Interdisciplinary Research (IDR) Origination Awards

Cover Page

Project Title

Title: Development of an organ-on-a-chip surface acoustic wave strain (Organ-SAWS) platform to apply heterogeneous, 3D matrix mechanostimulation for viable lung fibrosis tissue drug development models

Principal Investigator(s) (full-time faculty)

Name (PI listed first)	Department	College
Joseph Rich	Chemical and Biological Engineering	Engineering
Pam Van Ry	Chemistry and Biochemistry	Computational, Mathematical, and Physical Sciences
Greg Nordin	Electrical Engineering	Engineering
Brandon Gassaway	Chemistry and Biochemistry	Computational, Mathematical, and Physical Sciences

Track

Track one

Abstract

One of the biggest translational gaps in drug discovery is the use of animal models as surrogates for pre-clinical trials. Animal models, which have been considered the gold standard of the drug development pipeline, often are limited by interspecies differences, are poor predictors of human physiology responses, and are expensive in time and cost. With the increased investment in drug discovery (US$133 billion in 2021) and the increased drug attrition rate (95% in 2021) there arises a great need for more accurate human physiological models that are inexpensive, high-throughput, and accurate. To address the limitations of animal model systems, several approaches have been developed to accurately mimic human physiology *in vitro*, including 2D cell culture, 3D organoid culture, and organ-on-a-chip tissue models. Of these different microphysiological systems, organ-on-a-chip tissue models most accurately model the human physiological system due to their more native, heterogeneous, and dynamic representation of human physiology. However, 3D organs-on-a-chip models suffer from low yield and challenges in fabrication repeatability. The objective of this project is to develop an organ-on-a-chip surface acoustic wave strain (Organ-SAWS) platform to develop a heterogeneous active-3D matrix mechanostimulation platform to fabricate lung fibrotic tissue models for drug development assays. The Organ-SAWS system will implement the high spatial and temporal control of traveling surface acoustic waves (SAWs) with precision 3D printing and lung patient biomaterials and lung cell lines to develop an active, inexpensive, and viable microphysiological system. Furthermore, we will develop a proteomic protocol to analyze the organ-on-a-chip phenotype derived from Organ-SAWS.

Summary of Plans for External Funding

NIH R01 (NIGMS institute) – June 2027

1. SIGNIFICANCE

One of the biggest translational gaps in drug discovery to properly treat fatal diseases is the use of animal models as surrogates for pre-clinical trials. Animal models, which have been considered the gold standard of the drug development pipeline, often are limited by interspecies differences, are poor predictors of human physiology responses, and are expensive in time and cost[1]. With the increased investment in drug discovery (US$133 billion in 2021) and the increased drug attrition rate (95% in 2021) there arises a great need for more accurate human physiological models that are inexpensive, high-throughput, and accurate[1].

To address the limitations of animal model systems, several approaches have been developed to accurately mimic human physiology *in vitro*, including 2D cell culture, 3D organoid culture, and organ-on-a-chip tissue models[1]. Of these different microphysiological systems, 3D organoids and organ-on-a-chip tissue models most accurately model the human physiological system due to their more native, heterogeneous representation of human physiology. However, 3D organoids and organs-on-a-chip models suffer from low yield and challenges in fabrication repeatability[2]. Several different approaches to more accurately model the physiological system within organs-on-a-chip include chemical, biological, and physical control of the microenvironment that these organs-on-a-chip experience[3]. Of these difference approaches, physical altering methods to alter the microenvironment of organs-on-a-chip have particular advantages in their ability to induce a particular biological pathway in a reversible, biocompatible, and spatiotemporally controlled manner[4]. Some of the current methods for physically altering microphysiological systems include the use of fluidic (pneumatic or hydraulic)[5,6], tensile[7,8], viscoelastic[9-11], electromagnetic[12-14], optical[15-17], thermal[18-20], and acoustic forces[21-23].

Of the various physical active stimulation methods, fluidic and tensile forces demonstrate active manipulation with biomimetic relevance (Table 1). Particularly for lung physiology, tensile forces are very relevant, however controlling heterogeneous strains within 3D with high resolution in a microenvironment is difficult. Of the various mechanisms for physically altering microphysiological systems, acoustic forces have advantages in terms of active control, reversibility, high biocompatibility, appropriate spatial resolution, and force penetration depth[4,28], and have recently been shown to actively apply heterogeneous tensile strains in 3D within various biomaterials matrices[27].

