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CELL ORIENTATION RESPONSE TO CYCLICALLY DEFORMED SUBSTRATES: EXPERIMENTAL VALIDATION OF A CELL MODEL

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Abstract—We have developed a stochastic model that describes the orientation response of bipolar cells grown on a cyclically deformed substrate. The model was based on the following hypotheses regarding the behavior of individual cells: (a) the mechanical signal responsible for cell reorientation is the peak to peak surface strain along the cell's major axis (p–p axial strain); (b) each cell has an axial strain threshold and the threshold is normally distributed in the cell population; (c) the cell will avoid any direction where the p–p axial strain is above its threshold; and (d) the cell will randomly orient within the range of directions where the p–p axial strains are less than the cell's threshold. These hypotheses were tested by comparing model predictions with experimental observations from stretch experiments conducted with human melanocytes. The cells were grown on elastic rectangular culture dishes subjected to unidirectional cyclic (1 Hz) stretching with amplitudes of 0, 4, 8, and 12%. After 24 h of stimulation, the distribution of cell orientations was determined by measuring the orientations of 300–400 randomly selected cells. The 12% stretch experiment was used to determine the mean, 3.5%, and the standard deviation, 1.0%, of the strain threshold for the cell population. The Kolmogorov–Smirnov test was then used to determine if the orientation distributions predicted by the model were different from experimentally measured distributions for the 4 and 8% stretches. No significant differences were found between the predicted and experimental distributions (4%: $p = 0.70$; and 8%: $p = 0.71$). These results support the hypothesis that cells randomly orient, but avoid directions where the p–p axial strains are above their thresholds.

Keywords: Cell mechanics; Substrate deformations; Cell reorientation; Mathematical model.

INTRODUCTION

The loads imposed on extracellular matrix play an important role in the homeostasis and remodeling of connective tissues (Noyes, 1977; Tipton *et al.*, 1975; Wolff, 1986; Woo *et al.*, 1982). Many clinical problems, such as repetitive motion injuries (Davis, 1990), bone resorption after internal fracture fixation (Weinans *et al.*, 1993), total joint replacement (Schmalzried *et al.*, 1994), and disuse atrophy of ligaments and tendons (Noyes, 1977; Woo *et al.*, 1982), are believed to result from abnormal tissue loading. Although the connection between tissue loads and tissue remodeling has been known since the late 19th century (Wolff, 1892), the specific signal sensed by the tissue cells and the cascade of cellular events that lead to tissue remodeling remains unknown.

In an effort to elucidate the cellular mechanisms, researchers have increasingly used cell culture models to study cell response to various mechanical signals (Acevedo *et al.*, 1993; Banes *et al.*, 1985; Dartsch and Hammerle, 1986; Leung *et al.*, 1976; Ziegler and Nerem, 1994). These studies have shown that cyclic deformations of culture surfaces cause cell populations to become oriented away from the stretch direction (Buck, 1980; Dartsch and Betz, 1992; Shirinsky *et al.*, 1989), modify the cell's cytoskeleton (Dartsch and Betz, 1992), alter cell proliferation (Banes *et al.*, 1985; Neidlinger-Wilke *et al.*, 1994), and alter mRNA levels and protein synthesis (Carver *et al.*, 1991; Leung *et al.*, 1976).

The orientation response is important to understand for several reasons. First, the mechanisms responsible for the orientation response may be coupled with mechanisms that alter cellular mRNA levels and protein synthesis. Second, in many culture systems, reorientation of individual cells alters the deformations they experience. This occurs because the surface deformation field is anisotropic or direction dependent (Schaffer *et al.*, 1994). The substrate deformations are transmitted to the cell through focal adhesions (Burridge *et al.*, 1988). As a result, when a cell changes orientation, the deformations transmitted through the focal adhesions will change. Any cellular response that depends on the cell's deformation will thus also depend on the cell's orientation on the substrate.

Several hypotheses have been proposed to explain the orientation response to cyclic substrate deformations. Buck (1980) hypothesized that the response was a stretch avoidance reaction. Buckley *et al.* (1988) proposed that the cells reorient to minimize the strain acting on them. Neither investigator was specific about which feature of

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the substrate surface strain was the driving signal for the orientation response. Planar surface deformations can be completely described by two normal strains and one shear strain (Fung, 1994). Deciding, from experimental observations alone, which of these strains or their combination is the driving signal is difficult due to the anisotropy of the strain fields produced by most experimental apparatus. Theoretical modeling, when combined with experimental observations, can greatly aid in deciding between alternative hypotheses (Tran-Son-Tay, 1993).

