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The Strain Magnitude and Contact Guidance Determine Orientation Response of Fibroblasts to Cyclic Substrate Strains

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When grown in a substrate subjected to cyclic stretching, most types of cells change orientations. This cell orientation response (COR) has been shown to be driven by axial substrate strain (the strain beneath and along a cell's long axis). However, it remains unclear whether COR depends on the strain direction (tension vs compression). Furthermore, *in vitro* COR is paradoxical, since *in vivo* fibroblasts align along collagen fibers and hence the stretch direction. We hypothesized that COR does not depend on the surface strain direction, and that contact guidance provided by microgrooves can maintain cell alignment in the presence of cyclic stretching. Human skin fibroblasts were cultured on compliant smooth and microgrooved surfaces in silicone dishes. Cyclic uniaxial tensile and compressive strains (4%, 8% and 12%) were applied on the dishes at 1 Hz for 24 h. Cell orientation distributions were determined and compared using the Kolmogorov–Smirnov test. Significant differences were found between each of cell orientation distributions with the applied strains and that without strains ($p < 0.05$). Nevertheless, no significant differences were found between two cell orientation distributions for each pair of opposite strains applied (for 4%, $p = 0.33$; for 8%, $p = 0.18$; and for 12%, $p = 0.32$). Moreover, fibroblasts grown in microgrooves aligned in the groove direction and remained so after 8% cyclic stretch. Thus, this study showed that COR is the cells' avoidance to substrate deformation (i.e., strain-direction independent). It also suggested that the failure of fibroblasts to change orientations *in vivo* may result from the contact guidance provided by collagen fibers.

Keywords: Fibroblasts, surface strains, alignment, microgrooves, contact guidance

INTRODUCTION

It is well recognized that mechanical forces regulate connective tissue form and function.[10,17,20,28,29] However, little is known about cellular mechanisms through which mechanical forces bring about tissue physiological changes. To understand the mechanisms, researchers have been increasingly using cell

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culture models to study strain effects on mammalian cells. Many studies showed that cyclic stretching of cultured cells increases proliferation,[2,18] alters mRNA levels and protein synthesis,[4,15] modifies actin cytoskeleton,[7,13,22] and causes cell population to become oriented away from the stretching direction.[1,7,24] It has also been shown that this cell orientation response (COR) is driven by axial substrate strain, i.e., the strain beneath a cell and along the cell's long axis.[11] But it remains unclear whether COR depends on strain direction (tension vs compression).

Furthermore, orientation response of the fibroblasts *in vitro* appears to be paradoxical, since *in vivo* the cells align along collagen fibers and hence the stretching direction. One likely reason for the paradox is that contact guidance (substrate geometry influences orientation of cell locomotion) provided by collagen fibers may prevent change in cell orientation. It is known that cells grown in microgrooves *in vitro* align in the groove direction,[8,21] yet it is unknown whether the cell alignment in microgrooves can be maintained when cells are subjected to cyclic stretching.

Thus, the purpose of this study was to examine whether COR is dependent on the direction of substrate deformation, and whether this cellular response can be prevented by contact guidance. In addition, the role of actin cytoskeleton in COR was also examined, since the actin cytoskeleton is thought to be involved in a variety of cellular responses to mechanical forces.[16,26]

MATERIALS AND METHODS

Experimental Design

Human skin fibroblasts (HSFs) were used. The cells were grown on both smooth and microgrooved culture surfaces in custom-made silicone dishes. The dishes were subjected to cyclic uniaxial tensile and compressive strains, 0 (control), 4%, 8% and 12%, at 1 Hz for 24 h. To examine COR, cell orientation distribution for each of these strains was determined from measurements of individual cell orientations. The distribution was defined to be the percentage of cells that fell into 18 orientation intervals, 5° each, between 0° (the direction of applied strains) and 90° (the perpendicular to the applied strain direction). The Kolmogorov–Smirnov test[12] was used to determine whether two cell orientation distributions under a pair of opposite strains (e.g., 4% tensile strain vs 4% compressive strain) were significantly different. The difference was considered to be significant if $p < 0.05$. In addition, the response of actin cytoskeleton to applied strains was examined with fluorescence microscopy.

