Supporting Information for "Pneumatic microfluidic cell compression device for high-throughput study of chondrocyte mechanobiology"

Donghee Lee,[a, ‡] Alek Erickson,[b] TaeSun You,[c] Andrew T. Dudley[b,*] and Sangjin Ryu,[a,d,*]

[a]Department of Mechanical and Materials Engineering, University of Nebraska-Lincoln, Lincoln, NE 68588
[b]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198
[c]Houston District, Texas Department of Transportation, Houston, TX 77007
[d]Nebraska Center for Materials and Nanoscience, University of Nebraska-Lincoln, Lincoln, NE 68588
[‡]Current affiliation: Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198

I. Supplementary Note

1. SU-8 mold fabrication

The fabrication procedure of the microfluidic cell compression device is shown in Fig. S1. For fabrication of the pneumatic microfluidic unit of the cell compression device, an SU-8 mold with microchannel patterns of around 90 μm thickness was fabricated based on the conventional photolithography technique[1] as follows .

Sulfuric acid (H_2SO_4) and Hydrogen peroxide (H_2O_2) were mixed with a 3:1 volume ratio to make Piranha solution. A glass slide (2 in × 3 in, Fisher Scientific, Hampton, NH) was washed by being placed in 40°C Piranha solution for 30 minutes, and then rinsed with deionized water (diH_2O). Then, the glass slide was placed in acetone for 10 minutes and rinsed with isopropanol. The washed glass slide was dried with nitrogen (N_2) gas and baked at 200°C for 20 minutes.

Then, a thin seed layer of SU-8 5 was generated on the prepared glass slide for enhanced adhesion of SU-8 100 microfluidic channel patterns on the glass slide. All SU-8 products were purchased from MicroChem (Westborough, MA), and all SU-8 spin coating procedures contained an initial spinning cycle of 500 rpm for 35 seconds to evenly spread SU-8 on a substrate. First, SU-8 5 was spin coated on the glass slide at 2,500 rpm for 40 seconds. The SU-8 5 coated glass slide was baked at 65°C for 2 minutes and at 95°C for 5 minutes. Then, the SU-8 5 layer was exposed to UV light for 1 second. Post-exposure bake was done at 65°C for 2 minutes and then at 95°C at 5 minutes. The post-baked glass slide was immersed in the SU-8 developer for 2 minutes and washed with diH_2O and dried with N_2 gas. To harden the SU-8 seed layer, the glass slide was baked at 180°C for 20 minutes.

The microfluidic channel geometry was fabricated using SU-8 100 on the above glass slide with the SU-8 5 seed layer (Fig. S1a). SU-8 100 was spin coated on the glass slide at 3,000 rpm for 38 seconds. The SU-8 100 coated glass slide was baked at 65°C for 10 minutes and at 95°C at 30 minutes. Next, a photomask was placed on the SU-8 100 layer, and the SU-8 100 layer was exposed to UV light for 4 seconds. The transparency photomask was designed with AutoCAD (Autodesk, San Rafael, CA) and printed at 25,400 dpi (CAD/Art Services, Bandon, OR). After removing the photomask, the glass slide was baked at 65°C for 2 minutes and then at 95°C at 20 minutes. Then, the glass slide was kept in a Petri dish covered with aluminium foil

overnight at room temperature to stabilize the SU-8 100 layer. After the overnight curing, the glass slide was placed in the SU-8 developer for 15 minutes. After the development, the glass slide was washed with isopropyl alcohol, and was dried with N_2 gas.

