DLA Project Summary

Deciphering Whole Joint Crosstalk: Mechanotransduction Pathways in Healthy and Diseased States

A Novel On-Chip Mechanical Investigation

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01. Problem Statement & Research Objectives

Osteoarthritis (OA) is the third most rapidly rising cause of disability worldwide, affecting over 88% of adults aged 45 and older [1]. OA is characterized by the degeneration of articular cartilage, the progressive thickening of subchondral bone, and altered mechanical and biochemical signaling between tissues [2]. Joint-on-chip systems are being developed to replicate human joint physiology more accurately. Current experimental models, including 2D in vitro cultures, animal models, and 3D cell cultures and explants, remain limited in their ability to reproduce physiological mechanical stimuli, incorporate relevant immune interactions, capture human-specific physiological responses, and account for donor variability [3]. To address these challenges, this project introduces an on-chip platform engineered for controlled investigation of osteochondral biology, OA disease mechanisms, and tissue-level responses to mechanical and, eventually, chemical cues. The objectives are to (1) engineer and validate a mechanically tunable multi-compartment chip architecture, (2) establish sterile workflows for introducing and layering bone and cartilage tissues, and (3) evaluate tissue viability, morphology, and mechanobiological signaling to enable more accurate OA disease modeling and future drug-response studies.

02. Technical Background

Cartilage-bone crosstalk is central to joint homeostasis and is increasingly recognized as a driver of osteoarthritis (OA) progression [4]. However, most experimental models cannot replicate the physiologically relevant mechanical environment that governs these interactions. Native joints experience coordinated biochemical signaling alongside cyclic compression, articulation-induced shear, and load-dependent interstitial fluid pressurization [5]. Conventional 2D cultures and static 3D systems lack these dynamic features, while animal models introduce species-specific differences in joint structure and mechanobiology that limit their ability to model human OA [6]. These shortcomings underscore the need for a platform capable of delivering controlled, reproducible, human-relevant mechanical stimulation to cartilage and bone tissues.

Joint-on-chip (JOC) systems address these gaps by integrating microfluidics, mechanical actuation, and multi-tissue interfaces to recreate key aspects of the joint microenvironment. The platform developed in this project applies these principles through a mechanically tunable multi-compartment architecture designed to maintain distinct yet interacting cartilage and bone microenvironments. Separate media channels support sterile, layered introduction of each tissue type while preserving physiologically relevant gradients and crosstalk. Pressure-mediated chamber deformation generates lateral shear analogous to native articulation, enabling controlled investigation of mechanobiological signaling, tissue viability, and structural responses under joint-like loading. These capabilities position the device as a robust, human-relevant model for studying osteochondral biology and advancing OA disease modeling in line with the project's core objectives.

03. Methodology

The bulk shear device was fabricated using polydimethylsiloxane (PDMS) in a multilayer chip system (Figure 1). The top layer consisted of three chambers: two outer shear chambers (light blue) and a central compression chamber (dark blue). Each shear chamber contained a single microtube positioned at the chamber's midpoint, while the compression chamber housed two microtubes located at opposite ends to enable controlled pressurization and depressurization. The bottom layer contained a single tissue chamber designed to hold either a 50 µm elastic PDMS (10:1 ratio) membrane for mechanical testing or, in future experiments, bone and cartilage tissue co-cultures. No microtubes were inserted into this lower layer.

