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Supplemental Information

Positive Quantitative Relationship between EMT and Contact-Initiated Sliding on Fiber-like Tracks

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Supplemental Information

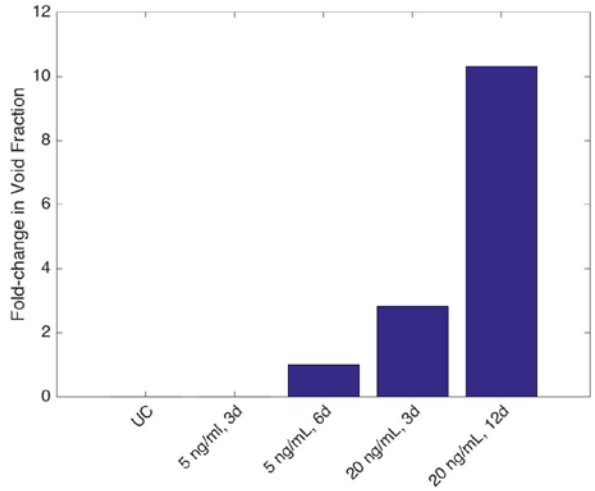
1. Quantification of TGFß-mediated morphological changes

Contours of the bodies of cells treated with different doses and duration of TGFß were manually outlined using Photoshop. Void regions where no cells were present were also outlined. Properties of these contoured regions-of-interest (ROI) were quantified using Matlab. From ROIs associated with cell bodies, the perimeter and area were quantified, and the shape factor was calculated as the Perimeter^2/(4π Area). A circle has a shape factor of 1, and shapes deviating from a circle have a shape factor greater than 1. Supplemental Table 1 summarizes the shape factor for cells left untreated or treated with different doses and duration of TGFß.

Treatment	Shape Factor	95% Confidence Interval
UC	1.42	0.08
5 ng/ml (3d)	1.51	0.07
5 ng/ml (6d)	1.63	0.10
20 ng/ml (3d)	1.57	0.11
20 ng/ml (12d)	1.52	0.11

Supplemental Table 1. Cells exhibit a more extended morphology following TGFβ treatment. The shape factor is higher among TGFβ-treated cells compared to untreated controls, consistent with an elongation of the cell body.

From ROIs associated with void regions, the void area was quantified, and the fraction of total surface area that was devoid of cells was calculated. The fold-change in the void fraction is depicted in Supplemental Figure 1. Longer exposure and higher doses of TGFß yielded higher fold-change in void fraction, consistent with the observed loss of cell-cell contacts with greater TGFß treatment.

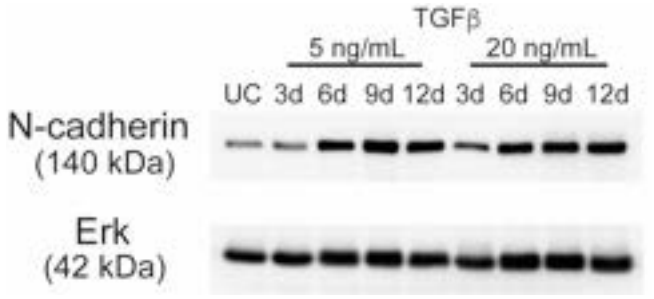


Supplemental Figure 1. Fold-change in void regions between cells. TGFβ-induced EMT results in loss of cell-cell contact. The amount of void areas between cells was quantified from a representative image of cells left untreated or treated with TGFβ at different doses

and duration. The untreated control and treatment with 5 ng/ml TGFβ for 3 d exhibited no void regions. For the other treatment conditions, the void fraction increased with higher exposure time and dose, with cells treated with 20 ng/ml TGFβ for 12 d exhibiting approximately 10-fold greater void regions than those treated with 5 ng/ml TGFβ for 6 d.

2. Upregulation of N-cadherin in response to TGFβ treatment

A characteristic marker of the mesenchymal state is the expression of N-cadherin. We assayed by Western blot the effect of TGFβ-mediated EMT on the expression level of N-cadherin (Supplemental Fig. 2). For both 5 ng/ml and 20 ng/ml of TGFβ, the level of N-cadherin increased gradually with time, and the final steady-state expression level of N-cadherin after 12 h of TGFβ treatment was clearly above the expression level in control untreated cells.



Supplemental Figure 2. N-cadherin expression was measured by Western Blot in TGFβ-treated and untreated (control) cells. Erk2 was probed as an equal-loading control.

3. Number of cell-cell collisions analyzed

In total, 7226 cell-cell encounters were analyzed across 50 combinations of 5 TGFβ treatment and 10 fiber-like micropattern widths. Supplemental Table 2 details the number of cell-cell collisions analyzed at each of the 50 conditions.