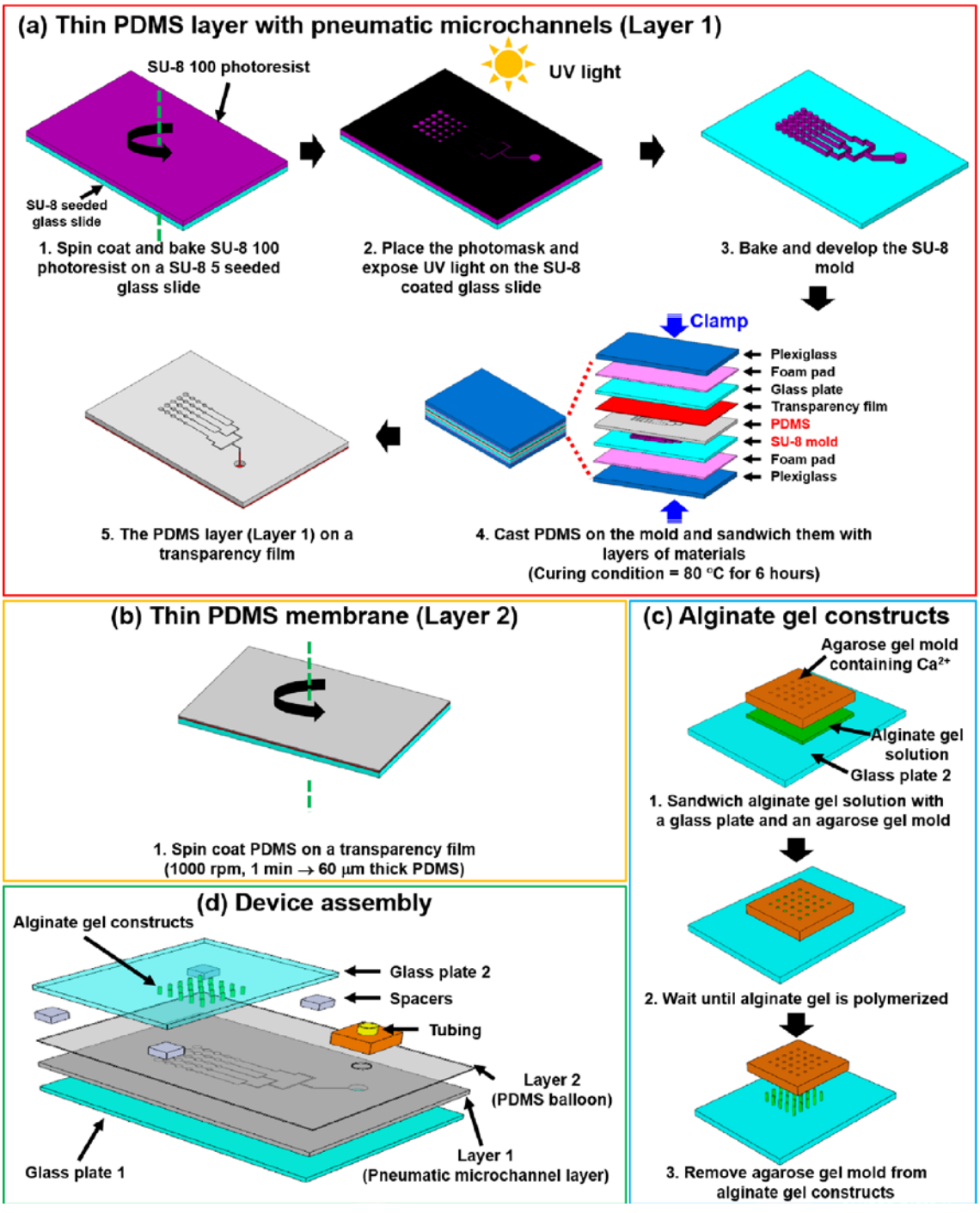

Fig. S1 Fabrication procedure of the microfluidic cell compression device. Fabrication of (a) a SU-8 mold and a PDMS layer with pneumatic microchannels (Layer 1), (b) thin uniform PDMS membrane (Layer 2) on a transparency film, (c) alginate gel constructs on glass (Glass plate 2), and (d) the microfluidic cell compression device.

2. Microfluidic unit of the cell compression device

The pneumatic part of the device consisted of two layers (Layer 1 and 2) of PDMS [Sylgard 184, Dow Corning, Midland, MI; 10:1 (weight ratio between prepolymer and curing agent)] on a glass slide (Glass plate 1, 2 in × 3 in) and a PDMS block with metal tubing (Fig. S1d).

