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DEVELOPMENT OF A DEFORMABLE MICROFLUIDIC CHIP TO REPLICATE TISSUE STRAINS IN SITU

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INTRODUCTION

Cells respond to mechanical and biochemical stimuli to maintain homeostasis; changes in the magnitude or type of stimuli can lead to various diseases. For example, the heart and lung tissue stiffen over time potentially leading to cardiovascular disease and lung fibrosis, respectively [1,2]. In this study, we focus on back and spine pathologies that affect ~8 million people in the United States and cost over $100 Billion annually [3,4]. The most identifiable and widely studied source of back pain is due to degeneration or failure of the intervertebral disc [5,6]. Disc degeneration, stemming from disruption of the annulus fibrosus (AF), leads to increased pain and reduced mobility [7,8]. To enable regenerative medicine strategies, a better understanding of AF cell mechanobiology during tissue development is needed. A method is required to quantitatively and reliably replicate intradisc loading to test potential disease mitigators at the cellular and nascent tissue levels. Advancements in microfluidics have enabled micromechanical testing of small cohorts of cells [9,10]. However, a chip that emulates more physiological strains observed in the AF is needed. Therefore, we present a deformable chip that emulates the AF's cyclical and multiaxial strains. We demonstrate a method to evaluate the effects of these strains on the initiation and propagation of AF tissue degradation. We bolster development of the chip by using the Finite Element Method (FEM) to validate lab measurements and examine strains within a cell monolayer.

METHODS

We designed a deformable microfluidic chip using principals of beam mechanics to target clinically relevant, multiaxial loads in the AF. Our previous work created a polydimethylsiloxane (PDMS) chip that enclosed a single microchannel (Figure 1A) [11]. The chip was deformed over a rigid cylinder (radius = 10.6 mm) to create uniform depth-dependent strains, due to bending. A microscope slide was positioned beneath the chip to help visualize cells throughout loading (Olympus CKX31 microscope; Figure 1B). When the chip is bent around the cylinder, uniform depth-dependent strains, ε, are assumed to follow the mechanics of a beam in pure bending: $\varepsilon = Y/\rho$, where Y is the vertical distance from the Neutral Axis (NA), and ρ is the radius of curvature [12]. We assumed that the NA bisects the chip. Two configurations of the chip were fabricated with differing Y-positions of the microchannel relative to the NA: 5% target strain (Y=0.63mm) and 10% target strain (Y=1.26mm) conditions as measured in the X direction. These represent the intradiscal radial strains observed in the AF during compression and bending [13,14]. We measured strain in the X direction by tracking the 1D displacements of the chip's channel walls using edge detection in MATLAB (MathWorks, Inc).

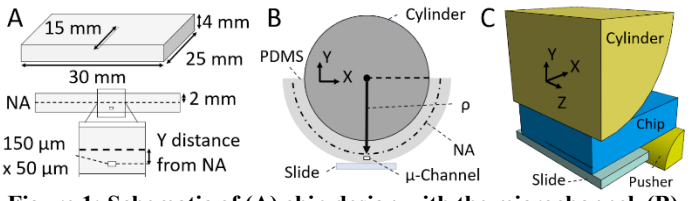

Figure 1: Schematic of (A) chip design with the microchannel, (B) loading setup, and (C) quarter computational model.

A solid model of a quarter symmetrical chip was created in Solidworks (Dassault Systemes) with the channel positioned for three configurations (0%, 5%, and 10% target strain). We imported geometries into GMsh [15] to construct meshes with tetrahedral elements. Then, the meshes were individually imported into Finite Elements for Biomechanics (FEBio) Preview 2.1.1 [16]. We then added the cylinder, the microscope slide, and a "pusher" (Figure 1C). All parts

except the chip used a rigid material model and were meshed using hexahedral elements. The PDMS chip used a Neo-Hookean material model with a Poisson's ratio of 0.45 and a Young's Modulus of 1.7 MPa [17]. We added symmetry boundary conditions and defined frictionless contacts between contacting surfaces. We applied a Y-displacement of 7 mm on the "pusher" to enforce the chip's curvature during the test cycle. The cycle is defined as when the chip starts to bend until it reaches its maximum curvature. We verified the curvature match between the model and the setup by comparing radii of curvature using ImageJ [18] (data not shown). Lab-based strain measurements at the chip's center were compared to beam-theory estimates and model predictions.

