Chapter 18

Fabrication and Mechanical Properties Measurements of 3D Microtissues for the Study of Cell–Matrix Interactions

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Abstract

Cell interactions with the extracellular matrix (ECM) are critical to cell and tissue functions involving adhesion, communication, and differentiation. Three-dimensional (3D) in vitro culture systems are an important approach to mimic in vivo cell–matrix interactions for mechanobiology studies and tissue engineering applications. This chapter describes the use of engineered microtissues as 3D constructs in combination with a magnetic tissue gauge (μTUG) system to analyze tissue mechanical properties. The μTUG system is composed of poly(dimethylsiloxane) (PDMS) microwells with vertical pillars in the wells. Self-assembled microtissues containing cells and ECM gel can form between the pillars, and generate mechanical forces that deform the pillars, which provides a readout of those forces. Herein, detailed procedures for microfabrication of the PDMS μTUG system, seeding and growth of cells with ECM gels in the microwells, and measurements of the mechanical properties of the resulting microtissues via magnetic actuation of magnetic sphere-tagged μTUGs are described.

Key words Cell–matrix interactions, Mechanobiology, Engineered microtissues, Microfabrication, Magnetic actuation

1 Introduction

Critical components of a cell's surfaceome are the receptors and transmembrane proteins that determine and control its adhesive and mechanical interactions with the surrounding extracellular matrix (ECM) [1–3]. These interactions are coordinated, and regulate a wide range of cellular functions [4–7]. Increasingly, it has been recognized that these interactions cannot be faithfully reproduced in two-dimensional cell culture, and there is a rapidly developing need for approaches that mimic more realistically the in-vivo cellular environment [8, 9].

This chapter describes use of a 3D magnetic microfabricated tissue gauge (μTUG) system that can be used to study cell-matrix interactions in engineered three-dimensional microtissue constructs

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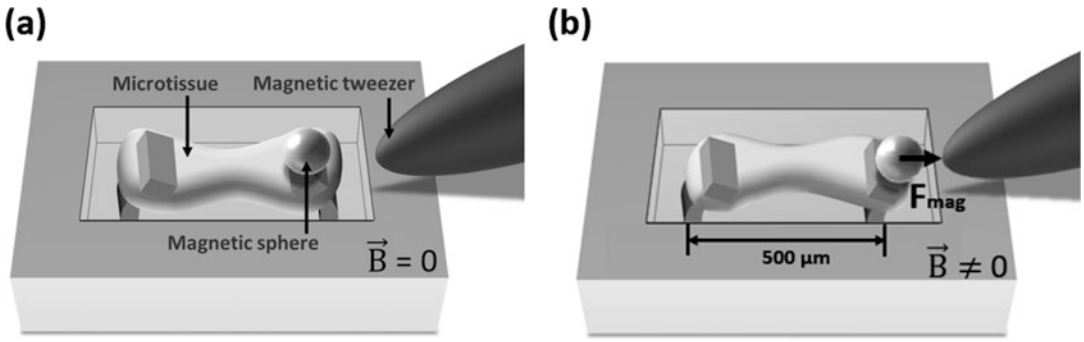

Fig. 1 Schematic of microtissue stretching using a magnetic tweezer. (**a**) Microtissue suspended between two flexible PDMS micropillars that are deflected by the microtissue's contractile force. (**b**) When a magnetic field is generated by the magnetic tweezer, a magnetic force F_{mag} is applied to the magnetic sphere bonded on the top of one of the pillars, and strains are exerted on the microtissue

[10–16]. The essence of the magnetically actuated microfabricated tissue gauge (μTUG) system is shown in Fig. 1. Cell-laden collagen gels are formed within poly(dimethylsiloxane) (PDMS) microwells that contain vertical PDMS pillars in the wells [10]. The microwells have typical dimensions 800 μm × 400 μm by 200 μm deep, and can contain 100–1000 cells each, depending on the application. As cells contract the collagen gels, the gels remain anchored by the pillars. This results in controlled self-assembly of cell–matrix "microtissues" suspended between the pillars (Fig. 1) [10, 11]. As the pillars are elastic, the forces of contraction of these microtissues can be monitored via the pillars' deflections. By incorporating magnetic material in the pillars, forces can be applied to the tissues [12–14, 17] to monitor tissue stiffness over time or to measure the dynamic response of the cells to external mechanical perturbations [15]. Arrays with >100 μTUG microwells in a standard P35 culture dish (25 per cm^2) can readily be produced, allowing multiple identical samples or samples with systematically varying properties to be prepared efficiently.

Procedures for fabrication, tissue seeding and growth, and tissue mechanical properties measurements are described in this chapter. Examples are drawn from work on fibroblast and smooth-muscle cell populated tissues, but the methods described herein can readily be extended to other cell types. This approach will also be useful for the evaluation of gene mutations of cell surface proteins or extracellular matrix components necessary for cell interactions and structural fidelity of tissues. The methods described in this section encompass several distinct processes. In Subheadings 3.1 and 3.2, we describe fabrication of the PDMS μTUG device arrays. "Master" versions of the devices are first produced in a thick photoresist on silicon wafers, using multilayer

photolithography techniques. The PDMS μTUG devices are then made via replica molding from the masters. Briefly, mirror inverses of the masters are cast in PDMS from the masters, and then these "negative molds" are used to cast the actual PDMS devices, which are thus exact copies of the masters. As the original masters are somewhat fragile, a replica-molding approach is also described to make more durable copies of the masters in plastic (Smooth-Cast), which can be used repeatedly without degradation. In Subheadings 3.3–3.5, we describe creation and growth of microtissues in the μTUG devices. This includes passivation of the devices' surfaces to prevent unwanted cell adhesion, preparation of the cell/ECM solutions that will make up the microtissues, insertion of these solutions into the microwells, and culture techniques to form and sustain the microtissues. In Subheadings 3.6 and 3.7, we describe data acquisition with the microtissue system. In Subheading 3.6, we discuss basic contractility measurements, wherein one measures the collective developed force produced by each microtissue by optically monitoring the deflections of the flexible pillars between which the microtissues are suspended. In Subheading 3.7, we describe approaches to measure the full mechanical properties of the microtissues via stress–strain measurements enabled by dynamic stretching of the tissue with magnetically applied external forces. Finally in Subheading 3.8, we describe approaches to analyze the images resulting from the above protocols to quantify physical properties of the microtissues. Examples described include the microtissues' developed force during tissue formation, stress–strain relations, and active cellular responses to applied forces.

2 Materials

2.1 Equipment and Instruments

1. Contact mask aligner (Model 200; Optical Associates, San Jose, CA, USA) or equivalent.
2. Programmable spin-coating system like the WS-400B-NPP-Lite system (Laurell Technologies Corporation, North Wales, PA, USA).
3. Vacuum desiccator capable of achieving $P = 25$ Torr pressure (Bel-Art 230 mm or equivalent).
4. Vacuum pump with ~140 L/min (5.0 Cfm) capacity (model 6912; FJC, Mooresville, NC, USA) or similar.
5. Oven: Set to 65 °C for PDMS curing.
6. Hot plates: One in cleanroom for photoresist processing and one for PDMS processing.
7. Stereo-microscope.
8. Cell culture hood.

