Modulation of Acute Inflammation and Keratocyte Death by Suturing, Blood, and Amniotic Membrane in PRK

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PURPOSE. To investigate the role of acute inflammation in keratocyte death, which may influence corneal haze after photorefractive keratectomy (PRK).

METHODS. Transepithelial PRK was performed on both eyes of 30 rabbits. Twenty-six rabbits were divided into 4 groups receiving autologous blood, suturing alone, suturing with amniotic membrane graft, or no treatment as the control. Twenty-four hours later, the ablated zone was analyzed for keratocyte death by TdT-dUTP terminal nick-end label (TUNEL) staining and transmission electron microscopy, for polymorphonuclear cell (PMN) infiltration by hematoxylin–eosin staining, and for oxygen radical–induced lipid peroxidation by malondialdehyde immunohistochemistry. The remaining four rabbits were subjected to PRK or mechanical scraping and analyzed immediately or after culturing for 24 hours.

RESULTS. Compared with the control group where TUNEL-positive keratocytes were found only in the superficial ablated stroma, blood application or suturing caused more and deeper keratocyte death and PMN infiltration (P < 0.05). The amniotic membrane graft group had less keratocyte death and PMN than the control or the suture group (P < 0.05 and P < 0.01, respectively). There was a strong correlation between keratocyte death and PMN infiltration (P < 0.01, correlation factor = 0.786). Transmission electron microscopy revealed that the majority of keratocyte death was due to necrosis. Amniotic membrane stroma trapped and prevented PMN infiltration into the stroma. Malondialdehyde-modified antigen was found on the ablated surface and around infiltrated PMN.

CONCLUSIONS. Transepithelial PRK causes oxygen radical–mediated lipid peroxidation on the superficial stroma and may contribute to superficial keratocyte death even in the absence of inflammation. Mechanical scraping leads to apoptosis without the participation of inflammation. Keratocyte death by necrosis spreads to the deeper part of the stroma and correlates with additional acute inflammation. Amniotic membrane precludes PMN infiltration and decreases lipid peroxidation and keratocyte death. Future studies are needed to discern whether prevention of inflammation-mediated keratocyte necrosis can reduce unwanted scarring caused by PRK. (Invest Ophthalmol Vis Sci. 2000;41:2906–2914)

Characteristics of initial cell death.

Corneal stromal wound healing after excimer laser photorefractive keratectomy (PRK) is a complex biological process. One undesirable outcome is the development of corneal haze and scar leading to refractive changes, loss of contrast sensitivity, or reduced visual acuity. The process of wound healing starts with injuries to epithelial cells, stromal keratocytes, and their surrounding stromal matrices. The source of injuries can directly come from thermal and acoustic damage caused by the laser irradiation and indirectly from ensuing inflammation. One way of exploring such a complex wound healing response is to understand the pathogenic role of initial cell death.

Two types of cell death have been recognized. Apoptosis, or programmed cell death, which exhibits characteristic morphologic changes, features a lack of inflammation, and plays an important role in a range of biological processes, including differentiation, development, response to infection, and wound healing. In contrast, necrosis, which is characterized by a rapid release of cellular contents, is invariably associated with, and frequently incites, inflammation. Wilson et al. first provided evidence that removal of the corneal epithelium by mechanical scraping can induce keratocyte apoptosis. This finding explains why there is an early loss of keratocytes in the anterior corneal stroma after epithelial scraping, an intriguing phenomenon first recognized by Dohlman et al. and subse-
In PRK, epithelial removal can be achieved by either mechanical scraping or transepithelial excimer ablation. Apoptosis of keratocytes caused by the mechanical scraping form of PRK has been recognized, and its extent is greater than that caused by transepithelial PRK. It is well established that a major component of post-PRK corneal haze is the enhanced backscattering of light from highly reflective intrastromal, migratory fibroblasts, which repopulate the zone of surgically induced keratocyte loss. Moller-Pedersen and coworkers, using in vivo confocal microscopy in a rabbit model, have recently reported that the initial keratocyte loss does not correlate with the subsequent increase in corneal haze. These new findings raise the issue of whether corneal haze is causatively linked with apoptosis and whether apoptosis alone accounts for the total keratocyte loss.

