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Wound Healing Mechanism in Epithelial Tissues Cell Adhesion to Basal Lamina

TATSUZO NAGAI and HISAO HONDA*
Kyushu Kyoritsu University
Kitakyushu, Fukuoka 807-8585
JAPAN
*Hyogo University
Kakogawa, Hyogo 675-0195
JAPAN

Abstract: - Mechanism of wound healing was explored in epithelial tissues (a 2D monolayered cell sheet). The wound means in this paper an area left after removing some cells from a normal tissue. Computer simulations were carried out applying the vertex dynamics cell model (T. Nagai and H. Honda, Phil. Mag. B, Vol. 81, No. 7, 2001, pp. 699-719.) with a certain modification. As a result, it was concluded that the driving force of wound healing is an adhesion to basal lamina acting on marginal cells minimizing cell-basal lamina interfacial free energy. This force governed the wound healing process competing with central forces of epithelial morphogenesis, i.e. cell-cell interfacial free energy and cellular elastic energy. Our simulations led to a picture that the wound healing is cellular erosion of wound. The process proceeded with parallel displacement of wound margin followed by intermittent decrease in the edge number of polygonal wound. The peripheral cells flowed into the wound from its side to reduce it approximately keeping its shape as if a water flow makes a land shape of erosion. The area of the wound decreased exponentially.

Key-Words: - Wound Healing Epithelial Cell Adhesion Basal Lamina Simulation Morphogenesis Erosion

1 Introduction

The spreading of epithelial cell sheets plays a main role of morphogenesis in animal embryos. This fundamental movement has been explored by observing wound healing [1]. Some important experiments elucidated the mechanism of wound healing at the molecular level, but the whole process of wound healing is poorly understood. The whole process can be grasped by investigating it at the cellular level including a wound area (exposed basal lamina), the marginal cell assembly and the surrounding fluid medium. However such study is little done and therefore that is the purpose of this paper. Throughout this paper the term, wound will be used in the meaning of an area left after removing some cells from a normal tissue.

Wound healing experiments important for our purpose are as follows. The attachment of rat hepatocytes to a serum-pretreated culture dish was observed by scanning electron microscopy [2], [3]. The resulting pictures showed that the cellular attachment to dish resembled liquid wetting to solid surface. That is, the cell with spherical initial shape spread radially and by 5 h after wounding became large enough and flattened on the culture dish. The spreading of epithelial cells in tadpole fins was observed in vivo during wound healing and recorded with a 16-mm film [4]. About 10 cells were removed from a normal tissue to make the initial wound. A series of time-lapse pictures obtained showed a detail of the wound closure by marginal cells and convinced us that the wound gradually contracted

approximately keeping its initial shape. The wound healing in cat corneal endothelia was observed in vivo during 100 days where about 180 cells were removed initially [5]. It was found that the marginal cell assembly flowed into the wound to cover it like 2D fluids. Those cell movements were interpreted as a result of the contractility of actin filaments contained in each cell. The stress-strain curves of the cytoskeletons of vertebrate cells, i.e. actin filaments, microtubles and intermediate filaments, were obtained in vitro by measuring the degree of deformation in each filament with the rheologic methods [6]. Those curves showed that the three filaments had their respective elastic regions and that the actin filaments among three had the least strain under an equal stress. This fact results in that the actin filaments play the leading part of maintaining cellular shape.

We proposed a model to describe morphogenisis of normal epithelial tissues (a 2D monolayered cell sheet) which will be called vertex dynamics cell model in the following [7]. This model expresses a cell by a prism and then describes the whole system by an assembly of vertices. These vertex movements are driven by thermodynamic force to minimize the total free energy of the system and then relaxed by viscous force. Our computer simulations by the model led to that the system formed the honeycomb like pattern seen universally in epithelial tissues even though starting with various intitial states.

In this paper we have apllied the vertex dynamics cell model to the wound healing with a certain modification. Firstly we examined as candidates for wound healing mechanism the following three, cellular elastic energy, cell-wound interfacial energy and cell-basal lamina interfacial energy. As a result, we concluded that the driving force in wound healing was an adhesion of cell to the basal lamina which intends to minimize the cell-basal lamina interfacial energy. In this paper we will follow this result to modify the vertex dynamics cell model and show our results obtained by computer simulations.

