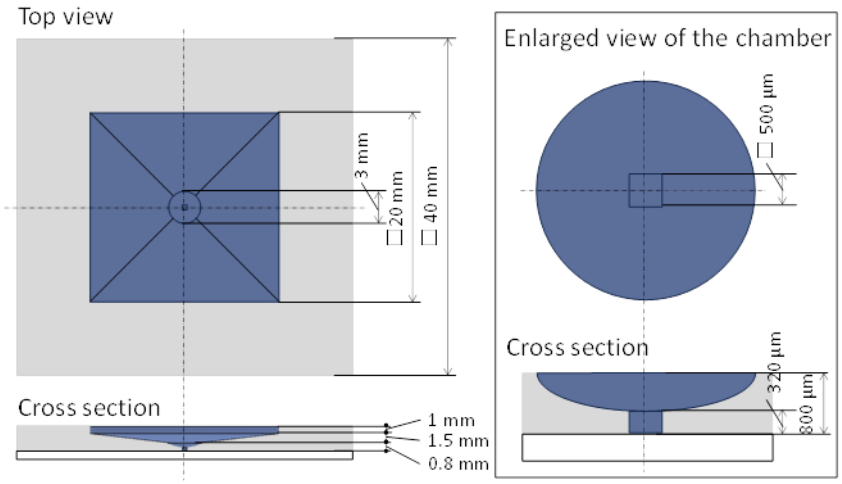


Figure S1. Dimensions of the chamber for 3D cultured cells.

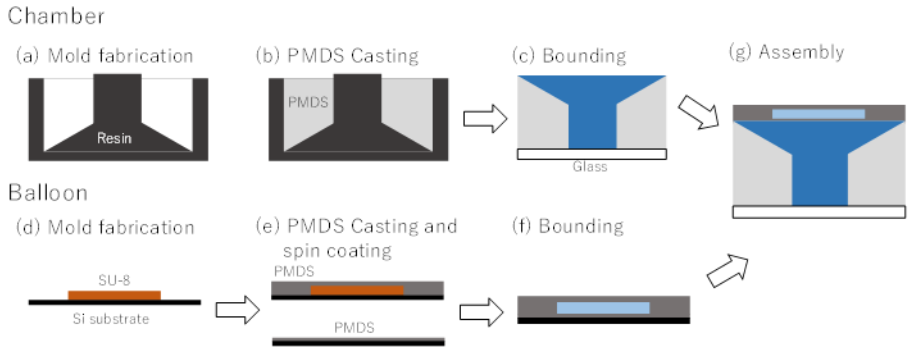


Figure S2. Fabrication process of the microfluidic device components. Schematic showing the stepwise fabrication of the (a–c) cell chamber and (d–f) balloon actuator, followed by (g) final assembly.

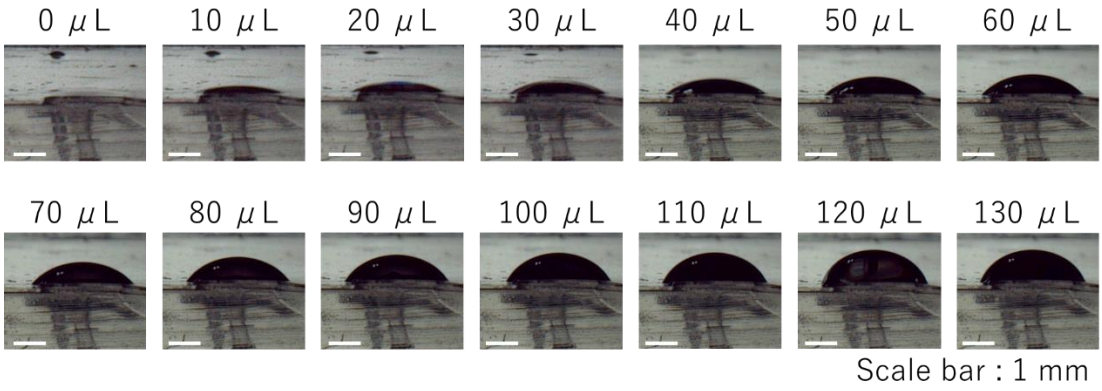


Figure S3. Photographs of balloons under different injected water volumes.

