Kinetics of Corneal Epithelial Maintenance and Graft Loss

A Population Balance Model

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We use a population balance model to study the mechanism and the rate of centripetal migration of epithelial cells, renewal of the corneal epithelial population by the cell derived from (progeny of) the limbal stem cells and the kinetics of the replacement of the donor corneal epithelium. The epithelial mass is constant under the normal circumstances and therefore the rate of cellular entry due to centripetal motion and mitosis into any epithelial volume must equal the cell loss from the same volume. The magnitude of the centripetal velocity and the rate of replacement of the donor tissue following keratoplasty are shown to depend only on the following directly quantifiable factors—the difference in the mitotic rates of the corneal and limbal epithelia and the radii of these two epithelia.

The a priori model predictions are found to be in very good agreement with the observed centripetal velocities and the rate of corneal graft replacement. The model provides an independent support for the hypothesis that the stem cells for the corneal epithelium are located in the limbus and are responsible for a slow replenishment of the corneal epithelial cell. The model suggests some factors that diminish the centripetal supply of cells and thus provides insights into the pathogenesis of persistent corneal defects and delayed reepithelialization of defects and grafts. The model is suitable for interpreting and quantitatively correlating the influence of some epithelial alterations and drugs on the centripetal supply of epithelial cells. Invest Ophthalmol Vis Sci 30:1962-1971, 1989

There are two modes of corneal epithelial maintenance. Friedenwald and his associates1-3 and other early studies4-6 of the normal corneal epithelial maintenance envisaged the process of epithelial renewal by mitosis and a relatively rapid vertical movement of basal cells to occupy increasingly superficial (differentiated) positions. That there is also a slow ongoing replenishment of the corneal epithelial cells due to the centripetal drift of the peripheral epithelial cells is supported by the following diverse observations: (1) formation of centripetal streaks by pigmented limbal cells7; (2) time-dependent loss of donor epithelium from corneal grafts8,9; (3) a significant correlation of hemidesmosome patterns with radial direction10 similar to that seen during wound healing11; (4) keratin expression data12 suggesting a limbal location of the corneal epithelial stem cells; (5) delay and even regression of corneal epithelial healing following a loss of limbal epithelium13,14; and (6) attrition of the central epithelial mass and thickening of the peripheral epithelium following the isolation of the central epithelium by a ring of glue.15 More directly, Buck16 showed a centripetal movement of peripheral epithelial cells at a rate of about 123 μm per week in the normal corneal epithelium of mouse.

The mechanism of this slow centripetal migration in normal corneas and after keratoplasty, as well as the modulating factors, however, remain obscure. Here we suggest a fresh approach for understanding and quantifying the dynamics and the renewal properties of the epithelial populations by formulating a simple population balance model which establishes the rate of centripetal migration. The basis for the population balance model is that the epithelial mass is constant and, as is shown in the next section, this implies certain relations between the velocity of centripetal migration and rates of cell proliferation and desquamation. The following are the objectives of the population balance model.

(1) To understand the mechanism of centripetal migration of cells and to test a hypothesis that this...
migration occurs due to population or mitotic "pressure."

(2) To trace the source of corneal epithelial maintenance, that is, to test whether the centripetal migration originates from the peripheral epithelium, limbal epithelium or possibly even from conjunctiva. An objective is to provide an independent test for the hypothesis that the stem cells for the corneal epithelium are located in the limbus.

(3) To estimate the magnitude of the centripetal velocity and the renewal time of the corneal epithelium, and to understand the physiologic and anatomic factors that modulate the normal rate of these processes. These issues are important for understanding the species differences and pathologic alterations that diminish the centripetal mode of supply. The extent of corneal epithelial loss following a reduction in the centripetal distribution is also evaluated.

The veracity of the proposed model is assessed by comparing the model predictions with the available data for the magnitude of centripetal velocity, distances travelled by the epithelial cells and the rate of replacement of lamellar keratoplasties.

(4) To explore whether the normal centripetal velocity varies with distance from the corneal center and thus to identify epithelial regions that may be particularly vulnerable to formation of defects.

(5) To derive estimates for the rate of replacement of the donor epithelium by centripetally drifting host cells after grafting. The a priori model predictions are compared with the data for a time-dependent replacement of lamellar keratoplasties. A match between the prediction and data would indicate that the observed normal loss of donor epithelium is a process similar, in mechanism and in rate, to the ongoing corneal epithelial maintenance.

Finally, a qualitative and quantitative understanding of processes involved in the centripetal supply and epithelial maintenance would provide insights into adverse epithelial responses (persistent defects, thinning, delayed reepithelialization, etc.) that may involve a deficiency of the centripetal supply. This should also suggest a basis for therapies aimed at restoring the corneal epithelial integrity by reestablishing the normal centripetal supply of cells.

