Vertical Movement of Epithelial Basal Cells toward the Corneal Surface during Use of Extended-Wear Contact Lenses

Patrick M. Ladage,1,2 James V. Jester,1 W. Matthew Petroll,1 Jan P. G. Bergmanson,2 and H. Dwight Cavanagh1

PURPOSE. To study the effects of extended contact lens wear (EW) on the movement of basal epithelial cells toward the corneal surface.

METHODS. Rabbits (n = 32) were injected with 5-bromo-2-deoxyuridine (BrdU) to label a group of proliferating basal epithelial cells, and, 24 hours later, one randomly chosen eye was fitted with a low- or medium-oxygen-transmissible (Dk/t) rigid gas permeable (RGP) contact lens, while the other eye served as the control (n = 28). Four rabbits were not fitted with any contact lens. Rabbits were euthanatized at different time points, and the corneal epithelium was immunocytochemically stained for BrdU and/or Ki-67, and counterstained with propidium iodide or Syto 59. Corneal flatmount tissues were examined three-dimensionally under a laser confocal microscope and the location of each BrdU-labeled cell in the corneal epithelium (basal or suprabasal) was determined.

RESULTS. Four days after injection of BrdU, both low- (P < 0.001) and medium-Dk/t RGP (P < 0.001) lens groups showed significantly more BrdU-labeled cells in the basal cell layer than in the control eyes. Six days after injection of BrdU, a small percentage of BrdU-labeled cells (<0.5%) were Ki-67 positive.

CONCLUSIONS. Within 6 days, the majority (80%) of BrdU-labeled basal cells became terminally differentiated and rarely divided secondarily in the central epithelium. Short-term use of low- and medium-Dk/t RGP EW contact lenses slows the normal movement of basal epithelial cells toward the surface in the central cornea. This is consistent with known EW-lens-induced decreases in corneal epithelial basal cell proliferation and surface cell exfoliation. Overall, the data suggest that EW lenses significantly inhibit the normal homeostatic turnover rate of the corneal epithelium.

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EXTENDED-WEAR (EW) contact lens users are exposed to a significantly higher risk of development of an infectious corneal ulcer than daytime lens wearers and non-contact lens users.1,2 One of the important components of the ocular surface’s defense against infection is the intact corneal epithelium, which forms a strong barrier against the penetration of infectious organisms. Increasing evidence in humans and rabbits suggests that EW contact lens use produces a decrease in basal cell proliferation and surface cell exfoliation of the corneal epithelium, causing an apparent stagnant ocular surface.3 There are, however, currently no reports on the effects of contact lens wear on the movement of epithelial basal cells toward the surface of the cornea. Based on previous studies, we have proposed that in the central corneal epithelium, continuous contact lens wear slows down the movement of basal epithelial cells toward the corneal surface.

Epithelial cells in the cornea are continuously in motion. There are two principal directions for the migration of epithelial cells: centripetal and vertical. Early observations with pigment and ink tracers have demonstrated the existence of centripetal movement in the corneal epithelium from the periphery to the center.4,5 It has also been shown that basal cells migrate centripetally at a speed of 1.7 to 32 μm a day.6 By contrast, vertical or upward cell movement occurs when basal cells leave the basal lamina and move toward the surface of the corneal epithelium, ultimately ending in apoptotic exfoliation.7–10 Using tritiated thymidine labeling to track the movement of basal cells toward the corneal surface, Hanna and O’Brien1,2 estimated the turnover rate of the epithelium to be 3.5 to 4 days in the rat and 6 to 7 days in the mouse. Beebe and Masters12 demonstrated that after a single-pulse injection of 5-bromo-2-deoxyuridine (BrdU), the first BrdU-labeled cells reach the rat corneal epithelial surface by days 3 to 4; however, some BrdU-labeled cells remain in the basal cell layer for up to 14 days. Overall, these findings suggest that the complete homeostatic turnover rate of the normal epithelium may be longer than previously suspected.

