Strain Scout: A novel device for the discovery and validation of novel mechano-pharmacological targets of disease

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Abstract

Mechanotransduction, defined as the conversion of mechanical stimuli like stretch and strain into biochemical cell signaling pathways, is an area of study of increasing importance. Understanding the signaling of healthy tissues when strain is applied can help to inform cellular targets for the treatment of disease in tissues that regularly stretch, such as the lungs, muscles, or vasculature. Previous devices used to study the effects of strain on cells were only able to culture cells under stretch or image cells under stretch and were not able to do both simultaneously. Therefore, the primary goal of this Capstone design is to develop a novel device, Strain Scout, that can apply mechanical stimuli to cells in a high-throughput, automation-oriented way. To iterate on previous models of Strain Scout, there were two aims. Aim 1 was to improve the Strain Scout user interface and make Strain Scout more compatible with current imaging techniques. Aim 2 was to make Strain Scout more high-throughput to test multiple experimental conditions in one cell culture. The device was modeled using Fusion 360 and 3D-printed in acrylonitrile butadiene styrene (ABS) and polylactic acid (PLA) to test various prototypes and designs. A membrane mold was also designed in Fusion 360 and printed in ABS and resin and was used to create a pliable polydimethylsiloxane (PDMS) membrane with a well-plate design for imaging tool compatibility. After several iterations and experiments, a successful, innovative device scaffold was made and PDMS was eliminated as a feasible material due to limited pliability. This new design of Strain Scout will provide a high-throughput and automated platform for drug screening and mechano-pharmacology that can potentially reduce the cost and time of drug development and discovery.

Keywords: 3D printing, mechanotransduction, strain, PD

Introduction

Mechanobiology explores the influence of mechanical forces on biological processes. It is a multidisciplinary field of biology, engineering, physics, and medicine. This field is gaining significant attention due to its potential to unravel the mysteries of how cells and tissues respond to mechanical stimuli, such as tension, compression, and shear stress. In a medical context, the human body is composed of many organ systems that are constructed to stretch as a part of their normal physiology, namely the lungs and alveoli which stretch as we breathe and heart chambers changing conformation as the heart pumps blood. As such, cellular mechanotransduction, the translation of mechanical stimuli into chemical signals between cells, is a topic of increasing importance in the studies of diseases in areas such as lung fibrosis, heart

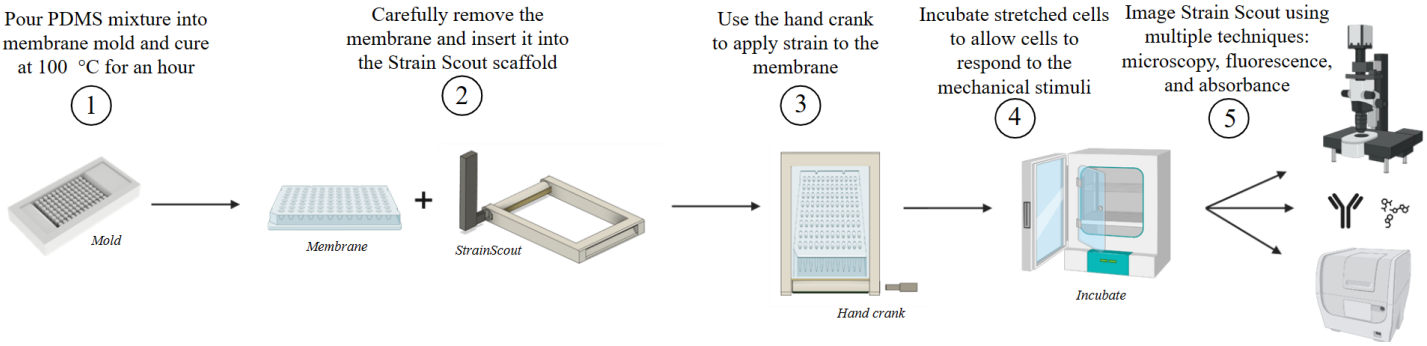

Figure 1. Intended procedure for Strain Scout. Utilize a mold to produce a PDMS membrane and insert the membrane into the Strain Scout Scaffold. Seed cells of choice and incubate before and after applying strain. Image with antibodies or small molecules to identify relevant mechanotransduction pathways.

disease, and cancer [1]–[3]. Breaking down the communication of physical signals to biochemical signals in a healthy model is integral to understanding how miscommunication of these signals can propagate disease [4]. Additionally, testing and observing the effects of relevant drugs on a microscopic, molecular level under a model of strain is necessary to create new treatments of previously undescribed pathways [5]–[7].