3D Microphysiological Mechanical Stimulation Systems				
Parameter	**Microfluidic Shear Stress Methods/Organ on a Chip [24]**	**Bioprinter Fabrication Methods [25]**	**Cyclic Stretching [26]**	**Proposed Organ-SAWS Platform [27]**
Controlled 3D strain Manipulation	No	No	No	Yes
Active or Passive Control	Active	Passive	Active	Active
Biomaterial Stiffness Dependent	Yes	Yes	Yes	No
Mechanism	Applied Fluidic Shear Stress	Applied Fluidic Shear Stress and Proximity	Material Deformation	Acoustic Traveling Wave-Induced Shear Torsion

Table 1: *Comparison between current 3D Microphysiological Mechanical Stimulation Systems and our proposed Organ-on-a-chip acoustic traveling wave induced shear torsion (Organ-SAWS) platform[24-27].*

The objective of this project is to develop an organ-on-a-chip surface acoustic wave strain (Organ-SAWS) platform to develop a heterogeneous active-3D matrix mechanostimulation platform to develop an inexpensive, high-throughput, and accurate physiological model of the mechanically sensitive lung fibrotic system for drug development assays. The Organ-SAWS system will implement the high spatial and temporal control of traveling surface acoustic waves (SAWs) with

precision 3D printing and lung patient biomaterials and lung cell lines to develop an active microphysiological system that accurately models the lung fibrotic disease models. We will further incorporate proteomic analysis to investigate the biological response to the mechanically induced force within the lung patient biomaterial to further understand the acoustic influence on the mechanical stimulation on the organ-on-a-chip. Particularly, the combination of inexpensive fabrication of custom SAW devices, the high yield and control of the 3D printed substrates, and the biomimetic application of lung patient biomaterials addresses the limitations of animal models and will enable the development of an inexpensive, high-throughput, and reliable fabrication of a lung fibrotic microphysiological system.

2. INNOVATION

The creation of Organ-SAWS requires greater mechanical stimulation control over the biomimetic 3D matrix than is currently possible. With regards to direct manipulation of materials, acoustic forces have been employed for the active and reversible release of material payloads within hydrogels for enhanced delivery[21,29,30], for direct modifications during hydrogel fabrication[31,32], and to deform hydrogels[33-35]. Most recently, Prof. Joseph Rich has developed an Acoustic Traveling Wave Induced Shear Torsion (A-TWIST) platform that enhanced endothelial cell vascularization with the application of 3D strain to collagen hydrogels via the focused traveling SAWs, demonstrating the first heterogeneous 3D strain platform capable of enhancing cell function[27]. However, there are still several limitations to this system, including hand-fabricated and hand-placed PDMS stimulation chambers, a limitation on the breadth of materials tested with the A-TWIST platform, and a deeper understanding of the cell phenotypic response to the acoustic forces in A-TWIST and the corresponding proteomic pathways.

The combination of high-precision 3D printing and the acoustic forces from A-TWIST will yield a highly repeatable and spatially controlled Organ-SAWS platform to further increase the spatial application of acoustic forces for the manipulation of 3D biomatrices. The incorporation of precision 3D printed chambers will allow for consistent chamber shape and for the continuous renewal of media. Furthermore, spatial design of the 3D microenvironment in which the acoustic waves can propagate through a microfluidic chamber could lead to novel focusing of acoustic waves for unique control of the acoustic field in 3D and the applied 3D strain.

With this added capability with the incorporated 3D printed chambers and designed traveling SAW shear force, we will investigate other biomaterial types in our Organ-SAWS platform. Particularly, we will leverage our expertise in biomimetic matrix fabrication techniques by investigating decellularized human lung fabricated hydrogels. This implementation of a biologically derived matrix creates both a chemical and physically relevant matrix for the organoids to form within our Organ-SAWS platform enabling the generation of repeatable biomimetic drug assays for lung fibrosis.

