Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Creased hydrogels as active platforms for mechanical deformation of cultured cells

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Dedicated to *Professor Priscilla M. Clarkson (1947-2013)* [b]

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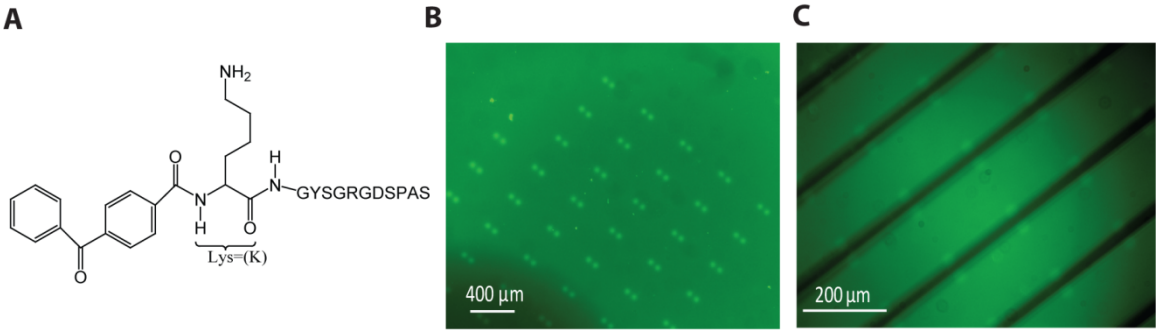

Fig. S1. (A) Chemical structure of the benzophenone-functionalized oligopeptide KGYSGRGDSPAS, (B) a FITC-labeled array of RGD circles patterned on a flat gel surface, (C) a FITC-labeled array of RGD circles patterned on a creased gel surface. The background fluorescence corresponds to FITC molecules that simply diffused into the hydrogels, while the dark stripes in (C) arise from the presence of the templating topographic patterns on the underlying glass substrate.

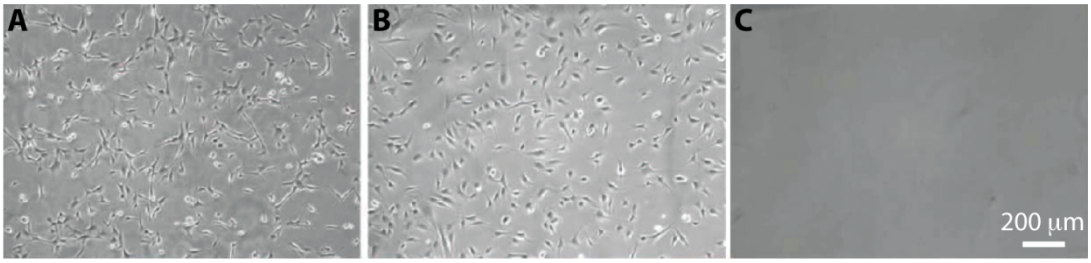

Fig. S2. (A) C2C12 mouse myoblasts cultured on a standard PS substrate as a positive control. (B) C2C12 cells adhered to, and spread on, uniformly RGD-coated gel surfaces, albeit at lower density than for PS. (C) A non-RGD coated gel surface used as a negative control did not promote cell attachment.

