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BIOPHYSICS OF COMPLEX SYSTEMS

Mathematical Modeling of the Stretching-Induced Elongation of the Embryonic Epithelium Layer in the Absence of an External Load

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Abstract—The problem of deformation of a planar embryonic epithelium layer that is unloaded after a short period of uniaxial stretching with subsequent fixation in the stretched state for different periods of time is solved. The initial conditions for solving this problem are derived from the previously discussed problem of the uniform stretching of a tissue fragment (explant) with subsequent fixation of the obtained length. In this study we used the previously developed continuum model that describes the stress–strain state of epithelial tissue taking the parameters that characterize the shape of the cells and their stress state into account, as well as the active stresses they exert when they interact with each other. The experimentally observed continuation of the deformation of a stretched tissue after the external force has ceased to act is described theoretically as a result of active cell reactions to mechanical stress. The duration of explant fixation is shown to have a strong effect on its further elongation and on the pattern of cell activity.

Keywords: cell systems, active media, embryonic epithelium

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INTRODUCTION

Rearrangements of cells in cell layers (their relative migrations with the reorganization of cell contacts and changes of neighbors) in response to active stresses in tissue play an important role in early embryonic development and form the basis of morphogenesis of all of the axial organs in vertebrates [1].

Cell rearrangements can be driven by two physically different mechanisms. On the one hand, external mechanical forces can rupture the existing cell contacts and induce the generation of new contacts following the relative repositioning of cells and the formation of a new configuration. In a way, this process is similar to the viscous flow of a material: tissue stretching occurs by relocation of some cells relative to others due to an external force. This process may be termed passive rearrangement.

On the other hand, at a certain stage of embryonic development, cells of the embryonic tissue acquire the ability to change their neighbors by developing active stresses. In epithelial layers, such stresses are usually created by lamellipodia, which are constantly occurring and disappearing projections of a cell. Contraction of lamellipodia attached to the surface of neighboring cells causes some cells to intercalate between others, which is accompanied by the reorganization of intercellular contacts. Such active cell repackaging is conventionally termed intercalation. The important difference between this process and passive rearrangements is that, as a result of intercalation, a tissue fragment can continue its deformation even after the external force has been removed. Such a behavior of embryonic tissues makes their rheological properties fundamentally different from those of a classical continuous media.

Several studies have experimentally investigated active reactions that were triggered in embryonic cells by explant stretching with subsequent fixation in the stretched state (see, for example, [2, 3]).

A relatively small number of works have considered continuum models that describe the mechanical behavior of cell layers [4–7]. In [4–6], the development of active stresses was not bound to the stress–strain state of the tissue at the cellular level, which made it necessary to introduce a homeostatic stress state that was described with an empirical equation that lacked a clear physical meaning [4, 5] or a specified direction in which active stresses develop [6]. These shortcomings were overcome in [7], which proposed a continuum model that describes the stress–strain state of a flat layer of epithelial cells taking the parameters that characterize both the shape of the

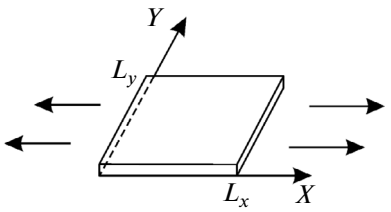

Fig. 1. The scheme of the experiment: the arrows indicate the directions of explant stretching.

cells and their stress state into account, as well as the active stresses that arise during the cell interactions.

The model that was proposed in [7] provided qualitative and quantitative agreement with the experimental data (according to the order of magnitude of the experimentally estimated values). In particular, it confirmed the experimentally observed phenomenon of continuing explant elongation after stretching has ceased [2]; however, further behavior of the explant after removal of the external stretching load was not analyzed. In this work, we consider the problem of the deformations of a flat layer of embryonic epithelium that is unloaded following uniaxial stretching and fixation of the stretched state for different periods of time. It was shown that the period of fixation in the stretched state has a significant effect on the magnitude of the fragment elongation that is induced by stretching.