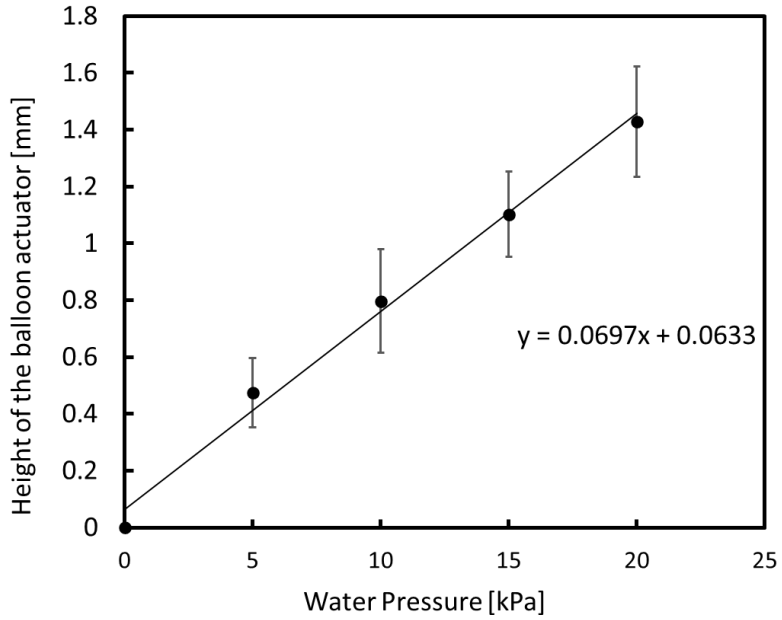


Figure S4. Correlation between the measured water pressure and balloon expansion height.
The pressure inside the balloon was measured using a pressure sensor (GC31-174-B7N3800, Nagano Keiki, Japan). The balloon height at each pressure was determined from photographs using ImageJ. Measurements were repeated three times under identical conditions. The line shown in the graph represents the linear interpolation calculated using the least-squares method.

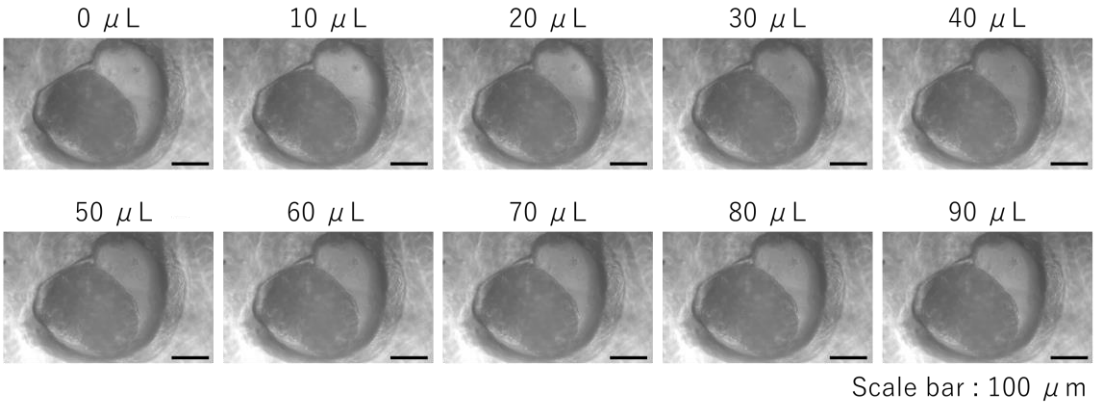


Figure S5. Micrographs of spheroids under different compression conditions.

