Supplementary Information

Hydrogel Microwell with Pneumatic Soft Actuator for Compression Formation of Three-Dimensional Cellular Tissue

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SI 1. Conditions in the simulation by COMSOL

In the case of Fig. 4a

Module: Structural Mechanics Module

Material characteristics:

	Density (kg/m^3)	Young's modulus (Pa)	Poisson's ratio
Agarose gel (A2576)	1000	43.1×10^3	0.45
Acrylic enclosure	1190	3.2×10^9	0.35
Cover glass	2203	73.1×10^9	0.17
PDMS chamber*	1100		

* Hyperelastic Material was used as a material for the PDMS chamber.

Boundary condition:

Boundary load (0.1 MPa inside the PDMS chamber)

In the case of Fig. S3

Module: Fluid-Structure Interaction (FSI) Module

Material characteristics:

	Density (kg/m^3)	Young's modulus (Pa)	Poisson's ratio
Acrylic enclosure	1190	3.2×10^9	0.35
Cover glass	2203	73.1×10^9	0.17
PDMS chamber*	1100		
Agarose gel (A9539)	1000	100×10^3	0.45
Culture medium	1000		
Cell	1000	500	0.38

* Hyperelastic Material was used as a material for the PDMS chamber.

Boundary condition:

Open boundary (The surface of the culture medium exposed to air)

Boundary load (0.06 MPa inside the PDMS chamber)

SI 2. The waveform of the air pressure applied to the pneumatic soft actuator in the cell culture (Fig. S1)

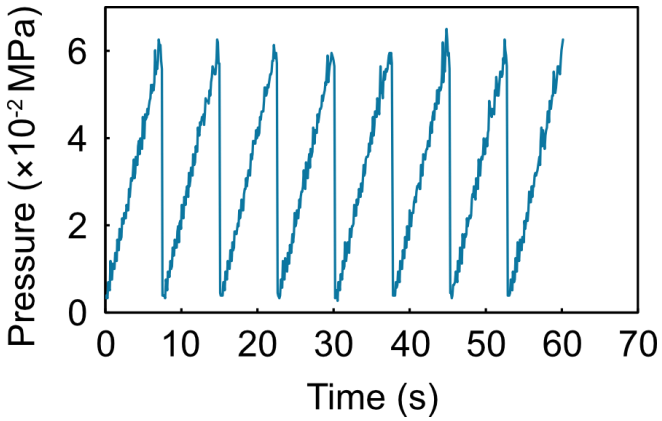

Fig. S1. The waveform of the air pressure applied to the pneumatic soft actuator in the cell culture. The air pressure ranged from 0 to 0.06 MPa, accuracy ±8% FS and 0.13 Hz.

SI 3. Cellular tissues cultured by W/HG_M, W/PM_M, and W/O HG_M (Fig. S2)

