Purpose. To compare the process of conjunctival epithelial regeneration after 3 types of pterygium excision procedures. Methods. Thirty-eight patients (45 eyes) with primary pterygium were randomly assigned to a bare-sclera procedure (BS, group 1, 15 eyes of 12 patients), BS with intraoperative mitomycin C (MMC 0.02% for 30 seconds, group 2, 15 eyes of 14 patients), or pterygium excision with conjunctival autografting (group 3, 15 eyes of 12 patients). Controls were healthy fellow eyes and 7 eyes of age- and sex-matched subjects. Impression cytology was performed preoperatively and 1 and 2 weeks, 1, 3, 6, and 12 months after surgery. The nucleus/cytoplasm (N/C) ratio of nongoblet epithelial cells and goblet cell density (GCD) in the pterygial area were calculated and compared over time across treatment groups. Results. Pterygium excision wounds healed in a similar 4-stage process in all groups but at different rates and different final results. N/C ratio was highest at about 1 month postoperatively in groups 1 and 2 and at 2 weeks in group 3 before gradually returning to control levels. Preoperatively, the GCD in treated eyes was almost twice that in control eyes (p = 0.001) but fell to zero immediately postoperatively. Goblet cells first appeared and its density increased most rapidly in group 3, followed by group 1. At 12 months, the mean GCD in groups 1 and 3 were not significantly different from those in controls, whereas the mean GCD in group 2 was still less than that of control (p = 0.048). Conclusions. Healing of conjunctiva is delayed by MMC and promoted by autografting. Even 1 year after surgery, the ocular surface remains abnormal with respect to epithelial phenotypes in eyes treated by any of the 3 techniques.

Key Words: Conjunctival epithelial phenotype - Impression cytology - Bare sclera - Intraoperative mitomycin C - Conjunctival autograft.
factor in the determination of clinical management of pterygium. To address this issue, impression cytology was used to (1) characterize the pterygial epithelium phenotypes and (2) to compare the healing conjunctival epithelium phenotypes on the ocular surface following bare-sclera pterygium excision with intraoperative MMC to that of bare-sclera procedure alone or pterygium excision with conjunctival autograft reconstruction.

PATIENTS AND METHODS
A total of 38 patients (45 eyes) who underwent treatment in our department for primary pterygium were enrolled in this study. Patients age ranged from 33 to 81 years (mean ± standard deviation, 57 ± 21 years).

Patients were randomly assigned to 1 of 3 treatment groups, denoted as groups 1, 2, and 3, such that there were 15 treated eyes in each group. For patients with a normal contralateral eye, it was used as the controls for the treated eye. For patients with bilateral pterygium, seven age- and sex-matched control subjects were recruited. Patients in group 1 underwent simple excision of pterygium (a bare-sclera procedure), those in group 2 underwent a bare-sclera procedure with low-dose intraoperative MMC (0.02% for 30 seconds) as described previously, and those in group 3 underwent pterygium excision followed by conjunctival autografting. The groups were similar in age and gender (Table 1).

For patients in the study, the first impression cytology specimen was obtained preoperatively from the pterygial surface of the affected eye and postoperative specimens were obtained from the wound surface at 1 and 2 weeks, 1, 3, 6, and 12 months. Control specimens were obtained from a corresponding site on fellow or control eyes. The techniques for impression cytology specimen collection, preparation, and examination have been described by Tseng. The nucleus-to-cytoplasm (N/C) ratio was used to compare cell sizes. The mean goblet cell density (GCD) was determined for each specimen by averaging the number of goblet cells in five 1-mm² areas defined by a calibrated grid under light microscopy at a magnification of 100.

RESULTS
Epithelial Phenotype of Pterygium
Preoperatively, the mean GCD in impression cytology specimens of pterygium was 546 ± 279 cells/mm², significantly (p = 0.001) higher than the mean GCD (190 ± 109 cells/mm²) in control eyes. However, mean epithelial cell size (N/C ratio) was similar (p = 0.31) for specimens of pterygium (1 : 1.3 ± 0.5) and of conjunctiva from control (1 : 1.2 ± 0.2) eyes.

Changes in Epithelial Phenotype after Pterygium Excision
In all eyes studied, wound healing progressed through a similar 4-stage process albeit at different rate. These 4 stages were characterized by the changes in N/C ratio of the nongoblet epithelial cells and GCD:

Stage 1. For the first few weeks after pterygium excision, only a few undifferentiated epithelial cells were present on the surgically denuded ocular surface. The N/C ratio was approximately 1 : 1 and goblet cells were essentially absent.

Stage 2. An increase in the number and size of the nongoblet epithelial cells is accompanied by aggregation of these cells into patches. The N/C ratio of these differentiated epithelial cells ranged from 1 : 2.5 to 1 : 3.5. Isolated goblet cells were seen, but the average GCD in this stage (~20 cells/mm²) was less than 10% of normal.