2 Modified Vertex Dynamics Cell Model for Wound Healing

In this section we extend the vertex dynamics cell model to treat the wound healing.

Curved faces of a real cell are coarse-grained in the model with an infinitesimal length Δ, which is assumed to be much shorter than the mean cell diameter, and then approximated by planes. As a result, each cell is expressed by a prism and our cellular system is described in terms of a set of 2D positional vectors of cell vertices $\{ \boldsymbol{r}_i \}$ and a set of cell heights $\{ h_i \}$.

2.1 Equations of Motion for Vertices

In order to describe motion of the system we use purely dissipative equations of motion that have been applied well to soap froth, emulsion and polycrystalline grains [8]. They are for vertices, assuming balance between the dissipative force and the potential force, given by

$$\eta \frac{\mathrm{d}\boldsymbol{r}_i}{\mathrm{d}t} = -\frac{\partial}{\partial \boldsymbol{r}_i} U \tag{1}$$

where U denotes the total free energy of the system assumed to be, in the case of wound healing,

$$U = U_{\mathrm{I}} + U_{\mathrm{D}} + U_{\mathrm{B}} \tag{2}$$

Here U_{I}, U_{D} and U_{B} are interfacial energies of cell-cell and cell-wound boundaries, deformaion energies of cells and cell-basal lamina interfacial energies, respectively. U_{I} is, defining $\sigma_{<ij>}$ as a free energy density of straight boundary $< ij >$, given in the form

$$U_{\mathrm{I}} = \sum_{<ij>} \sigma_{<ij>} r_{ij} \tag{3}$$

where $\sigma_{<ij>} = \sigma$ for a cell-cell boundary $< ij >$ and $\sigma_{<ij>} = \sigma^{\mathrm{F}}$ for a cell-wound boundary $< ij >$, and $r_{ij} \equiv |\boldsymbol{r}_i - \boldsymbol{r}_j|$. Denoting the area and its equlibrium value of cell α by S_α and S_α^0, respectively, U_{D} is

$$U_{\mathrm{D}} = \sum_{\alpha}^{(\mathrm{cell})} \rho (h^0)^2 (S_\alpha - S_\alpha^0)^2 \tag{4}$$

where ρ is a positive constant and h^0 the equilibrium cell height, assuming $h_i = h^0$. The equilibrium value of cell area S_α^0 is taken to be the mean value over cell α itself and its surrounding n_α^{C} cells, as called the local equilibrium dynamics in [7]. That is,

$$S_\alpha^0 = \frac{1}{1 + n_\alpha^{\mathrm{C}}} (S_\alpha + \sum_{\beta=1}^{n_\alpha^{\mathrm{C}}} S_\beta) \tag{5}$$

In Sec.1, we referred to the observation that a rat hepatocyte spreads over the bottom of a serum-pretreated culture dish just as wetting of liquids on a solid surface. In the wetting phenomena liquids cover the solid surface to minimize its interfacial energy. From this analogy we consider that cells cover the wound area, i.e. the exposed basal lamina, to minimize the cell-basal lamina interfacial energy. Then $U_{\rm B}$ can be written as, denoting the area of wound γ by $S_\gamma^{\rm F}$

$$U_{\rm B} = \sum_{\gamma}^{\rm (wound)} \sigma^{\rm B} S_\gamma^{\rm F} \tag{6}$$

where $\sigma^{\rm B}$ is a positive constant.

$U_{\rm B}$ gives rise to the driving force of wound healing. That is, if a wound γ is created the total free energy increases by $\sigma^{\rm B} S_\gamma^{\rm F}$. The surrounding cells intend to cancel the increment in the total free energy by covering the wound. However, their movements cause in general the increases in $U_{\rm I}$ and $U_{\rm D}$. Therefore the competition between the three energies, $U_{\rm B}, U_{\rm I}$ and $U_{\rm D}$ determines the speed of wound healing.