Layer 1 contained an array of air chambers and was prepared using the sandwich molding method[2] to prevent PDMS shrinkage.[3] The SU-8 mold was silanized with (Tridecafluoro-1, 1, 2, 2-Tetrahydrooctyl)-1-Trichlorosilane (T2492-KG, United Chemical Technologies, Bristol, PA) for 2 hours under vacuum, to facilitate the release of PDMS from the SU-8 mold.[4] Uncured PDMS was sandwiched between a transparency film and the SU-8 mold (Step 4 in Fig. S1a). The sandwiched structure was clamped with glass, foam pad and plexiglass to obtain a patterned PDMS layer. The sandwiched PDMS layer (Layer 1) was cured at 80 °C for 6 hours (or overnight) and Layer 1 on the transparency film was released from clamping. Layer 1 was then bonded on Glass plate 1 through plasma activation of PDMS and glass surface. Bonded Layer 1 and Glass plate 1 was placed in the oven at 80 °C for 30 min and the transparency film was removed.

For Layer 2, PDMS was spin coated on a transparency film (HP Transparencies for LaserJets, C2934A) at 1,000 rpm for 1 minute to obtain a thickness of 60 μm (Fig. S1b). The thickness of PDMS layer is shown in Fig. S2 as a function of spin coating rpm and PDMS composition. We used the equation derived by Emslie *et al*. to fit our data as shown below.[5]

$$t = \frac{a}{\sqrt{1 + b \cdot rpm^2}}, \tag{S1}$$

where t is the PDMS layer thickness, and a and b are fitting constants. The fitted values of a and b are shown in Fig. S2. The spin coated PDMS was cured in the oven at 80 °C for 20-30 minutes for partial cure. Layer 1 on Glass plate 1 and Layer 2 were bonded with plasma treatment and stored in the oven at 80 °C overnight. After cooling down the device, the transparency film was removed.

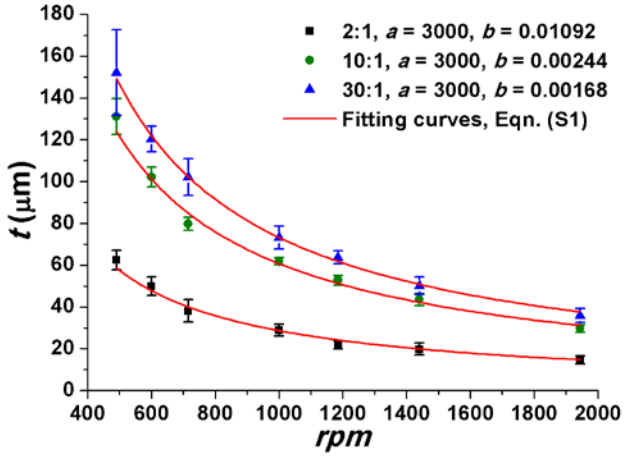

Fig. S2 Thickness of spin coated PDMS layer (t) as a function of rotational speed (rpm) and the mixing ratio (weight ratio between prepolymer and curing agent) of PDMS (Courtesy of Nick Bohlim). Error bar: standard deviation.

3. Aminopropyltriethoxysilane (APTES)-coated glass preparation

APTES-coated glass slides were prepared for Glass plate 2 to hold alginate gel constructs. Glass slides were shaken at 55 rpm in 0.2 M hydrogen chloride (HCl) solution overnight, and they were washed with diH_2O. Then, the glass slides were placed in 0.1 M sodium hydroxide (NaOH) solution for an hour at 55 rpm and rinsed with diH_2O. Finally, the glass slides were dispersed in 1% (v/v) 3-aminopropyltrimethoxysilane (APTES, Sigma-Aldrich, St. Louis, MO) in diH_2O for an hour at 55 rpm and then rinsed with diH_2O. The APTES-treated glass slides were dried in the fume hood before use.

