Time-Lapse Videomicroscopic Study of In Vitro Wound Closure in Rabbit Corneal Cells

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This study used video time-lapse recording to characterize the dynamic features of corneal epithelial cells and keratocytes during in vitro wound closure. Confluent cultures of these two cell types from rabbits were established in Rose chambers. A wound 4 or 10 mm in diameter was produced in the center of each culture by mechanical removal of cells. Wound closure was recorded by videomicroscopy for 2-3 days and reviewed at a playback speed of 400 times normal. The epithelial cells at the wound margin initiated migration by extending lamellipodia with undulatory motions. Successive tiers of cells moved as a continuous sheet in a unified and coordinated manner while maintaining intercellular linkage. The migration was unidirectional, toward the wound center. The mean migration rate of the leading cells was 104 μm/hr. The trailing cells migrated at successively slower rates, inversely proportional to their distance from the wound margin. Mitosis was rare during migration but did occur simultaneously. The mitotic rate was 3.7 mitoses/100 cells. The relative mitotic frequency was 0.23 mitosis/hr. By contrast, in keratocyte cultures, the cells around the wound margin migrated individually and asynchronously without intercellular connection. Initially the cells moved generally toward the wound space, but later, different cells migrated in different directions. The mean migration rate was 15 μm/hr. Mitosis occurred frequently. The mitotic rate was 25.3 mitoses/100 cells, and the relative mitotic frequency was 1.33 mitoses/hr. The cell cycle duration was 9.9 hr. Thus corneal epithelial cells and keratocytes showed fundamentally different characteristics and mechanisms of wound closure in vitro. Invest Ophthalmol Vis Sci 30:2488-2498, 1989

Materials and Methods

Cell Culture Techniques

Corneal cells were isolated from adult albino rabbits (2.0–2.5 kg). All experiments with these animals were performed according to the ARVO Resolution on the Use of Animals in Research. The epithelial cells were isolated by the dispase method as described previously. Keratocytes were isolated by collagenase digestion of the stroma. The purity of the cells isolated by these techniques has also been verified. To visualize the dynamic features of corneal epithelial cells and keratocytes during their migration in cell culture to close an artificially produced wound, part of the video recording has been presented previously.

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serum. Culture medium was replaced every 1–2 days. The cells grown in Rose chambers (sealed) were incubated at 37°C in humidified air.

Wounding of Cells

To produce a wound area in the confluent monolayer of epithelial cells or keratocytes, the Rose chamber was partially disassembled at room temperature inside a flow hood by removing the screws, the top stainless steel plate, and the top (plastic) coverslip. In the exposed culture chamber (containing culture medium), a plastic cylinder (10 mm in diameter) was positioned at the center of the culture surface and the cells inside the boundary of the cylinder were scrubbed away with a cotton applicator. To produce a 4-mm-diameter wound, the cells were scrubbed away with a cotton applicator by freehand. Complete removal of cells in the wound area was ascertained by microscopic examination. The culture chambers were rinsed with culture medium to remove cell debris. A new, glass coverslip was applied to the top of the silastic gasket, and the Rose chamber was reassembled. The interior of the chamber was filled with fresh culture medium, and incubation at 37°C was resumed.

Time-Lapse Videomicroscopy

Video recording of cell migration in the wounded cultures was started either immediately or a few hours after wounding. The Rose chamber was positioned on the stage of an inverted microscope and maintained at 37°C during the recording period (2–3 days). Culture medium was replaced daily as indicated by pH changes in the dye. A Dage MTI videocamera (Dage-MTI, Michigan City, IN) and a Gyry time-lapse videocassette recorder (Odetics, Anaheim, CA) were used to record images at one picture per 7 sec on VHS videotapes. Microscope objectives of 16X and 25X were used. The recording field either followed the same migration front (a wound margin) and so required manual repositioning of the field with time, or fixed at the same area on the growth surface and thereby showed a continuous passing of cells.

Data Analysis

The recorded image of cell migration was examined at a playback speed of 400 times normal. Calculation of time was based on the digital timer generated on-screen during each episode of recording. Distance was calibrated from the image of the divisions in a hemocytometer.