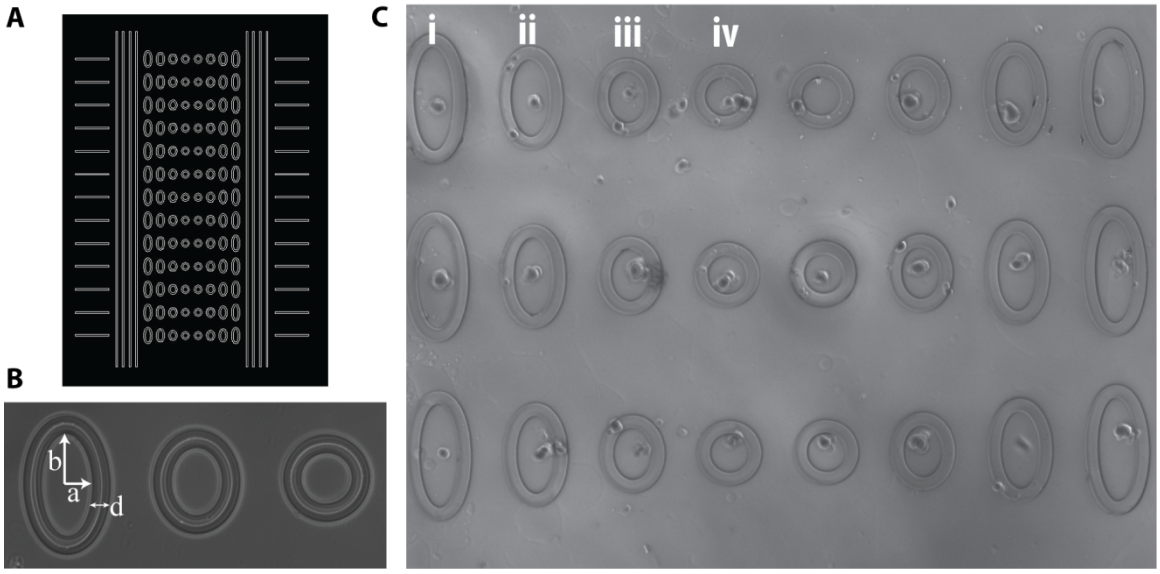

Fig. S3. (A) A photomask used to pattern templating topographic patterns consisting of parallel stripes and ellipses of different aspect ratios. (B) An optical micrograph of a single crease forming on the top of three different ellipses, with *a*, *b*, and *d* indicating the minor axis, major axis, and width of the feature, respectively, (C) An optical micrograph showing one to several cells seeded above each elliptical feature. All the patterns have d = 40 μm and a thickness of 40 μm. Ellipses with four different aspect ratios are patterned. (i) a = 60 μm, b = 160 μm, (ii) a = 60 μm, b = 120 μm, (iii) a = 60 μm, b = 80 μm, (iv) a = 60 μm, b = 60 μm.

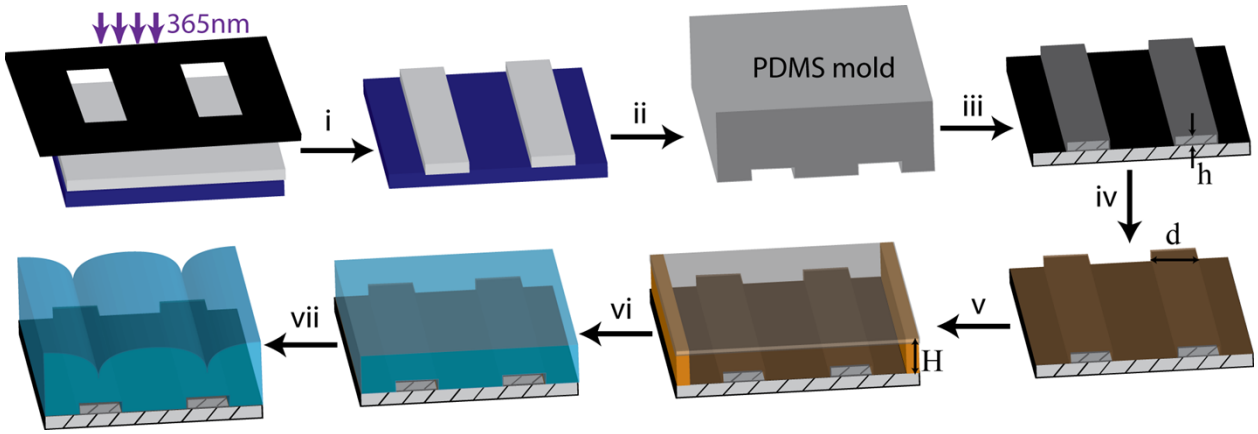

Fig. S4. A schematic illustration of the sample preparation procedure. (i) Photolithographic patterning of negative photoresist SU-8; (ii) casting of a PDMS mold, (iii) soft lithographic printing of Norland Optical Adhesive (NOA 81) topographic patterns with d = 40 μm and h = 40 μm on a glass substrate; (iv) modification of the substrate with 3-(methacryloxy) propyltrichlorosilane to promote covalent anchoring of the hydrogel layer; (v) assembly of a gelation cell from the patterned substrate, a bare glass coverslip, and polyimide film spacers that define the gel thickness of H = 76 μm; (vi) free radical polymerization to form a hydrogel; (vii) swelling-induced formation of creases with locations specified by the topographic substrate pattern.