4. Young's modulus of PDMS and alginate gel

The Young's modulus of 10:1 PDMS (E_{PDMS}) was obtained with the tensile test. Five PDMS samples were cast in poly(methyl methacrylate) mold (10 mm wide × 70 mm long × 1 mm thick) at 80 °C for one day, and they were stretched at a loading rate of 254 mm/min with Instron 5944 mechanical testing equipment (Norwood, MA).[6] The slope of the initial part (< 10% strain) of the stress-strain curve was calculated to determine E_{PDMS} (Fig. S3a, E_{PDMS} = 1.86 ± 0.22 MPa).

The Young's modulus of 1.5% (w/v) alginate gel (E_{gel}) was measured with the compression test. Nine cylindrical alginate gel samples (diameter = 9.7 mm, height = 6.2 mm) were harvested from the agarose gel mold containing 200 mM $CaCl_2$ (4 hour-long polymerization), and the alginate gels were immersed in the alginate gel cross-linking solution (50 mM $CaCl_2$ / 140 mM NaCl in diH_2O) for around 80 minutes for further polymerization. The alginate gel in the cross-linking solution was placed on the bottom plate of the mechanical testing equipment. Next, the top plate of the mechanical testing equipment was manually lowered until the alginate gel column and the top plate were close enough without contact to avoid pre-strain of the gel. Then, the alginate gel column was compressed at a loading rate of 1 mm/min. The E_{gel} was calculated by finding the initial slope (< 20% strain) of the stress strain curve (Fig. S3b, E_{gel} = 40.7 ± 3.2 kPa).

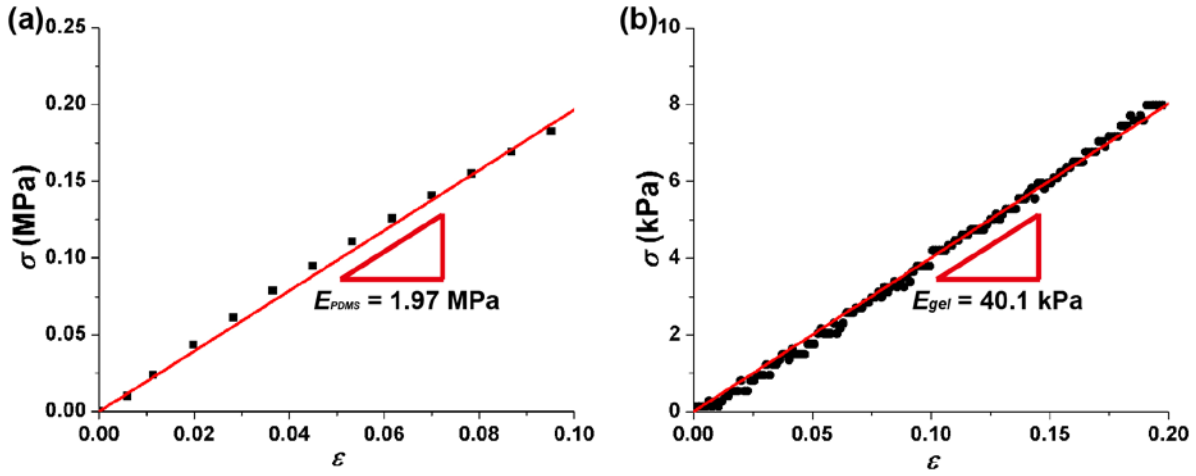

Fig. S3 Stress-strain (σ-ε) curve examples of 10:1 PDMS and 1.5% alginate gel. The slope of the curve is the Young's modulus (E_{PDMS} = 1.97 MPa and E_{gel} = 40.1 kPa).

5. Image processing

An image processing code[7] was developed with MATLAB (MathWorks, Natick, MA) to measure the height of a chondrocyte (Fig. S4). First, the *z*-stack images of a cell were imported, and the image center was determined in each *xy* image of the cell using circular fitting.[8] Then, the cell center was found by averaging the image center of all *xy* images (Fig. S4a). The fluorescence intensity profile in the *z*-direction was found along a virtual vertical line passing through the cell center (the red curve in Fig. S4b), and the cell height was measured based on the derivative of the intensity profile (the green curve in Fig. S4b). The top and bottom of the cell were determined to be the maximum and minimum gradient points (the red circles in Fig. S4b), and the *z*-distance between the top and bottom points was calculated as the cell height. The identified top and bottom locations were marked on the *xz* and *yz* cross sections of the cell to confirm the cell height measurement (Fig. S4c). The height of the alginate gel column was measured in the same way, using the intensity value of gel averaged in each *xy* cross section.