SETTING THE PROBLEM OF THE UNIAXIAL STRETCHING OF A CELL LAYER

The problem of the uniaxial stretching of an embryonic tissue layer was solved using the general equations that were proposed in [7]. The physical properties of the two-dimensional medium were assumed to be isotropic within the plane of the layer. At the initial moment, the layer occupies the $[0; L_x] \times [0; L_y]$ area in the viewer-centered system of coordinates (Fig. 1).

Stretching is assumed to be caused by surface forces that are applied at the moment $t = 0$ to the lateral surfaces $x = 0$ and $x = L_x$ along the X axis in such a manner that the left boundary of the layer remains fixed in the selected system of coordinates. The other boundaries are not loaded. Surface forces at the ends of the layer are assumed to be independent of the Y coordinate.

The equilibrium equations with the boundary conditions admit a solution that gives the following distribution of stresses within the layer: $\sigma_{11} = \sigma(t)$, $\sigma_{12} = \sigma_{22} = 0$, i.e., the distribution of stresses is spatially uniform and the axial stress is determined by the boundary conditions. Consequently, all of the variables in the equations below (except for velocities) will also be spatially uniform; thus, the equations for the compatibility of strain rates will be satisfied.

The principal equations of the continuum model [7], which describes the behavior of a flat layer of epithelial cells taking active deformations and cell rearrangements in the course of uniaxial stretching into account, are provided below without argument or discussion. The system of equations comprises the kinematic law of general medium deformation in the differential form for finite strains, equations that describe the force interactions in the medium, and relationships that define different components of the medium strain tensor and the strain rate tensor, as well as equations that describe time-dependant changes in active stresses. Taking the fact into account that the defining relationships are isotropic and the fact that for the postulated boundary conditions the system of coordinates that was chosen is the main system of coordinates for the cell strain tensor, we can write the complete system of equations as follows:

$$
\begin{gathered}
e = \frac{D\varepsilon^{(e)}}{Dt} + \frac{D\varepsilon^{(e)}}{Dt} + e^{(\mathrm{int})}, \quad e_y = \frac{D\varepsilon_y^{(e)}}{Dt} + \frac{D\varepsilon_y^{(a)}}{Dt} + e_y^{(\mathrm{int})}, \\
\sigma = \sigma^{(c)} + \tau, \quad \sigma_y^{(c)} + \tau_y = 0, \\
\varepsilon^{(c)} = \frac{1}{E}(\sigma^{(c)} - \nu\sigma_y^{(c)}), \quad \varepsilon_y^{(c)} = \frac{1}{E}(\sigma_y^{(c)} - \nu\sigma^{(c)}), \\
\frac{D\varepsilon^{(a)}}{DT} = \frac{1}{T_1}(k(\sigma^{(c)} - \nu_1\sigma_y^{(c)}) - \varepsilon^{(a)}), \\
\frac{D\varepsilon_y^{(a)}}{DT} = \frac{1}{T_1}(k(\sigma_y^{(c)} - \nu_1\sigma^{(c)}) - \varepsilon_y^{(a)}), \\
e^{(\mathrm{int})} = -G(\tau - \nu_2\tau_y), \quad e_y^{(\mathrm{int})} = -G(\tau_y - \nu_2\tau), \\
\frac{D_J\tau}{Dt} = m\frac{1 - 2\varepsilon^{(c)}}{\sqrt{(1 - 2\varepsilon^{(c)})(1 - 2\varepsilon_y^{(c)})}} - \frac{1}{T_2}\tau, \\
\frac{D_J\tau_y}{Dt} = m\frac{1 - 2\varepsilon_y^{(c)}}{\sqrt{(1 - 2\varepsilon^{(c)})(1 - 2\varepsilon_y^{(c)})}} - \frac{1}{T_2}\tau_y, \\
\varepsilon^{(c)} = \varepsilon^{(e)} + \varepsilon^{(a)}, \quad \varepsilon_y^{(c)} = \varepsilon_y^{(e)} + \varepsilon_y^{(a)}, \\
\frac{\partial\sigma}{\partial x} = 0, \quad e = \frac{\partial u}{\partial x}, \quad e_y = \frac{\partial u_y}{\partial y}.
\end{gathered}
\tag{1}
$$

In the proposed setting of the problem, the mixed components of the tensors are equal to zero. Notations without indices will be used for axial components of the tensors (along the X axis), whereas their normal components (along the Y axis) will be designated with lower y index.