**Materials and Methods**

The objective is to derive a population (or mass) balance model of epithelial dynamics that may be employed for evaluating the centripetal velocity in terms of directly quantifiable parameters, such as the mitotic activities of the corneal and limbal epithelium.

Figure 1A is a schematic diagram of the corneal and limbal epithelia, where $R_0$ is the radius of curvature of the cornea, $a$ is corneal radius and $b$ is the width of the limbal epithelium. Since the centripetal migration occurs along the spherical cap shown in Figure 1A, the true distances traversed per unit time can be measured only by flattening the epithelial surfaces; this was also performed in the experiments of Buck.

Figure 1B depicts a mid cross-section of flattened corneal and limbal epithelia that now constitute portions of a circular cylinder. From simple geometric considerations, the radius of this flattened corneal epithelium, $R_c$ is given by the following:

$$R_c = \theta R_0 = R_0 \sin^{-1} \left(\frac{a}{R_0}\right)$$

(1)

The limbal radius, $R_l$ is

$$R_l = R_c + b$$

(2)

where $R_c$ is about 7.5 mm and $R_l$ is about 8.5 to 9 mm for rabbit (limbus is considered to be about 1 to 1.5 mm wide, goblet cell–free zone).

**Model**

The population balance model rests on a simple observation that if the corneal and the limbal epithe-
A. Tear Film

B. (cell loss)

Mitotic rate in this region = M_{c}

Mitotic rate in this region = M_{c}

r < R_{c}

\( D \) (cell loss)

\( R_{c} < r < R_{e} \)

Fig. 2. Conservation of epithelial mass in an epithelial cylinder of radius \( r \). (A) For an epithelial cylinder located within the corneal region \((r < R_{e})\), the rate of cellular entry due to centripetal motion and corneal mitosis equals the rate of cell desquamation. (B) For an epithelial cylinder extending beyond the corneal region \((R_{c} < r < R_{e})\), the rate of cellular "entry" due to mitosis has contributions both from the entire corneal epithelium and a part of the limbal epithelium. Again, the total rate of cellular addition due to mitosis and centripetal drift balances the rate of cell desquamation.

Epithelial masses are to remain invariant under the normal circumstances, the rate of cellular entry (due to migration and mitosis) into any arbitrarily chosen epithelial volume must equal the rate of cellular desquamation from the same volume. This idea is reminiscent of the X, Y, Z hypothesis\(^{17}\) of corneal maintenance, which hypothesized a balance of cell proliferation \((X)\), centripetal movement \((Y)\) and cell loss \((Z)\), that is, \( X + Y = Z \) for the central corneal epithelium.

We now denote the velocity \((\text{distance per unit time})\) of centripetal migration of cells by \( V \) and the average rates of cell generation through mitosis in the limbal and corneal epithelia by \( M_{c} \) and \( M_{l} \), respectively. These mitotic rates are averaged rates including all cell layers and have units of volume generated per unit volume of the epithelium per unit time. That the peripheral and the limbal epithelia have a greater mitotic activity\(^{3,4,12,18,19}\) is also consistent with a possible nutritional role of perilimbal capillaries and, indeed, a study\(^{18}\) with rabbits has quantified the differences in the normal mitotic rates averaged over the corneal \((9.6\% \text{ per day})\) and limbal \((13.5\%)\) epithelia. The normal rate of cellular loss at the tear–epithelium interface is denoted by \( D \), which has units of cellular volume lost per unit area of the surface per unit time. Finally, the epithelial thickness is denoted by \( L \).

Consider now a hypothetical epithelial cylinder of radius \( r \) that is located entirely in the corneal epithelium, viz, \( r < R_{e} \) (Fig. 2A). The volume of such a cylinder is \( \pi r^{2}L \), the surface area available for cell desquamation is \( \pi r^{2} \) and the surface area available for the entry of cells due to centripetal motion in \( 2\pi rL \).

The steady-state population balance for this epithelial cylinder then dictates the following condition:

\[
\pi r^{2}LM_{c} + 2\pi rLV - \pi r^{2}D = 0
\]

\( (3) \)

Considering again a cylindrical volume with a radius that extends beyond the corneal epithelium \((r > R_{c}; \text{Fig. 2B})\), the balance condition is:

\[
\pi R_{e}^{2}LM_{c} + \pi (r^{2} - R_{c}^{2})LM_{c} + 2\pi rLV - \pi r^{2}D = 0
\]

\( (4) \)

Note that the first term of Equation (3) and the first two terms of Equation (4) reflect the rates of total cellular generation within the epithelial volumes considered here and the term \( 2\pi rLV \) is the added cellular volume per unit time due to the centripetal drift of the epithelial cells. The last term in Equations (3) and (4) model the rate of cell loss.

