Dynamic cell behavior on shape memory polymer substrates

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Abstract

Cell culture substrates of defined topography have emerged as powerful tools with which to investigate cell mechanobiology, but current technologies only allow passive control of substrate properties. Here we present a thermo-responsive cell culture system that uses shape memory polymer (SMP) substrates that are programmed to change surface topography during cell culture. Our hypothesis was that a shape-memory-activated change in substrate topography could be used to control cell behavior. To test this hypothesis, we embossed an initially flat SMP substrate to produce a temporary topography of parallel micron-scale grooves. After plating cells on the substrate, we triggered shape memory activation using a change in temperature tailored to be compatible with mammalian cell culture, thereby causing topographic transformation back to the original flat surface. We found that the programmed erasure of substrate topography caused a decrease in cell alignment as evidenced by an increase in angular dispersion with corresponding remodeling of the actin cytoskeleton. Cell viability remained greater than 95% before and after topography change and temperature increase. These results demonstrate control of cell behavior through shape-memory-activated topographic changes and introduce the use of active cell culture SMP substrates for investigation of mechanotransduction, cell biomechanical function, and cell soft-matter physics.

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1. Introduction

Cells are capable of surveying the mechanical properties of their surrounding environment [1]. This capacity is critical to events of embryogenesis, postnatal tissue development and maintenance, and the onset of certain pathological conditions [2–4]. In the synthetic realm, substrates of defined topography or elastic modulus have emerged as powerful tools in the investigation of the underlying cellular mechanisms. In particular, mesoscale, micro-scale, and nanoscale patterns of substrate topography have been shown to direct cell alignment, cell adhesion, and cell traction forces [5–12], while isotropic and anisotropic substrate elasticity have been used to direct cell lineage specification, growth, and migration [13,14]. Defined topographies have also found application in biomedical device design, for example to enhance implant cell adhesion and tissue ingrowth [15]. These findings have underscored the potential for substrates to control and assay the mechanical interactions between cells and their physical environment during cell culture; however, the substrates used to date have generally been passive, with substrate properties that could not be programmed to change significantly during culture. This physical stasis has limited the potential of substrates to control cells in culture and, therefore, to advance both fundamental understanding of cell biology and cell-based bioengineering applications.

To overcome the limitations of physically static systems, there has been growing interest in the development of stimuli-responsive biomaterials for cell culture, with several notable areas of progress. Poly(N-isopropylacrylamide) (PIPAAm) has been used to release cell layers [16] that can be used in laminar tissue engineering [17] by taking advantage of the material’s lower critical solution temperature (LCST) at 32 °C and a change in hydrophobicity around this temperature. PIPAAm’s thermo-responsive behavior has also been exploited in copolymer hydrogels designed to apply equibiaxial stretching to encapsulated cells when the temperature is lowered to 25 °C [18]. Elastin-like polypeptides (ELPs) are stimuli-responsive biomaterials that can exhibit an LCST in a physiologically relevant range [19] and have been used to capture and release proteins at surfaces [20], which can be used to control cell adhesion. Thermo-responsive ELP hydrogels for cell culture or tissue engineering applications have also been
developed [21,22]. To allow for spatial control of responsive biomaterials, UV-induced crosslink degradation has been used in a photo-responsive biomaterial to investigate the effects of substrate modulus change during cell culture [23]. This approach has been extended to three-dimensional spatial control with the development of a PEG-based hydrogel and two-photon photo degradation system to study cell response to selective removal of cell-adhesion sites [24].