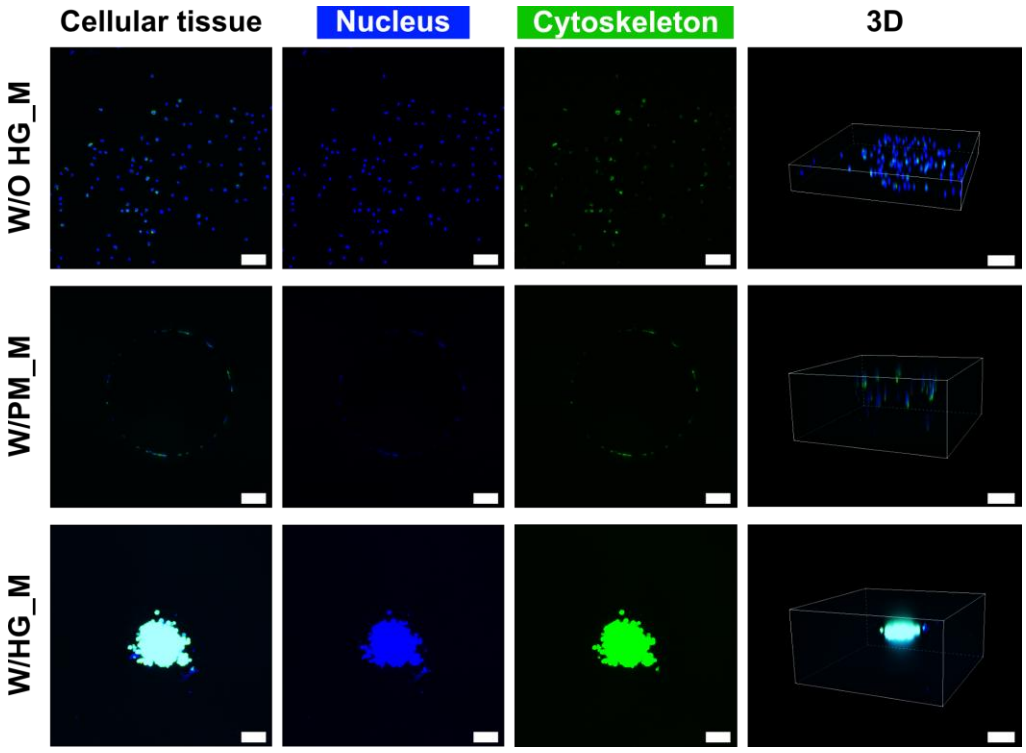

Fig. S2. Cellular tissues cultured by W/HG_M, W/PM_M, and W/O HG_M. The images show *z*-axis projections and three-dimensional shapes of immunostained cellular tissues after culturing for 6 h, acquired by a confocal microscope. The cell nucleus and cytoskeleton were immunostained with blue and green, respectively. All scale bars = 100 μm.

SI 4. The simulated results of the model of the cells compressed in the pneumatic soft actuator with the hydrogel microwell after inflating the pneumatic soft actuator at 0.06 MPa and 0.13 Hz (Fig. S3)

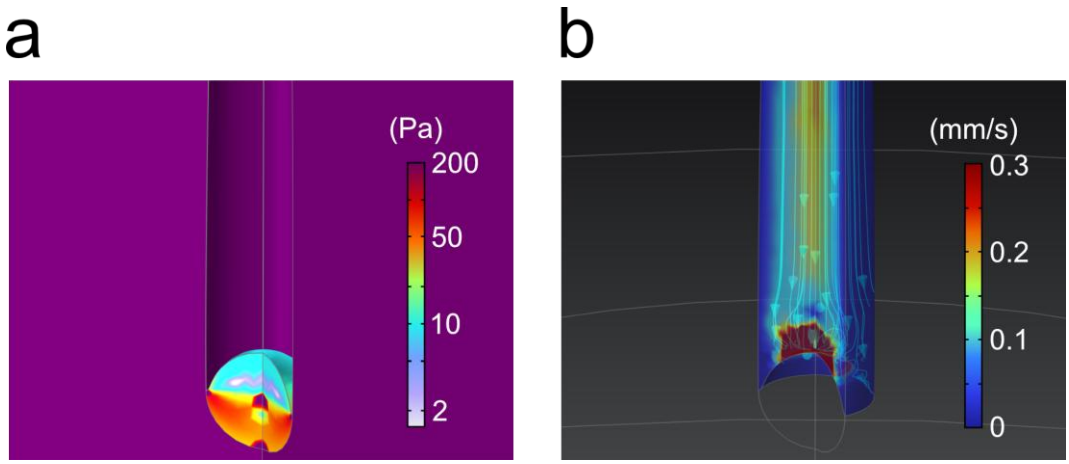

Fig. S3. The simulated results of the model of the cells compressed in the pneumatic soft actuator with the hydrogel microwell after inflating the pneumatic soft actuator at 0.06 MPa and 0.13 Hz. The sphere was assumed to be the cellular tissue after cell aggregation. (a) The stress distribution of the cellular tissue compressed by the MPHGS at the bottom of the hydrogel microwell. The color legend in the image is represented logarithmically. (b) The flows induced by the MPHGS around the bottom of the hydrogel microwell. The light blue arrows show the flows of the culture medium in the hydrogel microwell.

SI 5. Detailed values used in the graphs

Fig. 4b

	Contraction ratio (%)		
Pressure（MPa)	Device1	Device2	Device3
0.02	1.16	3.08	3.28
0.04	1.44	4.33	4.99
0.06	3.31	4.90	5.84
0.08	3.89	5.04	6.69
0.1	4.32	6.57	7.56

Fig. 4c

Device1	Pressure (MPa)	Contraction ratio (%)
	0.051	3.71
	0.1	3.71
	0.152	4.73
	0.196	5.35
	0.252	6.38

Device2	Pressure (MPa)	Contraction ratio (%)
	0.048	3.65
	0.099	3.65
	0.155	4.09
	0.2	4.09

Device3	Pressure (MPa)	Contraction ratio (%)
	0.05	3.28
	0.1	3.28
	0.15	3.28
	0.161	4.22

Fig. 5d

		Compressive strain (%)		
AG_{low}	Pressure (MPa)	Device1	Device2	Device3
	0.02	5.16	3.86	5.12
	0.04	11.90	3.86	8.27
	0.06	13.49	8.94	11.81

		Compressive strain (%)		
AG_{high}	Pressure (MPa)	Device1	Device2	Device3
	0.02	4.84	5.40	0
	0.04	8.51	8.80	5.28
	0.06	11.80	10.40	8.94

Fig. 6a

	Cellular tissue volume (μm^3)
Control	49974
	2991708
	2172414
Air pressure	3206859
	3798011
	9313952

Fig. 6b

	Porosity per area (%)
Control	67.87
	53.59
	59.70
Air pressure	48.43
	47.92
	47.69