To study how stretch affects cells, an elastic, biocompatible membrane can be used to culture cells, in which a stretch stimulus can be applied before incubation. This is a current model utilized in mechanobiology labs which mimics pathological states relating to stretch, like idiopathic pulmonary fibrosis [8]–[10]. Moreover, the Matrix Biology and Engineering Lab at the University of Virginia developed a device named Strain Scout which aims to accurately apply and maintain a stretch over a membrane. The original Strain Scout device applies strain to only one cell culture sample, is limited to two stretch settings (achieved by manually stretching) and requires complex technical knowledge to operate. The feasibility of the previous Strain Scout was low, given that labs and various pharmaceutical companies hope to test a very large number of therapeutics at once. Based on these flaws we created a prototype that introduces various advancements, including adjustments to the stretch length, automation, and the ability to perform in a high-throughput manner. Additionally, it allows for various lab techniques, such as imaging and assays, to be conducted after and during the stretch stimulus (**Figure 1**). Our model enhances efficiency and makes such scientific methods accessible to individuals with diverse skill sets. We also investigated the feasibility of fabricating a membrane utilizing polydimethylsiloxane (PDMS). The existing membrane configuration permits one single cell type at any given moment, which contributes to inefficiency. To address this, we have designed a 96-well plate mold that can facilitate the curing process of the membrane. This innovative approach could potentially enhance the overall efficiency of the system. Innovation in the field of mechanobiology in the form of Strain Scout is integral to discovering previously unknown pathological mechanisms in tissue of the body that regularly experience strain.

Results

To iterate on previous models of Strain Scout, design aims were two-fold:

- Aim 1: Improve the Strain Scout user interface and make Strain Scout more compatible with current imaging techniques.
- Aim 2: Make Strain Scout more high-throughput to test multiple experimental conditions in one cell culture.

To achieve Aim 1, a few specifications were outlined. The design must bring the membrane closer to the microscope lens for better, more efficient, imaging. The current design has material in between the membrane and the microscope lens, which makes the experiment itself less efficient as content visibility is decreased. The design must change to clearly observe all parts of a cell culture experiment. The membrane must be stretched with minimal human intervention, and when required, the human intervention is simple, short, and doesn't require advanced technical knowledge. The current design makes for a tedious experimental timeline, as only one condition can be tested at a time, making replication of experimental conditions almost impossible.

To achieve Aim 2, additional requirements were established. There is a wide variety of biosafe resins, and these resins can only be printed with SLA (stereolithographic) techniques. Therefore, SLA needs to be used to print a customizable membrane mold [8], [11]. The resin itself must be biosafe as it spends a significant amount of time in contact with the membrane. Cells will be cultured within the membrane and the environment needs to be free of cytotoxic chemicals. Additionally, the mold needed to maintain the dimensions of currently used microplates to ensure lab compatibility. It is important that the design of the membrane can be used with imaging instruments commonly found in labs to increase translatability. Strain Scout is meant to be easy for anyone to use and to make mechanotransduction studies more accessible. Lastly, the membrane will be printed out of PDMS, as that is the current standard material for soft membrane tools [8], [9]. It is hypothesized that there is an alternative design for the Strain Scout scaffold that maximizes visibility of the membrane for application in common microscopy techniques and that a customized, PDMS membrane can be made to make a high-throughput device.

Strain Scout Device

Previous models of strain simulation devices are only capable of testing one experimental condition at a time, and the work to strain the cell cultures is tedious and all done by hand. A pharmaceutical company must test multiple experiments at once and the current device would be impossible to scale to the level of testing needed. The current membrane being used in the Matrix Biology and Engineering Lab is limited as experiments are difficult to

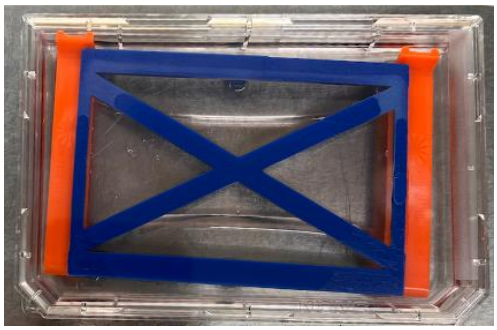

Figure 2. Previous Strain Scout Design. The membrane in this image is stretched. At the halfway point, posts protrude and act as an anchor for the membrane clips (orange) when the previous version of Strain Scout is in an unstretched state.

replicate with only one test able to be performed by the Strain Scout (**Figure 2**). Additionally, the current design has the membrane slightly lifted from the microplate making it harder to image the cells under a microscope.