The purpose of this study was to test a model of the equilibrium orientation response of cells subjected to cyclic substrate deformations. The model is based on the central hypothesis, that individual cells randomly orient, but avoid any direction where the peak-to- peak substrate strain along the cell's major axes is above some threshold value. The model also incorporates that the threshold varies among the cells, but is normally distributed in the cell population.

MODEL

This section is divided into three parts. In the first part we present our model of how bipolar cells respond to cyclic substrate deformations. In the second part, we describe how the substrate surface strains, as viewed by a cell, can be computed if the cell orientation is known. The third part describes the computer simulation we used to predict cell orientation distributions.

The proposed cell model describes how an individual cell responds to cyclic substrate strains. Neidlinger-Wilke *et al.* (submitted) have shown that without a stretch signal, fibroblasts and osteoblasts grown at low density are randomly oriented. This distribution of cell orientations can be described by a uniform random distribution, $u(\theta)$, within the range from 0 to 90°. Use of a 90° range, instead of a 360° range, follows from symmetry considerations. First, cells have no identifiable head and tail. Thus, if a cell is rotated 180°, it is not possible to distinguish a change in the cell's orientation. This reduces the range from 360° to 180°. The range is further reduced to 90° due to the symmetry of the substrate strains about the principal strain directions. This symmetry causes two cells, oriented $\pm\theta$ from a principal strain direction, to experience identical strains. The principal axes, for the dish geometry we use, are the stretch direction and its perpendicular, x and y axes in Fig. 1. Thus, we expect the cell orientation distributions should be symmetric about both directions.

Buck first reported that when the culture substrate was cyclically stretched, the cells oriented away from the stretch direction. As noted earlier, Buck believed this was an avoidance response of cells to stretching. Subsequently, Dartsch (1986) noted that the orientation response depended upon the applied stretch amplitude. As the stretch amplitude was increased, more cells were found oriented away from the stretch direction. Neidlinger-Wilke *et al.* (submitted) showed that the percent of cells, oriented within $\pm 2.5°$ of some direction, was strongly correlated with the substrate strain in that direction. We refer to this strain as axial strain because it is along the cell's major axis (Fig. 1). We have shown, in recent experiments (Wang *et al.* 1995), that the orientation distributions are the same whether the substrate is cyclically shortened or lengthened. These data led us to conclude that the substrate deformation signal responsible for cell reorientation was the peak-to-peak axial strain, i.e. the peak-to-peak strain that changed the cell's length.

Another important fact noted by Dartsch (1986) was that cells did not reorient under small ($< 2\%$) stretch amplitudes. Neidlinger-Wilke *et al.* confirmed Dartsch's results and further noted that few, if any, cells oriented in directions where the axial strain exceeded 5–6%. These experiments suggested that cells could tolerate the substrate deformation until some axial strain threshold level was reached. Based on these results, we postulated that each cell had an axial strain threshold and that the cell axial strain threshold was normally distributed in the cell population. The model thus had two free parameters, the mean and standard deviation of the axial strain threshold. These two parameters completely characterized the orientation response of a cell population.

The above description of the cell model can be summarized as follows:

1. The mechanical signal responsible for cell reorientation is the peak-to-peak surface strain along a cell's major axis (p–p axial strain).
2. Each cell has axial strain threshold.
3. The axial strain threshold varies among cells and is normally distributed in the cell population.
4. The cell will avoid any direction where the p–p axial strain is above its threshold.

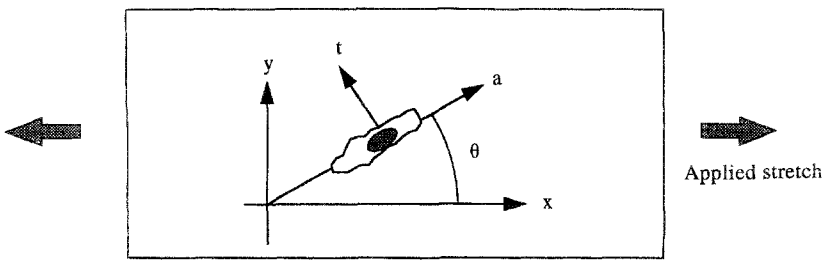

Fig. 1. A cell is shown on a rectangular substrate subjected to uniaxial stretching in the x-direction. The coordinate system in the cell is oriented along the cell's major axis, a, and the minor axis, t.

5. The cell will randomly orient within the range of directions where the p–p axial strains are less than the cell's axial strain threshold.

To test the cell model, we first determined the population parameters that produced a good fit to experimental data for a 12% p–p applied stretch. Then, using the same population parameters, we determined if the model agreed with experiments done at other stretch amplitudes. To make model predictions, however, it was necessary to describe the strain field produced on the substrate surface in the culture system we used.

