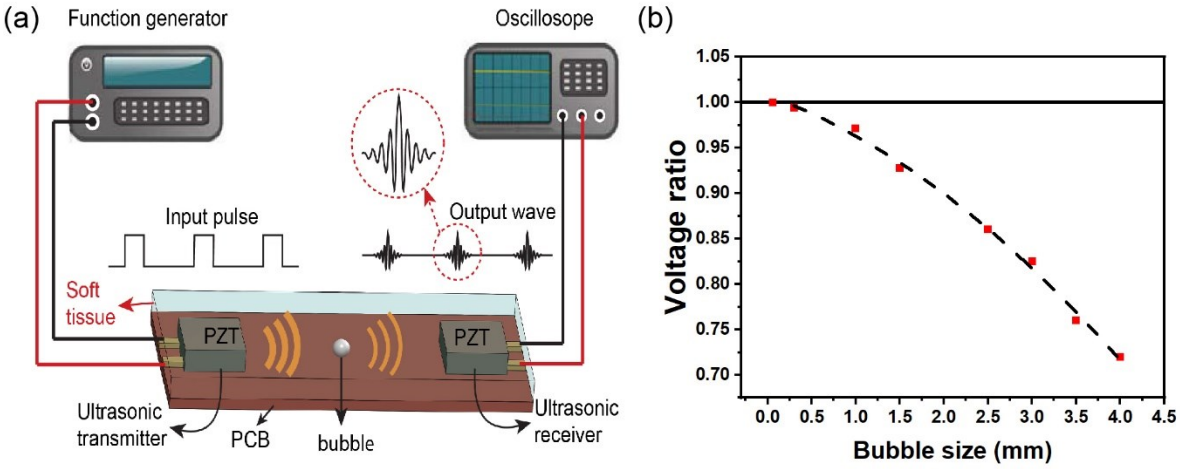

Fig. S1 (a) Schematic illustration of air bubble in the agarose gel casted on the platform, (b) relative change in maximum output voltage at the piezoelectric receiver with different bubble size disturbances in 1.5 %w/v agarose gel.

Ultrasonic Tomography

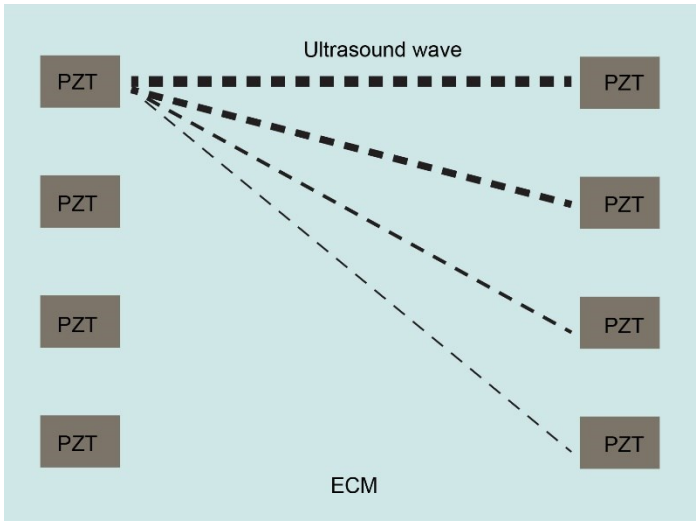

Fig. S2. Schematic illustration of arrays of PZTs for on-chip ultrasonic tomography assessment of heterogenous structures.

In order to be able to ultrasonically characterize heterogenous materials, an array of PZT materials can be used in the exterior circumference inside the cell culture chamber (**Figure S2**). The signals transmitted and received between each of piezoelectric transducers enables reconstruction of spatiotemporal distribution of mechanical stiffness. This modification can provide a new opportunity for future work for on chip stiffness assessment in heterogenous environment and compensating for bubble disturbances inside the hydrogel.

