Micro-Tug Device Fabrication as Platform for 3D Tissue Dynamical Construction and Magnetic Actuation

Zhuangyu Zhang

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Micro-Tug Device Fabrication as Platform for 3D Tissue Dynamical Construction and Magnetic Actuation

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Background

Tissue morphogenesis is an essential biological phenomenon describing tissue's geometrical change[2]. Mechanical forces play important roles in tissue morphogenesis. One of the examples is the activation of fibroblasts into myofibroblasts in response to mechanical forces in their environment, which includes the magnitude as well as the direction of the force. Another example is cardiomyocytes behave differently under the environment of hypertension.

In this study, I focused on fabricating and modifying the silicone-rubber-based platform that creates boundary conditions that control the geometry of engineered tissue.

First, I was looking for engineering improvements for fabricating the devices for tissue construction in a high-throughput manner. Second, I worked on modifying the device with magnetically responsive particles in order to generate stress, control the tissue geometry and morphogenesis, and mimic the environment of hypertension for cardiomyocytes. Third, I was involved in designing a static magnetic field-generating device to control the magnetically responsive platform in order to generate stress on the micro-pillar for tissue morphogenesis.

The goal of this project is to: 1) fabricate PDMS micro-tug devices at consistent success rates. These devices allow the tissue to grow to test mechanical properties under different growth conditions; 2) fabricate PDMS micro-tug devices with iron-containing pillars at consistent success rates. These devices allow the active control of stress to mimic different mechanical environments; and 3) produce a magnetic field generating device. This device works together with the iron-containing pillar micro-tug devices to generate different mechanical stresses.

In the past year in Huebsch Lab, I was involved in multiple experiments to 1) construct micro-tug devices, 2) use these devices as platforms for 3D tissue constructions, 3) quantify the tissue parameters through imaging and analysis, 4) modify the micro-pillars into magnetically responsive particles, 5) quantify the quality of the fabricated devices in order to study their mechanical properties, 6) design and fabricate larger scale devices for troubleshooting, and 7) design the prototype of the magnetic field generating device.

The first main project that I worked on involved micro-tug device fabrication. My mentor
has been making some sample devices by hydrogel casting, as shown in Figure 1.

*Figure 1. The Micro-Tug Devices with Micro-Pillars.* These figures were filmed with environmental scanning electron microscopy[2] from the top view of the device. There are three different types of designs: 2-pillar device, 4-pillar device, and 8-pillar device. The device properties are from anisotropy to isotropy.

There are three different types of devices: one with two pillars, one with four pillars, and one with eight pillars. Each micro-pillar on the device is designed to be straight up with a cover on the top, as shown in Figure 2[3]. In the left figure, the red part with the upward pillars is the “positive device,” and the white part with the tiny gaps is the “negative device.” The structure of the micro-pillars expects the tissue to grow around the pillars in both horizontal and vertical dimensions. Figure 3 shows the 2-pillar device dimensions with the unit in microns.

*Figure 2. The 2-Pillar Micro-Tug Device and the Micro-Pillars.*[3] These micro-pillars come from the base of the micro-tug device, having caps at the top to prevent tissue from getting out of the pillar. Tissues will be seeded at the bottom of the base, which would grow around the pillars to compact, generating stress between pillars that causes pillar bending. Such stress and compaction allows tissue to grow both horizontally and vertically. The right figure shows the Autodesk Inventor design of the micro-tug device as well as the micro-pillars with caps.
**Figure 3. The 2-Pillar Micro-Tug Device Dimensions.** The base plate is 1200 microns × 3260 microns large, with micro-pillars at the middle of the plate with a distance of 1000 microns between each other. The micro-pillars are 1125 microns tall with a diameter of 200 microns. There are also caps on the micro-pillars with a height of 125 microns and a diameter of 300 microns. There are also edges outside the base plate that are not counted in this dimension figure.

The previous methods of micro-tug device fabrication are by using casting with hydrogel\(^4\). However, this process has to be done in two days for each use. In the past semesters, I fabricated these micro-tug devices from 3D print models by surface chemistry onto Polydimethylsiloxane (PDMS) replica through fluoro-silane and parylene coating\(^1\) (see methods), which creates a higher throughput fabrication system with multiple times of usage for each device.

After the micro-tug device fabrication, NIH-3T3 fibroblast cells were seeded in these devices (see methods). NIH-3T3 cells have the ability to contract around the micro-pillars and the compaction of the tissue over time is expected\(^5\). Images were taken by Nikon during tissue growth from day 0 to day 4.

The transforming growth factor-beta (TGF-\(\beta\)) was treated as a comparison to the normal growing cell. On the one hand, TGF-\(\beta\) enhances the contractility of the tissue; on the other hand, it increases focal adhesion\(^6,7\). Two different treatments (TGF-\(\beta\) versus normal) of the cell were applied and compared.

