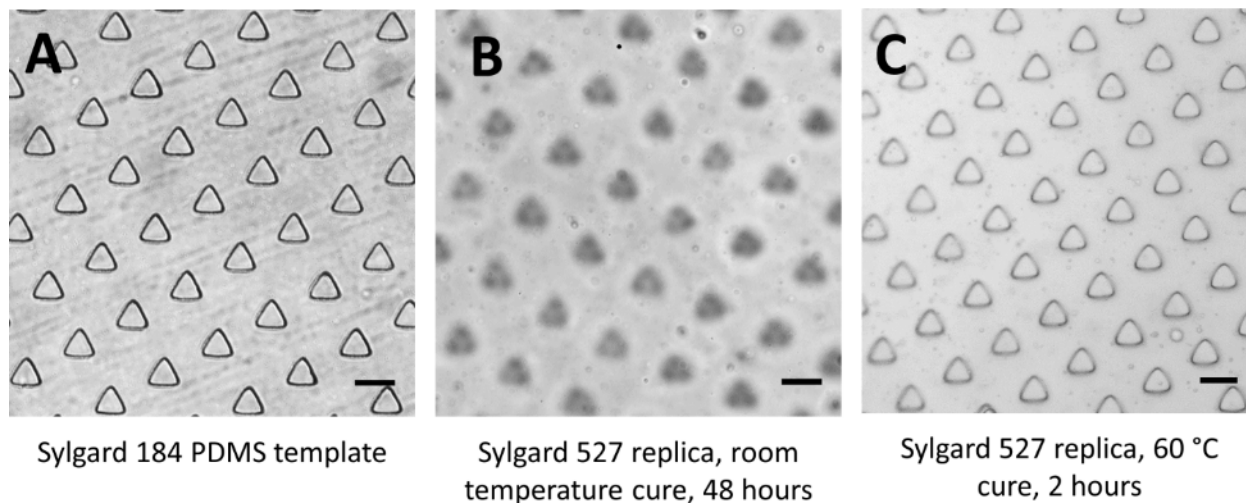
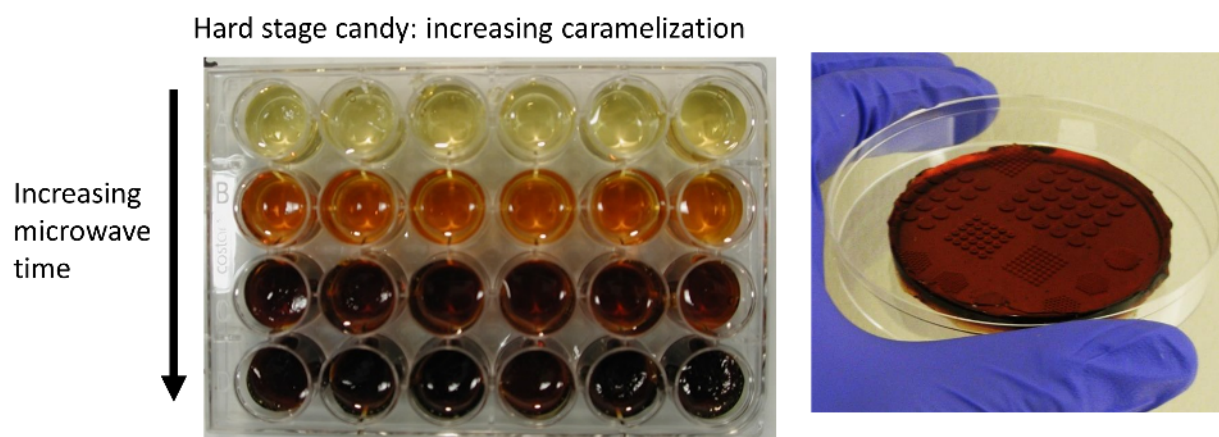