Finally, we note that Buck\(^{16}\) observed a lack of centripetal migration of the conjunctival cells into limbus. That under the normal circumstances conjunctival epithelium does not contribute to the corneal and the limbal epithelial mass is also supported by a possible limbal location of the corneal epithelial cells,\(^{12,14}\) lack of goblet cells in cornea, differences in the corneal and conjunctival cell phenotypes,\(^{20,21}\) and the fact that the resurfacing of a corneal defect is completed by the cells derived from the corneal and limbal epithelia and not the conjunctiva.\(^{21}\) Following a complete loss of the limbal epithelium however, the corneal reepithelialization from conjunctiva lacks the normal corneal morphology and cell phenotype and the possibility of delayed healing and recurrent epithelial breakdowns increases.\(^{13,14,20,22}\) We therefore hypothesize that the conjunctival epithelium does not contribute to the limbal and corneal epithelial masses for the normal ongoing maintenance of the corneal epithelium. This implies that the total rate of cell generation in the corneal and the limbal epithelia...
combined should equal the total rate of cell loss from the entire surface area of these two epithelia. This total population balance condition is represented as:

$$\pi R^2 L C + \pi (R_e^2 - R_c^2) L M_c = \pi R_e^2 D$$  \hspace{1cm} (5)

Note that in arriving at Equation (5), it is assumed that the conjunctival epithelium does not supply any cellular mass to the limbal and corneal epithelia under normal circumstances. Thus, if the predictions of the present population model match the experimental data, it would also provide an independent basis for supporting the hypothesis that the functional stem cell population for the corneal epithelium is located in the limbus.

**Centripetal Velocity**

A solution of Equations (3) and (5) by eliminating the unknown quantity, $D$ (rate of cell loss) gives the velocity of centripetal migration in the corneal region ($0 < r < R_c$) as:

$$V \text{(corneal)} = -\frac{r}{AM} (1 - x^2)$$  \hspace{1cm} (6)

where $AM = M_e - M_c$ is the difference in the mitotic rates of the limbal and the corneal epithelia and $x$ is the ratio of the corneal and limbal radii, viz, $x = (R_c/R_r)$. Similarly, a solution of Equations (4) and (5) yields the centripetal velocity within the limbal epithelium ($R_r < r < R_e$) as:

$$V \text{(limbal)} = \frac{1}{2r} \Delta M (R_r^2 - x^2 r^2)$$  \hspace{1cm} (7)

An inspection of Equations (6) and (7) reveals that the centripetal velocity is not expected to be uniform over the entire epithelium, but depends on the distance from the corneal center, $r$. If, however, experiments are performed over the entire corneal epithelium, the most probable (or average) velocity observed would be the following:

$$V_{av} \text{(corneal)} = \frac{R_c}{4} \Delta M (1 - x^2)$$  \hspace{1cm} (8)

Similarly, the average velocity over the entire width of the limbal epithelium ($R_e < r < R_r$) is:

$$V_{av} \text{(limbal)} = \frac{R_e \Delta M_x}{2(1 - x)} \left[ \ln \left( \frac{1}{x} \right) - \frac{1}{2} (1 - x^2) \right]$$  \hspace{1cm} (9)

where

$$\Delta M = M_e - M_c \quad \text{and} \quad x = (R_c/R_r)$$  \hspace{1cm} (10)

Equations (8) and (9) may be used for predicting the average centripetal velocity of cells and these predictions are compared with data in the next section.

**Rate of Renewal and Renewal Time**

Now the following question may be addressed: If we label a cluster of corneal or limbal epithelial cells with a marker that is passed on in cell division, where will the descendent or progeny (arising through repeated mitosis) of these cells be most likely found after a certain time?

The distance, $d$, traversed in time $T$ by the epithelial cells (and their progeny) that are located at a distance $R$ from the corneal center is derived from Equation (6) as:

$$d = R \left[ 1 - \exp \left( -\frac{\Delta M}{2} (1 - x^2) T \right) \right]$$  \hspace{1cm} (11)

Based on this, the percentage renewal of the corneal epithelial volume in time $T$ by the progeny of the limbal cells is given by:

$$P \text{(in percentage)} = 100 \left[ 1 - \exp \left( -\Delta M (1 - x^2) T \right) \right]$$  \hspace{1cm} (12)

Note that the above estimates hold also for the percentage dilution of donor corneal cells by the host cells if the process of the loss of donor tissue also arises due to the normal centripetal drift of the peripheral host cells.