One unsettling variable may be acute inflammation, which varies among different individuals during the early stage of wound healing. Hayashi et al. noted that the level of conjugated diene, a product of lipid peroxidation, is higher in the photoablated superficial stroma and postulated that this change is caused by oxygen radicals generated by infiltrating PMNs. However, it remains unclear whether such lipid peroxidation can also be caused by laser irradiation alone, whether PMNs indeed contribute to keratocyte death, or whether keratocyte death is mediated by necrosis or apoptosis when there is acute inflammation. The issue of acute inflammation is worth noting because its reduction by amniotic membrane transplantation has been correlated with reduced haze in a rabbit PRK model. In the present study we sought to investigate these questions in a rabbit model of transepithelial PRK. Acute inflammation was experimentally amplified by the application of autologous blood or 10-0 nylon sutures but reduced by amniotic membrane graft. Lipid peroxidation was monitored by immunohistochemistry with an antibody against malondialdehyde (MDA)-modified antigens. The nature of cell death was evaluated by both TdT-dUTP terminal nick-end label (TUNEL) staining and transmission electron microscopy.

METHODS

Animals and Surgery
All experiments were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and based on a protocol approved by the Animal Research Committee of the University of Miami. Adult male New Zealand albino rabbits, weighing 2 to 3 kg, were anesthetized with an intramuscular injection of 50 mg each of xylazine hydrochloride and ketamine hydrochloride.

Blood Application, Suturing, and Amniotic Membrane Graft
Both corneas of 26 rabbits received transepithelial PRK, consisting of 47 μm, 6-mm-diameter phototherapeutic keratectomy for epithelial removal and 9 D, 5-mm diameter PRK for refractive cut using a Kera Technology dual-beam 193 nm scanning laser (Model IsoBeam D200; Kera Technology, Orlando, FL). They were subdivided into the following four groups. Seventeen eyes were used as the control group without any additional treatment. Twelve eyes received application of autologous whole blood for 5 minutes immediately after PRK, and the blood clot was removed thereafter (the blood group). Nine eyes received 5 interrupted 10-0 nylon sutures placed outside of the ablation zone and also served as a control for the amniotic membrane graft group (the suture group). Nine eyes were covered with an 8-mm disc of preserved human amniotic membrane (kindly provided by Bio-Tissue, South Miami, FL), with the stromal side facing the corneal surface, on the ablated surface with five interrupted 10-0 nylon sutures placed outside of the ablation zone (the AM graft group). To prevent the amniotic membrane from being dislodged by the movement generated by the nasal nictitating membrane, we placed two sutures instead of one in that quadrant. All rabbits were killed 24 hours later.

Immediate Suturing
Four additional rabbits received transepithelial PRK in one eye and the fellow eye received either removal of a 6-mm-diameter area of the central corneal epithelium by mechanical scraping in two rabbits or no treatment as a control in the other two rabbits. These rabbits were killed immediately, and their cornescleral buttons were excised, rinsed in Hank's balanced salt solution with gentamicin (50 μg/ml), and cultured with the epithelial side up in a 30-mm petri dish containing supplemental hormonal epithelial media (SHEM; Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium with 5% fetal bovine serum, 5 μg/ml insulin, 100 ng/ml cholera toxin, 10 ng/ml epidermal growth factor, 0.5% dimethyl sulfoxide, and 50 μg/ml gentamicin). The dishes were incubated at 37°C in humidified air with 5% CO₂ for 24 hours before analysis.

