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On-Command On/Off Switching of Progenitor Cell and Cancer Cell Polarized Motility and Aligned Morphology Via a Cytocompatible Shape Memory Polymer Scaffold

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Abstract

In vitro biomaterial models have enabled advances in understanding the role of extracellular matrix (ECM) architecture in the control of cell motility and polarity. Most models are, however, static and cannot mimic dynamic aspects of *in vivo* ECM remodeling and function. To address this limitation, we present an electrospun shape memory polymer scaffold that can change fiber alignment on command under cytocompatible conditions. Cellular response was studied using the human fibrosarcoma cell line HT-1080 and the murine mesenchymal stem cell line C3H/10T1/2. The results demonstrate successful on-command on/off switching of cell polarized motility and alignment. Decrease in fiber alignment causes a change from polarized motility along the direction of fiber alignment to non-polarized motility and from aligned to unaligned morphology, while increase in fiber alignment causes a change from non-polarized to polarized motility along the direction of fiber alignment and from unaligned to aligned morphology. In addition, the findings are consistent with the hypothesis that increased fiber alignment causes increased cell velocity, while decreased fiber alignment causes decreased cell velocity. On-command on/off switching of cell polarized motility and alignment is anticipated to enable new study of directed cell motility in tumor metastasis, in cell homing, and in tissue engineering.

Keywords

Shape memory polymer; electrospun scaffold; polarized motility; cell alignment; polarized morphology; dynamic architectural change

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1. Introduction

Extracellular matrix (ECM) architecture plays a critical role in guiding cell motility during both tissue development and disease progression. During tissue development, the local ECM architecture can, for example, guide cells to migrate along elongated collagen fibers where tissue branching occurs [1], and fibrillar fibronectin is necessary to maintain cell polarity and guide many morphogenic movements [1–3]. Similarly, ECM fiber architecture has been implicated in the control of cell motility in diseases ranging from cancer to tissue hyperplasia [4] and fibrosis [5], with pregnancy-associated breast cancer being one of the most studied and well-understood examples. During breast cancer progression, radially aligned collagen fibers provide “tracks” for cancer cells to invade into the surrounding stroma [6–8]. Collagen alignment is thought to facilitate tumor cell invasion and, as a result, is being studied as a marker for diagnosis.

In vitro biomaterial models have been developed to study the architectural effects of the microenvironment on cell motility and cell morphology. These *in vitro* biomaterial models include naturally occurring polymeric three-dimensional (3D) matrices and synthetic polymeric two-dimensional (2D) substrates or 3D scaffolds. For example, collagen gels and cell-derived matrices are widely used natural polymeric 3D matrices [9–11]. With respect to synthetic models, electrospun scaffolds have been widely used as *in vitro* models due to their nano- to micro-fibrous architectures, which can mimic some aspects of the fibrillar structure of many native ECMs [12–15].

Both naturally occurring and synthetic matrices have been used to study cell motility. For example, Friedl and colleagues [16] showed that highly invasive melanoma cells in 3D collagen matrices follow the protrusion, attachment, and contraction three-step model of cell motility. Such invasive motility results in cell-driven reorganization of the ECM. Dubey and colleagues [17] found that magnetically aligned collagen fibrils can guide Schwann cell invasion into aligned collagen gel matrix. Such findings may provide improved methods of directing and enhancing axonal growth for nerve repair. Johnson and colleagues [18] used aligned and randomly oriented electrospun scaffolds to quantitatively study glioma cell motility on different fiber architectures. They found that cells would move along the highly aligned fibers in the aligned fiber architecture, while cells showed non-polarized motility on randomly oriented fibers. Lastly, Shao and colleagues [19] employed a polycaprolactone (PCL) electrospun mesh with a specific peptide sequence (E7) conjugated as an “MSC-homing device” to recruit mesenchymal stem cells (MSCs) for the application of tissue regeneration. Collectively, existing models such as these have proven successful in studying the response of cells to static matrices in which fiber alignment does not change.

Although many of the existing ECM models provide physiologically relevant fiber microarchitecture and biochemical composition, the models are limited by their fundamentally static nature, with reorganization of matrix architecture occurring only in models that permit cell-driven reorganization. Cells sense the surrounding matrix, and in return, remodel it by depositing additional ECM, by digesting it by secreting matrix metalloproteinase (MMPs), and also through their ability to attach to and actively pull on the fiber architecture, as is the case with cancer associated fibroblasts [20,21] Previous studies

have shown fibroblasts cultured *in vitro* can contract collagen fibers and remodel ECM architecture and density via collagen matrix remodeling through $\alpha_2\beta_1$ integrin and fibronectin matrix remodeling through $\alpha_5\beta_1$ integrin [22]. Cancer cell invasion has been found to be associated with increased collagenase activity, which digests collagen to assist cell translocation through the matrix [23,24]. Importantly, such cell-driven remodeling can result in changes in matrix biochemical composition. Many physical properties, including stiffness, are strongly coupled to the biochemical composition of the matrix. As a result, cellular remodeling of model matrices leads to changes in multiple physical properties, which are hard to predict, control, and characterize. Thus, the coupling of fiber alignment to biochemistry in models involving cell-driven reorganization confounds analysis of the role of fiber alignment in cell motility and polarity.