To study the dynamics of these 3D tissues, the analysis of tissue images was conducted. By measuring, quantifying, and analyzing the parameters of 3D tissue constructions within the
fabricated devices, I attempted to understand the tissue compaction, the effects of the growth factor, and the micro-pillar deflection. These parameters include the horizontal area occupied by the tissue, the width of the occupied area, the length of the occupied area, the deflection of the micro-pillar, and other parameters in different dimensions.

Moreover, I was also involved in fabricating magnetic responsive micro-pillars. Unlike the passive platforms, these magnetic responsive elements can actively generate actuation by magnetic forces\(^{[8,9]}\). With the fabricated magnetic responsive elements in the pillars, the mechanical force can be applied to those micro-pillars by manually handling the magnetic tweezers near the pillar to provide a magnetic field\(^ {10}\). The magnetic sphere at the top of the pillar senses the magnetic contraction or repulsion, resulting in pillar bending and stress applied to the tissue\(^ {10}\). The current method is to fabricate each device manually (see methods). There is a new plan to first fabricate micro-pillars and then add iron particles to a batch of micro-pillars. This plan will be tested by using large-scaled device fabrication. More engineering improvements can be done in the future.

To actively generate stress on the tissue and achieve tissue morphogenesis, one can use magnetic tweezers near the pillar manually. However, this method is time-consuming and unreliable, and it is extremely hard to keep consistency in experiments. Therefore, there is a need for a device to generate magnetic fields to first consistently control the movement of iron-particle-containing pillars in the micro-tug tissue platform to achieve morphogenesis, and second be able to change such the field to apply different stresses in order to mimic different environments.
Methods

1. Micro-Tug Device Fabrication

The straightforward approach to fabricate these micro-tug device is through double molding. The purpose of the double molding process is to make PDMS positive devices from the 3D-print positive devices, as shown in Figure 4.

The main idea of double molding is to create agar negative devices from the 3D-print positive models, then fabricate PDMS positive devices from the agar negatives.

![Fabricated PDMS Micro-Tug Positive Devices](image)

*Figure 4. The Fabricated PDMS Micro-Tug Positive Devices.* The left part of the figure shows the 2-pillar devices, the right part of the figure shows the 4-pillar devices, and the bottom part of the figure shows the 8-pillar devices.

The first step of double molding is to make agar negatives off the 3D-print positive models. Procedures are:

[1] Mix 3g of agar with 200mL of water in the measuring glass;
[2] Microwave the solution until agar is fully dissolved and the solution is exactly boiled.
Observation is required during the entire microwave process to avoid overflowing.

[3] Add 2mL of 20% Triton-X to the mixed solution. For other amounts of the agar mixture or other concentrations of Triton-X, keep the overall concentration of Triton-X in the mixture at 0.2%. This step improves the final quality of the micro-pillars.

[4] Place the agar solution on the top of the 3D-print model in the dish to cover the positive surface. Pipetting the solution to get rid of bubbles. Then, wait until the agar solution cools down and gets more solidified.

[5] After the solution becomes more solidified, place the dish in the 4°C fridge for about 20 minutes to allow the complete solidification of agar solution.

After these steps, a dish of the 3D-print positive model with contact to the full solidification agar negative device is produced. Then, carefully remove the 3D-print model from the solidified agar.

The next step is to make PDMS positive devices off the agar negatives. Procedures are:

[1] Mix 30g of Sylgard 184 PDMS and 3g of curing agent in a plate. Then place the plate in the vacuum desiccator to degas PDMS. It usually takes 30-60 minutes to get rid of all the bubbles. Also, it would be helpful to frequently open the vacuum desiccator to allow the partial removal of the bubbles from the PDMS.

Note that for other amounts of the PDMS, keep the amount of curing agent at 10% of the amount of PDMS.

[2] After getting rid of the bubbles, carefully pour the cured 184 PDMS onto the surface of the agar negatives in the agar dish. Be careful to rise the pouring altitude so that the pouring flow diameter can be small enough to fill the small micro-pillar negative gaps.

[3] Place the dish in the vacuum desiccator to degas PDMS and to allow PDMS to cure. This process usually takes more than 3 hours. It would be helpful to frequently open the vacuum desiccator to allow the partial removal of the bubbles before leaving it in the vacuum desiccator overnight.

When the PDMS is fully cross-linked, carefully remove the PDMS from the agar base or
melt the agar in the oven. Now, the positive PDMS device is produced from the initial 3D-print positive model. Some example devices are shown in Figure 5.

Figure 5. Examples of Positive PDMS Micro-Tug Devices. There is a mix of all 2-pillar, 4-pillar, and 8-pillar devices. These devices can be cut as sections from the batch, and unsuccessful devices can be discarded.

2. PDMS Reusable Template

The double molding process allows straightforward fabrication. Nevertheless, agar materials are fragile; Moreover, in order to make the device that can be reusable, it is more effective to fabricate the PDMS negative template instead of the agar template. However, the positive PDMS device cannot be used directly as a template to make PDSM off PDMS because the PDMS material would cross-link together. Thus, it is important to conduct the fluorosilane treatment in order to make reusable PDMS templates.