Further, to determine whether contact guidance can prevent cells from change in orientation, HSFs were grown in microgrooved surfaces in silicone dishes subjected to 0 (control) and 8% cyclic stretch. Cell orientations before and after the stretches were examined with phase-contrast microscopy.

Detailed Methods

Cell Culture

HSFs were grown in 75 cm^2 flasks (Falcon) containing growth medium made of DMEM supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/ml epidermal growth factor (Gibco Labs), 5 µg/ml insulin, 0.5 µg/ml hydrocortisone (Sigma), and 1% penicillin/streptomycin (Gibco Labs). Cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% carbon dioxide. Cells grown to subconfluence in flasks were trypsinized (0.25% trypsin) and plated to silicone dishes. For all experiments, cells from passage 2 to 5 were used.

Smooth and Microgrooved Silicone Dishes

Silicone dishes were used as substrates for growing cells and applying strains to the cells. The dishes were made from silicone (RTV ME 601 A + B, Wacker Chemie, Munich, FRG) by a molding process. Briefly, two fluid components (601A and 601B), with a ratio of 10 to 1, were mixed in a glass container. The mixture was casted into a six-dish mold made of Plexiglas. After curing, silicone dishes with smooth culture surfaces were obtained.

Two types of dishes were made by the molding process. One was referred to as the tensile dish, which was used in all the experiments where tensile strains were applied. The dish had a rectangular shape (10 cm long × 3.5 cm wide × 1.75 cm high), with a well having a smooth culture surface (6 cm × 3 cm), in the middle. The other was the compressive dish, which was used only in the experiments where 4% compressive strain was applied. The dish also had a rectangular shape, but its dimensions were smaller. The length was 9 cm, and the well culture surface was 2 cm × 1.5 cm. The bottom thickness of the well was 3 mm (Fig. 1).

Since the compressive dish buckled under large compressive strains, 8% and 12%, we used the following approach to applying the large compressive strains to cells. Using a custom-made device, the tensile dish was statically stretched to 8% or 12%. Under the prestretched state, cells were plated and grown overnight (12–16 h). These cells adhered to the dish culture surface, and were therefore subjected to the compression when the dish was cyclically released and stretched.

Silicone dishes with microgrooved culture surfaces were made as follows. First, microgrooved silicone membranes were fabricated by molding silicone against a silicon wafer with 18 patches. Fifteen of them had microgrooves, 1.6 μm deep, with widths ranging from 1 to 6 μm, separated by 2 to 6 μm wide ridges. Three patches had only smooth surfaces. Next, the microgrooved membranes were bound to the bottoms of the tensile dishes whose smooth surfaces had been removed. The direction of the microgrooves in the dishes was along the dish long axis, i.e., the same direction in which cyclic stretching was applied.

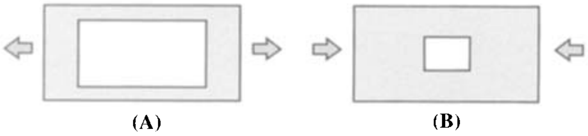

FIGURE 1 Two types of elastic silicone dishes were used in experiments: the tensile dish (A), and the compressive dish (B). The blank areas in the two illustrations were wells, where cells were plated in the central regions of culture surfaces, which were 6 cm × 3 cm (A) and 2 cm × 1.5 cm (B), respectively. Dish (A) was used in the 4%, 8% and 12% stretch, and 8% and 12% prestretch (i.e., compression to cells adherent to a culture surface) experiments, whereas dish (B) was used in only 4% compression experiment.

All dishes were thoroughly washed with 95% ethanol and then rinsed with double distilled water. The dishes were then placed in a laminar hood under UV light for at least 2 h. After the UV treatment, dish culture surfaces were coated with 20 μg/ml ProNectin-F (Protein Polymer Technologies, Inc., San Diego, CA), which is a bioengineered polymer containing RGD ligand of human fibronectin that can promote cell attachment.