The rate of cell migration was calculated based on the distance traveled per unit time (μm/hr). The same marker point in the nucleus or cell membrane of a migrating cell was tracked across the screen of the monitor, from one side of the screen to the opposite side, using the freeze-frame technique. The distance traveled on the screen was measured and converted to the actual distance in micrometers. Travel time was noted from the on-screen timer. In each image field, four representative cells were measured (10–15% variability), each with duplicate measurements (1–2% variability). The mean rate was calculated for each field. When the same migration front was tracked with time, the image field was repositioned at appropriate time intervals. When the field was fixed (see previous section), sequential groups of migrating cells were measured as they appeared in the field, starting with the migration front. The separation between adjacent groups was less than the width of the field. The distance of each group from the migration front was estimated by summation of the distance between adjacent groups.

Cell mitosis was evaluated by three measurements. Mitotic cells were identified by specific features in each phase of mitosis (see Results). The relative frequency of mitosis (mitoses/hr) was determined by the number of mitoses occurring in a given field within a given period (5–18 hr). Mitotic rate (mitoses/10C cells) was determined by the number of mitoses occurring in a cell population which was enumerated in a given field. The duration of a cell cycle (in hours) was determined from the time taken by the daughter cells of one mitosis to undergo another mitosis, with cytokinesis used as a marker of mitosis.

Statistical analysis was based on the student t-test.
Fig. 2. Phase micrograph of corneal epithelial cells during in vitro wound closure. The leading cells surrounding the wound margin (W) typically extend lamellipodia (arrows), and the nucleus (star) is displaced to the posterior. Sometimes intercellular linkage appears as a broad band of membranous structure (arrowhead) between cell borders. Calibration bar = 50 μm.

Results

Epithelial Cells

The use of Rose chambers for videomicroscopy necessitated the culturing of rabbit corneal epithelial cells on gelatin-coated glass coverslips. The growth and morphology of these cells were similar to the previously studied cells grown in plastic tissue culture dishes. During confluency, the monolayered cells did not show any gross migratory movements, except for an infrequent, transient shifting motion of local cell groups suggesting minor adjustment of cell positions on the substratum.

When a cell-free area (a wound) was artificially produced in the center of the confluent cells, cell migration was initiated around the wound margin. This occurred from 1 half hour to several hours after wounding. The first tier of cells surrounding the wound margin (the leading cells) extended broad, fanlike lamellipodia toward the open wound area (Fig. 2). The nucleus of these cells was located off-center and posterior to the lamellipodia (Fig. 2). Cell diameter was 50–80 μm, depending on the extent of lamellipodia. In the compressed video playback time-frame (equivalent to fast-motion), the lamellipodia undulated in quick successions like waves. The trailing cells also exhibited undulating and streaming motions, although the forward position of the lamellipodia and dislocation of the nucleus were more difficult to distinguish. Cell diameter was 30–40 μm. The migration of the tiers of cells was unidirectional, toward the wound center. The cells moved as a continuous sheet in a unified and coordinated manner while maintaining intercellular linkage and relative position to one another (Fig. 3). This linkage sometimes appeared as a broad, membranous structure at the cell boundaries (Figs. 2, 3). During cell migration, intracellular organelles (refractive vesicles of various
Fig. 4. Videomicrographs showing the reunion of a separated leading epithelial cell. The migration front is moving from right to left. (A) A leading cell (large arrow) is separated from the others (relative time, min:sec = 0). (B) Numerous smaller lamellipodia (arrowheads) are extended as the cell turns around to move back (18:34). (C) A filopodium (small arrow) is extended to anchor the cell onto the closest neighbor (39:44). (D) The cell pulls itself back to join the other leading cells. The filopodium (small arrow) is still visible (62:16). (E) The cell attempts to establish normal cellular contacts (83:19). (F) The cell finally reestablishes intercellular linkage and extends typical lamellipodia (large arrow) (111:17). Asterisks indicate air bubbles. Calibration bar = 50 μm.
sizes) also moved about inside the cytoplasm. Infrequently, a leading cell moved ahead of the migration front and became separated from other leading cells (Fig. 4A). When this occurred, the cell moved back, using lamellipodia (Fig. 4B). A filopodium was then extended to contact the closest cell in the migration front (Fig. 4C), which the separated cell used as an anchor to pull itself back into the group (Fig. 4D). The effort to reestablish contact lasted approximately 1 hr. Another 40 min were required for the cell to reestablish normal cellular linkage with the surrounding cells (Fig. 4E, F). In some cases, the separated cell rejoined the group using lamellipodia action alone, without extending a filopodium. On another occasion, a separated leading cell attempted to rejoin the group but failed to establish sufficient contact with the migrating cells. The cell became spherical and was finally ejected into the culture medium.