Ultrasonic Stiffness Assessment of Gelatin

In order to assess whether the initial calibration curve holds for commonly used hydrogels, we designed a new set of experiments on gelatin gel with concentration ranging between 0.75 %w/v to 2.5 %w/v. Standard compression test performed on gelatin samples demonstrated a relatively linear increase of 3.62 kPa/%w/v in elastic modulus as the gelatin concertation increased with stiffness range of 34.73 kPa to 99.67 kPa which was within working range of the previously characterized Agarose gel samples, **Figure S3a**. The standard compression test was compared with ultrasonic stiffness measurements that was obtained using the calibration curve made with agarose gel (**Figure 4d**). Results of this comparison revealed a high level of accuracy (>93%) and consistency between the stiffness measured with the ultrasonic on-chip platform and the standard compression test, **Figure S3b**.

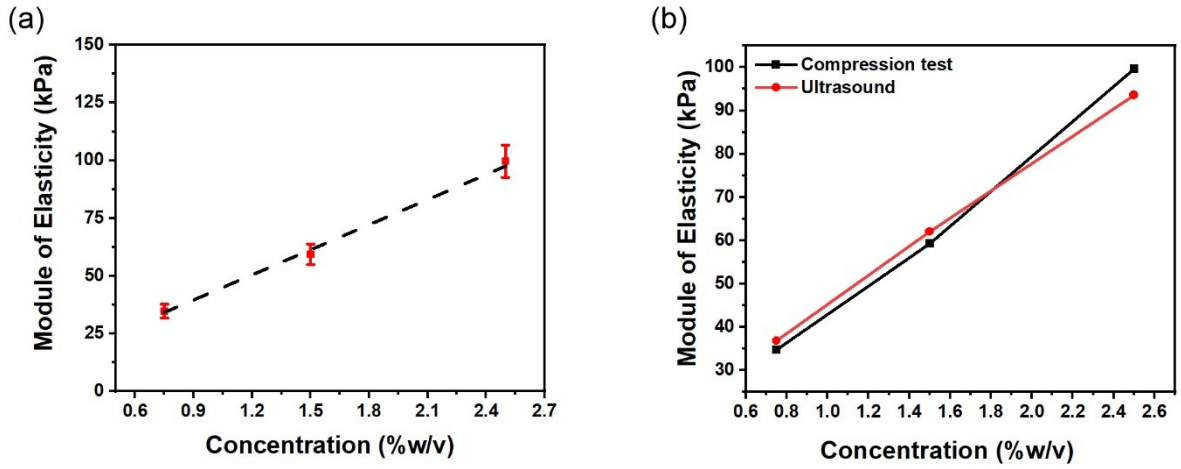

Fig. S3 (a) Stiffness measurement of different concentration of gelatin by compression test, (b) comparison of change in elastic modulus of gelatin using ultrasonic and compression test.

Measurement Accuracy Assessment

Measurement accuracy of the on chip ultrasonic stiffness measurement during the real-time stiffness changing experiment was reported as the maximum error between all the recorded datapoints with using the ultrasound and compression test based on the following equation

$$RE = \frac{|Ultrasound - Compression\ test|}{Compression\ test} \times 100 \qquad (1)$$

The results of this comparison between experimental data revealed relative error of approximately 8%.

Overall the demonstrated approach provides a high level of accuracy with comparable, and in many cases better performance in stiffness calculation of the hydrogel than optical and imaging-based approaches summarized as follows:

1. Inverted microscopy indentation method with a steel ball indentor on 10 AA/%w/v poly-N-isopropylacrylamide (PNIPA): ~ 23.3 kPa showing relative error of 15.92% comparing to tensile test [1].

2. Confocal microscopy indentation method using confocal laser fluorescence microscopy and automated image processing with submillimeter tungsten carbide spherical ball indentor on 10% acrylamide and 0.1% bis-acrylamide polyacrylamide (PAAM) gel: 13.17 kPa [2] showing relative error of 24.12% comparing to AFM [3].

3. Optical coherence tomography on 2% agarose: ~ 43 kPa [4] showing relative error of ~14% comparing to depth-sensing micro-indentation.

4. Atomic force microscopy on polyacrylamide gel with 10% acrylamide and 0.1% bis-acrylamide: ~13.5 kPa [5] showing relative error of ~ 32.5% comparing to rheometric method.

References

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