Stage 3. Islands of nongoblet epithelial cells proliferate to form an intact cell sheet that was easily harvested by conjunctival impression. As they proliferate, nongoblet epithelial cells returned to their normal size (N/C ratio of 1 : 1.2). In contrast, the number of goblet cells, as indicated by
GCD, increased only slightly, to about 50 cells/mm$^2$ or 25% of normal.

**Stage 4.** In stage 4, nongoblet epithelial cells remained relatively stable both in number and size while goblet cells began to proliferate markedly. However, GCD ($\sim$ 100 cells/mm$^2$) was still subnormal.

**Time Change of Phenotype Population after Surgery**

The mean N/C ratio from those in group 3 (conjunctival autograft) increased at 2 weeks and then was the first to drop (at 1 months) and reached near-normal value 12 months postoperatively. The greatest N/C ratio was measured in group 2 (bare sclera procedure with MMC) and the next greatest in group 1 (bare-sclera procedure without MMC). N/C ratios in groups 1 and 2 both increased at 1 month after pterygium excision and for group 2 it was still greater than normal at the end of study (Fig. 1). Goblet cells appeared earliest (about 2 weeks postoperatively) in eyes from group 3 and the mean GCD in this group increased steadily towards a normal value by 6 months after surgery. Goblet cells were noted in eyes from groups 1 and 2 at 1 month after surgery and the GCD increased at the same rate toward normal values at 12 months. However, the GCD appeared to be higher in eyes from group 1 at each evaluation than those from group 2. At 1 year postoperatively, only group 2 has a GCD significantly below that of normal controls ($p = 0.048$) (Fig. 2).

**DISCUSSION**

**Epithelial Phenotype of Pterygium**

Using impression cytology, we have documented a statistically significant 2-fold to 3-fold increase in the density of goblet cells on the surface of pterygium compared with normal conjunctiva. We speculate that goblet cell hyperplasia plays some role in the stringy mucus occasionally seen in eyes with inflamed pterygium. Although its pathophysiological significance has not been addressed, goblet cell hyperplasia in other diseases has been reported in the literature.

**Alterations in Epithelial Phenotypes During Wound Healing**

The paucity of goblet cells on impression specimens obtained during stages 1 and 2 of re-epithelialization suggested that epithelial differentiation was altered during this postoperative period. The improvement in the rate of conjunctival wound healing with autografting may be explained by the presence of the basement membrane of the graft that serve as a substrate for the regenerating epithelium.

**Conclusion**

Ptetrigial epithelium is characterized by goblet cell hyperplasia. Following pterygial excision by a bare-sclera procedure with or without an intraoperative dose of MMC or conjunctival autografting, the wound heals by a 4-stage process with appearance and proliferation of nongoblet epithelial cells in the first 3 stages and marked proliferation of goblet cells in stage 4. Application of the anti-proliferative agent MMC retards the rate at which the mixed keratinocyte-goblet cell phenotype of normal conjunctiva is restored, whereas conjunctival autografting expedites the regenerative process. Even 1 year after surgery, however, conjunctiva in the wound area was not completely normal in eyes treated with low-dosage of MMC. These results highlight the importance of longer follow-up to monitor the progress of the surgical wound after pterygium excision, particularly for patients treated with MMC.

**REFERENCES**

2. Tseng SCG. Staging of conjunctival squamous metaplasia by impression cytology.
TABLE 1. Characteristics of Pterygium Treatment Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of eyes</th>
<th>Number of patients</th>
<th>Age* (years)</th>
<th>Gender (male/female)</th>
</tr>
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<tbody>
<tr>
<td>Group 1</td>
<td>15</td>
<td>12</td>
<td>59.2 ± 15.6</td>
<td>7/5</td>
</tr>
<tr>
<td>Group 2</td>
<td>15</td>
<td>14</td>
<td>61.6 ± 13.6</td>
<td>7/7</td>
</tr>
<tr>
<td>Group 3</td>
<td>15</td>
<td>12</td>
<td>54.7 ± 13.4</td>
<td>8/4</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation (range); □ Kruskal-Wallis test; # Chi-square test.

Fig. 1. Time-courses of changes in nucleus-to-cytoplasm ratio of nongoblet epithelial cells after a bare-sclera procedure alone or with intraoperative mitomycin C (MMC) or followed by conjunctival autografting, compared to controls. The differences among the three treatment groups were compared at each follow-up.

Fig. 2. Time-course of changes in goblet cell density after a bare-sclera procedure alone or with intraoperative mitomycin C (MMC) or followed by conjunctival autografting, compared to controls. The differences among the three treatment groups were compared at each follow-up.