Histology and TUNEL Staining
After euthanization, cornescleral rims were excised, flattened by four radial relaxing cuts, and bisected into two halves through the center of the ablated center at the 12 and 6 o’clock axis. Bisected corneas were embedded in OCT compound (Sakura Finetek, Torrance, CA) for frozen sections. Serial 6-μm-thick sections were cut, and sections were stained with hematoxylin and cosin (H & E) alone until both epithelial edges of the ablation zone were identified together with the ablation zone in the center. From there on, the 5th section was selected for the H & E staining alone, and the 6th section was stained for the TUNEL assay. TUNEL staining, which detects cell death by apoptosis predominantly and necrosis to a lesser extent, used an in situ fluorescein-based Apoptosis Detection Kit and followed procedures recommended by the manufacturer (Oncor, Gaithersburg, MD). An epifluorescence microscope (Carl Zeiss, Thornwood, NY) with appropriate excitation and emission filters was used to photograph all propidium iodide-stained cell nuclei (as red fluorescence) and the fluorescein-stained apoptotic nuclei (as green fluorescence). The counting of cells was performed (by WCP) and verified (by SCGT) in a masked fashion. The H & E slide was used for counting total number of PMNs; the TUNEL slide was used for counting apoptotic cells. The counting was conducted under 400× magnification of a high power field (HPF), which covers the stromal area bordered by the epithelium superiorly and the endothelium inferiorly. A total of four HPFs, with each tangential to the other and together spanning the majority of the ablated stroma, were counted for each slide.
TUNEL-positive cells were distinguished as keratocytes by their slender and scanty cytoplasm and round nuclei, but as PMNs by their multi-lobed nuclei. When in doubt, we performed on the 4th section fluorescein-based TUNEL staining without adding propidium iodide and double-labeled it with a rhodamine-conjugated anti-vimentin antibody, which stains mesenchymal cells including fibroblasts and keratocytes. If TUNEL-positive cells (green nuclear fluorescence) also showed vimentin-positive staining (red cytoplasmic fluorescence), these cells were counted as apoptotic keratocytes (an example is given in Figs. 5G and 3H). For identifying PMNs, we relied primarily on the nuclear characteristics as described above (see Figs. 4C and 4D for an example). When in doubt, on the 4th section we also performed fluorescein-based TUNEL staining without adding propidium iodide and double-labeled it with a rhodamine-conjugated anti-myeloperoxidase antibody (Sigma, St. Louis, MO), which stains the granules of PMNs.

**Transmission Electron Microscopy**

The other half of the specimen was fixed in 2% glutaraldehyde and processed for conventional transmission electron microscopy. Samples were rinsed in 0.1 M phosphate buffer (pH 7.3), postfixed in 1% osmium tetroxide and embedded in Epon. Semi-thin sections were stained with 1% methylene blue, 1% Azure II, and 1% borax. Ultrathin sections were cut and conventionally stained with uranyl acetate and lead citrate and then examined with a Philips EM 420 electron microscope (Philips, Eindhoven, The Netherlands). For the purpose of determining the proportion of apoptosis and necrosis, the entire section of the central ablated zone was sampled, and all abnormal cells were photographed from the ablated surface to Descemet’s membrane.

**MDA Immunohistochemistry**

To evaluate the involvement of oxygen radical–mediated lipid peroxidation, immunohistochemical staining was performed with a rat polyclonal antibody raised against MDA-modified albumin using a Vectastain Elite ABC kit in combination with an affinity-purified biotinylated goat anti-rat IgG secondary antibody (Vector, Burlingame, CA) according to the manufacturer’s recommendation, followed by color reaction with diaminobenzidine tetrahydrochloride as the peroxidase substrate. We developed such an antibody using a previously reported protocol by Hall et al. In brief, MDA was prepared by acid-catalyzed hydrolysis of MDA bis dimethyl acetal (Sigma). This solution was then mixed with rat serum albumin (RaSA; Sigma) at 57°C for 72 hours. The reaction mixture was then dialyzed against a total 4 liters of phosphate-buffered saline with 6 changes over a 72-hour period. Six rats were immunized by an intramuscular injection of 0.1 mg of MDA–RaSA antigen mixed with complete Freund’s adjuvant (Sigma). Ten days later, 0.05 mg of MDA–RaSA antigen was similarly administered in incomplete Freund’s adjuvant (Sigma) and repeated twice at 10-day intervals. The specificity of these antisera obtained at 7 days after the last injection was tested by demonstrating their immunoreactivity with MDA–RaSA but not with unmodified RaSA, using an enzyme-linked immunosorbent assay identical to that previously described. Furthermore, its specificity was identical to that of a similar antibody from a commercial source and confirmed by a well-described rat model of mid–cerebral artery occlusion–induced infarction, which is known to produce MDA-modified antigens via lipid peroxidation (unpublished observation by B. Watson, PhD, Department of Neurology, University of Miami, Florida, 1998).

**Statistical Analysis**

Statistical comparisons between groups were performed using the Mann–Whitney and Wilcoxon signed rank tests with the help of the Department of Biostatistics, Bascom Palmer Eye Institute, University of Miami. P < 0.05 was considered statistically significant. All results are expressed as mean ± SD.