Exploitation of shape memory polymers (SMPs) has also been proposed as a possible solution to current limitations of static, unresponsive substrates, medical devices [25], and tissue engineering scaffolds [26,27]. SMPs are a class of active materials that have the ability to memorize a permanent shape through cross-linking, be manipulated and then fixed to a temporary shape by an immobilizing transition, such as vitrification or crystallization, and then later recover to the permanent shape by a triggering event, such as a thermal, electrical, or solvent activation [28]. This phenomenon is known as one way shape memory, since activity is witnessed in one direction. Although most previous work has centered on bulk one way shape memory behavior, surface (or topographical) shape memory is also possible [29,30]. In the case of thermal triggering, an SMP frozen in a stressed configuration will return to its equilibrium configuration upon the action of network enthalpy (i.e., the energy upon heating through the glass transition or melting point, termed the recovery temperature. Traditional biocompatible SMPs, including crosslinked (to confer shape memory properties) PCL, PLGA, and polystyrene, feature recovery temperatures too high for cells to maintain viability during the thermal trigger [28]. Considerable effort has been focused on this issue of thermal cytocompatibility and, very recently, having observed dynamic mechanical characteristics indicating the potential for shape memory behavior [31], one of us has reported bulk and surface shape memory behavior of a commercially available, non-cytotoxic [32] optical adhesive [33], Norland Optical Adhesive 63 (NOA-63, Norland Products, Cranbury, NJ, USA), with a recovery temperature that can be tuned by the crosslinking conditions [33].

The goal of this study was to develop temperature-responsive SMP substrates that can be programmed to change topography under cell culture conditions. We further sought to test the working hypothesis that a shape-memory-activated change in substrate topography can be used to control cell behavior. To test this hypothesis, we programmed SMP substrates to transition from a micron-scale grooved surface to a flat surface and analyzed cell alignment before and after the topographic transition.

2. Materials and methods

2.1. Substrate preparation

To enable surface shape memory during cell culture, we prepared a glassy SMP from the NOA-63 formulation (Lot # 111). To prepare shape memory substrates in their permanent, flat surface, NOA-63 was used as received and injected between an aluminum block and a glass slide with a 1 mm thick Teflon spacer (Supplementary Data Fig. S1). NOA-63 is supplied as a photocrosslinkable solvent-free prepolymer with a photoinitiator and, although its exact composition is not disclosed by the supplier, it is known to be a polyurethane that is end-linked by a thiol-based crosslinker using photoinitiated thiol-ene crosslinking [34]. NOA-63 exhibits one way shape memory around its glass transition temperature (Tg; Supplementary Data Fig. S2) [33]; moreover, the Tg of NOA-63 can be manipulated by controlling the temperature during photo-curing, which alters the vitrification event. The effective Tg of NOA-63 when wet (e.g., during cell culture) is lower than the dry Tg due to plasticization by water. To address the shift in Tg caused by hydration, we adjusted the wet Tg to fall in a cell-compatible range, approximately 30 °C–37 °C, by choosing a cure temperature of 125 °C, which resulted in a dry Tg of 51 °C above the cell-compatible range. The prepolymer was photocured for 20 min on a hot plate at 125 °C with a 1000 W Spectroline SB-100PC 365 nm UV light source placed 6.5 cm above the top glass surface of the mold described above. The film was removed from the mold, placed on the hot plate, and post-cured with heat and UV light for an additional 3.75 h with the light placed 6.5 cm above the NOA-63 surface. The cured material was stored in a desiccator at −20 °C until further processing. Next, the NOA-63 films were readyed for embossing by cutting into approximately 3 mm × 3 mm rectangles. These pieces were then fixed to a temporary shape, such as an engraving surface (Supplementary Data Fig. S3), which was the negative impression of a vinyl record (Supplementary Data Method 1), and compressed using a Carver 3851-0 hydraulic press with heating platens. A compressive stress of 4.9 MPa was applied to the samples at 90 °C and held for 1 min. Water cooling of the platens was then started and the embossing stress was released at 20 °C after approximately 5 min of cooling. Samples prepared in this manner thus had a temporarily grooved topography that could be triggered to transition to the permanent flat surface by heating. To produce a static control substrate with a permanently grooved topography (i.e., to produce a substrate that has the grooved topography after curing, instead of after embossing into a temporary shape), we replicated equilibrated samples (described under Substrate Surface Equilibration) in silicone (Supplementary Data Method 2), which then served as a mold to prepare permanently grooved SMPs. NOA-63 was poured onto the mold and cured following the heat and UV protocol described above. Finally, to produce a static control substrate with a flat topography, temporarily grooved samples were recovered in air at 90 °C for 5 min before sterilization to recover the permanent flat topography. Sample topography was measured by profilmometry (Supplementary Data Method 3) before sterilization.