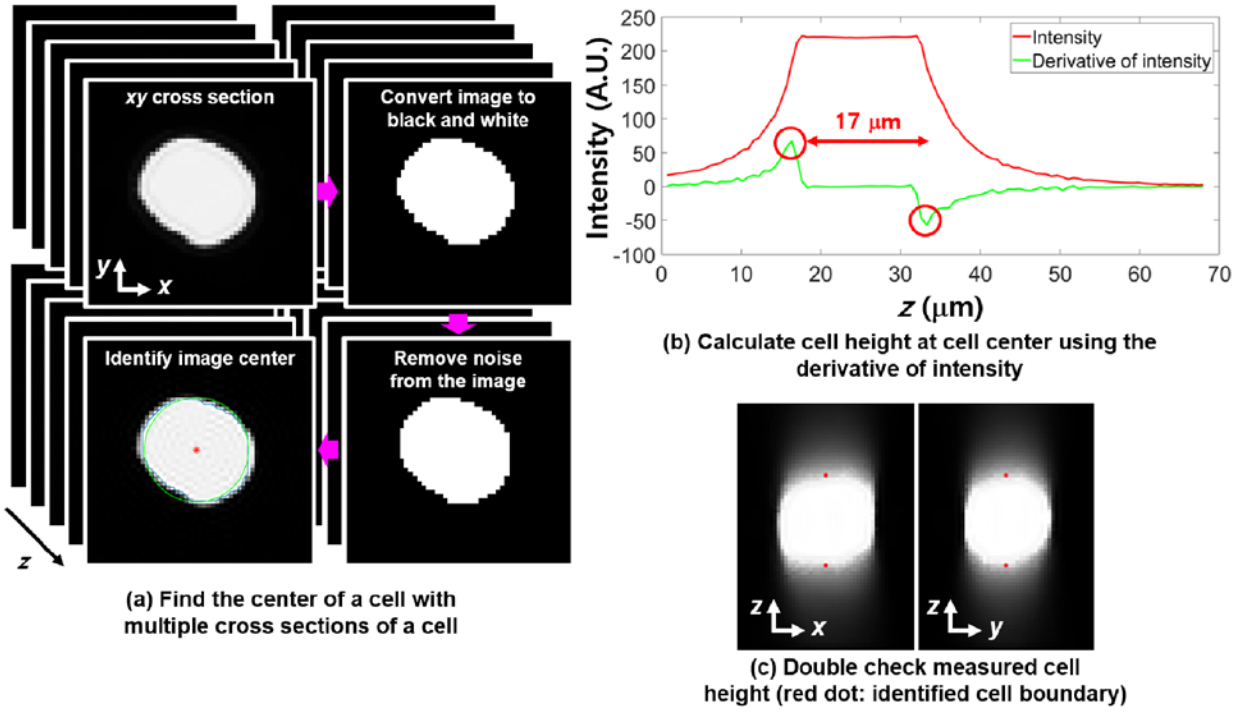

Fig. S4 Image processing procedure for cell height measurement. (a) *z*-stack images of a cell were imported and converted into black and white image. After noise removal, the image center (red) was determined by circular fitting for each *xy* images (green). Next, the cell center was found by averaging the image centers. (b) The cell height was measured based on the derivative (green) of fluorescence intensity profile (red) along the *z* direction passing through the cell center. The cell's top and bottom were identified as the maximum and minimum fluorescence gradient points (red circles). The distance between the two points was calculated as the cell height. (c) The identified cell's top and bottom locations agree well with the image, which confirms the cell height measurement results.