9. Cold ice pack: Corning™ XT Starter Ice-free cooler (catalog number 432015; Corning, Corning, NY, USA).
10. Temperature-controlled centrifuge: e.g., Sorvall RT1 or Eppendorf 5810R with swing-bucket rotor capable of holding P35 dishes, and microtiter plate buckets (ThermoFisher Scientific, Grand Island, NY, USA).
11. Epifluorescence microscope: Zeiss Axiovert 200M, Nikon Eclipse Ti, Nikon TE-2000, or equivalent equipped with 4× and 10× objectives, and a live cell chamber (37 °C and 5–10% CO_2). A programmable *xyz*-stage is helpful but not essential.
12. Fluorescence microscopy camera: CoolSnap HQ (Photometrics, Tucson, AZ, USA) or equivalent.
13. High-speed microscope camera with up to 100 frames/s capability in 8 bit mode, and at least 1 MPixel image size: Prosilica, GX (Allied Vision Technologies, Exton, PA, USA) or equivalent.
14. Magnetic tweezer: homebuilt (*see* **Note 1**).
15. Computer Aided Design (CAD) software (AutoCAD) or Illustrator (Adobe) for mask design.
16. Computer with multi-channel ADC/DAC card and LabVIEW software or other instrument control software (catalog number NI PCIe-6321; National Instruments Corporation, Austin, TX, USA).
17. Computer with software to control fast camera capable of recording multi-minute movies at 100 frames/s. StreamPix (Norpix, Montreal, Quebec, Canada), or equivalent.
18. Programmable bipolar power supply capable of supplying up to 2 A current: (model BOP 50-2; Kepco Power Solutions, New York City, NY, USA).
19. Hall probe: to monitor field of magnetic tweezer (catalog number HGT-2100-10; Lakeshore Cryotronics, Westerville, OH, USA).
20. Image analysis software: Igor Pro (Wavemetrics Inc., Lake Oswego, OR, USA), Matlab (Mathworks, Natick, MA, USA), ImageJ (https://imagej.nih.gov/ij/) with Spot tracker plugin (http://bigwww.epfl.ch/sage/soft/spottracker/).

2.2 Photolithography

1. Mylar masks: patterns printed at 40,000 dots per inch (DPI).
2. Blank glass mask plate: 4″ × 4″ to fit in mask aligner (*see* **Note 2**).
3. Silicon wafers: ⟨100⟩ Si wafers with 3″ diameter and thickness 350–400 μm (*see* **Note 2**).
4. Precoated glass plate: 4″ × 4″ glass plate precoated with chromium and 0.5 μm of positive photoresist, AZ 1518 (Nanofilm Inc., Westlake Village, CA, USA).

5. Cleaning agents: acetone, isopropyl alcohol (IPA), ethanol, deionized water, and low-pressure nitrogen gas (~350 mbar, 5 pounds per square inch (PSI)).
6. Chromium etchant (catalog number 651826; Sigma-Aldrich Chemical Company, St. Louis, MO, USA).
7. Photoresists: SU-8 2002 (catalog number Y111029; MicroChem Corp, Westborough, MA, USA), SU-8 2050 (catalog number Y111045; MicroChem Corp), and SU-8 2010 (catalog number Y111058; MicroChem Corp) mixed with Microposit S1813 photoresist (catalog number 41280; Shipley Co Inc., Atlanta GA, USA), 70/30 v/v ratio.
8. Photoresist developers: AZ 400K (1:4 dilution in deionized water), Propylene glycol monomethyl ether acetate (PGMEA) SU-8 developer (catalog number Y020100; MicroChem Corp).
9. Precision tweezers and glass petri dishes large enough to hold Si wafers.

2.3 Replica Molding and μTUG Device Fabrication

1. Poly(dimethyl siloxane) (PDMS) and curing agent Sylgard 184 elastomer kit (catalog number 2065662; Dow Corning Corporation, Auburn, MI, USA).
2. Smooth-Cast® 305, Parts A and B (Smooth-On Inc., Macungie, PA, USA).
3. Tridecafluoroctyltrichlorosilane (catalog number 78560-45-9; UCT Specialties, Bristol, PA, USA).
4. Nickel spheres: Ni powder 74–116 μm (mesh size: −150 + 200; catalog number 7440-02-0; Alfa Aesar, Ward Hill, MA, USA).
5. Fluorescent beads: Fluoresbrite YG carboxylate microspheres 2 μm diameter (catalog number 09847-5; Polysciences Inc., Warrington, PA, USA) diluted 1:3000 in 100% Ethyl Alcohol 200 proof, Absolute, Undenatured, A.C.S./USP Grade).
6. Plastic culture dishes and plates: P35 (35 mm) culture dishes (catalog number 353001 or similar), 12-well plates (catalog number 353043 or similar), 24-well plates (catalog number 353226 or similar; BD Biosciences, Bedford, MA, USA).
7. 44 mm aluminum weighing dishes (catalog number 25433-016; VWR International, Radnor, PA, USA).
8. Precision nonmagnetic tweezers with 50 μm tips, razor blades, 50 mL centrifuge tubes.

2.4 Microtissue Seeding and Culture

1. 0.2% Pluronic® F-127: Prepare from mixing Pluronic® F-127 powder (catalog number P2443; Sigma-Aldrich Chemical Company) or 10% Pluronic® F-127 solution (catalog number P6866; Thermo Fisher Inc.) with deionized water or Phosphate-Buffered Saline (PBS, pH 7.4) and followed by

sterilizing with 0.22 μm filter (catalog number SCGP00525) (Merck Millipore KGaA, Darmstadt, Germany).

2. UV sterilizer.
3. 70% Ethyl Alcohol: 100% Ethyl Alcohol (200 Proof, Absolute, Undenatured, A.C.S./USP Grade) diluted with distilled water.
4. Phosphate-buffered saline (PBS), pH = 7.4 (catalog number 10010-023; ThermoFisher Scientific).
5. Cell dissociation agents: Trypsin (0.05% w/v)-EDTA (2 mM) (catalog number 25300054; ThermoFisher Scientific), Accutase™ (catalog number AT104; Innovative Cell Technologies, San Diego, CA, USA) or TrypLE™ Express (catalog number 12604013; ThermoFisher Scientific).
6. Growth medium: Dulbecco's Modified Eagle Medium (high glucose) (catalog number 11965092; ThermoFisher Scientific), supplemented with 10% Fetal Bovine Serum or as appropriate for cell type in use.
7. Extracellular matrix buffer components: Prepare stock solutions of 1 M NaOH (Sigma-Aldrich Corp), 10× Medium 199 (catalog number 11825015; ThermoFisher Scientific), 5% (w/v) $NaHCO_3$ (catalog number S5761; Sigma-Aldrich Corp), and 1 M HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (catalog number 15630-80; ThermoFisher Scientific) diluted in water to 250 mM.
8. Extracellular matrix: Rat Tail type I Collagen (catalog number 354236, 100 mg; Corning®, Corning, NY, USA), human fibrinogen (catalog number F3879; Sigma-Aldrich Corp), bovine fibrinogen (catalog number F8630; Sigma-Aldrich Corp) and other matrix components as needed.
9. Extracellular matrix solution: Calculate the volumes of the following components to reach their desire final concentrations and mix them thoroughly. *See* example in Table 1.
   (a) 10× M199: to reach final concentration of 1× in the final matrix solution.
   (b) 5% $NaHCO_3$: to reach final concentration of 0.035% (w/v).
   (c) 250 mM HEPES: to reach final concentration of 10 mM.
   (d) S-DI water: to bring the solution to final volume.
   (e) Collagen type I: to reach desired concentration.
   (f) Fibrin or fibrinogen (if needed): to reach desired concentration.
   (g) 1 M NaOH: adjust the volume ratio of the NaOH to stock collagen solution to be 0.022:1.
10. Sterilized Deionized Water (S-DI water).

