present study, little phagocytosis of the fresh autologous GBCs was seen, and in the rare finding of GBC phagocytosis, the endothelial cells appeared normal, indicating little role of a secondary effect. This contrasts with earlier studies in which glutaraldehyde-fixed GBCs caused some phagocytosis and endothelial swelling.4 Perhaps the fixation of the cells accounts for this difference. The acute, one-time injection of GBCs into the anterior chamber to create GCG differs from the actual clinical setting, where ghost cells present more slowly. This difference could mean that the experimental findings may differ from the human and pathologic findings. From the present study, however, it is clear that the intertrabecular spaces become acutely blocked by GBCs with little phagocytic response. The effect of chronic exposure has yet to be studied.

GBCs were found to be far more effective in causing trabecular meshwork obstruction in vivo than RBCs. Although four times more RBCs than GBCs were injected, GBCs caused a higher and longer elevation of IOP. As has been shown, this is most likely due to decreased pliability.1 Previous studies using glutaraldehyde-fixed GBCs showed an in vivo pressure elevation lasting many days in the rabbit and cynomologous monkey.7,8 This difference in duration appears to be due to the fixation of the GBCs; it has been shown that glutaraldehyde can cause RBC membranes to become rigid.6,7 Specifically, in the progression from fresh RBCs to GBCs and fixed GBCs, decreased pliability parallels a decrease in ability to pass through the small intertrabecular spaces of the meshwork, resulting in a longer duration of elevated IOP.

The finding that GCG may be due to primary obstruction of the intertrabecular spaces rather than secondary effects may mean that future treatment could be aimed at dislocation of the cells, perhaps by ultrasound or biochemical means, to render the cells more pliable.

Key words: ghost cell glaucoma, trabecular meshwork, glaucoma mechanisms, blood cell deformability

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References

A Larger Corneal Epithelial Wound Closes at a Faster Rate

Momoru Marsuda,* John L. Ubbels,** and Henry F. Edelhauser†

In order to evaluate relationships between healing rates and initial wound area, epithelial wounds were made on rabbit corneas by scraping the epithelium within a 4-, 6.5-, or 8-mm trephine mark. The wounds were stained with fluorescein and photographed during healing. The wounded areas were measured by planimetry. Although larger wounds closed later than smaller wounds, all of the healing curves appeared to be linear. The mean healing rate of the 8-mm diameter wounds (0.91 mm²/hr) was significantly greater than that of the 6.5-mm diameter wounds (0.80 mm²/hr). The 4-mm diameter wounds healed at a significantly slower rate (0.37 mm²/hr) when compared to the 6.5-mm diameter wounds. The authors found a strong positive correlation between the healing rates and the initial wound areas. By comparison, regardless of the initial wound area, the wound diameter decreased at a rate of approximately 0.1 mm/hr, which may explain the dependency of the healing rate on the initial wound area. The healing rate varied considerably
Table 1. The epithelial healing rates and initial wounded areas

<table>
<thead>
<tr>
<th>Wounded area (mm²)</th>
<th>Healing rate (mm²/hr)</th>
<th>r</th>
<th>Wounded area (mm²)</th>
<th>Healing rate (mm²/hr)</th>
<th>r</th>
<th>Wounded area (mm²)</th>
<th>Healing rate (mm²/hr)</th>
<th>r</th>
</tr>
</thead>
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<tr>
<td>Rabbits</td>
<td></td>
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<td>Rabbits</td>
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<td>Rabbits</td>
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<tr>
<td>OD</td>
<td>11.9</td>
<td>0.39</td>
<td>0.997</td>
<td>a OD</td>
<td>30.1</td>
<td>0.84</td>
<td>0.997</td>
<td>i OD</td>
</tr>
<tr>
<td>OS</td>
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<td>0.997</td>
<td>a OS</td>
<td>31.1</td>
<td>0.83</td>
<td>0.987</td>
<td>i OS</td>
</tr>
<tr>
<td>P</td>
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<td>0.997</td>
<td>b OD</td>
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</tr>
<tr>
<td>c</td>
<td>13.9</td>
<td>0.43</td>
<td>0.997</td>
<td>c OD</td>
<td>28.9</td>
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</tr>
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<td>0.999</td>
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<tr>
<td>Mean</td>
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<td></td>
<td>30.0</td>
<td>0.80*</td>
<td>45.3</td>
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<td>SEM</td>
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<td>0.02</td>
<td></td>
<td>0.9</td>
<td>0.04</td>
<td>0.8</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

r: correlation coefficient.
* Significant (P < 0.01) from 4-mm scraping.
† Significant (P < 0.05) from 6.5-mm scraping.

