

PERSPECTIVE

Photorefractive keratectomy: implications of corneal wound healing

Photorefractive keratectomy (PRK) with an argon fluoride laser (193 nm) was developed to alter the refractive power of the eye predictably and permanently. At PRK the central corneal epithelium is debrided mechanically and a disc 4 to 6 mm in diameter is ablated into the anterior stroma using computer controlled algorithms to control the depth of the wound. Theoretically for corrections of up to -7 dioptres less than 10% of the axial corneal thickness is removed.¹ As there is little loss of corneal strength and minimal tissue distortion during surgery, reproducibility is enhanced, producing a potentially superior refractive technique to radial keratotomy.

Initial enthusiasm for PRK was based on the remarkable precision and smoothness of the ablated surface²⁻⁴ which it was hoped would be translated to an accurate change in refraction. An ideal result would be achieved if the corneal epithelium then assumed the contour of the ablated surface and there was no stromal regeneration. However, it is now apparent that an initial period of overcorrection is followed by regression to a relatively stable refraction and that in a proportion of cases stromal scarring can produce glare and a loss of best corrected visual acuity.⁵⁻⁸ Despite the precision of the PRK ablation the accuracy of the final refraction is at present only comparable to radial keratotomy. Accordingly 5 months after PRK approximately 55% of eyes have an unaided vision of 6/6, while 95% of eyes with -2.00 dioptres of error are within 1 dioptre of their intended refraction,⁷ but with considerable variation between individuals.^{5,7,8} This error in final refraction is due to the normal response of the cornea to an anterior keratectomy, which is to regenerate tissue to restore the integrity of the eye. Anterior stromal haze after PRK is thought to result from light scattered by scar tissue and has been shown to be more severe with increased ablation depth.^{5,7-11} Gartry *et al* detected a reduction in corneal transparency in 92% of patients, of whom 18% had lost one to two lines of best corrected Snellen acuity 1 year after surgery.⁷ Additionally, if a small ablation zone (4 mm) is used positive spherical aberration (creating a halo effect) occurs in conditions of reduced illumination as the pupil dilates past the edge of the ablation.^{5,7} Although these halos can be eliminated by using a larger ablation zone,⁷ this entails an increased depth of ablation with its associated increased risk of haze.⁸

A potential loss of transparency in the optical axis of an otherwise healthy eye must give cause for concern. Unfortunately, topical corticosteroid therapy following PRK has not been demonstrated to reduce subepithelial haze or regression significantly.⁸ Trials are therefore in progress to evaluate different postoperative regimens to minimise scar formation. As modification of the wound healing response may become an integral part of the management of patients following PRK it is opportune to review our current concepts of corneal scar formation.

Wound healing after PRK

Corneal wound healing passes through a series of well defined stages which are similar following laser or mechanical

keratectomy. Tissue destruction stimulates a directed migration towards the wound of activated epithelial cells, fibroblasts, and macrophages which participate in the removal of damaged tissue and the de novo synthesis of ground substance; finally, there is a period of wound remodelling and increased collagen cross linkage in the mature scar. Elements of the healing process can be considered separately.

EPITHELIAL WOUND HEALING

Epithelial cell slide begins at the edge of the wound after a short latent phase during which structural proteins are synthesised, actin microfilaments polymerised, and damaged cells desquamated.¹² Hemidesmosomal attachments to the basement membrane are released in a zone of 50 to 70 μm from the wound edge allowing these cells to move as a monolayer to cover the defect. This early movement is independent of cellular division^{13,14} but cell numbers are subsequently replaced by division and centripetal migration from a population of slow cycling stem cells in the basal layer at the limbus.¹⁵⁻¹⁷

Although permanent epithelial cell attachments are released during migration, adhesion to the corneal surface is maintained via transient macromolecular focal contacts. The principal components of these transient attachments are the dimeric glycoproteins, fibronectin and laminin, which bind to the type IV collagen of the basement membrane and to an integrin molecule in the cell membrane which in turn is linked to intracellular actin. Sliding cells release plasminogen activators which cleave these temporary focal contacts,¹⁸ and permanent anchoring filaments of type VII collagen are regenerated when movement is terminated by contact inhibition.¹⁹⁻²² Finally, the epithelial layer thins over protuberances and thickens over defects in the stroma – an overall effect that tends to smooth out irregularities in the corneal surface.