Table 1
Example of creating 2 mL of extracellular matrix solution with 2.5 mg/mL of collagen and 2 mg/mL of fibrinogen

Component	Volume (μL)	Final concentration
Water	307.8	
10× M199	200	1×
HEPES (250 mM)	83	10 mM
$NaHCO_3$ (5% w/v)	14	0.035% (w/v)
NaOH (1 M)	29	
Collagen (3.77 mg/mL)	1326.2	2.5 mg/mL
Fibrinogen (100 mg/mL)	40	2 mg/mL
Total	2000	

11. Detergent: Triton X-100 (catalog number H5142; Promega Corporation, Madison, WI, USA).
12. Lids from P35 dishes filled with PDMS (*see* **Note 3**).

3 Methods

3.1 Master Fabrication

This section describes fabrication of original "master" versions of μTUG device arrays out of SU-8 photoresist using multilayer photolithography (*see* **Note 4**). Three major steps are involved: Design, production of chromium-on-glass masks, and fabrication of the master device. Production of the masks and masters should ideally be carried out in a cleanroom setting. The master μTUG device is made from sequential multilayer deposition of photoresists of varying thickness on a silicon wafer, followed by UV exposure of each layer and final development in SU-8 developer (*see* **Note 5**). The process flow is shown in Fig. 2.

1. Design patterns for the two masks needed using CAD software, and print on Mylar sheets at 40,000 DPI (*see* **Note 6**). Use one mask to define the pillars' stems and the second mask to define their heads. As negative photoresists are used for device fabrication, transparent regions in the masks correspond to protruding or raised features in the masters and the resulting devices. An example of patterns for a mask set is shown in Fig. 3.
2. To make a chromium-on-glass mask, attach the Mylar mask on a blank glass plate and expose the AZ1518 coated chromium-on-glass plate with UV light under a mask aligner using a total dose of 70 mJ/cm^2 (*see* **Note 7**).

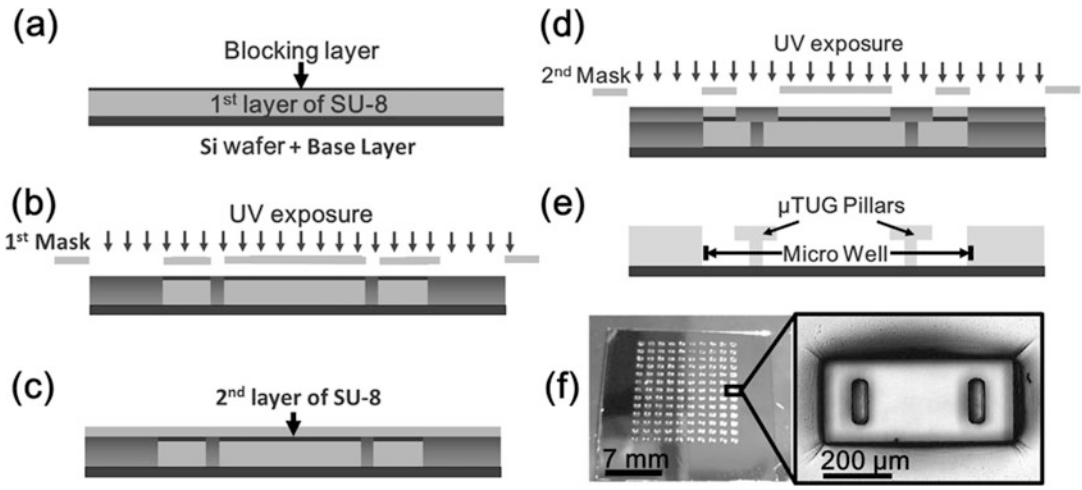

Fig. 2 Schematics of the fabrication of SU-8 master mold. (**a**) Deposit photoresist base layer, first layer and blocking layer on Si wafer. (**b**) Expose under first layer mask. (**c**) Deposit second layer of SU-8. (**d**) Expose under second layer mask after alignment. (**e**) Develop with SU-8 developer. (**f**) Image of an SU-8 master mold array on a silicon wafer and a magnified image of one microwell

3. Develop the plate for 45 s in 1:4 diluted solution of AZ 400K in DI water (*see* **Note 8**).
4. Immediately wash the plate with DI water and dry with nitrogen gas.
5. Remove the visible chromium sections on the plate, by immersing it into chromium etchant. Keep it immersed until the transparent areas through which light is allowed to pass can be seen by the naked eye (typically 1–2 min).
6. Wash and dry again with DI water and nitrogen gas (*see* **Note 9**).
7. Remove the leftover developed AZ 1518 by washing the plate in acetone.
8. Clean the plate using IPA and dry with nitrogen gas.
9. The chromium-on-glass mask is now ready to be used for μTUG master fabrication, which is described in the subsequent steps (*see* **Note 10**).
10. Repeat **steps 2–9** for the second mask.
11. Prior to resist deposition, clean the silicon wafer with acetone, followed by IPA.
12. Dry the wafer at 200 °C for 2 min on a hot plate. After drying, allow it to cool to room temperature (~2 min).
13. ***Preparation of base layer***: Create a 2.4 μm thick layer of SU-8 2002 on the wafer using a spin coater with the following settings: 10 s at 500 rpm + 30 s at 2000 rpm (*see* **Note 11**).

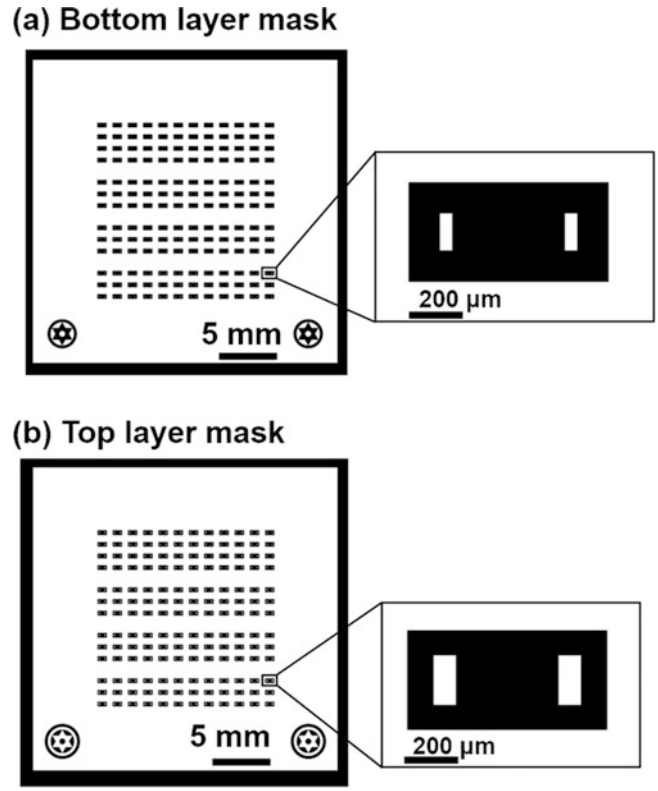

Fig. 3 Mask set to produce an array of μTUG devices in a P35 culture dish. (**a**) *Bottom layer mask* used to define the pillars' stems. (**b**) *Top layer mask* that defines the pillars' heads. A magnified view of one microwell on each mask is shown. The complimentarily shaded, circumscribed star patterns in the bottom corners are used for mask and substrate alignment as described in **Note 16**. The micro-wells are 800 μm × 400 μm in size, and the horizontal pillar-to-pillar spacing is 500 μm. In the well shown magnified, the pillar stems' cross-sectional dimensions are 150 μm × 50 μm, and those of the heads' are 200 μm × 90 μm. This yields pillars with effective spring constants $k = 2$ μN/μm. The other three groups of μTUGs (listed top to bottom) had pillar stem dimensions of 150 μm × 26 μm, 150 × 33 μm, and 150 μm × 42 μm, pillar head dimensions of 200 μm × 65 μm, 200 μm × 75 μm, and 200 μm × 80 μm, and corresponding spring constants $k = 0.25$ μN/μm, 0.5 μN/μm, and 1 μN/μm, respectively. All quoted k values assume a 10:1 PDMS mixing ratio (Young's modulus = 1.6 MPa)

14. Bake on a hot plate at 95 °C for 2 min (soft bake), followed by UV exposure under a mask aligner with a total dose of 100 mJ/cm^2.
15. Do a post exposure bake (PEB) at 95 °C for 2 min just after the exposure (*see* **Note 12**).
16. ***Preparation of first layer***: Deposit 130 μm of SU8 2050, using the following spin coater settings: 60 s at 500 rpm + 60 s at 900 rpm (*see* **Note 13**).