**RESULTS**

**Effects of Autologous Blood, Suturing, and Amniotic Membrane Graft on Acute Inflammation and TUNEL-Positive Cell Infiltration**

The total number of TUNEL-positive cells was measured and compared in the stroma (Fig. 1A). Compared with the control group, the blood application group had significantly more TUNEL-positive cells (P < 0.05). Compared with the suture group, the AM graft group had significantly fewer TUNEL-positive cells (P < 0.01). These TUNEL-positive cells were further separated into keratocytes and PMNs, based on nuclear morphology. Cells were considered keratocytes if they were slender with minimal cytoplasm and round nuclei, and PMNs if they had prominent multi-lobed nuclei. This criterion was further confirmed by double labeling with anti-vimentin antibody because the former express vimentin in the cytoplasm but the latter do not (see Figs. 3G and 3H). Based on this criterion, the TUNEL-positive keratocytes were counted and compared (Fig. 1B). Compared with the control group, the blood application and suture groups had significantly more TUNEL-positive keratocytes (P < 0.05). Compared with the suture group, the AM graft group had significantly fewer TUNEL-positive keratocytes (P < 0.01). The total number of PMNs was counted from H & E slides and was compared among groups (Fig. 1C). Compared with the control group, the blood application and suture groups had significantly more PMNs (P < 0.05), whereas the AM graft group had significantly fewer PMNs (P < 0.05). Compared with the suture group, the AM graft group had significantly fewer PMNs (P < 0.01).

When the number of TUNEL-positive keratocytes was compared with the number of PMNs, we noted that there was a strong correlation between keratocyte death and PMN infiltration (Fig. 2, P < 0.01, correlation factor = 0.786). In other words, the higher the PMN counts in the stroma, the higher the amount of keratocyte death.

The above trend and relationship between keratocyte death and acute inflammation are further illustrated in Figure 3, with corresponding slides with in situ TUNEL staining and H & E staining, respectively, of a representative example of each group. In the control group, H & E staining showed a minimal amount of PMNs in the superficial ablated surface (Fig. 3A). TUNEL staining showed positive cells (stained with green fluorescence) at the ablated surface and occasionally the superficial stroma (Fig. 3B; the latter is indicated by arrowheads). In the blood application group, H & E staining showed more cells, which under higher magnification indicated PMNs (see below), in the superficial and deep stromata of the ablated area (Fig. 3C; the latter is included by brackets). TUNEL staining showed positive cells at the surface and in the superficial and
Compared with the suture group (Figs. 3E and 3F), the AM graft group showed a reduced number of PMNs and TUNEL-positive cells in the stroma (Figs. 4A and 4B). In this particular example, only two keratocytes were TUNEL-positive (indicated by arrowheads, Fig. 4B), whereas the remainder of TUNEL-positive cells was found in the amniotic membrane itself (also see the higher magnification shown in Figs. 4C and 4D). This notion was confirmed by partially detaching the amniotic membrane from the underlying corneal stroma before embedding and staining (Fig. 4E). In the latter situation, the majority of the TUNEL-positive cells were noted in the amniotic membrane predetached from the ablated surface before embedding and corresponded to PMNs. As a control, cells normally present in the amniotic membrane did not reveal any TUNEL positivity before transplantation (Fig. 4F). Under high power magnification (Fig. 4C), we confirmed that PMNs in contact with the amniotic membrane stromal matrix underwent cell death (i.e., mostly exhibiting green florescence) were more frequent than those in contact with the basement membrane side (i.e., mostly exhibiting red florescence; Fig. 4D).

Investigation of Cell Death by Transmission Electron Microscopy

Compared with the morphology of the normal keratocyte that was present in the unablated region of the control cornea (Fig. 5A), numerous keratocytes found in the superficial stroma of the blood group showed disruption of the cell membrane and intracellular organelles and dissolution of the chromatin matrix (Fig. 5B). Throughout the entire section from the superficial stroma to the deep posterior stroma, we did not find chromatin condensation characteristic of apoptosis in any of the keratocytes surveyed. Scattered in the superficial stroma were some multinucleated PMNs, some of which were adjacent to the keratocytes showing the above degenerative changes (Fig. 5C). Compared with the PMNs in the stroma of the blood group (Fig. 5C) or the suturing group (not shown), PMNs trapped in the amniotic membrane stroma showed marked vacuolation of...
the cytoplasm, and swollen mitochondria and chromatin condensation at the nuclear membrane characteristic of apoptosis (Fig. 5D, see arrows).

