Supplementary Information

In Vitro Wrinkle Formation Via Shape Memory Dynamically Aligns Adherent Cells

Supporting Methods 1: Example Formulation for the tBA/BA Substrate

An SMP film with a hydrated Tg of ~37 °C was achieved when using a copolymer composition of 95 wt-% tBA and 5 wt-% BA, with a constant 5 wt-% TEGDMA and 0.5 wt-% DMPA, computed relative to the weight of co-monomers in the formulation. For example, tBA (1.5 g), BA (78.9 mg), TEGDMA (61.5 mg), and DMPA (8.2 mg) were mixed together and syringed between two glass slides with a 1 mm thick teflon spacer. The samples were UV cured for 1 h, extracted in methanol for 6 h, and dried in a vacuum oven at 55 °C overnight. After soaking in water for 24 h, DSC was performed and the resulting T_g of the hydrated copolymer was 37 °C.

Supporting Methods 2: Substrate Characterization

Linear viscoelastic thermomechanical properties of the substrate were determined using a dynamic mechanical analyzer (DMA) (TA Instruments Q800). A rectangular bar of the copolymer film was cut and placed in the tensile grips of the DMA and loaded in multi-frequency strain mode with oscillation amplitude of 10 μm and an oscillation frequency of 1 Hz. The sample was heated to 70 °C to erase all thermal history and then cooled down to -90 °C at 3 °C/min. Once thermally equilibrated, the sample was then heated to 100 °C at 3 °C/min. The second heating trace was recorded and used to determine storage modulus as a function of temperature.

Supporting Methods 3: Cell Staining

After incubation, cells were fixed in 3.75% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100 for 5 min, and blocked with 1% bovine serum albumin for 30 min. Cells were then stained with Alexa Fluor 647 conjugated phalloidin (Invitrogen) for 30 min, followed by staining with DAPI for 5 min. All steps were performed at room temperature. After staining, samples were mounted in Prolong Gold. The Alexa Fluor 647 enabled imaging of the actin cytoskeleton whereas the DAPI enabled imaging of the cell nucleus.

Supporting Methods 4: Cell Nuclear Alignment Determination

The nuclear angle for each cell was determined using ImageJ v1.44 (U.S. http://rsbweb.nih.gov/ij/). A threshold was applied to each image to isolate cell nuclei and a binary image was created. The Analyze Particles function was then used to fit an ellipse to each nucleus and determine the corresponding angle, ranging from 0° to 180°. These angles correspond to an arbitrary reference angle of 0°, which is subsequently refined as described below. A value of 90° was subtracted from each cell angle to adjust the range of angles to -90° to +90°, centered around 0°. The truncated standard deviation[1] of nuclear angles about 0° was then calculated, yielding the angular spread. The reference angle was then incremented by 1° and the resulting truncated standard deviation calculated for all angles up to a reference angle of 180°. To normalize between wrinkled and non-wrinkled substrates, the reference angle that yielded the minimum standard deviation, or highest degree of alignment, was the angle used for statistical comparison. The reference angle yielding the minimum standard deviation was compared to angle of wrinkle direction for wrinkled substrates and was always within ±3° of the wrinkle direction, meaning the angle of orientation was well aligned with the wrinkle direction.

Statistical analyses were then performed on the truncated standard deviations, or angular spread, for the different groups.

Supporting Methods 5: Statistics

Statistical analysis was performed using a similar method as reported in our previous study.[2] Resampling statistics were used rather than parametric statistics since sample size was small. One factor ANOVA was used to compare all groups in a given experiment using the Design5 Excel Macro [3]. For the previously wrinkled substrate experiment, the one factor was substrate prestrain with 6 groups (0%, 2%, 7%, 12%, 17%, and 23%), and for the actively wrinkling substrate experiment the one factor was topography with 3 groups (flat, wrinkled, and flat control). Each group was then compared to every other group using permutation testing on the raw data of angular spread for each replicate in a group. This was performed using the Design5a Excel Macro.[3] Significance was determined on the $P<0.05$ level.

