Theoretical study of intracellular stress fiber orientation under cyclic deformation

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Abstract

We studied stress fiber orientation under a wide range of uniaxial cyclic deformations. We devised and validated a hypothesis consisting of two parts, as follows: (1) a stress fiber aligns to avoid a mechanical stimulus in the fiber direction under cyclic deformation. This means that, among all allowable directions, a stress fiber aligns in the direction which minimizes the stimulus, i.e., the summation of the changes in length of the stress fiber over one stretch cycle; and (2) there is a limit in the sensitivity of the cellular response to the mechanical stimulus. Due to this sensing limit, the orientation angle in stress fibers is distributed around the angle corresponding to the minimum stimulus. To validate this hypothesis, we approximated an anisotropic deformation of the membrane on which cells were to be cultured. We then obtained the relationships between the stretch range and the fiber angle in the undeformed state which minimize the mechanical stimuli, assuming that the membrane on which stress fibers and cells adhered was homogeneous and incompressible. Numerical simulation results showed that the proposed hypothesis described our previous experimental results well and was consistent with the experimental results in the literature. The simulation results, taking account of the second part of the hypothesis with a small value for the limit in sensitivity to the mechanical stimulus, could explain why cell orientation is distributed so widely with cyclic stretch ranges of less than 10%. The proposed hypothesis can be applied to various types of deformation because the mechanical stimulus is always sensed and accumulates under cyclic deformation without the necessity of a reference state to measure the stimulus.

Keywords: Cell mechanics; Mathematical model; Stress fiber; Orientation; Mechanical stimulus

1. Introduction

The orientation of cultured cells under cyclic stretch has been investigated in order to reveal the mechanism of cell alignment from the mechanical point of view in the endothelium and the smooth muscles of the artery, which are subjected to periodic blood pressure. Various hypotheses have been proposed to explain the alignment of cultured cells and intracellular stress fibers under cyclic deformation (Buck, 1980; Ives et al., 1986; Buckley et al., 1988; Wang et al., 1995; Takemasa et al., 1998). Buck (1980) hypothesized that cells oriented as a stretch avoidance reaction. His focus was on the elongation, not the compression, of a cell in its axial direction. Ives et al. (1986) proposed that the purpose of cellular alignment was to reduce the stress or strain energy on cells and to minimize the stretch of microtubules or other cytoskeletal networks. Buckley et al. (1988) hypothesized that cells oriented so as to minimize the strain, explaining from their experimental results that cells tended to align in the perpendicular direction of a uniaxial stretch. On the other hand, Wang et al. (1995) proposed a new hypothesis stating that cellular alignment occurred to avoid an orientation in any direction where the maximum axial strain in the cell was above a given threshold. The maximum axial strain was obtained when the silicone substratum was elongated or compressed maximally. The threshold value of the maximum axial strain and the assumption of a normal probability distribution determined the distribution of the cell's direction. Takemasa et al. (1998) also hypothesized that stress fibers orient so as to minimize the change in their length. Both Wang et al. and Takemasa et al. studied the deformation of cells and intracellular stress fibers under cyclic deformation, which is quite important and was not taken into account in the earlier hypotheses mentioned above.

Experimental evidence on the orientation of cells and intracellular stress fibers in endothelium, smooth muscle and melanocytes under cyclic deformation has been reported in the literature (Buck, 1983; Ives et al., 1986; Dartsch & Hämmerle, 1986; Sumpio and Banes, 1988; Shirinsky et al., 1989; Dartsch and Betz, 1989; Iba and Sumpio 1991; Wang et al., 1995; Takemasa et al., 1997; Takemasa et al., 1998). Initially, cellular orientation was investigated with respect to the direction of stretch of a membrane on which cells were cultured (Buck, 1983; Ives et al., 1986; Dartsch & Hämmerle, 1986; Sumpio and Banes, 1988; Shirinsky et al., 1989; Dartsch and Betz, 1989; Iba and Sumpio 1991). More recently, orientation was investigated with regard to the deformation of a substratum (Wang et al., 1995; Takemasa et al., 1997; Takemasa et al., 1998), and the importance of the deformation of the substratum rather than the direction of stretch has been realized.

