Collagen Shields as a Vehicle for Collecting and Studying Migratory Cells on Human Corneas

Sharon D. Geasey, Manuel del Cerro, Michael D. DePaolis, James V. Aquavella, and Ralph S. Viola

Collagen shields have been studied in the enhancement of the initial healing of epithelial defects, as an adjunct in the treatment of dry eye, and as a reservoir and delivery system for topical ocular medications. The authors used collagen shields to collect information on the numbers and types of free cells populating the normal and postoperative ocular surface. In addition, correlative microscopic techniques were used to study details of the mechanisms responsible for the dissolution of the shields when applied to the human eye. Collagen shields were applied as a bandage lens on the eyes of patients who underwent extracapsular cataract extraction (n = 10) or penetrating keratoplasty (n = 10) and on normal volunteers (n = 10). The shields were collected at the 1-day postoperative examination and fixed in aldehyde mixtures. Specimens then were processed for correlative light (LM), transmission (TEM), and scanning (SEM) microscopy. Cell accumulation was shown by SEM on both anterior and posterior shield surfaces. Cell adherence occurred primarily on the posterior shield periphery for approximately 2 mm, with the central zone relatively clean. Both LM and TEM evaluation revealed cell counts ranging from 0.066 cells/10^4 mm^2 (standard deviation, ± 0.256) in healthy eyes compared with shields placed on postoperative eyes (194.25 ± 7.32 cells/10^4 mm^2). Various correlative microscopy techniques revealed that most cells were polymorphonuclear leukocytes with a low number of other hematogenous (lymphocytes and monocytes) and exfoliated epithelial cells. The ability of collagen shields to serve as a substrate for cellular accumulation may allow them to serve as a device for studying the dynamics of migratory cells on the ocular surfaces of healthy and inflamed eyes. It may also aid in documenting the role these cells play in the dissolution of the shields. Invest Ophthalmol Vis Sci 33:298–303, 1992

Materials and Methods

Patients who underwent extracapsular cataract surgery (n = 10), those who underwent penetrating keratoplasty (n = 10), and normal volunteers (n = 10) were fitted with 24-hr cross-linked collagen shields (Bausch and Lomb, Clearwater, FL) as bandage lenses. The shields were removed carefully and collected 24 hr after application. They were bisected, and one half of each shield was fixed in 2% glutaraldehyde and 2% paraformaldehyde solution in cacodylate buffer. They were processed for correlative light (LM), transmission (TEM), and scanning electron microscopy (SEM). This process, after initial fixation, included a 1-hr immersion at 4°C in 2% osmium tetroxide, rinsed in 0.85% NaCl, and then dehydrated in a graded ethanol series. The other half of the shield was fixed in 4% paraformaldehyde solution in cacodylate buffer. They were processed for correlative light (LM), transmission (TEM), and scanning electron microscopy (SEM). This process, after initial fixation, included a 1-hr immersion at 4°C in 2% osmium tetroxide, rinsed in 0.85% NaCl, and then dehydrated in a graded ethanol series. The other half of the shield was fixed in 4% paraformaldehyde solution for histochemical studies. An avidin–biotin complex kit (Vector, Burlingame, CA) with a monoclonal mouse antineutrophil primary antibody (Chemicon, Temecula, CA) was used for the immunoperoxidase staining. Care was taken to prepare the sample properly by quenching the endogenous peroxidase activity with 3% hydrogen peroxide for 5 min before starting the immunochemical procedure. The immunochemical
procedure was used according to the manufacturer's instructions, except for an incubation time of 1 hr in primary antibody diluted in 1.5% normal blocking serum and then washing in phosphate-buffered saline three times, each for 5 min. To test the specificity of the reaction, two sets of blood smears (n = 10) were handled identically, but specific binding steps were eliminated. For example, the primary antibody was

Fig. 1. Light microscopic view of a postoperative shield. Numerous cells identified as polymorphonucleocytes (PMNs) adhere to the shield surface. Toluidine blue stain (×750).

Fig. 2. Light microscopic view of a tangential cut through a postsurgical shield showing PMNs (arrowheads) interspersed with masses of dissolving collagen matrix (C), and exfoliated epithelial cells. Other mononuclear cells are present in low numbers. Toluidine blue stain (×750).
withheld from the first control, the secondary antibody from the second control, the avidin–biotin complex from the third control, and so on, until the final control, which was processed through every step and then evaluated for the staining of neutrophils. Analysis of the shields and cells involved observations of surface characteristics, cell distribution on the shields, and external and internal characteristics of both the cells and shields. Cell counts using SEM and LM were completed by measuring square sample areas (100 μm in size; area, = 10,000 μm²) then quantifying the cells.