The synthesis of new, functional attachment complexes is complete 4 weeks after PRK but in monkey corneas there are still missing, fragmented, and thickened areas of basement membrane after 18 months²³⁻²⁵ with regions of the epithelium devoid of type VII collagen anchoring fibrils.²⁶ A failure to re-establish firm anchoring points contributes to epithelial breakdown²⁷ which occurs in 3% of eyes after PRK,⁷ and may contribute to the small number of patients who have either a foreign body sensation, watering, or tenderness on rubbing the eye up to 2 years following surgery.^{7,8}

STROMAL WOUND HEALING

Stromal regeneration depends upon a coordinated interaction between epithelial cells and keratocytes. Epithelial cells maintain a protective environment suitable for stromal cell proliferation²⁸ and release cytokines that stimulate keratocyte migration and division.^{29,30} Fibrin and fibronectin are deposited from the tear film onto the wound and act as a scaffold for the adhesion of neutrophils, macrophages, and lymphocytes. Simultaneously, keratocytes die back in a 200 μm zone from the exposed wound surface,³¹ and migrate back to the surface

of the wound only after an intact epithelial surface has been re-established. The remaining keratocytes undergo fibroblastic transformation, and expansion of the fibroblast population by cell division begins after approximately 48 to 72 hours and reaches a peak after 3 to 6 days as new connective tissue is synthesised.³²⁻³³ Epithelial cells are then gradually displaced upward and their original attachments become incorporated within the scar.²⁶ Keratocyte activity returns to normal after 3 months, although hypercellularity of the wound may persist for years.

Corneal transparency is generally believed to depend upon a restriction in the cross sectional diameter and spacing between collagen fibrils to form a regular lattice. This produces destructive interference of scattered incident light but allows transmission of light in a forward direction.³⁴⁻³⁶ An alteration in collagen or proteoglycan structure would alter this lattice structure and degrade the optical properties of the cornea. An alternative theory suggests that corneal transparency results from the close similarity of the refractive indices of the collagen fibrils and of the ground substance.³⁷ The human corneal stroma contains collagen types I, III, V, and VI.³⁸⁻⁴¹ Although early immunohistochemical electron microscope studies either failed to detect type III collagen in human cornea⁴² or located it only in isolated fibrils,⁴³ more recent work suggests that types I, III, and V collagen copolymerise within the same fibrils throughout the stroma.⁴⁰⁻⁴¹ This hybrid nature has led to speculation that fibril diameters in the cornea are controlled by the inclusion of one or more of the minor collagens within the predominantly type I fibrils.⁴⁴ Type VI collagen is also a major constituent of the stroma, comprising up to 30% of the total collagen content, and immunolabelling has identified type VI filaments interconnecting the collagen fibrils throughout the stroma.⁴¹ Bowman's layer consists of an irregular meshwork of filaments containing collagen types I, III, V, and VI⁴⁰⁻⁴¹ and possibly type IV.⁴²

Corneal scar tissue is hazier, less elastic, and has a lower mechanical strength than normal adult stroma. The structural basis for the haziness of corneal scars is disputed, but may derive from an increased cellularity of the scar, vacuolation, or light scattering as a result of altered collagen fibril arrangement. A recent study of laser ablated rabbit corneas suggests that the relative contribution of each of these factors may depend on the age of the scar tissue.⁴⁵ In the electron microscope, early corneal scar has a vacuolated appearance; while the collagen fibrils have a normal mean diameter but a greater range.⁴⁶⁻⁴⁷ After an extended period, transparency improves as the spacing between the fibrils approaches normal⁴⁸⁻⁴⁹ but vacuoles persist in the scar tissue,⁴⁵ and even after 18 months immunohistochemical studies in monkeys indicate increased levels of type III collagen.²⁶ Normal collagen cross linkage is not restored⁵⁰ and the remodelling of the scar is therefore never complete.