17. Soft bake at 95 °C for 3.5 h, and then allow the wafer to cool to room temperature.
18. ***Preparation of blocking layer***: Deposit a 20 μm thick layer from a v/v mixture of 30% S1813 in SU-8 2010 on the wafer via the following spin coater settings: 10 s at 500 rpm + 30 s at 1000 rpm.
19. Soft bake at 95 °C for 30 min. Remove and allow to cool to room temperature.
20. Once the sample cools to room temperature, expose it under the mask aligner using the first layer chrome mask. Keep the total dose at 700 mJ/cm^2 and insert a low pass filter in the path of the light to block out UV rays of wavelength below 350 nm (*see* **Note 14**). The high dose penetrates the blocking layer and exposes the first layer.
21. Do a post-exposure bake at 95 °C for 12 min (*see* **Note 15**).
22. ***Preparation of final layer***: Deposit the final layer of 50 μm of SU-8 2050 using the following spin coater settings: 60 s at 500 rpm + 60 s at 1500 rpm.
23. Soft bake at 95 °C for 3 h.
24. Once the sample cools to room temperature, expose the rest of the wafer using the second layer mask. Use the mask aligner to align patterns on the mask to the patterns on the wafer (*see* **Note 16**).
25. The UV light dose at this step should be between 100–110 mJ/cm^2 in the presence of the low pass filter (*see* **Note 17**).
26. Follow this exposure with a post exposure bake at 95 °C for 12 min. After a few minutes of cooling down, the wafer is ready for development.
27. Develop the wafer in a glass petri dish filled with PGMEA for 45 min on a stirrer or shaker (*see* **Note 18**).
28. Once the development is done, wash the wafer in IPA for 90 s, and then dry using nitrogen gas (*see* **Note 19**).
29. The master fabrication is now complete.

3.2 Replica Molding and PDMS Device Fabrication

1. ***Generation of negative molds***. The 'negative' molds required to make the final devices are made from PDMS. The process is sketched in Fig. 4a–c.
2. Combine PDMS and curing agent in a 20:1 ratio (*see* **Note 20**), and mix thoroughly for 20 min to obtain a uniform solution.
3. Centrifuge the solution in a 50 mL tube at 720 × *g* (*see* **Note 21**) for 1 min to remove any bubbles.

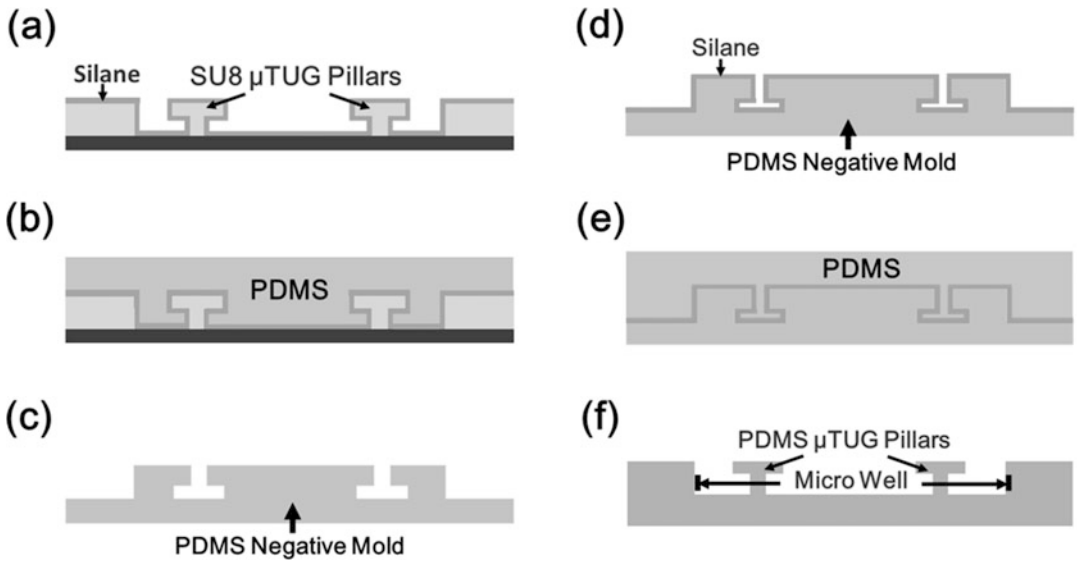

Fig. 4 Schematics of replica molding and PDMS device fabrication. (**a**) Silanization of SU-8 master molds. (**b**) Cast PDMS and cure at 65 °C. (**c**) Peel off PDMS negative mold. (**d**) Silanization of PDMS negative mold. (**e**) Cast PDMS and cure at 65 °C. (**f**) Peel off the PDMS devices

4. Place the μTUG master flat in a large plastic weighing dish, and add the PDMS + curing agent solution on top to a depth of 3–5 mm.
5. Insert the entire dish into a vacuum desiccator, and hold at $P = 25$ Torr for 1 h to remove all trapped air.
6. Bake in a 65 °C convection oven for 24 h to cure the PDMS.
7. Separate the PDMS mold from the master (*see* **Note 22**) and trim away excess PDMS around the features to form squares ~1.75 × 1.75 cm^2.
8. ***Generation of Smooth-Cast® master copies.*** To make these durable masters, attach negative molds (pattern side facing up) in 44 mm aluminum weighing dishes using double-sided tape (*see* **Note 23**).
9. Prepare a 1:1 volume mixture of Smooth-Cast 305 A and 305 B, mix for 30 s, add on top of the molds to cover them, and degas for 5 min using a vacuum desiccator at $P = 25$ Torr (*see* **Note 24**).
10. The Smooth-Cast mixture hardens within 3–4 h at room temperature and the molds can be peeled off (use ethanol for lubrication) (*see* **Note 25**).
11. ***μTUG device production from molds.*** The final μTUG devices are made from PDMS, as outlined in Fig. 4d–f and described in subsequent steps**.** The PDMS to curing agent ratio may be varied to control the pillars' stiffness.