between animals with the same diameter wounds, but both eyes of each animal showed a similar healing rate. Invest Ophthalmol Vis Sci 26:897-900, 1985

Wound closure of the corneal epithelium occurs in an essentially linear manner, allowing calculation of a wound healing rate (mm²/hr) by linear regression analysis.1-9 Many investigators have measured the healing rate for a quantitative evaluation of the effects of various substances on the epithelial healing process.1,3-7,9 Most of these studies used the rabbit, and the normal healing rate ranges considerably from 0.69 mm²/hr to 1.46 mm²/hr,2 depending on the methods used for wounding (mechanical scraping1,4,7 or chemical debridement5,7,9), the size of the wound, and the vital dyes (fluorescein or Richardson’s stain) used to stain the wound.3 When we review and compare data obtained by similar techniques, a larger wound appears to close at a faster rate than a smaller wound. However, this has not yet been clearly demonstrated. The present study was undertaken in order to evaluate whether there is a difference in the epithelial healing rate between large and small wounds.

Materials and Methods. New Zealand white rabbits (2 to 2.5 kg) were anesthetized with ketamine HCl (30 mg/kg) and xylazine (5 mg/kg), and each eye received a drop of proparacaine HCl (0.5%). Central epithelial wounds were made by scraping with a mini-blade (Beaver; Belmont, MA) to remove the epithelium within a 4-, 6.5-, or 8-mm trephine mark. The same sized trephine was used for both corneas of each animal. These methods adhered to the ARVO Resolution on the Use of Animals in Research.

The corneal wounds were stained with fluorescein and photographed at 0, 6, 24, 30, 48, 54, and 72 hr after wounding. The photographs of the wounds were projected, and the wound perimeters were traced onto paper. The wound areas were determined by computerized planimetry, as previously described.5-8

Results. The initial wound areas were 11.3 ± 0.5 mm² (mean ± SEM) for the 4-mm diameter wounds (8 eyes), 30.0 ± 0.9 mm² for the 6.5-mm diameter wounds (6 eyes), and 45.3 ± 0.3 mm² for the 8-mm diameter wounds (6 eyes), respectively (Table 1). All of the corneas with 4- and 8-mm diameter wounds were healed completely between 30 and 48 hr and 54 and 72 hr, respectively. After 6.5-mm diameter wounding, four corneas of two animals (rabbits a and b) were healed between 48 and 54 hr, whereas both corneas of one animal (rabbit c) were healed between 54 and 72 hr.

A healing curve for each cornea was obtained by plotting the area of the wound as a function of time.* All of the 20 healing curves appeared to be linear. We calculated the healing rate of each cornea by linear regression analysis of the data at 0, 6, 24, and 30 hr after wounding because these data were commonly available in any cornea studied.

The healing rate for the 4-mm diameter wounds was 0.37 ± 0.02 mm²/hr (mean ± SEM), which was significantly slower (P < 0.05) than that for the 6.5-mm diameter wounds (0.80 ± 0.04 mm²/hr) (Table 1). The 8-mm diameter wounds closed at a significant
cantly faster rate (0.91 ± 0.02 mm²/hr) when compared to the 6.5-mm diameter wounds (P < 0.05). A strong correlation was found between the healing rate and the initial wound areas (r = 0.901, P < 0.05) (Fig. 1A).

There was a considerable variation in the healing rate between animals with the same diameter wounds. However, a strong correlation was found between the healing rates of both eyes of each animal (r = 0.991, P < 0.05) (Fig. 1B).

Wound diameter was plotted vs time and appeared to be a linear function. The rate of decrease in wound diameter was calculated by linear regression and was found to be about 0.1 mm/hr regardless of initial wound size (Table 2).

Discussion. The results of this study clearly demonstrate that larger wounds of the corneal epithelium close at a faster rate than smaller wounds when this healing is expressed in the commonly used units of mm²/hr. By comparison, Ho et al.1 and our previous experiment5 have shown no significant correlation between the healing rate and the initial areas of the wound. In these studies, however, the wound was made by scraping the epithelium within a uniformly-sized trephine mark where the range of the wounded areas was smaller (40-60 mm²) when compared to that of the present study (9.6-47.2 mm²).