The constituents of the ground substance are also altered in scar tissue.⁵¹⁻⁵² The ground substance between the collagen fibrils consists mainly of proteoglycans, type VI collagen filaments,³⁹ and a number of other proteins and glycoproteins. Proteoglycans (PG) are acidic macromolecules that are formed of at least one sulphated glycosaminoglycan (GAG) bound to a protein core and associated with collagen fibrils at specific axial locations.⁵³ The main proteoglycans in the human stroma are keratan sulphate proteoglycan (KSPG, lumican) and dermatan sulphate proteoglycan (DSPG, decorin) with trace amounts of heparan sulphate proteoglycan (perlecan) and laminin.⁵⁴ GAGs are extremely hydrophilic and most of the water present in the intercellular stroma is in the form of solvation water of GAG molecules. They thus play a vital role in the regulation of tissue hydration, collagen fibril diameter,⁵⁵ spacing,⁵⁶ and hence tissue transparency.

Changes in the ground substance in corneal scars can be demonstrated by staining the proteoglycans in full thickness⁵⁷⁻⁵⁸ or superficial keratectomy wounds.⁴⁷ In early scar tissue the proteoglycan filaments are much larger than normal and contain mostly highly sulphated, high iduronate DSPG,⁵⁹ which persists for about 3 months and is associated with a reduction in the concentration of the KSPG.⁵⁷⁻⁶⁰ These abnormal proteoglycans may contribute to the high water content and vacuolated appearance, which in turn contributes to the corneal haze. There are other changes in structure. Hyaluronic acid is present in only small quantities in human and rabbit cornea but is deposited in the rabbit cornea after laser keratectomy,⁶¹ and chondroitin-6-sulphate is not found in normal stroma but is uniformly distributed with chondroitin-4-sulphate in all scar tissue. The significance of these observations has not been established.

In some respects scar formation recapitulates fetal corneal formation. Fibrils secreted in the early stages of scar formation³¹ are similar in diameter to the parallel bundles of fibrils secreted in embryogenesis.⁶² However, there is no longer domain structure superimposed on newly synthesised collagen in scar tissue⁵⁰ and the ratio of interfibrillar type VI collagen to type I collagen is reduced compared with developing cornea.⁶³ Activated keratocytes also express fetal surface antigens for approximately 6 weeks after injury.⁶⁴ During the development of the cornea there is normally a change in the component GAGs from DSPG in the fetus to KSPG which is the predominant proteoglycan in the adult of most species.

Resorption and remodelling of damaged collagen is an integral component of wound repair.⁶⁵ Resorption is initiated by proteolytic enzymes from polymorphonuclear leucocytes and is continued by macrophages, epithelial cells, and keratocytes.⁶⁶ The degradation of collagen is catalysed at neutral pH by a group of structurally related metalloproteinases (collagenase, stromelysin, gelatinase). Only very small quantities of type IV collagenase and gelatinase have been detected in the unwounded cornea, suggesting they play a role in the maintenance of the extracellular matrix,⁶⁷⁻⁶⁹ but following injury the expression of these enzymes is increased for prolonged periods as they are secreted into the extracellular matrix by epithelial and stromal cells or by invading inflammatory cells.⁷⁰

Regulation of repair and remodelling

The factors that control the maturation of scar tissue have become the subject of intense scrutiny. In the uninjured cornea there is a balance between cell production and loss, and the levels of stimulatory and inhibitory growth factors. Cells that are injured become activated and produce cytokines. These are potent peptide or glycoprotein regulatory factors that can act as autocrine or paracrine agents to mediate communications between cells and coordinate chemotaxis, cellular proliferation, and protein synthesis. By acting as competence factors or promoters of DNA synthesis they may have a primary action or they may allow a secondary effect. Signal transduction by cytokines is believed to require binding to a cellular receptor, where an active agent may be cleaved, producing stimulation of the cytoplasmic domain of the receptor and activation of cytoplasmic tyrosine kinase.⁷¹ The signal thus stimulates the transcription of other signalling molecules and mediates the complex biological responses associated with inflammation and wound healing. Indeed, the pattern of cytokines may influence the degree of cellular infiltration in wounds, and the effect of a single agent may vary according to the cellular environment.⁷²⁻⁷³

In the avascular cornea cytokines are probably derived from macrophages and fibroblasts.⁷⁴ Platelet derived growth factor (PDGF), transforming growth factor beta (TGF- β), basic fibroblast growth factor (FGF), the interleukins (II-1,

II-6, II-8), and tumour necrosis factor alpha (TNF- α) all modulate wound healing.^{74,75} TGF- β and PDGF in particular are strong chemotactic agents and mitogens for fibroblasts, but they can also block cell differentiation and stimulate gelatinase production. TGF- β is auto-inductive in that it is a chemotactic factor for fibroblasts that in turn secrete more TGF- β ³⁰; thus regulatory processes must exist which terminate stromal repair.

