Electrospun Fibrous Scaffolds Promote Breast Cancer Cell Alignment and Epithelial–Mesenchymal Transition

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ABSTRACT: In this work we created electrospun fibrous scaffolds with random and aligned fiber orientations in order to mimic the three-dimensional structure of the natural extracellular matrix (ECM). The rigidity and topography of the ECM environment have been reported to alter cancer cell behavior. However, the complexity of the in vivo system makes it difficult to isolate and study such extracellular topographical cues that trigger cancer cells’ response. Breast cancer cells were cultured on these fibrous scaffolds for 3–5 days. The cells showed elongated spindle-like morphology in the aligned fibers, whereas they maintained a mostly flat stellar shape in the random fibers. Gene expression profiling of these cells post seeding showed up-regulation of transforming growth factor β-1 (TGFβ-1) along with other mesenchymal biomarkers, suggesting that these cells undergo epithelial–mesenchymal transitions in response to the polymer scaffold. The results of this study indicate that the topographical cue may play a significant role in tumor progression.

INTRODUCTION

Evidence accumulating from recent literature makes scientists believe that the biophysical properties of extracellular matrix (ECM) have a major impact on cancer cell survival, morphogenesis, invasion, and metastasis.¹,² During tumor progression, ECM components modulate cell phenotype by generating tensile forces within the matrix, or spatial orientation of matrix fibrils.³ Cells respond to these geometric cues by restructuring their cytoskeleton also known as “contact guidance”,⁴ which is further translated to biochemical signals within a cell, altering its gene and protein expressions. Several studies show that substrate topography can guide differentiation of neural stem cells,⁵ Schwann cell maturation,⁶ skeletal muscle constructs,⁷ and restoration of tissue architecture⁸ through contact cue. Many recent works also reported that cell adhesion to a patterned surface depends largely on the surface architecture of the physical patterns.⁹–¹¹

In the developmental process of tumor metastasis, ECM remodeling occurs where the epithelial cancer cells undergo a phenotypic change to obtain a more migratory, invasive form also known as epithelial–mesenchymal transitions (EMTs), promoting directed cell invasion into the vessels.¹² However, the complexity of the in vivo system makes it difficult to isolate and study those ECM topographical cues that affect such cellular transitions and behaviors. Therefore it is necessary to employ an in vitro biomimetic scaffold to investigate such cell–matrix interactions. In this paper, we exploit the method of electrospinning to obtain random and aligned poly(ε-caprolactone) (PCL) fibers and use it as a model to evaluate cell–substrate response.

Electrospinning is a simple and very economical method to produce biomaterials with large surface area, controllable mechanical properties, and tunable surface chemistries.¹³,¹⁴ It is a promising technique in the field of tissue engineering, as the nonwoven fibrous mats produced in the submicrometer range can mimic the structure and topography of the ECM. Among many synthetic polymers that have been used in electrospinning, PCL is known for its excellent biocompatibility and good support for cell growth.¹³,¹⁵–¹⁷ Moreover, the alignment of electrospun fibers can be readily achieved by improved fiber collection method, which has been employed to dictate neuron cells, stem cells, and fibroblast growth by providing contact cue guidance.⁵,⁶,¹⁸–¹⁹ Here we report that a contact cue can induce the elongation and alignment of a variety of breast cancer cells with aligned PCL fibers as culturing substrates. Furthermore, such morphological change may indicate an EMT-like transition in breast cancer cells, regulated by the transforming growth factor β (TGFβ) signaling pathway. Our result implies that the biophysical attributes of a tumor microenvironment like ECM alignment may be sufficient to induce the occurrence of EMT in cancer cells.

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**EXPERIMENTAL SECTION**

**Materials.** PCL (Average Mn ca. 60 kDa, Sigma), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were obtained from Sigma Aldrich. TGFβ-1 was purchased from Stentgem Inc. Deionized (DI) water was produced from a Millipore Purification System (18 MΩ cm at 25 °C). H605 is a mammary tumor cell line that was isolated from the primary tumor of the MMTV-Her2/neu transgenic mouse as described in our previous publications. MTCL and 4T1 cell lines were obtained from Dr. Xiaoming Yang of the School of Medicine, Columbia, SC. MDAMB-231 cell line was a kind gift from Dr. Shaojin You from the Atlanta VA Medical Center. MCF-7 and NMuMG cell lines were purchased from ATCC.