Our results also demonstrate that, when a standardized wound is made, the corneal healing rates are more uniform within animals than between animals. These results agree with the findings of our previous study7 and those of Moses et al.2 As will be shown, these observations suggest the existence of small differences in the velocity of cell migration between animals. Therefore, the experiments that can be analyzed on a paired basis may be preferable to the use of separate treated and untreated groups.7

In this study, all of the healing curves appeared to be linear. However, our observations of the larger (8-mm) wounds were not made at frequent enough intervals after 30 hr to detect a possible slowing of healing. Indeed, if the healing occurs at a rate of 0.91 mm²/hr, 8-mm diameter wounds should be healed in 49.8 hr, whereas they actually closed between 54 and 72 hr after wounding. It appears that the rate of reepithelialization may decrease late in the healing process.10 Therefore, the present data as calculated by linear regression may represent only the average rate of healing that occurs during the first 30 hr after wounding.

Of particular interest is the observation that the wound diameter decreased at a rate of 0.1 mm/hr regardless of the initial wound area, suggesting a constant velocity of cell migration to cover the defect. By assuming that the cell migration toward the center of the wound occurs at a rate of k (mm/hr) and that the wounded area is a perfect circle, the wound diameter, D (mm), at any time, t, can be calculated as follows (Fig. 2):

\[ D = D_0 - 2kt \]  

where \( D_0 \) is diameter of the initial wounded area and \( t \) is time after wounding. The healing rate, R (mm²/hr), can also be calculated as follows:

Table 2. Rate of decrease in wound diameter

<table>
<thead>
<tr>
<th>Wound diameter (mm)</th>
<th>Rate (mm²/hr)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.096 ± 0.005</td>
</tr>
<tr>
<td>6.5</td>
<td>0.107 ± 0.008</td>
</tr>
<tr>
<td>8.0</td>
<td>0.096 ± 0.001</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

Differences are not significant.
constant for both wounds, shaded area A is greater than shaded area B at time t. Therefore, $R_A > R_B$.

$R = -\frac{d}{dt} \pi \left( \frac{D}{2} \right)^2$  \hspace{1cm} (2)

from the equation (1)

$R = -\frac{d}{dt} \pi \left( \frac{D}{2} - kt \right)^2$

$= \pi k D_0 - 2\pi k t$.  \hspace{1cm} (3)

This equation indicates that the rate of healing decreases with time. However, the value of $k$ would be small (approximately 0.05 mm/hr) because the wound diameter decreased at a rate of 0.1 mm/hr. Therefore, the second factor, $2\pi k t$, in equation (3) is minimal during the healing of small wounds and during the early healing process of larger wounds where $t$ is relatively small. In such an instance, the healing curve would appear to be linear and the healing rate (mm$^2$/hr) for larger wounds would also be greater than that for smaller wounds. We have observed that 8-mm diameter wounds close at twice the rate of the 4-mm diameter wounds over a 30-hr period as calculated by linear regression. This difference can easily be explained by equation (3), and therefore the difference in healing rate (mm$^2$/hr) between large and small corneal epithelial wounds can be explained mathematically rather than on the basis of any physiologic difference in the healing process for large and small wounds. The decrease in healing rate and delay in wound closure when the healing process extends beyond 48 hr can also be explained mathematically as $t$ in the second term of equation (3) begins to have a significant effect on $R$.

The dependency of the healing rate on the initial wound area indicates that one should make wounds as uniform as possible when evaluating the effects of drugs or chemical substances on the epithelial healing process. Moreover, the dependency must be taken into account when a testing substance causes further loss of epithelial cells. This enlarged wound might close at a faster rate than the wound of untreated (control) eyes. In such an instance, therefore, direct comparison of the healing rate between treated and untreated eyes can give us the underestimation of actual effects of the substance.

In conclusion, we have shown that observed differences in the healing rates (mm$^2$/hr) of large and small, circular corneal epithelial wounds can be explained by the fact that velocity of cell migration (mm/hr) toward the center of the wound is the same, regardless of wound size.

**Key words**: corneal epithelium, rabbit, corneal wound healing

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