ORIENTATION OF STRESS FIBERS IN A CULTURED ENDOTHELIAL CELL UNDER CYCLIC DEFORMATION TAKING ACCOUNT OF ITS NONUNIFORM DEFORMATION: STRAIN LIMIT HYPOTHESIS AND NUMERICAL SIMULATIONS

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INTRODUCTION

Vascular endothelial cells are subjected to fluid shear stress and cyclic deformation in the circumferential direction of the vascular wall which are caused by periodic blood flow. Intracellular stress fibers (SFs), i.e., bundles of actin filaments, are observed to orient in the longitudinal direction of the vessel under these conditions.

To reveal the mechanism of the orientation of SFs from a mechanical viewpoint, Takemasa et al. measured the angle of SFs in cultured endothelial cells by applying cyclic simple elongations to cells through a substrate with constant strain ranges [1]. These SFs orient along the surface of the substrate. Wang et al. observed that actin cytoskeletons were remodeled into three-dimensional "tent-like" actin structures in a cultured endothelial cell under cyclic equibiaxial stretch [2]. In this case, probably one end of a SF attaches to the apical membrane and the other to the basal membrane. Kano et al. also observed SFs with one end at the apical membrane and the other end at the basal membrane or the lateral cell border under fluid shear stress [3]. From these observations, a SF is categorized into three cases; spanning the cytoplasm (a) between points on the basal membrane, (b) between the apical and basal membranes, and (c) between points on the apical membrane (See Figure 1).

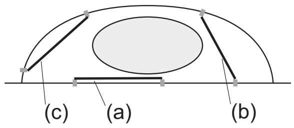

Figure 1. Three cases of a SF spanning the cytoplasm

We proposed a strain limit hypothesis to explain the orientation of SFs caused by cyclic deformation which had another concept from the previous hypothesis [4, 5]. The hypothesis states that a SF orients itself only in the direction in which the strain in the fiber direction does not exceed the strain limit in the maximum deformed state of the substrate [5]. In this study we predicted the orientation of three types of SFs categorized as (a) to (c) for cyclic deformations taking account of nonuniform and uniform deformations of a cell. We also evaluated the effects of the deformation field and the position of a SF's end in the cell on the orientation of SFs.

METHODS

We assumed the stretch ratios as a function of height:

$$\begin{aligned} &\lambda_x(z) = f(z)\{\lambda_x(0)-1\}+1, \\ &\lambda_y(z) = g(z)\{\lambda_y(0)-1\}+1, \quad 0 \le z \le z_{\max} \\ &\lambda_z(z) = 1/\left\{\lambda_x(z)\cdot\lambda_y(z)\right\}, \end{aligned} \tag{1}$$

to approximate the cellular deformation in the case of pure uniaxial stretch. In Eq. (1), λ_x, λ_y and λ_z are stretch ratios in the *x*, *y* and *z* directions, respectively, and $f(z)$ and $g(z)$ are approximation functions, *z* is a height from the substrate surface, and z_{max} is the cellular height (See Figure 2). The boundary conditions of the substrate are shown in Table 1 where σ_z is a Cauchy stress component in the *z* direction. The approximation functions were validated by comparing the positions from Eq. (1) with a result from a finite element analysis of a cell [5].

Table 1. Boundary conditions of the substrate deformation

Deformation type	*x*-axis	*y*-axis	*z*-axis
Pure uniaxial stretch	$\lambda_x > 1$	$\lambda_y = 1$	$\sigma_z = 0$
Equibiaxial stretch	$\lambda_x > 1$	$\lambda_y = \lambda_x$	$\sigma_z = 0$

Table 2. Conditions of the numerical simulation

Deformation type (Strain range)	Case of a SF (See Figure 1)	Location of one end of a SF
Cyclic pure uniaxial stretch, nonuniform deformation (10%)	(a), (b)	P, Q, R
	(c)	P' Q' R'
Cyclic equibiaxial stretch, uniform deformation (10%)	(b)	Cell height: 5 μm, 8 μm

We carried out numerical simulations of the mechanically permissible orientations of SFs under the conditions summarized in Table 2. Six points were chosen on a cellular membrane as one end of a SF. The cyclic pure uniaxial stretch was applied as a physiological condition of vascular endothelial cells. Two heights were also chosen for the simulation of the orientation of SFs under cyclic equibiaxial stretch with an assumption of uniform deformation of a cell. The result was compared with the observation result by Wang et al. [2]

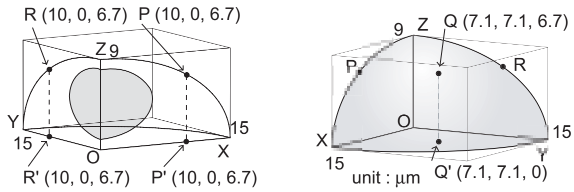

Figure 2. Positions of an end of a SF for the numerical simulation of its orientation under cyclic deformations

RESULTS AND DISCUSSION

Under pure uniaxial stretch we obtained approximation functions

$$\begin{aligned} f(z) &= 0.921 + 0.079\exp(-0.322z), \\ g(z) &= 1 - 0.166\{1-\exp(-0.508z)\}(\lambda_x(0)-1) \end{aligned} . \qquad (2)$$

Figure 3 shows a comparison of the predicted result by the hypothesis and the experimental result by Takemasa et al. [1] for SFs in cultured endothelial cells subjected to cyclic simple elongations. The hypothesis reproduced the experimental result quite well.