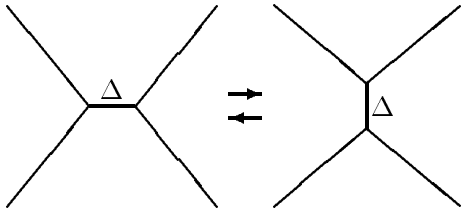
The explicit form of the force due to $U_{\rm B}$ acting on vertex i on the wound margin is , substituting Eq. (6) into Eq. (1)

$$\boldsymbol{f}_i^{\rm B} = \frac{\sigma^{\rm B}}{2}[\hat{\boldsymbol{k}} \times \boldsymbol{r}_{j'j''}] \tag{7}$$

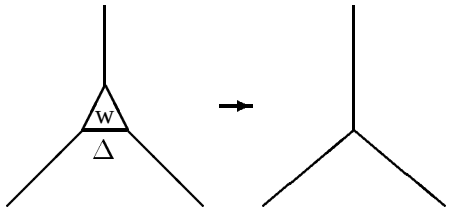
where $\hat{\boldsymbol{k}}$ is a unit vecter which is perpendicular to the paper and points from the back page to the surface page and $\boldsymbol{r}_{j'j''} \equiv \boldsymbol{r}_{j'} - \boldsymbol{r}_{j''}$. The two vertices, j' and j'' are nearest neighbors of vertex i on the wound margin. The order of (i, j', j'') is that in rotating anticlockwise within the wound along its margin. This force always points to the wound and proportional to $|\boldsymbol{r}_{j'j''}|$.

2.2 Elementary Processes for Topological Change

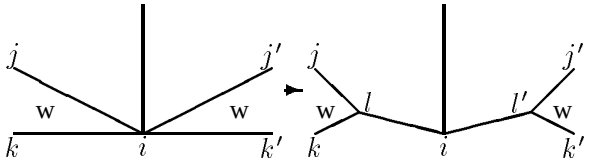
In addtion to the equations of motion Eq. (1) we introduce the following three elementary processes shown in Fig. 1, to describe the topological change of geometrical patterns composed of cell boundaries. 1) Switching (T1): Switch the connection between two neighbor vertices if they come close at a distance of Δ as shown in Fig. 1(a). 2) Wound annihilation (T2): Annihilate the wound if it is an triangle with edge Δ as shown in Fig. 1(b). 3) Adhesion (T3): Make the two cells adhere to each other if a vertex meets a wound margin on the other side within the same wound as shown in Fig. 1(c). The T2 and T3 are newly added to the elementary processes.



(a) Switching (T1)



(b) Wound annihilation (T2)



(c) Adhesion (T3)

Fig. 1: Elementary processes for topological change in wound healing.

3 Simulation

3.1 Dimensionless Quantities and Parameter Values

We make all quantities dimensionless to carry out computer simulation, using a characteristic length R_0 and a characteristic time τ_0 as new units, where $R_0 \equiv \sqrt{S_0}$ (S_0 the mean cell area at an initial state) and $\tau_0 \equiv \eta R_0/\sigma$. Furthermore, we define new dimensionless parameters as follows:

$$\tilde{\rho} \equiv \rho (h^0)^2 (R_0)^3/\sigma, \;\; \tilde{\sigma}^{\rm F} \equiv \sigma^{\rm F}/\sigma, \;\; \tilde{\sigma}^{\rm B} \equiv \sigma^{\rm B} R_0/\sigma \tag{8}$$

Here the new energy unit is taken to be the cell-cell interfacial energy per the new unit length, $\varepsilon_0 \equiv \sigma R_0$. Then the linear energy densities become

$$\begin{aligned} \tilde{\sigma}_{<ij>} \equiv \sigma_{<ij>}/\sigma = 1 \quad & \text{for cell-cell boundaries} \\ = \tilde{\sigma}^{\rm F} \quad & \text{for cell-wound boundaries} \end{aligned} \tag{9}$$

In the following we drop out for simplicity the symbol tilde on letters and use the original letters.