Finally, further proteomic investigation is required to understand the cellular and organoid phenotype response to the applied mechanical stimulation from Organ-SAWS. Several mechanosensitive pathways, such as Piezo1 or integrin-focal adhesion pathways could be incorporated with the applied strain from the acoustic stimulation. By leveraging our experience in thermal proteomics, and previous organoid proteomic experience, we will further develop a novel proteomic protocol to isolate relevant proteins within the stimulated organoid from the proteins of the matrix.

At the completion of this project, we will yield the following novel capabilities: **(1) High resolution 3D heterogeneous strain platform of biomimetic materials**. Current mechanical stimulation platforms are limited by 2D strain platforms. Using state-of-the-art additive manufacturing and high-resolution traveling SAW platforms, we will enable a repeatable 3D heterogenous strain platform for mechanical stimulation. **(2) Organoid 3D mechanical strain platform for lung fibrosis drug delivery models**. We will further investigate material types, particularly decellularized lung samples to generate 3D organoids which we will then stimulate within the Organ-SAWS platform to develop biomimetic lung fibrosis tissue models. **(3) Proteomic understanding of the cellular**

and organoid phenotypic response to the acoustic stimulation. For both cellular networks (fibrotic cells) and lung fibrosis organoids (multi-cellular) we will investigate the underlying mechanostimulation pathway to the acoustic strain forces. (**4) Organoid isolation and proteomic profiling protocol**. Post-stimulation of the acoustic forces, a developed isolation protocol is required to isolate the organoid proteins from the biomatrix proteins.

Together these innovations will establish a biocompatible, high spatial and temporal heterogenous 3D strain platform to develop a more accurate, active biomimetic lung fibrosis model to enable repeatable, high yield drug discovery assays. This could be further applied to other strain sensitive organoid models, including cancer, immune responses, and cardiac models for further disease modeling and potentially for therapeutic tissue development as well[36].

3. APPROACH

3.1. Overview and Team

Fig. 1 shows an overall schematic of the proposed Organ-SAWS platform to enable the development of an active lung fibrosis microphysiological system that utilizes precise application of 3D strain via traveling SAWs within a 3D printed chamber, followed by subsequent proteomic analysis to characterize the organoids phenotype with the applied stress. Through multi-resolution and high-resolution 3D printing we'll enable the fabrication of repeatable and high-resolution stimulation of lung-derived biomaterials and organoids to construct robust organ-on-a-chip platforms for drug delivery assays. By further implementing proteomic analysis of the Organ-SAWS phenotype, we will further elucidate the mechanosensitive response between the cell and the acoustic strain forces and validate the phenotype of lung fibrotic or healthy lung cell models.

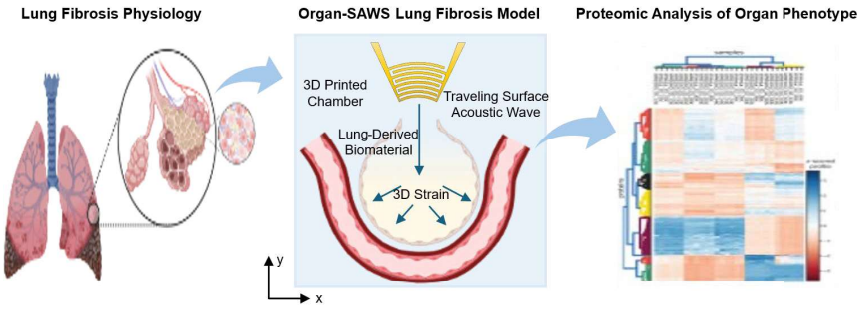

Figure 1: *Schematic of a fabricated Organ-SAWS lung fibrosis model that will enable the high fidelity fabrication of biomimetic microphysiological system for drug discovery assays[37].*

Our interdisciplinary team includes investigators at Brigham Young University with the corresponding expertise required to see the completion of this project, including expertise in acoustofluidics, surface acoustic wave transducer fabrication, and biomedical microsystems (Prof. Joseph Rich)[27,38-56], high and multi-resolution 3D printing (Prof. Greg Nordin)[57-66], pulmonary fibrosis in vitro models (Prof. Pam Van Ry)[63,67-74], and thermal proteomic methods and protocol development (Prof. Brandon Gassaway)[75-82]. By leveraging our collective experience, we will establish a team to complete the outlined aims and lead future collaborative efforts.