Supporting Video 1: Wrinkle Formation in Water

To investigate the time scale of wrinkle formation and simulate the actively wrinkling experiment, a gold coated SMP substrate with a prestrain of 12% was soaked in 30 °C water for 5 h and then transferred to 37 °C water and imaged over time. A Leica DMI 6000B inverted microscope was used in phase contrast along with an Andor Luca-R camera using a 10x/0.22 NA objective. Imaging began 10 min after transferring the sample to the 37 °C water due to setting up the image acquisition parameters. At each time point a built-in autofocus was used to focus on the surface of the sample and a z-stack was performed to acquire all focal planes of the substrate. Images were post-processed in ImageJ to obtain the image with optimal focus for each time point. These images were then stacked and an .avi with 5 frames per second was created. From the video, wrinkling had begun to occur within the first 10 min of transferring the sample to the 37 °C water as can be noted by the onset of wrinkle formation in the first frame. The wrinkles grow quickly over the next 10-15 frames (40-55 min after placed in 37 °C water) and crack formation follows. It appears wrinkle formation occurs once bulk SMP recovery begins and grows until reaching a saturation point, after which the substrate still recovers and cracks continue to propagate.

Figure S1. Compositional effect on the T_g of the copolymer substrate: The weight fractions of tBA and BA were systematically varied and the T_g of each copolymer (without methanol extraction) was determined by DSC. Increasing the weight fraction of tBA causes the Tg of the copolymer to increase.

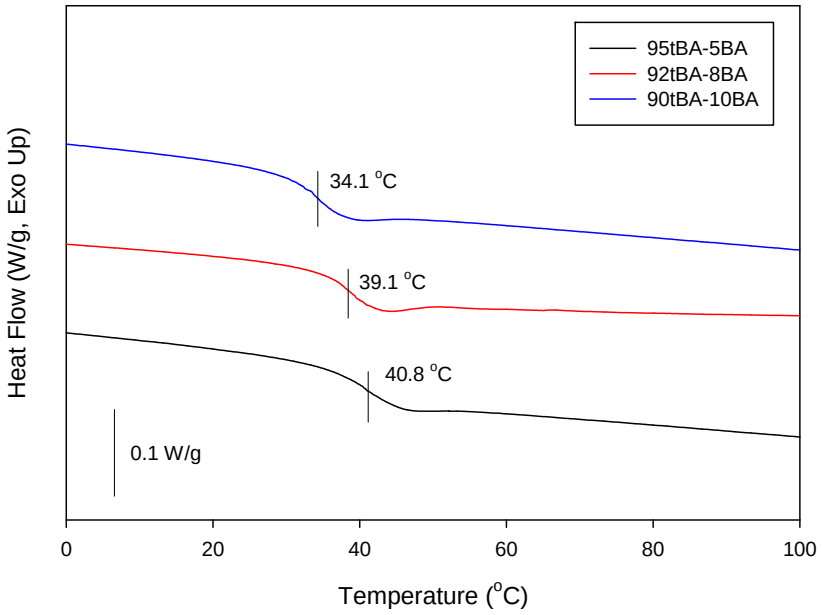


Figure S2. Sputter time effect on gold coating thickness: Increase in sputter time led to a linear increase in gold coating thickness

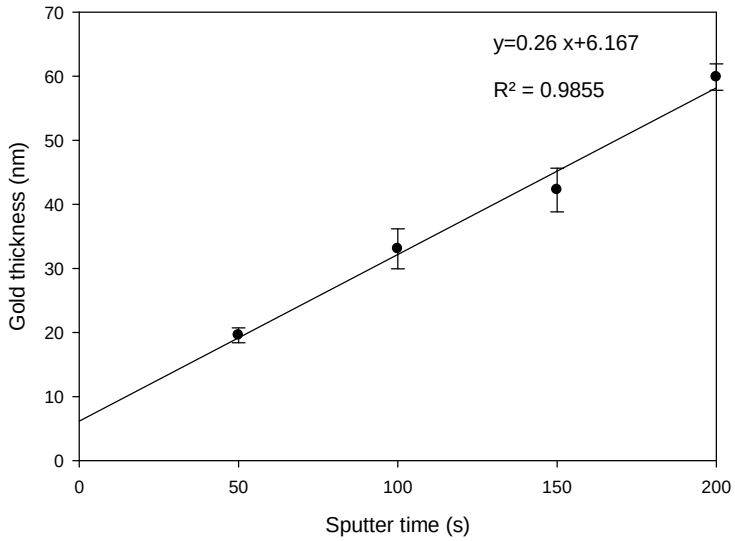


Figure S3. Water plasticization lowers the T_g of the copolymer: a 95tBA-5BA sample was hydrated in water at 37 °C for 24 h and the effect of water plasticization on T_g was determined using DSC. Water lowered the T_g of the copolymer by 3 °C.

