Could Apoptosis Change the Way We DO PRK?
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Just when we thought photorefractive keratorectomy (PRK) had achieved a level of safety and predictability in correcting refractive errors in our patients, new wounding healing information regarding apoptosis (programmed cell death) surfaces to show us that the way we remove the corneal epithelium matters a great deal in how the corneal stroma responds to our treatment. Excimer laser manufacturers have previously adjusted the algorithms for PRK to include anti-central island software, multizone treatments, and astigmatism conversion factors. LASIK surgeons have created their own algorithms for correcting refractive errors under a flap because of perceived benefits of LASIK over PRK, including the absence of central epithelial and stromal wound healing. Now PRK may be in store for yet another change in algorithms to compensate for the regional variation in epithelial thickness when performing transepithelial ablation.

Kim and coauthors (pg. 526-533), describe the relative absence of kerocyte apoptosis following transepithelial excimer laser ablation compared to transepithelial ablation followed by mechanical scraping prior to excimer laser photorefractive keratotomy. This relative paucity of apoptosis with transepithelial ablation means that fewer kerocytes die and fewer need to be replaced by activated kerocytes that produce collagen and proteoglycans that are involved in wound healing, and that lead to regression and haze. These results suggest that minimizing apoptosis would improve the outcome and stability of excimer laser PRK by reducing the variable of wound healing which is experienced differently among patients. This leads to the question: Could apoptosis change the way we do PRK?

WHAT IS APOPTOSIS?

When a cell undergoes ordinary cell death, the cell contents including the proteolytic enzymes are released into the surrounding area, inciting an inflammatory response and causing further damage to adjacent cells and tissue. Apoptosis differs in that it is programmed cell death, and the cells die in a complex and controlled way without damage to surrounding corneal tissue.

Kerocytes that undergo apoptosis do so in response to corneal epithelial injury. The injured cells release cytokines such as interleukin (IL)-1 and FAS/FAS ligands that mediate this involution process of cell destruction. These cytokines bind to the receptors in the underlying kerocytes and trigger their death within a few minutes. The process results in fragmentation of cellular DNA into thousands of small pieces—detected as densely stained cromatin by electron microscopy—by isolating the DNA fragments by electromorphosis or by staining histological sections of the cornea with a technique called TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-digoxin nick and labeling assay), which is sensitive to the loose ends of the fragmented DNA. It is this latter technique, together with electron microscopy, that is used in detecting apoptosis in the study reported in this issue of the Journal of Refractive Surgery.

Although kerocytes have been seen to disappear following corneal epithelial wounding in the past, apoptosis was not understood until recently. The process has been seen in association with herpes simplex virus, an observation that may explain why apoptosis exists: in triggering the death of underlying kerocytes, the injured epithelium protects the underlying cornea from infectious extension of the virus. Hence, apoptosis is part of the cornea's defense mechanism.

When apoptosis is associated with a refractive corneal procedure, activated kerocytes repopulate the anterior corneal stroma within days and produce collagen and other components associated with stromal remodelling and a refractive change. Whether the amount of apoptosis is correlated with the amount of stromal remodeling is unknown.

WHY DOES TRANSEPITHELIAL ABLATION MINIMIZE APOPTOSIS?

Epithelial injury, from scraping the epithelium or from laser-scrape, leads to the release of cytokines in the apoptosis process. In laser-scrape, transepithelial ablation is performed until the anterior stroma is reached in the periphery of the treatment area. The remnant of cells or fragment of cells still remaining in the center are then scraped away rather easily with a metal blade; it appears that the removal of these remaining cells is where cytokine release occurs. With transepithelial ablation alone, the level of apoptosis is significantly less and somehow cytokines are prevented from reaching the

494
keratocytes during the excimer laser ablation process. Why this occurs is unknown, but we can formulate several theories. One theory is that the cytokines are destroyed by the laser before they reach the keratocytes. Not all of the cytokines may be destroyed so that some apoptosis may still occur. Another theory goes back to our understanding of excimer laser pseudomembrane formation. Following excimer laser ablation, a thin pseudomembrane (approximately 200 μm thick) remains. The first observation of a pseudomembrane and its membrane-like properties was by Marshall and colleagues when an epithelial cell was cleaved into two along the border of excimer laser ablation.\(^5\) This half epithelial cell, although pale with staining, was seen to maintain its integrity because of an electron dense border, termed “pseudomembrane” (Figure). It is possible that cytokines are not released because the pseudomembrane, in part, prevents their liberation.