Stretch and Compression Experiments

Cells were plated in central regions ($3 \times 10^3/\text{cm}^2$) of both the tensile and compressive dishes. The central regions were about 3 cm × 2 cm for the tensile dish, and 1.2 cm × 0.8 cm for the compressive dish (see Fig. 1). After overnight incubation (12–16 h), dishes were cyclically stretched or compressed to specified strains (4%, 8% and 12%) at 1 Hz for 24 h using the stretching apparatus,[18] which was modified so that it could apply both cyclic stretch and compression.

Immediately after the end of mechanical stimulation, the dish was put on the stage of a Nikon inverted phase-contrast microscope. Two stop ends were used to make sure that the dish long axis was parallel to the stage edges. Without staining the cells, microphotographs (Kodak 200 slide films) were taken at 16–24 regions in the central culture surface of the dish. The microphotographs were projected onto the digitizer table of the Zeiss interactive digital analysis system (ZIDAS, Carl Zeiss, Inc., Thronged, NY). To reduce possible bias in defining cell orientations, only bipolar fibroblasts were used in measurement of cell orientations. This was done by manually tracing images of the fibroblasts through their midways with a stylus. Orientations of 122–200 cells were measured and then used for the construction of cell orientation distributions, which were determined by calculating percentages of cells that fell into 18 orientation intervals, 5° each, between 0° (the stretching direction) and 90° (the perpendicular to the stretching direction).

For cells grown in microgrooves, both before and after stretching, the cells were observed and photographed on the inverted phase-contrast microscope.

Staining Actin Microfilaments

Briefly, actin filaments were stained as follows. Fibroblasts were washed twice with phosphate buffered saline (PBS, 1X), fixed in 3.7% formaldehyde for 30 min, permeabilized in 0.25% Triton X-100 for 10 min, and finally incubated with 0.165 μM rhodamine phalloidin or fluorescein phalloidin in PBS for 1 h. After extensive washing with PBS, the stained cells were viewed on a Zeiss fluorescence microscope, and photographed with Kodak 200 color slide film.

RESULTS

Without substrate deformation, cell orientations were distributed in the range from 0° to 90° (Fig. 2A). This cell orientation distribution was not significantly different from the uniform orientation distribution within the same range ($p = 0.31$), indicating that the cells randomly oriented on a static surface. In contrast, after 4%, 8% and 12% tensile and compressive strains (Fig. 2B–D), cells oriented away from the direction of the applied strains (i.e., 0°). Furthermore, the resulting orientation distributions from the application of 4%, 8% and 12% tensile and compressive strains for 24 h were significantly different from the distribution without strain ($p < 0.05$). But two cell orientation distributions for each pair of applied strains (e.g., 4% tensile strain vs 4% compressive strain) showed a similar distribution pattern and were not significantly different (for 4%, $p = 0.33$; for 8%, $p = 0.18$; and for 12%, $p = 0.32$).

A representative phase-contrast microphotograph of cell orientation response is given in Fig. 3. It shows that after 12% cyclic compression for 24 h, the fibroblasts oriented almost uniformly to about 60° with respect to the stretching direction. Without strain, however, the cells randomly oriented (not shown).

Representative fluorescence microphotographs of actin filaments of the fibroblasts are given in Fig. 4. It can be seen that after 12% cyclic compression, bundles of actin filaments (stress fibers) formed in the direction of around 60° about the stretching direction, regardless of the shape of the cells (Fig. 4A). In contrast, without substrate strains, actin filaments of individual cells had no apparent specific orientations (Fig. 4B).

The fibroblasts grown in microgrooves without stretching strongly aligned along the direction of all microgrooves. Furthermore, after 8% cyclic stretching for 72 h, these cells remained aligned in the microgrooves. This was in contrast to the cells on smooth surfaces next to the microgrooves, which changed orientations, as shown in the representative phase contrast microphotograph (Fig. 5).