6. Uncertainty analysis

The derivation of eqn (2) is shown below:

$$\begin{aligned}\frac{\sigma_h}{m_h} &= \sqrt{\left[UMF_t \cdot \frac{\sigma_t}{m_t}\right]^2 + \left[UMF_{E_{PDMS}} \cdot \frac{\sigma_{E_{PDMS}}}{m_{E_{PDMS}}}\right]^2} \\ &= \sqrt{\left[\left(\frac{\partial h}{\partial t} \cdot \frac{t}{h}\right) \cdot \frac{\sigma_t}{m_t}\right]^2 + \left[\left(\frac{\partial h}{\partial E_{PDMS}} \cdot \frac{E_{PDMS}}{h}\right) \cdot \frac{\sigma_{E_{PDMS}}}{m_{E_{PDMS}}}\right]^2} \\ &= \sqrt{\left[-\frac{1}{3} \cdot \frac{\sigma_t}{m_t}\right]^2 + \left[-\frac{1}{3} \cdot \frac{\sigma_{E_{PDMS}}}{m_{E_{PDMS}}}\right]^2}\end{aligned} \quad \text{(S2)}$$

where UMF_t and UMF_{EPDMS} are uncertainty magnification factor with respect to the thickness (t) and Young's modulus (E_{PDMS}) of PDMS, respectively. σ and m are the standard deviation and mean values, respectively.

II. Supplementary Figures

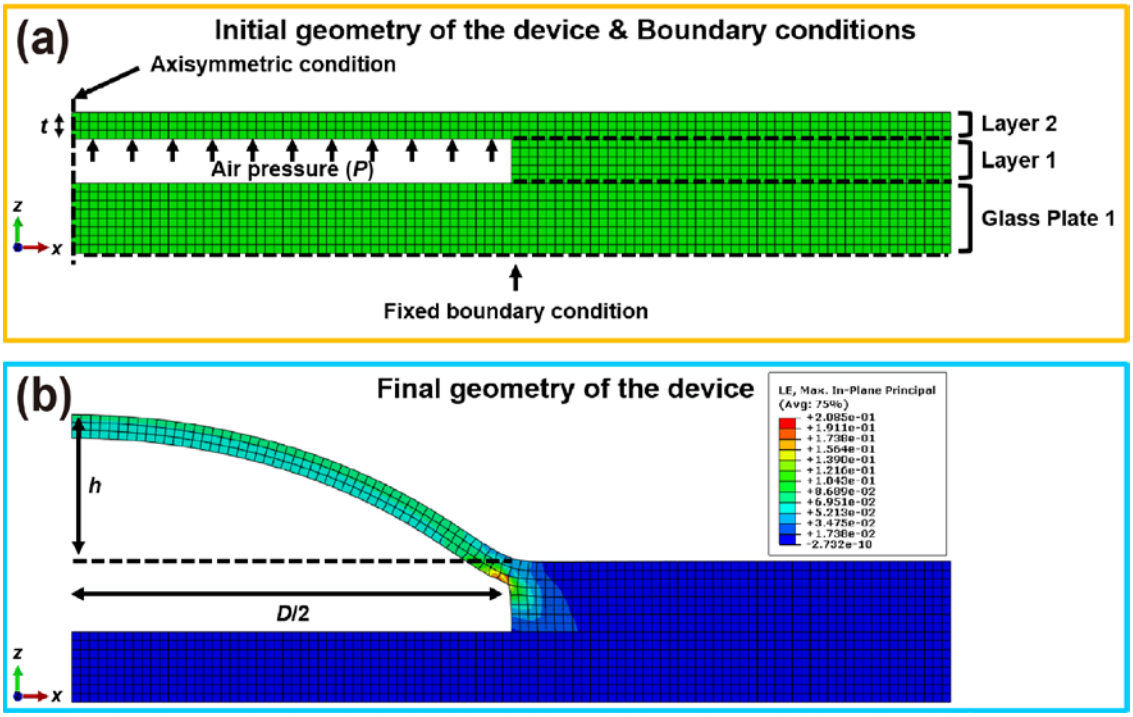

Fig. S5 Finite element method (FEM) model for simulating PDMS balloon deformation under pressurized air. (a) Initial geometry of the model with assigned boundary conditions. A two-dimensional (2D) axisymmetric model consisting of quadrilateral elements was developed, and the axisymmetric boundary condition was applied along the axis of symmetry of the model while the bottom of the model was fixed. (b) Deformed geometry of the model. The 2.0 mm-diameter PDMS balloon (Layer 2) was expanded by air pressure (P = 14 kPa). t: PDMS balloon thickness. h: the center height of PDMS balloon. D: the diameter of the PDMS balloon.