In contrast to the static nature of most natural and synthetic materials employed in the study of cell motility and polarity, shape memory polymers (SMPs) are a class of “smart materials” that can demonstrate dynamic change in shape on command. SMPs achieve the shape memory effect by “memorizing” a permanent shape through chemical or physical cross-linking, then being manipulated and fixed to a temporary shape by an immobilizing transition, such as vitrification or crystallization, and then later recovering to the permanent shape by a triggering event, such as thermal, electrical or solvent activation [25–31]. A number of recent breakthroughs in the area of cytocompatible SMPs [32–39] have enabled application of SMP on-command functionality in cell culture application. Although SMPs have not previously been applied in the study of cell motility and polarity switching, we have recently demonstrated the feasibility of employing SMPs in the study of cell motility in a 2D system [40].

To address limitations of current static *in vitro* ECM models used in the study of cell motility and polarity, the objective of the present study was to develop a synthetic 3D biomaterial scaffold that can, with cells present, undergo programmed increases or decreases in fiber alignment on command. Furthermore, we sought to use the scaffold to demonstrate successful on-command on/off switching of cell polarized motility and aligned morphology, with decrease in fiber alignment resulting in a change from polarized motility along the direction of fiber alignment to non-polarized motility and from aligned to unaligned morphology, and increase in fiber alignment resulting in a change from non-polarized motility to polarized motility along the direction of fiber alignment and from unaligned to aligned morphology. In addition, we hypothesized that an increase in fiber alignment would cause increased cell velocity, while a decrease in fiber alignment causes decreased cell velocity, as quantified by cell average velocity. To achieve these objectives and test this hypothesis, our approach was to tailor for this purpose a 3D SMP nano-fibrous scaffold. The SMP selected was one recently demonstrated to be suitable for shape change under cytocompatible conditions, being triggered by increasing the incubation temperature from 30 °C to 37 °C when hydrated [31]. To study the cellular response of both cancer cells and progenitor cells, the human fibrosarcoma cell line HT-1080 and the multipotent murine mesenchymal stem cell line C3H/10T1/2 were chosen, as they demonstrate multipotentiality with highly metastatic cancer cell motility and classic fibroblastic motility, respectively [41,42]. Analysis of cell motility was enabled by a recently developed cell tracking algorithm [40].

2. Methods and Materials

2.1 Study Design

Scaffolds of four different architectures, two static and two dynamic, were developed and used in this study (Figure 1). The two control scaffolds featured static architectures of unidirectional aligned fibers and of randomly oriented fibers, respectively. The two dynamic scaffolds featured architectures that dynamically increase unidirectional alignment and dynamically decrease unidirectional alignment, respectively, when warmed from 30 °C to 37 °C under cell culture conditions. To determine whether successful on-command on/off switching of cell polarized motility and aligned morphology was achieved, both the human fibrosarcoma cell line HT-1080 and the multipotent murine mesenchymal stem cell line C3H/10T1/2 were studied, as they demonstrate multipotentiality with highly metastatic cancer cell motility and classic fibroblastic motility, respectively. Cell motility and morphology were qualitatively and quantitatively assessed before and after thermal triggering by time-lapse imaging, computational cell tracking, and fixed time point staining.

2.2 Shape Memory Polymer Synthesis

To produce a shape memory 3D electrospun scaffold capable of dynamical increase or decrease in fiber alignment on command, a thermoplastic polyurethane (TPU) featuring shape memory was synthesized as previously described ([31] and Supplemental Method 1). One TPU batch, which had a molecular weight of 130 kg/mol, was used for this entire study.

2.3 Scaffold Fabrication

To prepare the fibrous 3D architecture that forms the basis of both the static control architectures and the dynamic architectures, scaffolds were fabricated by electrospinning the SMP TPU. Electrospinning was performed as previously described [31], but with the spinning parameters optimized to achieve sub-micron fibers of ~400 nm diameter (Supplemental Method 2). The two control static scaffold architectures were prepared by electrospinning unidirectional aligned fibers (as-spun static aligned scaffolds, “A”) or randomly oriented fibers (as-spun static unaligned scaffolds, “U”). These static control scaffolds will not change fiber architecture regardless of culture temperature. The two control scaffold architectures were used in cell culture experiments without additional modification. The two dynamic scaffold architectures were prepared by an additional programming step, as described in the next section.

2.4 Scaffold Programming and Characterization

The two dynamic scaffold architectures used in the study were prepared by programming as-spun scaffolds, as follows. To prepare the scaffold architecture that dynamically increases unidirectional alignment (U-to-A) when warmed from 30 °C to 37 °C under cell culture conditions, as-spun aligned scaffolds were stretched uniaxially in the direction *perpendicular* to the fiber alignment direction at 50 °C using a dynamic mechanical analyzer (DMA; TA Instruments). The stretching was programmed to stop when strain exceeded 95 %, resulting in scaffolds having a final strain of approximately 100 % (94—115 %). The resulting temporary architecture in which unidirectional alignment was disrupted was fixed by cooling

the sample to 0 °C. To prepare the scaffold architecture that dynamically decreases unidirectional alignment (A-to-U) when warmed from 30 °C to 37 °C under cell culture conditions, as-spun unaligned scaffolds were stretched uniaxially to approximately 100 % strain at 50 °C using a DMA. The resulting temporary architecture in which unidirectional alignment is increased was fixed by cooling the sample to 0 °C. Both programmed scaffolds could then be triggered to recovery back to their permanent architectures at 37 °C when hydrated. After programming, scaffolds were characterized to assess their shape memory functionality, fiber architecture, and fiber diameter before and after thermal triggering (Supplemental Method 3). Fixing ratio and recovery ratio were calculated as described previously [26,31].