We adopt values of parameters as

$$\rho = 5.5, \ \ \sigma^{\mathrm{F}} = 0.5, \ \ \sigma^{\mathrm{B}} = 2.0 \qquad (10)$$

The value of ρ in Eq. (10) was obtained by our previous simulations without wound [7] and will work also in the present case with wound, because it denotes the elasticity of cell. The value of σ^{F} in Eq. (10) means that the linear energy density at cell-wound interfaces is a half of the one at cell-cell interfaces. This is because bundles of actin filaments exist only inside cells not wound. The value of σ^{B} in Eq. (10) was determined by the present simulations imposing two conditions, i.e. the wound healing finishes completely at the end and wound margins move in a uniform and smooth way. These are considered to be reasonable from results of real observations [3], [4]. The first condition gives the lower limit of σ^{B} while the second the upper limit of it. Its value of Eq. (10) is one of those. The values of above qunatities should be determined in the future from comparison with observation results according to the system of interest individually.

3.2 Initial Wound and Time Development

The initial wound for computer simulation is shown in Fig. 2(1) where a long and narrow white region is a wound with area 21.6 in the system with size 10×10. That was made by removing 19 cells from a normal equilibrium state with 100 cells obtained by our previous computer simulation of the vertex dynamics cell model [7]. Starting with this figure we solved Eq. (1) numerically using the Runge-Kutta-Gill method and obtained a set of vertex positional vectors $\{\boldsymbol{r}_i(t)\}$. We calculated each edge length at every time step of size $\delta t = 0.01$ and then compared it with $\Delta = 0.2$. If the edge length was longer than Δ we proceeded to the next step, while it was shorter than Δ we performed T1-process shown in Fig. 1(a).

As a result we have obtained a series of time development of wound healing shown in Fig. 2. With increasing time the wound contracts approximately keeping its long and narrow shape, as seen from Fig. 2. Two margins of wound met at a middle part at $t = 2.91$ as shown in Fig. 2(3) and then adhered to each other and divided into two wounds according to T3 process shown in Fig. 1(c). After that the two wounds contracted individually and then the upper wound disappeared at $t = 4.30$ as shown in Fig. 2(4) while the lower one disappeared at $t = 6.21$ as shown in Fig. 2(5).

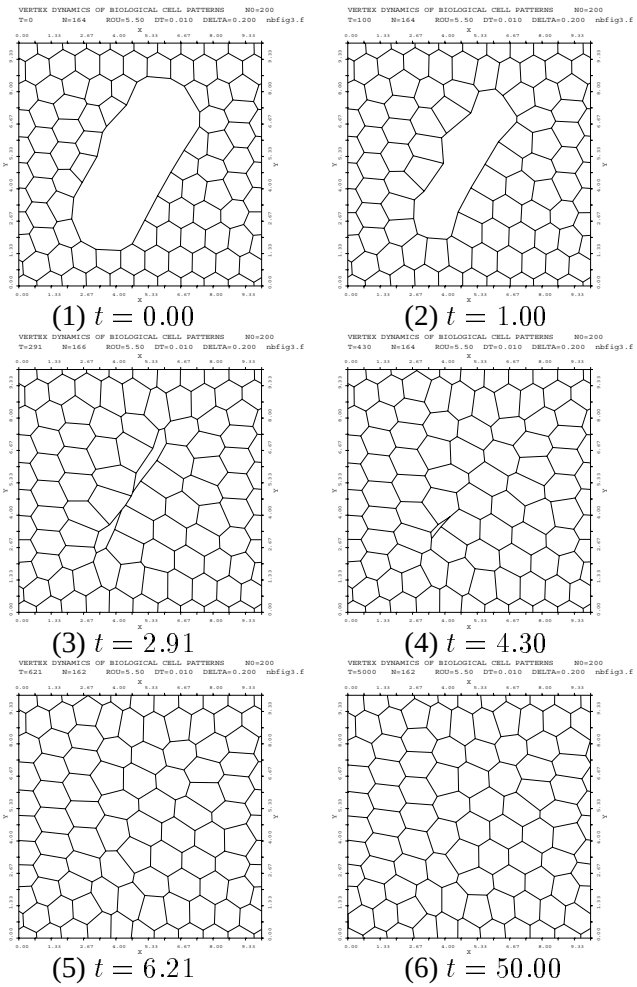


Fig. 2: Snapshots of wound healing.