Finally, if the initial graft radius is $R_d$ but the sampling of the host cells is performed only within an epithelial circle of radius $R_s$ ($R_s < R_d$), the average retention of the donor cells (denoted by $P_d$) within the sampling region after time $T$ may be obtained from the following:

$$\% \text{retention of the donor cells within a radius of } R_s:$$

$$P_d = \pi (R_d - R_s) \frac{\Delta M (1 - x^2)}{R_s^2}$$  \hspace{1cm} (13)

Substituting for $d$ from Equation (11) in the above gives the following relation.

$$P_d = \frac{100 R_d^2}{R_s^2} \exp \left[ -\Delta M (1 - x^2) T \right] \leq 100\%$$  \hspace{1cm} (14)

If $P_d$ is determined experimentally, the mean distances travelled by the progeny of host cells may be evaluated by rearranging Equation (13) in the following form:

$$d = R_d - R_s \sqrt{P_d/100}$$  \hspace{1cm} (15)

Formulas (14) and (15) will be useful for comparing the predictions of the present model with the sex chromatin data of Kinoshita et al. for the rate of replacement of the donor epithelium. This test of the model is reported in the next section.

Formulas (6) through (15) are the predictions of the population balance model that relate the various characteristics of centripetal migration to directly
Table 1. Predictions of the average centripetal velocity of migration of the normal corneal and limbal epithelial cells in rabbit

<table>
<thead>
<tr>
<th>Width of limbus (mm)</th>
<th>Average centripetal velocity (μm/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assumining a uniform epithelial thickness. $L_{c} = L_{c}$</td>
</tr>
<tr>
<td>Corneal</td>
<td>Limbal</td>
</tr>
<tr>
<td>1</td>
<td>112</td>
</tr>
<tr>
<td>1.5</td>
<td>154</td>
</tr>
</tbody>
</table>

quantifiable factors such as mitotic activities and radii of the corneal and the limbal epithelia. This also provides a basis for understanding species differences.

It may be noted that in the above model, only the currently known average values of the rates of mitosis in the corneal and limbal epithelia are employed and the epithelial thickness and desquamation rate are assumed to be uniform. In the future a better characterization of the subtle spatial variations of the mitotic rates may become available; the general population balance model incorporating these variations of mitotic rates and epithelial thickness is presented in the Appendix. The model formulated in the Appendix incorporates different epithelial thicknesses and different rates of cell loss for the corneal and limbal regions. While these details do not alter the conclusions in an essential way, they are included here for completeness and their potential for future applications.

Finally, the proposed model provides an average description of epithelial dynamics, but does not incorporate poorly understood, relatively short-term statistical variations that may arise due to the complexities of the processes of cell desquamation, displacement and mitosis at the cellular level. Hence, the model’s predictions are anticipated to be the most meaningful for time periods longer than a few days.

Results

An important conclusion from Equations (6) and (7) is that notwithstanding the dynamic cellular events (mitosis, loss, movement) and nonuniformity of mitotic rate, the corneal and limbal epithelial masses are maintained by an ongoing, nonuniform centripetal distribution of cells due to mitotic pressure.

We now explore the qualitative and quantitative predictions of the model and compare the quantitative results with the relevant data to evaluate the veracity of the proposed approach.

Estimates of Centripetal Velocity

The average mitotic rate, $M_c$, for the entire corneal epithelium (averaged over all cell layers) indicates an average cell generation of 9.6 cells per 100 epithelial cells per day. The average mitotic rate for the limbal epithelium is about 13.44% per day. The mitotic rate (14.5%) of the rat corneal epithelium obtained by metaphase arrest method is also somewhat similar.

Table 1 summarizes the predicted centripetal velocities for the rabbit. The predictions in the first two columns of Table 1 are based on Equations (8) and (9) and those in the third and fourth columns are obtained from Equations (A.7) and (A.8) of the Appendix. The width of the limbal epithelium in the study of Danjo et al was about 1.5 mm, and thus the most probable average value of the corneal centripetal velocity is predicted to be about 150 μm to 200 μm per week in the rabbit. The centripetal drift in the limbal region is predicted to be somewhat slower, averaging about 130 to 150 μm per week. The migration of epithelial cells is predicted to be the most vigorous in the vicinity of the corneolimbal junction, of the order of about 300–400 μm per week in the rabbit (cf. Equations (6) and (7) for $r = R_c$). This centripetal drift decreases both towards the corneal center and towards the far periphery of the limbus and becomes vanishingly small near the conjunctival epithelium. Absence of conjunctival epithelial drift noted by Buck and a noted predilection of the persistent defects for the central cornea are in agreement with these model predictions.