2.5 Cell Culture

To determine whether successful on-command on/off switching of cell polarized motility and aligned morphology was achieved, both the HT-1080 and C3H/10T1/2 cell lines (both from ATCC) were cultured on the four different scaffold architectures. For all experiments, cells were seeded on scaffolds at a density of 2500 cells/cm² for HT-1080 cells and 4000 cells/cm² for C3H/10T1/2 cells (Supplemental Method 4; Supplemental Figure 1).

2.6 Time-Lapse Imaging

To image cells for tracking analysis (Supplemental Method 4), cell nuclei of both HT-1080 and C3H/10T1/2 cells lines were labeled with Hoechst 33342 nuclear dye (Invitrogen). One set of “before” samples were imaged for 24 h at 30 °C prior to thermal triggering scaffolds, and a second set of “after” samples were imaged for 24 h at 37 °C after thermal triggering. Z-stacks of images were taken at each position from a relative -40 µm to 40 µm with 20 µm increments to capture cells that had infiltrated multiple cell diameters into the scaffolds. Eight different positions were imaged per sample. Time-lapse imaging was independently repeated three times (n = 3).

2.7 Cell Tracking and Motility Analysis

To analyze cell motility before and after thermal triggering, images within each z-stack were first processed using the Extended Depth of Field ImageJ macro [43] to generate a single plane maximal projection image. Time-lapse videos consisting of 288 frames per video, representing 24 h of real time, were then analyzed by Automated Contour-based Tracking for *In Vitro* Environments, *ACTIVE*, a computational algorithm we recently introduced for tracking large numbers of cells for long time periods that provides qualitative and quantitative analysis of cell motility [40]. *ACTIVE* was used to analyze cell motility in each time-lapse video and output cell motility tracks, diffusion plots, average and decomposed mean square displacement (MSD) plots and metrics, and average velocity plots and metrics (Supplemental Method 5).

2.8 Cell Staining and Imaging for Morphometric Analysis

In addition to the cell tracking and motion analysis, cell staining and imaging at the time point equivalent to the end point of time-lapse imaging were performed to assess cellular and nuclear alignment in both HT-1080 and C3H/10T1/2 cells. Cell staining was performed

to fluorescently label the F-actin cytoskeleton by Alexa Fluor 568 Phalloidin (Invitrogen) and cell nuclei by DAPI (Invitrogen), following manufactures' protocols. Samples were imaged using a Leica DMI 6000B inverted microscope with 20X and 40X objectives. Z-stacks of images were taken at each position from relative $-20\text{ }\mu\text{m}$ to $20\text{ }\mu\text{m}$ with $8\text{ }\mu\text{m}$ increments. Acquired z-stacks were further processed to obtain a single all focused image merged from images from different focal planes using the ImageJ Extended Depth of Field plugin [43]. Images at twenty different positions per sample were taken. Six independent samples ($n = 6$) were used for statistical analysis.

Confocal fluorescent microscopy (Leica SP5) was additionally performed on scaffolds with HT-1080 cells at the same time points as mentioned above. In addition to phalloidin and DAPI labels as described above, an antibody to paxillin (clone 349; BD Pharmingen) was used to label focal adhesion proteins to further examine the cell attachment. Confocal Z-stack images were taken at $0.13\text{ }\mu\text{m}$ intervals. Focused images from each channel were obtained from images from different focal planes as mentioned above using ImageJ.

2.9 Quantification of Cellular and Nuclear Morphology

To quantify cellular alignment and nuclear alignment when the HT-1080 and C3H/10T1/2 cells were cultured on the scaffolds of four different architectures, actin cytoskeleton images from phalloidin staining and nuclear images from DAPI staining were acquired with a 20X objective. Actin and nuclear images were analyzed and cellular alignment and nuclear alignment were quantified as previously described [44], with the long axis of the cells and of the nuclei, which are oval in shape, used in determining alignment. Alignment was quantified in terms of the truncated standard deviation, with possible values from 0° to 52° , with a decrease in truncated standard deviation indicating an increase in alignment, and vice versa ([45,44], Supplemental Method 5).

Both cellular alignment and nuclear alignment were also quantified by the mean resultant vector length (R), calculated by averaging the vector summation of angles treated as unit vectors over the total number of cells [46,47], as an indicator of level of alignment. Perfectly aligned cells or nuclei would produce an R of 1.0, while randomly distributed cells or nuclei would have an R of ~ 0.63 [48].

In addition, for the HT-1080 human fibrosarcoma cells, the total area, circularity, and aspect ratio values of cells and nuclei were calculated from an ellipse by the Analyze Particles function and further analyzed to quantify the extent to which scaffold architectural change affects cancer cell polarized morphology. Total area represents the total area of the cell or nucleus. Circularity of a value 1.0 indicates a perfect circle, while a value approaching 0.0 indicates an increasingly elongated polarized morphology. Aspect ratio is calculated as the ratio of the major and minor axis of a cell or nucleus. Collectively, a larger total area, a smaller circularity, and a higher aspect ratio together indicate a more polarized morphology [49,50]. Unfocused or overlapped cells and nuclei were excluded from analysis.

HT-1080 cell nuclear alignment during time-lapse imaging, before and after thermal triggering, was also quantified in the same way as mentioned above to further assess the effect of dynamic scaffold fiber architectural change on cell nuclei orientation. Briefly, cell

nuclear alignment angles were collected from all cells appeared in all frames in time-lapse videos, and plotted as histograms of total cell count at different alignment angles. Narrow distribution with distinct peak at certain angle indicates high level of alignment; while widespread distribution with non-distinct peak indicate non-specific alignment.

2.10 Statistics

Data used for statistical analysis were first tested for normal distribution using the Shapiro-Wilks test. Parametric or non-parametric tests were then used depending on whether data sets passed the assumption of normal distribution. Statistical significant difference was determined at $p < 0.05$ for all the above tests (Supplemental Method 6).