The objective of the updated scaffolding, as described by Aim 1, is in part to bring the membrane closer to the microscope lens. Moreover, the device should be able to stretch the membrane with minimal human intervention. The design should be durable as the parts will be under significant stress. Lastly, the device function should be compatible with an automated system for future research.

Serval prototypes of the design scaffolding were modeled in Autodesk Fusion 360 until we landed on the final design (**Figure 3a**). The final design fits in a microplate for culturing and imaging the cells. Additionally, the membrane is raised so it sits at the top of the device for better imaging results. The scaffolding can now be adjusted depending on the level of strain needed. The design is also compatible with a control interface by connecting a motor to the crank/roller region. Lastly, the stretching mechanism is easy to take apart for sterilizing the device through an autoclave (**Figure 3b**).

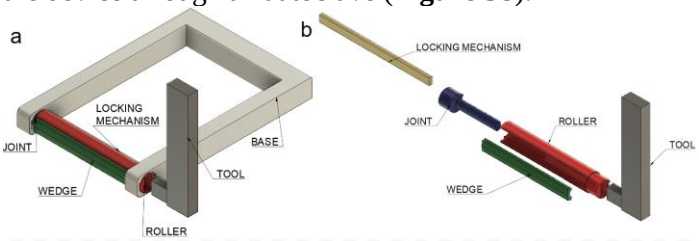

Figure 3. Final Strain Scout Scaffold Design. a) Final labeled drawing of Strain Scout device using Fusion 360 b) Exploded drawing of stretching mechanism using Fusion 360

The first iterations of Strain Scout scaffolding were 3D printed in PLA, although the material is insufficient in that it has limited flexibility and low-temperature resistance. The final design of Strain Scout was printed in ABS as the material is durable, heat resistant, and inexpensive to produce relative to other materials.

We conducted various tests in Autodesk Fusion 360 on different metals for the final scaffolding of Strain Scout. Strain Scout needs to be autoclavable, and 3D-printed ABS parts cannot withstand constant reuse, therefore alternative metal materials were reviewed. Silver was first considered as it is antimicrobial, durable, and can withstand high temperatures for autoclaving. However, silver scaffolding would be too expensive to mass produce for pharmaceutical companies. Aluminum was ultimately chosen as it is inexpensive, durable, and can withstand high temperatures for autoclaving [12]. Stress testing was conducted in Autodesk Fusion 360 with aluminum scaffolding using a 10 Newton force applied to both sides of the device (**Figure 4**). This will be more than enough strength for what is required of the Strain Scout device.

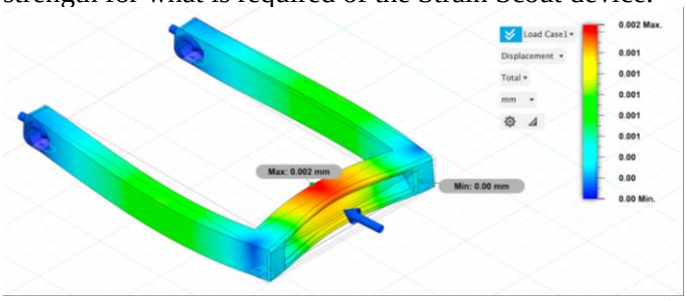

Figure 4. **Strain Testing.** Finite element analysis (displacement with strain of membrane) with aluminum scaffolding.

PDMS Membrane

To create a PDMS membrane to fit inside the Strain Scout scaffold, molds were designed. The membrane design aimed to make the Strain Scout device high-throughput to test multiple conditions in one cell culture, as previous models of Strain Scout only allow for testing of one experimental condition, which wastes time and resources. This design also has limited reproducibility and is therefore impractical for pharmaceutical testing. The purpose of Aim 2 is to streamline the process of applying strain to a variety of different experimental conditions, all at one time. To do this, the PDMS membrane mold was printed using SLA 3D printing from Tough 1500, a specialized resin. The mold was printed in a few designs that have several wells to isolate regions of the membrane for different conditions, similar to current microplate designs. The similarity of the well designs to current microplates allows the membrane to be compatible with popular and relevant imaging devices such as plate readers and confocal microscopy.