The first step is to conduct the plasma treatment to create silanol, which then can bind to
other silanes in the PDMS, making it more reactive so that it can better bind with the fluorosilane. After that, the fluorosilane is treated in the PDMS positive device so that it helps the PDMS to become hydrophilic. The fluorosilane treatment usually takes about 48 hours.

After that, a plate of the cured 184 PDMS is poured on the surface of the fluorosilane-treated PDMS positive device in order to make the PDMS negative device. Finally, another plate of the cured PDMS is poured on the surface of the PDMS negative device to get the final product. During these processes, the dish should always be placed in the vacuum desiccator to degas PDMS, and the pouring process should always be careful to rise the pouring distance so that the pouring flow diameter can be small enough to fill the gaps.

As the result, the final PDMS positive product would be used for tissue construction. Because PDMS is usually hydrophobic, it is really easy to pull out the final PDMS positive product from the PDMS negative devices. That said, those PDMS negative devices made in the second last step can be reused for future micro-tug device fabrication.

3. Fabrication Success

To check the fabrication success rate, the dissection microscope was used to capture pictures with focal views on the bottom and the top plate. The top focal view would indicate whether all the pillars are formed, while the bottom focal view would indicate if the micro-pillar deflection happens. Figure 6 shows the example of the top and the bottom focal views.

![Figure 6. The Top Focal View and the Bottom Focal View of the 4-Pillar Micro-Tug Device.](image)
The left figure of the top focal view indicates that the micro-pillars are well-formed with caps. The right figure of the bottom focal view indicates that these four pillars are formed straight up without deflections.

4. Seeding the Tissue in the Micro-Tug Device

To seed the tissue in the device, the first step is to prepare the sterilized well plate with micro-tug devices. Procedures are:

[1] Glue the micro-tug devices on the well plate by 184 PDMS, then let it cross-links. The well plate can be the normal 6-wall plate. However, to make PDMS material better mix with the PDMS material, the well plate can be fabricated by PDMS through double molding as shown in Figure 7.

![Figure 7. The Fabricated PDMS Well Plates](image)

*Figure 7. The Fabricated PDMS Well Plates.* The PDMS micro-tug devices can be glued on these PDMS well plates by PDMS cross-linking. These well plates are also fabricated by double molding from the 3D-print models.

[2] Prepare 0.3% poloxamers 188 in Dulbecco's phosphate-buffered saline (dPBS);
[3] Fill the sterile well plate with 0.3% Pluronic, then let it sit for 20 minutes;
[4] After sitting for 20 minutes, aspirate the Pluronic solution; Rinse 3 times with dPBS and
sit for 10 minutes each time.

After preparing the well plate, passage the cell and centrifuge the NIH-3T3 fibroblast live cells in the desired amount. Then calculate all the liquid amounts and prepare liquids in the cool-downed device plate. After that, add cells and collagen, then add some NaOH. Procedures are:

[1] Prepare collagen and well plate: 1M NaOH, N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES buffer), dPBS and collagen tubes on sterile ice.

[2] Start to passage the cells: first use dPBS to rinse cells in the flask; then aspirate the liquid and add 0.5% trypsin before putting the flask into the 37°C incubator. This allows the cells to detach from the surface.

[3] After leaving the flask for 3-5 minutes, take out the flask and add some media to the tube. Centrifuge the tube by 300RCF for 5mins. Then aspirate the media.

[4] Count the cells and calculate the amount of needed liquids.

[5] Cell seeding: add 2μL of HEPES, 38μL of dPBS, 50μL of collagen, and then 1μL of NaOH. After that, seed proper amounts of cells in the micro-tug device.

When finishing the cell seeding procedures, remember to add media in the well plate. To compare the effects of the growth factor, one plate used normal media and the other plate used the media with TGF-β.

5. Quantification of Tissue Parameters Using ImageJ

After seeding the cell, these NIH-3T3 fibroblast live cells were placed in two-pillar, four-pillar, and eight-pillar devices for two days. A Nikon microscope was used to capture the pictures after day one and day two for the high density of the tissue. Only day-one pictures were captured for low-density tissues. Both high-density and low-density tissues had another comparing group treated with TGF-β.

ImageJ was used to measure the tissue parameters, including the horizontal area occupied by the tissue, the width of the occupied area, and the length of the occupied area. ImageJ was also
used to measure the micro-pillar deflection. This can be done by measuring the positions and displacements among each center or mass center of the micro-pillars on different days of tissue growth.

6. Magnetic Micro-Pillar Fabrication

The micro-tug device with magnetic micro-pillar fabrication involves two main parts: 1) soft magnetic PDMS pillar fabrication and 2) hard PDMS plate fabrication. The purpose of the first step is to fabricate the soft micro-pillar with iron particles, while the second step connects the iron-containing micro-pillar to the entire micro-tug device. Because of the anisotropy and unidirectional control, only two-pillar devices were fabricated, and a new design that has a higher potential deflection rate was used.