## Supplemental Material

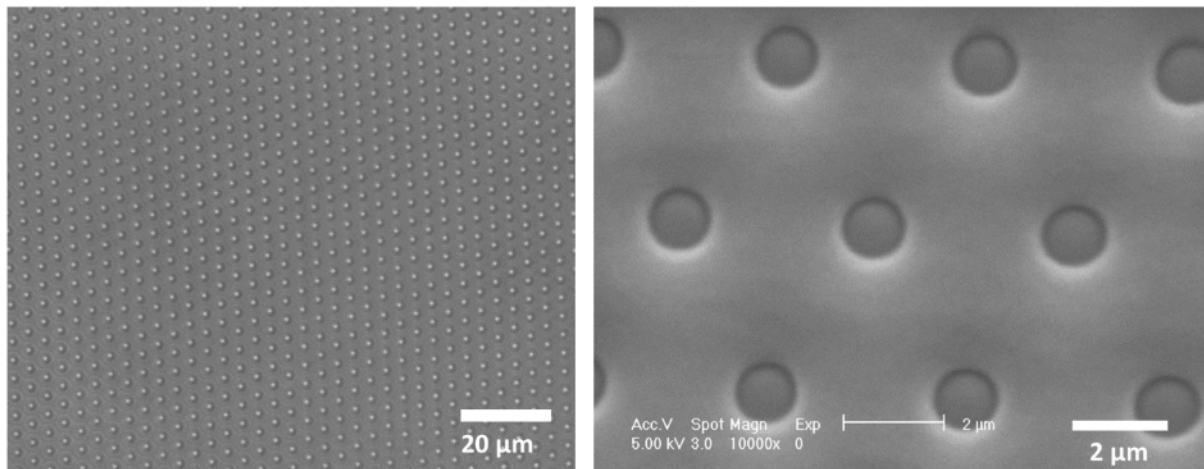
### Supplemental Figures



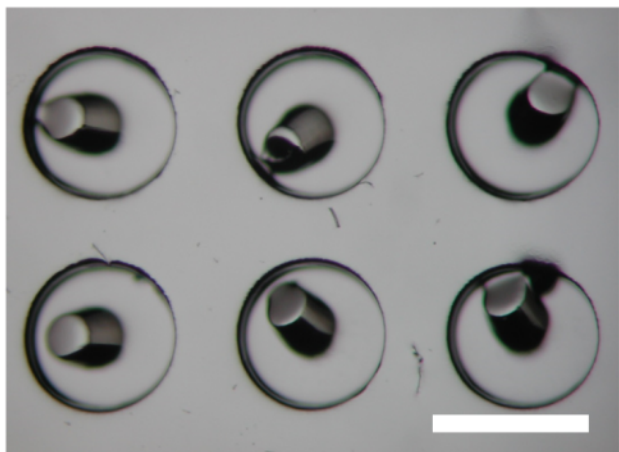
**Figure S1.** Pattern transfer of microfeatures into (A) Sylgard 184 (conventional) PDMS, (B) Sylgard 527 (soft) PDMS, cured at room temperature over 48 hours, and (C) Sylgard 527 cured rapidly at elevated temperatures. These differences are likely due to increased exposure to humidity during the long cure-times required for room temperature cure protocols, making it necessary to cure PDMS rapidly at elevated temperatures. Scale bar = 50  $\mu\text{m}$ .



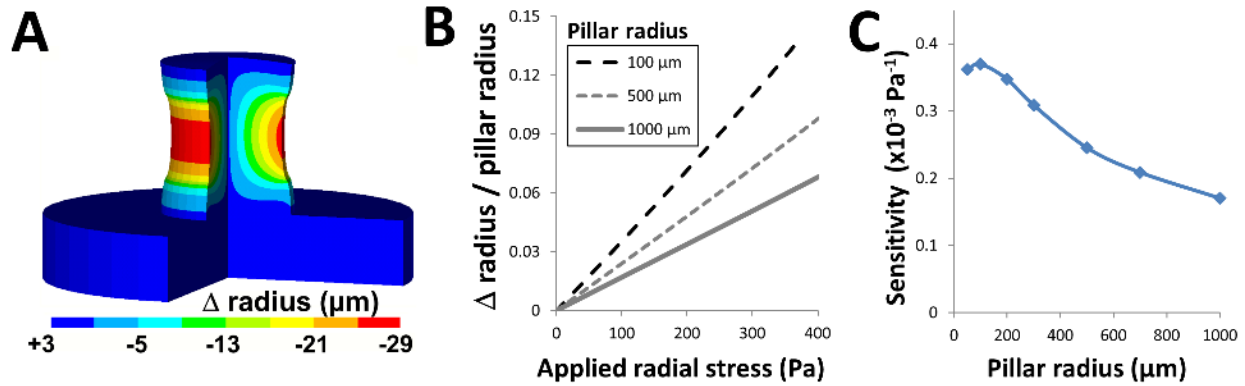
**Figure S2.** Increasing caramelization changes the candy color, but has minimal impact on the supersoft lithography process: candy pucks maintain microscale features for replica molding.