DISCUSSION

This study showed that for three pairs of opposite strains, cells oriented away from the direction of applied strains, and the three pairs of resulting cell orientation distributions were not significantly different. According to strain theory,[9] if applied tensile strain is changed to compressive strain, or vice versa, the direction of surface strains, which act on adherent cells, will be reversed. Moreover, it has been shown that axial surface strain is responsible for COR.[11] Taken together, the results of this study suggest that COR is the cells' avoidance response to both tensile and compressive axial surface strains.

COR has been observed in many types of cells before.[1,7,23] It was thought to be cell avoidance to stretch.[1] This implies that the cells favor compression. Our results, however, indicated that the cells actually avoid both, the stretch and the compression. One possible reason for the discrepancy may be due to the subjective interpretation, since under the uniaxial stretching cells oriented at *nearly* perpendicular to the stretching direction.[7]

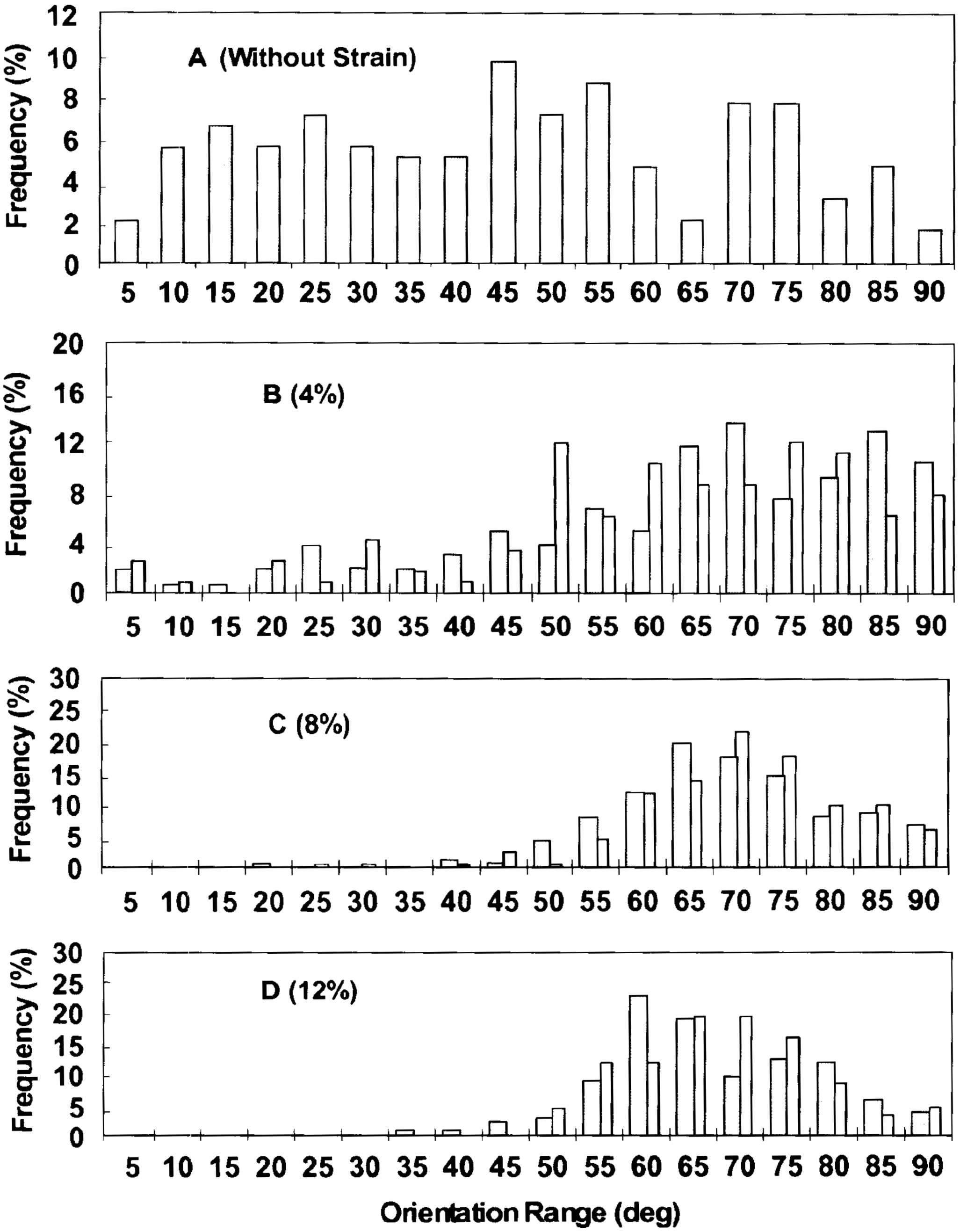

FIGURE 2 Orientation distributions of the fibroblasts without strain (A) and with the tensile (black bars) and compressive (blank bars) strains 4% (B), 8% (C) and 12% (D). The cell orientation distribution without strain (A) was not significantly different from the uniform distribution within the same range ($p = 0.31$). This indicated that the fibroblasts randomly oriented on the static surface. Furthermore, it is seen that with increased tensile and compressive strains, the orientation distributions skew to the right, meaning that the cells oriented away from the