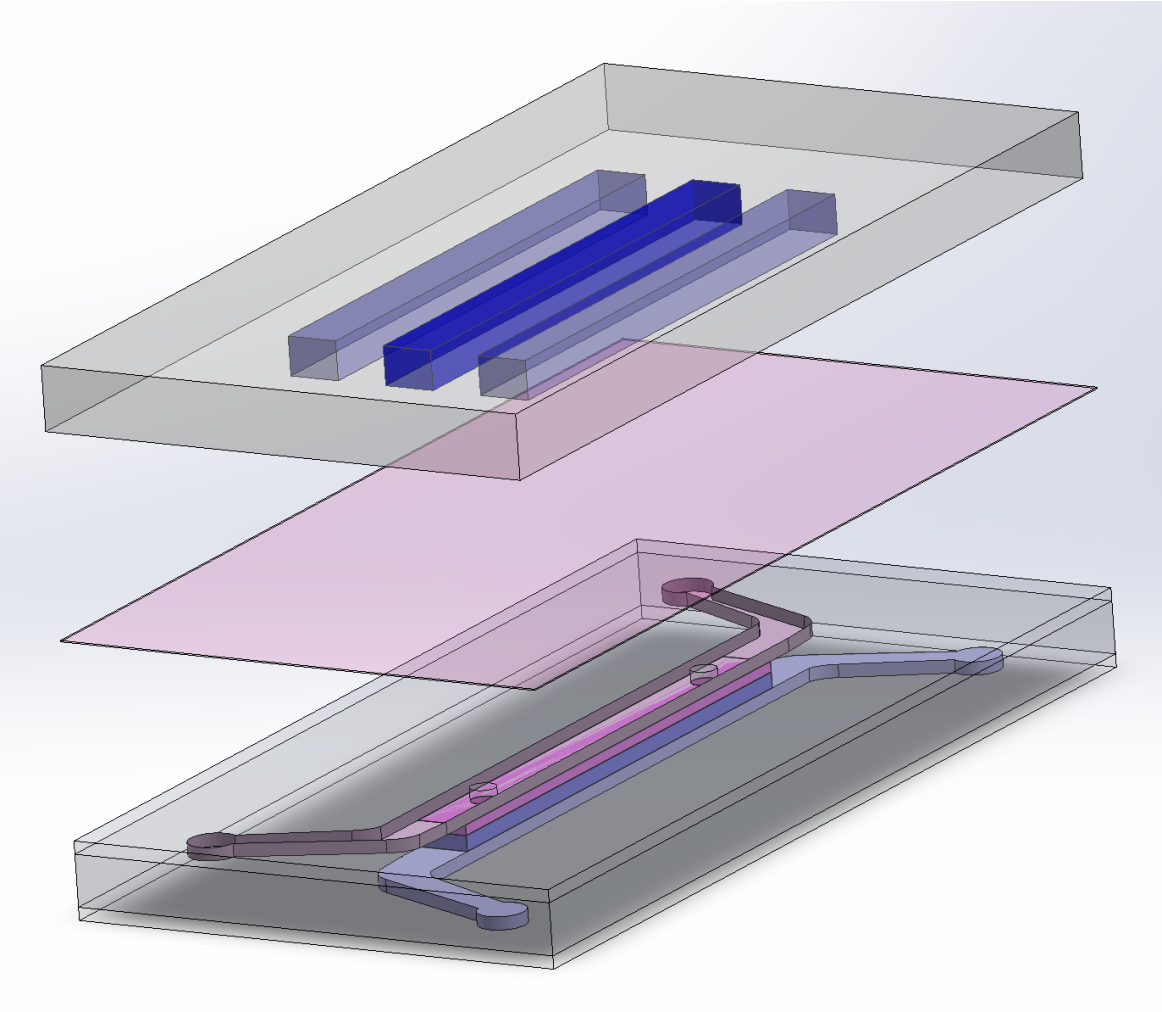

Figure 1. (Top) Top layer of PDMS chip with outer shear chambers (light blue) and central compression chamber (dark blue). (Middle) 50 µm membrane layer. (Bottom) Bottom layer of the chip, showing the single chamber; the version depicted here illustrates the advanced design incorporating separate bone and cartilage tissue chambers with dedicated media channels.

Bulk shear was integrated into the device to replicate the lateral sliding motion that occurs during physiological joint articulation, enabling controlled investigation of shear-dependent

mechanotransduction pathways known to influence cartilage-bone communication and OA progression [5]. Because abnormal shear stresses are linked to early matrix degradation, incorporating shear inputs provides a more physiologically relevant stimulus than compression alone. Microtubes were placed at the midpoint of each shear chamber to reduce edge effects, achieve symmetric pressure propagation, and maximize lateral displacement of the PDMS membrane. This configuration ensures consistent and repeatable shear deformation across the tissue interface, improving experimental reproducibility and enabling more accurate modeling of joint-like loading patterns.