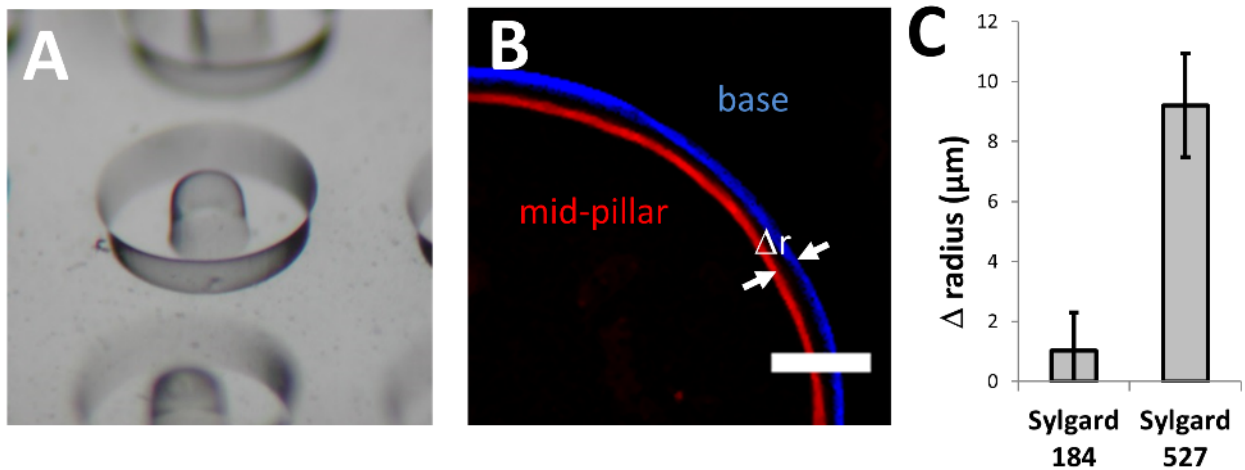
**Figure S3.** The sacrificial candy material can maintain extremely small microfeatures in the surface. An array of microposts (diameter = 1.83  $\mu\text{m}$ , height = 8.3  $\mu\text{m}$ ) is faithfully replicated in the candy. However, in transferring features of this size to soft PDMS, the resulting structures were too flexible to remain upright and collapsed with even slight fluid movement.



**Figure S4.** Microstructures created in soft PDMS collapse due to surface-tension driven forces during evaporation of liquid at room temperature and pressure. Features of this size in conventional PDMS would not collapse due to drying. Collapse of structures can be avoided using processes such as critical point drying or lyophilization. Scale bar = 1 mm.

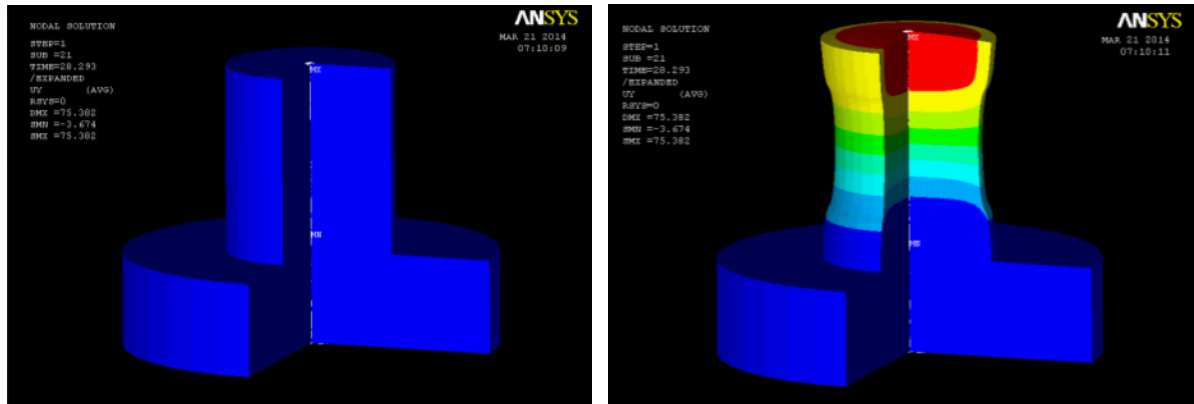


**Figure S5.** (A) Finite element models are used to relate measured radial deformations with applied radial stresses. (B) Fractional change in pillar radius for several sizes of micropillars. (C) The sensitivity of the pillar to applied stress decreases with increasing pillar diameter.



**Figure S6.** (A) Toroid-shaped cavities to mold cell-laden collagen microgels. (B) Comparing the diameter of the pillars in Sylgard 527 at the base and the mid-section demonstrates that (C) pillars fabricated in soft Sylgard 527 PDMS deform under microtissue-generated applied compression. It should be noted that the small change in dimensions between the bottom and middle of the pillar in the conventional Sylgard 184 PDMS replica was due to a small sidewall angle of the pillar ( $< 1 \mu\text{m}$  over  $200 \mu\text{m}$  of thickness), caused by inaccuracies in the fabrication process. This error was less than the observed standard deviation in measurements of post contraction. Scale bar =  $200 \mu\text{m}$ .

## Supplemental movies



**Supplemental Movie 1.** Finite element simulation of axial displacement as a result of radial compression. Colour scale indicates y-displacement (in the vertical direction) and ranges from blue (min) to red (max).