The first step is to fabricate the micro-pillars with magnetically responsive particles. Procedures are:

[1] Mix 0.4g of Sylgard 527 PDMS A and 0.4g of Sylgard 527 PDMS B in one plate A, and make sure the mass of soft PDMS A to B is in the one-to-one ratio.

[2] Prepare some Sylgard 184 hard PDMS mixed with the curing agent in another plate B (for example, 30g of 184 PDMS and 3g of the curing agent, the ratio is one to ten, and the excess can be used later). Add 0.2g of the 184 hard PDMS mixture in the 527 soft PDMS mixture in Plate A. Make sure the mass of the 184 hard PDMS to the 527 soft PDMS is in the one-to-four ratio.

[3] Add 0.25g of 200 mesh iron powder into the PDMS mixture in Plate A. Make sure the mass of the iron powder to the total PDMS is in the one-to-four ratio. Mix them together.

[4] Place both Plate A and B in the vacuum desiccator to degas them.

[5] Prepare the agar negatives. Then carefully drop the PDMS-Iron mixture from Plate A into the desired negative “holes” of the agar. Be careful to keep the other areas clean (do not leave any PDMS outside of the micro-pillar negatives).

[6] Place the agar dish in the vacuum desiccator to degas them. Add more drops when
necessary. Place some magnetic fields around the agar dish to depolarize the iron.

[7] Put the agar plate in the oven to allow PDMS to cross-link. It worked well when leaving the plate in the 37°C oven overnight.

[8] After the cross-link of the magnetic micro-pillars, carefully pour the 184 PDMS onto the surface of the agar negatives in the agar dish. Place the dish in the vacuum desiccator to degas PDMS and to allow PDMS to cure.

7. Large-Scale Device Fabrication

The current method to fabricate iron-containing pillars is to add iron particles manually into the device negatives. There is a new plan to first fabricate micro-pillars and then add iron particles to a batch of micro-pillars. However, this plan could not work with the current 2-pillar device design with edges. Therefore, I designed and fabricated some large-scale devices to troubleshoot potential issues. Some dimensions are shown in Figure 8 and Figure 9.

**Figure 8. A Large-Scale Device Design with All Dimensions Doubled.** All the dimensions (in microns) are double in this design, including the base plate dimensions, the distance between two pillars, the distance between the pillar and the edge of the base plate, the height and diameter of the micro-pillars, and the height and diameter of the pillar cap.
Figure 9. A Large-Scale Device Design with only Pillar Diameter Doubled. The unit is in microns. Only the diameter of the micro-pillars and pillar caps are doubled, and the rest of the dimensions remain the same.

While these designs did not keep the original bending moment, it should be noted in the later design that:

\[ M \propto \text{Height}^3, \text{ and } M \propto \frac{1}{\text{Diameter}^4} \]

8. Magnetic Field Generating Device

There is a need for a device to generate magnetic fields in order to consistently control the movement of the magnet-containing pillar in the micro-tug tissue platform to examine the effects of mechanical cues on tissue cells.

This device should be able to generate a consistent magnetic field to keep the stress consistent during imaging or measurement. This device should also be able to adjust different magnetic field strengths depending on the need or the stiffness of different iron-particle-containing PDMS posts. The advantage of using this device is that such magnetic attraction is free of contamination for the tissue.

Therefore, the need is a magnetic field-generating device, which can be able to stay in one place to give a constant magnetic field, but also able to move back and forth to change the magnetic field strength.

To produce this device, the majority of the device can be fabricated by 3D-prints. The
magnetic source could come from permanent magnet, electromagnet, or a constant magnetic field generating device from the market. The design is shown in Figure 10 with Autodesk Inventor Graphics shown in Appendix A. A user manual is provided in Appendix B.

**Figure 10. The Designed Device.** This device consists of five main components: (1) Main Base holder (Part A): provides a general base structure and supports other components. (2) Base holder (Part B): another half the base plate, which can be attached to the part A. (3) Magnet basket: for holding the magnets and sliding on the base plate. (4) The Screw: The screw can move the basket along the roads with high accuracy. (5) Fixation rods: These two identical cylindrical rods provide the track for the basket to slide.

The fundamental concept of this design is to have a strong magnet installed in the magnet basket as shown in Figure 11a, which can be accurately slid on the base holder when rotating the micro-manipulator knob. By changing the distance between the magnet and iron-containing micro-pillar, the magnetic force exerted on the iron-containing micro-pillar becomes adjustable; thus, changing the force exerted on the iron-containing micro-pillar and actively applying the mechanical cues to the tissue (Figure 11b-e). The key advantage of utilizing magnetic force is to
avoid contamination, and the key success of the device is providing researchers with a reliable and low-cost tool for applying tissue morphogenesis with minimal variance.

Figure 11. Qualification View of Device Prototype in Applying Stresses to Iron-Containing Micro-Pillar: (a) general setup of the device; (b) when the magnet is close to the micro-pillar platform, the micro-pillar was bent; (c) when the magnet is far away from the micro-pillar platform, the micro-pillar was not bent; (d) an enlarged view of (b), showing the bending of iron-containing micro-pillars under magnetic force; (e) an enlarged view of (c), showing the non-bent iron-containing micro-pillars.