Here, e and e_y are the components of the strain rate tensor of medium, $e^{(\mathrm{int})}$ and $e_y^{(\mathrm{int})}$ are the components of the strain rate tensor defined by cell rearrangements resulting from intercalations (active cell motion), $\varepsilon^{(e)}$

and $\varepsilon_y^{(e)}$ are the components of the tensor of elastic strains of the medium as determined by elastic deformation of the cells that the medium is composed of; $\varepsilon^{(a)}$ and $\varepsilon_y^{(a)}$ are the components of the tensor of the active strains of the medium associated with changes in the unloaded state of cells due to the rearrangement of the cytoskeleton and the cell membrane; σ is the axial (the only nonzero) component of the tensor of the full stresses in the medium; $\sigma^{(c)}$ and $\sigma_y^{(c)}$ are the components of the tensor of cell stresses (transmitted via contacts of neighboring cells); τ and τ_y are the components of the tensor of active stresses produced by cytoskeleton contractions in lamellipodia, D/Dt is the Oldroyd lower derivative, and D_J/Dt is the Jaumann upper derivative [8].

To satisfy the condition of two-dimensional incompressibility of the medium as a whole, let $\nu = \nu_1 = \nu_2 = 1$. However, incompressibility is not assumed for cellular and intercalational deformations that are considered individually.

SOLVING THE PROBLEM OF A CELL LAYER THAT IS SUBJECTED TO CONTINUOUS UNIAXIAL STRETCHING WITH SUBSEQUENT REMOVAL OF THE EXTERNAL LOAD

Let us consider the problem of the changes of the length of a tissue fragment after the removal of the external mechanical force, which depends on its duration.

The solution of the general modeling problem is composed of the solutions of several problems that correspond to different stages of the experiment. The first step is solving the problem on the specimen stretching along the X axis with subsequent fixation of its length for different periods of time.

Let us consider the modeling problem, which will enable us to describe the experimental technique in a more adequate manner than that proposed in [7]. The requirement to maintain the integrity of the explant forbids instant stretching that would induce deformations that are too strong; therefore, considerable stretching is attained by moderate stepwise elongations (e.g., within a period of 30 min with 5-min intervals between subsequent elongations) [2, 3]. This gradual stepwise stretching is naturally described as a continuous process. After the stretching has stopped, the explant is fixed in the stretched state for different periods of time.

We assume that a tissue fragment with the initial length L_x is exposed to continuous stretching along the X axis that is induced by moving its boundary $x = L_x$ with a constant velocity u_0 for a period of T and that its length is subsequently fixed for the period of time $T < t < T_f$. At the same time, the boundary $x = 0$ remains immobile.

Due to the spatial uniformity of strain rates, their axial component will be described with the following expression: $e(t) = u_0/(L_x + u_0 t)$ for $t < T$. For $T < t < T_f$, the strain rate is equal to zero: $e(t) = 0$. The condition of uniformity of the strain rates together with the condition of immobility of the $x = 0$ boundary imply that the axial component of the medium relocation velocity u depends only on time and on the X coordinate, while the normal component u_y depends only on time and the Y coordinate. Due to the uniformity of active stresses, the relative derivative D_J/Dt becomes a partial derivative. Taking the fact into account that the mixed components of the elastic strain tensor are equal to zero, we obtain:

$$\frac{D\varepsilon^{(e)}}{dt} = \frac{\partial\varepsilon^{(e)}}{\partial t} + 2\varepsilon^{(e)}e(t).$$

Let us introduce the following dimensionless values:

$$\sigma^* = \frac{\sigma}{mT_2},\ \Delta\tau^* = \frac{\Delta\tau}{mT_2},\ t^* = \frac{t}{T_2},$$

$$T^* = \frac{T_1}{T_2},\ k^* = kE,$$

$$G^* = Gm(T_2)^2,\ E^* = \frac{E}{mT_2},\ u_0^* = u_0T_2/L_x,$$

$$e^* = T_2e,$$

where $\Delta\tau = \tau_y - \tau$, and T_1 and T_2 are the characteristic times of development of active cell deformations (rearrangements of internal cell structures and cell membrane in response to an external force) and of active stresses (formation, attachment, and contraction of lamellipodia), respectively.