In the following section, we limit our discussion to substrate strains produced in the experimental apparatus we employ. The system has been previously described by Neidlinger-Wilke *et al.* (1994, submitted). The basic geometry is a rectangular substrate stretched along its length, and allowed to freely change its width. This produces planar deformations and experimental measurements have shown the strains are uniform over the surface (Neidlinger-Wilke, *et al.*, 1994). As noted earlier, substrate surface deformations can be described by two normal strains and one shear strain. The normal strains quantify surface length changes in two mutually perpendicular directions, while the shear strain describes the change in angle between two initially perpendicular lines. Our goal was to describe these strains as observed from a coordinate system located on a cell. The coordinate axes were selected to correspond to the cell's major and minor axes (Fig. 1). We refer to the normal strains directed along the cell's axis as axial strain, and the normal strain across the cell's width as transverse strain.

If the strains observed by a cell oriented in the stretch direction are known, and expressed in proper tensorial form, the strains observed by a cell oriented in any other direction can be readily computed using tensorial coordinate transformation equations (Fung, 1994):

$$\varepsilon_{aa}(\theta) = \varepsilon_{xx}\cos^2(\theta) + \varepsilon_{yy}\sin^2(\theta) + \gamma_{xy}\sin(2\theta)/2, \quad (1a)$$

$$\varepsilon_{tt}(\theta) = \varepsilon_{xx}\sin^2(\theta) + \varepsilon_{yy}\cos^2(\theta) - \gamma_{xy}\sin(2\theta)/2, \quad (1b)$$

$$\gamma_{at}(\theta) = (\varepsilon_{yy} - \varepsilon_{xx})\sin(2\theta) + \gamma_{xy}\cos(2\theta). \quad (1c)$$

The parameters ε_{xx}, ε_{yy}, and γ_{xy} are the axial, transverse, and shear strains observed by a cell oriented in the stretch direction (x-axis). The variables $\varepsilon_{aa}(\theta)$, $\varepsilon_{tt}(\theta)$, and $\gamma_{at}(\theta)$ are the axial, transverse and shear strains observed by a cell oriented at an angle, θ, about the stretch direction (Fig.1).

To use equation (1a) to describe the axial strain for a cell, it is necessary to find the three strains, ε_{xx}, ε_{yy} and γ_{xy}, for a cell oriented in the stretch direction. This can be done by direct experimental measurement of the culture dish deformation that results from an applied stretch. Let the relative length change, $\Delta L/L_0$, of the substrate in the x and y direction be designated by δ_x and δ_y, respctively. Further, let the angle change between two initially perpendicular lines, one oriented in the stretch direction, be designated by φ_{xy}. Using the Green strain definition, the tensorial strains ε_{xx}, ε_{yy}, and γ_{xy} can be computed from these experimental measures by (Fung, 1994)

$$\varepsilon_{xx} = \delta_x + \delta_x^2/2, \quad (2a)$$

$$\varepsilon_{yy} = \delta_y + \delta_y^2/2, \quad (2b)$$

$$\gamma_{xy} = (\delta_x + 1)(\delta_y + 1)\sin(\varphi_{xy})/2. \quad (2c)$$

For the rectangular substrate used in our experimental system, the stretch direction and its perpendicular are axes of symmetry. As a result, these directions must also be principal strain directions and the shear strain γ_{xy} will vanish ($\gamma_{xy} = 0$). Further, δ_y can be expressed as a function of δ_x and the Poisson's ratio, v, of the substrate material, $\delta_y = -v\delta_x$. Substituting it into equation (1a) yields

$$\varepsilon_{aa}(\theta) = \delta_x[(1 + v) + (1 - v^2)\delta_x/2]\cos^2(\theta) - v\,\delta_x + v^2\delta_x^2/2. \quad (3)$$

Equation (3) expresses the direction dependence of the axial strain in terms of the applied stretch, δ_x, and the substrate Poisson's ratio, which we measured to be $v = 0.38$.

To use the cell model to make predictions, we rearrange equation (3) to determine the orientation, θ, for a specified axial strain, ε_{aa}, and obtain

$$\theta = \cos^{-1}\left(\sqrt{\frac{2(\varepsilon_{aa}/\delta_x) + 2v - v^2\ \delta_x}{2\ (1 + v) + (1 - v^2)\ \delta_x}}\right). \quad (4)$$

The following section describes how we used the cell model and equations that describe the substrate strain field to predict cell orientation frequency distributions. Because we were unable to obtain a closed form solution for the cell orientation probability distribution, we decided to do computer simulation of experiments to obtain the cell orientation frequency distributions. The first step of the simulation was to obtain a sample of cell strain thresholds, from a normal distribution having specified mean axial strain threshold and standard deviation. This was accomplished using random number generator (Excel 5.0, Microsoft Corp, Redmond, WA). The second step was to compute, for each cell strain threshold, the range of orientations where the peak-to-peak axial strains were below the threshold. The last step was to randomly specify an orientation within the range. This process produced a sample of orientations that could be analyzed in the same manner as experimental cell orientation data.