9. Calculation of Stress in Magnetic Actuation

Because there are two micro-pillars in the 2-pillar device, the magnetic actuation would only need one micro-pillar to contain iron particles. The total stress applied on the tissue could be calculated based on the material and deflection of the other micro-pillar without the iron particles. The stress can be calculated by the following equation[12]:

\[
f = \frac{\Delta}{\left\{ \frac{l^3}{3EI} + \frac{d^2(l + \gamma)l}{4EI} \right\} + \frac{l^2}{2EI}(h - l)}
\]

where f represents the force applied, \( \Delta \) is the displacement, I is the area moment of inertia, \( \gamma \)
is the Poisson’s ratio for PDMS, \( l \) is the pillar length in the direction of force exerted, \( d \) is the pillar diameter, \( h \) is the pillar height, and \( E \) is the PDMS Young’s Modulus.

For ease of usage, a Python-based user interface as shown in Figure 12 was generated by Zhongli Tong for instantly calculating the force experienced by the microtissue by using the equation above, with its python code available in Appendix C.

![Python User Interface for Calculating the Mechanical Force on the Micro-Pillar](image)

**Figure 12. Python User Interface for Calculating the Mechanical Force on the Micro-Pillar:** Users can give the input, and the algorithm will automatically return the calculated force for users.
Results & Discussion

1. Micro-Tug Device Fabrication

A total of 13 sets of the pre-fluorosilane-treated PDMS positive devices was made during the semester, each set of devices contains 192 walls of two-pillar devices, 192 walls of four-pillar devices, and 192 walls of eight-pillar devices. Among 13 sets of pre-fluorosilane-treated devices, 3 sets of the devices was checked and ready for the fluorosilane treated; among those 3 sets of the devices, 2 sets of devices underwent the fluorosilane treatments and 1 set of post-fluorosilane-treated PDMS positive devices was produced.

The success rate from the 3D-print positive model to the pre-fluorosilane-treated PDMS positive devices is shown in Figure 13; The success rate from the well-formed chosen pre-fluorosilane-treated PDMS positive devices to the final products is shown in Figure 14.

![Success Rate of Double-Molding Products](image)

*Figure 13. Fabrication Success Rates of Double Molding.* Success rates from the 3D print models to the pre-fluorosilane-treated PDMS positives for different designs. The standard deviation comes from different batches.
Figure 14. Fabrication Success Rates of Fluorosilane Devices. Success rates from post-fluorosilane-treated PDMS positive devices to the final products. The standard deviation comes from different batches.

2. The Effects of Triton-X

In the double molding procedure 3, 20% Triton-X was added into the agar solution before the solidification step. To investigate the effects of Triton-X, I compared 4 different sets of fabricated micro-tug devices with and without the addition of Triton-X under the same environment. The PDMS positive success rate is shown in Figure 15.

Figure 15. The Use of Triton-X Comparison. Comparing the pillar quality with and without
Triton-X for 2-pillar device fabrication. The standard deviation comes from different batches.

Besides, through observation, the addition of Triton-X helps to produce the firmer and straighter micro-pillars in 2-pillar devices; the device becomes very sticky in the absence of Triton-X.

Furthermore, in 4-pillar and 8-pillar devices, the success rate of double molding is 0% without the addition of Triton-X in the agar mixture.

3. Quantification of Tissue Parameters Using ImageJ

Figure 16 shows the NIH-3T3 cell horizontal growth area in the fabricated devices with versus without the TGF-β treatment in the following three conditions: high density of the cell on day one, high density of the cell on day two, and low density of the cell on day one.

![Figure 16A. 2-Pillar Device Area Compaction: Control vs TGF-β](image-url)
Figure 16B. 4-Pillar Device Area Compaction: Control vs TGF-β

Figure 16C. 8-Pillar Device Area Compaction: Control vs TGF-β
Likewise, Figure 17 shows the NIH-3T3 cell horizontal growth width in the fabricated devices with versus without the TGF-β treatment in the following three conditions: high density of the cell on day one, high density of the cell on day two, and low density of the cell on day one.

Note that for the 8-pillar devices, the horizontal width and length measurements are the same as the horizontal diameter measurements.

Figure 17A. 2-Pillar Device Width Compaction: Control vs TGF-β

Figure 17B. 4-Pillar Device Width Compaction: Control vs TGF-β
Likewise, Figure 18 shows the NIH-3T3 cell horizontal growth length in the fabricated devices with versus without the TGF-β treatment in the following three conditions: high density of the cell on day one, high density of the cell on day two, and low density of the cell on day one.

**Figure 18A. 2-Pillar Device Length Compaction: Control vs TGF-β**

**Figure 18B. 4-Pillar Device Length Compaction: Control vs TGF-β**
4. Magnetic Micro-Pillar Fabrication

A total of 6 sets of magnetic 2-pillar devices were fabricated. Some example devices are shown in Figure 19.