12. Place a mold made from the masters or the Smooth-Cast copies in a glass petri dish (pattern side up), clean with ethanol on a shaker for 5 min, and dry with nitrogen gas (*see* **Note 26**).
13. To remove any organic impurities and to make the surface hydrophilic, clean the molds using an oxygen plasma prior to each use. We recommend a power of 25 W and pressure of 450 mTorr for 60 s.
14. To "silanize" the molds, place the plasma-cleaned molds in a vacuum desiccator, together with a few drops of tridecafluoroctyltrichlorosilane on a separate coverslip.
15. Seal the desiccator under vacuum ($P = 25$ Torr) and expose the molds to the silane fumes for at least 12 h. This creates a thin layer of silane over the PDMS mold to facilitate separation of the device from the mold.
16. Prepare a mixture of PDMS and curing agent. Together with the pillar geometry, the PDMS to curing agent ratio determines the pillar stiffness of the μTUGs [10]. The Young's modulus for 20:1, 10:1, and 4:1 mixtures is 0.54, 1.6, and 4 MPa, respectively.
17. To produce μTUG devices in a P35 culture dish, add 2 mL of PDMS solution to the dish, followed by quick degassing (10 min at $P = 25$ Torr).
18. Heat the dish on a hot plate at 65 °C for 20 min. This step is done to harden this bottom layer of PDMS somewhat (*see* **Note 27**).
19. While the bottom layer of PDMS in the petri dish is curing, pour 1 mL of PDMS on top of the silane-treated mold and degas (~15 min at $P = 25$ Torr).
20. Invert the mold and gently push down through the liquid PDMS and onto the slightly hardened PDMS in the petri dish.
21. Top off the sides of the mold with excess PDMS. It may be necessary to degas the dish for 2–3 h to remove air bubbles.
22. After all the bubbles are removed, cure the PDMS in a 65 °C oven for 24 h.
23. Once the dishes are cured, the molds are peeled off slowly from the device. This is done by making incisions along the edge of the mold device interface from the top using a thin tweezer or a razor blade, and then prying off the mold from one or multiple sides using the flat edge of the tweezer or blade. Squirting ethanol in the fissures is highly recommended as it facilitates the separation and prevents the μTUG pillars from breaking.
24. To create μTUG devices in 12- or 24-well plates, cut the molds into smaller cubes with surface area near 1 cm^2, and employ the

process described in **steps 12–23** above with the following modifications.

25. Use 1 mL of PDMS solution per well for 12-well plates, and 500 μL per well for 24-well plates.
26. For **steps 17–21**, to harden the bottom layer of PDMS in 12- and 24-well plates, place the plates on a hot plate at 65 °C for 30 min (*see* **Note 27**).
27. If desired, the pillars in the μTUG wells can be labeled with fluorescent microbeads to aid in tracking their deflections. To accomplish this, after plasma cleaning and silanizing the molds (**step 14** above), pour a solution of ethanol and fluorescent beads on top of the molds, degas for 15 min, and centrifuge at 605 × *g* for 3 min to drive the beads into the pillar forms of the molds (*see* **Note 28**).
28. Remove the excess ethanol and dry the molds overnight in a 65 °C oven.
29. Resume the device fabrication protocol from **step 16** above.
30. ***Attaching magnetic spheres to pillars.*** To enable magnetic actuation of the μTUG pillars, glue magnetic nickel spheres on top of them. Nickel particles of mesh size 150–200 (74–116 μm) are used for this step.
31. Working under a stereomicroscope, immerse a few such particles in PDMS solution (10:1 ratio), select for sphericality using precision tweezers, and place them on top of the desired pillars.
32. Cure the PDMS solution to bond the spheres to the pillars (*see* **Note 29**). For 2-pillar μTUGs, our standard approach is to bond Ni spheres to one pillar per microwell.

3.3 Passivation of Arrays

1. Sterilize the devices by placing them in a UV chamber or hood. Alternatively, incubate with 1 mL of 70% ethanol for 10 min, and dry with nitrogen gas.
2. Add 1 mL of freshly prepared 0.2% Pluronic® F-127 to the devices.
3. Transfer to desiccator and degas at $P = 25$ Torr until air bubbles are visible in the microwells of the devices.
4. Centrifuge the devices at 420 × *g* for 30 s to get rid of bubbles in the microwells.
5. Transfer the devices to the cell culture hood and let the Pluronic® incubate at room temperature for 10 min (or longer) (*see* **Note 30**) while preparing collagen solution in the seeding steps below (*see* **Note 31**).
6. To fully remove the residual Pluronic®, aspirate the Pluronic® out of the devices.
7. Rinse once with 1 mL of PBS, and dry with nitrogen gas.

8. Let the devices stand on ice while finishing the preparation of the cells + collagen solution.

3.4 Cell Seeding

1. Detach and dissociate the cells by Trypsin, Accutase or TrypLE, neutralize with fresh media and count the cells (*see* **Note 32**. For fibroblasts, adjust the cell concentration to ~300,000 cells/mL and take 1 mL of cell solution (*see* **Note 33**). This volume varies for 12- and 24-well plates, depending on the device size. Centrifuge at 180 × *g* for 5 min, resuspend the cells in fresh media and keep the cells on ice).
2. Place all the ECM buffer components and the soluble ECM (1 M NaOH, 10× M199, 5% $NaHCO_3$, 250 mM HEPES, S-DI water, collagen and (if needed) fibrin or fibrinogen) on ice.
3. Place μTUG device(s) on a cold ice pack.
4. Determine the desired concentration of matrix components (collagen, fibrin and/or fibrinogen.) Add calculated volumes of each component to a 15 mL conical tube (*see* **items 8** and **9** of Subheadings 2.4 and Table 1) and mix thoroughly by repeatedly and gently pipetting (*see* **Note 34**).
5. Add 1 mL of ECM mixture to μTUGs in P35 dish or 250 μL per well in 12-well or 24-well plates.
6. Place μTUGs along with ice pack in a vacuum desiccator to degas (2–3 min at $P = 25$ Torr) (*see* **Note 35**), or pipet up and down swiftly to destroy any air bubbles that may have formed in the collagen solution.
7. Spin down the cell solution from **step 1** at 180 × *g* for 3 min, and replace the media solution with 500 μL of the ECM mixture for P35 dishes to reach the desired cell number. This volume varies for 12- and 24-well plates, depending on the device size (*see* **Note 36**).
8. Transfer the above cell-ECM solution to the μTUGs and mix by pipetting (*see* **Note 37**).
9. Spin the μTUGs at 237 × *g* for 90 s at −9 °C (*see* **Note 38**) to pull the cells down into the wells.
10. For P35 dishes, rotate the dishes by 90° and spin at 237 × *g* for 90 s at −9 °C to even out effects on the distribution of the solution due to the tangential acceleration. This additional spinning step is optional for 12- and 24-well plates.
11. Transfer the μTUGs on ice pack to the cell culture hood.
12. Hold the ice pack slightly tilted towards you.
13. Aspirate the collagen mixture. Start with the aspirator at one of the top corners of the device, and draw the aspirator horizontally to the other top corner to detach the liquid from the upper sidewall of the dish. Then gradually slide the aspirator

down along the side, staying away from the microwells (*see* **Note 39**).

14. Invert the dish or plates so that they are now upside down, and spin at 40–60 × *g* for 15 s at −9 °C (*see* **Note 40**).
15. Keep the μTUGs inverted on an ice block when transferring from centrifuge to hood.
16. Add 500 μL of H_2O or PBS in the lid of the P35 dish or 3 mL to 12-well or 24-well plates.
17. Incubate the μTUGs with the lid down (inverted) for 10–15 min at 37 °C until the collagen is polymerized (*see* **Note 41**).
18. Aspirate water and gently add medium at the corner (1 mL for P35 dish and 250 μL per well for 12-well or 24-well plates).
19. Place the dishes or plates in the incubator for culturing to allow the microtissues to form.

3.5 Culturing Microtissues

1. For fibroblast microtissues, incubate the microtissues at 37 °C with 5% CO_2 (*see* **Note 42**).
2. Replace medium for fibroblast microtissues every day (*see* **Note 43**).
3. Change medium one device at a time. This is important as PDMS is hydrophobic, and total removal of medium will result in the tissues drying out rapidly. Therefore, do not aspirate all of the medium, but leave a thin layer to keep the microtissues moist.
4. During microtissue growth, when replacing medium, aspirate medium and gently add an adequate volume of fresh medium at the corner of the device. Typical volumes for devices in P35 dishes range between 1 to 2 mL.