The migration rate was analyzed by following the same migration front with time or by fixing the frame at one area to monitor successive tiers of cells trailing behind the migration front. The migration rate of the leading cells was $104 \pm 7 \mu m/hr$ (mean $\pm$ SEM; N = 11 fields, from four cultures). The trailing cells moved successively more slowly the farther they were from the wound margin (Fig. 5). The half-maximal rate occurred at 264–438 $\mu m$ behind the wound margin, as calculated by regression analysis of the two cultures shown in Figure 5. It is unlikely that this differential migration rate was dependent on time (eg, aging of cells during culture or fatigue of migrating cells) rather than distance from the wound margin. As shown in Figure 5, multiple measurements that spanned several hours on the leading cells yielded similar migration rates, whereas measurements of a similar time span on the trailing cells resulted in differential rates. In addition, as shown in Figure 5B, the leading cells migrated on the second day at a rate similar to that of the first day. During completion of wound closure, the leading cells from all sides converged at a similar rate (Fig. 5B). On visual inspection, the leading cells merged together centripetally to cover the remaining open space. Even after complete closure, the trailing cells still moved centripetally for approximately 1 hr with gradual reduction in speed, until all movement stopped. This residual motion caused some compression of cells in the center, which appeared to have a higher cell density, as shown previously (Fig. 7F in ref. 9). Afterwards, the cells did not move any further, with the exception of some transient shifting motion as was noticed in normal confluent cells.

Time-lapse videomicroscopy of wound closure also offered an opportunity to visualize mitosis of corneal epithelial cells. Figure 6 shows an epithelial cell undergoing mitosis during cell migration. This cell was located a few cells behind the migration front. During prophase, the nucleus rotated a few degrees, after which the cytoplasm retracted centripetally approximately one third of the way toward the nucleus. This
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Fig. 6. Videomicrographs of epithelial mitosis during wound closure. The migration front is moving toward the lower right corner. The leading cells show lamellipodia (arrow) and a posteriorly located nucleus (arrowhead). (A) Metaphase in a dividing cell (asterisk). Note the alignment of chromosomes (relative time, min:sec, 0:0). (B) In anaphase, two sets of chromosomes separate (asterisk) (9:22). (C) In telophase, two daughter cells are formed (stars) (11:56). Calibration bar = 50 μm.

partial rounding produced a refractive outline (Fig. 6A). Then, in sequence, the chromosomes arranged themselves linearly along the equator (metaphase, Fig. 6A); the chromosomes migrated toward opposite poles (anaphase, Fig. 6B); and the cytoplasm divided (cytokinesis) to form two daughter cells (telophase, Fig. 6C). During the whole process, the dividing cell apparently maintained the same rate of migration as the adjacent migrating cells. Figure 7 shows a rare occasion when two adjacent cells divided simultaneously. These cells maintained their relative positions just behind the leading cells during the whole process. Mitosis usually lasted 15–30 min. The relative frequency of mitosis in cultured epithelial cells was 0.23 ± 0.09 mitoses/hr (mean ± SEM; N = five fields, from four cultures). The mitotic rate was 3.7 ± 1.2 mitoses/100 cells (mean ± SEM; N = five fields, from four cultures).

Keratocytes

When wound closure was studied on keratocytes, different features were observed. Approximately 1 half hr after wounding, only the first few tiers of cells at the wound margin migrated into the wound space (Fig. 8A, B). The cells moved individually and asynchronously. The movement involved sliding of the whole cell, which maintained its spindle shape. There was no pronounced undulation in the filopodium or in the flattened cytoplasmic process. The soma diameter was under 20 μm. None of the cells was interconnected. Although the general direction of migration was toward the wound center, individual cells could move sideways or even backwards (Fig. 8). As the cells spread out in the wound space, they did not cover the substratum completely, but rather left pockets of blank space behind. The cells behind the migration front remained confluent and stationary, until 5–6 hr later, when they began to move into the wound area. The migration rate of the leading cells (generalized over an imaginary front) was 15 ± 1.5 μm/hr (mean ± SEM; N = four fields, from two cultures).