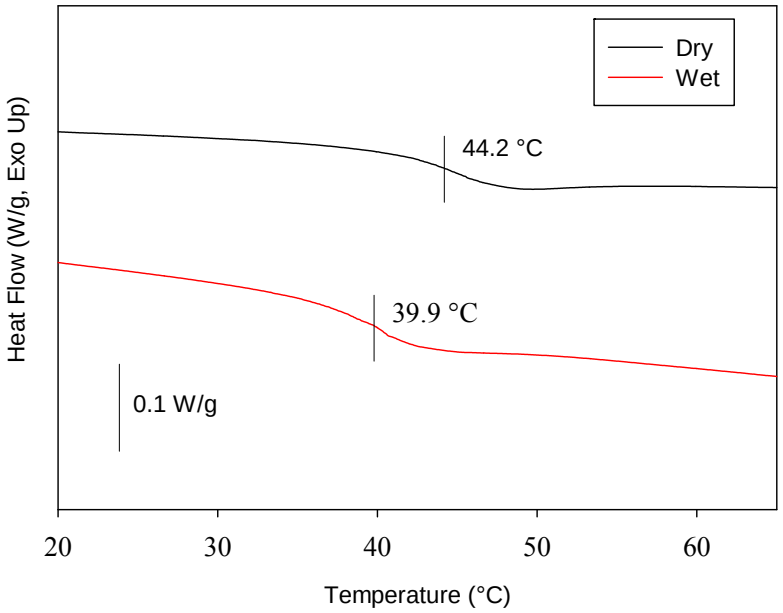


Figure S4. Bulk recovery profile of the hydrated copolymer substrate at 37 °C: A rectangular bar was uniaxially fixed with 12% strain and held isothermal at 37 °C in a submersion clamp fixture on the DMA. The sample recovered 80% after 6 h with 50% of the recovery coming within the first hour.

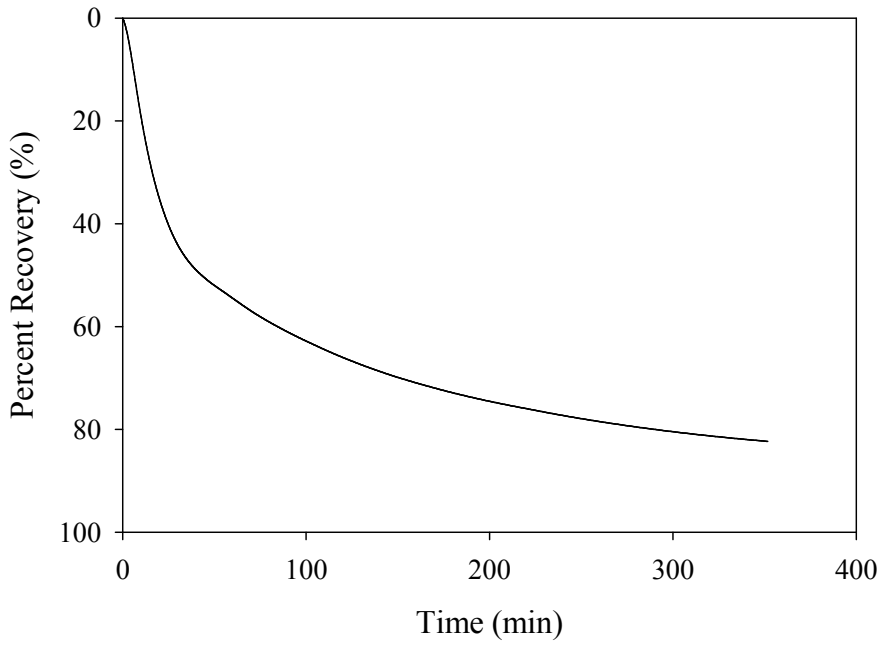


Figure S5. Cell viability on wrinkled substrates. LIVE/DEAD assay performed on cells seeded on a) non-wrinkled, b) 2%, c) 7%, d) 12%, e) 17%, f) 23% prestrain. (Scale bar is 200 μm). Green cells represent live cells while red cells represent dead cells. Cells showed >95% viability for all samples.