2.2. Substrate surface equilibration

SMPs based on Tg triggers (Class I SMPs [35]) exhibit a temperature range over which recovery from the temporary to the permanent shape can occur. For a thermally triggered SMP surface to function as an active cell culture substrate, the substrate must maintain a stable temporary topography during an initial phase of cell culture and then recover its permanent topography following a temperature increase. We developed such an equilibration protocol and found that approximately 30 h of equilibration at 30 °C in complete growth medium (but before cell-seeding) was required to reach a relatively stable topography where topographic features of initial amplitude 25.6 ± 0.8 μm recovered to a nearly 28.5 h at 30 °C the amplitude decreased 4.5 μm to 8.1 ± 1.1 μm (Supplementary Data Fig. S4) for an average rate of change of 0.16 μm h−1.

2.3. Cell culture

Prior to active cell culture experiments, C17/1017/2 mouse embryonic fibroblasts were expanded in complete growth medium: Basal Medium Eagle supplemented with 10% fetal bovine serum (Invitrogen, Gibco, Lot #502439), 1% penicillin/streptomycin, and 1% Glutamax (Invitrogen) in a 37 °C humified incubator with 5% CO2. Cells were cultured on a T182ask with 35 mL of complete growth medium. The medium was changed every four days and cells were passaged at 80% confluence using 0.25% trypsin-EDTA solution. Cells were collected at passage 14 for active cell culture experiments.

2.4. Active cell culture experiments

To determine whether a change in topography can be used to control cell behavior, we plated C17/1017/2 mouse embryonic fibroblasts on the temporary topography and analyzed cell alignment before and after triggering the shape memory activated topographic transition. Temporarily grooved samples, grooved control samples, and flat control samples were cleaned by vortexing in 70% ethanol for 20 s and then sterilized by exposure to UV light for 12 h (Supplementary Data Method 4). Samples were then placed in a 96-well plate and 150 μL of complete growth medium was added. The plates were placed in a 30 °C incubator for 30 h to equilibrate the temporarily grooved samples. After 30 h, the existing medium was removed and replaced with 150 μL of a cell solution at a concentration of 20,000 cells mL−1 in complete growth medium. Cells on temporarily grooved samples, grooved control samples, and flat control samples were stained and imaged after one of two endpoints: before transition (9.5 h in a 30 °C incubator) and after transition (9.5 h in a 30 °C incubator followed by 1 h in a 37 °C incubator). Three cured NOA-63 substrates were prepared identically. For each such substrate, two sets of temporarily grooved samples were embossed by independent shape fixing cycles. The active cell culture experiment was run for each cycle of each substrate for an n of 6, except where contamination caused a reduction in sample number as indicated.

2.5. Cell alignment quantification

Cells were stained with Cilengitide Deep Red Plasma Membrane stain (Invitrogen). The Cilengitide stain was used at 5 μL mL−1 and all incubation steps were performed at 30 °C. Fixed Cilengitide stained samples were mounted in Prolong Gold (Invitrogen). Cell morphometric data was quantified by manually tracing cell outlines (30 isolated cells per replicate) from Cilengitide stained samples using ImageJ v1.43 (U.S. NIH http://rsb.info.nih.gov/ij). The traces were filled and binary images were created. The Analyze Particles function was used to determine cell area (A),...
Fig. 1. Active cell culture substrate transitions from a grooved topography to a flat surface. Cells were plated on an equilibrated temporarily grooved sample and allowed to attach for 9.5 h. The substrate was then triggered to transition to the flat surface by moving the samples to a 37 °C incubator. After 19 h, the cells were assayed again to detect any changes due to the topographic transition. Traces below images are profilometry scans of representative samples. SEM images are representative samples without cells to highlight the change in topography.