A cell and its intracellular stress fibers orient approximately in the same direction under cyclic deformation. According to a previous experimental study (Takemasa et al., 1998), cells respond in a time frame of hours while stress fibers respond in minutes in the early stage of cyclic deformation. There is also some difficulty in determining the axial direction of a cell from its shape. Therefore, we have focused our attention on stress fibers rather than the cell.

In a previous study, Takemasa et al. (1998) mathematically analyzed the deformation of stress fibers on a silicone membrane, hypothesizing a minimal length change of a stress fiber. In the current study, we propose a new hypothesis for a mechanical stimulus under cyclic deformation. This hypothesis consists of two parts: (1) a stress fiber aligns to avoid a mechanical stimulus in the fiber direction under cyclic deformation. This means that, among all allowable directions, a stress fiber aligns in the direction which minimizes the stimulus, i.e., the summation of the length change in the stress fiber over one stretch cycle; and (2) there is a limit in the sensitivity of the cellular response to the mechanical stimulus. Due to this sensing limit, the orientation angle in stress fibers is distributed around the angle corresponding to the minimum stimulus. The hypotheses proposed in previous studies (Wang et al., 1995; Takemasa et al., 1998) can be mathematically well described, but a question remains: Why would only the undeformed state and the maximally deformed state play important roles in determining the cell's orientation? The hypothesis proposed in the present study is based on the idea that a cell senses a mechanical stimulus with any degree of deformation, and that the cell orients itself to minimize the overall effect of the stimulus.

2. Kinematics of a stress fiber under uniaxial stretch

A stress fiber on a silicone membrane is modeled mathematically as a line element. In the present study, we assume that the silicone membrane is a homogeneous, nonlinear elastic and incompressible material (Fung, 1994b). We also assume a uniform deformation of the membrane based on the experimental setup in a previous study (Takemasa et al., 1997). The center of the membrane is taken as the origin *O* of the Cartesian coordinate system (*X, Y, Z*) and *o* of (*x, y, z*), respectively. The coordinates *X, Y* and *Z* denote coordinates in an undeformed state in the direction of uniaxial stretch, in the perpendicular direction along the membrane, and in the normal direction of the membrane surface, respectively, and *x*, *y* and *z* denote the same in a deformed state. Under uniaxial cyclic stretch, a membrane deforms symmetrically with respect to the three coordinate axes *X*, *Y* and *Z* with the origin *O* kept in the same position. Therefore, the three principal axes in the strain field of the membrane coincide with the three coordinate axes *X*, *Y* and *Z*. A typical example of how a line element deforms under uniaxial stretch is shown in Fig. 1. This shows the change of length and angle in a line element which has an angle of $\theta_y = 22°$ with respect to the *Y* axis in the undeformed state. This also shows how a unit circle in the undeformed state deforms with the uniaxial stretch of $\lambda_x = 1.0$, 1.5 and 2.0, representing the deformation of the membrane.

Denoting the stretch ratios, i.e., the ratio of the length of a line element in the deformed state to its length in the undeformed state, in the three principal directions *X*, *Y* and *Z* as λ_x, λ_y and λ_z, respectively, we consider a uniform deformation of the membrane to be expressed as

$$x = \lambda_x X, y = \lambda_y Y, z = \lambda_z Z . \tag{1}$$

Each stretch ratio can be expressed in terms of a technical strain ε_x, ε_y or ε_z as

$$\lambda_x = 1 + \varepsilon_x, \lambda_y = 1 + \varepsilon_y, \lambda_z = 1 + \varepsilon_z . \tag{2}$$