In detail, a sample of 10,000 random numbers that represent cell strain thresholds was generated using Excel's random number generator. Then, for each strain threshold we determined the range of orientations [θ_a, θ_b] within which the peak-to-peak substrate strains were less than the strain threshold, and outside which the peak-to-peak axial strains were greater than the threshold. Three cases existed in the determination of θ_a and θ_b. If the cell's axial strain threshold was greater than applied substrate strain ε_{xx}, then θ_a and θ_b were specified to be 0 and 90°, respectively (Fig. 2, case I). If the cell's threshold was less than the applied strain ε_{xx} but greater

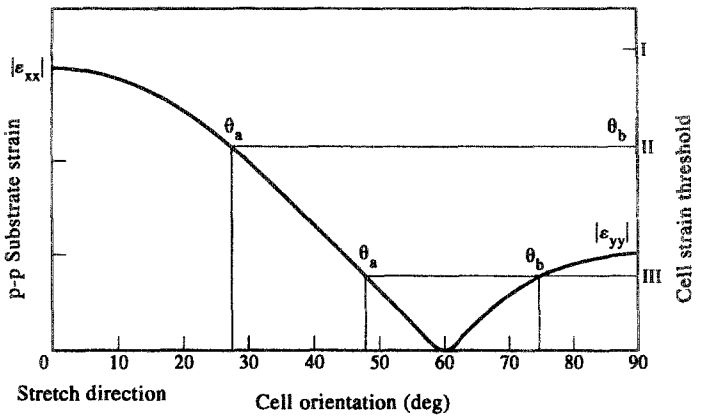

Fig. 2. The solid line shows the variation of peak-to-peak (p–p) axial strain magnitude, $|\varepsilon_{aa}|$, with orientation. An applied stretch, δ_x, produces the Green's strain, ε_{xx}, in the stretch direction. The p–p axial strain equals the magnitude of ε_{xx}, $|\varepsilon_{xx}|$, at 0° and decreases to zero near 60°. The angle at which the p–p axial strain is zero, $\varepsilon_{aa} = 0$, depends primarily on the Poisson ratio of the substrate, equation (4). For example, if $\nu = 0$, the location is 90°; and if $\nu = 0.5$, it is about 55°. Above 60°, the p–p axial strain again increases even though the sign of the Green's strain, ε_{xx}, changes. The p–p axial strain continues to increase until, at 90°, it becomes equal to the magnitude of the lateral strain $|\varepsilon_{yy}|$. $\varepsilon_{yy} = -\nu\delta_x + \nu^2\delta_x^2/2$. The range of orientations, $[\theta_a, \theta_b]$, which can be assumed by a cell, is shown for three cases, I, II, and III. Case I, if the cell's strain threshold is greater than the applied strain magnitude $|\varepsilon_{xx}|$, the cell can assume any orientation, then $\theta_a = 0$ and $\theta_b = 90°$. Case II, if the cell's threshold is less than $|\varepsilon_{xx}|$ but greater than $|\varepsilon_{yy}|$, θ_a is determined from equation (4), and $\theta_b = 90°$. Case III, if the cell's threshold is less than both $|\varepsilon_{xx}|$ and $|\varepsilon_{yy}|$, θ_a and θ_b are both determined from equation (4).

than the lateral strain ε_{yy} (Fig. 2, case II), the lower orientation limit, θ_a, was computed from equation (4) with ε_{aa} replaced by the cell's threshold. The upper orientation limit, θ_b, was set to be 90°. Finally, if the cell's threshold was less than both ε_{xx} and ε_{yy} (Fig. 2, case III), both θ_a and θ_b were determined from equation (4).

The last step in the simulation was to randomly specify each cell's orientation, θ, within its permissible orientation range, $[\theta_a, \theta_b]$. This was accomplished using the following equation:

$$\theta = \theta_a + u(\theta_b - \theta_a), \tag{5}$$

where u was a random number generated from a uniform distribution within the range [0, 1]. The cell orientations obtained in this manner were then used to determine the orientation frequency distribution for the cell population. This was accomplished by dividing the 0–90° range into eighteen 5° intervals and counting the percent of cells whose orientations were within each interval.

To examine how the shape of predicted frequency distributions changed with different values of the two model parameters, i.e. the mean and standard deviation of the cell axial strain threshold, we performed a parametric study in which we varied the mean from 3.0 to 4.0%, and the standard deviation from 0.5 to 1.5%.