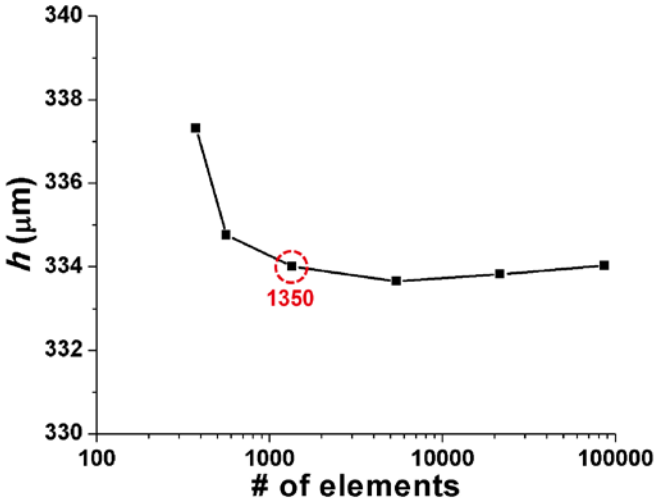

Fig. S6 Mesh dependency test result: Simulated h values versus the number of element in the FEM model shown in Fig. S5. The h value became nearly constant when the number of element was 1350.

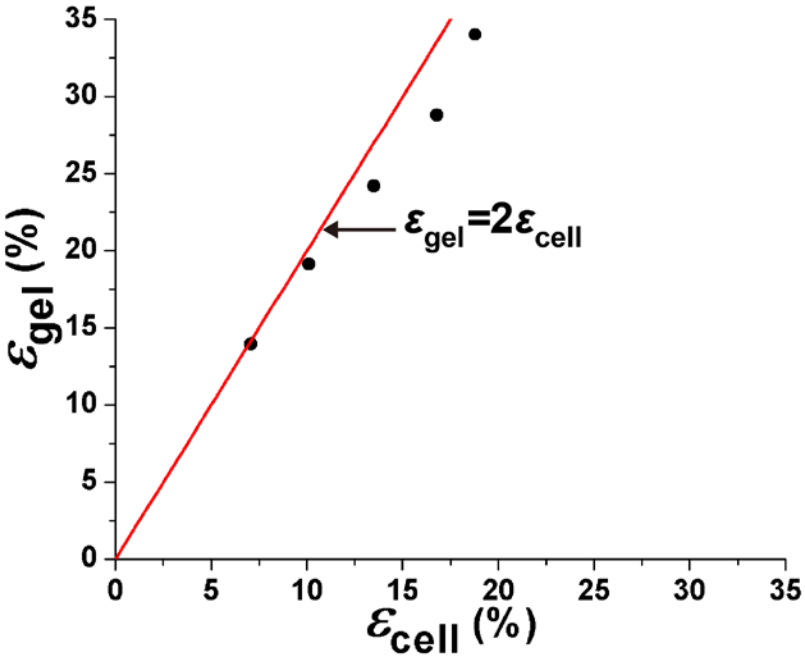

Fig. S7 Relationship between gel strain (ε_{gel}) and chondrocyte strain (ε_{cell}): $\varepsilon_{gel} \approx 2\varepsilon_{cell}$.