Mitosis was commonly observed in the keratocytes. The cell, normally spindle-shaped (Fig. 8D), rounded up completely into a refractive sphere (Fig. 8E), which subsequently divided into two (Fig. 8F). Sometimes a filopodium remained unretracted during the process (Fig. 8E, F). The daughter cells flattened into the normal spindle shape after division and moved away from one another (Fig. 8G, H). Under favorable optical conditions, detailed observation of each phase of mitosis was possible on some keratocytes. The events were similar to those described above for epithelial cells, except that each phase, including cytokinesis, occurred while the cell was rounded. The duration of keratocyte mitosis was 15–30 min. The relative frequency of mitosis was...
1.33 ± 0.17 mitoses/hr (mean ± SEM; N = six fields, from two cultures). In some episodes of videomicroscopy, the daughter cells from an earlier mitosis remained in the same field and underwent another mitosis at a later time. Thus, it was possible to determine the duration of a cell cycle in keratocytes, which was 9.9 ± 0.6 hr (mean ± SEM; N = six cells). Some daughter cells divided at exactly the same time, while some divided within 1 half hr of each other.

Table 1 summarizes the different features of in vitro wound closure in corneal epithelial cells and keratocytes.

Discussion

This study has characterized certain dynamic features of corneal cell migration during in vitro wound closure. Numerous studies have provided useful information on the cellular and structural features associated with corneal cell migration during either in vivo or in vitro wound healing. However, most of these studies employed conventional techniques, such as gross measurement of change in wound size, and various conventional microscopic methods, such as light, transmission, or scanning microscopy. We used videomicroscopy to record cell migration in real time and obtained information that confirms some aspects of the hypothesis raised in previous studies. In addition, our data provide new information not available previously.

This study shows that the hallmarks of epithelial cell migration during wound closure include lamellipodia, sliding in sheets, and interconnection between cells. Previous studies have described surface ruffling, lamellae, and filopodia on the marginal cells of a wound, and have suggested that these are responsible for cell movement. Our data confirm that lamellipodia provide cell motility. The ruffles are caused by the undulatory motion of the lamellipodia. The filopodia described previously are small in size and are different in appearance from the larger filopodium we observed when a separated cell rejoined the migration front. This study also provides direct evidence to support the previous suggestion that epithelial cells migrate as a continuous sheet while maintaining interconnections between cells. Previous ultrastructural studies have shown a reduction of microvilli, a widening of interdesmosome space, and a lack of hemidesmosomes on marginal cells. In addition, our observation shows that each tier of cells migrates within a narrow range of rates (10–15% variability). We also observed flexibility between the cell borders while the cells undulate individually and yet slide together. Thus it appears that during mass migration, the intercellular linkage between epithelial cells is transformed into a flexible condition. Whether this
Fig. 8. Videomicrographs of keratocyte migration and mitosis during wound closure. (A) The freshly made wound is located in the upper half of the frame (relative time, hr:min:sec, 0:0:0). (B) The first few tiers of cells move into the wound space (2:10:13). (C) General movement of the cells begins to appear more variable in direction and speed (4:35:35). For example, the cell marked with the arrowhead is moving sideways toward the right in subsequent frames (C–H). (D) Another cell (arrow) moves towards the left in subsequent frames (D–H). The cell marked with an asterisk undergoes mitosis in subsequent frames (5:49:33). (E) A keratocyte rounds up and appears refractive during mitosis (asterisk). Note that the filopodium is not completely retracted (relative time of mitosis, min:sec, 0:0). (F) Cytokinesis in anaphase (asterisk) (time of mitosis 9:02). (G) Two daughter cells separate (stars) (mitosis time 14:36). (H) The daughter cells flatten and lose refractivity (stars). Calibration bar = 50 μm.
condition compromises the normal barrier function of epithelial layers is not clear.

Previous studies have discussed whether the sliding cells push or pull during wound closure.\textsuperscript{10,13} Two observations in our study suggest that this may be a complex issue. First, starting with the leading cells at the wound margin, the migration rate was inversely proportional to the distance of cells from the wound margin. If an open space provides a signal for the marginal cells to initiate migration, this inverse relationship ensures that the trailing cells do not outrun the leading cells. The differential rates may be a consequence of an intercellular signal mechanism and the location of open space in one direction. Thus, the leading cells may constitute a pulling force. Second, after completion of closure at the center of the wound, when the leading cells essentially stopped, the trailing cells continued to migrate with reducing speed for 1 hr before stopping completely. Thus, the trailing cells may exert a pushing force. Alternatively, the slowdown time may be related to the time required for execution of a signal mechanism for cessation of cell motility. It is noteworthy that a previous study, in which trailing cells were monitored with ink marks, described a crowded appearance of cells in the center of a completed closure.\textsuperscript{13} The dynamic significance of these images is resolved by the video technique used in this study.