Fig. 2. Change in substrate topography controls cell behavior. a, Cells stained with Cellmask plasma membrane stain on a temporary grooved topography are aligned with groove direction (white arrow) before transition. After transition, cells are randomly oriented. The angular histogram shows small dispersion and long average mean resultant vector length (orange line extending radially from origin), $R$, before transition. After transition, cell angles are widely dispersed and the average $R$ has shortened significantly ($p = 0.001$, $n = 6$). Bins are 12° and bin length is normalized by the bin with the highest frequency of cell angles. Color indicates contribution from each sample ($n = 6$). Scale bar is 200 µm. Traces are as in Fig. 1. b, Cells on flat control substrates are randomly oriented before and after temperature increase with large dispersion and short average $R$ ($n = 6$). c, Cells on grooved control substrates are aligned with groove direction before and after temperature increase with small dispersion and long average $R$ ($n = 4$).

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perimeter (p), shape index \((p_2/4\pi A_1)\), and angle. Cell angles were defined from \(0^\circ\) to \(180^\circ\). Cell angles on grooved samples were rotationally shifted so that the groove direction corresponded to an angle of \(90^\circ\). Cell alignment was quantified by the mean resultant vector length \((R)\) calculated using the vector summation of individual cell angles treated as unit vectors and normalized by total cell number \([36]\). Uniformly distributed cell angles feature an \(R = 0.63\) when defined from \(0^\circ\) to \(180^\circ\), and cells aligned parallel to a biasing angle have an \(R\) of 1.0.

2.6. Cell viability

Cells were stained with LIVE/DEAD cell viability stain (Invitrogen); LIVE/DEAD stain was used at a concentration of 2 \(\mu\)M for both Calcein AM and EthD-1. Samples were stained following manufacturer protocol, except that the incubation steps were performed at 30 °C. Cell viability was calculated by counting the number of live and dead cells for a 2052 \(\mu\)m \(\times 1539\) \(\mu\)m area of the sample and dividing the number of live cells by the total number of cells.

2.7. Actin cytoskeleton staining

Cells were stained with Alexa Fluor 647 conjugated phalloidin (Invitrogen), enabling imaging of the actin cytoskeleton. Fixed phalloidin stained samples were mounted in Prolong Gold.

2.8. Cell imaging

LIVE/DEAD and Cellmask stained cells were imaged on a Leica DMI 4000B inverted microscope with a Leica DFC 340FX camera using a 3x/0.15 NA objective. Phalloidin stained cells were imaged on a Zeiss LSM 710 confocal laser scanning microscope using a 20x/0.8 NA air or a 40x/1.30 NA oil objective with Zeiss Immersol 518 F immersion oil. ImageJ was used to apply the red or green lookup table to all micrographs. Histogram stretching was applied to Cellmask images in order to maximize image contrast.

2.9. SMP recovery kinetics

Temporarily grooved samples were incubated simultaneously with cell-seeded samples and removed from the heated incubators at the pre-selected intervals to determine substrate topography recovery kinetics (Supplementary Data Fig. S4). Topographic amplitude was measured for each sample by profilometry before and after thermal treatment (Supplementary Data Method 3). Relative amplitude was calculated as the average amplitude measured after thermal treatment divided by the average amplitude measured after embossing, this measure ranging from 1.0 for no recovery to 0.0 for complete recovery. Representative samples were also imaged using scanning electron microscopy (Supplementary Data Method 5) for qualitative comparison.

2.10. Statistics

The 95% confidence intervals of the mean average resultant vector length were calculated using a bootstrap method (Supplementary Data Method 6). A two factor generalized linear model permutation-based ANOVA was used to compare all groups with controls followed by multiple comparisons. The two factor ANOVA test was performed using the DISTLM software and permutation tests were performed using a Minitab macro for multiple comparisons (Supplementary Data Method 7).