3.6 Contractility Measurements

The contractile forces exerted by the microtissues are obtained by tracking the deflection of the pillars to which the tissues are adhered. This is achieved by measuring the positions of the fluorescent microbeads on the top of the pillars or by tracking the edges of the pillars through imaging.

1. To record the initial compaction and formation of the microtissues, which typically takes 12–24 h, mount the P35 dish or multi-well plate containing the microtissues in a live-cell chamber mounted on top of the microscope stage at 37 °C with the CO_2 concentration set between 5–10% depending on the cell type used (*see* **Note 44**).
2. To maximize the ability to resolve the pillars' motion, choose a microscope objective so that one microtissue fills the field of view (typically 4× or 10×). However, make sure that static

features, such as the boundaries of the well, are visible to provide reference points for the measurement the absolute deflection of the pillars, and to use in registering successive images should unwanted horizontal motion occur.

3. Record images for each well in the device every 1–2 h.
4. At each time point, record a white light (phase contrast) image of each tissue, focused on the tops of the pillars. If the pillars are labeled with fluorescent beads, record a fluorescent image as well.
5. For longer time-lapse studies (from 2–3 days to >1 week) that record the long-term tissue growth and development, culture the microtissues in a conventional incubator, and bring the devices to a microscope periodically (*see* **Note 45**) to record single white-light (or white light and fluorescence) images at 12 or 24 h intervals (*see* **Note 46**).
6. At the end of the data acquisition, replace the medium by either trypsin, collagenase or a detergent, such as Triton X-100, for 10 min, and take a final set of images to determine the baseline position of the pillars for each microtissue.
7. Analyze the data to obtain the force and stress on each microtissue, following the procedures of Subheading 3.8.

3.7 Magnetically Actuated Stress–Strain Measurements

The system for obtaining stress–strain measurements of microtissues via actuation of the magnetic sphere-tagged pillars includes a magnetic tweezer and associated electronic controls to generate local magnetic fields to apply forces to the magnetic spheres (*see* **Note 1**). The field-dependent magnetic force acting on the nickel spheres can enable multiple different types of stretching experiments. Quasistatic stretching, stretch-recovery and dynamic stretching are described, along with associated image analysis and data reduction methods.

1. ***Design of the magnetic tweezer system***. A magnetic tweezer consists of a sharpened soft iron core inserted into a solenoid and mounted on a three-axis micromanipulator (Fig. 5) (*see* **Note 47**).
2. Mount the micromanipulator/tweezer assembly on a fixed plate mounted above the microscope's moveable stage. The tweezer's sharp pole tip should project through a hole in this plate and down into the culture dish containing the microtissues, which sits on the moveable stage. This allows the pole tip of the tweezer to remain in the field of view of the microscope, while microtissues are successively brought into proximity of the tip for measurements. Mount a Hall probe at the blunt end of the iron core away from the sample to monitor the magnitude of the field (*see* **Note 48**).

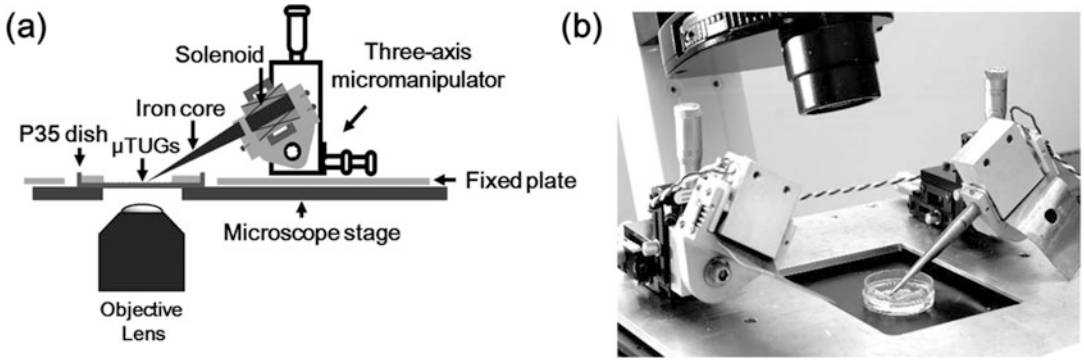

Fig. 5 Magnetic tweezer system. (**a**) shows a schematic and (**b**) shows a photograph of the unit mounted on a microscope. The long, tapered rod is an iron core that acts as a magnetic pole tip projecting into a P35 μTUG sample dish. The tweezer solenoid surrounds the core between the arms of the aluminum bracket on a 3-axis micromanipulator and is encased in an aluminum heat-sinking block. A second manipulator and coil assembly (unused in this application) is shown at left in (**b**) without its core. The image in (**b**) is reproduced from A. S. Liu, Ph.D. Thesis, the Johns Hopkins University (2015). Used by permission

3. ***Electronic control system***. Use a bipolar power supply to provide the electric current that is passed through the solenoid coils to generate a magnetic field.
4. Control the bipolar power supply with a computer-controlled digital to analog converter (DAC) card. The Hall probe is read with an analog to digital converter (ADC) channel in the same card.
5. ***Magnetic tweezer positioning and local magnetic force adjustment***. Position the tweezer with its tip at the edge of the microwell under study closest to the magnetic pillar (Fig. 1), using the microscope's stage for coarse motion, and the micromanipulator for fine alignment (*see* **Notes 49** and **50**).
6. Raise the tweezer tip when moving between microwells to avoid damaging the devices.
7. ***Magnetic Stretching Protocols and Data Acquisition***. The force on the nickel sphere is given by $\boldsymbol{F} = \nabla(\boldsymbol{\mu}(\boldsymbol{B})\cdot\boldsymbol{B})$, where $\boldsymbol{\mu}(\boldsymbol{B})$ is the field-dependent magnetic moment of the sphere induced by the magnetic field $\boldsymbol{B}$ produced by the magnetic tweezer tip (*see* **Notes 51** and **52**). For fields $B < 100$ mT, $\mu \sim B$, and hence $F \sim B^2$ [12, 13]. Hence to obtain a linearly increasing applied force with time $F \propto t$, the magnetic field has to increase as $B \propto \sqrt{t}$, which is achieved with solenoid currents $I \propto \sqrt{t}$.
8. The field-dependent magnetic force acting on the nickel spheres can enable multiple different types of stretching experiments, including Quasistatic stretching, stretch-recovery and dynamic stretching.

(a) ***Quasistatic stretching***. To acquire basic stress–strain data, e.g., to determine a microtissue's elastic modulus, increase the magnetic field in a stepwise linear fashion. After each step, record a phase contrast image and, if the pillars are fluorescently labeled, an epifluorescence image of the microtissue. One should confirm that there is no creep of the microtissue during imaging (*see* **Note 53**).

(b) ***Stretch-recovery***. To study the cells' active response to stretch, for example in smooth muscle microtissues [15], one may use a triangle-shaped stretch-relaxation protocol wherein the magnetic force is increased to 25–35 μN over 120 s, and then decreased to zero over a similar time interval. The microtissues are then observed for an additional 10–15 min at 5–10 frames per min as they recover to their baseline states due to the active cytoskeletal dynamics of the cells (*see* **Note 54**).

(c) ***Dynamic stretching***. For higher strain rates, or where higher temporal resolution is desired, use a fast camera and record white light images only.