The migration rate (104 \(\mu m/hr\)) of the leading epithelial cells measured in this in vitro model is the highest ever recorded. Buck has summarized the rates obtained in various studies through 1979 (in vivo wounds produced by various methods): the rates range within 60–70 \(\mu m/hr\).\textsuperscript{13} Recently, a more elaborate study yielded a rate of 64 \(\mu m/hr\).\textsuperscript{16} There has been speculation that neutrophils, including polymorphonuclear leucocytes, retard the initial rate of epithelial migration.\textsuperscript{12,14} It is possible that the simplified environment of our in vitro model avoided this type of interfering factor and therefore permitted the maximal migration rate. In a model of cultured epithelial cells similar to the one in this study, with the exception that wounding was produced by freezing, the migration rate was approximately 70 \(\mu m/hr\) (recalculated from mm\(^2/hr\)).\textsuperscript{17} However, in organ culture studies in which the sliding of epithelium towards the cut edge of excised cornea blocks was measured, rates of 20–26 \(\mu m/hr\) were obtained.\textsuperscript{11,18,19} In the present study, we sometimes encountered lower rates of 50–60 \(\mu m/hr\) in the leading cells. In each of these instances, however, the cells either quickly changed from confluency to a differentiated state, or appeared unhealthy. Thus it is likely that subtle changes in the culture condition or differentiation of cells in vitro\textsuperscript{9} affects the migration rate.

Also relevant to the above discussion is the possibility that a latent period occurs before the initiation of epithelial migration. Croson et al\textsuperscript{16} have made a definitive measurement of the lag period (5.5 hr) that previous in vivo studies have repeatedly encountered.\textsuperscript{11,13} In our in vitro study, initiation of epithelial migration was also variable among different cultures and among different locations of wound margins in the same culture. It is possible that under in vivo or in vitro conditions, biologic variability is an intrinsic problem and may not be related directly to the mechanism of cell migration. However, since the minimum time required in our studies for the initiation of migration was approximately 1 half hour for both epithelial cells and keratocytes, a minimum period for the induction of cell migration may be required. Under in vivo conditions, the presence of damaged tissues and cell debris, the variable nature of the exposed basal lamina or stroma surface, and the influence of inflammatory reaction may all contribute to lengthen the latent period.

Perhaps the most surprising observation in this study is the ability of migrating epithelial cells to undergo mitosis without missing a step in the sheetform migration. Previous studies in this laboratory have indicated that cultured epithelial cells are derived from basal and wing cells, with very little contribution from superficial cells.\textsuperscript{7} Although the mitotic rate of the cultured cells was low (similar to in vivo epithelium), our observation indicates that an epithelial cell can execute both cell migration and mitosis almost simultaneously, even at the migration front. This concept has not been recognized previously.\textsuperscript{1} One unique feature of epithelial mitosis was the partial and limited retraction of perikaryon throughout the

### Table 1. Summarized features of in vitro wound closure in rabbit corneal cells

<table>
<thead>
<tr>
<th>Features</th>
<th>Epithelial cells</th>
<th>Keratocytes</th>
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<tbody>
<tr>
<td><strong>Directionality</strong></td>
<td>Unidirectional</td>
<td>Multidirectional</td>
</tr>
<tr>
<td><strong>Cellular relation</strong></td>
<td>Interconnected</td>
<td>Separate</td>
</tr>
<tr>
<td><strong>Timing</strong></td>
<td>Synchronous</td>
<td>Asynchronous</td>
</tr>
<tr>
<td><strong>Motility structure</strong></td>
<td>Lamellipodia</td>
<td>Nonspecific</td>
</tr>
<tr>
<td><strong>Migration rate ((\mu m/hr)) (leading cells)</strong></td>
<td>104 ± 7*</td>
<td>15 ± 1.5</td>
</tr>
<tr>
<td><strong>Mitotic rate (mitoses/100 cells)</strong></td>
<td>3.7 ± 1.2†</td>
<td>25.3 ± 5.2</td>
</tr>
<tr>
<td><strong>Relative mitotic frequency (mitoses/hr)</strong></td>
<td>0.23 ± 0.09*</td>
<td>1.33 ± 0.17</td>
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<tr>
<td><strong>Cell cycle duration (hr)</strong></td>
<td>—</td>
<td>9.9 ± 0.6</td>
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The values are mean ± SEM. See text for N.

* \(P < 0.001\).
† \(P < 0.005\) when compared to keratocytes.
To maintain an interconnection with adjacent cells completely during mitosis. Thus, the epithelial cell is able to maintain an interconnection with adjacent cells and to slide forward while dividing.