3. Results

3.1 Scaffold Properties

The shape memory e-spun scaffold demonstrated excellent shape memory functionality in a dry state, as shown in a one-way shape-memory cycle and strain versus time plot (Supplemental Figure 2). The scaffold showed a high fixing ratio of 99 %, indicating that 99 % of the strain was maintained by cooling down the sample below glass transition temperature when the applied force was removed. Upon trigger, the scaffold showed a high recovery ratio of 99 %, indicating that the scaffold recovered 99 % of the initial strain after shape memory recovery.

When hydrated under simulated cell culture conditions, the scaffolds remained highly stable at 30 °C, showing only approximately 5 % pre-recovery of the programmed strain within 24 h. When hydrated scaffolds were triggered to recover at 37 °C, full recovery completed in 5 h with only approximately 1 % programmed strain left unrecovered (Supplemental Figure 3).

Internal architectural change of the scaffold before and after thermal triggering visualized by SEM and further quantified by 2D FFT image analysis revealed that static scaffolds (A and U) maintained a stable scaffold architecture after thermal triggering. In contrast, dynamic scaffolds changed from unaligned to aligned (U-to-A) or from aligned to unaligned (A-to-U) after thermal triggering (Figure 2; Supplemental Figure 4; Supplemental Table 1).

Measurement of fiber diameter before and after thermal triggering showed no significant change of fiber diameter due to shape memory thermal triggering (Supplemental Table 2).

3.2 Cell Motility

Qualitative analysis of cell tracks over 24 h indicates that unidirectional fiber alignment promotes polarized cell motility, that random fiber alignment results in non-polarized motility, and that dynamic increase and decrease in unidirectional fiber alignment results in on-command increase and decrease of polarized cell motility, respectively (Figure 3: HT-1080 cells; Supplemental Figure 5: C3H/10T1/2 cells). Briefly, on static aligned scaffolds (A) cells moved preferentially along the direction of fiber alignment both before and after thermal triggering, while on static unaligned scaffolds (U) cells moved randomly without a preferential direction both before and after thermal triggering. On dynamic

scaffolds that increase in unidirectional alignment (U-to-A), polarized cell motility increased after thermal triggering, while on dynamic scaffolds that decrease in unidirectional alignment (A-to-U), polarized cell motility decreased after thermal triggering. These findings were further observed in the diffusion plots (Supplemental Figure 6), which showed an elongated distribution on as-spun static aligned scaffolds (A), indicative of polarized motility along the direction of fiber alignment, a circular distribution on as-spun static unaligned scaffolds (U), indicative of non-polarized motility without preferential directionality, and a change from more circular distribution to more elongated distribution and from more elongated distribution to more circular distribution on dynamic scaffolds that increase unidirectional alignment (U-to-A) and decrease unidirectional alignment (A-to-U), respectively, indicative of on-command increase and decrease of polarized cell motility, respectively.

Analysis of decomposed MSD (Figure 4: HT-1080 cells; Supplemental Figure 7: C3H/10T1/2 cells) provided qualitative and quantitative confirmation of the motility behaviors qualitatively observed in the cell tracks and diffusion plots. Qualitatively, cells on static aligned control scaffolds (A) showed non-overlapping decomposed MSD curves, which indicates that cells moved with polarized motility along the direction of fiber alignment both before and after thermal triggering. Cells on static unaligned control scaffolds (U) showed overlapping decomposed MSD curves, which indicates cells moved randomly with non-polarized motility both before and after thermal triggering. Cells on the scaffold architecture that dynamically increases unidirectional alignment (U-to-A) showed a change from overlapping to non-overlapping decomposed MSD curves, which indicates polarized cell motility increased after thermal triggering. Cells on the scaffold architecture that dynamically decreases unidirectional alignment (A-to-U) showed a change from non-overlapping to overlapping decomposed MSD curves, which indicates polarized cell motility decreased after thermal triggering.

To assess polarized cell motility quantitatively, x-long timescale intercept and y-long timescale intercept within each scaffold architecture were compared (Supplemental Table 3: HT-1080 cells; Supplemental Table 4: C3H/10T1/2 cells). Briefly, static aligned control scaffolds (A) had significantly higher x-long timescale intercept than y-long timescale intercept, which indicates cells moved with polarized motility along the direction of fiber alignment both before and after thermal triggering. Static unaligned control scaffolds (U) had comparable x-long timescale intercept and y-long timescale intercept, which indicates cells moved randomly with non-polarized motility both before and after thermal triggering. The scaffold architecture that dynamically increases unidirectional alignment (U-to-A) showed significantly increased difference in x and y long timescale intercept (x-long timescale intercept – y-long timescale intercept), which indicates polarized cell motility increased after thermal triggering. The scaffold architecture that dynamically decreases unidirectional alignment (A-to-U) showed significantly decreased difference in x and y long timescale intercept, which indicates polarized cell motility decreased after thermal triggering.