In this study, the proliferation marker BrdU was selected to label a group of basal epithelial cells in both cornes of each rabbit before application of a contact lens. Once a cell takes up BrdU, the label remains detectable in the nucleus over time, even if the cell exits the cell cycle or is not actively undergoing cell division. BrdU-labeled cells can then be monitored over time as they move upward toward the surface of the corneal epithelium. This article reports for the first time that short-term EW contact lens wear inhibits the movement of basal cells into the suprabasal cell layer.

METHODS

In the first part of the study, the experimental purpose was to determine the vertical movement of basal epithelial cells at three different corneal locations (study I). A low-Dk/t rigid gas-permeable (RGP) test contact lens was chosen, because it is known to cause maximum suppression of basal cell proliferation.13,14 In the second part, a me-
Rabbit corneal epithelium. Four NZW rabbits with intact lenses were used to study the basal cell vertical movement at different time points in the central corneal epithelium. In addition, Ki-67 was used to detect possible secondary divisions in the BrdU-labeled cell group (study II).

### Animals

Thirty-two New Zealand White (NZW) rabbits weighing 3.0 to 4.0 kg and of either sex were used in the study. All rabbits were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animals were screened for ocular disease with a handheld biomicroscope before any experimental procedure and were excluded if necessary. The rabbits were housed in individual cages at 19°C to 25°C room temperature, under relative humidity of 30% to 50% and maintained in a 12-hour light-dark cycle. Food and water were provided ad libitum.

To facilitate RGP lens wear, a standard bilateral partial nictitating membraneectomy was performed on both eyes (control and lens-wearing eyes) in the RGP lens groups (n = 28). Rabbits were anesthetized with 30 to 50 mg/kg ketamine (Ketaset; Fort Dodge, Fort Dodge, IA) and 3 to 5 mg/kg xylazine (Rompun; Bayer, Shawnee Mission, KS) and two topical anesthetic drops of tetracaine ophthalmic solution USP 0.5% (Bausch & Lomb, Tampa, FL). The nictitating membrane was removed by pulling away from the eye globe with forceps and partly (30%) to 50% and maintained in a 12-hour light-dark cycle. Food and water were provided ad libitum.

To facilitate RGP lens wear, a standard bilateral partial nictitating membraneectomy was performed on both eyes (control and lens-wearing eyes) in the RGP lens groups (n = 28). Rabbits were anesthetized with 30 to 50 mg/kg ketamine (Ketaset; Fort Dodge, Fort Dodge, IA) and 3 to 5 mg/kg xylazine (Rompun; Bayer, Shawnee Mission, KS) and two topical anesthetic drops of tetracaine ophthalmic solution USP 0.5% (Bausch & Lomb, Tampa, FL). The nictitating membrane was pulled away from the eye globe with forceps and partly (30%–40%) cut with scissors. Thereafter, prophylactic antibiotic ointment (AK-Polybac; Akorn, Decatur, IL) and drops (gentamicin sulfate ophthalmic solution USP 0.5%; Bausch & Lomb) were applied to the rabbits’ eyes. This minor surgical intervention was needed to prevent the adhesion of the nictitating membrane to the RGP lens surface, which can cause lens loss from blinking. The rabbits were allowed to recover for at least 1 week. This procedure has been reported not to alter either lens-related changes in basal cell proliferation or surface cell apoptosis in the rabbit corneal epithelium. Four NZW rabbits with intact nictitating membranes served as an additional control.

### Contact Lenses

Table 1 shows the parameters of the test lenses used. The spherical RGP lens was specially developed for the rabbit cornea, a diameter of 14.0 mm and a uniform thickness of 0.15 mm. The best fitting base curve was selected after trial fitting with radii of 7.60, 7.80, and 8.00 mm using fluorescein and cobalt blue light.

### Experimental Design

All rabbits were anesthetized and injected with BrdU (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS; 30.5 mg/mL). BrdU is incorporated in nuclear DNA as a substitute for thymidine during the S-phase of DNA replication. The injection was performed in the lateral ear vein with a 25-gauge needle between 9:00 and 9:15 AM, to control for circadian effects. Exactly 24 hours later, rabbits were fitted unilaterally with either a low-O2 RGP lens (Dk/t = 10; n = 7, study I) or medium-O2 RGP lens (Dk/t = 27; n = 21, study II), and the other eye served as the control. The contact lens-wearing eye was randomly chosen by coin toss. Four days (n = 7, low-Dk/t RGP lens) and 2, 4, or 6 days after BrdU injection (n = 21, medium-Dk/t RGP lens), rabbits were euthanized with an overdose of pentobarbital (0.4 mL/kg body weight; Abbott Laboratories, North Chicago, IL). In addition, a control group (n = 4) was included with no contact lens in either eye to control for possible sympathetic effects in the non-lens-wearing eye caused by the contact lens in the contralateral eye. These control rabbits were killed 4 days after injection of BrdU.