Results

The shields placed on normal volunteers were relatively free of cells compared with those placed on postoperative eyes. Most cells from both normal and postoperative subjects were found on the posterior shield surface, and they were located primarily toward the periphery of the shield, leaving the central area relatively cell free. Actual cell counts of the normal volunteer shields revealed 0.066 cells/10⁴ μm² (± 0.256). On the postoperative shields, we found 194.25 cells/10⁴ μm² (± 7.32). Using LM, most cells adhering to the posterior surface of the shield were identified initially as polymorphonuclear leukocytes (PMNs, Fig. 1). Small subpopulations of other cell types, 2% lymphocytes, 1% monocytes, and exfoliated epithelial cells were observed intermixed between the PMNs and dissolving collagen matrix. This may indicate further the interaction between the shield and the cells adhering to it (Fig. 2). Immunocytochemical evaluation was done using a primary antibody specific for neutrophils to confirm the nature of the cells on the shields. Smears of human blood used to test the specificity of the antibody showed a positive staining of only the PMNs. All other cells were counterstained and identified with Wright's Giemsa stain. The postoperative and volunteer shields underwent the same staining procedure, resulting in clearly stained PMNs with little-to-no background staining (Fig. 3). Both experimental groups and the different surgical procedures showed PMNs to be the overwhelming (97%) major cell type, and our histochemical results confirmed this. Because populations of other cell types were virtually nonexistent in the presence of the PMNs, immunohistochemical procedures were not done to identify them further.

Interesting morphologic aspects of the cells were revealed by TEM. On first observation, it could be seen that the PMNs contained many intracellular vacuoles filled with a substance of the same electron density as amorphous collagen. This observation suggests that the cells not only adhere to the shield, but they also may be phagocytizing the shield itself. Closer examination of the material surrounding the intact PMNs revealed cellular debris, indicating that the cells are undergoing various stages of disintegration and releasing their contents into the collagen matrix (Fig. 4). Correlative SEM also showed the presence of leukocytes and epithelial cells (Fig. 5). The irregularity of the shield edge (Fig. 6) may indicate the dissolution of the shield, not only by tear enzymes and the mechanical action of the eye lid, but also by cellular activity.

Discussion

The observation of cells intermixed with the collagen matrix indicated that the shields were acting as a substrate for cellular adherence and colonization. In addition, using TEM, we observed numerous PMNs undergoing cell dissolution while embedded in the collagenous matrix of shields worn by postoperative patients. This showed the existence of a highly dynamic process of cell extravasation, activation, and final disintegration of the PMNs patrolling the human ocular surface during the immediate postoperative period. Vacuoles filled with electron-dense material, com-

Fig. 3. Immunoperoxidase staining on the postoperative shield reveals only PMNs stained (×350).
Fig. 4. Cross-sectional TEM view of a postoperative shield. PMNs and cellular debris (CD) are present, interspersed between collagen matrix masses (×5510). Inset: Vacuoles (V) within the PMNs are filled (×17,530) with electron-dense material similar to that of the surrounding collagen.

parable to the surrounding collagen evident by TEM, suggest that, not only are the cells adhering and dissolving into the collagen shield, but they also may be phagocytizing the shield. In concordance with our microscopic findings, other investigators have shown that PMNs contain different proteinases that degrade collagen types I-IV and XI. Furthermore, positive correlation between infiltration of PMNs in the cornea and collagen degradation has been reported, which may support the finding that the amorphous material found in the vacuoles of these cells is phagocytized collagen matrix. It also has been observed in an evaluation of the role of PMNs in stromal ulcerations that there are many collagenases and other hydrolases in the cytoplasmic granules of the PMNs, which can cause corneal ulcerations and degradation of the extracellular matrix of the corneal stroma.

This increase in inflammatory cells that can cause stromal disintegration may correlate with the dissolution of the shield by collagenases in the cells phagocytizing the shield. The irregularity of the shield edge also may indicate that dissolution is a function, not only of eyelid mechanics and cellular activity, but also of ocular enzymes. This is consistent with clinical observations that the dissolution rate generally was higher in postoperative eyes with increased inflammatory cell counts compared with that of the normal volunteers. In addition, PMNs play a role in stromal matrix destruction in corneal ulcerations. When hydrophilic bandage lenses are used as protective devices after alkali burns, they prevent PMN infiltration of the stroma and subsequent ulceration. This is consistent with our observation that most free cells remain on the periphery of the shield, suggesting a
protective role of the shield by restricting the migration of the inflammatory cells to the incision site. Studies show that collagen shields aid in corneal healing\textsuperscript{1-4,11,16-21} by sequestering these cells.\textsuperscript{22}

Our correlative microscopic results showed that the therapeutic collagen shields used on human patients are an efficient, inexpensive, and safe device for collecting samples of the free cells populating the corneal surface. Bandage collagen shields offer a unique means of studying the cell populations that are active on the normal and postoperative ocular surface and analyzing their characteristics and their responses to surgical or pharmacologic treatments.

**Key words:** collagen shield, cell migration, cornea, inflammatory response, electron microscopy

**References**