**Figure 19. Examples of Iron-Containing Micro-Pillar Devices.** The dark black parts are iron particles. Some devices have both pillars containing iron particles.
The following rubric was used to score the quality of magnetic micro-pillars:

[1] Score of 0: There are bubbles in the device or no micro-pillars are formed.

[2] Score of 1: Micro-pillars are formed.

[3] Score of 2: There are iron particles presented in the micro-pillar.


[5] Score of 4: The quality between 3 and 5; Can be attracted by magnet but iron particles are not evenly distributed at the top of the micro-pillar.

[6] Score of 5: The pillar can be attracted by the magnet to deflect, and the iron particles are evenly distributed at the top of the micro-pillar.

Table 1 indicates the average scores for the six sets of fabricated devices.

The problem of the fabrication is mainly because of bubbles. There is also a problem due to the different compositions of the pillar (soft PDMS) and the base plate (hard PDMS). These problems would be tested by large-scale devices.

Table 1. Magnetic Micro-Pillar Fabrication Scoring

<table>
<thead>
<tr>
<th>Set</th>
<th>Average Score</th>
<th>Main Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>The iron particles are presented on the edges.</td>
</tr>
<tr>
<td>2</td>
<td>2.72</td>
<td>The iron particles are not at the top of the pillar.</td>
</tr>
<tr>
<td>3</td>
<td>2.17</td>
<td>There are many bubbles in the device.</td>
</tr>
<tr>
<td>4</td>
<td>3.08</td>
<td>There are some bubbles in the device.</td>
</tr>
<tr>
<td>5</td>
<td>3.89</td>
<td>There are some bubbles in the device.</td>
</tr>
<tr>
<td>6</td>
<td>4.22</td>
<td>The vacuum desiccator sucks out some soft pillars entirely.</td>
</tr>
<tr>
<td>7</td>
<td>4.31</td>
<td>Most devices look good. Occasion bubbles exist.</td>
</tr>
</tbody>
</table>

5. Large-Scale Device Fabrication

I first fabricated both the double-scaled device and the double-diameter device each model twice. Both double-diameter devices formed well, but most double-scaled devices did not. There
may be some reasons for the double-scaled device not forming well:

(1) No base plate. Because I did not create a lower plate of edge, peeling up the 3D print models may hurt the agar negatives.

(2) The bending moment has changed because I did not follow the ratio of $\frac{\text{Height}^3}{\text{Diameter}^4}$ when scaling for both devices.

(3) The micro-pillars were too tall, and it would be too deep for PDMS to enter the negative devices in the double-scaled model.

After the second trial, I added new PDMS onto the used agar, after one PDMS product has been removed from the agar and added some new PDMS onto it. The resulting device has a problem with degassing. This may be because the temperature of agar was still high after getting out of the oven, while the new PDMS is relatively cold.

After melting the agar, no PDMS can be found. Therefore, for those pillars not formed tall or deep enough in the previous cases, they are not stuck in the agar but just not formed.

6. Magnetic Field-Generating Device Verification

The primary design specification for the magnetic field-generating device is the capacity to apply forces on the iron-containing micro-pillar, leading to the bending of the micro-pillar, through a non-contact way.

To ensure this, the ability of the 3D printed prototype to achieve the desired bending moment was first tested and quantified. The 3D printed device demonstrated success in leading to bending of the iron-containing micro-pillar for the application of tissue morphogenesis as shown in Figure 20.

Other specifications are also examined: The device should be highly effective in preventing contamination for bio-tissue research. Utilizing this device eliminates the need to open the six-well plate throughout the entire experiment. The device utilizes magnetic force to apply mechanical cues without any physical contact to the tissue inside the six-well plates or other types of contamination-free container, ensuring a contamination-free environment. As a result,
the device successfully demonstrated its capacity to prevent contamination.

Besides, the design drawings by Autodesk Inventor are shown in Appendix A and are ready to be printed. Utilizing a Form 3 printer with resin, each device takes approximately 15 hours to print, while using PLA material can be even faster.

Assembly of the device is simple and can typically be completed within a few minutes by following the user manual provided in Appendix B.

![Figure 20. Quantification of Bending Displacement of Pillar's Cap in three micro-pillars with Different Amounts of Iron Embedded:](image)

it was observed that when the distance between the magnet and pillar becomes smaller, the bending of the micro-pillar becomes more significant.

Furthermore, to ensure the reasonable long duration of usage, the durability of this device has been examined using two methods:

Firstly, the 3D-printed device was dropped from a height of 50 centimeters onto a table five times to examine if any components are broken. The test results showed that for prototypes printed with PLA, while some components may become disassembled, these components can be reassembled without impacting the device’s usability. No significant glass-like breakage, which may lead to potential harm, was observed in PLA-printed device. However, the prototype printed with resin is broken apart immediately after falling to the ground.