3. Results

3.1. Substrate material properties

The SMP substrate employed in this study featured a stepwise decrease in tensile storage modulus at about 51 °C, with a glassy modulus of about 2200 MPa below this temperature and a rubbery plateau of about 25 MPa above this temperature (Supplementary Data Fig. S5). Embossing to fix a temporary topography produced rounded peaks with a peak to trough amplitude of 25.6 ± 0.8 \(\mu\)m.

3.2. Active cell culture experiments

Cells were seeded on equilibrated temporarily grooved samples and allowed to adhere and spread for 9.5 h at 30 °C before imaging. The topographic change was activated and allowed to recover to completion for a second 9.5 h time period and cell morphology was allowed to stabilize for a final 9.5 h time period before final imaging (Fig. 1). Shape memory activation induced a topographic transition (Supplementary Data Movie 1) that was found to cause cells to change from preferential alignment along the grooves to a more random, unaligned orientation (Fig. 2a). Prior to shape memory activation, cells were elongated and aligned parallel to the direction of the grooves with an average \(R\) of 0.85 ± 0.04 (Fig. 3, Supplementary Data Fig. S6). Following shape memory activation, however, cells became randomly oriented with an \(R\) of 0.67 ± 0.05 \((p = 0.001, n = 6)\). Control groups in which the substrate did not change topography confirmed that the observed change in cell alignment was a result of the change in topography rather than the change in temperature (Fig. 2b,c, Supplementary Data Fig. S7). Angular histograms show clearly the large dispersion of cell angles on flat and recovered-to-flat samples and the small dispersion of cell angles on grooved and temporarily grooved samples (Supplementary Data Fig. S8). In contrast to the observed changes in cell alignment, the projected cell area, cell perimeter, and cell shape index were found to be similar for all groups (data not shown) and we observed qualitatively that cell shapes were similar among all grooved and flat groups regardless of alignment angle, except for

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**Fig. 3.** Cell angle dispersion increases following topographic transition. Box plot of average \(R\) from angular histogram data. Orange (uppermost) shading indicates the total range of confidence intervals for all grooved samples and red (bottommost) shading indicates the total range of confidence intervals for flat and recovered to flat samples. Orange dashed-dotted line indicates greatest possible \(R\), alignment to a single angle, and red dashed line indicates a uniform distribution of angles from \(0^\circ\) to \(180^\circ\). Boxes display interquartile range with median center line; diamonds indicate mean; capped whiskers indicate 95% confidence interval of the mean; and uncapped whiskers indicate minimum and maximum data values. Star indicates significance \(p = 0.001\) \((n = 6)\), permutation test. A two-factor generalized linear model, permutation-based ANOVA (Supplementary Data Method 7) test that this thermal protocol (9.5 h at 30 °C followed by 19 h at 37 °C) subject to either initial topography (flat or grooved) had identical distributions of \(R\) was statistically significant \(p = 0.001\) \((n = 6)\), indicating that for the given thermal protocol, the initial topography had a significant effect on \(R\).

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Fig. 4. Cell actin cytoskeleton rearranges following topographic transition. a, Confocal images of cells stained with phalloidin on a temporary grooved topography show microfilaments aligned with groove direction (white arrow) before transition and temperature increase. After transition, microfilaments have rearranged, are randomly oriented, and exhibit apparent stress fiber formation. b, Cells on flat control substrates show randomly oriented microfilaments before and after temperature increase. c, Cells on grooved control substrates show microfilaments aligned with groove direction before and after temperature increase. Scale bar is 100 μm. Traces are as in Fig. 1.
some cells that are very highly elongated along the direction of the grooves. The lack of apparent difference may also be due to the effect of the 2D projected image of cells on the 3D micron-scale topography.

Supplementary videos related to this article can be found at doi: 10.1016/j.biomaterials.2010.12.006.