Following transformations, system (1) can be presented as a system of three equations in three unknowns σ, $\Delta\tau$, $\varepsilon^{(c)}$:

$$\frac{\partial\sigma^*}{\partial t^*} = -\left(\frac{1}{T^*}(k^* + 1) + 2e^*(t^*)\right)\sigma^* + \left(\frac{E^*}{T^*} - \frac{4}{\sqrt{1 - 4(\varepsilon^{(c)})^2}}\right)\varepsilon^{(c)}$$

$$-\left(\frac{k^* + 1}{T^*} + G^*E^* - 1 + 2e^*(t^*)\right)\Delta\tau^* + E^*e^*(t^*), \quad (2)$$

$$\frac{\partial\Delta\tau^*}{\partial t^*} = \frac{4\varepsilon^{(c)}}{\sqrt{1 - 4(\varepsilon^c)^2}} - \Delta\tau^*,$$

$$\frac{\partial\varepsilon^c}{\partial t^*} = e^*(t)(1 - 2\varepsilon^{(c)}) - G^*\Delta\tau^*.$$

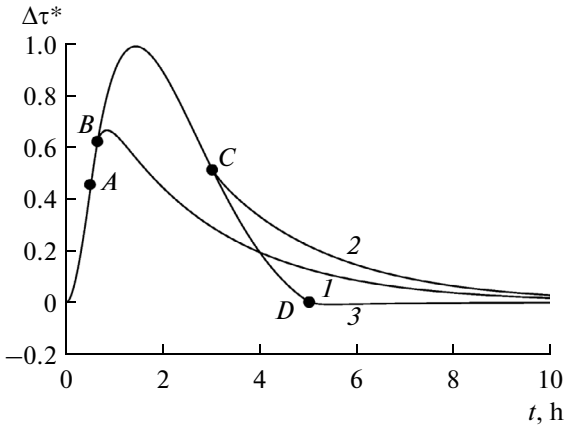

Fig. 2. The evolution of $\Delta\tau^* = \tau_y^*$ for different periods of fixation in the stretched state: *1*, $T_f = 40$ min; *2*, $T_f = 3$ h; *3*, $T_f = 5$ h; point *A* corresponds to the moment when the stretching was discontinued; *B*, *C*, and *D* are the release moments for each curve.

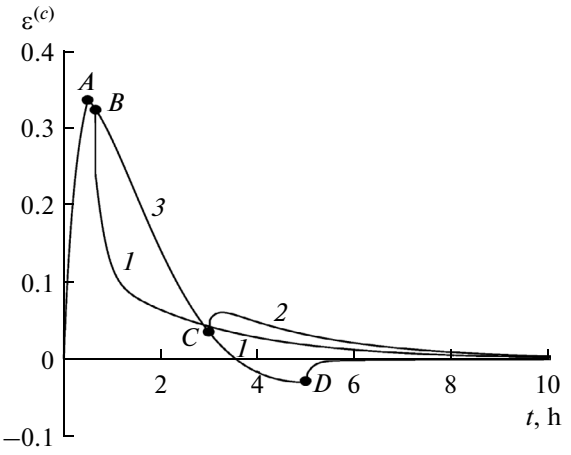

Fig. 3. The evolution of the cell strain $\varepsilon^{(c)}$ for different periods of fixation in the stretched state. Notation as in Fig. 2.

Here $e^*(t^*) = \dfrac{u_0^*}{1 + u_0^* t^*}$ for $t^* < T/T_2$, and $e^*(t^*) = 0$ for $T/T_2 \le t^* \le T_f^*$, where T_f^* is the dimensionless moment at which the external force that maintains the explant in the stretched state ceased to act.

The initial conditions for system (2) at $t^* = 0$ were as follows: $\sigma^* = 0$, $\Delta\tau^* = 0$, and $\varepsilon^{(c)} = 0$.