Secondly, the device usage durability was evaluated. Two hundred times of applying tissue morphogenesis were simulated by rapidly moving the magnet basket back and forth 200 times.
After completing this simulation, the device's ability to attract the iron-containing micro-pillar was tested and was compared with its performance before the simulation. For both the resin and PLA printed device, even though there are some abrasions on the device structures, they can still be used for holding the magnet and applying forces leading to the bending of the iron-containing micro-pillar.

Moreover, both the resin and PLA-printed prototypes were tested for their waterproof capacity. After immersing the 3D-printed prototypes in the water for 3 minutes, no significant structural changes were observed in the device, and the usability was also not affected. Additionally, since the device is mainly used for force generation on tissues seeded in the six-well plates or other types of contamination-free container, it will not be placed in an overly humid place; Thus, a general waterproof ability is sufficient.
Conclusion & Acknowledgments

The success rate of double molding is always higher than 80%. The fluorosilane treatment process involves careful pouring of the PDMS so that the success rate of the final reusable device is around 50% to 80%. It is notable that four-pillar devices have a higher success rate than the two-pillar and eight-pillar devices.

Besides, the addition of Triton-X in the agar solution, which plays a role in lowering the surface tension barrier, increases the success rate for device fabrication through double molding. For four-pillar and eight-pillar devices, it is essential to add Triton-X for the device formation. The absence of Triton-X may result in a zero success rate for device fabrication.

The imaging data indicates that the tissue keeps compacting over time, while TGF-β plays a complex role that increases the focal adhesion in general. Further measurements of different dimensions should be conducted. More robust blockage of cell adhesion to the PDMS is needed.

In the magnetic iron-containing micro-pillar fabrication, though the overall quality in improving through practice, it is not effective to manually add each magnetic pillar drop. Since agar materials are fragile, it would be helpful to introduce PDMS templates instead of agar negatives to fabricate magnetic micro-pillars.

During large-scale device fabrication, there are problems with designing parameters and degassing the PDMS material. Future directions may include designing some new 3D print models with lower plate edges, following the original bending moment, and keeping the micro-pillar height smaller. A potential future direction is to print some models without the device wells but only the pillars and attempt to fabricate these large-scale devices to troubleshoot and prepare for the new plans of global magnetic pillar fabrication.

To make it easier and more consistent to apply morphogenesis to tissue, the magnetic field generating device is needed and I have been working on this project with my colleague Zhongli Tong. We designed the device and verified the device capability to apply static magnetic field on the iron-containing pillars.

Overall, this is a productive experience and I have been learning a lot in Huebsch Lab.
At the end, I want to thank to Dr. Huebsch for accepting me into the lab, helping me to choose research topics, and giving me feedback on the progress of experiments.

Besides, thanks to my graduate mentor Ghiska Ramahdita who helped me from the beginning of the project in the past semesters and instructed me on the procedures of device fabrication as well as tissue seeding processes.

I would also like to express my special thanks of gratitude to my colleague Zhongli Tong who worked on the magnetic field generating device project with me together.
Appendix

1. Appendix A: Autodesk Inventor Graphics

Figure 21. The Mechanical Drawing of the Base Hold (Part A): a critical component that functions as the primary support for other components.
**Figure 22. The Mechanical Drawing of the Base Holder (Part B):** This component serves as the second half of the base plate and can be assembled together with the base plate.
Figure 23. Mechanical Drawings of Magnet Basket (for holding the magnet and slide along the base plate)
Figure 24. Mechanical Drawings of the Screw (designed to move the basket with high precision)
Figure 25. Mechanical Drawing of the Cylindrical Fixation Rods Component Design: the fixation rod serves as a track for the basket to slide, ensuring smooth movement of magnet basket.

User Manual for the Magnetic Field-Generating Device

I. Introduction

This is the user guide for the Magnetic Field-Generating Device, which provides step-by-step instructions for the assembly, operation, and maintenance.

The magnetic field-generating device is designed to apply mechanical forces to biological tissues in a contamination-free manner through magnetic force interactions. By adjusting the distance between the magnet and the iron-containing micro-pillar, users can control the stress that leads to bending of the iron-containing micro-pillar and actively apply mechanical cues to the microtissue implanted surrounding two micro-pillars.

II. Components

This device consists of five main component:

1. Base holder (Part A): provides a general base structure and supports other components.
2. Base holder (Part B): another half the base plate, which can be attached to the part A
3. Magnet basket: for holding the magnets and sliding on the base plate.
4. The Screw: The screw can move the basket along the roads with high accuracy.
5. Fixation rods: These two identical cylindrical rods provide the track for the basket to slide.

III. Assembly

To assemble the device, follow the following steps:

1. Place the base plate (part A) on a stable, flat surface.
2. Insert the two cylindrical fixation rods into the designated side holes on the base plate (part A), and then insert the screw into the base plate (part A).
3. Install the magnet basket and ensure the two holes in the basket can slide smoothly through
the fixation rods.

(4) Assemble the base plate (part B) to the base plate (part A), aligning the holes on base plate (part B) with the rods and screw.