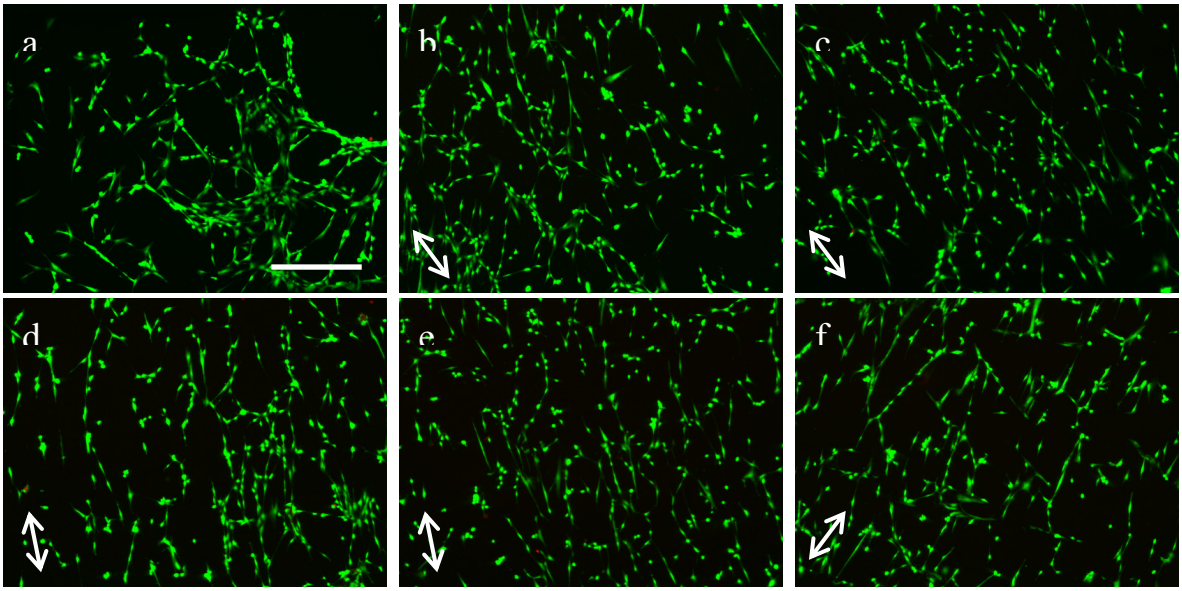


Figure S6. Nuclear alignment on flat control: Phalloidin and DAPI assays performed on cells seeded at 30 °C for 29 h (Scale bar is 200 μm). Qualitatively there is no preferential orientation of the cell cytoskeleton; the angular histogram shows a random distribution of nuclear angles.