The initial design of the mold was in a 96-well plate format that had flaps on either end to be inserted into the Strain Scout scaffold (**Figure 5a**). The wells were ovular in shape so that when strain was applied, they expanded to a circular shape to mimic standard wells.

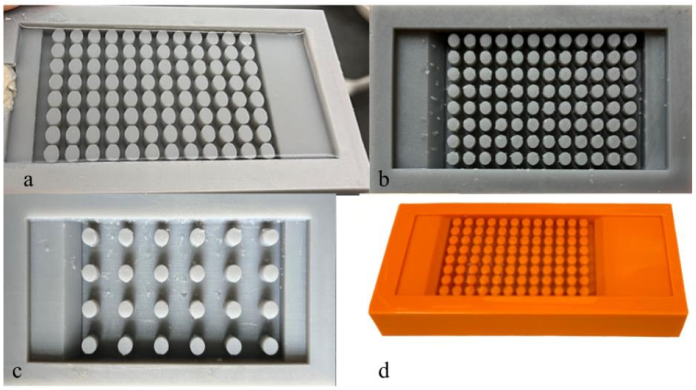

Figure 5. Iteration of the Strain Scout membrane mold. a) Original large 96-well plate design with PDMS and made out of resin b) Scaled down design (0.7 scale) c) 24-well plate membrane mold d) Scaled down (0.7 scale) mold made out of ABS and with increased tab length.

PDMS elastomer and curing agent were prepared in a ratio of 7:1, poured into the first iteration of the mold, and cured in an incubator at 45 ºC for four hours. Upon removal of the membrane from the mold, the PDMS broke into pieces. To determine the cause of the brittleness, various crosslinking ratios were tested and poured onto glass slides to test elasticity. Crosslinking ratios of 5:1, 10:1, 15:1, 20:1 were cured at room temperature for 24 hours (**Figure 6**). Ratios of 15:1 and 20:1 were determined to have too little curing agent, as the PDMS was sticky to the touch, soft, and tore upon removal. Stretching of 5:1 and 10:1 ratios revealed that 5:1 is too tough for stretching in the scaffold, as a lot of force would be required to move it. The ratio of 10:1 was selected for following procedures as it did not rip and succumbed to strain when stretched.

Figure 6. Crosslinking experiment. Elastomer to curing agent ratios tested were 5:1, 10:1, 15:1, 20:1.

The crosslinking ratio of 10:1 was used again in the initial mold and cured at 10ºC for an hour. The same results occurred when the PDMS was removed, as it broke and came off in chunks. The surface of the mold material was tested as a variable and soapy water was brushed through the surface and dried to leave a layer of soap molecules in between the PDMS and resin mold. This also did not help with the removal of the membrane. The geometry of the well design was tested to determine if the well shape was a cause for the difficulty in removal. Two new designs were proposed: a 24-well mold and a scaled-down version of the 96-well plate (0.7 scale) (**Figure 5b, c**). PDMS was prepared at a 10:1 ratio and cured in both molds at 100ºC for an hour. In the case of the 24-well design, the spaces in between the wells were too large, and the PDMS blocks were even stronger and harder to remove in one piece. In the scaled-down 96-well plate, the membrane did start to peel out in a much larger section than in previous iterations, although it did rip and ruin the integrity of the wells.

The final iteration of the mold was printed in ABS, scaled down at a 0.7 scale, and edited to fit the final version of the Strain Scout scaffold (**Figure 5d**). The previous attempt of a scaled-down model was tested and determined to be unsuccessful, therefore this final attempt to make a membrane was used to determine if mold material had any effect on the ability to remove a cured membrane. An elastomer to curing ratio of 10:1 was poured into the mold and cured for 1 hour at 100ºC. The membrane was still extremely difficult to remove. The difficulties experienced with the pliability of PDMS created with SYLGARD™ 184 Silicone Elastomer Kit indicate that this is not a suitable material to use with the molds to make a Strain Scout membrane moving forward.