direction of applied strains (i.e., 0°). The shape of the distributions under 8% and 12% strains were similar, but the highest frequencies were moved from the range 65°-75° (C) to the 60°-70° (D). This indicated that increased tensile or compressive strains made the cells to orient toward the directions with smaller axial surface strains. No significant differences for each pair of the distributions were found (Kolmogorov-Smirnov test, for 4%, $p = 0.33$; for 8%, $p = 0.18$; and for 12%, $p = 0.32$). Note that the number of cells used in the distributions ranged from 122 to 200.

It can be shown that under the uniaxial stretch or compression, the direction of zero-axial substrate deformation is about 60° relative to the stretching direction, although the exact zero-deformation direction depends on the material property of the substrate used.[25] So the question is: If both stretch and compression are unfavorable signals to cells and under them cells would change orientations, why did cells orient in the directions other than 60° (Fig. 2)? We reason that cells may have strain thresholds, and therefore they can tolerate certain magnitudes of strains before they change orientations. Indeed, the notion of cell strain threshold is supported by previous observations. For example, Dartsch *et al.* (1986) found that for a stretch below

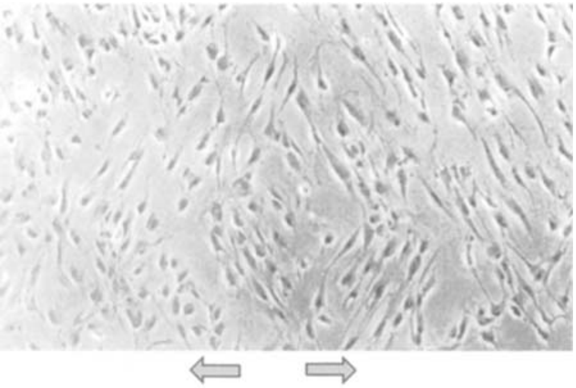

FIGURE 3 Representative phase-contrast microphotographs of the fibroblasts with cyclic compression (12%) at 1 Hz for 24 h. It is seen that the cells appeared to orient uniformly around 60° with respect to stretching direction, which is indicated by arrows. Note that the fibroblasts without strains orient randomly (not shown).