MATERIALS AND METHODS

This section describes stretch experiments we performed to validate predictions of the presented cell model. Human melanocyte culture was established using shave biopsies from the forearm of normal subjects. Briefly, the biopsies were placed in trypsin (2.5 mg l^{-1}) and incubated for 2 h at 37°C. The trypsin was then replaced with MCDP-153 medium added with fibroblast growth factor, insulin, and other supplements (Boissy *et al.*, 1991). The melanocytes in the culture had well-defined, nearly linear shape, which reduced the uncertainty in measuring cell orientations. Cells from the 12th to 15th passage were used in all experiments.

The established cultures of melanocytes were transferred into elastic silicone dishes. The dishes had a rectangular shape (10 × 3.5 cm), with a 3 × 6 cm well in the middle. To promote cell attachment, all dishes were coated with 20 μg ml^{-1} ProNectin-F (Protein Polymer Technologies, Inc., San Diego, CA) that contains the RGD ligand of human fibronectin. The cells were plated in low densities (2×10^3 cm^{-2}). Cells were grown in the dishes for 48–72 h before initiating the stretch stimulus.

Using a stretching apparatus described previously (Neidlinger-Wilke *et al.*, 1994), cyclic stretches of 0, 4, 8 and 12% were applied to the culture dishes at 1 Hz for 24 h. Prior studies had demonstrated that this was sufficient time for the cells to reach a near equilibrium distribution of orientations. Immediately after stopping the stimulation, 35 mm photomicrographs were taken at 25× of 16–24 regions in the central areas of dishes. The photomicrographs were projected onto a digitizer table of the Zeiss interactive digital analysis system (ZIDAS, Carl Zeiss, Inc., Thornwood, NY). Cell images were manually traced along their axial directions by a stylus, and the cell orientations were automatically printed out. By this method, orientations of 300–400 cells in two replicate experiments were measured. From the orientation measurements, cell orientation frequency distributions