### Statistics

Two-way analysis of variance (ANOVA) combined with a post hoc multiple-comparison Student-Newman-Keuls test or a paired t-test was applied at a significance level of α = 0.05 (SigmaStat 2.05 software; SPSS Science, Inc., Chicago, IL).

### Study I: Low-Dk/t RGP Lens

**Immunocytochemistry.** Corneas were fixed in situ with 1% paraformaldehyde in PBS for 3 minutes, excised with a scleral rim, and cut in a vertical line from the superior to the inferior rectus muscle. Subsequently, the tissues were processed through a series of staining and washing, as has been described elsewhere in more detail. Briefly, tissues were washed in TD buffer (PBS with 1% dimethyl sulfoxide [DMSO], 1% Triton X-100), placed in acetone, washed in TD buffer, placed in 0.3 N HCl, washed in TD buffer, incubated in whole goat serum diluted 1:10 in PBS for 30 minutes at 37°C, stained overnight in mouse monoclonal anti-BrdU antibody diluted 1:20 in washing buffer (Roche Molecular Biochemicals, Indianapolis, IN) at room temperature with agitation (100 turns per minute), washed with PBS three times for 30 minutes, placed in FITC-conjugated goat anti-mouse secondary antibody (ICN, Costa Mesa, CA) overnight at room temperature with agitation (100 turns per minute), and stained with propidium iodide (PI) 5 μg/mL PBS (Sigma) for 1 minute to label all epithelial cell nuclei.

**Laser-Scanning Confocal Microscopy.** The tissues were mounted epithelium down on a mylar petri dish (Backhofer GmbH, Reutlingen, Germany) and scanned with the confocal laser scanning microscope (Leica, Heidelberg, Germany). An Ar-Kr laser (488 nm and 543 nm) was used to excite the fluorescein labeled cells (BrdU) and PI-stained cells. The examined area of the z-axis and y-axis was 125 × 125 μm, whereas the z-axis covered the full thickness of the corneal epithelium (40× objective). During the procedure, the image was focused on the anterior keratocytes and slowly moved outward until the first image of PI-labeled epithelial cell nuclei (basal cells) was visible. All BrdU-labeled basal cells were counted manually (Fig. 1). Thereafter, all the BrdU-labeled cells were determined for the combined wing and superficial cell layers by focusing the image farther outward. Three corneal locations were assessed: the central, midperipheral, and peripheral epithelium. Ten images were obtained per location and per eye. Every image contained approximately 300 basal cells; thus, in total, approximately 9000 basal cells were surveyed per cornea. The percentage of BrdU-labeled cells in the basal cell compartment was determined for the low-Dk/t RGP lens and contralateral control groups.

### Table 1. Parameters of the Test Contact Lenses

<table>
<thead>
<tr>
<th>Lens Type*</th>
<th>Dk/t †</th>
<th>EOP ‡</th>
<th>Thickness §</th>
<th>Water Content</th>
<th>Base Curve</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Dk/t RGP</td>
<td>10</td>
<td>5.76</td>
<td>0.15</td>
<td>&lt;1</td>
<td>7.60–8.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Medium Dk/t RGP</td>
<td>27</td>
<td>11.28</td>
<td>0.15</td>
<td>&lt;1</td>
<td>7.60–8.00</td>
<td>14.00</td>
</tr>
</tbody>
</table>

* Classification according to Benjamin. † Oxygen transmissibility measured in saline at 35°C by the polarographic method/edge effect correction (International Standard Organization 9913). ‡ Equivalent oxygen percentage (21% is normal at sea level). § Thickness at plano power.
labeled cells (BrdU), and the He-Ne (635 nm) to excite the Syto 59. Three images of the same field were scanned sequentially to optimize excitation and to prevent any possible cross talk from one fluorophore to another. In each eye, two three-dimensional image stacks were taken in 1-μm z-steps with a 20× objective through the full thickness of the corneal epithelium, starting at the corneal surface epithelium and ending in the corneal stroma. The x and y dimensions of each image measured 375 × 375 μm and contained approximately 5700 to 6000 basal cells.