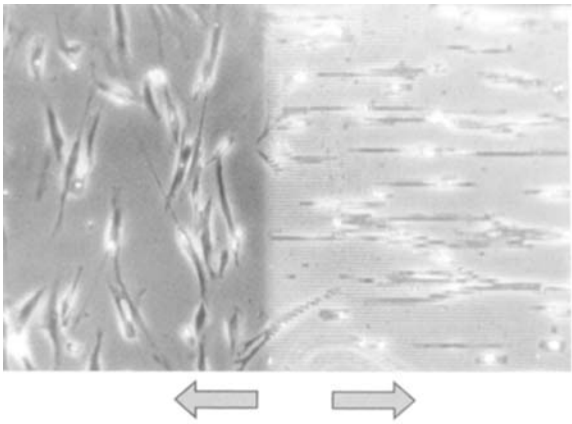

FIGURE 5 A representative phase-contrast microphotograph of the fibroblasts in microgrooves (1.6 μm deep, 5 μm in groove width, and 2 μm in ridge width) and on a smooth surface next to the microgrooves. It is seen that the cells in the microgrooves remained aligned after 8% cyclic stretch for 72 h (the stretching direction is horizontal, as indicated by arrows). In contrast, the fibroblasts on the smooth surface, which was next to the microgrooves, oriented away from the stretching direction. This result suggested that the geometric shape of a surface may play a dominant role in determining cell behavior, such as the cell orientation response, in response to mechanical forces.

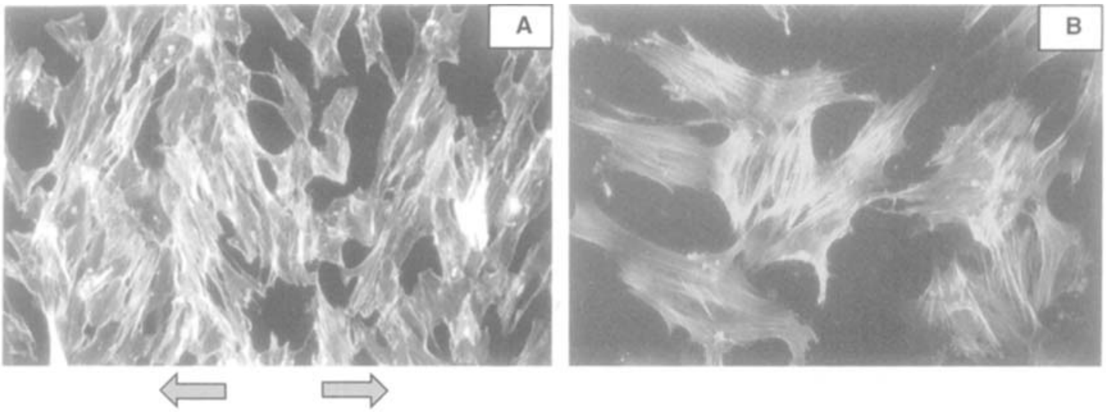

FIGURE 4 Representative fluorescence microphotographs of the actin filaments of the fibroblasts with 12% cyclic compression (A) and without strain (B). Note that without strain, the actin filaments of the cells had *no apparent specific orientation* (B), but after the compression, the filaments became oriented parallel to about 60° direction with respect to the direction of the applied strain (arrows).

2%, arterial smooth muscle cells do not change orientations. Grood *et al.*[11] showed that axial strain limits existed for fibroblasts and osteoblasts, above which few of these cells were found. Furthermore, using the notion of the cell strain threshold, we established a cell model whose predictions on orientation response of melanocytes match well with experimental results.[25]

How do the cells sense the signal of mechanical deformation? Since cells avoid both stretch and compression, the mechano-sensing mechanism, at least for the COR, must work for both stretch and compression signals. One likely signaling pathway may be from focal adhesions to actin cytoskeleton. The focal adhesions attach to substrate and also link with actin cytoskeleton. Therefore, substrate deformation can be transmitted to the actin cytoskeleton and this would change tension in actin filaments,[14] resulting in change in equilibrium between actin filament polymerization and depolymerization.[3] Moreover, it has been shown that the actin polymerization is deformation dependent:[22] The larger the applied deformation, the more actin depolymerization it produces. Thus, we speculate that large substrate deformation would result in actin depolymerization of actin filaments. Under the mechanical deformation, the cells could only form actin stress fibers in the direction with minimal substrate deformation. Ultimately, the formation of stress fibers in new direction drives cells to orient in the same direction as the stress fibers. These speculations await further study.

