Histopathologic Features of Conjunctival Filtering Blebs

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Objectives: To characterize and compare the histological features of conjunctiva obtained during the repair of trabeculectomy and inadvertent blebs and to assess the effects of antifibrotic agents.

Methods: We used routine paraffin processing and light microscopy to examine conjunctival specimens from 28 eyes undergoing late bleb revision: 19 leaking trabeculectomy blebs, 5 nonleaking trabeculectomy blebs, and 4 inadvertent blebs. Quantitative analysis of histological characteristics included conjunctival epithelial thickness, stromal vascularity, squamous metaplasia, stromal inflammatory cell infiltrate, and goblet cell density. The type of surgery, presence of leaks, prior topical medication, and antifibrotic use were correlated with histological findings.

Results: Trabeculectomy blebs (leaking and nonleaking) showed decreases in overall epithelial thickness ($P<.02$), goblet cell density ($P<.001$), and vascularity ($P<.001$) compared with inadvertent bleb controls. Conjunctiva exposed to antifibrotics had fewer layers of epithelial cells than those with no prior exposure ($P<.05$).

Conclusions: Histological analysis of thin blebs excised following trabeculectomy demonstrated decreases in epithelial thickness and goblet cell density compared with inadvertent blebs. Both leaking and nonleaking trabeculectomy blebs exhibited decreases in localized stromal vascularity and increases in surrounding stromal vascularity compared with normal conjunctiva.

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CONJUNCTIVAL FILTERING blebs can develop aqueous leaks during the early or late postoperative period following a trabeculectomy. Adjunctive antifibrotics such as mitomycin C (MMC) and 5-fluorouracil (5-FU) have increased the prevalence of late-onset leaks (>3 months after surgery) that are apparently related to ischemia and the breakdown of the conjunctiva.

Both MMC and 5-FU reduce fibroblast proliferation in the subconjunctival space as well as in the Tenon capsule; they are used in trabeculectomy to prevent episcleral fibrosis of the filter. The pyrimidine base analog 5-FU is incorporated into replicating nucleic acid strands and is active only in the synthesis phase of the cell cycle. When applied via postoperative subconjunctival injections, it has been shown to significantly improve the success rate of trabeculectomy in eyes at a high risk for failure. Mitomycin C, an antibiotic secreted by Streptomyces caesposus, acts as an alkylating agent that inhibits the synthesis of protein and DNA. Mitomycin C also has potent antiangiogenic properties. Clinically, the use of either 5-FU or MMC increases the likelihood that blebs will be thin, cystic, and avascular: characteristics that increase the risk of conjunctival breakdown and aqueous leakage.

We present the histopathological findings, including a quantitative analysis of histologic features of conjunctival blebs, in 3 groups: inadvertent blebs following cataract surgery, nonleaking cystic trabeculectomy blebs, and late-leaking trabeculectomy blebs. Our hypothesis was that leaking blebs would show a decreased epithelial thickness, decreased goblet cell density, and decreased vascularity. We also postulated that the use of antifibrotics would result in greater decreases in these parameters. To our knowledge, this is the largest such series reported to date and the first that incorporates quantitative statistical analysis and a control and comparison group.
for the study and a waiver of informed consent from the institutional review board. We used 4 inclusion criteria after trabeculectomy: late onset of aqueous leak or thin, cystic, dysesthetic bleb; intact primary conjunctival wound closure; no related eye trauma; and no clinical signs of associated blebitis or endophthalmitis. Among these patients, 19 had discrete, active flow of aqueous through the bleb surface, which was demonstrated by Seidel testing using a fluorescein strip at the slitlamp. Nine had nonleaking dysesthetic blebs, including the 4 postcataract cases used as controls.