Average velocity results provided further insight into on-command on/off switching of cell polarized motility, while also providing quantitative assessment of both the extent to which

the thermal trigger affects motility and the hypothesis that an increase in fiber alignment would cause increased cell velocity. Mean velocity was higher after thermal triggering for all groups, but the increase was only statistically significant in some groups (Figure 5). Most notably, a statistically significant increase in mean velocity was observed for HT-1080 cells on dynamic scaffolds that increase unidirectional alignment (U-to-A; before = 1.295 ± 0.241 $\mu\text{m}/\text{min}$; after = 1.799 ± 0.049 $\mu\text{m}/\text{min}$; $p = 0.048$). A statistically significant increase in mean velocity was observed for C3H/10T1/2 cells on static aligned scaffolds (A; before = 1.353 ± 0.262 $\mu\text{m}/\text{min}$; after = 1.785 ± 0.289 $\mu\text{m}/\text{min}$; $p = 0.044$) and on dynamic scaffolds that increase unidirectional alignment (U-to-A; before = 1.583 ± 0.020 $\mu\text{m}/\text{min}$; after = 2.413 ± 0.308 $\mu\text{m}/\text{min}$; $p = 0.038$). In terms of the effect of the thermal trigger on cell motility, these findings suggest that a small but real increase in average velocity may be due to the thermal trigger, particularly in the C3H/10T1/2 cells. In terms of the hypothesis being tested, in both cell types the largest increase in average velocity was observed in the dynamic scaffolds that increase unidirectional alignment (U-to-A) and the smallest (and non-statistically significant) increase was observed in the dynamic scaffolds that decrease unidirectional alignment (A-to-U). When the small but significant increase in average velocity due to the thermal trigger is taken into account, these findings are consistent with the hypothesis that the increase in fiber alignment causes increased cell velocity, while the decrease in fiber alignment causes decreased cell velocity.

3.3 Cellular and Nuclear Morphology

Representative merged fluorescence micrographs of the cell actin cytoskeleton and nuclei from both cell lines (Figure 6: HT-1080 cells; Supplemental Figure 8: C3H/10T1/2 cells) qualitatively show that before thermal triggering cells and nuclei preferentially aligned in a direction parallel to the dominant fiber direction when on scaffold architectures possessing unidirectional fibers (A, A-to-U) and did not show preferential alignment and were more randomly distributed when on scaffold architectures without unidirectional fibers (U, U-to-A). After thermal triggering, cells and nuclei remained preferentially aligned on the static aligned scaffolds (A) and continued to show no preferential alignment and more random distribution on static unaligned scaffolds (U). Importantly, the two dynamic scaffolds demonstrated successful on-command on/off switching of cellular and nuclear alignment. After thermal triggering, cells and nuclei showed a change from preferential alignment to lack of preferential alignment on the dynamic scaffolds that decrease unidirectional alignment (A-to-U) and a change from no preferential alignment to preferential alignment in a direction parallel to the dominant fiber direction on the dynamic scaffolds that increase unidirectional alignment (U-to-A). Representative confocal fluorescence images of HT-1080 cell actin, nuclei, and paxillin focal adhesion protein (Supplemental Figure 9) show the same trend of cell alignment change before and after thermal triggering. Abundant paxillin staining at focal adhesions is indicative of robust integrin-mediated cell-ECM attachment.

These qualitative findings were reflected in quantitative angular histograms of cellular alignment and nuclear alignment in fixed scaffold samples, as well as cell nuclei alignment histograms examined during time lapse imaging (Figure 7: HT-1080 cells; Supplemental Figure 10: C3H/10T1/2 cells; Supplemental Figure 11: HT-1080 cells in time lapse). Briefly, cells on static aligned control scaffolds (A) showed narrow cellular and nuclear angular

distribution, which indicates both cells and nuclei aligned along the direction of fiber alignment both before and after thermal triggering. Cells on static unaligned control scaffolds (U) showed much broader cellular and nuclear angular distribution, which indicates neither cells nor nuclei were preferentially aligned in a particular direction either before or after thermal triggering. Cells on the scaffold architecture that dynamically increases unidirectional alignment (U-to-A) showed a change from broad to narrow cellular and nuclear angular distribution, which indicates cells and nuclei reoriented to align along the direction of fiber alignment after thermal triggering. The scaffold architecture that dynamically decreases unidirectional alignment (A-to-U) showed a change from narrow to broad cellular and nuclear angular distribution, which indicates cells and nuclei lost their alignment along the fibers after thermal triggering.

Statistical analysis of angular standard deviation (Figure 8: HT-1080 cells; Supplemental Figure 12: C3H/10T1/2 cells) and the mean resultant vector length (R) (Supplemental Figure 6) confirmed that the observed changes in cellular alignment and nuclear alignment represent successful on/off switching of cellular alignment and nuclear alignment. In this analysis, in which an increase in standard deviation and a decrease in R indicate a decrease in alignment, and *vice versa*, the interquartile range of standard deviations and of R of cells on the control static aligned scaffold architectures (A) and on the static unaligned scaffold architectures (U) was used to define the effective range of standard deviation for cells exhibiting unidirectional alignment and disrupted unidirectional alignment (lack of alignment) for the given cell type under the present experimental conditions (Figure 8; Supplemental Figure 10; aligned = red shading, or dark gray shading when in gray scale; unaligned = blue shading, or light gray shading when in gray scale). Thus, a statistically significant increase in standard deviation and decrease in R with a corresponding move from the red shading to the blue shading after thermal triggering would indicate “switching off” of cellular alignment or nuclear alignment. Conversely, a statistically significant decrease in standard deviation and increase in R with a corresponding move from the blue shading to the red shading after thermal triggering would indicate “switching on” of cellular alignment or nuclear alignment. When analyzed in this way, we found that interquartile ranges did not overlap, thereby defining clear ranges of standard deviation and of R for alignment and for lack of alignment. Triggering of the dynamic scaffolds that decrease fiber alignment (A-to-U) was found to turn off alignment, with both mean cellular alignment and mean nuclear alignment showing a significant change and a switch from the red shading to the blue shading after thermal triggering. Similarly, triggering of the dynamic scaffolds that increase fiber alignment (U-to-A) was found to turn on alignment, with both mean cellular alignment and mean nuclear alignment showing a significant change and a switch from the blue shading to the red shading after thermal triggering. The changes in R were all consistent with and supported these findings (Supplemental Figure 13).