Three-dimensional image stacks were reconstructed with image-management software (MetaMorph; Universal Imaging Corp., Downingtown, PA) and color coded: Green was used to detect all Ki-67− positive cells, blue for BrdU, and red for Syto 59 (Molecular Probes). All nuclei in the corneal epithelium were labeled with red fluorescence, facilitating the determination of the exact location of each BrdU-labeled cell in the corneal epithelium (Figs. 2A, 2B). Each BrdU-labeled cell was placed in one of two locations: basal or suprabasal. In addition, it was verified whether the BrdU-labeled cells were Ki-67 positive or negative, to detect possible secondary divisions. Triple-labeled cells (BrdU, Ki-67 and Syto 59) showed up as white cells (Fig. 2C).

RESULTS

Study I: Low-Dk/t RGP Lenses

All BrdU-labeled cells counted were subdivided into two compartments: basal and suprabasal. The percentage of BrdU-labeled cells present in the basal cell layer was calculated for the control and contact lens–wearing corneas. There was no statistically significant difference in the total number of BrdU-labeled cells between the experimental and control corneas in the same rabbits (P = 0.5489, two-way ANOVA).

Table 2 shows the percentage of BrdU-labeled cells present in the basal cell layer in the low-Dk/t RGP lens compared with the control group in the central, midperipheral, and peripheral layers of the corneal epithelium 4 days after single-pulse injection of BrdU. Two-way ANOVA with the factors group (P < 0.001, power 0.973) and location (P = 0.216, power 0.137) revealed a significantly higher percentage of BrdU-labeled cells retained in the basal cell layer of the low-Dk/t RGP lens group than in the control. The increase was significant in the central (P = 0.006) and midperipheral (P = 0.021), but not in the peripheral (P = 0.148), epithelium (multiple-comparison Student-Newman-Keuls [SNK] test).

Increased numbers of BrdU-labeled basal cells were found in the central (43.6%), midperipheral (35.6%), and peripheral (18%) corneal epithelium, compared with control labeling, taken as 100%.

Study II: Medium-Dk/t RGP Lenses

BrdU-Labeling. There was no statistically significant difference in the total (basal + suprabasal) number of BrdU-labeled cells between control and contact lens–wearing corneas (P = 0.411, two-way ANOVA). Figure 3 shows the percentile data for the control and contact lens groups over time. A significant difference was found for the factors time (P = 0.029) and group (P < 0.001; two-way ANOVA). Within the control, the multiple-comparison SNK method showed a significant difference between day 2 and days 4 (P < 0.001) and 6 (P = 0.002) but not between days 4 and 6 (P = 0.114). However, within the contact lens group, all three time points were significantly different: day 2 versus day 4 (P < 0.001), day 2 versus day 6 (P = 0.008), and day 4 versus day 6 (P = 0.001). Multiple comparison revealed a significantly higher percentage of BrdU-positive basal cells in the contact lens group at day 4 than in the control (P = 0.013), but not at days 2 and 6 (P = 0.628 and P = 0.532, respectively). There was no significant difference (P = 0.730) at day 4 in percentage of BrdU-positive basal cells...
between control eyes of rabbits wearing a contact lens in the contralateral cornea (31.0% ± 5.4%) and rabbits wearing no contact lens in either eye (32.5% ± 8.1%).

The total number of BrdU-labeled cells in the basal cell layer declined over time, as expected, in both the control and contact lens groups (Fig. 4). However, there was a significant difference between control and contact lens wearers at day 4 (P = 0.015), with the contact lens group retaining more BrdU-labeled cells in the basal cell layer. By contrast, there was no significant difference at days 2 (P = 0.287) and 6 (P = 0.684; two-way ANOVA, multiple-comparison SNK method).