The light microscopy histopathological results of excised bleb samples were reviewed with the observer masked to the clinical and surgical characteristics of the patients. Specimens in all cases included the entire bleb and a surrounding rim of normal conjunctiva and underwent routine formalin fixation and paraffin processing. Step-sectioning at 5-µm intervals was performed, and the first 3 sections and every 10th section thereafter were stained with hematoxylin-eosin. Periodic acid–Schiff and Masson trichrome stains were also done on representative sections. All stained sections, approximately 15 slides per specimen, were studied by light microscopy.

Quantitative measurements included the following:

- Subconjunctival cystic areas (0=None, 1=minimal, 2=easily seen, 3=obvious in most of field, 4=almost total field)
- Squamous metaplasia (0=None, 1=just identifiable, 2=obvious, 3=in every field)
- Epithelial thickness: number of cell layers from basal to surface (counted in 3 areas: thickest, thinnest, and most cystic)
- Subconjunctival vascularity: small vessels, including capillaries present, compared with normal conjunctiva (1=none, 2=decreased, 3=normal, 4=minimal increase, 5=moderate increase, 6=vessels in every high-power field; ×40 objective; counted in the thickest, thinnest, and most cystic areas)
- Goblet cell density: number of cells per low-power field (×10 objective; counted in 5 fields and averaged)

Epithelial thickness and stromal vascularity measurements were obtained in each specimen in 3 areas: where the epithelium was thickest, where it was thinnest, and where it was adjacent to cystic areas. We chose this method to minimize bias from nonrandom sampling for measurements because of the variability of these parameters in different areas of the specimen. For measuring stromal vascularity, we created an objective scale of what constituted normal, avascular, and hypervascular conjunctiva by examining 5 normal conjunctival specimens selected from unrelated cases. The 3 groups were then compared with this scale and objectively graded for comparison. We performed statistical analysis using the paired t test when comparing 2 measures and performed analysis of variance when comparing more than 2 parameters. When an analysis of variance reached statistical significance, individual t tests were carried out using the appropriate Bonferroni corrections.

Table 1. Leaking vs Nonleaking vs Inadvertent Blebs

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD Epithelial Thickness</th>
<th>Mean ± SD Goblet Cell Density</th>
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<tbody>
<tr>
<td></td>
<td>Thickest Area, µm</td>
<td></td>
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<tr>
<td>Leaking trabeculectomy (n=19)</td>
<td>7.8 ± 2.6</td>
<td>2.9 ± 5.3</td>
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<tr>
<td>Nonleaking trabeculectomy (n=5)</td>
<td>7.9 ± 2.2</td>
<td>3.8 ± 4.8</td>
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<tr>
<td>Inadvertent bleb (n=4)</td>
<td>12.3 ± 6.7</td>
<td>20.3 ± 12.9</td>
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<td>P value</td>
<td>.02</td>
<td>&lt;.001</td>
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RESULTS

The 28 eyes fell into 2 general groups based on the presence (n=19) or absence (n=9) of active leaks. When separated according to surgery type, the 28 eyes fell into 3 groups: trabeculectomy with MMC or 5-FU (n=15), trabeculectomy without antifibrotics (n=9), and inadvertent blebs after cataract surgery (n=4). Statistical analyses compared the effects of 2 variables on histopathological appearance: bleb leakage and antifibrotic use. We performed the first analysis with respect to the presence or absence of leaks, regardless of surgery type or antifibrotic use. We did the second analysis with respect to the presence or absence of antimetabolite use, regardless of bleb leakage. In both cases, we used histological findings in the 4 inadvertent bleb specimens for comparison.

Analysis of prior topical medication usage was calculated by the number of medication years per person. The leaking trabeculectomy group showed a mean of 21±7.1, the nonleaking trabeculectomy group 24±6.4, and the inadvertent blebs 3±1.2. There was a statistically significant difference between the inadvertent bleb group and the trabeculectomy groups (P<.001). We saw no differences in squamous metaplasia or the presence of inflammatory cells in conjunctival stroma across groups.