Both PDMS layers were plasma-treated on their bonding surfaces and joined using a thin layer of uncured PDMS, which simultaneously sealed the interface and secured a flexible membrane between the layers. This bonding strategy ensured full adhesion across the membrane, preventing air leakage and minimizing unintended membrane drift during actuation. After alignment, each fabrication stage was followed by curing at 80°F for 30 minutes to ensure complete crosslinking and structural stability.

For bulk shear characterization, microtubes in the outer shear chambers were connected to syringes to generate cyclic lateral deformation. The withdrawing syringe (“negative”) was initialized at 0 mL, and the advancing syringe (“positive”) was initialized at 2 mL of air. Under confocal microscopy, each trial was recorded for 1 minute and 45 seconds. Air volumes were adjusted in synchronized 0.2 mL increments every 15 seconds, producing a total displacement of 0.8 mL before returning both syringes to baseline. The 0.2 mL step size was selected because it produced measurable, uniform membrane deformation without introducing abrupt pressure changes, allowing consistent shear generation and clear visualization of deformation under confocal imaging. This protocol was repeated three times per chip across four chips, yielding 12 experimental trials. Center effects were tested across three chips, and edge effects on only one to ensure data accuracy.

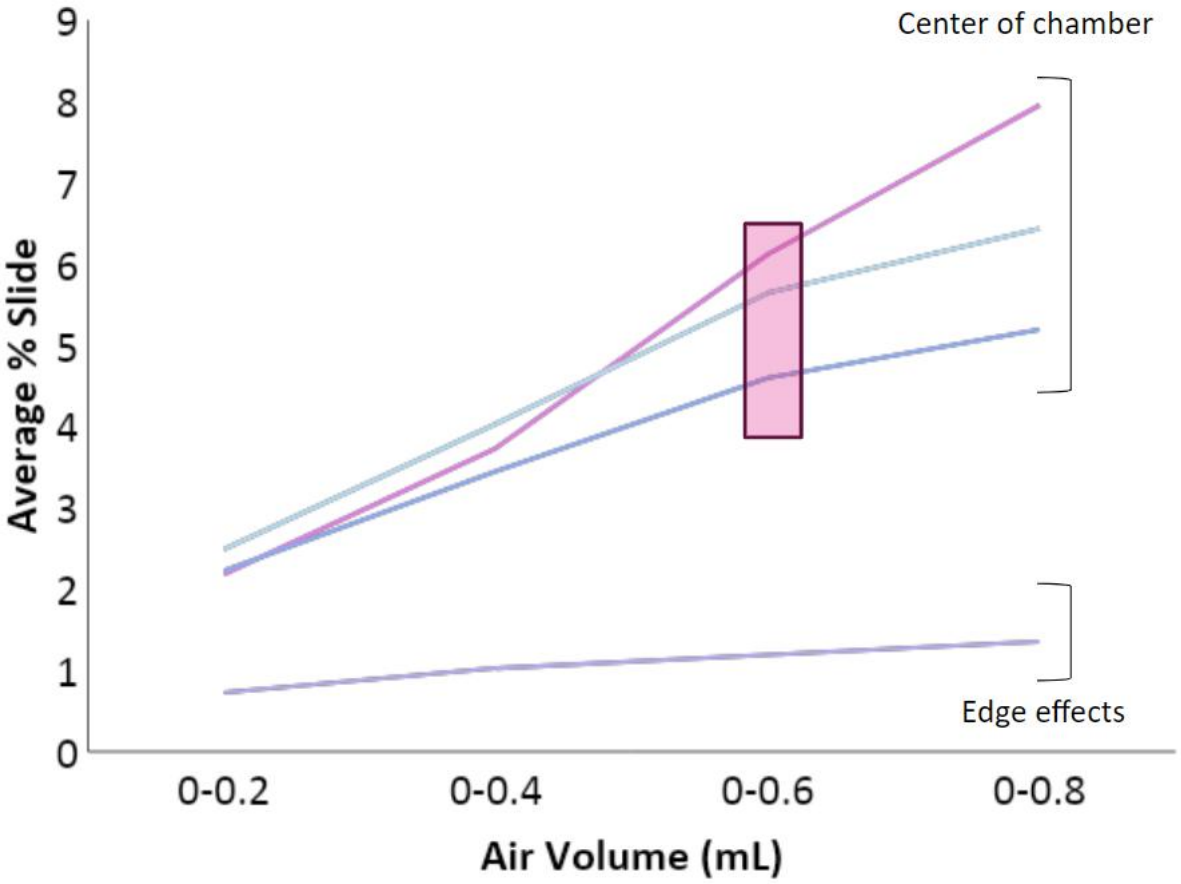

Figure 2. Bulk shear strain data for Chips 1-3 and 5 under cyclic air-pressure loading, showing average displacement across trails for each chip, with Chip 4 excluded due to membrane rupture. This figure demonstrates the reproducibility of mechanical deformation across multiple devices.

04. Resources/Equipment

The joint-on-chip devices were fabricated in the Neu Laboratory for Cell and Tissue Engineering using polydimethylsiloxane (PDMS, Sylgard 184, 10:1 ratio) molded in laser-cut acrylic forms. Device bonding was achieved via plasma treatment (machine specs) and cured in an 80°F oven between fabrication steps to ensure complete adhesion.

Mechanical actuation was generated using 10 mL syringes connected via Tygon ® tubing (0.02” ID), providing controlled positive and negative pressure to the shear chambers. Testing and visualization were performed using a Nikon A1R confocal microscope (10X objective), with ImageJ/FIJI employed for image processing and displacement analysis. Supporting tools included a vacuum desiccator for degassing PDMS, 50 µm membranes fabricated in the Whiting Laboratory, sterile tools, and ABAQUS/SOLIDWORKS for computational modeling and device design.