3.3. Actin cytoskeleton remodeling

Analysis of microfilament organization revealed profound remodeling after shape memory activation. Microfilaments that initially aligned along the direction of the grooves reorganized to a randomly distributed organization following transition to the flat surface (Fig. 4a). The observed microfilament organization before and after transition was comparable to that observed in cells on grooved and flat static controls, respectively (Fig. 4b,c). Cells on static controls exhibited no comparable reorganization following the temperature change (Supplementary Data Fig. S9).

3.4. Cell viability

For all groups, cell viability remained greater than 95% and no cell detachment was observed (Fig. 5; Supplementary Data Figs S10,S11), including those cases where SMP substrates with

Fig. 5. Cell viability is high on active cell culture substrates. All groups show many viable (green) cells and very few dead (red) cells. The high background is from substrate auto fluorescence in both channels, allowing the grooves on grooved control and temporarily grooved substrates to be visualized. Scale bar is 200 μm.
adherent cells went through a simultaneous topography and temperature change.

3.5. SMP recovery kinetics

Profilometry analysis of unseeded SMP samples collected in parallel with cell experiments revealed the topographic recovery kinetics experienced by the cells. Activation of the SMP substrate by increasing the temperature to 37 °C resulted in rapid onset of topographic change. Equilibrated temporarily grooved samples (Fig. 1, Equilibrated Shape; Supplementary Data Fig. S12), of initial amplitude 12.6 ± 1.5 μm showed an amplitude decrease of 3.4 μm to an amplitude of 9.2 ± 1.0 μm after 9.5 h at 30 °C. Upon heating to 37 °C by incubator transfer, the amplitude decreased still further to 0.2 ± 0.1 μm within 9.5 h. No detectable decrease over the next 9.5 h was observed (Fig. 6). Thus, adherent cells were expected to experience a large and rapid substrate topography change (flattening) when heated from 30 °C to 37 °C. The final topography had 0.2 μm deep and 40 μm wide grooves spaced 80 μm apart (Supplementary Data Fig. S4).

4. Discussion

Here we have demonstrated a temperature-responsive cell culture system, active cell culture substrates, which can be used to control cell behavior via surface shape memory. The work presented here demonstrates the first application of surface shape memory to cell culture. Shape memory activation caused changes in cell morphology and remodeling of the actin cytoskeleton while maintaining excellent cell viability. Previous research has firmly established substrate topography as a useful tool for investigating multiple aspects of cell biology, including cell motility, cell–cell interaction, the effect of cell shape on differentiation and cell viability, and cell traction. The approach described in this paper expands the potential of this tool by allowing the unprecedented ability to apply programmed changes in topography during culture.

This first successful demonstration of shape memory activation at 37 °C (body temperature) with attached and viable cells was achieved through manipulation of an end-linked thiol-ene system, and there will now be several immediate opportunities for improvement and diversification of active cell culture substrates through materials design. First, researchers have demonstrated shape memory activation triggered by phase transitions in semi-crystalline polymers [37] and liquid crystalline elastomers [38], with light activated and degraded crosslinks [39], and with Joule-heating from electrical current [40] and near infrared heating for doped Tg-based systems [41]. Use of such triggers may allow for isothermal recovery or may reduce the cell culture temperature change needed to trigger meaningful recovery. Second, the shape recovery is slower the lower the modulus of the activated SMP state [42], so recovery could be prolonged by designing SMPs of lower modulus. Indeed, comparing cell behavior for varying shape memory recovery rates may provide distinct insights into cell mechanobiology and the theoretical understanding of the mechanisms underlying the soft-matter physics of cells. In the present work, for example, we observed that the actin cytoskeleton remodeling profoundly after topography change, which suggests Rho activation [43]. Third, increasing fixed strain is expected to increase shape recovery rate [42], so the temporal recovery profiles will differ for different fixed topographies. Lastly, the temperature at which an SMP is deformed or fixed will influence the temperature at which recovery begins [44], suggesting yet another level of active cell culture control.