A comparison of the different features of epithelial cells and keratocytes during in vitro wound closure indicates that the process in each cell type is based on a fundamentally different biologic program. The epithelial cells normally are interconnected by desmosomes in confluent cultures and show little motility. Upon induction by unknown mechanisms to migrate toward a wound space, the cells were mobilized in coordination, with their interconnections intact but flexible. In essence, these cultured cells behaved as an organized tissue layer rather than as a collection of individual cells. In contrast, keratocytes are a collection of separate cells in confluent cultures. Even though they responded to a migration induction, the movement was not coordinated and lacked a common directionality. Previous in vivo studies have suggested that keratocytes align themselves in the wound, and that in unwounded conditions, keratocyte processes interconnect to form a network structure. In this regard, it is possible that the in vitro keratocyte model is far different from keratocyte wound healing in vivo. Another difference between epithelial and keratocyte migration is the difference in migration rate. Epithelial cells moved as much as seven times faster than keratocytes. On the other hand, the mitotic rate and relative frequency of mitosis in migrating keratocytes were approximately six times higher than those of migrating epithelial cells. Thus, epithelial wound closure is based predominantly on cell migration, whereas keratocyte wound closure relies heavily on cell replication. This conclusion is consistent with the concept derived from in vivo wound healing studies. The present study did not evaluate the aspect of increased epithelial mitosis after the wound closure is completed.

Numerous studies have focused on the effect of extracellular matrix proteins on epithelial migration and on the role of actin in epithelial migration. In light of the above discussion on the susceptibility of the latent period and migration rate to variability according to experimental conditions, some precautions in the interpretation of data are worthy of mention. For example, the organ culture model used in some studies attained a migration rate of only 20 μm/hr, which was enhanced by fibronectin or epidermal growth factor (EGF) to a maximum of 30 μm/hr. These values are only one third to one half of those reported in animal studies. It appears that this model does not provide optimal conditions to attain the normal rate of epithelial migration.

Thus, whether fibronectin or EGF remains effective at the maximal migration rate of epithelial cells is yet to be concluded. Another precaution involves the localization of actin filaments in migrating epithelial cells. Several studies have identified actin filaments in the basal cell membrane of marginal cells surrounding a wound. It is crucial in studies of this type to ascertain that the cells being examined are actively migrating. The hallmark features of a migrating epithelial cell identified in our study would be useful for this purpose. In one study, which evaluated the difference between rat and rabbit epithelial migration in vitro, the rabbit cells, shown in a micrograph, did not exhibit lamellipodia and ruffles typical of migrating cells. It is possible that the lack of stress fibers and the perikaryal location of actin observed in these cells were not related to migration. In addition, the migration rate measured in the model was 17–22 μm/hr.

The model itself was based on the movement of epithelial cells during subconfluent culture and did not involve a deliberate wounding of confluent cells. Thus, it is unclear whether the findings in that study are applicable to normal wound healing. From the large body of information obtained from previous studies, some common criteria for various model systems (whether in vivo or in vitro) may be established for studying epithelial wound healing in cornea. Such criteria should include the presence of lamellipodia and ruffles, movement in sheetform, and a migration rate of at least 60–70 μm/hr in the leading cells. The lag period should not be significantly longer than 6 hr. Adherence to these criteria would ensure that the model system operates in optimal conditions in a way similar to the natural process of wound healing itself.

The dynamic features of corneal cell migration shown in this study emphasize the difficulty and incompleteness of studying wound healing when static techniques are used. A leading epithelial cell (50–80 μm in diameter) migrating at 104 μm/hr will move a distance equivalent to its diameter in 30–45 min. Each cycle of undulation in the ruffles of lamellipodia possibly happens in minutes, if not seconds. It is likely that the structural and molecular components that form the basis of epithelial migration are changing rapidly with time and space. It will be necessary to employ dynamic techniques, such as time-lapse recording, to investigate the dynamic components of wound closure in the same time frame as the phenomenon itself. This study has further recognized the various finer components of epithelial migration, such as initiation of migration (signal transduction), modification of intercellular linkage, intercellular communication (synchronization among cells and...
reunion of out-of-step cells), lamellipodia motility, and termination of migration (slowdown and cessation). To understand fully the migratory function of corneal epithelium, it may be necessary in future investigations to differentiate between the above components when determining the effect of various substances (eg, matrix proteins, growth factors, drugs) on wound closure.

Key words: cell migration, corneal epithelium, keratocytes, time-lapse technique, wound healing

Acknowledgments

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References