## Supplemental Methods

### Silicon mold fabrication

The initial silicon mold was fabricated using standard SU-8 photolithographic techniques. Briefly, a 4" polished silicon wafer (University Wafers) was rinsed in acetone, and spin-coated with a negative photoresist (SU-8 2075, Microchem), and pre-baked on a hot-plate using a two-step heating protocol (at 65 °C and at 95 °C), according to the manufacturers' instructions. A second layer of SU-8 2075 was spin coated on top of this first layer, to raise the thickness of the resist to ~400  $\mu\text{m}$ . The pre-baking procedure was repeated, and the sample was exposed to UV light (Karl Suss Mask Aligner) through an AutoCAD-designed mask printed on a transparent film (CAD/ART Services; Bandon, OR). The wafer was then post-baked following the same two-stage heating protocol, and developed in SU-8 Developer

(Microchem) to remove the uncrosslinked material. The mold was then hard-baked at 120 °C for 2 hours, silanized with (tridecafluoro-1,1,2,2-tetra-hydrooctyl)-1-trichlorosilane (United Chemical Technologies, Bristol, PA) in a vacuum dessicator for 30 minutes, and replica molded using standard PDMS soft lithography techniques.

#### Inverted sugar candy recipe

The standard inverted-sugar candy recipe used in the confectionary industry is included in this supplemental material for completeness: as shown in Figure 2A, this recipe is not suitable for supersoft lithography. Inverted sugar candy was prepared by preparing a mixture of 65% (w/w) sucrose, 5% (w/w) light corn syrup, and 0.02% (w/w) glacial acetic acid in water, and bringing the mixture to a boil on a hotplate. Temperature is monitored using a digital thermometer, and the beaker was removed from the hotplate at 150 °C. This process took between 25 and 35 minutes. Once cooled to 120 °C, the mixture was poured over the PDMS templates, as outlined in the methods section of the manuscript.

#### Mechanical characterization

Compressive tests were conducted using a XT-PLUS Texture analyzer (TA instruments), set up with a 30 kg load cell. 5-10 mm thick PDMS and candy samples were cast and cured in 22 mm diameter containers. A stainless steel spherical indenter of radius 6.35 mm was used to apply a deformation of 250  $\mu\text{m}$  into the material. The resulting force-displacement curves were fitted to a Hertzian spherical indentation model to calculate the modulus of the material. Four indentation curves were generated from each of at least three independent samples, and averaged. Data reported as means  $\pm$  standard deviation.

Shear rheometry to characterize softer samples was performed on an ARES-LS rheometer (TA Instruments), using a parallel plate geometry. PDMS samples were dispensed onto a 25 mm diameter

glass slide, covered with a second circular coverslip, and cured at 60 °C overnight. The glass slides were loaded in the testing plates and attached with double-sided tape. The shear rheometer was operated in dynamic mode at a frequency of 0.7 rad/s. No slippage of the sample was observed at this frequency, as confirmed by no variation in the loss modulus results. Samples approximately 1 mm in thickness were tested from strains of 10-250 % in increments of 5 %. Data reported as means  $\pm$  standard deviation.

### Cell culture

All cell culture agents were purchased from Invitrogen Life Technologies and from Sigma-Aldrich, unless otherwise stated. HS-5 cells (ATCC) were maintained in Dulbecco's modified eagle medium (DMEM; supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics-antimycotics). Cells were sub-cultured by standard trypsinization protocols at 1:4 ratios when confluence was reached. Briefly, confluent cells were incubated with 0.25% trypsin for 5 minutes until they detached from the surface. Supplemented media was added to neutralize the trypsin, and the number of cells was assessed manually using a hemocytometer. The neutralized trypsin solution was centrifuged at 200 RCF for 5 minutes. The supernatant was aspirated and the cell pellet was resuspended in media, and either re-plated for further culture, or used to form collagen microtissues.

### Finite element simulations

Computational models of the deforming PDMS pillar were constructed in ANSYS (Canonburg, PA), a commercially available finite element modelling software package. A simple 2D axisymmetric model was constructed with rectangular PLANE183 elements. Boundary conditions were established based on the model symmetry and radial pressure was applied via an attached line section. Data was extracted based on nodal displacements, and video sequences created using a  $\frac{3}{4}$  axisymmetric expansion.

### Image analysis

For each fluorescent image of the PDMS pillars, three points were picked on the boundary of the pillar, and the diameter of the circle was calculated using a simple geometric analysis. Briefly, the intersection of perpendicular bisectors of lines connecting the three selected points indicates the center of the circle. The radius can then be calculated based on the positions of the calculated center and the points on the pillar boundary. The change in pillar radius was then used to calculate microtissue stresses, using the FEA (SI Fig S5), and mechanical characterization data (Figure 1B).