3.8 Data Reduction and Analysis

For both contractility measurements (Subheading 3.6) and stress–-strain measurements (Subheading 3.7), the tissue force is measured from the deflection of the nonmagnetic pillar(s), using the relation $F = k\delta$, where k is the pillar's bending constant, and δ is the measured pillar deflection. The value of k is calculated for small deflections from Euler-Bernoulli beam theory. For pillars with heads, the bending constant is given by, $k = \frac{6EI}{a^2(3L-a)}$, where E is the Young's modulus of PDMS, I is the area moment of inertia of the pillar stem's cross section, a is the height measured to the center of the pillar head, and L is the total height of the pillar (*see* **Note 55**) [10]. Strain is measured either from the length change of the microtissue, or from measurements of the local strain in the central region of the microtissues via a texture correlation algorithm (*see* **Note 56**) [18].

1. ***Pillar tracking: fluorescent labeling***. When relatively small numbers of images (<~150) are acquired, and the pillars are fluorescently labeled, import images into ImageJ and convert images into a stack.
2. Use the Spot Tracker plugin [19] to analyze the changes of particle locations at different time point.
3. For larger fluorescently labeled data sets, use automated particle tracking approaches [20].
4. ***Pillar tracking: white light***. We use custom image analysis written in the software package Igor Pro (Wavemetrics) to track the motion of identifiable features, such as the edge of a moving pillar (*see* **Note 57**).

5. Define a region of interest (ROI) for each pillar that encompasses the horizontal motion of the feature of interest.
6. Average the pixel intensity values along the *y*-direction of the ROI (perpendicular to the motion) and assign a single value to the corresponding *x*-position (*see* **Note 58**).
7. Fit the positions of the features (edges or peaks) for each frame to determine the pillars' motion.
8. ***Microtissue stress determination***. Stress is calculated by dividing the force measured by the nonmagnetic pillar by the cross-sectional area of the tissue.
9. The cross-sectional area is estimated by finding a linear relation between the width and thickness of the tissue at its central position from confocal imaging of selected microtissues.
10. ***Microtissue strain determination***. Rudimentary deformation is calculated by finding the separation of the current positions of the pillars and subtracting the original unstretched pillar separation. Dividing the deformation by original pillar separation provides the strain induced in the tissue.
11. For more precise strain measurements, a texture correlation approach may be used [18].
12. Assign a grid of nodes with spacing ~10 μm over the central region of the microtissue, and track the displacement of each node in the stretched images by examining the correlation of the intensity pattern surrounding the node in the stretched and unstretched configurations.
13. Calculate the strain of each grid element in the stretching direction as $\varepsilon = \Delta L/L$, where ΔL is the distance change between adjacent nodes.
14. The strain field may be averaged over the central region of the tissue to report an average strain.
15. The tissues' elastic moduli are determined from the slopes of the resulting stress–strain plots.

4 Notes

1. Complete details on the construction of the magnetic tweezer system are beyond the scope of this methods chapter. The basic principles involved and a description of key aspects of the system in the authors' lab are given in Subheading 3.7. Fabrication of mechanical components and assembly of the system requires access to a standard machine shop. Further information on how to construct such a system may be obtained by contacting the authors.

2. All photolithography descriptions assume use of a 3″ mask aligner. Adjust accordingly for a different system.
3. Make lids of P35 dishes filled with cured PDMS to use as covers during time lapse microscopy. Because PDMS is gas permeable but not water permeable, PDMS-filled lids will prevent drying out of the samples.
4. A complete guide to photolithography techniques is beyond the scope of this methods chapter. However, the facilities and technical expertise needed to support this part of these Methods should be available at most universities or laboratories, or through national facilities such as the US National Nanotechnology Coordinated Infrastructure network (http://www.nnci.net).
5. The main challenge in fabricating the μTUGs is that the heads of the "T-shaped" pillars are typically larger than their stems by ~50 μm in each dimension. This necessitates multilayer photolithography with separate photomasks used to define the pillar stems and heads, and careful alignment of the corresponding exposures.
6. Mylar masks are printed by commercial vendors using high-resolution printers.
7. It is recommended, but not crucial, to make chromium-on-glass copies of the original Mylar masks for better pattern transfer to the Si wafer.
8. Stirring the petri dish that contains the developer and the sample by hand or using a shaker is recommended for uniform development of samples.
9. Rinse in DI water for 5 s and blow dry with nitrogen gas until all the water droplets are removed.
10. The shapes in the chrome masks should be accurate to at least 10 μm.
11. Since wafers are typically centered on the axis of rotation of the photoresist spinner, spinning speeds are most naturally given in rpm rather than in equivalent "g" forces, as is customary for centrifugation.
12. The spin speeds, times, and UV exposure doses need to be calibrated for every new bottle of photoresist and for the particular mask aligner used.
13. After pouring SU8 2050 on the wafer, it is recommended to wait for 5–10 min before spinning to release any trapped bubbles.
14. If UV light below 350 nm is allowed to expose the substrate, then the features typically end up being somewhat curved

instead of straight. Hence it is recommended to use a low pass UV filter to block UV rays <350 nm.

15. If the exposure is sufficient, then a visible latent image of the pattern is seen in the resist within 5–15 s after being placed on the PEB hotplate.
16. It is recommended to include alignment marks on the masks, e.g., additional shapes that have distinct symmetries, such as stars and crosses. This facilitates alignment of features on the second mask to the corresponding features on the wafer produced by the first mask.
17. This particular step is exposure sensitive. If the exposure is low, then the top layer can peel off from the blocking layer during development. If the exposure is high, then the light can 'bleed into' the first layer. Hence the exact exposure dose needs to be optimized such that it is enough to get into the blocking layer, but does not bleed through it.
18. While developing, the speed of the shaker/stirrer has to be low so that the features do not break from the agitation. Also, the development times need to be calibrated, as development is dependent significantly on agitation. The use of an ultrasonic bath is not recommended as the vibrations can break features in the patterns.
19. Drying must be done gently to avoid breaking fragile features in the SU8 structures.
20. 20:1 PDMS to curing agent ratio is used because the standard 10:1 ratio yields molds that are too stiff, leading to pillar heads tearing off when molds are peeled off μTUG devices.
21. We use rotors A-4-44 (rotor radius = 16.1 cm) A-2-DWP (rotor radius = 14.7 cm). The relative centrifugal force (RCF) is related to the rotor radius r and the rotation speed of the centrifuge. The g force to *rpm* conversion formula is as follows:

    $\mathrm{RCF\ (g)} = 1.118 \times 10^{-5} \times r \times (\mathrm{rpm})^2$, where r is in cm.
22. While removing the mold from the master, it is recommended to cut the plastic weighing dish off the PDMS mold, and then slowly pour ethanol into the interface between the master and mold. This prevents the master from breaking.
23. The SU-8 masters on Si wafers are quite brittle, and tend to break after a few repetitions of mold fabrication. Thus, stronger masters should be made from Smooth-Cast®.
24. While degassing, the Smooth-Cast® liquid mixture starts to boil, and so the vacuum has to be turned on and off a few times to let the liquid settle.