3.2. Preliminary Results

Recently, our team has demonstrated preliminary results in high-resolution 3D printing, and in demonstrating the acoustic induced 3D strain of various materials, including collagen, GelMA, collagen methacrylate, PEGDA[27]. Furthermore, with the acoustic traveling wave induced shear torsion (A-TWIST) platform we demonstrated that we could enhance endothelial colony forming cell vascularization with the applied mechanical strain from A-TWIST (Fig. 3)[27].

Furthermore, our team has recently demonstrated the successful fabrication of lung-on-a-chip organoids by talking lung samples of fibrotic and healthy patients and demonstrating that decellularized lung samples reconstituted in a gel matrix enhanced the pathway of cells to the corresponding physiological response. Finally, our research group has also demonstrated the proteomic analysis of cells within these reconstituted lung samples.

3.3. Aim 1: Develop Organ-SAWS platform with advanced 3D printing methods and traveling surface acoustic wave technology to enable high spatial and temporal resolution application of 3D strain on mechanosensitive cell models

3.3.1. Rationale

In Aim 1, we will develop a reliable, functional, and precise 3D mechanical strain platform with the following features:

Acoustic applied heterogeneous 3D strain with increased precision for mechanical cell stimulation: Our preliminary results demonstrate that we can control the 3D strain with the design of the SAW acoustic transducer. By further incorporating 3D fabrication techniques, we can precisely control the boundary conditions of the system to control the acoustic strain applied on the system.

Universal 3D strain platform for a variety of biomaterials of varying stiffness: Several of the current mechanical stimulation or strain platforms are limited to gels that can withstand the physical force applied to it, limiting the softer range of biological materials[10]. By utilizing acoustic forces, we can highly tune the applied force for stimulation of softer biomaterials.

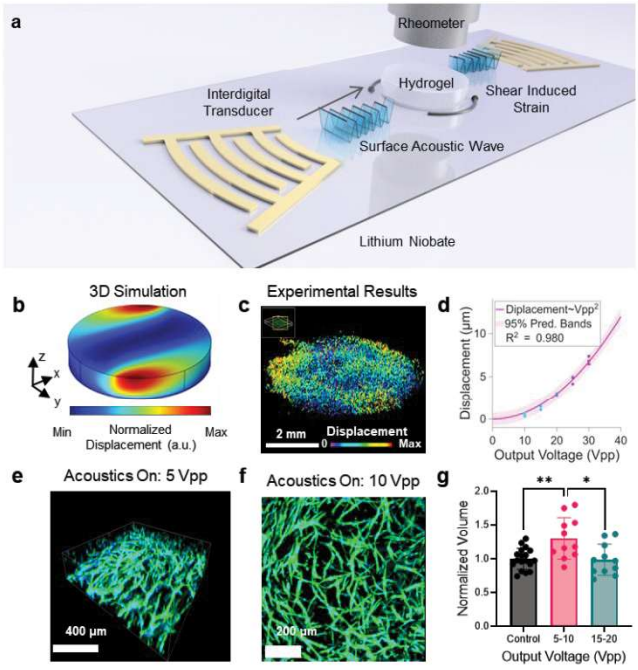

Figure 2: ***(a)*** *Illustration of SAW strain generation via the A-TWIST device.* ***(b)*** *Displacement simulation by the A-TWIST device.* ***(c)*** ***C****onfocal image showing 3D particle tracking of a gel with A-TWIST.* ***(d)*** *Displacement as a function of applied voltage (RM=3).* ***(e)*** *Confocal image of the endothelial cells vasculature at 5 Vpp.* ***(f)*** *2D confocal image at 10 Vpp.* ***(g)*** *Comparison of the vasculature volume for each low (5-10 Vpp) and high (15-20 Vpp) applied voltage conditions (n=11-18). Data analyzed via a Welch t-test[27].*

3.3.2. Aim 1.1.: Develop Organ-SAWS platform for the investigation of spatial controlled 3D strain on fibrotic cell lines within varying biomaterials

In this sub-aim we will develop a variety of chamber designs to control the 3D strain of the hydrogel laden with mechanosensitive fibroblasts. With this platform we will validate the acoustic effect on fibrotic cell alignment of the hydrogel network and investigate how the acoustic strain changes fibrotic cell phenotype. This will further validate the efficacy of this platform for translational applications for active microphysiological control for drug development assays.