We compared at day 4 the control corneas of rabbits wearing either the low- or medium-Dk/t RGP lens in the contralateral cornea with control corneas of rabbits wearing no contact lenses to test for possible sympathetic effects of the contralateral eye. No significant differences between the three groups was found (P = 0.936, one-way ANOVA; Fig. 5).

**KI-67 Labeling.** The proliferation marker Ki-67 was used to detect secondary divisions in the BrdU-labeled cell population of the central corneal epithelium. At day 2, 1 of the 12 corneas (contact lens group) had only one triple-labeled cell in the suprabasal cell layer, which represented 0.45% of all BrdU-labeled cells (Fig. 2C). At day 4, 1 of the 14 corneas (control group) had one triple-labeled cell (0.8%). By day 6, two of the seven control corneas contained triple-labeled cells, and five of seven corneas in the contact lens test group had triple-labeled cells. The mean percentage (±SD) of all BrdU-labeled cells in the control group that were Ki-67 positive at day 6 was 0.25% ± 0.49%. In the contact lens group at day 6, the percentage was 2.19% ± 4.56%. When an obvious outlier with 12.5% triple-labeling was omitted, this percentage was 0.47% ± 0.43%. Without the outlier, the data were normally distributed, and a paired t-test was applied. There was no statistically significant difference in triple-labeled cells between control and contact lens groups at day 6, although the power is too low to produce a definite conclusion (P = 0.492, power 0.05). In the two groups that had only one triple-labeled cell at days 2 and 4, we performed an analysis with a single-group t-test showing that the data were not statistically different from zero (P = 0.363 for the contact lens group at day 2 and P = 0.336 for the control group at day 4) and therefore, these two time points were not included in the overall analysis.

Most of the Ki-67-positive nuclei were found in the basal cell layer (Table 3). Figure 6 shows the total number of Ki-67-positive cells at each time point for the contact lens and control groups. With the relatively small sample size (n = 6 at day 2, n = 7 at day 4, and n = 6 at day 6) used in this study, no statistically significant difference was found (P = 0.059, two-way ANOVA).

**DISCUSSION**

Previous clinical studies in humans have shown an increase in superficial cell size of the corneal epithelium after overnight lens wear.17-22 It has been hypothesized that this increase in cell size can be caused by a prolonged residence time of epithelial cells on the ocular surface and that overnight contact lens wear delays the corneal epithelial turnover rate.17 We have demonstrated in this study for the first time a direct inhibitory effect of short-term use of extended-wear contact lenses on the vertical movement of epithelial cells from the basal cell layer to the suprabasal compartment (wing and superficial cell layers) in the rabbit cornea. Basal epithelial cells of predominantly the central cornea in the contact-lens-wearing eye remained present longer in the basal cell compartment compared with the control eye. At day 2 (2 days after injection of BrdU and 1 day of contact lens wear), there was no statis-
tically significant difference between the control and lens-wearing group but at day 4 (4 days after injection of BrdU and 3 days of contact lens wear) significantly more BrdU-labeled cells had moved out of the basal cell layer in the control group compared with the medium Dk/t RGP contact lens group. At day 6 (6 days after injection of BrdU and 5 days of contact lens wear), there was no significant difference between the lens group and control, but these results should be interpreted carefully, particularly because BrdU-labeled cells were regularly found on the corneal epithelial surface at that time point compared with only an occasional cell at day 4. This demonstrates that the first wave of BrdU-labeled cells had begun to exfoliate into the tear film between days 4 and 6, contradicting the findings of Haddad who reported that it takes at least 14 days before cells reach the corneal surface in the rabbit. Thus, after one test labeling, the data at day 6 or later began to underestimate the ratio, because an unknown number of BrdU-labeled cells had exfoliated. Another consideration is that BrdU-labeled cells were only subdivided into basal or suprabasal compartments and not into each epithelial single-cell layer. Consequently, even though the ratio was almost equal between lens-wearing and control groups at day 6, possible differences within the suprabasal cell layers of the two groups may have gone undetected. Nevertheless, both study I with a low-Dk/t RGP lens and study II with a more physiological medium-Dk/t RGP lens clearly showed that 4 days after injection of BrdU there was a significant decrease of cells moving out of the basal cell layer compared with movement in the control. This new finding of decreased vertical movement of basal cells is consistent with previously established concomitant contact lens-induced decreases in basal epithelial cell proliferation and surface epithelial cell death. Taken together, these data demonstrate that the overall corneal epithelial turnover rate is reduced during short-term wear of EW RGP contact lenses.