Figure 4 shows numerical simulation results of the positions (gray color in Figure 4) of an end of a SF under cyclic pure uniaxial stretch with 10% strain range in cases that the other end was located at either one of the Points P, Q, R, P', Q' and R' in Figure 2. The deformation of the basal membrane was assumed to be the same as that of the substrate. Figure 4 (a) and (b) indicated that SFs with one end at the apical membrane were allowed to orient in a wide region when the strain limit was assumed to be 5%.

Figure 5 shows the theoretical prediction of the position of an end at the basal membrane for a SF which has the other end at the apical membrane at the height of 5 and 8 μm with an assumption of uniform deformation of a cell. The result was compared with the observation by Wang et al. [2]. Form their microscopic image data we measured the relative location of the end of actin filaments and plotted in the figure assuming that the other end was located at (x, y, z) = (0, 0, 5) or (0, 0, 8) [μm]. The comparison shows that the prediction has a good agreement with the tendency of the alignment of actin cytoskeletons.

CONCLUSIONS

Based on a strain limit hypothesis we carried out numerical simulations of the orientation of SFs located at various regions in a cell under cyclic biaxial deformations. The predictions showed a good agreement with observation results in the literature.

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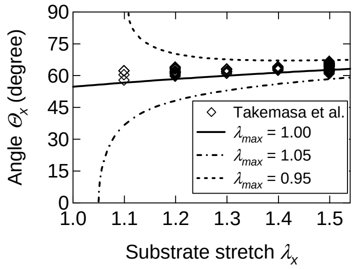


Figure 3. Numerical simulation result of the orientation of SFs under cyclic simple elongations [1, 5]

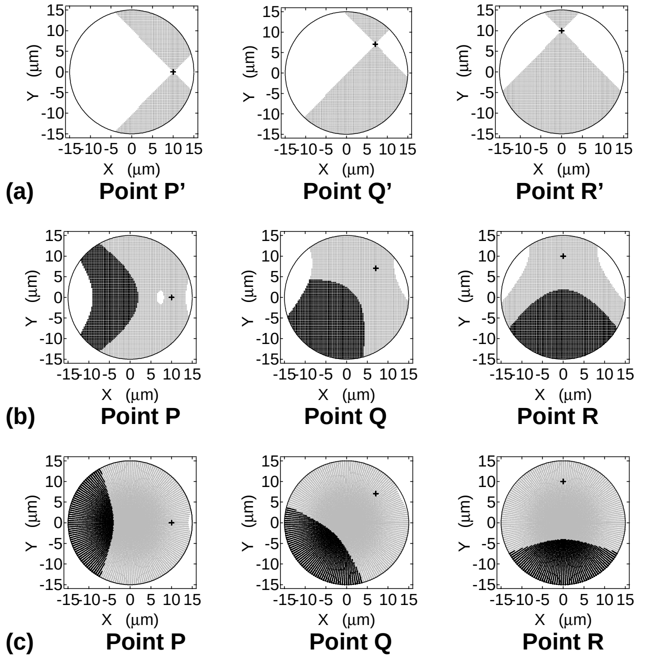
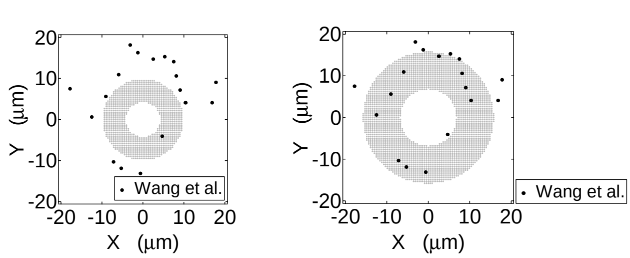


Figure 4. Prediction of the position of an end of a SF (gray color in the figure) located on the basal membrane (Case (a), (b)) or on the apical membrane (Case (c)) for SFs (a) on the basal membrane, (b) between apical and basal membranes, and (c) between points on apical membrane for the three positions of a SF's end under cyclic pure uniaxial stretch with 10% strain range. Dark color denotes the region in which a SF cannot orient due to the nucleus. The symbol (+) denotes either point among P', Q' and R'.



(a) Height 5 μm **(b) Height 8 μm**

Figure 5. Numerical simulation result of the position of an end on the basal membrane for a SF which has the other end on the apical membrane at the height of (a) 5 μm and (b) 8 μm under cyclic equibiaxial stretch [2]