LEAKING VS NONLEAKING TRABECULECTOMY BLEBS

The first analysis (Table 1) defined 3 groups according to the presence of leaks: 19 eyes with leaking trabeculectomy blebs (with or without antifibrotics), 5 eyes of nonleaking trabeculectomy blebs, and 4 inadvertent blebs. Findings of interest included epithelial thickness and goblet cell density.

When we compared the thickest areas from each eye, the epithelial layer was less thick in trabeculectomy eyes than in those with inadvertent blebs (P<.02). There was no difference in epithelial thickness between leaking and nonleaking trabeculectomy blebs.

Goblet cell density was significantly higher in the leaking trabeculectomy group than in the nonleaking trabeculectomy blebs (P<.001) (Figure 1 and Figure 2). We noted a trend for the leaking trabeculectomy group to have a lower goblet cell density than the nonleaking trabeculectomy group did, but it did not reach statistical significance.

ANTIFIBROTIC VS NONANTIFIBROTIC TRABECULECTOMY BLEBS

The next analysis (Table 2) defined groups according to use or nonuse of antifibrotic agents: 15 eyes had antifibrotic trabeculectomy blebs, 9 eyes had nonantifibrotic trabeculectomy blebs, and 4 eyes had inadvertent blebs. Once again, there were statistically significant differences in epithelial thickness and goblet cell density between groups. The epithelial thickness measured in the thickest area was thinner in trabeculectomy blebs with antimetabolites than in inadvertent blebs (P<.05). Epithelial thickness measured in the thinnest area was lower in trabeculectomy blebs with antifibrotics than without (P<.05). Goblet cell density was significantly reduced
in trabeculectomy (with or without antifibrotics) groups compared with the inadvertent bleb group (Figure 3).

Regarding conjunctival vascularity (Table 3), all of the trabeculectomy blebs had a mean vascularity of 1 by our definition (completely avascular) in the cystic area of the bleb. Vascularity was decreased when compared with a normal control vascularity of 3 (P<.001).

**COMMENT**

The development of late-onset filtering bleb leaks following glaucoma filtering surgery is a potentially serious complication. Full-thickness surgery, in the preantifibrotic era, had a reported incidence of late bleb leaks of 3.3%, which decreased significantly with the advent of partial-thickness, guarded trabeculectomy. With the use of antifibrotics, the incidence of leaks has risen to as high as 30% in some series but is usually in the 5% to 10% range. The reported frequency of leaks might vary according to the detection method and selection bias and especially whether the eye is pressurized at the time.

Normal bulbar conjunctiva includes a stratified squamous epithelium resting on a basal cell membrane with an underlying loose connective tissue stroma, the substantia propria. Normally, there are 4 layers of epithelial cells in the epithelium of the bulbar conjunctiva, increasing to 10 to 15 layers in the pericorneal region. The midepithelial and superficial cells progressively flatten as they migrate toward the surface in an orderly progression of maturation and normally do not keratinize. Goblet cells differentiate from the basal layer and typically appear within the middle epithelial layers, unless they reach the surface where they may express their contents. Goblet cells secrete mucus and are essential to the maintenance and protection of the ocular surface, which is the major portion of the deepest layer of the normal tear film. Epithelial cells are also partially responsible for mucus secretion. The substantia propria, or stroma, consists of a superficial lymphoid layer and a deeper, fibrous layer. The fibrous layer contains elastic and collagenous tissue, lymphatic channels, and blood vessels.

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**Table 2. Antimetabolite vs Nonantimetabolite vs Inadvertent Blebs**