Future work will incorporate dehydrated cartilage and bone tissues, prepared within the Neu Laboratory, while continuing to utilize the validated PDMS chip design established in this study. Using dehydrated tissues offers several advantages, including improved handling during insertion, reduced viability in the initial hydration state, and enhanced capability with confined microfluidic environments where fully hydrated tissues are difficult to position without damage or deformation. Upon rehydration within the chip, the tissues can recover native structure while maintaining consistent geometry, enabling more reproducible mechanical loading and imaging.

05. Timeline

Month	Objectives
August - September	• Conducted background research on osteoarthritis and cartilage-bone crosstalk. • Began PDMS fabrication protocol optimization and chip preparation.
October - November	• Completed multilayer chip fabrication and membrane integration. • Established pressure-driven shear testing setup using syringe system. • Conducted membrane-based bulk-shear experiments under confocal microscopy. • Collected and analyzed displacement data to validate mechanical deformation. • Built 3D CAD models of the fully assembled chips in SOLIDWORKS. • Began researching sterile workflows for introducing tissue into the chip and strategies for sequential layering.
December - January	• Develop a 3D model in Abaqus using experimental data to replicate chip deformation under realistic loading conditions. • Begin integrating tissue constructs into the model to predict tissue response within the on-chip platform. • Validate model using real tissue constructs in the PDMS device.
February - March	• Integrate cartilage and bone tissue constructs into the PDMS chip. • Develop and refine sequential tissue layering protocols. • Conduct experimental testing to evaluate tissue viability, morphology, and mechanical response within the chip. • Optimize sterile handling workflows and chip-tissue interface conditions based on experimental results.
April	• Finalize experimental trials and data analysis. • Complete figure preparation, presentation poster, and written report. • Prepare for DLA research symposium presentation and submit final documentation.

06. References

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