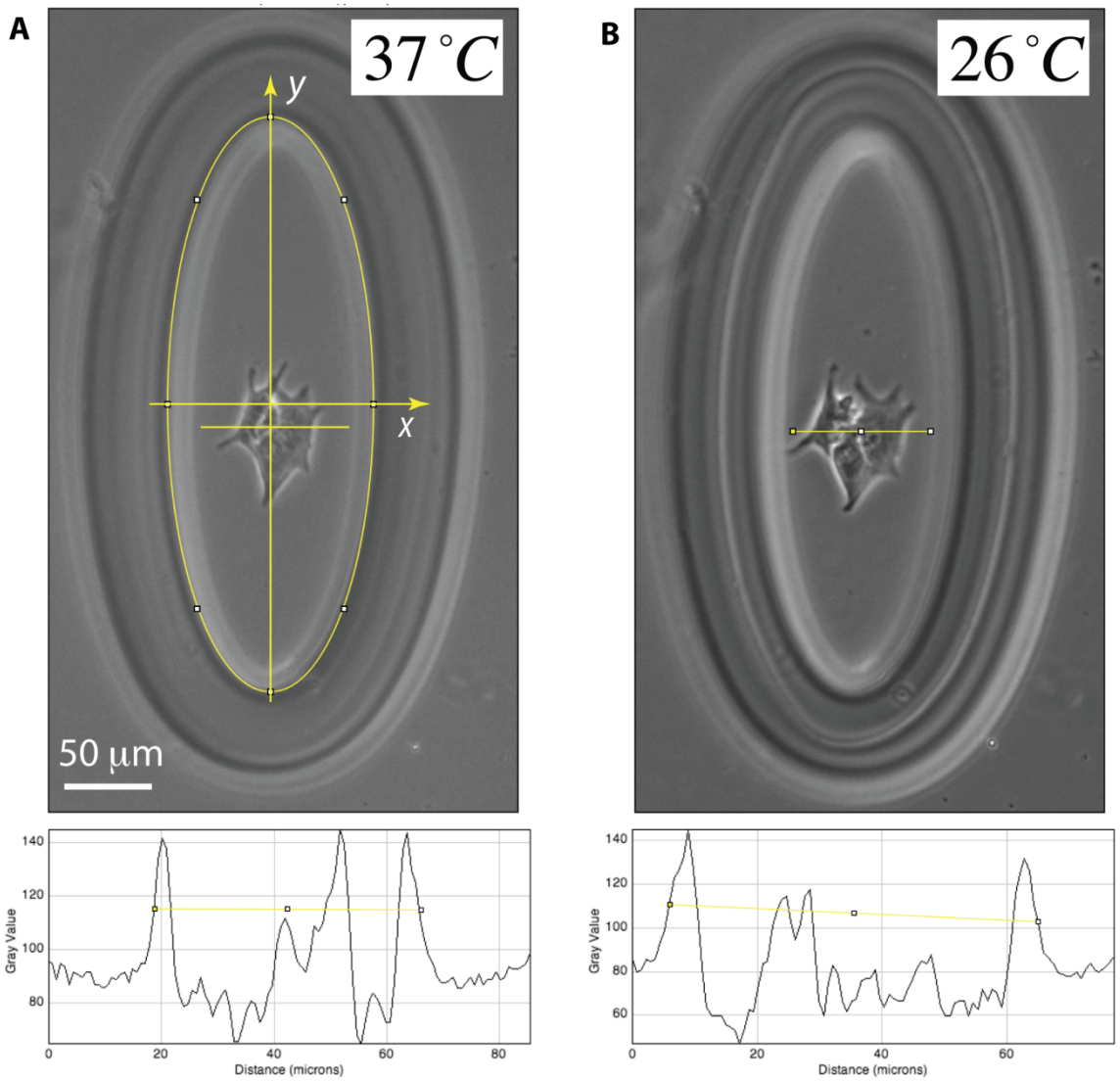

Fig. S5. An example of the image analysis procedure. (A) At 37 °C, a cell cluster is seeded within a elliptical crease directed by an elliptical topographic feature on the substrate. Using ImageJ software, the center of the ellipse is first identified and a Cartesian coordinate system is constructed with the origin at the center of the ellipse, and x and y axes in parallel with the short and long axes of the ellipse, respectively. One line profile is drawn at $y = -20$ μm across the cell cluster along the x direction and the cell cluster dimension is determined by plotting the grayscale intensity profile (shown at bottom). Specifically, we measure the distance between the half-maximum points at the outside edges of the cluster, here determined to be $l_x = 47.5$ μm. (B) At 26 °C, for the same cell cluster, the same procedure is applied and the stretched cell cluster dimension at $y = -20$ μm is determined to be $l_x^{'} = 58.5$ μm. The tensile strain is then calculated by $\varepsilon_x^1 = (l_x^{'} - l_x)/l_x = 0.23$. Following the same procedure, two more line profiles along the x direction are drawn and the tensile strain of $\varepsilon_x = 0.22 \pm 0.01$ is taken from the average value of three measurements. The tensile strain in the y direction is obtained in the same way.

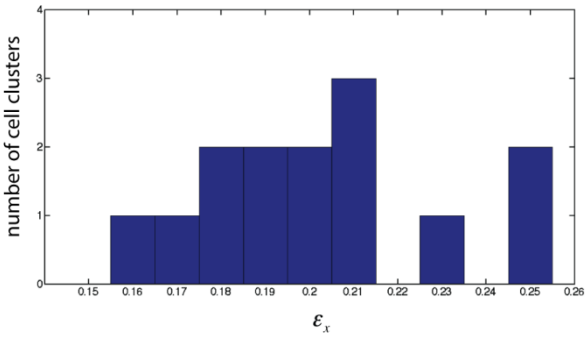

Fig. S6. A bar graph showing the distribution of cell cluster deformations measured under plane strain condition as shown in Fig. 3. The tensile strain is calculated to be $\varepsilon_x = 0.20 \pm 0.03$.

Movie 1. Several cells are seeded between two parallel creases at 37 °C. Reducing the temperature to 26 °C causes creases to grow in depth, stretching the cells under plane strain deformation. Raising the temperature to 37 °C leads to recovery of the initial cell size. The total observation time is ~ 30 min.

Movie 2. A single cell is seeded at 37 °C in the center of an elliptical crease (type ii). Reducing the temperature to 26 °C subjects the cell to a biaxial stretch, while raising the temperature to 37 °C leads to recovery of the initial cell size. The total observation time is ~ 30 min.

Movie 3. A control experiment is performed on the flat unpatterned region of the same hydrogel surface coated with the same RGD peptide density. Over the same period of ~ 30 min, a change in temperature from 37 °C to 26 °C does not induce significant changes in the size of cells.