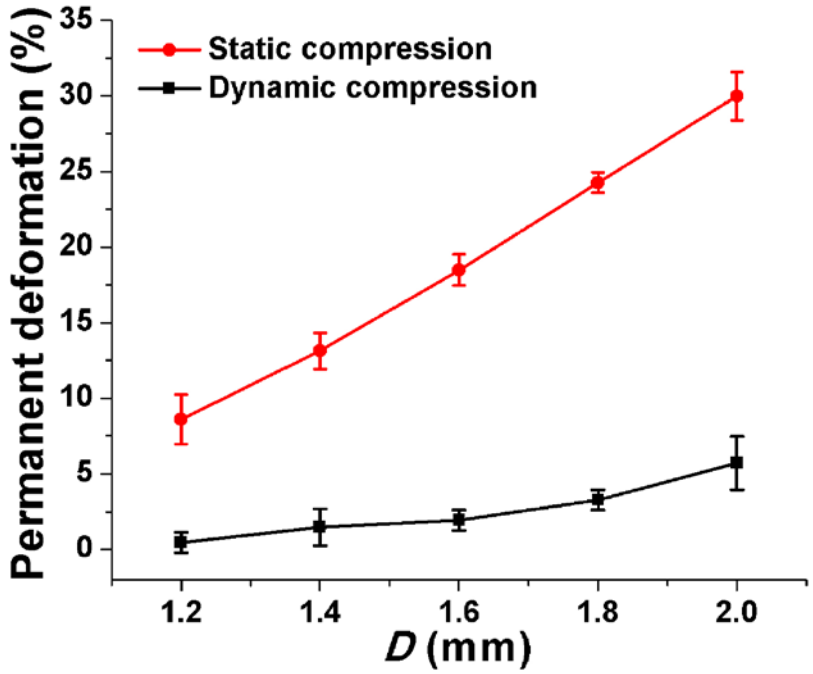

Fig. S8 The permanent deformation of alginate gel constructs after 1 hour-long static and dynamic compression (P = 14 kPa). D: the diameter of the PDMS balloon.

III. Supplementary Tables

Table S1 Mean value (m_h) of center height (h) of inflated PDMS balloons (unit: μm).

Balloon diameter	D = 1.2 mm	D = 1.4 mm	D = 1.6 mm	D = 1.8 mm	D = 2.0 mm
Device 1	167.2	214.3	250.9	291.0	342.1
Device 2	146.5	193.5	231.8	273.6	327.4
Device 3	151.9	198.1	241.3	283.1	336.6
Mean of m_h ± standard deviation of m_h	155.2 ± 10.7	202.0 ± 11.0	241.3 ± 9.6	282.6 ± 8.7	335.4 ± 7.4
RSD_h	6.9%	5.4%	4.0%	3.1%	2.2%

Table S2 FEM simulation results.

Balloon diameter (mm)	Maximum von Mises stress (kPa)	Maximum in-plane principal strain (-)	Decrease in the center thickness of PDMS balloon (μm)
2.0	434.8	0.209	7.19
1.8	399.2	0.192	7.15
1.6	362.3	0.175	6.23
1.4	323.6	0.157	6.44
1.2	282.4	0.137	5.23

IV. References

1. H. Lorenz, M. Despont, N. Fahrni, J. Brugger, P. Vettiger and P. Renaud, *Sensors and Actuators A: Physical*, 1998, **64**, 33-39.
2. B. H. Jo, L. M. V. Lerberghe, K. M. Motsegood and D. J. Beebe, *J. Microelectromech. Syst.*, 2000, **9**, 76-81.
3. C. Moraes, Y. Sun and C. A. Simmons, *J. Micromech. Microeng.*, 2009, **19**, 065015.
4. J. R. Anderson, D. T. Chiu, R. J. Jackman, O. Cherniavskaya, J. C. McDonald, H. Wu, S. H. Whitesides and G. M. Whitesides, *Anal. Chem.*, 2000, **72**, 3158-3164.
5. A. G. Emslie, F. T. Bonner and L. G. Peck, *J. Appl. Phys.*, 1958, **29**, 858-862.
6. I. D. Johnston, D. K. McCluskey, C. K. L. Tan and M. C. Tracey, *J. Micromech. Microeng.*, 2014, **24**, 035017.
7. D. Lee, M. M. Rahman, Y. Zhou and S. Ryu, *Langmuir*, 2015, **31**, 9684-9693.
8. I. Kasa, *IEEE T. Instrum. Meas.*, 1976, **IM-25**, 8-14.