In this study, BrdU labeling was not used as a proliferation marker but rather to label basal epithelial cells and to follow the upward movement of these labeled cells. Deliberately, the contact lens was placed on the eye 24 hours after the BrdU injection, allowing enough time to reduce the concentration of BrdU in the blood to minimum levels, thus preventing an effect of the contact lens on primary basal cell divisions. The success of this approach is demonstrated by the fact that there were an equal number of BrdU-labeled cells in both control and experimental corneas. It is also important to recognize that BrdU is passed on to the next generation of daughter cells (secondary divisions), which could have affected the study results, because contact lens wear suppresses corneal epithelial cell proliferation and surface epithelial cell death.

### Table 2. Percentage of BrdU-Labeled Cells in the Basal Cell Layer Four Days after BrdU Injection

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Central Epithelium</th>
<th>Midperipheral Epithelium</th>
<th>Peripheral Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RGP A</td>
<td>Control</td>
</tr>
<tr>
<td>R1</td>
<td>43.96</td>
<td>50.33</td>
<td>34.83</td>
</tr>
<tr>
<td>R2</td>
<td>28.91</td>
<td>55.24</td>
<td>23.02</td>
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<tr>
<td>R3</td>
<td>18.4</td>
<td>50.87</td>
<td>41.51</td>
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<tr>
<td>R4</td>
<td>45.61</td>
<td>40.49</td>
<td>40.49</td>
</tr>
<tr>
<td>R5</td>
<td>30.39</td>
<td>46.73</td>
<td>32.56</td>
</tr>
<tr>
<td>R6</td>
<td>35.04</td>
<td>43.4</td>
<td>27.45</td>
</tr>
<tr>
<td>R7</td>
<td>26.03</td>
<td>38.46</td>
<td>28.95</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>32.35 ± 9.3</td>
<td>46.45 ± 6.15</td>
<td>32.69 ± 6.81</td>
</tr>
</tbody>
</table>

**FIGURE 3.** To monitor over time the movement of basal BrdU-labeled cells into the suprabasal cell layers, the ratio of BrdU-labeled cells in the basal cell layer versus all counted BrdU-labeled cells was determined at different time points. Day 2 means the BrdU injection was performed 48 hours before the count and the contact lens was fitted 24 hours before. Most BrdU-labeled cells were still considered part of the basal cell layer. One day of contact lens wear did not have a significant impact. However, at day 4, clearly the contact lens-wearing corneas contained more BrdU-labeled cells in the basal cell layer than did the control. Between days 4 and 6, the control eyes were losing BrdU-labeled cells through surface cell exfoliation, and therefore the ratio at day 6 should be interpreted carefully.
lial proliferation. However, the proliferative marker Ki-67 used at each time interval showed no positive triple-labeled cells (BrdU, Ki-67, and red fluorescence) at days 2 and 4 in the central cornea. This means that the observed group of BrdU-labeled cells in either control or contact lens–wearing eyes did not significantly divide a second time during the first 4 days after injection of BrdU, and therefore secondary divisions could not have influenced the data. Our Ki-67/BrdU double-labeling data also confirm the earlier reported findings of Lehrer et al., who studied the time intervals in the mouse cornea between primary and secondary epithelial cell divisions with BrdU and 3H-TdR double labeling. Based on double-labeled cell counts in corneal sections, they concluded that in a time span of 72 hours, only 8% of all epithelial cells in the corneal periphery had undergone two divisions, whereas in the central epithelium only rarely was a double-labeled cell observed, even 96 hours after injection of the first label.