For the additional analyses performed for the HT-1080 human fibrosarcoma cells, the polarized morphology of cells and nuclei induced by dynamic scaffold architectural change were reflected by significant changes in total area, circularity, and aspect ratio in the cells (Table 1 top) and nuclei (Table 1 bottom), respectively, before and after thermal triggering in dynamic scaffolds. Briefly, the scaffold architecture that dynamically increases fiber alignment (U-to-A) showed significant increase in cellular area and nuclear area, significant

decrease in cellular circularity and nuclear circularity, and significant increase in cellular aspect ratio and nuclear aspect ratio. These changes indicate that increased scaffold fiber alignment induces more elongated and polarized cells. In contrast, the scaffold architecture that dynamically decreases fiber alignment (A-to-U) showed significant decrease in cellular area and nuclear area, significant increase in cellular circularity and nuclear circularity, and significant decrease in cellular aspect ratio and nuclear aspect ratio. These changes indicate that decreased scaffold fiber alignment induced less elongated, non-polarized cells.

4. Discussion

Here we have developed a synthetic biomaterial scaffold that can, with cells present, undergo programmed increases or decreases in fiber alignment on command. The results demonstrate successful on-command on/off switching of cell polarized motility and alignment, with a decrease in fiber alignment resulting in a change from polarized motility along the direction of fiber alignment to non-polarized motility and from aligned to unaligned morphology, and an increase in fiber alignment resulting in a change from non-polarized motility to polarized motility along the direction of fiber alignment and from unaligned to aligned morphology. In addition, the findings are consistent with the hypothesis that an increase in fiber alignment causes increased cell velocity, while a decrease in fiber alignment causes decreased cell velocity.

Such a dynamic system, which not only supports the study of cell motility but also can change cell motility polarity on command, can be further applied to study cell motility and polarity in cancer and stem cell-based therapies. With the help of the dynamic SMP 3D fibrous scaffold described herein, further investigations could be made to study cancer cell motility in such dynamic systems in which cell polarized motility and velocity could be directed by the change of the scaffold architecture. Cancer cell invasive phenotype and cell and internal organelle polarization could be studied to determine the extent to which matrix architecture affects cancer cell invasiveness. Such finding would improve understanding of cancer cell motility, metastasis, and tumor formation. In previous studies, cancer cell morphology has been shown to be controlled by key regulators of focal adhesion, such as paxillin, which is known to guide cancer cell polarization, tumor invasion, and metastasis [11,51]. Cancer cell morphology is also an indicator of the mesenchymal versus amoeboid modes of motility in 3D matrices [52–54], and increased ECM density and fiber alignment correlate with increased tumor formation and metastasis [55,56]. Highly aligned collagen fibers are thought to provide “tracks” for cancer cells to invade into the surrounding tissue in breast cancer [7]. Molecular mechanisms, involving, for example paxillin [11] and the Rho GTPase family [57], which underly the observed on/off switching of cell polarized motility and elongated morphology could be dissected to identify potential therapeutic targets. As cell adhesion and morphology is regulated by focal adhesions [58–60], predictable changes in cell morphology and polarized motility may be an indication of changes in focal adhesion turnover and signaling, which could be evaluated further in such a controlled and dynamic *in vitro* microenvironment. More complex scaffold architecture could also be incorporated, such as locally varied fiber alignment, to study preferential cell polarized motility to remote locations with different matrix architectures. These scaffolds are also promising candidates

for mimicking the invasion of cancer cells into stroma and subsequent metastasis events *in vivo*.

Besides cancer cell motility study for the application of cancer therapy, stem cell motility has been extensively studied for application in stem cell-based therapies, with the goal of delivering stem cells to a specific site to promote local tissue repair by inducing stem cells to differentiate down specific lineages. The present SMP system could be further investigated and applied for study of guided stem cell migration, which could not only direct stem cells to traffic towards the desired site, but at the same time having stem cells as delivery vehicles could also potentially deliver molecules as chemical cues to induce differentiation or deliver drugs for specific treatments [61–63]. However, without incorporation of growth factors, guided cell migration directed by scaffold architecture, or particularly the dynamic change of scaffold architecture, remains largely untested. With the help of the dynamic SMP 3D scaffolds described herein, specifically, with a highly aligned scaffold architecture, stem cells may preferentially migrate along the fiber alignment direction, then be further recruited to the injured sites potentially with the help of chemokines. We have previously demonstrated the application of SMP scaffold in regenerative medicine approaches [34], lending confidence to the feasibility of such a line of inquiry.

Limitations inherent in the materials and methods employed in this first demonstration of shape memory enabled on-command on/off switching of cell polarized motility and alignment provide opportunities for further innovation. The electrospinning technique employed in the present study allows for tuning of fiber diameter beyond the single fiber diameter distribution employed here. In addition, in the present work, cell motility occurred in a 3D environment, but we restricted our analysis to 2D, as we projected cells from different focal planes onto one common plane. However, the ACTIVE analysis algorithm could, with modification, be capable of analyzing three-dimensional cell motility. Furthermore, for before and after thermal triggering groups, scaffolds were imaged at 30 °C and 37 °C respectively. An alternative approach would be to return the imaging temperature to 30 °C to eliminate imaging temperature difference once the “after triggering” dynamic scaffolds had fully recovered. Moreover, other non-ambient thermal triggering mechanisms, such as photo-thermal triggering [64], could also be employed to maintain the cell culture temperature at a constant 37 °C throughout the entire study.