Subsequently the system proceeded to the honeycomb like pattern in the same way as morphogenesis process. All vertices ceased moving at $t = 50.00$ shown in Fig. 2(6) and therefore it is the final pattern. From this figure we can see that the system has recovered in the sense of statistics the initial normal state from which the initial wound state shown in Fig. 2(1) was made, except 23% increase in mean cell area.

As seen from Fig. 2, the contraction of wound causes movements of the marginal cells. The marginal cells move into the wound and their displacements depend on the distance from the wound margin at t=0.00. The nearer to the wound cells are located, the longer their displacements become.The marginal cells elongate to

the wound at an initial stage and round at a late stage. The degree of deformations also depends on the cell position, just as the cell displacement. Such responses of the marginal cell assembly to wound healing were observed in cat corneal endothelia [5].

3.3 Characteristic Wound Contraction

Fig. 3 is a picture where instantaneous wound margines are superimposed in the snapshots of Fig. 2. They are plotted from the outside to the inside at equal time intervals of $\delta t = 0.5$.

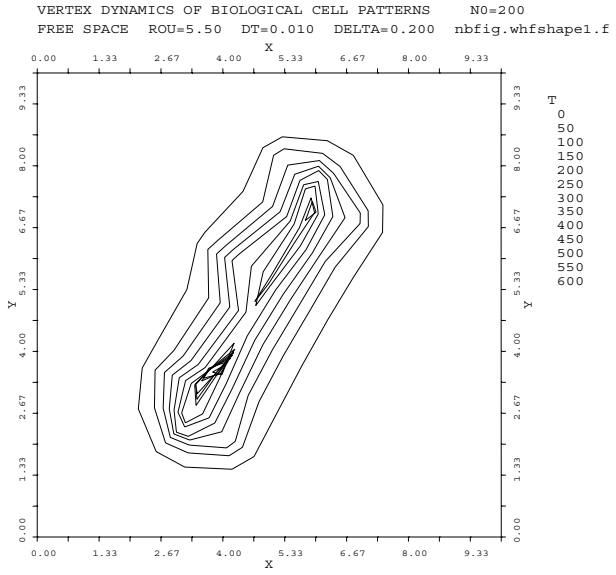


Fig. 3: Superimposed wound margins obtained by the present simulations: from the outside to the inside $t = 0.0 \rightarrow 6.0$, equal time interval $\delta t = 0.5$.

This figure shows that the edges of polygonal wound move with parallel displacements to contract the wound and then the shortest edge disappears to reduce the number of edges and so on. That is, the parallel displacements of wound edges and the reduction of the number of wound edges occur alternately. Fig. 3 reminds us about the formation of erosive land figure by water flows. Thus it is possible to say that the wound healing is an erosion process of wound by marginal cells and that the assembly of marginal cells gradually flows into and covers the wound from its sides just as a water flow.

This erosion process of wound by marginal cells is caused by the mechanism minimizing cell-basal lamina interfacial free energy. Fig. 3 strikingly resembles a trace figure of wound healing in the fin of *Xenopus laevis* tadpoles, Fig. 2 of [3] which was drawn from the observation [4]. This fact supports the above mechanism of wound healing.

The wound area obtained by our simulations was well fitted by the exponential function in the form

$$S^{\mathrm{F}}(t) = S^{\mathrm{F}}(0)\exp(-t/\tau_s) \tag{11}$$

where $\tau_s = 1.68$. Eq. (11) was applicable until $t \sim 0.7t_c$ with the adhesion time $t_c = 2.91$. As time approached t_c the wound area decreased more rapidely.