A reduced renewal rate of the corneal epithelium may have significant implications for the health and maintenance of the ocular surface. The relatively rapid epithelial renewal rate in primary and secondary epithelial cell divisions with BrdU and 3H-TdR double labeling. Based on double-labeled cell counts in corneal sections, they concluded that in a time span of 72 hours, only 8% of all epithelial cells in the corneal periphery had undergone two divisions, whereas in the central epithelium only rarely was a double-labeled cell observed, even 96 hours after injection of the first label.

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normal corneas can be regarded as a safety measure against mechanical damage and the continuous barrage of potentially adherent and infectious organisms present in the external environment. Presumably, this reduces the time and opportunity of microbial organisms to attach to surface cells and penetrate deeper into the epithelium as cells are continuously shed into the tear film. A delayed corneal epithelial renewal rate associated with contact lens wear inherently leads to cells with increased lifespans, and cells on the epithelial surface are therefore presumably older than cells of non-lens-wearing corneas. There is no direct evidence that aged surface epithelial cells are less resistant against infectious intruders; however, several studies have shown that corneal surface epithelial cells of overnight contact lens wearers tend to bind more Pseudomonas aeruginosa (PA) bacteria, as is the case in the normal non-lens-wearing cornea. Ren et al. hypothesized that an increase in PA binding may lead to an increased chance of development of a corneal infection. Furthermore, epithelial defects associated with lens wear (mechanical, postlens tear film debris, necrosis caused by hypoxia) may take longer to heal if a contact lens is decelerating the normal corneal epithelial renewal rate.

The non-lens-wearing control eyes showed differences, although not statistically significant, in Ki-67 labeling at the three measured time points ($P = 0.059$). The rabbits wearing the contact lens for three continuous days (4 days after BrdU injection) showed more Ki-67-positive cells in the control eye than did the control subjects at days 2 and 6, suggesting an increase in cell proliferation at day 4 or a decrease at days 2 and 6. This potentially important finding, which should be studied further, suggests that the manipulation of one eye with a contact lens may indirectly affect the corneal epithelial proliferation rate of the contralateral eye. We not only observed such a sympathetic response in this experiment but also in some of our prior proliferation experiments with BrdU labeling. The control eyes of the high-oxygen-transmissible lenses clearly contained more BrdU-labeled cells than did the control eyes of the low-oxygen-transmissible lenses. Comparable observations have been made with corneal swelling in control eyes. Fonn et al. found that the corneal thickness was significantly different between the control eyes of patients wearing unilaterally a high-oxygen-transmissible lens compared with the control eyes with the low-oxygen-transmissible lens. Furthermore, inducing a wound in one cornea resulted in an upregulation of proliferation in the contralateral control cornea, and even tear film osmolality in the contralateral control eye can be affected after monocular soft lens wear.

Thus, corneal epithelial homeostasis in both eyes appears to be linked systemically through mechanism(s) that remain to be established.

In conclusion, short-term wear of EW RGP contact lenses of various oxygen transmissibility inhibited the differentiation and upward movement of basal epithelial cells into postmitotic suprabasal cells. This is consistent with the hypothesis that contact lens wear slows down the overall corneal epithelial renewal rate. Future studies are needed to examine the long-term effects of overnight contact lens wear on the renewal rate, especially because adaptive effect(s) of lens wear on the corneal epithelium are known to occur.

### TABLE 3. Distribution of Ki-67-Positive Cells in the Central Corneal Epithelium

<table>
<thead>
<tr>
<th>Time of Death after BrdU Injection</th>
<th>Control</th>
<th>Medium-Dk RGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>94.7 ± 9.1</td>
<td>94.7 ± 3.1</td>
</tr>
<tr>
<td>Day 4</td>
<td>98.7 ± 1.8</td>
<td>97.3 ± 2.1</td>
</tr>
<tr>
<td>Day 6</td>
<td>96.5 ± 3.7</td>
<td>95.5 ± 5.6</td>
</tr>
</tbody>
</table>

Data are the mean percentage ± SD of all Ki-67-positive cells in the basal cell layer of the central corneal epithelium.

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**FIGURE 6.** Total number of Ki-67-positive cells in the corneal epithelium. The control corneas reacted differently over time, although the difference was not statistically significant with the current sample size, suggesting that contact lens wear in one eye affected the corneal epithelial homeostasis of the control eye.
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References