The incompressibility condition is expressed as

$$\lambda_x \lambda_y \lambda_z = 1 . \tag{3}$$

Substituting Eq. (3) into Eq. (1), we obtain the relationship

$$x = \lambda_x X, \quad y = \lambda_y Y, \quad z = \frac{Z}{\lambda_x \lambda_y}. \tag{4}$$

Because the membrane is in a plane stress state, stress fibers or line elements in the *X*-*Y* plane always exists in the same plane during deformation. We focus on the deformation of a stress fiber in the *X*-*Y* plane which is obtained by

$$z = Z = 0. \tag{5}$$

Because of the anisotropy between the direction of membrane thickness and any direction along the surface of the membrane, we assume that the membrane is a transversely isotropic material. Under a uniaxial stretch in the *X* direction, the relationships

$$\lambda_y = \lambda_x^{-1/2+\alpha}, \quad \lambda_z = \lambda_x^{-1/2-\alpha} \tag{6}$$

were assumed for a nonlinear and incompressible deformation without using a stress-strain relationship for the membrane where α is a constant of anisotropy.

The lengths L_0 and L of a line element in the undeformed state and the deformed state are expressed as

$$\begin{aligned} L_0 &= \sqrt{X^2 + Y^2} = 1 \\ L &= \sqrt{x^2 + y^2} = \sqrt{\lambda_x^2 X^2 + \lambda_y^2 Y^2} = \sqrt{\lambda_x^2 X^2 + \lambda_x^{-1+2\alpha} Y^2}, \end{aligned} \tag{7}$$

respectively. The total length change $T(\lambda_x)$ for a uniaxial stretch with a stretch ratio λ_x is estimated by the summation

$$T(\lambda_x) = \sum_{t=1}^{t=t_f} \left\{ L[1 + t\Delta\lambda] - L[1 + (t-1)\Delta\lambda] \right\}, \quad t_f = (\lambda_x - 1)/\Delta\lambda \tag{8}$$

where a sufficiently small $\Delta\lambda$ should be selected, for example, 0.005.

By obtaining the intersection of a circle in the undeformed state with a normalized length of 1 in a line element

$$X^2 + Y^2 = 1 \tag{9}$$

and an ellipse in the deformed state (λ_x, λ_y) with a length of 1 + Δr in the corresponding line element (Fung, 1994a)

$$\frac{X^2}{\left(\frac{1}{\lambda_x}\right)^2}+\frac{Y^2}{\left(\frac{1}{\lambda_y}\right)^2}=(1+\Delta r)^2, \tag{10}$$

the angle θ_y ($0^\circ \le \theta_y \le 90^\circ$) of the line element in the undeformed state is expressed as

$$\theta_y=\begin{cases}\tan^{-1}\sqrt{\dfrac{(1+\Delta r)^2-\lambda_y{}^2}{\lambda_x{}^2-(1+\Delta r)^2}}, & \lambda_x \neq 1\\ \tan^{-1}\sqrt{\dfrac{1-2\alpha}{2}}, & \lambda_x = 1\end{cases}. \tag{11}$$

3. Results and Discussion

To validate the proposed hypothesis, we carried out numerical simulations. Because no difference was observed in the experimental results between elongation and compression of a stress fiber in a previous study (Takemasa et al., 1998), we compared the results from numerical simulations with the experimental results for elongation only. In the experiment, primary cultured cells, which were observed to strongly adhere on the surface of a silicone membrane, were utilized. Under 110% (λ_x = 2.1) cyclic stretch at a 1 Hz frequency, these cells adhered on the membrane surface for one hour, which was long enough to enable the cells to deform with the membrane and to complete orientation. This behavior was obtained by preparing a belt-like membrane which could be deformed uniformly by stretching it over bar without clamped support (Takemasa et al., 1997).