First introduced by Weiss,[27] contact guidance has been shown to induce alignment of cells on a variety of materials.[5,6,8] Our observation that the fibroblasts aligned in the direction of silicone microgrooves is consistent with the findings of these studies. Furthermore, we found that the cells could maintain this alignment, in spite of cyclic substrate deformation (8%). However, we noticed that the cells in small microgrooves (1 μm in the groove width and 2 μm in the ridge width) changed orientations under larger applied strain (12%). This suggests that both the dimension of the microgrooves and the strain magnitude are two important factors in determining cell alignment in microgrooves subjected to cyclic stretching.

In vivo, fibroblasts are lined in rows between collagen fibers. The cells are elongated in shape, and presumably adhere to the collagen fibers through focal adhesions.[19] In the present experimental model, silicone microgrooves was used to mimic collagen fibers surrounding the tendon/ligament fibroblasts. The results with the model showed that the fibroblasts aligned in microgrooves without deformation, due to the contact guidance by the microgrooves, and furthermore, the cells remained aligned in the presence of cyclic stretching. Therefore, it seems likely that collagen fibers *in vivo* may also provide similar contact guidance, so that fibroblasts align with collagen fibers even under mechanical stretching. However, differences exist between the present experimental model and *in vivo* environment of the fibroblasts. For example, the silicone microgrooves we used in the experiments, were coated with ProNectin-F containing RGD sequences, which bind with integrins on the fibroblast surface. However, collagen molecules are known to contain both RGD and RGE sequences.[29] Therefore, the attachment of the fibroblasts *in vivo* to collagen fibers may be different from *in vitro*. Besides, fibroblasts *in vivo* are also surrounded by other matrix proteins such as proteoglycans, which were not used in our basically two-dimensional model system. All these may influence cell behavior, including the alignment of fibroblasts along the direction of collagen fibers, in response to mechanical forces.

In summary, this study showed that cell orientation response is the cells' avoidance reaction to both excessive tensile and compressive strains. This study also suggested that the failure of fibroblasts to change orientations *in vivo* may result from contact guidance provided by collagen fibers.

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References

[1] Buck, R.C. (1980). Reorientation response of cells to repeated stretch and recoil of the substratum. *Exp. Cell Res.*, **127**, 470–474.

[2] Buckley, M.J., Banes, A.J., Levin, L.G., Sumpio, B.E., Sato, M., Jordan, R., Gilbert, J., Link, G.W. and Tay, R. (1988). Osteoblasts increase their rate of division and align in response to cyclic mechanical tension *in vitro*. *Bone Miner.*, **4**, 225–236.

[3] Buxbaum, R.E. and Heidemann, S.R. (1988). A thermodynamic model for force integration and microtubule assembly during axonal elongation. *J. Theor. Biol.*, **134**, 379–390.

[4] Carver, W., Nagpal, M.L., Nachtigal, M., Borg, T.K. and Terracio, L. (1991). Collagen expression in mechanically stimulated cardiac fibroblasts. *Circ. Res.*, **69**, 116–122.

[5] Clark, P., Connolly, P., Curtis, A.S., Dow, J.A. and Wilkinson, C.D. (1987). Topographical control of cell behavior. I. Simple step cues. *Development*, **99**, 439–448.

[6] Clark, P., Connolly, P., Curtis, A.S., Dow, J.A. and Wilkinson, C.D. (1990). Topographical control of cell behavior. II. Multiple grooved substrata. *Development*, **108**, 635–644.

[7] Dartsch, P.C., Hammerle, H. and Betz, E. (1986). Orientation of cultured arterial smooth muscle cells growing on cyclically stretched substrates. *Acta Anatomica.*, **125**, 108–113.

[8] Dow, J.A., Clark, P., Connolly, P., Curtis, A.S. and Wilkinson, C.D. (1987). Novel methods for the guidance and monitoring of single cells and simple networks in culture. *J. Cell Sci. Suppl.*, **8**, 55–79.

[9] Fung, Y.C. (1994). *A First Course in Continuum Mechanics*. 3rd edn., Prentice-Hall, Inc.