**Electrospinning PCL Fibers.** PCL was dissolved in HFIP to form a 15% w/w solution. The polymer solution was transferred to a 1 mL syringe connected to a 21G blunt needle (BD precision guide), which was also the positive electrode. The polymer was dispensed using a syringe pump (KD scientific) at a constant flow rate of 15 μL/min. The polymer solution was transferred to a 1 mL syringe connected to a 21G blunt needle (BD precision guide), which was also the positive electrode. The polymer was dispensed using a syringe pump (KD scientific) at a constant flow rate of 15 μL/min. The distance of 10 cm from the tip of the needle and turned at 1000 rpm for collecting aligned fibers. Electrospinning of the polymer was carried out by applying a positive voltage of 6 kV employing a high voltage supply (HVR Orlando, FL) between the needle tip and the collector was placed at a distance of 15 cm from the tip of the needle for obtaining random fibers. A rotating mandrel was placed at a distance of 10 cm from the tip of the needle and turned at 1000 rpm for collecting aligned fibers. Electrospinning of the polymer was carried out by applying a positive voltage of 6–8 kV employing a high voltage supply (HVR Orlando, FL) between the needle tip and the collector. The electrospun PCL scaffold was kept overnight on a vacuum-dry oven in order to remove residual solvents. The PCL fiber scaffold was sterilized by immersing in 70% ethanol for 20 min and following UV irradiation in a laminar flow hood for 20 min.

**Cell Culture and Seeding.** Mouse mammary tumor cell H605, mouse mammary epithelial cells (NMuMG), and human breast cancer cell line MCF-7 were maintained in Dulbecco’s modified Eagle’s medium (DMEM) and supplemented with 10% fetal bovine serum (FBS), 10 μg/mL insulin, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM l-glutamine. Mouse breast cancer cells 4T1 and MTCL were cultured in DMEM, supplemented with 10% FBS, 2 mM l-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Human breast cancer cell line MDA-MB-231 was cultured in RPMI 1640 and supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM l-glutamine. All cells were cultured in a 5% CO2 humidified incubator at 37 °C. All media components were obtained from Hyclone laboratories, Inc. Sterilized PCL fiber scaffolds were washed with phosphate-buffered saline (PBS) twice and soaked in media overnight prior to cell seeding. Cells at their growth phase of 80% confluency were detached from the plate using 0.25% trypsin-EDTA and seeded on a fiber mat at a density of 1.3 × 10^4 per cm^2. Cells were maintained in 5 ng/mL (final concentration) of TGFβ-1 in complete medium for one week. Cells were subcultured at 80–90% confluence once in one week.

**EMT Induction in NMuMG Cells.** NMuMG cells were seeded at a density of 2.6 × 10^4 per cm^2 on aligned PCL fiber mat. The higher seeding density for NMuMG cells was employed to make sure cell density was still in an appropriate range since the addition of TGFβ-1 would cause cell death and reduce cell number significantly. Twenty four hours post seeding, 5 ng/mL (final concentration) of TGFβ-1 was added to media for EMT induction. Cells on the PCL fiber mat without TGFβ-1 treatment were used as a control.

**Microscopy.** For fluorescence microscopy, cells on the fiber mat were rinsed with PBS and fixed with 4% paraformaldehyde for 10 min at room temperature. Actin cytoskeleton was stained with Rhodamine-phalloidin (Cytoskeleton Inc.), and the nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, Invitrogen). The fluorescence was visualized under a microscope (Olympus IX81) with DAPI filter and Cy3 filter set. Signals from the DAPI filter set were assigned blue, and those from the Cy3 filter set were assigned green. A mercury lamp was used as the light source. Nucleus orientation analysis was performed for 15–50 cells using ImageJ software, keeping the fiber directionality as the reference point. For scanning electron microscopy (SEM), the PCL fiber mat was dried with nitrogen, sputter coated with gold for 30 s, and imaged at 20–25 kV with a Zeiss UltraPlus FESEM. The diameter of approximately 10 fibers was calculated with ImageJ software (NIH, USA). Cells on the PCL mat were fixed with 2.5% glutaraldehyde for 2 h at room temperature. Postfixation, cells were treated with 1% OsO4 in 0.1 M cacodylate buffer, the cell samples were dehydrated in a series of ethanol solutions (30%, 50%, 70%, 80%, 95%, and 100%) for 10 min each. The samples were processed through a critical point drying apparatus (Ladd Research Industries, Inc.). A thin layer of gold (20 nm) was sputter-coated on the samples and imaged with a Zeiss UltraPlus FESEM.