Although direct measurements of centripetal velocity in the rabbit are lacking, the data of Kinoshita et al may be used to evaluate the time dependence of the mean distance travelled by the host epithelial cells after grafting. Thus, based on their $|D - H|$ counts (see Table 2 and next section), the percentage retention of donor cells may be computed from Equation (16). The experimental mean distance travelled by the progeny of host cells is now determined from Equation (15). The experimental mean distances are thus evaluated to be 0.92 mm and 1.37 mm at 6 and 12 weeks, respectively. If the model predictions for the velocity are correct, the theoretical distance travelled is given by Equation (11). This equation predicts the mean distance travelled by host cells to be 0.87 mm and 1.55 mm at 6 weeks and 12 weeks, respectively. It is thus concluded that the a priori model predictions are in good agreement with the data.

Buck demonstrated a centripetal movement at a rate of about 123 μm per week in the mouse. This magnitude is also in remarkable agreement with the predictions of the present model, especially so be-
Table 2. Time course of the replacement of donor tissue by the peripheral host after keratoplasty: A comparison of model predictions with the observations of Kinoshita et al.

| Postoperative time (weeks) | Observed\(a\) dilution in 6.5 mm central donor button, average \(|D - H|\) | Model predictions, \(|D - H|\) (limbal width, \(b\)) |
|---------------------------|-------------------------------------------------|--------------------------------------------------|
|                           |                                                 | \(b = 1 \text{ mm}\)                           | \(b = 1.5 \text{ mm}\)                        |
| 3                         | 38.8 ± 1                                        | 37.9 ± 3.3\(†\)                               | 37.0 ± 3.3\(‡\)                              |
| 6                         | 34.0 ± 4.5                                      | 37.9 ± 3.3\(†\)                               | 35.0 ± 3.3\(‡\)                              |
| 12                        | 24.8 ± 4                                        | 27.9 ± 2.4\(†\)                               | 21.5 ± 1.9\(‡\)                              |
| 18                        | *                                               | 19.5 ± 1.7                                    | 13.1 ± 1.2                                   |
| 24                        | *                                               | 13.6 ± 1.2                                    | 8.0 ± 0.7                                    |
| 30                        | *                                               | 9.5 ± 0.8                                     | 4.9 ± 0.4                                    |
| 36                        | *                                               | 6.6 ± 0.6                                     | 2.0 ± 0.2                                    |
| 42                        | *                                               | 4.7 ± 0.4                                     | 1.8 ± 0.2                                    |

\(a\) Data not available.
\(†\) Correlation coefficient with the data is 0.9179.
\(‡\) Correlation coefficient with the data is 0.9843.

cause the centripetal velocity in the mouse is expected to be slower than the rabbit due to the smaller corneal dimensions of the mouse cornea (cf. Equations (8) and (9), where, among other things, velocity is predicted to be proportional to the corneal radius, \(R_c\)). In contrast to the rabbit, exact predictions for the mouse are not possible due to the current lack of the mitotic rates data for the limbus. A good match between the model prediction and data\(^9,16\) indicates that the normal proliferative capacity of the presumptive stem cells in the limbus is alone sufficient for maintaining the observed magnitudes of centripetal velocity, without the aid of concurrent migration from the conjunctival epithelium.

Qualitatively, Equations (6) through (9) show that the centripetal velocity under the normal circumstances depends only on three different factors—differences in the mitotic rates of the corneal and limbal epithelia, \(\Delta M\), the ratio of the corneal and limbal radii, \(x\), and the corneal radius, \(R_c\). Due to the similarity of these quantities in humans and rabbits, the centripetal velocity in humans is anticipated also to be of a similar magnitude. However, other factors being somewhat comparable, the velocity is expected to be slower for the smaller animals.

Time Course of Dilution of Donor Epithelium: Comparison with Sex Chromatin Data

Kinoshita et al\(^9\) measured the extent of replacement of the rabbit donor tissue by the so-called \(|D - H|\) value, which is the absolute difference between the percentage of sex chromatin-containing cells in the donor button (\(D\)) and the percentage in the host contralateral eye (\(H_2\)). If the donor (male/female) epithelium is completely replaced by the host (female/male) cells, the \(|D - H|\) value is zero, whereas this value approaches the normal difference between male and female sex chromatin counts (about 37.9 ± 3.3)\(^9\) if no dilution occurs. Following a 8 mm diameter lamellar keratoplasty, the sex chromatin frequency was sampled in 5 mm diameter donor central button and a doughnut-shaped ring of donor tissue between 5 and 6.5 mm in diameter.\(^9\) The second column in Table 2 shows the observed \(|D - H|\) values that we have calculated from the original data\(^9\) by the area (or volume) averaging of the two different sampling areas. These \(|D - H|\) values are directly related\(^9\) to the percentage retention of the donor epithelium within a total sampling diameter of 6.5 mm:

\[
|D - H| = \left(\frac{37.9 \pm 3.3}{100}\right) P_d \tag{16}
\]