**Study of Lipid Peroxidation**

Immunohistochemical staining for MDA-modified antigen was then performed to study the damaging effect mediated by oxygen radical-induced lipid peroxidation. A positive finding, evidenced by reddish-brown staining, was noted on the ablated surface of the control group immediately (not shown), at 4 hours (not shown), and at 24 hours (Fig. 6A) after photobleaching. In contrast, such positive staining extended to the deep stroma and was exhibited as diffuse dots, corresponding to the PMN infiltration, in both the blood application and suture groups (Figs. 6B and 6C, marked by brackets). Such a correlation was confirmed with an antibody to myeloperoxidase, a marker for PMNs (not shown). In contrast to the suture group with a high MDA activity in the deep stroma, MDA-positive staining was detected only in PMNs that had infiltrated and were trapped in the grafted tissue.

**FIGURE 3.** Correlation between PMN infiltration and cell death among the control, blood application, and suture groups. PMN infiltration was detected by H & E staining (**left column**), and cell death was detected by TUNEL staining (**right column**) among the control group (**A, B**), blood application group (**C, D**), and suture group (**E, F**) at 24 hours after ablation. The **black arrows** and **white arrows** are used in the H & E and TUNEL slides, respectively, to mark the epithelial growing edge. **White V-shaped arrowheads** indicate TUNEL-positive green fluorescent nuclei on the ablation surface and **arrowheads** indicate TUNEL-positive green fluorescent nuclei in the superficial and deep stromata of the ablated area. Brackets include PMN infiltration in the H & E slide and corresponding TUNEL-positive cells. TUNEL staining without propidium iodide shows green fluorescent dead cells (**G**) and double staining with rhodamine-conjugated anti-vimentin antibody. Among them, the **arrowhead** points at a wave-shaped green fluorescent apoptotic nucleus of a keratocyte, for which the identity is proven by the positive vimentin staining. In contrast, the **arrow** points at a small, round-shaped green apoptotic nucleus of a PMN, for which the identity is proven by the negative vimentin staining. Photographs were taken at the same magnification for (**A**) through (**F**) and the higher magnification for (**G**) and (**H**). Scale bar, (**A** and **G**) 50 μm.
amniotic membrane, whereas the ablated stroma did not show any staining (Fig. 6D).

The control cornea analyzed immediately after transepithelial PRK did not reveal any TUNEL-positive cells (Fig. 7A), whereas a positive MDA staining similar to that shown in Figure 6A was already noted on the ablated surface. To determine whether such lipid peroxidation leads to acute inflammation and whether subsequent acute inflammatory response played a role in inducing the expression of TUNEL-positive cells, the cornea was removed immediately after transepithelial PRK and cultured for 24 hours. We noted that TUNEL-positive cells became detectable on the ablated surface in a pattern similar to that shown in Figure 6B, whereas positive MDA staining similar to that shown in Figure 6A was still observed (not shown). This result indicated that cell death of superficial keratocytes progressed without tissue inflammation after transepithelial PRK. Under this circumstance, TUNEL-positive cells were not noted in the deeper part of the stroma as shown in Figures 3D and 3F, indicating that the spread of keratocyte death to the deep stroma was a result of acute inflammation incited by blood application or suturing. For comparison, corneas analyzed after 24 hours of culturing after mechanical scraping showed a marked intensity of TUNEL-positive apoptosis in the superficial and deep stromata (Fig. 7C). This result supports the notion that keratocyte death owing to apoptosis in the deep stroma can occur after mechanical scraping without a PMN response. In the latter situation, no MDA staining was noted (Fig. 7D), further confirming that MDA-altered tissue changes were specific to excimer laser irradiation.

**DISCUSSION**

This study demonstrated that transepithelial PRK alone caused minimal and superficial (i.e., limited to the ablated surface) keratocyte death in rabbits. Besides thermal and acoustic effects that have been reported as potential insults, lipid peroxidation is another excimer laser irradiation-facilitated insult that may damage the stroma. Lipid peroxidation may lead to additional tissue damage and incite inflammation. In the present study, we noted that there was a low grade of PMN infiltration in the ablated stroma of the control group at 24 hours (Fig. 2A). We also showed that TUNEL-positive keratocytes were not present immediately but developed 24 hours later after culturing (Fig. 7), indicating that superficial keratocyte death after transepithelial PRK can occur without the...
participation of PMNs. Therefore, it is likely that acute inflammation, even if triggered by lipid peroxidation, does not lead to keratocyte death, for which the mechanism has been determined to be apoptosis.4,9 Future studies are needed to resolve whether lipid peroxidation alone is the sole cause of such superficial keratocyte death as suggested by a previous article.21

