

Mechanically Active Microfluidic Device to Mimic Tensile Hoop-Strains in the Annulus Fibrosus

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Introduction: Mechanically active microfluidic devices or organ-chips are used to study disease progression and organ function in skin, lungs, and articular cartilage¹⁻⁴. Organ-chips with more complex mechanical activity, such as hoop tensile strains, are needed to study mechanobiology of the annulus fibrosus. In this study, we developed an easily controlled, mechanically actuated organ-chip that can apply low (5%) and high (10%) tensile hoop-strains, representing intradiscal strains observed during compression and bending of the disc joint.⁵⁻⁶

Materials and Methods: Devices were manufactured by bonding two sheets of poly-di-methyl-siloxane (PDMS) (25 x 45 mm) to enclose a chamber with a rectangular cross section (300 x 40 x 1500 μm)⁷. Strain applied to the chamber was controlled by altering the location of the chamber within the device and with respect to a barrel-like structure by varying the thickness of the two sheets while maintaining an overall thickness of 4 mm ($t_1 + t_2 = 4$ mm, Fig. 1A). Therefore, tensile hoop-strains (ϵ_x) were increased by positioning the chamber further from the center of the barrel (greater t_1 , Fig. 1A - black circle). Strains were applied by deforming the device around a barrel ($R = 10.6$ mm radius; Fig. 1A & 1B). Two sets of devices were manufactured to apply low (5%) or high-tensile (10%) strain ($n = 5$ per group). Tensile strain across the chamber was predicted using beam theory with an ideal geometrical model as a function of the total device thickness ($T = t_1 + t_2$), the radius of curvature (R), and the distance (Y) between the chamber and the midway thickness ($T / 2$) called the neutral axis of the device (Fig. 1A)⁸. Experimentally, strain was measured by imaging the chamber before and after bending with a 20X objective lens on an Olympus CKX31 microscope. Strains along the x-axis were calculated by tracking movement of the chamber walls (MATLAB, MathWorks Inc.). Thirty sequential measurements were acquired and tests were performed in triplicate (90 data points to assess repeatability). Between each test, the device was uninstalled and reinstalled to mimic laboratory use. Additional testing for extended use was performed (100 cycles).

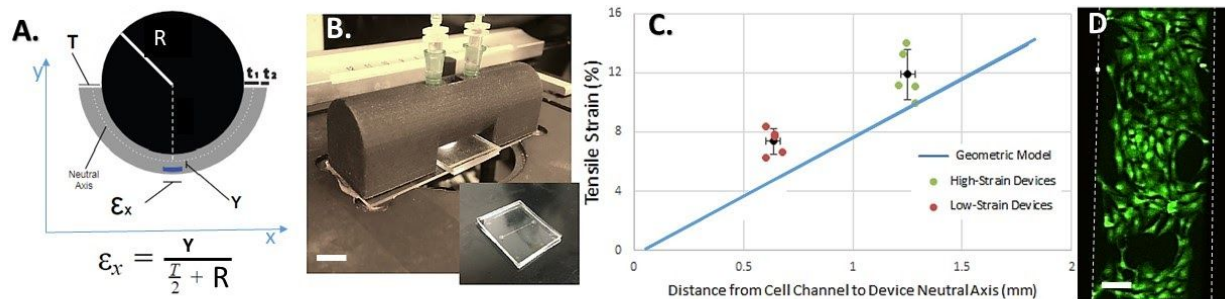


Fig. 1: (A) Beam theory, or geometric model, used to predict applied strains along the x-axis. (B) PDMS device shown beneath barrel-like structure (unloaded). Bar = 10mm. (C) Measured tensile strains (dots) compared to model prediction (blue line). Bars represent standard deviation. (D) Fibroblast viability after 7 days in culture. Bar = 50 μm .

Results and Discussion: Measured strains were $7.38\% \pm 1.69\%$ for the low-strain group and $11.90\% \pm 0.88\%$ for the high-strain group. These strains were greater than the predictive model by 2.65% and 2.30%, respectively (absolute values; Fig. 1C). The standard error ranging between ~10-20% was just above that of a comparable device, yet the range of strains applied still fell within the range reported for native disc tissues⁹. Drift in strain values over 100 cycles was negligible ($\Delta \epsilon_x = -0.1\%$). Ongoing work includes both device fatigue analysis during multi-week cellular studies and radial compressive strain measurements due to Poisson's effect. Lastly, live-dead analysis demonstrated that bovine fibroblasts were viable after 7-days in culture (Live/Dead ThermoFisher, Waltham, MA; Fig. 1D). Therefore, future work will evaluate changes in gene expression with tensile strains.

Conclusion: Initial testing of an organ-chip that can apply tensile hoop-strains provides promising data for developing a platform to evaluate annulus fibrosus cell mechanobiology.

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