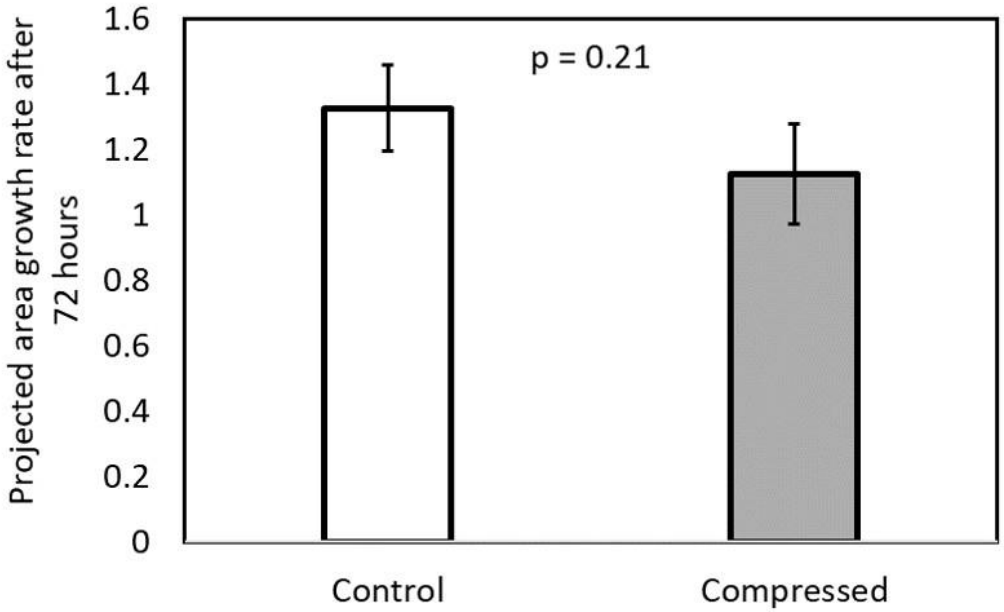


Figure S6. Differences in spheroid proliferation rates with and without pressure application. Spheroids subjected to 30 cycles of compression using the balloon actuator and uncompressed control spheroids were cultured for 72 h, and the increase in projected area was quantified from captured images. Control spheroids exhibited a 1.3-fold increase (n = 5, s.d. = 0.13), whereas spheroids exposed to compression showed a 1.1-fold increase (n = 5, s.d. = 0.15). Statistical analysis indicated no significant difference between the two groups ($p = 0.21$).

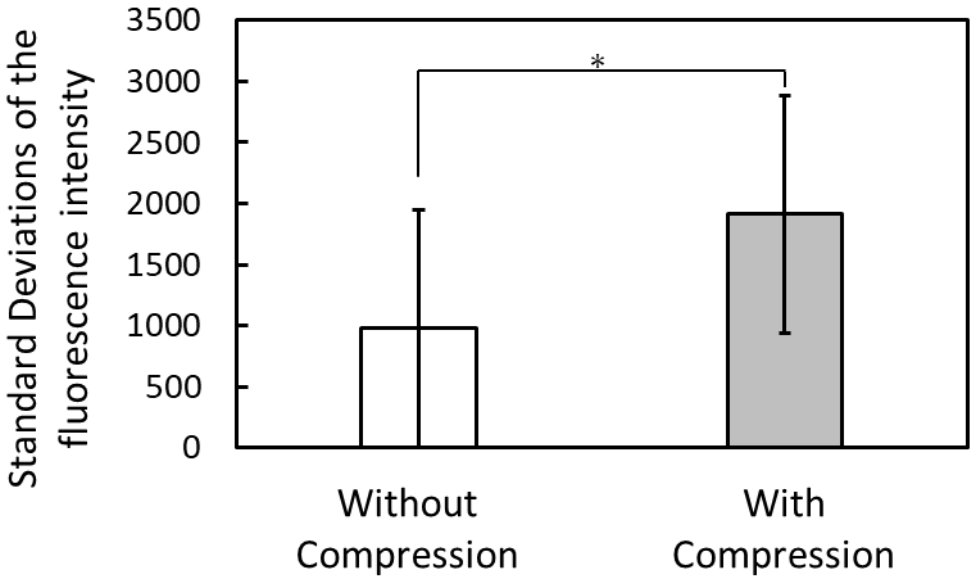


Figure S7. Standard deviation of TIRF fluorescence intensity without and with balloon compression.

The standard deviation (S.D.) of fluorescence intensity in TIRF images was measured within cell-containing regions of membrane-stained spheroids (n = 2, 12 ROIs). A statistically significant difference was observed between the uncompressed and compressed conditions (p = 0.027, Student's t-test). Error bars represent the standard deviation.