One way to induce keratocyte death in the deeper part of the stroma by transepithelial PRK is to create acute inflammation caused by the application of autologous blood or corneal sutures. Once PMNs appear in the deeper part of the stroma, they can release agents to cause more damage to cells and their matrices. Oxygen radicals generated by PMN respiratory burst or degranulation are such agents.22,23 In this study we did find positive MDA staining in the region where there was intense PMN infiltration, supporting the idea that lipid peroxidation by oxygen radicals released by PMNs indeed can occur as speculated by Hayashi et al.14 and reported by others.19,20 Future studies are still needed to resolve whether oxygen radicals, lipid peroxidation, or both are directly responsible for such keratocyte death in the deeper part of the stroma.

Another way to induce more keratocyte death in the deeper part of the stroma is by mechanical scraping to remove the epithelium as demonstrated in Figure 7C. This result is consistent with a recent report.40 It has been reported that mechanical scraping–PRK induces more inflammation than transepithelial PRK.24 This study further showed keratocyte death even after the cornea was removed immediately for 24 hours of culturing. This result clearly indicates that this process of cell death, characterized as apoptosis,9 does not require the participation of PMNs. Because mechanical scraping did not generate MDA staining, we also conclude that lipid peroxidation is not involved.

In contrast to the apoptosis explanation for keratocyte death caused by mechanical scraping, necrosis was identified as the major mechanism for keratocyte death associated with acute inflammation (Fig. 5). In this study we distinguished keratocytes from PMNs by nuclear morphology and double-labeling; we cannot be absolutely certain that the process of counting TUNEL-positive keratocytes may have included some PMNs. Furthermore, TUNEL assay detects cell death by apoptosis predominantly and necrosis to a lesser extent. Therefore, necrotic keratocyte death induced by PMNs might have been underrepresented in this study. Moreover, our study draws a correlation but not a causative relationship between inflammation and keratocyte loss. Necrosis involves the rapid release of intracellular contents and is invariably associated with inflammation and the incitement of inflammation. It has been reported that PMNs first appear at the ablated margin at 6 hours and increase at 24 hours after PRK.25 The finding of intense MDA staining around the infiltrating PMNs in the stroma is important because oxidized lipids act as chemoattrac-

Figure 5. Transmission electron microscopy. Normal keratocytes were found in the unablated control cornea (A; magnification, ×6300), whereas disruption of cell membrane, cytoplasm, and nuclear matrix was noted in the necrotic keratocytes in the ablated superficial stroma of the blood group (B; magnification, ×4000). A healthy PMN was found adjacent to the necrotic keratocytes (C; magnification, ×8000); chromatin condensation (arrow C), swollen mitochondria (arrow M), and vacuolation of cytoplasm (arrow V) were noted in PMNs adherent to the amniotic membrane stroma (D; magnification, ×8000; †, membrane stroma).
tants and may help amplify inflammation. It remains to be determined whether keratocyte necrosis, but not apoptosis, is responsible for subsequent fibroblast activation and transformation into scar-forming myofibroblasts.

One effective measure to suppress keratocyte death is to use amniotic membrane as a temporary patch. Within 24 hours, the suture-induced PMN influx from the denuded stroma was mitigated, and as a result keratocyte death was significantly reduced and MDA staining was limited to the PMN-infiltrated amniotic membrane. The finding that PMNs are excluded by amniotic membrane has previously been observed. We further noted that more TUNEL-positive PMNs were adherent to the amniotic membrane stromal matrix than those adherent to the basement membrane. Transmission electron microscopy revealed these PMN undergoing apoptosis (Fig. 5). Because facilitation of PMN apoptosis is an effective and physiological way of suppressing tissue inflammation, future research is needed to determine whether amniotic membrane stromal matrix promotes PMN adherence and subsequent apoptosis. Such an action better explains how amniotic membrane transplantation reduces inflammation as a graft and reduces PRK-induced corneal haze in rabbits as a temporary patch used for 2 days or 1 week. Research in this direction may uncover other new applications.
References

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