IV. Operation

To operate this adjustable magnetic field-generating device, follow these steps:

(1) Place the plate (6 well-plate or other sizes of plates) containing the microtissue platform on the designated area of the base plate.

(2) Ensure the magnet basket is at the initial position (far side from the microtissue platform) so that there is no magnetic force applied to the iron-containing micro-pillar at the beginning.

(3) Then, place the magnet in the magnet basket.

(4) Rotate the screw to move the magnet basket along the cylindrical fixation rods, to adjust the distance between the magnet and the iron-containing micro-pillar.

(5) Monitor the stress experienced and the bending moment of the iron-containing micro-pillar.

(6) The force encountered by the tissue can be calculated based on the mechanical profile of the non-iron-containing micro-pillar. This can be calculated by using the software provided.

V. Disassembly

To disassemble the device:

(1) First, dis-attach the base holder (part B) from the base holder (part A).

(2) Then, get rid of the magnet basket, followed by disassembling of cylindrical fixations rods, and the screw from the base holder (part A).

VI. Reassembly

To reassemble the device, follow the assembly instructions outlined in the Assembly Section.
VII. Maintenance

Regular maintenance and cleaning of the device are crucial for its long-term usage. Below are some suggestions for the device’s maintenance:

(1) Clean the device with a soft napkin for cleaning after each time of usage.
(2) Use the lubrication oil when experiencing the sliding resistance on the magnet basket.
(3) The device can be disassembled, and any component that looks abrasives can be substituted.
3. Appendix C: Python Code for Software

```python
import tkinter as tk

def calculate_output():
    try:
        displacement = float(entry_input1.get())
        inertia = float(entry_input2.get())
        ratio = float(entry_input3.get())
        length = float(entry_input4.get())
        diameter = float(entry_input5.get())
        height = float(entry_input6.get())
        modulus = float(entry_input7.get())
        Part1 = (length**3)/(3*modulus*inertia)
        Part2 = ((diameter**2)*(length+ratio)*length)/(4*modulus*inertia)
        Part3 = ((length**2)/(2*modulus*inertia))/(height-length)
        output = (displacement)/(Part1+Part2+Part3)
        output_label.config(text=f"Output: {output:.2f}"),
    except ValueError:
        output_label.config(text="Invalid input. Please enter numbers only!"

root = tk.Tk()
root.title("Calculator for Mechanical Force Experienced by Microtissue")

label_input1 = tk.Label(root, text="Please Enter Pillar Head's Displacement (m):")
entry_input1 = tk.Entry(root)
label_input2 = tk.Label(root, text="Please Enter Area Moment of Inertia (Kg·m^2):")
```

entry_input2 = tk.Entry(root)

label_input3 = tk.Label(root, text="Please Enter PDMS's Poisson's Ratio:")

entry_input3 = tk.Entry(root)

label_input4 = tk.Label(root, text="Please Enter Micropillar's length (m):")

entry_input4 = tk.Entry(root)

label_input5 = tk.Label(root, text="Please Enter Micropillar's diameter (m):")

entry_input5 = tk.Entry(root)

label_input6 = tk.Label(root, text="Please Enter Micropillar's height (m):")

entry_input6 = tk.Entry(root)

label_input7 = tk.Label(root, text="Please Enter Young’s Modulus of PDMS Material (Pa):")

entry_input7 = tk.Entry(root)


calculate_button = tk.Button(root, text="Calculate", command=calculate_output)

output_label = tk.Label(root, text="The Force Experienced by Microtissue:")

label_input1.grid(row=0, column=0, padx=(10, 0), pady=(10, 0))
entry_input1.grid(row=0, column=1, padx=(0, 10), pady=(10, 0))

label_input2.grid(row=1, column=0, padx=(10, 0), pady=(10, 0))
entry_input2.grid(row=1, column=1, padx=(0, 10), pady=(10, 0))

label_input3.grid(row=2, column=0, padx=(10, 0), pady=(10, 0))
entry_input3.grid(row=2, column=1, padx=(0, 10), pady=(10, 0))

label_input4.grid(row=3, column=0, padx=(10, 0), pady=(10, 0))
entry_input4.grid(row=3, column=1, padx=(0, 10), pady=(10, 0))

label_input5.grid(row=4, column=0, padx=(10, 0), pady=(10, 0))
entry_input5.grid(row=4, column=1, padx=(0, 10), pady=(10, 0))

label_input6.grid(row=5, column=0, padx=(10, 0), pady=(10, 0))
entry_input6.grid(row=5, column=1, padx=(0, 10), pady=(10, 0))
label_input7.grid(row=6, column=0, padx=(10, 0), pady=(10, 0))
entry_input7.grid(row=6, column=1, padx=(0, 10), pady=(10, 0))

calculate_button.grid(row=7, column=0, columnspan=2, pady=(10, 0))
output_label.grid(row=8, column=0, columnspan=2, padx=10, pady=(10, 0))

root.mainloop()

# The python tkinter toolkit was learned from the following websites[13-15].
References