4 A Simple Picture of Wound Healing

In this section we consider a relatively large polygonal wound and assume marginal cells are identical within each wound edge, where cell-cell boundaries are perpendicular to the wound edge. The normal velocity of the i th vertex on the k th wound edge is given by, taking account of the symmetry of the configuration in Eq. (1),

$$\eta v_{in} = -\sigma - \rho(h^0)^2(\Delta S_k)2r_k + \frac{1}{2}\sigma^{\mathrm{B}}2r_k \tag{12}$$

where $\Delta S_k = S_k - S_k^0$ and r_k denotes the length of cell edge. All quantities are expressed by their original units in and after this section. The first two terms give inhibition of the wound contraction while the last term drives it. Now we notice the first term being constant and the last two terms proportional to the legth of cell edge r_k. The condition of complete wound healing is that v_{in} remains positive until $r_k = \Delta$. Therefore, we can neglect the first term in the case of complete wound healing and then simplify Eq. (12) as follows.

The normal velocity of the k th edge of polygonal wound in the direction of wound is written as

$$v_k = \mu^* r_k \tag{13}$$

where μ^* is a positive constant. We note in Eq. (13) that r_k is the length of cell edge but not the length of wound edge and so that all wound edges move with the same normal velocity if all marginal cell edges have an equal length. We can cosider that if the wound has several 100 of cells along its periphery r_k is equal to the mean length of cell edges.

Now we consider a regular polygonal wound with n edges and r edge length, where only one cell exists on one wound edge. The area of the wound S_n is then

$$S_n = \beta r^2, \quad \beta = \frac{n}{4}\cot(\frac{\pi}{n}) \tag{14}$$

Since Eq. (13) gives the normal velocity of edge $v = \mu^* r$ the rate of the area change is

$$\frac{\mathrm{d}S_n}{\mathrm{d}t} = -nrv = -\frac{n\mu^*}{\beta}S_n \qquad (15)$$

The solution to Eq. (15) is obtained in the form

$$S_n = S_n(0)\exp(-t/\tau_s), \quad \tau_s \equiv \frac{1}{4\mu^*}\cot(\frac{\pi}{n}) \quad (16)$$

where $S_n(0)$ is the initial area and τ_s gives a characteristic time needed for wound healing. Eq. (16) agrees with Eq. (11) obtained by our simulations. If we apply the above simple picture to the initial state of the simulation Fig. 2(1) then we obtain $\mu^* = 0.987/\tau_0$ using $n = 21$、 $\tau_s = 1.68\tau_0$ for Eq. (16). This means one wound edge advances toward the center of wound by the order of cell edge length in τ_0. The wound healing of regular polygonal wound is the completely similar contraction process in this simple picture.

5 Discussion and Conclusion

Bereiter-Hahn listed unsolved problems of the wound healing after he had approved the fundamental role of cell migration for the wound healing [1]. That is, the initiative migration mechanism, the role of a basal lamina in the migration, the cause of cell's ceasing the migration after wound closure and the formation mechanism of new cell boundaries.

Now we can answer these questions at the cellular level, not the more microscopic level or not the molecular level. The adhesive force due to the basal lamina initiates the cell migration. The basal lamina plays the most important role of drawing the marginal cells in the cell migration. The cause of cell's ceasing the migration is that the exposed area of basal lamina disappeared after wound closure. New cell boundaries are established when two margins of a wound meet and adhere to each other during the wound contraction.

We conclude that the driving force of wound healing is the force which intends to minimize the cell-basal lamina interfacial free energy. This force can explain the observation that the wound contracts approximately keeping its shape [4]. On the other hand the cell-wound interfacial tension may be a candidate for the driving force of wound healing. However we think that it cannot explain the above observation and gives wound contraction going toward a roundish shape.

The wound healing has an analogy with the formation of erosive land figure by water flows. The healing process of wound can be called "the erosion of wound by marginal cells", in which the parallel displacements of wound margin and the reduction of the edge number of wound polygon occur alternately. The assembly of cells surrounding the wound flows toward its inside and gradually covers its area, just as a water flow. However there is a difference between them in action principle. The geological erosion is destruction by water flows while the erosion of wound is a thermodynamic action by marginal cells.

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