The next stage can be described by the solution of the problem of the deformation of the explant that occurs in the absence of external forces following continuous stretching and subsequent length fixation for a certain period of time. Taking the spatial uniformity of the strain rates into account, we obtain that $e = \partial u/\partial x = d\ln L/dt$, where $L = L(t)$ is the fragment length at the moment t. Then, the deformation of the unloaded explant can be described with equations that were obtained from (2) by substituting $\sigma^* = 0$ and $e^*(t^*) = d\ln L^*/dt^*$, where $L^* = L/L_x$. As a result, we obtain the following system of three equations to find the unknowns L^*, $\Delta\tau^*$, and $\varepsilon^{(c)}$:

$$\begin{aligned}(E^* - 2\Delta\tau^*)\frac{d\ln L^*}{dt^*} &= -\left(\frac{E^*}{T^*} - \frac{4}{\sqrt{1 - 4(\varepsilon^{(c)})^2}}\right)\varepsilon^{(c)} \\ &+ \left(\frac{k^* + 1}{T^*} + G^* E^* - 1\right)\Delta\tau^*, \\ \frac{\partial\Delta\tau^*}{\partial t^*} &= \frac{4\varepsilon^{(c)}}{\sqrt{1 - 4(\varepsilon^{(c)})^2}} - \Delta\tau^*, \\ \frac{\partial\varepsilon^{(c)}}{\partial t^*} &= \frac{d\ln L^*}{dt}(1 - 2\varepsilon^{(c)}) - G^*\Delta\tau^*.\end{aligned} \tag{3}$$

The values that were obtained in the solution of (2) for the moment $t^* = T_f^*$ were used as the initial conditions for solving system (3). It is also necessary to account for instant changes in elastic strain and the explant length after removal of the external load. Under the assumption that the elastic deformations at the moment of unloading are small, we can write the initial conditions as $L^* = L^*(t^*)(1 - \varepsilon^{(e)}(t^*))\big|_{t^* = T_f^*}$ (since under this assumption $\dfrac{L(T_f^*) - L}{L(T_f^*)} = \varepsilon^{(e)}$, where $L(T_f^*)$ is the fragment length that is fixed at the end of the stretching stage and L is it length after unloading) and $\Delta\tau^* = \Delta\tau^*\big|_{t^* = T_f^*}$, $\varepsilon^{(c)} = (\varepsilon^{(c)} - \sigma^*/E^*)\big|_{t^* = T_f^*}$.

Based on the estimates that were obtained in [3], in our previous work [7] we chose the following values for the characteristic times of the development of active cell strains and active stresses: $T_1 = 10$ min, $T_2 = 1$ h. According to the results that were described in [9], the modulus of elasticity was taken for $E = 3$ kPa for a 30-μm-thick layer (in fact, it is only the product of these values that is of importance [7, 9]).

Direct estimates for m, G, and k from experimental data are unavailable. Based on the results that described in [7], we will assume $m = 0.15$ kPa h^{-1}, $G = 1$ kPa^{-1} h^{-1}, $k = 0.6$ kPa^{-1}.

The calculations were performed using the following values for the explant size and the setting of the stretching experiment: $L_x = 0.6$ mm, $u_0 = 0.96$ mm h^{-1}, and $t = 0.5$ h. For these values, stretching resulted in fragment elongation by 80%.

The calculations were performed using the fourth-order Runge–Kutta method.

RESULTS

The results of our computations are presented in Figs. 2–4. Each figure shows the evolution of one of the principal parameters that characterize the system. The period under analysis starts from the moment when the stretching begins and includes the stages of stretching, fixation (fragment length does not change), and subsequent evolution after the removal of the stretching load. We analyzed the following parameters: the effective active stress $\Delta\tau^* = \tau_y^* - \tau^*$ (Fig. 2), the cell strain $\varepsilon^{(c)}$, which describes changes in the cell shape and is equal to zero when the cell shape reverts to normal (Fig. 3), and the logarithm of the ratio of the current explant length to its initial length, $\ln(L/L_x)$ (Fig. 4). For each of these parameters, its evolution is shown for three different periods of fixation: T_f = 40 min, 3 and 5 h. In all of the charts, the point *A* corresponds to the moment when stretching was discontinued. Obviously, until the moment $t = 40$ min (point *B*, whose abscissa is close to that of point *A*), the behavior of each parameter is described with a unique curve; at $t = 40$ min (point *B*), branch *1*, which corresponds to the behavior of the explant that is released at this moment, separates, and at $t = 3$ h (point *C*), the curve splits into branch *2* (representing the explant that is released at this point of time) and branch *3* (the explant released at $t = 5$ h). At $t = 5$ h, (point *D*), the behavior of branch *3* exhibits a qualitative change.