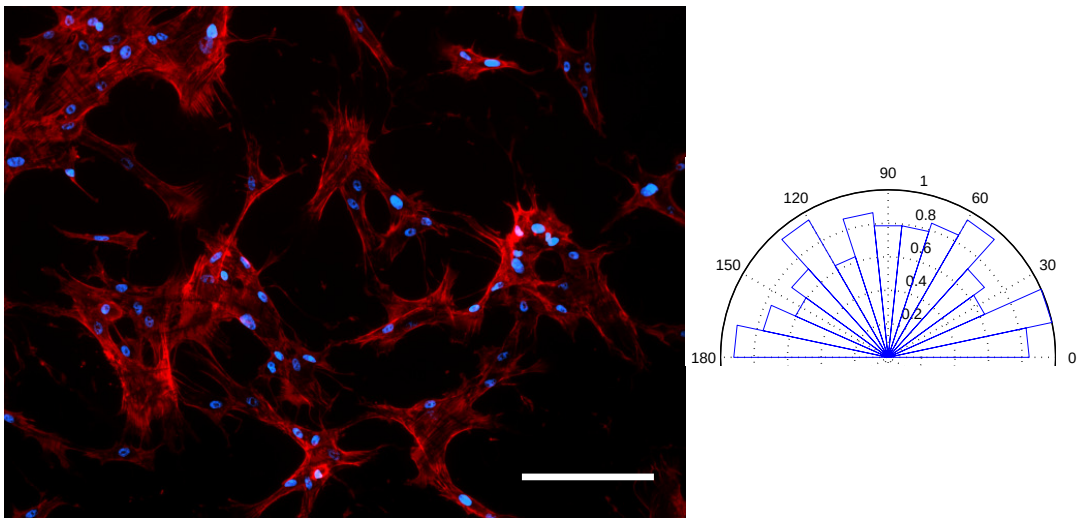


Figure S7: Cell viability during wrinkle formation. LIVE/DEAD assay performed on cells seeded a) at 30 °C for 5 h, b) at 30 ° C for 29 h, c) at 30 ° C for 5 h and 37 °C for 24 h. (Scale bar is 200 μm). Green cells represent live cells while red cells represent dead cells. Cells showed >95% viability for all samples

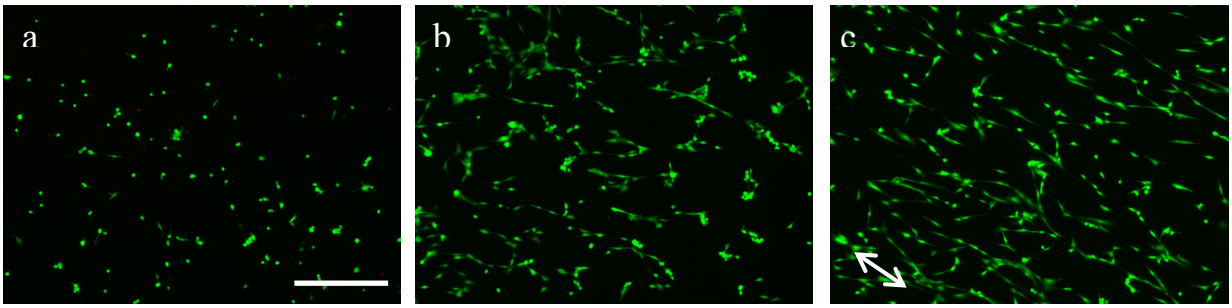


Figure S8. Optical microscopic images of wrinkles and cracks formed under various prestrain a) 2% b) 7% c) 12% d) 17% e) 23%. Scale bar is 200 μm.

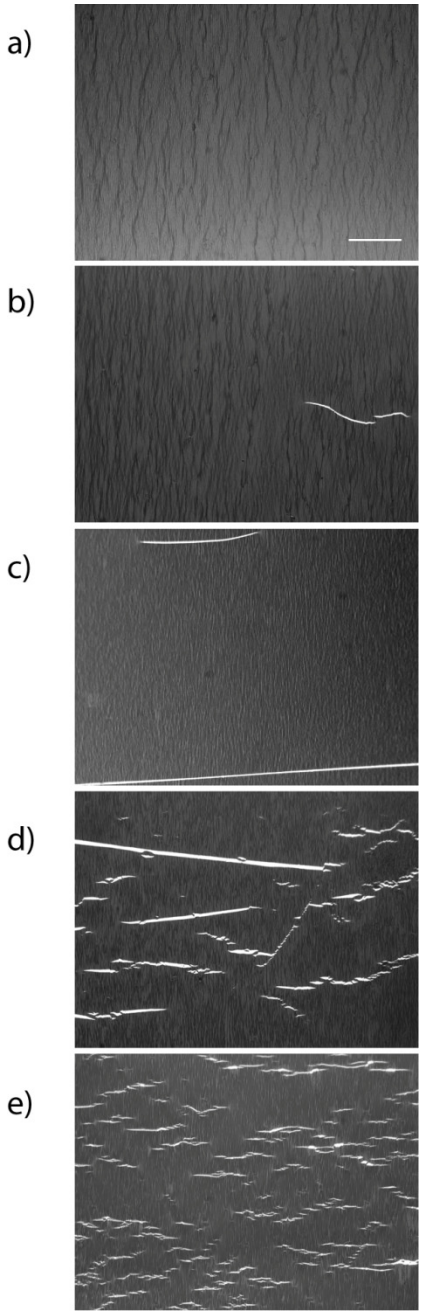


Figure S9. Correlation between wrinkle aspect ratio (amplitude/wavelength) and cell nuclear alignment.