The model predictions for the percentage retention of the donor cells (\(P_d\)) within the central 6.5 mm diameter of the 8 mm lamellar keratoplasties are obtained from Equation (14) with \(R_c = 3.25 \text{ mm}\) and \(R_h = 4 \text{ mm}\). In order to compare these with the experimental \(|D - H|\) values, the predicted percent retention values are converted to \(|D - H|\) values with the help of correlation (16). The model predictions for these \(|D - H|\) values are displayed in the third column of Table 2. A comparison reveals a very good (corr. coefficient > 0.9) agreement between the a priori model predictions and the observed rates of replacement of the donor tissues for up to 12 weeks after keratoplasty. While the experiments\(^9\) were discontinued after 12 weeks, the model predictions (Table 2, column 3) are that an essentially complete (more than 95%) replacement of the central 6.5 mm donor epithelium would take longer than about 42 weeks.

An important conclusion here is that the mechanism and the rate of donor invasion by host tissue are similar to that of normal corneal maintenance by centripetal migration of the peripheral and limbal cells. It is however also possible that the normal mitotic rates of both the donor and host tissues are si-
Fig. 3. Percentage (on volume basis) of the corneal epithelium renewed by the cells derived from the limbus within a time period of T months. These predictions are based upon Equation (12) and the mitotic activity data for rabbit. An essentially complete renewal (greater than 95%) of the corneal grafts and the normal corneal epithelium occurs in more than 9 months.

It is also concluded (within the limits of statistical errors) that the donor corneal epithelium is replaced only by cells derived from the limbal epithelium and not from the conjunctiva.

Renewal Times of the Corneal Epithelium

The percentage, P, of the corneal epithelial volume replaced by the progeny of the limbal cells in time T is obtained from relation (12). Figure 3 displays this correlation for the mitotic rates data of Danjo et al\textsuperscript{18} for rabbit (R = 7.5 mm and b = 1.5 mm). The half-life (time needed for replacing half of all preexisting cells) for the corneal epithelium is predicted to be about 9 weeks. Also, the normal corneal epithelium of rabbit is expected to undergo an almost complete (95 to 99%) renewal by the progeny of the limbal stem cells in about a 9- to 12-month period (Fig. 3). Such a long-term survival\textsuperscript{9,23,24} of the corneal epithelium is indeed suggested by studies of the donor epithelium, and thus the model provides quantitative support for the hypothesis\textsuperscript{17} that a relative absence of epithelial graft reactions more than 13 months after keratoplasty\textsuperscript{25} is indicative of a complete replacement of the donor by the host tissue.

It is also interesting to note that while the centripetal velocity depends explicitly on the corneal radius, the renewal time for the corneal epithelium (Equation (12) and Fig. 3) does not. It is in view of this that the renewal times are expected to display less species differences.

Mitosis in the corneal basal cells, as well as the centripetal supply from limbus, contribute to corneal epithelial maintenance. The “renewal” time corresponds to the replacement of the corneal epithelial cells by the progeny of the limbal cells due to a slow, ongoing centripetal motion, and this is a quantity distinct from the so called “turnover” time for the corneal epithelial cells. Turnover is associated with a rapid vertical movement of basal cells to occupy increasingly differentiated positions until they are shed from the superficial squamous layer about 4 to 11 days later. Within this short lifetime of an average epithelial cell, only about 10% of the corneal mass is infused by cells derived from the limbal epithelium (Fig. 3). Thus, while the corneal basal cells are more effective for relatively short-term maintenance of the epithelium, a longer-term replenishment of the corneal mass (including basal cells) is achieved by the centripetal mode of supply. For example, if the centripetal supply from the limbus ceases without any concurrent elevation in the rate of corneal mitosis, the corneal epithelium would lose about one-third of its volume in a 1 month period (Fig. 3).

The turnover time or the average residence time of an epithelial cell is defined from the proposed model as:

\[ T_t = (L/D) = (1/M_x - x^2 AM) \quad \text{(from Equation (5))} \]

This together with the mitotic rate data\textsuperscript{18} predicts the expected turnover time for the rabbit to be about 9-10 days. Thus, the physical picture at the cellular level is that of an epithelial cell undergoing a slow centripetal drift while migrating to the superficial layers (Fig. 4). The cell eventually desquamates from the squamous layer, but its daughters similarly continue their migration. This steady motion occurring across all corneal cross-sections and cell layers then results in a gradual replenishment of the corneal epithelium by the descendents (progeny) of the limbal cell. This mechanism is qualitatively similar to that proposed by Schermer et al\textsuperscript{12} based on their keratin expression data.