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<th></th>
<th>Mean ± SD</th>
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<th>Mean ± SD</th>
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<tr>
<td></td>
<td>Epithelial Thickness</td>
<td>Epithelial Thickness</td>
<td>Goblet Cell Density</td>
</tr>
<tr>
<td></td>
<td>Thickest Area, µm</td>
<td>Thinnest Area, µm</td>
<td></td>
</tr>
<tr>
<td>Trabeculectomy</td>
<td></td>
<td></td>
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<tr>
<td>with MMC/5-FU</td>
<td>7.0 ± 2.1</td>
<td>2.1 ± 0.6*</td>
<td>3.4 ± 5.7</td>
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<td>(n = 15)</td>
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<tr>
<td>Trabeculectomy</td>
<td>9.2 ± 2.5</td>
<td>4.1 ± 2.1</td>
<td>2.2 ± 3.9</td>
</tr>
<tr>
<td>without MMC/5-FU</td>
<td>(n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadvertent</td>
<td>12.3 ± 6.7*</td>
<td>3.3 ± 1.3</td>
<td>20.5 ± 12.9*</td>
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<td>(n = 4)</td>
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Abbreviations: 5-FU, 5-fluorouracil; MMC, mitomycin C.

*P<.05.

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**Figure 1.** Conjunctival epithelium obtained during surgical repair of a nonleaking, inadvertent, filtering bleb (superior nasally) following cataract surgery. Goblet cells are prominent in the mid and upper levels of the epithelium. The stroma is normal with the exception of moderate numbers of migrating and marginating inflammatory cells, which is consistent with preoperative irritation and surgery (periodic acid–Schiff, original magnification ×10, Doheny Pathology #99367A).

**Figure 2.** Conjunctival epithelium obtained during repair of a leaking, inadvertent, filtering bleb following cataract surgery. The epithelium is irregular in thickness but attenuated in focal areas, and goblet cells are present. Scattered chronic inflammatory cells are present in the stroma (periodic acid–Schiff, original magnification ×10, Doheny Pathology #96-447A).

**Figure 3.** Conjunctival epithelium obtained during excision of a leaking, filtering bleb many months after a trabeculectomy that included intraoperative application of mitomycin C. The epithelium is attenuated and lacks goblet cells. Scattered chronic inflammatory cells are present in the stroma that appears edematous (periodic acid–Schiff, original magnification ×10, Doheny Pathology #00-238).
One previously published case series with 3 patients analyzed the histopathological appearance of late-onset leaking blebs following trabeculectomy with postoperative injections of 5-FU. These specimens showed focal areas of thinned or absent conjunctival epithelium with underlying stromal degeneration. The conjunctival substantia propria showed minimal scarring with focal collagen degeneration and scattered inflammatory cells.

A report of 7 cases of leaking blebs before the advent of antimetabolites included a case with ingrowth of the conjunctival epithelium into the subconjunctival tissue. Ingrowing conjunctiva can create a fistulous tract, which is responsible for aqueous leakage. In our 20 specimens of leaking trabeculectomy blebs, we did not identify any cases of epithelial ingrowth and a fistulous tract. Based on the differences in goblet cell density and epithelial thinning, we theorize that in our series, surface conjunctival breakdown, rather than epithelial ingrowth, was responsible for bleb leakage. Several publications present small clinicopathological series or individual case reports of nonleaking filtering blebs that were partially or totally excised because of hypotony, large size, or irritation. These overfiltering blebs showed deformed collagen, and few fibroblasts and inflammatory cells. Consistent with the clinically observed avascularity of many blebs, blood vessels were rarely visible.

Our study includes analysis of late-onset leaking trabeculectomy filtering blebs with or without antifibrotics, compared with nonleaking, thin, cystic trabeculectomy blebs and inadvertent blebs following cataract surgery. We observed that the conjunctiva adjacent to leaks and in thin, cystic, nonleaking blebs showed decreased epithelial thickness and decreased goblet cell density compared with inadvertent blebs. The trabeculectomy blebs also exhibited decreased vascularity compared with normal conjunctiva.

The breakdown of conjunctival surface integrity might be exacerbated by goblet cell loss, which might also explain the frequent aggravation of dry eye after trabeculectomy. Conjunctival specimens from cystic trabeculectomies (with or without antimetabolites) showed abnormal thinning of the epithelium and decreased conjunctival goblet cell density compared to inadvertent blebs. We expected to see greater goblet cell loss with the use of antifibrotics than without but did not. Perhaps any bleb with conjunctival thinning significant enough to develop a leak has a large degree of goblet cell loss. If we had compared goblet cell densities of nonleaking trabeculectomy blebs with and without antifibrotics, perhaps a difference would have been evident.