**RNA Extraction and Reverse-Transcription Quantitative Polymerase Chain Reaction (RT-qPCR).** Total RNA was extracted from H605 cells after 5 days of culture in aligned fibers, random fibers, and a tissue culture plate (TCP) using an RNeasy mini purification kit (Qiagen), and subsequently reverse-transcribed with a qScript cDNA synthesis kit (Quanta Biosciences Inc.). RT-qPCR was carried out for 45 cycles of PCR (95 °C for 1 s, 58 °C for 15 s, and 72 °C for 30 s) with iQ5 SYBR Green supermix (Biorad) using the primers indicated in Table 1. In a 25 μL total volume of reaction mixture, 200 nM of both forward and reverse primers (Integrated DNA Technologies, Inc.) and the cDNA template at a final concentration of 0.25 ng/μL were employed. Data analysis was performed using the 2^−ΔΔC_T method for relative quantification based on three replicate measurements, and all samples were normalized to Gapdh expression as the internal control. Statistical analysis on normalized expression was performed using a “pair-wise fixed reallocation randomization test” on each sample, and a value of p < 0.005 was considered significant.

### Table 1. Primer Sequences for RT-qPCR Experiments

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<th>gene name</th>
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<th>reverse sequence 5′-3′</th>
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RESULTS

PCL Fiber Fabrication. Electrospun PCL fibers were readily fabricated using a homemade apparatus. With a stationary collector placed at 15 cm underneath the tip, a random fiber mat was obtained, and no preferred fiber orientation was observed under SEM (Figure 1b). When the collector was replaced with a rotating mandrel, the well-oriented fiber mat showed alignment of fibers along the direction of the rotation of the collecting mandrel (Figure 1a). When fibers reach the edge of the rotating collector during electrospinning, it is stretched by the tangential force and forms aligned fibers on the collector surface. The solution concentration and solvent have significant effects on the diameter of the fibers, as previously reported in literatures.26 Various PCL solution concentrations (9%, 12% and 15%) were used to test the fiber formation. The 15% PCL solution product homogeneous fibers with no beading, which was used in the cell studies. The average fiber diameters for a 15% PCL solution in HFIP for aligned and random fibers were calculated to be 1.8 ± 0.46 μm and 2.0 ± 0.65 μm, respectively. The aligned fibers were produced at a rotation speed of 1000 rpm of the rotating collector. The degree of alignment was calculated by measuring the orientation angles of the fibers with respect to the direction of the rotating collector. More than 75% of the fibers lie within 10° of the direction of the rotating axis, thus showing good alignment (Figure 1c).

Cellular Response to Aligned and Random PCL Fiber Scaffolds. The PCL fiber scaffold was optimized for cell studies. A mammary tumor cell line, H605, which was isolated from the primary tumor of the MMTV-Her2/neu transgenic mouse, was first investigated.22 Due to the high hydrophobicity (Table S1, Supporting Information, water contact angle 121.5°) of PCL material, cells attach but do not spread immediately. To promote cell attachment and spreading, a sterilized PCL fiber scaffold was soaked in media overnight at 37 °C prior to cell seeding.27 No cell death was observed in both random and aligned fiber scaffolds after 1 day of cell seeding, and the increase in cell number upon culturing from 3 to 5 days suggested cells were viable in the PCL scaffold (data not shown). In the literature, electrospun fibrous scaffold with diameter ranging from micrometer to nanometer have not been shown to hinder cell growth.28 Figure 2 shows morphological differences in these cells when cultured on different PCL fiber mats for 5 days. When cells were cultured on aligned fibers, the cytoskeleton and nuclei align and elongate along the fiber axes (Figure 2a,b). A similar phenomenon has been reported with fibroblasts, Schwann cells, and neural stem cells before.5,6,21 When cells were cultured on a random PCL fiber, its cellular cytoskeleton was stretched to cross multiple fibers (Figure 2d,e). The orientation of the cells related to the fiber scaffold was not dependent on the size of the electrospun fibers but may be specific to certain cell types.28,29 The cell alignment on different scaffolds was evaluated by measuring the nucleus orientation and the elongation factor.
The elongation factor of each cell is defined as the ratio \( \frac{X}{Y} \), where \( X \) is the major axis of the nucleus, which runs along the aligned fiber direction, and \( Y \) is the minor axis of the nucleus, which runs perpendicular to the \( X \)-axis. Longer and thinner, the spindle-morphology of cells will give a higher elongation factor of nuclei, which normally indicates the tensional effect of the fibers in stretching the actin cytoskeleton of the cells. The angle created between the major axis of the cell nucleus (\( X \)-axis) and the direction of the aligned fibers represents the orientation angle (Figure 2c). Lower orientation angle indicates better cell alignment along the fiber direction. On the basis of the fluorescence microscopy images of cells on PCL fibers, elongation factors (Figure 2f) and degrees of orientation of at least 50 nuclei were calculated. The average elongation of cells on aligned fibers is shown to be significantly higher than cells on random fibers, while the average of the orientation angle of cells on aligned fibers is \( 8.5 \pm 9.27^\circ \), which indicates a very good cell alignment.