Discussion

The proposed population balance model is an a priori physical model, in that it relates the centripetal velocity and the rate of epithelial renewal to directly quantifiable quantities, without any adjustable parameters. A good agreement of the model’s predictions with the observed centripetal velocity, renewal time of the corneal epithelium and the rate of replace-
ment of the donor epithelium suggests that these three phenomena are governed essentially by the same mechanism. Potential quantitative uses of this model lie thus in assessing and correlating the kinetics of donor tissue replacement and in evaluating the differences between the corneal and limbal mitotic rates from the simple measurements\(^{16}\) of the magnitude of the centripetal velocity. The differential responses of the corneal, peripheral and limbal epithelia following perturbations of mitotic rates (eg, nutrition, UV irradiation, drugs, anesthetics, etc.), desquamation rate (adhesion failure, preservative, etc) and the centripetal motion may also be studied using the population balance approach.

It is shown that the limbal epithelium alone is sufficient for perpetuating the observed centripetal velocities of up to a few hundreds of microns per week without any drift of conjunctival cells onto the limbus. The conjunctival migration, if it occurs at all under normal circumstances, is so slow as to be statistically indistinguishable or insignificant compared to the supply from the limbal epithelium. This conclusion provides an independent support for the hypothesis\(^{12,14,22}\) that the functional stem cell population for corneal maintenance and corneal graft replacement is located in the limbus. Limbal cell descendents are displaced progressively towards the corneal center and divide (in the basal layer) as they move. Hence the corneal epithelium is composed of a hierarchical lineage of cells which originates in the limbus. Whether the expression of corneal phenotype is a result of an asymmetric division of the limbal stem cells or whether it arises due to changes in the cell microenvironment coupled possibly with effects of repeated divisions is, however, the questions that remain. More attention should be paid to the limbal mitotic activity, because a mere 30 to 50% rise in the rate of cellular proliferation in limbal epithelia compared to cornea is sufficient for an efficient maintenance of normal corneal epithelial integrity.

Origin of the cellular supply from the limbus supports the suggestion that there are transcorneal epithelial diseases\(^{15}\) that may gradually involve the central cornea, and thus central keratoplasty and corneal epithelial scraping may be relatively short-term therapies for diseases of limbal origin.

A dynamic balancing of the rates of cell generation and cell loss in the corneal and limbal epithelia reveals the genesis of corneal maintenance and centripetal migration in a redistribution of epithelial mass from regions with higher supply (limbus) to regions with higher demand and lower supply (cornea). Indeed, the model predicts that an arrest of steady centripetal supply results in a loss of about 10% volume (one to two superficial cell layers) of the corneal epithelium, even within a relatively short time of 1 week (Fig. 3). This prediction agrees with the extent of cell loss and central punctate staining witnessed in normal, as well as freshly healed corneas 8 days after their centripetal supply was terminated.\(^{15}\)

These model predictions suggest that corneal attrition and formation of persistent defects of the central cornea may be due to inhibition of centripetal migration in some conditions. Based on the proposed model, we now enumerate factors that inhibit or reduce the centripetal supply of epithelial cells to the central cornea.

(1) An elevation in the rate of cell loss (adhesion failure) and reduction in cell proliferation decrease the centripetal supply. This conclusion holds regardless of whether the abnormalities of cell loss and cell proliferation occur uniformly over the corneal and limbal epithelia or are confined only to the limbal and peripheral epithelia. The maximum epithelial attrition in either case is expected to occur over the central cornea that is dependent on the "external" supply for its maintenance. For example, vitamin A affects mitotic rate\(^{3,26}\) and synthesis of cell surface glycoconjugates\(^{27}\) that participate in the process of cell adhesion. It thus appears that erosion and defects of central corneal epithelium observed in keratoconjunctivitis sicca\(^{28}\) and vitamin A deficiency may be due in part to a compromised centripetal supply.

(2) The normal slow migration of cells implies the repeated dislocation and reformation of junctional complexes including hemidesmosomes.\(^{16}\) Thus, abnormalities of hemidesmosome relocation and abnormally strong or long-lasting adhesion of cells may also conceivably inhibit their motion despite the mitotic pressure.
Based on the following reasoning, we speculate that an arrest of the centripetal supply is possible in pemphigus vulgaris and ocular pemphigoid due to abnormal tenacity of the limbal (and possibly corneal) epithelial cells.

Agglutination (adhesion) of various cells, by antibody molecules that specifically attach to the antigenic determinants on the cell surface, is a well known process. Similarly, cross-binding of autoantibodies present in the sera of these patients to the target antigens located at the surface of epithelial cells (in pemphigus) and the epithelial–stromal junction (in pemphigoid) may render the limbal cell relatively immobile. Indeed, a substantial increase in the junctional complexes resembling desmosomes has been demonstrated in ocular pemphigus.