The use of MMC or 5-FU did result in decreased layers of conjunctival epithelium compared with trabeculectomy alone. However, antifibrotic use was not associated with greater decreases in conjunctival vascularity. All of the thin, cystic blebs showed pronounced avascularity in the central portion of the bleb but normal vascularity elsewhere.

Some of the observed differences between trabeculectomy and inadvertent blebs might come from differences in surgical procedure or preoperative exposure to medications. In particular, we found a significant difference in prior use of topical medications (and, by association, preservatives) in trabeculectomy patients vs cataract patients. The long-term use of medications might in itself cause a decrease in goblet cell density but probably not changes in epithelial thickness. In addition, the surgical procedure is different for trabeculectomy and cataracts, with greater posterior dissection of the conjunctiva in the former. However, all cases of inadvertent bleb formation were with a superior scleral tunnel incision, which indicates significant conjunctival dissection underlying the surgical incision and eventual bleb site. Another limitation of the current analysis is the relatively small number of inadvertent bleb controls.

Late-onset bleb leaks following filtration surgery can lead to devastating complications if left untreated. We routinely check for aqueous leakage with a fluorescein strip even in the late postoperative period because many of these leaks are occult. Conservative approaches include topical antibiotics and observation for possible spontaneous healing, aqueous suppression, tissue adhesive, autologous blood injection, and laser revision. In cases with a leak sufficient to cause anterior chamber shallowing or hypotony, we recommend partial or complete excision of the bleb with conjunctival advancement and, if necessary to close the conjunctival surface, autologous grafting or inclusion of amniotic membrane in the wound closure. In our experience with the latter, amniotic membrane is useful as a substrate to encourage conjunctival epithelial repair but is not a substitute for conjunctival graft or flap. This practice conforms with the study by Budenz et al, who evaluated the success of different methods of bleb repair. In cases with partial or complete melting of the original scleral flap, we used donor sclera or pericardial tissue grafting over the original flap remnants.

More judicious use of antifibrotics and alterations in surgical technique to create more diffuse, succulent blebs can probably reduce the incidence of late-onset bleb leaks following trabeculectomy. In any case, examining physicians should adopt a high index of suspicion regarding the potential for epithelial breakdown and late leaks and counsel patients accordingly.

**Table 3. Subconjunctival Vascularity**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD Cystic Area</th>
<th>Mean ± SD Thickest Area</th>
<th>Mean ± SD Thinnest Area</th>
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<tr>
<td><strong>Trabeculectomy</strong></td>
<td></td>
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<tr>
<td>with MMC/5-FU</td>
<td>1.0 ± 0.0</td>
<td>3.3 ± 1.2</td>
<td>3.1 ± 1.3</td>
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<tr>
<td>(n = 15)</td>
<td></td>
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<tr>
<td>without MMC/5-FU</td>
<td>1.0 ± 0.0</td>
<td>3.9 ± 0.9</td>
<td>3.4 ± 1.0</td>
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<tr>
<td>(n = 5)</td>
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<tr>
<td><strong>Inadvertent</strong></td>
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</tr>
<tr>
<td>(n = 4)</td>
<td>1.0 ± 0.0</td>
<td>4.25 ± 0.1</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td><strong>Normal controls</strong></td>
<td></td>
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<tr>
<td>(n = 5)</td>
<td>3.0 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>3.0 ± 0.0</td>
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<tr>
<td><strong>P value</strong></td>
<td>&lt;.001</td>
<td>.27</td>
<td>.61</td>
</tr>
</tbody>
</table>

Abbreviations: 5-FU, 5-fluorouracil; MMC, mitomycin C.

*Vascularity was measured on a scale from 1 to 6. A score of 3 was considered normal.
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