**Response of Different Types of Breast Cancer Cells on Aligned PCL Fiber Scaffold.** The dramatic change of the H605 cell line on different fiber scaffolds inspired us to investigate the effect of other breast cancer cell lines on these scaffolds. We assume cancer cells with different subtypes, such as luminal (nonaggressive)\(^3\) or basal (aggressive),\(^3\) would behave differently in these aligned fiber mats. To test this hypothesis, we cultured MCF-7 (luminal),\(^3\) 4T1, MTCL, and MDA-MD-231 (basal)\(^3\) on an aligned PCL fiber mat for 3 days. The breast cancer cell lines with basal phenotype showed similar morphology on aligned fibers, where the cytoskeleton and nuclei of individual cells aligned and elongated along the fiber axis (Figure 3a–c). In contrast, the luminal-type cell line MCF-7 maintained cell–cell contacts showing less impact of the aligned fibers on the cells (Figure 3d). Average orientation angle of basal type cells are lower than \( 10^\circ \), which indicates good alignment with the fibers. In comparison, the orientation angle of MCF-7 is much higher than the other three (Figure 3e), indicating a random orientation of MCF-7 on aligned fibers. All four breast cancer cell lines showed random...
orientation when cultured on tissue culture plastic (data not shown).

**PCL Fiber-Induced EMT-like Phenotype.** Real-time PCR was performed on H605 cells after 5 days of culture on random and aligned fibers, keeping H605 on tissue culture plates as the control. Gene expression analysis of cells on aligned fibers showed significant upregulation of Cytokeratin 14 (Ck14), Smooth muscle actin (Sma), TGFβ-1 (Tgfb1), Snail, Fibroblast specific protein-1 (Fsp1), and Smad3 (Figure 4a). Similar gene expression pattern with less fold difference was observed in the cells cultured on random PCL fibers. Upregulation of Smad3 (downstream of TGFβ-1) suggests the cellular signaling by the TGFβ pathway.32 TGFβ-1 and Snail are well-known EMT inducers,33,34 therefore higher expression of these genes shows that the cells are undergoing EMT-like transitions. Ck14 has been known to be activated in basal like breast cancer cells, with enhanced migratory and invasive phenotype.35 We hypothesize that the increase in the Ck14 indicates that H605 is undergoing more aggressive and motile phenotype. Sma and Fsp1 have been well reported as EMT markers in fibroblasts and epithelial cells, thus increased expression of these genes also highlights the EMT-like phenotype.36 According to Zeisberg et al., there exist three types of EMT, i.e., Type-1 (mesenchymal), Type-2 (fibroblast), and Type-3 (metastatic).37 Enhanced upregulation of cytoskeletal markers such as Ck14, Sma, Fsp1, and transcriptional factors such as Snail clearly indicates that H605 on aligned fibers are undergoing Type-3 EMT in comparison to H605 on random fibers and in control experiments. However, cell surface proteins such as Fibronectin, N-cadherin (Ncadh), and E-cadherin (Ecadh) did not show significant changes in the gene profile (data not shown), possibly because these cells had been cultured on the fibers for a relatively shorter period of time. More importantly, chemical induction in H605 with TGFβ-1 also showed similar gene expression pattern with upregulation of Bone morphogenetic protein (Bmp7), Ncadh, Smad3, and Sma, corroborating our hypothesis that Type-3 EMT on PCL fibers was induced via the TGFβ pathway (Figure 4b).

EMT in a tumor environment is thought to be a force-dependent phenomenon.38 Dynamic compression and contraction of cell–matrix interaction leads to the activation of latent ECM-bound TGFβ, which thereafter stimulates a feedback loop to induce EMT in the cell itself.36 Cell alignment along the fibers can be explained, considering the mechanical strength of the fibers stretching the cytoskeleton of the cell, giving rise to tension and tensile forces, which further induce actomyosin reorganization and result in a change in cell shape.39,40 These conformational changes in the cells can influence the biochemical signaling cascade within the cell by integrin clustering. Thus we can conclude that contact cue guidance of the cells, giving spine morphology along the fibers, may induce an EMT-like phenotype through the TGFβ pathway.