[10] Gillard, G.C., Reilly, H.C., Bell-Booth, P.G. and Flint, M.H. (1979). The influence of mechanical forces on the glycosaminoglycan content of the rabbit flexor digitorum profundus tendon, *Conn. Tiss. Res.*, **7**, 37–46.

[11] Grood, E.S., Neidlinger-Wilke, C. and Claes, L. (1994). The *in vitro* alignment of fibroblasts and osteoblast in response to cyclic surface deformation, *40th Annual Meeting, ORS*, February 21–24, New Orleans, Louisiana.

[12] Hoel, P.G. (1971). *Introduction to Mathematical Statistics*. John Wiley & Sons, Inc., San Diego.

[13] Iba, T. and Sumpio, B.E. (1991). Morphological response of human endothelial cells subjected to cyclic strain *in vitro*. *Microvascul. Res.*, **42**, 245–254.

[14] Ingber, D. (1991). Integrins as mechanochemical transducers. *Curr. Opin. Cell Biol.*, **3**, 841–848.

[15] Leung, D.Y.M., Glagov, S. and Mathews, M.B. (1976). Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells *in vitro*. *Science*, **191**, 475–477.

[16] Malek, A.M. and Izumo, S. (1996). Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress. *J. Cell Sci.*, **109**, 713–726.

[17] Martin, R.B. and Burr, D.B. (1989). Structure, function, and adaptation of compact bone, Raven Press, New York.

[18] Neidlinger-Wilke, C., Wilke, H.J. and Clase, L. (1994). Cyclic stretching of human osteoblasts affects proliferation and metabolism: A new experimental method and its application. *J. Orthop. Res.*, **12**, 70–78.

[19] Nirschl, R.P. (1989). Rotator cuff tendonitis: Basic concepts of pathoetiology. *Inst. Course Lect.*, **38**, 439–445.

[20] Noyes, F.R. (1977). Functional properties of knee ligaments and alterations induced by immobilization: A correlative biomechanical and histological study in primates. *Clin. Orthop.*, **123**, 210–242.

[21] Ohara, P. and Buck, R.C. (1979). Contact guidance *in vitro* – A light, transmission, and scanning electron microscopic study. *Exp. Cell Res.*, **121**, 235–249.

[22] Pender, N. and Mcculloch, C.A.G. (1991). Quantitation of actin polymerization in two human fibroblast sub-types responding to mechanical stretching. *J. Cell Sci.*, **100**, 187–193.

[23] Shirinsky, V.P., Antonov, A.S., Birukov, K.G., Sobolevsky, A.V., Romanov, Y.A., Kabaeva, N.V., Antonova, G.N. and Smirnov, V.N. (1989). Mechanochemical control of human endothelium orientation and size. *J. Cell Biol.*, **109**, 331–339.

[24] Sumpio, B.E. and Banes, A.J. (1988). Response of porcine aortic smooth muscle cells to cyclic tensional deformation in culture. *J. Surg. Res.*, **44**, 696–701.

[25] Wang, H.C., Ip, W., Boissy, R. and Grood, E.S. (1995). Cell orientation response to cyclically deformed substrates: Experimental validation of a cell model. *J. Biomech.*, **7**, 130–138.

[26] Wang, N., Butler, J.P. and Ingber, D.E. (1993). Mechanotransduction across the cell surface and through the cytoskeleton. *Science*, **260**, 1124–1127.

[27] Weiss, P. (1945). Experiments on cell and axon orientation *in vitro*: The role of colloidal exudates in tissue organization. *J. Exp. Zool.*, **100**, 353–386.

[28] Wolff, J. (1986). *The Law of Bone Remodeling*, Maquet, P. and Furlong, R. Trans., Springer-Verlag Berlin (Original work published 1892).

[29] Woo, S.-L.Y., Gomez, M.A., Woo, Y.K. and Akeson, W.H. (1982). Mechanical properties of tendons and ligaments: II. The relationships of immobilization and exercise on tissue remodeling. *Biorheology*, **19**, 397–408.