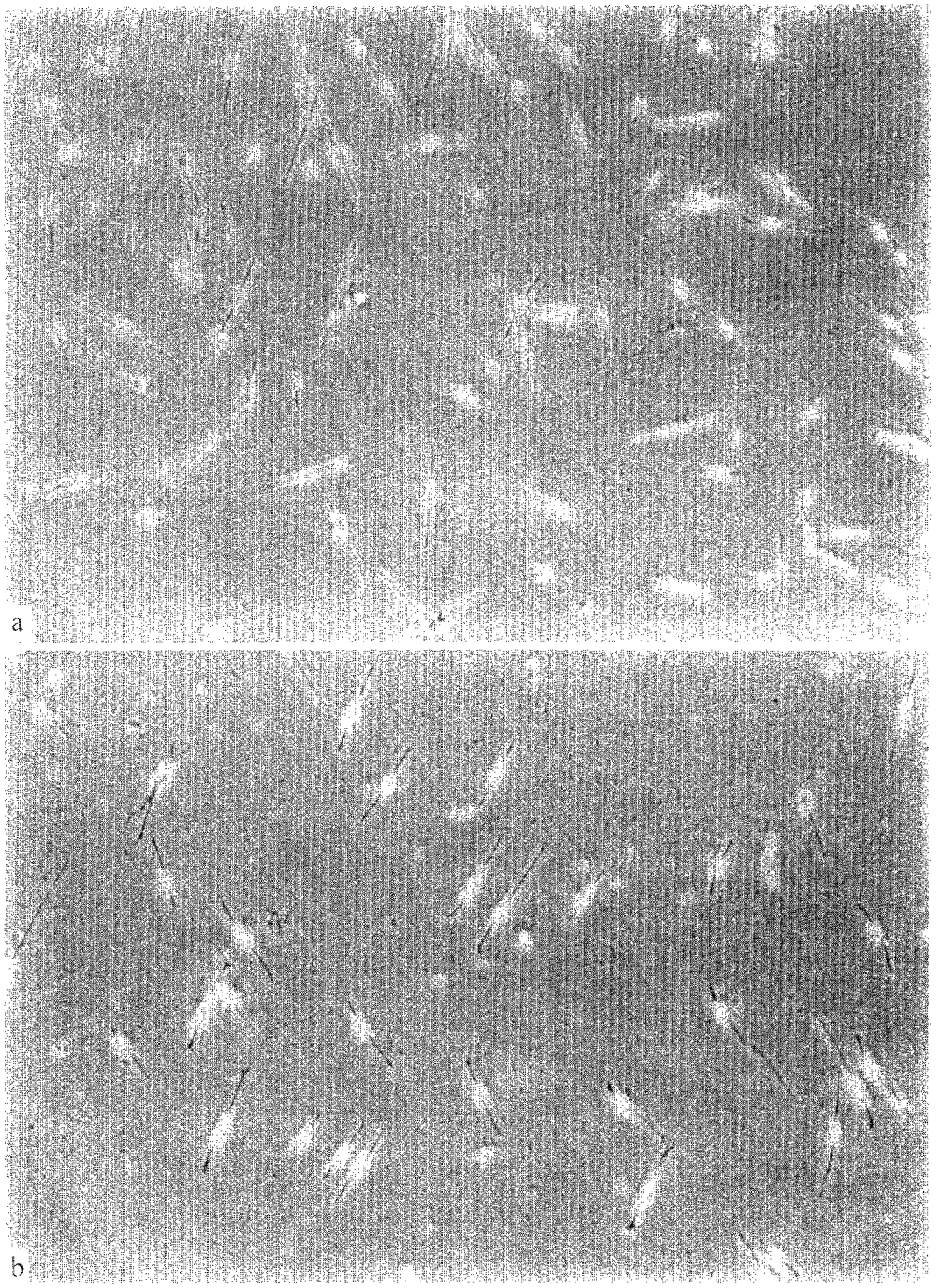

Fig. 3. (a) Photomicrograph (50 ×) of human melanocytes grown at low density in the absence of a stretch stimulus. (b) A similar population of cells grown on a substrate subjected to a 1 Hz 12% peak-to-peak applied stretch for 24 h. Note that the cells tend to align at 60° to the applied stretch direction (horizontal), near the direction of zero axial surface strain.

were determined by calculating percentages of cells that fell into 18 orientation intervals (5° each) between 0 and 90°. Finally, the Kolmogorov–Smirnov test was used to determine if the experimental distributions were significantly different from those obtained from the computer simulation.

RESULTS

Cells grown without a stretch stimulus appeared to randomly orient [Fig 3(a)] and the cell orientation distribution was not significantly different from a uniform random distribution ($p = 0.81$). When a 12% cyclic stretch was applied, the cells oriented away from the stretch direction and appeared to orient near 60°, the direction where the peak-to- peak axial strain was zero [Fig. 3(b)]. A good fit between the predicted and experimental frequency distributions was obtained using a mean axial strain threshold of 3.5% and a standard deviation of 1.0% [Fig. 4(a)]. There was no significant difference between the two distributions ($p = 0.19$), and the model explained 96.5% of the variance in the experimental data.

The predicted distributions under the 4 and 8% stretches [Fig. 4(b) and (c)] were not significantly different from the experimental distributions ($p = 0.70$ for the 4%; and $p = 0.71$ for the 8%). The two predicted distributions explained 75.4% of the variance (the 4%) and 91.2% (the 8%), respectively.

A parametric study showed that varying the mean and standard deviation of the axial strain thresholds in a cell population, changed the shape of the predicted frequency