To further confirm the correlation between cell alignment and an EMT-like phenotype, a well-studied inducible EMT model was tested with a mouse mammary epithelial cell line (NMuMG) on aligned PCL fibers. TGFβ-1 has been shown to induce EMTs in NMuMG cells.39 Without any treatment, these cells exhibit typical epithelial cell morphology having compact colonies on the aligned fibers. Although the cells on the exterior of the colonies showed partial alignment with the fibers, cells in the interior of the colonies showed strong resistance to align and maintained their cuboidal and clustered morphology (Figure 5a). Upon TGFβ-1 treatment, NMuMG cells are malleable in response to the contact cue given by the aligned fiber mat and appear spindle shaped and elongated (Figure 5b,c). These morphological changes are classic features of EMT.40 Consistent with this observation, rearrangement of a cytoskeleton as a signature of the transition was observed by phalloidin staining. The control cells without TGFβ-1 treatment exhibited a peripheral F-actin staining with slim central stress fibers, while the cells with TGFβ-1 treatment showed a decrease in marginal F-actin but contained much thicker central stress fibers (Figure 5a,b). Besides the induced morphological changes and cytoskeleton rearrangement, cells located both in the interior and exterior of the colonies showed good alignment along fiber direction with average orientation angle of 12.86° (Figure 5b,d). This observation suggests that the EMT-like changes are at least partially induced by the contact cues.

**DISCUSSION**

In this paper, two types of PCL fiber mats with different orientations were fabricated by electrospinning technique to study the influence of contact cue guidance on breast cancer cell behavior. Different morphologies of the primary tumor cell line H605 were observed when it was cultured on fiber mats with different orientations. H605 showed spindle-like shape on aligned fiber and stellar-like shape on random fiber. After testing four more cell lines, we observed that only the breast cancer cell lines that have basal-like phenotype, show spindle-like...
like morphology on aligned fiber. Several Type-3 EMT\textsuperscript{36,37} related genes were up-regulated when H605 cells were cultured on aligned PCL fibers. Increase of cell tension due to cell alignment may cause the up-regulation of TGFβ-1, which further promotes an EMT-like phenotype. This assumption was further confirmed by gene expression analysis and TGFβ-1 treatment of H605 and NMuMG. This work provided a useful in vitro model to study the interplay between ECM and cancer cells during tumor progression.

A parallel fiber alignment was observed previously as a characteristic of ECM produced by primary carcinoma-associated fibroblasts of the skin.\textsuperscript{31} However, the consequence of this fiber arrangement for tumor cell behavior was unknown. Our study identifies ECM architecture as a direct inducer of EMT, suggesting that the biophysical attributes of tumor microenvironment may play a critical role in cancer progression. The parallel fiber architecture is reminiscent of the collagen fiber signature identified in the transgenic Wnt-1 mouse mammary tumor model by Provenzano and colleagues.\textsuperscript{42} This group identified parallel collagen fibers perpendicular to the advancing edge of the tumors. Interestingly, local cell invasion was found to be predominantly oriented along certain aligned collagen fibers, suggesting that radial alignment of collagen fibers relative to tumors facilitate invasion. Because of the inherent limitations of the model system, it was not clear whether the invasion was a consequence or cause of the fiber arrangement. Our data clearly demonstrate that ECM alignment can directly induce an EMT-like phenotype, which is known to be a critical step in tumor invasion and metastasis.

Evidence supporting the importance of the biophysical attributes of the ECM for breast carcinoma cell behavior is accumulating. Besides ECM architectures, dense rigid physical properties are also known to suppress tubulogenesis and stimulate invasion of well-differentiated breast carcinoma cells.\textsuperscript{43} Due to the lack of a good system to mimic the in vivo tumor microenvironment, the contribution of individual microenvironmental cues to tumor initiation and progression remains largely unknown. The electropun fibers used in our study have dimensions similar to those of ECM fibers in natural tissue matrix and various fiber attributes in a spatial arrangement that correspond with normal tissue structure in vivo. Most importantly, they are amenable to modulation of the mechanical properties by cospinning with biopolymers and to the integration of defined ECM ligands, and bioactive environmental materials thus provide an ideal model system to assess three-dimensional matrix effects on breast carcinoma cell behavior. This study provides proof-of-principle experiments demonstrating that this model system can be used to address the contribution of microenvironmental cues to tumor progression, which is otherwise difficult to be evaluated due to the complexity of cancer cell–microenvironment interaction in vivo.

ASSOCIATED CONTENT

Supporting Information

Fluorescence microscope images of H605 cells cultured on aligned fibers for 3 and 5 days, and water contact angle measurements of the PCL scaffold. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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REFERENCES

(16) Pham, Q. P.; Sharma, U.; Mikos, A. G. Tissue Eng. 2006, 12, 1197–1211.
(26) Pham, Q. P.; Sharma, U.; Mikos, A. G. Tissue Eng. 2006, 12, 1197–1211.