Figure 2 shows the relationship between the stretch ratios of the silicone membrane, λ_x in the direction of stretch and λ_y in the perpendicular direction, under uniaxial stretch. The constant α in Eq. (6) is determined to be 0.03 to fit the experimental result of a silicone membrane in our previous study. The theoretical curve nearly describes the relationship of the silicone membrane in the stretch ratio range of λ_x = 1.0 to 2.2. The discrepancy in the stretch ratio between the experimental result and the theoretical approximation in the range of $1.0 < \lambda_x < 1.2$ may be the result of a reduction in accuracy in the measurement with small length changes.

Under the hypothesis of the minimum stimulus, we obtain the relationship between the stretch ratio λ_x and the angle θ_y in the undeformed state with uniaxial stretch, which is shown by the solid line in Fig. 3. The theoretical prediction slightly overestimates the angle for the range of smaller stretch ratios, $\lambda_x \leq 1.4$, and underestimates it for the range of larger stretch ratios, $\lambda_x \geq 1.8$. Besides the human vascular endothelial cells compared in this study, we have obtained the same results for the behavior of the stress fiber orientation of rat aorta smooth muscle cells, newborn rat calvarial cells (osteoblast) and chicken embryo femoral muscle cells (myoblast) under cyclic uniaxial stretch of $\lambda_x = 1.3$, 1.5 and 1.8 (unpublished data).

We also took account of the small value of the limit in sensitivity to the mechanical stimulus, which was defined as the stretch ratio of 0.02 for a normalized line element over one period of stretch. Figure 3 shows that the width of the distribution of angle θ_y, which results from this sensitivity limit, is almost constant for the range of stretch ratios $1.1 \leq \lambda_x \leq 2.1$. This limit in sensitivity covers almost all the experimental data in Fig. 3. In the range of smaller stretch ratios $\lambda_x < 1.1$, the width of the distribution of the angle with the same value of the limit in sensitivity increases markedly, which is consistent with the tendency of the width of the distribution in cell angles in the literature to increase. Wang et al. (1995) demonstrated the effect of the threshold in the stimulus of axial strain on a cell on the cell distribution through experiments and numerical simulations based on a linear elastic theory with three ranges of uniaxial stretch, $\lambda_x = 1.04$, 1.08 and 1.12. In the current study, extending of the uniaxial stretch range to $1 \leq \lambda_x \leq 2.2$ clearly demonstrates how the minimum stimulus and the limit in sensitivity, which is on the order of one percent of fiber length, determine the distribution range of a stress fiber's orientation for each stretch range. It is also possible that a wide distribution of cell orientations under a small amplitude of cyclic stretch is attributable to a small variance in threshold level or a small variance in mechanical stimulus level in different cells on a substratum.

We also carried out a numerical simulation under the previous hypothesis, i.e., that there is no length change in a stress fiber in the maximally deformed state, with the limit in sensitivity defined in terms of stretch. Figure 4 shows the relationships between the stretch ratio λ_x and the fiber angle θ_y for a line element, the length of which is normalized as 1 in the undeformed state and 0.98, 1.00

and 1.02 in the maximally deformed state. A 2% length change in the maximally deformed state covers most of the experimental data points in our previous study. Variation in a stress fiber's length by 2% also has a large effect on the distribution of fiber orientation when the uniaxial stretch range is as small as $\lambda_x = 1.1$, while the variation in the angle with a 2% length change is less than 5° for $\lambda_x > 1.4$.

Comparing the angle widths in Figs. 3 and 4, it is seen that both the present and earlier hypotheses are able to predict the orientation of stress fibers. The proposed hypothesis, however, has the advantage that it can be applied to numerical simulations of intracellular orientation under various conditions of deformation, while the previous hypothesis needs other criteria in order to be extensively used.