5. Conclusions

We have reported successful on-command on/off switching of cell polarization and polarized motility. By thermally triggering a shape memory 3D electrospun scaffold to increase or decrease fiber alignment, we found that both the human fibrosarcoma cell line HT-1080 and the multipotent murine mesenchymal stem cell line C3H/10T1/2 could be switched between polarized motility along the direction of fiber alignment and non-polarized motility and between aligned and unaligned morphology. We anticipate that on-command guidance of polarized cell motility and alignment via SMP scaffolds will enable new study of polarized cell motility in tumor invasion and cancer metastasis and enable new control over polarized cell motility in tissue engineering, cell homing, and regenerative medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Ⓐ Scaffold Preparation

Ⓑ Experimental Phases

Figure 1. Study Design

Static aligned (A) and static unaligned (U) scaffolds were prepared by electrospinning. These two types of scaffolds could be further programmed to Unaligned-to-Aligned (U-to-A) and Aligned-to-Unaligned (A-to-U) scaffolds, which increase or decrease fiber unidirectional alignment, respectively. Two sets of samples, to be imaged before thermal triggering and after thermal triggering, were prepared with each set including four different scaffold architectures, A, U, U-to-A, and A-to-U. A and U were used as static controls that will not change fiber architecture upon a trigger. Cells from both murine C3H/10T1/2 mesenchymal stem cell line and human fibrosarcoma HT-1080 cell line were seeded on both sets at the same time. The before triggering set of samples were imaged at 30 °C. The after triggering set of samples were triggered to achieve full recovery at 37 °C then followed by imaging at 37 °C. Terminal analysis was performed to assess cell motility and cell body and cell nuclear morphology.

Figure 2. Scanning Electron Microscopy (SEM) reveals scaffolds architecture

Static aligned (A) scaffolds showed highly unidirectionally aligned fibers and static unaligned (U) scaffolds showed randomly oriented fibers without specific alignment both before (top row) and after (bottom row) thermal triggering. Dynamic Unaligned-to-Aligned scaffolds (U-to-A) changed from unaligned to unidirectionally aligned architecture after thermal triggering. Dynamic Aligned-to-Unaligned scaffolds (A-to-U) changed from unidirectionally aligned to unaligned architecture after thermal triggering. Double-headed arrow indicates fiber alignment direction. Scale bar is 40 μm .

Figure 3. Qualitative analysis of cell tracks of HT-1080 cells

Cell tracks were plotted by ACT/VE with each line represents a cell's moving path over 24 h. For HT-1080 cells, on static aligned scaffolds (A), cells moved preferentially along the unidirectional fiber alignment direction both before (top row) and after (bottom row) thermal triggering. On static unaligned scaffolds (U), cells moved randomly without a preferential direction both before and after thermal triggering. On dynamic scaffolds that increase in unidirectional alignment (U-to-A), cells showed increased directional cell motility after thermal triggering. On dynamic scaffolds that decrease in unidirectional alignment (A-to-U), cells showed decreased directional cell motility after thermal triggering. The cartoon on top right corner of each track plot indicates the associated fiber architecture, either aligned or unaligned. Double-headed arrow indicates the principle fiber alignment direction in the scaffold, if one existed.

Figure 4. Decomposed mean square displacement (MSD) analysis of HT-1080 cell motility
 For HT-1080 cells, static aligned scaffolds (A) had non-overlapped decomposed MSD curves, which indicates that cells showed directional motility preferentially along the unidirectional fiber alignment direction both before (top row) and after (bottom row) thermal triggering. Static unaligned scaffolds (U) had overlapped decomposed MSD curves, which indicates that cells showed non-directional motility both before and after thermal triggering. Dynamic scaffolds that increase in unidirectional alignment (U-to-A) showed a change from overlapped to non-overlapped decomposed MSD curves, which indicates that cells had increased directional motility. Dynamic scaffolds that decrease in unidirectional alignment (A-to-U) showed a change from non-overlapped to overlapped decomposed MSD curves, which indicates that cells had decreased directional motility. The cartoon on top right corner of each MSD plot indicates the associated fiber architecture, either aligned or unaligned.

Figure 5. Average cell velocity of HT-1080 cells and C3H/10T1/2 cells

Average cell velocity ($\mu\text{m}/\text{min}$) of both HT-1080 cells (top) and C3H/10T1/2 cells (bottom) was compared within each scaffold architecture before (open circles) and after (closed circles) thermal triggering, and also among different scaffold architectures either before or after thermal triggering. For HT-1080 cells, cells on dynamic scaffolds that increase in unidirectional alignment (U-to-A) scaffolds showed significantly higher average velocity after thermal triggering comparing to before thermal triggering. After thermal triggering, cells on U-to-A scaffolds showed significantly higher average velocity than the cells on dynamic scaffolds that decrease in unidirectional alignment (A-to-U). For C3H/10T1/2 cell

line, cells on static aligned scaffolds (A) and dynamic scaffolds that increase in unidirectional alignment (U-to-A) showed significantly increased average velocity after thermal triggering than before thermal triggering. After thermal triggering, cells on U-to-A scaffolds showed significantly higher average velocity compared to the cells on static aligned (A) and static unaligned (U) scaffolds; and cells on static aligned (A) scaffolds showed significantly higher average velocity compared to the cells on static unaligned (U) scaffolds. Within each scaffold architecture group, one circle represents one biological replicate. Horizontal line represents the average of three biological replicates. Asterisks indicate significant difference ($p < 0.05$; $n = 3$).