Discussion

In conclusion, we have made significant strides in the development of Strain Scout, a high-throughput tool designed to investigate the effects of mechanical strain on cells cultured *in vitro*. Our work reveals three significant findings: the successful development of a new Strain Scout design, an innovative approach to membrane creation, and new developments for experimental techniques in the mechanobiology field. In our exploration of the viability of a PDMS membrane material, our hypothesis was ultimately disproved that PDMS is a suitable material for an elastic cell culture membrane. Curing the membrane into a 96-well plate shape was successful, however elastic properties of the PDMS made with SYLGARD™ 184 Silicone Elastomer Kit membrane were not suitable with our parameters. Despite various attempts to optimize its properties through different cross-linking ratios, PDMS membranes consistently exhibited excessive brittleness, making it difficult to remove from the mold and therefore unsuitable for our purposes. This finding creates a foundation for further exploration of other suitable materials with desirable mechanical properties.
The creation of a versatile 96-well plate mold was successful and is a breakthrough in our ability to cure membranes from a range of materials, therefore our mold can be used for future work of membrane exploration.

While PDMS was excluded due to its limitations, this mold has proven itself as an opportunity to cure other substances, laying the groundwork for future research to identify materials with the ideal combination of mechanical properties, such as elasticity and durability, necessary for our experimental conditions. Additionally, we tested various materials for the mold including a proprietary resin and ABS. Interestingly, both materials worked during the curing process of the PDMS membrane. Lastly, the most critical achievement of our project is the successful design and implementation of a scaffold that meets all our operational criteria. The Strain Scout device, created via 3D printing, excels in its imaging and cell culturing capabilities. The device is high-throughput, easy to use, and can be adjusted to the desired membrane strain. It has been proven that the scaffold will be successful with various 3D materials, however, to prevent bacterial contamination we recommend aluminum printing in the future. This innovative device aligns perfectly with our goal of facilitating the study of mechanotransduction in a controlled laboratory setting, thereby advancing our understanding of cellular responses to mechanical stimuli. The insights gained from our research will contribute to the field of mechanobiology and pave the way for novel therapeutic approaches in the treatment of diseases where mechanotransduction plays a key role. PDMS has been eliminated as a viable membrane option, a viable 96-well plate mold has been created, and most importantly, a scaffolding device able to stretch and hold a membrane has been created. Whilst future work should explore other membrane materials, we've paved a path for future efforts with this novel device. Our new Strain Scout has the potential for interdisciplinary collaboration and innovation in the field of biomedical engineering.

Materials and Methods

Designing Strain Scout Scaffolding and PDMS Mold

Parts were modeled using Autodesk Fusion 360 and exported as STL files to be 3D printed. For the mold specifically, a third-party 3D printing application, PreForm, was used as an interface between the print files and the 3D printer.

Printing Strain Scout Scaffolding

The Strain Scout Scaffolding was 3D printed in ABS plastic using a Fused Deposition Modeling (FDM) 3D printer, Stratasys F170. After printing the parts in ABS, they were sanded to make the components fit properly.

Printing the PDMS Mold Using SLA

A customized PDMS mold was printed out of Tough 1500, a Formlabs resin, in a Form 3 3D printer, which utilizes stereolithographic printing technology. After printing, supports were removed from the mold and it was sanded to create smooth surfaces across the mold. Extra material was removed from between the wells with a sharp blade. As the piece was being refined, the mold was rinsed in isopropyl alcohol with the Form Wash for 10 minutes at a time to remove the excess material dislodged from the scraping and sanding. Finally, the mold was cured in the Form Cure for one hour at 70 ºC.

Printing the PDMS Mold in ABS

The Fusion 360 mold model was printed in ABS with the University of Virginia's Rapid Prototyping Lab, which utilizes Fused Deposition Modeling (FDM) 3D printers, specifically a Stratasys F170.

Creation of PDMS Membrane

The SYLGARD™ 184 Silicone Elastomer Kit was utilized to create the PDMS mixture and was prepared according to previous procedures [13]. The elastomer and curing agent was mixed in several ratios by weight during experimentation: 5:1, 7:1, 10:1, 15:1, 20:1. The mixture was stirred for ten minutes and then desiccated in a desiccator to pop bubbles. Bubbles that remained in the mixture after desiccation were popped by poking them with the tip of a micropipette.

End Matter

Author Contributions and Notes

The authors declare no conflict of interest.
J.S. and M.K. made designs, J.S., M.K. and D.A. performed research, M.K. and D.A. performed experimentation, J.S. analyzed designs; and J.S., M.K. and D.A. wrote the paper.

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