The orientation of cells under a combination of flow shear stress and cyclic stretch was also investigated with cultured cells from bovine pulmonary arterial endothelium and bovine aortic endothelium (Moore et al. 1994; Zhao et al. 1995). The results reported by Zhao et al. (1995) showed that the addition of cyclic stretches of 3.9–4.3% and 6.8-7.3% to any level of cyclic shear stress (means 1.9–2.2%, 3.2–3.4%, 5.2–6.4%) enhanced the cell alignment in the flow direction, whereas the addition of 1.9–2.2% cyclic stretch did not. From a macroscopic viewpoint, the mechanism of orientation reported for vascular endothelial cells when they are subjected to cyclic flow and stretch needs to be described in terms of both the proposed stimulus and an additional one, or a common measure to quantify both stimuli. From a microscopic viewpoint, an internal factor in the tissue, e.g., a transmembrane protein (Civelekoglu et al., 1998), may sense and transduce an external stimulus into cells. Further investigation is also required for a more precise validation of the mechanical stimuli and the sensing mechanism in cells.

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Captions

Fig. 1. Change in a line element, representing a stress fiber, which has an angle with respect to the Y axis of θ_y = 22° in the undeformed state, and the deformation of a unit circle in the undeformed state with an increase in the range of uniaxial stretch, $\lambda_x = 1.0$, 1.5 and 2.0, which represents the deformation of the substratum.

Fig. 2. Relationship between the stretch ratios of the silicone membrane, λ_x in the stretch direction and λ_y in the perpendicular direction, under uniaxial stretch.

Fig. 3. Relationship between the stretch ratio λ_x in the stretch direction and the angle θ_y in the undeformed state of a line element which corresponds to the minimum total length change during the uniaxial stretch. The dashed lines show the relationships, taking the threshold as 2% of the line element length over one stretch cycle.

Fig. 4. Fiber angle in the undeformed state for a line element, the length of which is 1 in the undeformed state and 0.98, 1.00 and 1.02 in the maximally deformed state.

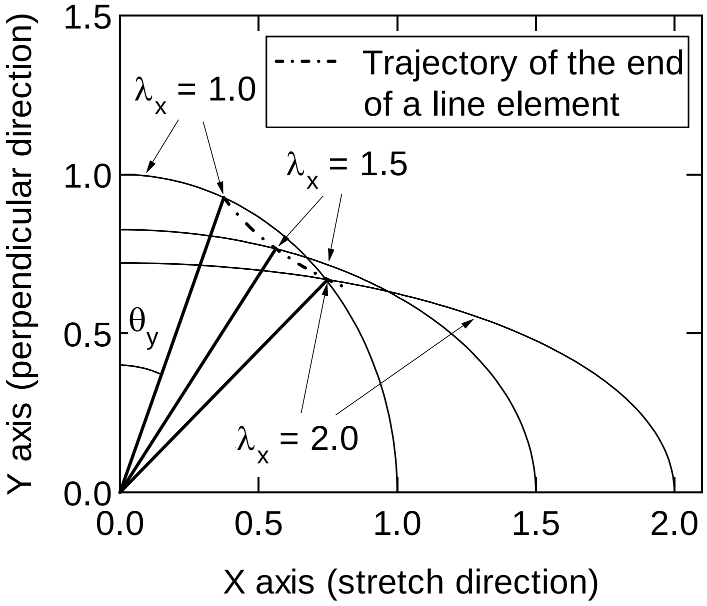
Trajectory of the end of a line element
λ_x = 1.0
λ_x = 1.5
λ_x = 2.0
θ_y
Y axis (perpendicular direction)
X axis (stretch direction)
0.0
0.5
1.0
1.5
2.0
0.0
0.5
1.0
1.5