Although it has been reported that scraping and even alcohol debridement\(^6\) results in cell injury and the release of cytokines leading to apoptosis, we are unaware of the impact of other modes of epithelial removal, such as a motorized brush or rapid erosion of epithelial cells by a waterjet.

**DOES CLINICAL EXPERIENCE SUPPORT THE EXPERIMENTAL EVIDENCE?**

So far, apoptosis has been demonstrated only in experimental animal models (rabbits or mice) and not in human tissue. The human cornea, unlike that of other mammals, contains a Bowman’s layer, which may impact the role of cytokines reaching and binding to keratocytes beneath the surface. Studies of apoptosis in nonhuman primates may help to validate conclusions about transepithelial ablation and need to be considered in addition to clinical outcomes analysis.

Clinical studies have compared transepithelial ablation to epithelial scraping. Gimbel and coauthors\(^7\) reported that transepithelial ablation with the Summit Apex laser had a statistically similar refractive end point and haze score at 6 months after PRK for myopia compared with manual removal, even though a trend toward undercorrection was observed in the latter. Johnson and coauthors\(^8\) demonstrated good results using transepithelial ablation with a multizone, multipass VISX algorithm in 120 myopic eyes. Transepithelial ablation was performed using a 6 mm excimer laser phototherapeutic keratectomy (PTK) while monitoring epithelial fluorescence. Because of the VISX beam profile and ablation characteristics, the peripheral epithelium was first to disappear in all eyes. The PTK was continued until all the central epithelium was removed, as noted by an absence of fluorescence. Multizone PRK was then performed with an additional -1.00 diopter (D) added to the refractive algorithm to compensate for the steepening effect of peripheral stromal ablation. The results were divided into three treatment groups: 1) -1.00 to -3.00 D, 2) -3.12 to -5.00 D, and 3) -5.12 to -7.00 D; there was a slight overcorrection in the lower myopic groups with good overall uncorrected visual acuity (86% ≥ 20/25) at 6 months. Haze scores were 0 or trace in all eyes except one (mild, grade 1). Although a future algorithm adjustment will be needed, these results are promising, and support further investigation of transepithelial ablation in comparative clinical studies.

**WOULD TRANSEPITHelial ABLATION BE A WELCOME CHANGE?**

Despite the fact that transepithelial ablation seems to minimize keratocyte apoptosis, and that early clinical experience of transepithelial ablation seems to show favorable outcome, there may be
resistance to changing the way we do PRK to a laser only procedure. Most physicians have grown accustomed to manual removal of the epithelium prior to PRK, and although transepithelial ablation may make this simpler and decrease the time needed for epithelial removal, the perceived necessity for change may not be apparent. Additionally, variability in epithelial thickness and in the endpoint of the epithelial ablation add to the uncertainty of outcome in eyes treated with transepithelial and stromal ablation compared to stromal ablation only. Accuracy may require preoperative regional measurement of epithelial thickness (possible but cumbersome and expensive) using high frequency ultrasound or confocal microscopy. Yet, it is variability of outcome that we are trying improve by studying corneal wound healing in relation to PRK. Wound healing is an important part of outcome, and finding a way to minimize the variability of wound healing by minimizing apoptosis would be a progressive step.

The creation of new laser algorithms by the manufacturers may be necessary to bring both transepithelial and stromal ablation as a "laser only" procedure into widespread acceptance. This places the burden of further development on manufacturers. Just as anti-central island software and multizone software have been improvements in PRK ablation patterns, so to could more accurate transepithelial and stromal ablation software. Alternatively, newer devices may be introduced that monitor regional fluorescence to control epithelial ablation or preoperatively display a profile map of epithelial thickness by very high frequency ultrasound. These technologies could be coupled to the laser software to assure accuracy and uniformity of epithelial ablation.

Could apoptosis change the way we do PRK? Possibly. But increasing acceptance of laser in situ keratomileusis (LASIK) with its absence of disruption of the central epithelium, preservation of Bowman's layer, absence of central corneal haze, and presumed absence of disruption of the central keratocytes, may render discussion of epithelial removal irrelevant.

REFERENCES