SUPPORTING INFORMATION

Effects of physiologic mechanical stimulation on embryonic chick cardiomyocytes using a microfluidic cardiac cell culture model.

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ABSTRACT

The stress, strain, membrane deflection, and Young's modulus of the PDMS membrane of the cardiac cell culture model was calculated from experimental data for membrane thicknesses of 250 µm, 400 µm, and 500 µm. These results demonstrated that the cardiomyocytes experienced physiologic levels of stretch and circumferential and radial strains during the mechanical stimulation.

METHODS

Tensile test for Young's modulus of the PDMS membrane

The fabrication of thin PDMS membrane was accomplished using standard soft lithography techniques.[1,2] This thin cured PDMS sheet was then cut into strips (0.5 inch x 4.0 inches) and vertically mounted to the fixtures of the RSA III Rheumatics System Analyzer. Zero force was initialized. The membrane was pulled slowly with the rate of 0.5 mm/s until it reached a 50% displacement (2 inches). The stress, strain, and Young modulus of the PDMS were calculated for three different thicknesses (250 µm, 400 µm, and 500 µm).

Experimental quantification of membrane deflection:

A Finite Element Analysis (FEA) was used to estimate the membrane deflection and strain.[1] Additionally, Visual Sonics Imaging System (VisualSonics Inc, a subsidiary of Fuji-FILM, Toronto, Canada) with a MS-400 probe (30MHz) was used to examine the displacement of the membrane inside the CCCM set-up system (Fig. S1). 500 µm thick PDMS membranes were used. The CCCM was operated with a flow rate of 44µl/cycle, peak cyclic pressure of 10mmHg, and a frequency of 2 Hz with 40% diastolic fraction. Further, the deflecting movement of the PDMS membrane was recorded for ejection volumes of 25, 50, and 75 µl to determine the membrane deflection and the strain values.

RESULTS

Young's modulus of the PDMS membrane

The stress-strain relationship of the PDMS membrane were similar for PDMS thicknesses of 250 µm - 500 µm (Fig. S2) and yielded a Young's modulus of approximately 1.0 MPa. These results were in agreement with values published in literature.[3-5]

Membrane Deflection and Strain Estimation

Chick embryonic left ventricle cardiomyocytes were used in the CCCM and thus, the system was characterized based on the strain of the *in-vivo* chick embryonic left ventricle cardiomyocytes on the Hamburger Hamilton stage 31; which is approximately 0.10-0.20. [6,7] For *in-vitro* culture, a strain of 8-15% was applied.

Figure S3 demonstrates the deflection of the membrane based on different fluid volume loaded to the cell culture chamber. To ensure that the cells experienced suitable stretches during the mechanical stimulation, both circumferential and radial strain of the membrane were determined via videos of the experimental membrane deflection obtained from the Ultra Sound system (Fig. 4). For each run, the deflecting movement of the PDMS membrane was converted to single moving slides (30 slides/sec). Referent points from the base line and the displacement points along the deflected membranes were created evenly along the membrane diameter using m-files written in Matlab (MathWorks, Natick, MA). The deflection profile was then created to calculate the strains.[5] The strains obtained in this real set-up CCCM system for chick embryonic culturing are shown in Figure S4.

FIGURE LEGENDS:

Figure S1: Images of experimental set up for making movies of the PDMS membrane deflections using high frequency ultrasound. [A] Image of the Vevo2100 VisualSonics high frequency ultrasound system used for the PDMS strain measurement. [B] Experimental set-up for the strain measurement with [1] probe and [2] CCCM platform where the CCCM culture chamber was installed.

Figure S2: Graphs of stress versus strain of thin PDMS sheets. [A] Plot of engineering stress versus engineering strain of the thin PDMS sheets. [B] Plot of the true stress versus true strain of the thin PDMS sheet; this plot was used to determine the Young's Modulus of the PDMS membrane. N= 5

Figure S3: Images of PDMS membrane deflections and the graph of the strain at 0, 25, 50 and 75 µl fluid loaded to the cell chamber along with the amount of fluid loaded in the real set-up experiement for chick embryonic ventricle myocyte experiment. From these images, the level of membrane stretch from the real chick embryonic culturing experiment is located between 25µl and 50µl injections.