Fig. 1

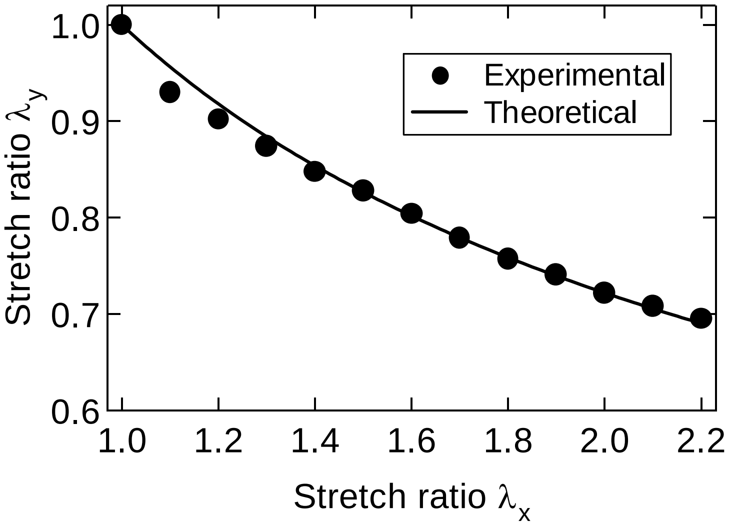
Experimental
Theoretical
Stretch ratio λ_y
1.0
0.9
0.8
0.7
0.6
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Stretch ratio λ_x

Fig. 2

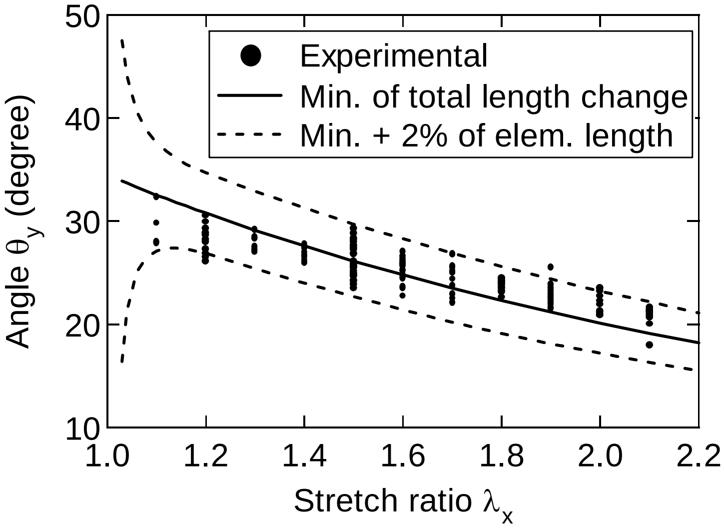
50
40
30
20
10
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Angle θ_y (degree)
Stretch ratio λ_x
● Experimental
Min. of total length change
Min. + 2% of elem. length

Fig. 3

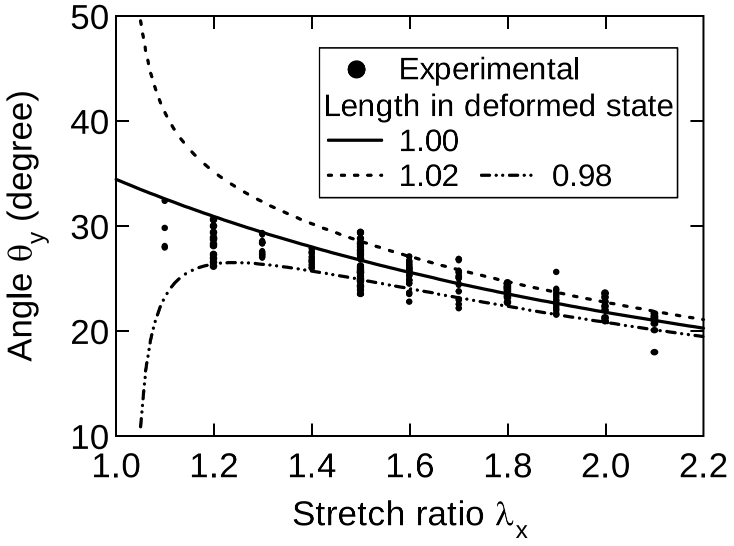
Experimental
Length in deformed state
1.00
1.02
0.98
Angle θ_y (degree)
Stretch ratio λ_x
10
20
30
40
50
1.0
1.2
1.4
1.6
1.8
2.0
2.2

Fig. 4