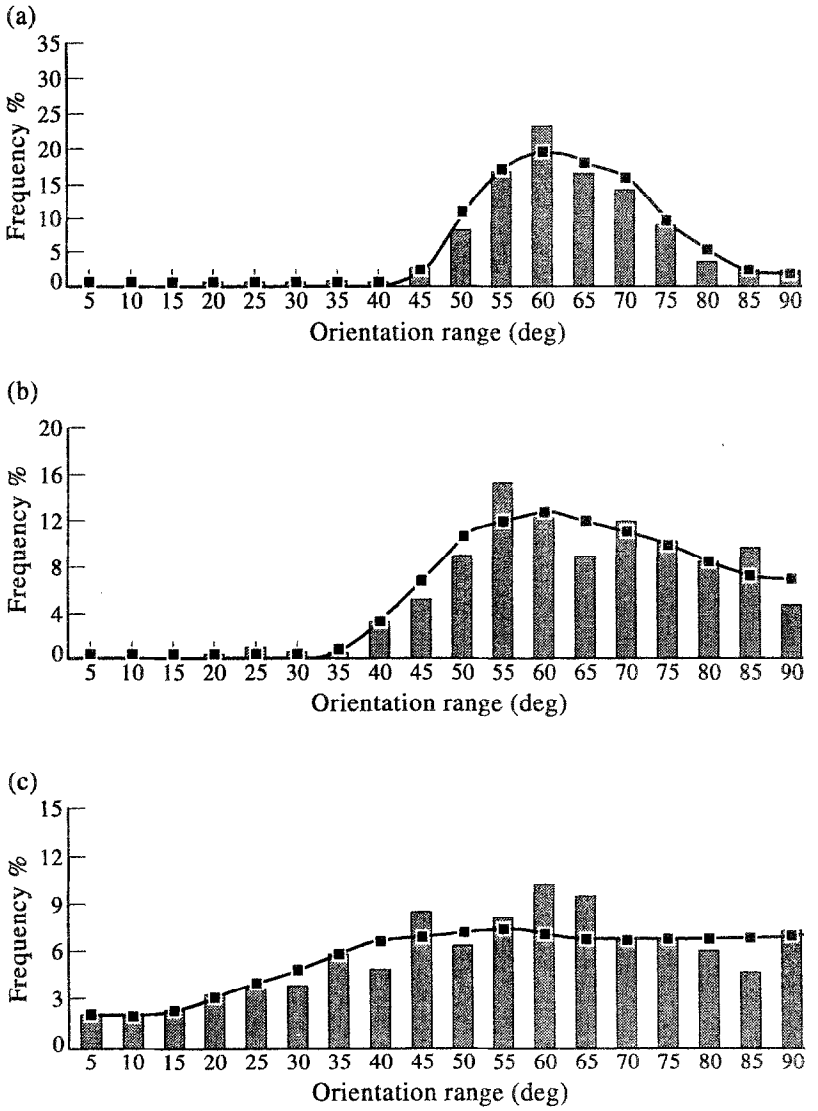

Fig. 4. Comparisons between experimental frequency distributions (bars) and predicted frequency distributions (curves). (a) 12% peak-to-peak applied stretch. These data, $n = 333$, were used to determine the model parameters that provided a good fit (mean axial strain threshold = 3.5%, S.D. = 1.0%). (b) 8% stretch, $n = 307$. (c) 4% stretch, $n = 392$.

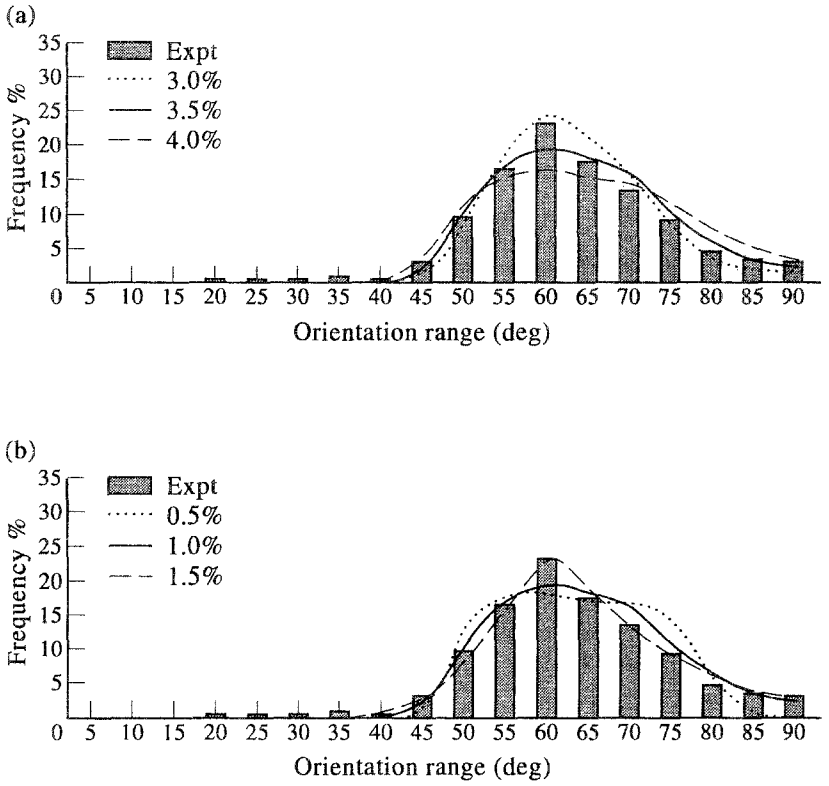

Fig. 5. Effect of varying model parameters on the predicted frequency distributions for a peak-to-peak applied stretch of 12%. (a) The mean axial strain threshold of the cell population is varied with a fixed standard deviation of 1%. (b) The standard deviation is varied with a fixed mean axial strain threshold of 3.5%.

distributions. Specifically, decreasing the axial strain threshold mean from 3.5 to 3.0% caused an increase in cell frequency near 60°, the zero axial strain direction [Fig. 5(a)]. Decreasing the standard deviation from 1.0 to 0.5% caused the distribution to become more uniform (flatter), while increasing the standard deviation to 1.5% created a more peaked distribution [Fig. 5(b)].

DISCUSSION

This study supports the hypothesis that cell reorientation is a response to excessive cell length change (i.e. peak-to-peak axial strain). The hypothesis that cells reoriented to avoid stretch was first proposed by Buck (1988), who suggested that the cell reorientation is "... an avoidance reaction to stretching based on cells' adhesion by linear focal contacts, which run parallel to the long axis of the cells, and which are associated with microfilaments."