Modeling of the observed explant reactions to unloading after different periods of fixation in the stretched state showed that after short-termed stretching (T_f = 40 min), active stresses at the moment of unloading were relatively weak (Fig. 2, point *B*), while the cells were strongly elongated along the stretching axis (Fig. 3, point *B*). After unloading, the cells gradually regained their initial isotropic shape ($\varepsilon^{(c)}$ tends to zero) due to continuing intercalating deformations (Fig. 3, branch *1*). During this time, the active stresses diminished considerably (Fig. 2, branch *1*) and cell rearrangements weakened. After unloading, the fragment length dropped sharply due to the immediate decrease in the axial elastic strain and slightly increased subsequently (Fig. 4, branch *1*). If the external force acted for an even shorter time, the fragment length decreased nearly to the initial values.

If a specimen was fixed in the stretched state for a longer period of time (T_f = 3 h), active stresses that existed at the moment of unloading could be higher than or similar to those that were observed in the previous case (Fig. 2, point *C*). However, cell deformations were much less pronounced than in the previous case (Fig. 3, point *C*) due to well-developed intercalation deformations. Further evolution of intercalations resulted primarily in an increase of the fragment length (Fig. 4, branch *2*) rather than in the cell shape reversal to the initial isotropic form. Immediately after unloading, the specimen length increased slightly, because by this moment the intercalations had generated a contracting axial stress ($\sigma < 0$).

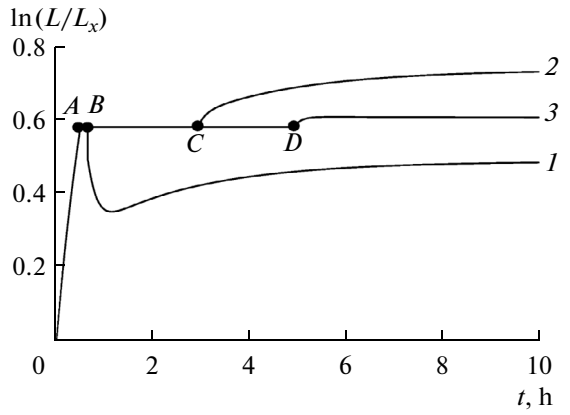

Fig. 4. The evolution of $\ln(L/L_x)$ for different periods of fixation in the stretched state. Notation as in Fig. 2.

In the cell layer that had been loaded for T_f = 5 h, active stresses (and accordingly, the strain rate of intercalations and the change of the fragment length) were weakened (Figs. 2 and 4, branch *3*). A very slight increase in the fragment length was due to the fact that the cell shape, which was initially oblate along the stretching axis ($\varepsilon^{(c)} < 0$), was restored to isotropic (Fig. 3, branch *3*). The contraction of cells along the stretching axis results from the hyper-restoration reaction [10], which was discussed in more detail in [7].

CONCLUSIONS

The obtained solution of the model problem demonstrates that the rheological properties of embryonic epithelium are different from those of a conventional continuum media in that the deformation of tissue continues in the absence of an external force. This phenomenon is determined by active cell reactions that arise in response to a mechanical force.

Active stresses that are triggered during stretching are maintained for a certain period of time after the mechanical force that was applied to the explant has ceased to act. As a result, the strain rate of intercalations remains nonzero and induces a continuation of weakening elongation of the stretched fragment after the removal of the external load. The behavior of an explant that is released from a stretching force essentially depends on the period of time that the tissue remained in the stretched state.

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