Figure 6. HT-1080 cell body and cell nuclear fluorescent staining images before and after thermal triggering

Representative cell body (red) and cell nuclei (green) merged fluorescent images of HT-1080 cell line qualitatively show the cell alignment before (top row) and after (bottom row) thermal triggering. On static aligned scaffolds (A), cells remained preferentially aligned in the directional parallel to the dominant fiber direction. On static unaligned scaffolds (U), cells remained randomly distributed. On dynamic scaffolds that increase unidirectional alignment (U-to-A), cells showed a change from random distribution to preferential alignment along the fiber alignment direction. On dynamic scaffolds that decrease unidirectional alignment (A-to-U), cells showed a change from preferential alignment along the fiber alignment direction to random distribution. White double-headed arrows indicate the fiber principle alignment direction, if one existed. The cartoon on top right corner of each fluorescent image indicates the associated fiber architecture, either aligned or unaligned. Scale bar is 200 μm .

Figure 7. Angular histograms qualitatively show HT-1080 cell body and cell nuclear alignment on different scaffold architectures

HT-1080 cell body (solid line box) and cell nuclei (dashed line box) showed the same trend of alignment. Static aligned scaffolds (A) showed narrow cell body and cell nuclear angular distribution, which indicates that both cell body and cell nuclei aligned along the unidirectional fiber alignment direction before and after thermal triggering. Static unaligned scaffolds (U) showed much broader cell body and cell nuclear angular distribution, which indicates that both cell body and cell nuclei had no apparent preferential alignment before and after thermal triggering. Dynamic scaffolds that increase unidirectional alignment (U-to-A) showed a change from broad to narrow cell body and cell nuclear angular distribution, which indicates that both cell body and cell nuclei increased preferential alignment along the unidirectional fiber alignment direction. Dynamic scaffolds that decrease unidirectional alignment (A-to-U) showed a change from narrow to broad cell body and cell nuclear angular distribution, which indicates that both cell body and cell nuclei decreased alignment. The red line represents the mean resultant vector length (R). The cartoon on top right corner of each angular histogram plot indicates the associated fiber architecture, either aligned or unaligned.

Figure 8. HT-1080 cell body and cell nuclei angular standard deviation comparison within the same scaffold architecture before and after thermal triggering

HT-1080 cells showed that the cell body (left column) and cell nuclei (right column) angular standard deviation decreased in dynamic U-to-A scaffolds after thermal triggering, which indicates that cell body and cell nuclei reoriented to be preferentially aligned along the fiber unidirectional alignment direction when fiber unidirectional alignment increased. Cell body and cell nuclei angular standard deviation increased in dynamic A-to-U scaffolds after thermal triggering, which indicates that cell body and cell nuclei reoriented to be preferentially aligned along the fiber unidirectional alignment direction when fiber unidirectional alignment increased. Boxes display interquartile range with black median center line, red lines indicate mean, and capped whiskers indicate 95% confidence interval of the mean. Red shading (or dark gray shading when in gray scale) represents the range of unidirectionally aligned architecture; while blue shading (or light gray shading when in gray scale) represents the range of unaligned architecture. Asterisks indicate significant difference ($p < 0.05$; $n = 6$). Statistically significant differences exist among the architectural groups before or after recovery, but now shown to highlight the key difference of interest.

Table 1

HT-1080 cell and nuclei polarized morphology analysis

Cell	Before					After				
	A	U	A-to-U	U-to-A	A	U	A-to-U	U-to-A	A	U
Area (pixels ²)	864.303 ± 358.897^A	493.066 ± 143.109^B	783.836 ± 203.261^C	646.382 ± 120.554^F	1291.697 ± 326.322^A	799.446 ± 254.256^B	565.181 ± 65.790^C	1489.334 ± 148.587^F		
Circ.	0.286 ± 0.027	0.441 ± 0.093	0.304 ± 0.041^D	0.440 ± 0.033^G	0.228 ± 0.055	0.391 ± 0.052	0.347 ± 0.027^D	0.204 ± 0.054^G		
Aspect Ratio	3.682 ± 0.547	1.869 ± 0.141	3.552 ± 0.684^E	2.017 ± 0.195^H	3.863 ± 0.295	1.947 ± 0.197	2.346 ± 0.394^E	4.756 ± 0.731^H		

Nuclei	Before					After				
	A	U	A-to-U	U-to-A	A	U	A-to-U	U-to-A	A	U
Area (pixels ²)	226.827 ± 46.297	175.936 ± 59.305	169.529 ± 23.772^A	187.388 ± 40.503^D	255.520 ± 16.654	234.831 ± 53.561	119.210 ± 9.886^A	263.90 ± 20.110^D		
Circ.	0.618 ± 0.157	0.631 ± 0.157	0.396 ± 0.047^B	0.715 ± 0.060^E	0.682 ± 0.040	0.683 ± 0.092	0.565 ± 0.050^B	0.564 ± 0.104^E		
Aspect Ratio	1.516 ± 0.094	1.3580 ± 0.069	1.543 ± 0.191^C	1.439 ± 0.071^F	1.593 ± 0.191	1.423 ± 0.087	1.383 ± 0.043^C	1.730 ± 0.081^F		

Total area, circularity and aspect ratio values of HT-1080 cell (top) and nuclei (bottom) were compared within the same architectural group before and after thermal triggering. Data are reported as average ± standard deviation. Within each table, bolded groups that share the same letter labels are significantly different with *p* value less than 0.05. Statistically significant differences exist among the architectural groups before or after recovery, but are not shown to highlight the key difference of interest.