The ability of SMPs to undergo programmed changes in shape is entirely distinct in several important ways from other stimuli-responsive biomaterials based on photo degradation, hydrogel swelling, or LCSTs. For example, SMPs are unique in that they are able to store energy that can later be triggered to do work, they do not undergo changes in surface or bulk chemistry during the transition, and they are able to change modulus and molecular orientation [45] in addition to changing shape. Furthermore, SMPs can also be fabricated into a variety of morphologies by a number of methods such as electrospinning, casting, fiber drawing, salt leaching, direct writing, UV patterning, and photolithography to allow for a wide range of bulk or topographic transitions such as pillars or microwells; and shape fixation of SMPs can be accomplished by custom mechanical devices, manual embossing, micro- and nano-indentation [30], or mechanical testing apparatus. These unique properties of SMPs allow for dynamic control of modulus and shape which can be used to accomplish and extend the capabilities of many existing stimuli-responsive systems. For Tg-based SMPs, the modulus decreases by as many as 3 orders of magnitude, which is reversed by decreasing the temperature below Tg. Topographical transitions may also allow for release of confluent cell layers, which could also be patterned spatially simply by fabrication of an appropriate embosser. The SMPs can be stretched or compressed to apply uni- or biaxial strain to cells without the need for expensive or custom mechanical devices. Finally, with appropriate design, a single substrate could simultaneously exhibit many of these capabilities.

Fig. 6. SMP activation results in a rapid topographic change. Equilibrated (recovered 52%) from initial embossed amplitude) temporarily grooved samples of amplitude 12.6 ± 1.5 μm that were incubated for 9.5 h at 30 °C (black ○) showed an amplitude decrease of 3.4 μm to 9.2 ± 1.0 μm. Once heated by incubator transfer to 37 °C, samples showed an amplitude decrease of 9.0 μm to 0.2 ± 0.1 μm within 9.5 h (red ▲), and no detectable decrease over the final 9.5 h was observed. Error bars represent one standard deviation (n = 4–6). Relative amplitude (defined as the final amplitude/initial embossed amplitude) over time is fit well by a multiple exponential decay (Supplementary Data Method S8). Thus, adherent cells experience a large rapid decrease in amplitude (red line) when moved to 37 °C. The final topography (*) has 0.2 μm deep and 40 μm wide grooves spaced 80 μm apart (Supplementary Data Fig. S4). Inset traces are profilometry profiles of representative samples. Scale for all traces is the same. The maximum represented amplitude is 14.9 μm and the length of the traces is 1200 μm.

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An intriguing extension of the present work would be application of the shape memory principles and approaches presented here to three-dimensional cell culture, particularly tissue engineering. The stasis of most current tissue engineering scaffolds prevents investigation of dynamic biological processes and hinders therapeutic applications. The potential for SMPs to address these challenges was recognized as early as 2006 [26], but no approach to date has successfully employed an SMP as a tissue engineering scaffold. Cui et al. [46] achieved recovery of a porous SMP at 37 °C, but no cell culture was performed. To evaluate the suitability of a poly-(caprolactone)-dimethylacrylate network as a tissue engineer-
ing scaffold, Neuss et al. [27] cultured cells on a contracting film of the SMP, but cell viability was poor due to the need to heat to a hyperthermic 54 °C to trigger shape change. In the present study, cells remained healthy for extended periods of time at both a pre-
activation temperature and the activation temperature and while undergoing a change in topography, supporting the feasibility of the development of SMP scaffolds.

5. Conclusions

We have developed active cell culture substrates that provide programmed topographical changes and showed that the substrates can be used to control cell alignment. Alignment of the cell body and microfilaments along the grooves of the temporary topography was significantly reduced upon transition to a flat permanent topography. Furthermore, we presented an SMP system and programming cycle that can provide a topography change between two topographies that have distinct affects on cell morphology under cell culture conditions while maintaining high cell viability and attachment, which should enable the study of cell responses to dynamic mechanical stimuli.

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Appendix

Figures with essential color discrimination. Figs. 1–6 in this article have parts that are difficult to interpret in black and white. The full color images can be found in the online version, at doi:10.1016/j.biomaterials.2010.12.006.

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biomaterials.2010.12.006.

References


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