	Width of fiber-like micropattern (μm)										
Condition	6	9	12	15	18	21	24	27	30	33	Marginal total
Untreated	38	74	103	117	117	95	112	125	112	112	1005
TGFβ 5 ng/ml, 3 d	118	159	138	101	84	124	127	102	78	65	1096
TGFβ 5 ng/ml, 6 d	168	202	207	155	185	202	249	211	185	193	1957
TGFβ 20 ng/ml, 3 d	142	133	140	137	138	152	146	127	137	110	1362
TGFβ 20 ng/ml, 12 d	83	129	119	176	177	195	169	202	257	299	1806
Marginal total	549	697	707	686	701	768	803	767	769	779	7226

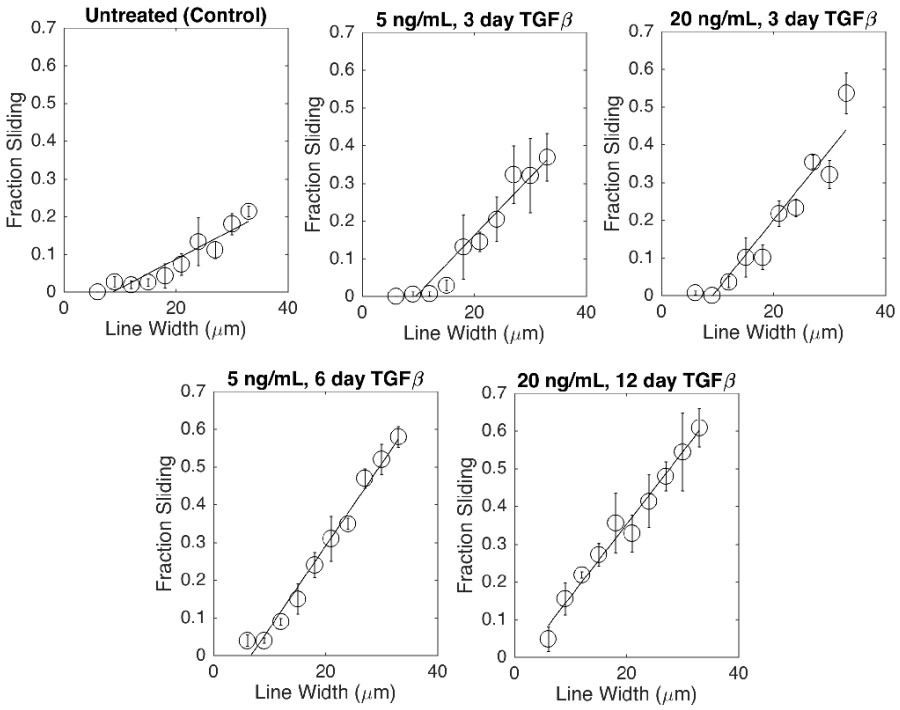
Supplemental Table 2. Number of collisions analyzed at every combination of ten fiber-like micropattern widths and five TGFβ treatment conditions.

4. Statistical analysis of sliding response

Two-way ANOVA was performed to test the null hypothesis that fraction sliding does not depend on either TGFß treatment or the width of the fiber-like micropattern (data shown in Figure 3A). The analysis was performed using statistical analysis software R (v3.3.1) and shows that fraction sliding exhibits a statistically significant dependence on TGFß treatment ($p<2e-16$) and line width ($p<2e-16$). The interaction between these factors is significant ($p<0.05$); therefore, we conducted detailed analysis of simple effects by 1-way ANOVA, with the Sidak-Bonferroni correction for familywise error. For each TGFß treatment condition, including control, the dependence of fraction sliding on line width was statistically significant ($p<0.001$). Meanwhile, the dependence of fraction sliding on TGFß treatment was statistically significant at line widths of 12 μm ($p<0.001$, ***) and 9, 15, 21, 27 and 33 μm ($p<0.05$, *). The dependence of fraction sliding on TGFß treatment at line widths of 6, 18, 24 and 30 μm were not statistically significant by ANOVA (ns).

5. Characteristic fiber-like dimension (CFD)

We previously defined the CFD as the hypothetical line width required to support moderate sliding (i.e. 25% sliding) on fiber-like micropatterns (20). Here, we applied the same linear model to fraction sliding data for untreated and TGFß-treated MCF-10A cells (Supplemental Fig. 3). Increasing the dose and duration of TGFß treatment progressively reduced the calculated value of the CFD compared to untreated (control) cells.



Supplemental Figure 3. A simple linear model was applied to fraction sliding data for untreated and TGFβ-treated MCF-10A cells. The CFD was calculated for each condition by determining the line width required to achieve an intermediate level of sliding.