25. The resulting hardened plastic shapes have the same pattern as the original masters and can be utilized to make new molds following the procedure given in **step 1** above.
26. Typically, between four to eight molds are treated simultaneously to make multiple μTUG devices in P35 dishes.
27. If the PDMS at the bottom of the dish is not cured well, then the mold will sink all the way to the bottom of the dish when inverted onto the dish. This makes peeling off the mold from the completed devices difficult. On the other hand, if the PDMS is over-cured, then inverting the mold onto the surface can often lead to trapping of air bubbles.
28. Some optimization of this process is needed, as tracking is easiest with a small number of isolated beads in each pillar. As an alternate approach, fluorescent beads can also be glued to the pillar tops by hand with PDMS after the μTUGs are fabricated.
29. Dissolution of nickel into cell culture media was measured and the levels does not show any negative effects on biocompatibility of the tissues [12].
30. The duration of Pluronic® treatment varies between 1–30 min depending on the contractility of the cells and the concentration of ECM. Shorter times can be used when working with more contractile cells, such as fibroblasts, which start compacting the matrix within a few minutes after polymerization. In contrast, longer duration Pluronic® treatments are needed for low contractility cell types, such as smooth muscle cells, that compact tissues over the course of up to 48 h. For example, it takes 1–2 min of treatment for 3T3 fibroblasts in 1 mg/mL of collagen, while 7–8 min treatment is needed for the same cells in 2.5 mg/mL of collagen. Note that the microtissues may slip off the pillar caps if the treatment is excessive.
31. Although passivation of the devices and seeding of the cells are presented as separate methods, we suggest combining both procedures in order to shorten the time. Typically, we start with sterilizing the devices for 10–15 min. During this time, we trypsinize and count the cells, and aliquot the number of cells needed for the experiment. Then, we treat the devices with Pluronic®, and during the incubation time we make the collagen solution.
32. Cells may require different dissociation reagents. We use Accutase for SMCs and cardiac fibroblasts, TrypLE for cardiomyocytes, and Trypsin for fibroblasts.
33. In this step, one needs to define the total number of cells needed for the experiments. For fibroblasts, 1 mL of 300,000 cells/mL is enough for one μTUG array in a P35

dish. For smooth muscle cells, one may require 1 mL of 400,000–500,000 cells/mL. For cardiomyocytes, one may require 1 mL of 10^6/mL. For devices in 12-well or 24- well plates, 250 μL of the cells with the same concentrations can be used.

34. Solubilized collagen can be buffered in different ways. We use the formula published by Saltzmann [21]. We use an Excel spreadsheet to facilitate computation of reagent quantities. Typical concentrations are 2–3 mg/mL (collagen) and 0.5–1 mg/mL of fibrin or fibrinogen. Tissues with low concentrations of collagen are more easily torn apart around the edges of the pillars during microtissue formation. Fibrin and fibrinogen are not necessary for fibroblasts, but are typically used for smooth muscle or cardiac microtissues.
35. The collagen mixture sometimes freezes during degassing. One can add a small amount of additional collagen mixture to help it defrost quickly.
36. Cell seeding concentrations vary considerably, depending on the application. Examples include 4.1 million/mL for 3T3 fibroblasts [12–14] and for smooth muscle cells [15], and 1 million/mL for wound healing assays [16]. Further, one can adjust the number of cells seeded into each microwell by the following equation: Total number of cells per μTUG = $\frac{\text{Cell number per microwell}}{\text{surface area of microwell}} \times$ surface area of μTUG
37. Dropping the cell-ECM solution at the edges and also the center. Hold the p1000 pipette perpendicular to the surface of the well, and pipette up and down from dish edges to the dish center to let the cells evenly distributed.
38. Since the ECM solution forms a gel if it warms up, setting a relatively low temperature (−9 °C) for the centrifuge will ensure the ECM solution stays sufficiently cold during centrifugation.
39. Do not aspirate everything at once. Allow the remainder of the collagen/cell mixture to run to the bottom of the device while aspirating. The goal is to avoid having a layer of the mixture polymerize in between the μTUG microwells.
40. This step allows the cell-ECM solution be slightly lifted to a position around the heads of the pillars. The spinning time needs to be optimized so that the solution does not escape from the microwells. In some cases it may be helpful to do this step a second time after rotating the device 90°.
41. Check for polymerization of the ECM by putting the tube with the remaining ECM inside the same incubator. If the ECM inside the tube is not fully polymerized, allow the μTUGs incubate for a longer time.

42. We culture microtissues at the same CO_2 concentration as used when the cells are in ordinary 2D culture. For the cells mentioned here, all are cultured at 5% CO_2, except for bovine pulmonary artery SMCs, for which we use 10% CO_2 [15].
43. For other cell types, such as SMCs, we find that the media can be changed every other day.
44. While recording time-lapsed images or videos, make sure the P35 dish or plate containing the microtissues sits firmly in its holder on the microscope stage. Otherwise, the devices may shift during the time-lapse measurements, which makes it necessary to register the images (e.g., by aligning successive images based on the position of static feature) in order to track the pillars' deflections accurately.
45. As it is impossible to position the devices identically on the microscope stage each time images are recorded, including static features on the devices in the images to provide reference points to measure the pillar deflections is essential.
46. For such longer term time-lapse studies, we generally do not monitor the initial formation of the microtissues (**steps 1** and **3**).
47. We use a commercial relay solenoid. The coil has a resistance $R \sim 18\ \Omega$, and the coil + core have a self-inductance $L \sim 160$ mH. The micromanipulator should have travel of ~2 cm in each direction. We use a manual unit, but an automated, joystick-driven manipulator could be convenient.
48. In our system, the core is 7.94 mm (5/16″) in diameter, and extends 70 mm out of the solenoid. It tapers to a ~75 μm diameter tip, which serves as a magnetic pole to concentrate the local magnetic field near an individual μTUG. The pole tip projects into the culture media at an angle of ~35° relative to the plane of the μTUG. The solenoid/core assembly is attached to the micromanipulator via an aluminum mounting bracket. This bracket has a U-shaped extension, and each arm of the U has a hole that fits the core snugly and holds it with set screws. The solenoid sits on the core between the arms of the U, and is encased in an aluminum block to provide heatsinking.
49. The magnetic field depends linearly on the current in the solenoid, and falls off at least as $1/r^2$, where r is the distance from the sphere to the pole tip. This strong localization of the field ensures that only one μTUG is actuated at a time.
50. The spacing between the magnetic pillar and the tweezer tip should be more than 50 μm, because, if the magnetic sphere gets too close to the tweezer tip, then the pillar can be pulled all the way to the microwell wall, which can damage the microtissue.

51. To determine the magnetization curves of single nickel sphere, encase in 0.1 mL of epoxy (e.g., Araldite 502) and measure at room temperature with a vector vibrating sample magnetometer (VSM) (DMS Model 10; ADE Technologies, Westwood, MA). For nickel sphere of 100 μm diameter, a field $B = 100$ mT yields a magnetic moment of ~0.15 μA m^2, resulting in a magnetic force of ~40 μN [12, 13].
52. The tweezer's magnetic field can be calibrated with a magnetic field sensor. We use a magnetic tunnel junction (MTJ) sensor with the structure CoFeB/MgO/CoFeB, and with an active circular area 7 μm in diameter, defined by photolithography and ion beam etching [12, 13].
53. We typically use strain rates of 0.02% per s.
54. It is frequently useful to separate the cell and ECM contributions to the microtissues' stiffness and/or dynamic response. To do so, after the initial measurements of the as-grown tissues, treat with 0.1% Triton-X solution for 10 min to lyse the cells and remeasure [12, 15].
55. Procedures for calibrating pillar spring constants are given in Ref. [10]. For large deflections, effective spring constants may be modeled using finite element software [14].
56. The texture correlation approach is preferable, as nonuniformities in the microtissues' structure near the pillars can lead to nonuniform strains near the microtissues' ends.
57. Igor procedures to measure the pillar displacements are available from the authors upon request.
58. If the μTUG arrays are lined up so that the motion of the pillars is only in the *x*-direction, averaging the pixel intensity values along the *y*-direction improves signal-to-noise for tracking features, such as the pillar edges, that are extended in the *y*-direction.

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