Figure S4: Graphs of circumferential strain [A] and radial strain [B] of the 500µm PDMS membrane in the real set-up CCCM system. The center lines of both graphs were the strain values used for the chick embryonic ventricular cardiomyocyte culturing experiment. The bottom

and the top line were the strain values of the PDMS membrane obtained from manual fluid injection (25µl and 50µl). N = 3.

Figure S1A:

Figure S1B:

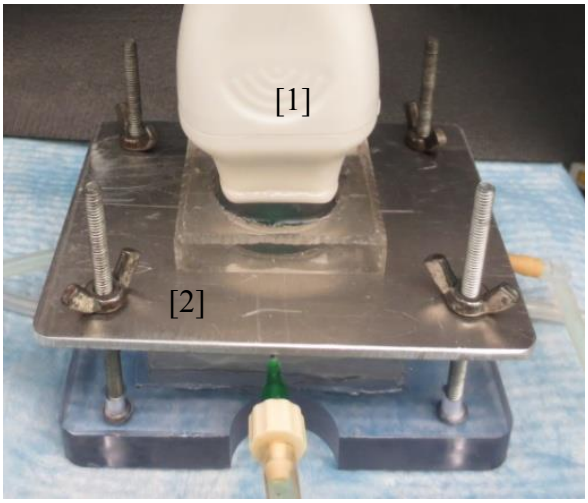


Figure S2A:

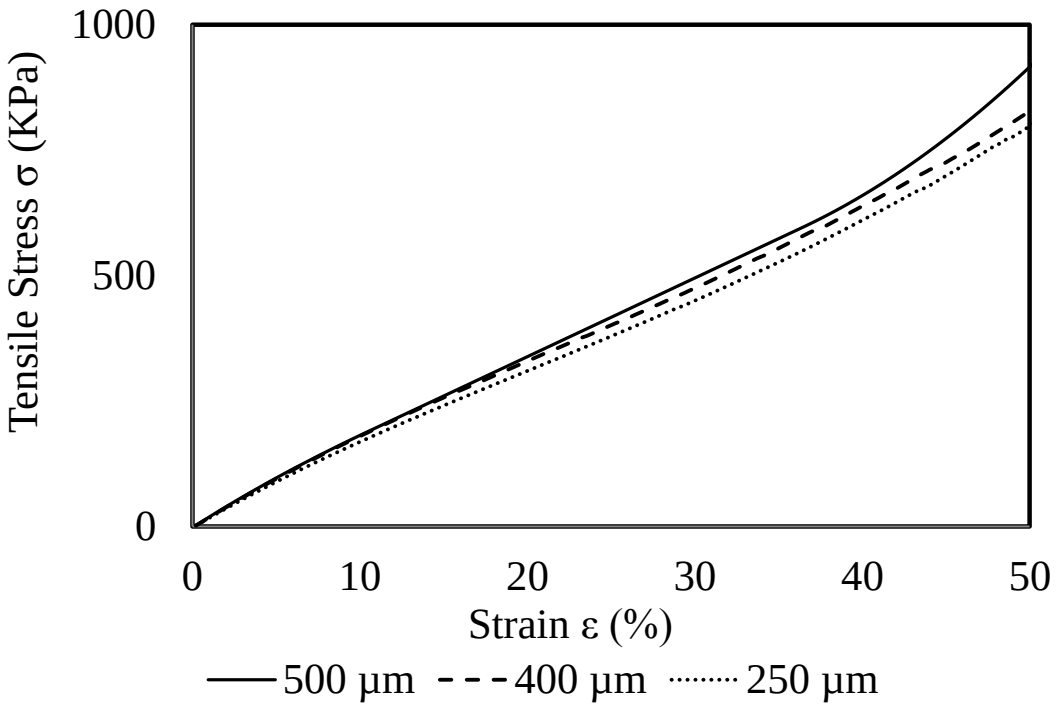


Figure S2B

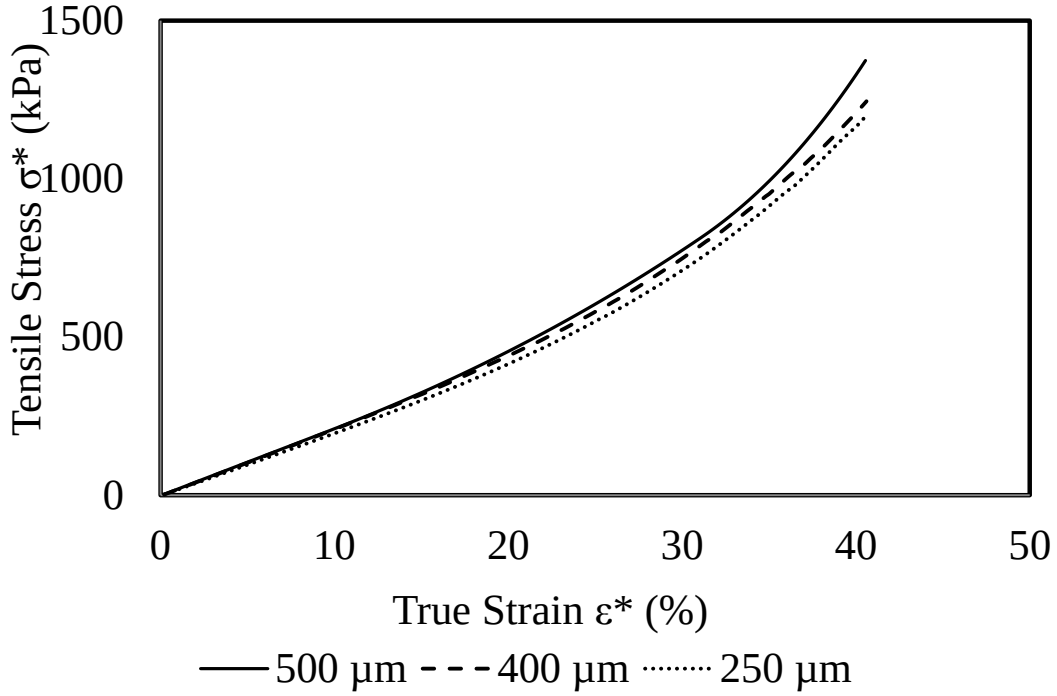


Figure S3:

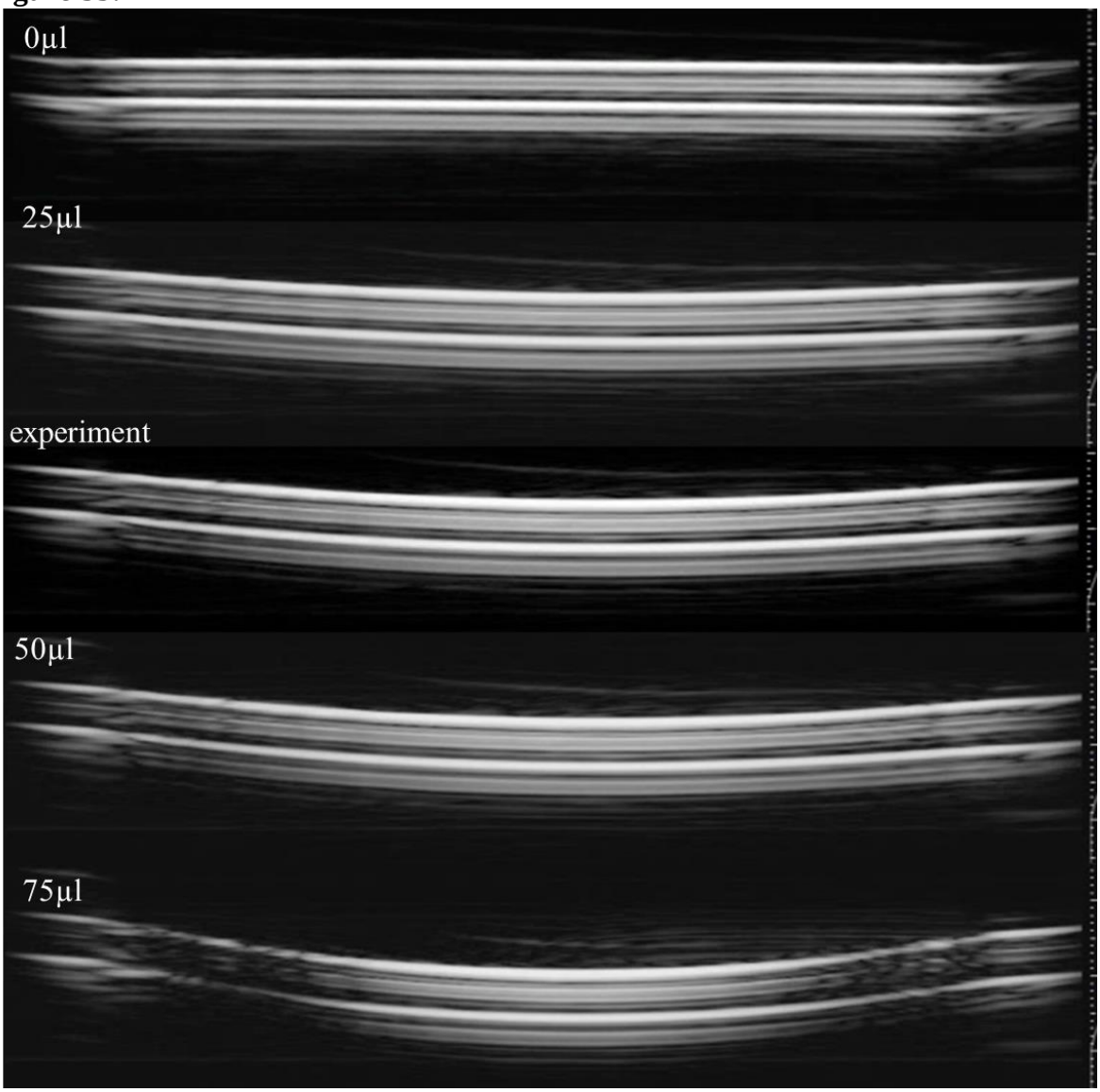


Figure S4A:

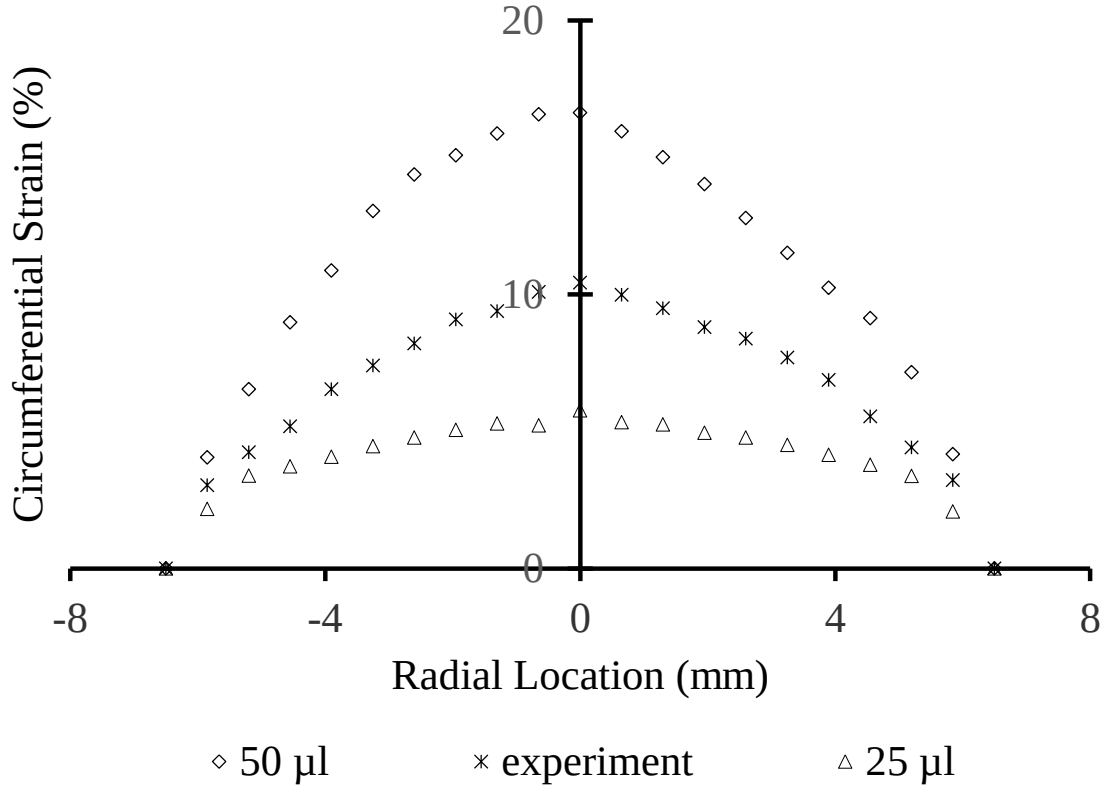
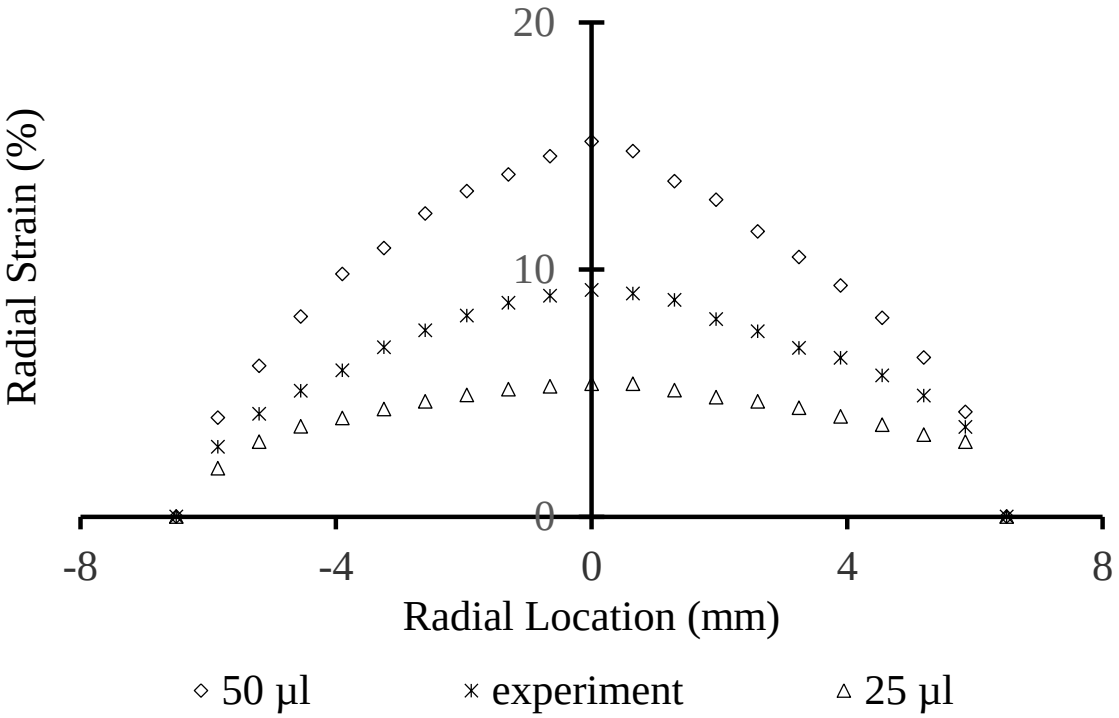


Figure S4B:



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