However, the therapeutic endeavors directed towards correcting the cell production (mitogens, nutritional factors, etc.) and cell loss (soft lenses, tarsorrhaphy and adhesive agents) would not be effective in treatment of persistent corneal defects that are secondary to the tenacity or strong adhesion of epithelial cells. This may be a factor in the failure of topical Dextran (an adhesive) therapy of persistent corneal defects in patients with ocular pemphigoid, whereas it had about 90% success rate in other patients.

To conclude, in either of the three aforementioned deficiencies, (ie, cell loss, proliferation and abnormal adhesion), it is the central corneal epithelium that is rendered most vulnerable to erosions and widening of intercellular spaces and this explains a noted prediction of defects for the central cornea. Thus, an understanding of the abnormalities associated with a particular epithelial disease would suggest a more specific therapeutic approach for epithelial defects and erosions.

Finally, the model may prove to be useful for understanding and quantitatively correlating the influence of mitogens (eg, growth factors), antiproliferative drugs (eg, 5-FU, steroids, cytotoxic drugs, etc.), adhesive agents (eg, fibronectin, Dextran, etc.) and of some other physiologic alterations on the rate of centripetal migration of cells.

Key words: corneal epithelial maintenance, centripetal migration, corneal epithelial stem cells, corneal graft replacement, epithelial dynamics

References

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Appendix

If the mitotic rate, M(r) and the epithelial thickness, L(r) are known as functions of the distance from the corneal center, r, then the general population (mass) balance equations are the following:

\[
\int_0^{R_c} 2\pi rL(r)M(r)dr = \int_0^{R_c} 2\pi D(r)rdr
\]

(A.1)

and

\[
d\left[rL(r)V(r)\right]/dr = r[D(r) - L(r)M(r)]
\]

(A.2)

The first balance equation simply states the steady condition that the total rate of generation in the corneal and limbal epithelia combined should equal the total rate of desquamation. The second balance is a differential conservation of mass over any epithelial ring from which the integral forms of the balance equations (3) and (4) of the text may be readily obtained by integration.

For example, let us relax the assumptions of uniform epithelial thickness and rate of cell loss, and denote the corneal and limbal epithelial thicknesses by \(L_c\) and \(L_e\) and the rates of cell loss in these two epithelia by \(D_c\) and \(D_e\), respectively. The modified mass balances corresponding to conditions (3), (4) and (5) of the text are now the following:

\[
\pi L_c L_c^2 M_c + 2\pi rL_c V - \pi r^2 D_c = 0 \quad (A.3)
\]

\[
\pi R_c^2 L_c M_c + \pi (r^2 - R_c^2) L_c M_r + 2\pi rL_c V
\]

\[
- \pi r^2 D_e - \pi (r^2 - R_e^2) D_e = 0 \quad (A.4)
\]

\[
\pi R_e^2 L_e M_e + \pi (R_e^2 - R_c^2) L_e M_r - \pi R_c^2 D_c
\]

\[
- \pi (R_e^2 - R_c^2) D_e = 0 \quad (A.5)
\]

The average residence times (denoted by \(T_t\)) of cells in the corneal and limbal epithelia are not expected to be very different and, hence, we also have the following approximation (see Equation (17)).

\[
T_t = \frac{L_c}{D_c} = \frac{L_e}{D_e} \quad (A.6)
\]

Solutions of Equations (A.3) and (A.4) with the help of conditions (A.5) and (A.6) give the following formulae for the centripetal velocities:

\[
V_{av}(\text{corneal}) = \frac{R_c \Delta M(1 - x^2)}{4(1 + C x^2 - x^2)}
\]

(A.7)

\[
V_{av}(\text{limbal}) = \frac{R_e \Delta M C}{2(1-x)(1+C x^2 - x^2)}
\]

\[
\times \left[\ln \left(\frac{1}{x}\right) - \frac{1}{2} (1-x^2)\right]
\]

(A.8)

where

\[
C = \frac{L_c}{L_e} < 1.
\]

A comparison with Equations (3) and (4) suggests that effects of a thicker limbal epithelium are to increase the corneal velocity by a factor of \(1/(1 + C x^2 - x^2)\), to decrease the limbal velocity by a factor of \(C/(1 + C x^2 - x^2)\) and to increase the rate of cell loss from the limbal epithelium by a factor of \(1/C\) compared to the corneal epithelium. Numerical values of the centripetal velocity are summarized in the last two columns of Table 1 for \(L_L = 1.5 L_c\) (or \(C = 0.67\)). Note that the velocity increases somewhat compared to the case of uniform epithelial thickness, even though the rate of cell loss from the limbal epithelium is 50% higher than the corneal cell loss (Equation (A.6)).