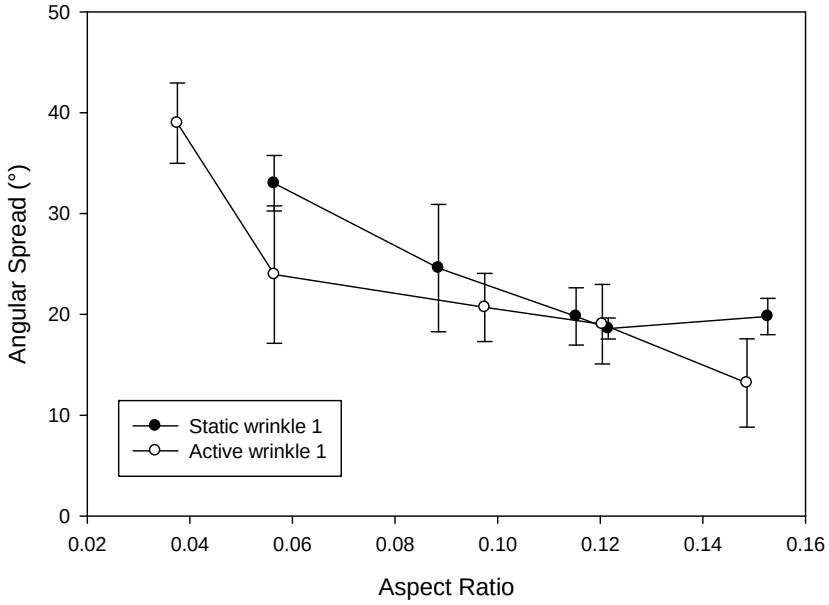
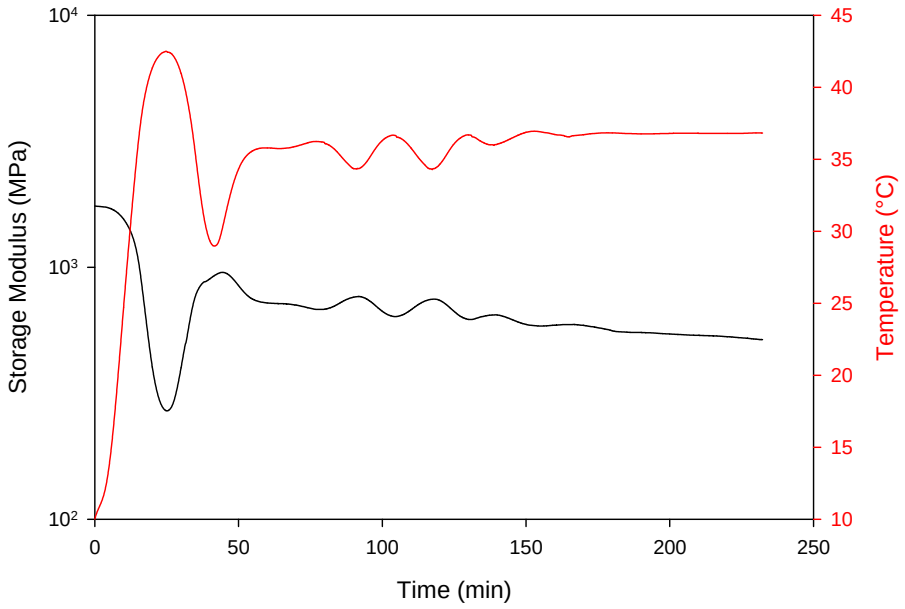


Figure S10. Thermomechanical properties of the hydrated tBA/BA substrate: A fully hydrated rectangular sample was ramp to 37 ºC and held isothermal for 3h on a DMA submersion clamp fixture.



References

1. P. Davidson, M. Bigerelle, B. Bounichane, M. Giazzon and K. Anselme, *Acta Biomater.*, 2010, **6**, 2590-2598.
2. K. A. Davis, K. A. Burke, P. T. Mather and J. H. Henderson, *Biomaterials*, 2011, **32**, 2285-2293.
3. J. B. Todman and P. Dugard, *Single-case and small-n experimental designs: A practical guide to randomization tests*, Lawrence Erlbaum Associates, 2001.