3.4. Aim 2: Develop Organ-SAWS platform for the application of 3D strain on lung fibrotic models and reconstituted materials to fabricate viable biomimetic systems

3.4.1. Rationale

Organoids within 3D culture often struggle with robust and repeatable fabrication techniques. This is largely due to the complexity that is associated with controlling the 3D forces experienced within the microenvironment. By implementing the combined precision of acoustic strain stimulation and 3D printing we'll achieve the precise control to actively strain the lung models for accurate biomimicry of the lung tissues.

3.4.2. Aim 2.1: Investigate the Organ-SAWS effect on lung fibrotic organoid models and lung reconstituted materials

The transition between matrix manipulation from single cell formed networks and the matrix

manipulation of multi-cellular organoids we hypothesize will present a series of challenges that will require the dynamic stimulation and quick iteration design nature of 3D printed chambers. Iteration across different sized chambers, acoustic stimulation magnitude, and stimulation start time and duration will be investigated for optimal organoid formation. We hypothesize that acoustic strain magnitudes and time of duration similar to lung stimulation within the alveoli will create the optimal lung tissue model. We will also incorporate a chemical matching scaffold of the lung tissue models of interest (healthy and fibrotic) by utilizing patient derived, reconstituted lung biomaterials. To validate the integrity of the fabricated organoids we will first investigate the viability and stained organoids for junction density within the various tissue models.

3.4.3. Aim 2.2: Develop a proteomic protocol for the investigation of Organ-SAWS organoid phenotype

To understand the phenotypic effects of the Organ-SAWS device on the cellular response to the mechanical stimulation of this 3D acoustic strain, proteomic analysis will be implemented to isolate cellular pathways of interest. Particularly, we will investigate pathways, such as Piezo1 or integrin junction pathways to elucidate if acoustic stimulation is affecting the membrane of the cells within the organoid (Piezo) or if this mechanical stimulation is isolated to the matrix strain on the focal adhesions of the cell (integrin junction pathways). However, in order to analyze this, we must develop a protocol to isolate the organoid from the biomaterial matrix, to get sufficient proteomic data to make a conclusion.

3.5. Potential Pitfalls and Alternative Solutions

Temperature Control: Depending on the acoustic intensity required to achieve organoid stimulation, the temperature could increase. To mitigate this, we could implement a Peltier cooling system to maintain temperature within 0.1°C.

3.6. End-point objectives and measures of success

We have determined 3 parameters (Table 2) as measures of success of this project. Our goal is to attain and surpass the outlined performance metrics. Once we have demonstrated the completion of these parameters, we will consider the project complete and developed to a point that we could move on validating Organ-SAWS for drug delivery assays. Furthermore, the development of this project would provide a tool for future researchers to actively stimulate organ-on-a-chip models for disease mechanism discovery and tissue therapy generation.

Parameters	Target Value	Acceptable Value	Benchmark Value
3D Strain Resolution	100 µm	500 µm	~1 mm*
Minimum Stiffness of Biomaterial	5 Pa	20 Pa	200 Pa†
Organoid 3D Strain Platform	Yes	Yes	No*

Table 2: *Performance measures for this proposal. Benchmarks are based off *A-TWIST[27] and †cyclic stretching[26].*

3.7. Research and scholarly activities

The funding of this research will let us get some critical preliminary data for the application of an NIH R01 grant. The agency we hope to target is NIGMS for the development of this platform. The funding for this project would provide the funding for student support to help acquire the data required for to make this proposal competitive to successfully receive an NIH R01 grant, where there currently is a lack of funding for students on this project (both graduate and undergraduate). Furthermore, there are several impactful manuscripts (at least 3) and patents (at least 3) that could result in the funding of this project.

3.8. Schedule and Expected Milestones

The schedule for this project is to apply for an NIH R01 grant in June of 2027. Depending on our ability to get preliminary data, we anticipate receiving feedback to then further revise and resubmit. Expected milestones and their schedule includes: 1. Aim 1 completion (December 2027), Aim 2.1 completion (June 2028), and Aim 2.2 completion (December 2028).