For the 10% target configuration, we placed an idealized bovine chondrocyte monolayer, in the shape of a rectangular prism, at the base of the channel to model the strain transfer from the chip to the cells. We filled the channel with a 9 µm tall monolayer and applied a Neo-Hookean material model with a Young's Modulus of 14 kPa and a Poisson's ratio of 0.34 [19]. We added a no-slip contact between the channel bed and the monolayer. We neglected any adhesion between the channel walls and the monolayer. Lastly, we applied a structured hexahedral mesh to the monolayer. We compared the monolayer model's strains to those observed in the AF via magnetic resonance imaging (MRI) [20]. X and Y direction strains in the model correspond to radial and axial strains reported in the AF, respectively. We ran all models in FEBio 2.9.1, then analyzed outputs in FEBio Postview 2.5.

RESULTS

A linear fit of the model results ($\varepsilon = 8.8*Y+1.74$) showed that the NA was 0.198 mm above the midline and not at the midline as predicted by beam mechanics (Figure 2). Compared to the measured strains, the beam mechanics and model predictions had a 24.1% and 4.55% average percent error, respectively. The range of radial and axial strains in critical locations of the AF, as measured by MRI [20], are approximately -4 to 10% and -10 to 0%, respectively (Figure 3). The monolayer model covered a range of 0 to 15.1% and -6.9 to 0% strain in the radial and axial directions, respectively. The monolayer model overshot the positive radial strains by 5.1%. The model was unable to cover -10 to -6.9% axial strain or any negative radial strain.

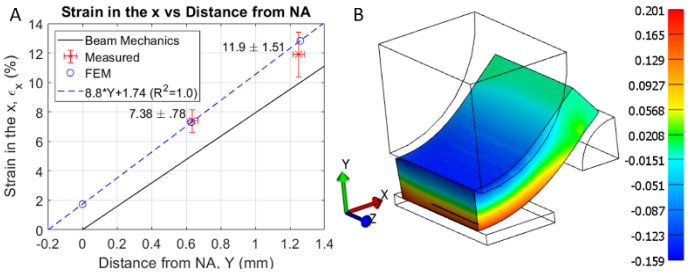

Figure 2: (A) Comparison plot of models to measurements. (B) The model strain results for the 10% target strain chip.

DISCUSSION

The work presented here builds upon our previous development of a deformable organ-chip system that aims to replicate strains experienced by AF cells *in situ*. Results from this study indicate that the computational model provided a more accurate approximation of chip strains than simple beam theory. To cover the full range of axial and radial strains observed *in situ*, we can use modeling to inform us how to scale our chip, position our channel, and alter the chip's bending magnitude.

While many microfluidic platforms exist to evaluate the effects of fluid shear stress on cell behavior [9,10], evaluation of physiologically relevant applied multiaxial strains on AF cells remains unexplored. Our chip provides a controllable method of applying these strains. Since the applied strain in the monolayer model follows a predictable curve over the test cycle (Figure 3), we can adjust bending amplitude to meet target strains. Also, while modeling is often used to characterize factors like mechanical or fluid loading applied by the chip onto the cells, modeling of the cells themselves is often missing. By modeling the cellular mechanics and confirming them empirically, we can estimate internal cellular stresses and strains in three dimensions which otherwise would be difficult to observe. While the current application of our chip is for the AF, biological systems that undergo similar stretching patterns, such as cardiovascular walls, the cervix, or the bladder, can also be investigated by altering the chip geometry to represent healthy or diseased loading conditions. For example, the maximum absolute strain in the left ventricle is 20% in healthy tissues but decreases to 15% right before heart failure [1]. Ongoing work is focused on directly measuring strains in the monolayer to validate model predictions. We will also evaluate cellular responses to different applied strain conditions representative of healthy and degenerated discs.

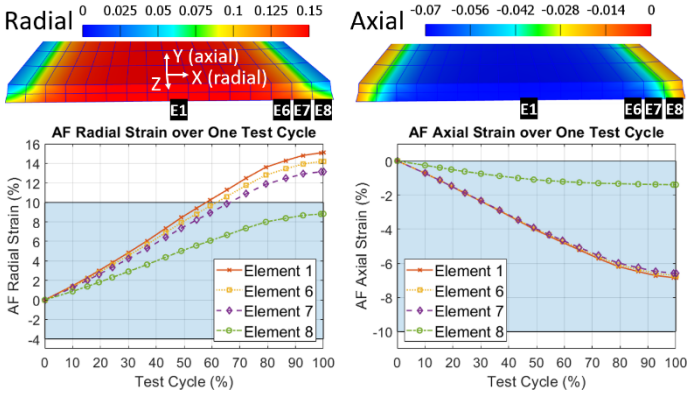

Figure 3: (Left) Radial and (Right) axial direction strains in the chip model were compared to the range of strains reported during AF flexion and extension (shaded region) [20].

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