In this study, we did not address the pathways by which axial strain is sensed by the cell. Several potential pathways exist, including transmission of the substrate strain to the cytoskeleton via integrin receptors and activation/or inhibition of membrane channels (Ingber, 1991; Sumpio and Banes, 1988; Wang *et al.*, 1993). Several investigators have shown that cytoskeleton reorganization begins prior to cell reorientation and that drugs, like forskolin and cytochalasin B, which disrupt the actin cytoskeleton, also inhibit the orientation response (Iba and Sumpio, 1991; Pender and McCullogh, 1991; Shirinsky *et al.*, 1989). However, further studies are required to find out if the cytoskeleton reorganization results directly from the deformation transmitted via integrins or by a second messenger, like Ca^{2+}, which enters the cytoplasm via membrane channels.

The proposed cell model can account for a variety of previously observed cell behavior. For example, Dartsch *et al.* (1986) reported that a stretch threshold existed for arterial smooth muscle cells. Below 2% stretch, no orientation was found. In the present model, over 93% of the cells had thresholds above 2% peak-to-peak axial strain. The small number of cells with low thresholds makes it unlikely that an orientation response could be detected at low stretch levels. At higher stretch levels, Neidlinger-Wilke *et al.* (submitted) noted that few fibroblasts were found anywhere where the axial strains were above ~ 4.5%. Osteoblasts tolerated larger axial strains of about 6.5%. In the present model for melanocytes, only 2.3% of the cells had strain thresholds above 5.5%.

Cyclic substrate deformations are known to produce a variety of cell responses besides cell reorientation. The cell responses include altered proliferation (Banes *et al.*, 1985; Brighton *et al.*, 1991; Buckley *et al.*, 1988; Neidlinger-Wilke *et al.*, 1994), altered mRNA levels, and

synthesis of matrix proteins (Carver *et al.*, 1991; Leung *et al.*, 1976, 1977) and degradative enzymes (Lambert *et al.*, 1992). These cell responses depend on the amount of the substrate deformation, and therefore are altered by cell reorientation that changes the cell's deformation. Further, even without reorientation, the substrate deformation sensed by each cell will depend on the cell's orientation. These facts make it difficult for an experimenter to control the mechanical signals delivered to the cells. There are two ways to avoid this problem the first is to apply a uniform biaxial strain to the substrate (Schaffer *et al.*, 1994). Here all cells see the same deformation, independent of their orientation. A second potential approach is use contact guidance (i.e. cell orientation is determined by substrate surface geometry) to align the cells in the desired direction. It has been shown that a variety of cell types align along the direction of grooves fabricated on the substrate surface (Dow *et al.*, 1987). It has yet to be shown if cell orientation will be maintained by contact guidance in the presence of substrate deformation.

We used the Green's strains to describe substrate deformations. This was done both to provide an accurate description of large substrate deformations and to allow the use of orthonormal tensorial coordinate transformations to compute the orientation dependence of the strains. We found that for the maximum stretch of 12% we employed in our experiments, the use of Green's large strain definition, as opposed to Cauchy's infinitesimal strain definition, had only a small influence on the model predictions.

One underlying assumption in the cell model was that cells reorient in response to the p–p strain magnitude along their major axes. Thus the direction of the substrate strain is not important. This is reflected in Fig. 2, which shows only positive strains. For a 12% applied stretch, the actual substrate strain is positive from 0 to 60°, and negative from 60 to 90°. The successful experimental validation of the model supports the hypothesis that the cells respond to the p–p strain magnitude and not to the strain direction. Recent experiments also confirm this hypothesis (Wang *et al.*, 1995).

The proposed cell model was tested using data on human melanocytes. These cells were selected because they have a high length to width ratio. This made it easier to identify the orientation of individual cells and helped insure the assumption that transverse and shear strains could be neglected. Most cell types, e.g. fibroblasts, osteoblasts, and endothelial cells, are not as highly elongated and are more two-dimensional in shape. It is possible, because of their shape, that these cell types might also be responsive to the transverse and shear strains. However, we expect the two dimensional effects would be small if the cells are primarily bipolar in shape.

Another assumption used in our model was that cells respond independently to the substrate strain. This required that we compare model predictions to experiments where the cells were plated and grown at low density to reduce cell–cell contact. This is because, at high cell densities, locally oriented groups of cells develop without any stretch stimulus. Consequently, the orientation response of a high density cells will depend on both the substrate strains and cell–cell communication.

We proposed a model that describes how individual cells within a cell population respond to cyclic substrate strains. The resulting model, which had two free parameters, could accurately predict the response of human melanocytes grown on cyclically stretched rectangular culture dishes. We concluded that the cell orientation response is the cell's attempt to reduce its axial deformation to the level below its axial strain threshold.

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