The inhibitory processes of corneal repair are understood poorly and may depend on whether the epithelium is stable or injured.⁷⁶ Keratocytes probably secrete factors which inhibit their growth, and secreted TGF- β may both stimulate the deposition of extracellular matrix and suppress matrix degrading collagenases.⁷⁷ The complexity of these interrelationships suggests that any modulation of cytokines to control healing would require the use of combination therapies.

The clinical effect of wound healing

It has been demonstrated in rabbits,⁷⁸ monkeys,⁷⁹ and humans^{80,81} that scar tissue is formed following PRK and that the depth of scar tissue is roughly proportional to the depth of the ablation.^{82,83} The scarring is also proportional to ablation depth^{84,85} and, although there are large individual variations,^{5,7,8} the haze is maximal at 4 to 6 months and then diminishes.^{5,7,8} Any figure for the incidence of haze depends upon the definition of the term; Seiler *et al* reported 'scarring' in as few as 1.8% of patients with <6.0D correction,⁸³ while Gartry *et al* noted the development of 'haze' in as many as 92% of eyes after <7.0D correction, of whom 18% lost Snellen acuity.⁷ Forward scatter from haze results in loss of low (5%) contrast sensitivity.⁶

Although a large number of patients have undergone PRK for treatment of myopia, the presentation of systematic follow up data of refraction results is less common.^{5,7-11,83,85-89} It appears that after an initial overcorrection, which is proportional to the attempted correction, there is a rapid regression in the first 1 to 2 months (owing to epithelial hyperplasia); haze begins to develop 4 to 6 weeks after PRK (possibly as a result of stromal regeneration) and a relatively stable refraction is achieved by 3 to 5 months.^{5,7,8,84} The accuracy of the procedure is then usually defined as the percentage of eyes within the band ± 1.0 dioptres of the intended refraction; this definition produces a natural bias toward greater accuracy for the correction of low refractive error. It can thus be expected that 95% of patients with 2 dioptres of error will achieve this target, but this figure falls to 20% when 7 dioptre corrections are attempted.⁷ Thus the greater the attempted correction the smaller the number of patients within these limits. There is a relationship between the amount of haze and the regression of refraction (mean correlation=0.68),⁸⁴ suggesting that stromal regeneration is a major source of residual refractive error following PRK. The intensity of the haze is proportional to loss of contrast sensitivity,⁸ moderately correlated with Snellen acuity loss but not proportional to any halo effect.⁷ It is possible that regression is greater when a smaller diameter is used. Sher *et al*⁸⁴ reported encouraging results for errors of up to - 14.5 dioptres using beam diameter up to 6.0 mm and a cut depth as great as 230 μ m; other studies in which regression has been a problem have used 3.5 to 4.8 mm discs.^{5,90,91}

Seiler *et al* have claimed that undercorrection can be treated and scars removed by reablation after 6 months with a low incidence of recurrence, although their figure (1.8%) for scarring after PRK was already remarkably low.⁸³ However, early repeat PRK (3 months post PRK) induced exaggerated response and opacification in monkeys.⁹²

The prevention of fibrosis

A number of approaches have been suggested to control scar formation after PRK. Corticosteroids are known to inhibit corneal stromal healing⁹³⁻⁹⁹ and to reduce the deposition of scar tissue in experimental models of PRK.^{78,100} Topical steroids have been incorporated in most postoperative regimens.^{5,9,86-89} It has even been suggested that the administration of steroid can be titrated against the extent of haze and the regression.^{9,89} The mechanism of this steroid effect is uncertain but may be by prevention of the recruitment of macrophages and lymphocytes to the wound, or via a distinct anti-anabolic effect by reducing DNA synthesis and collagen deposition in activated fibroblasts.^{96-98,101} In a randomised, double masked, prospective study examining the effect of topical corticosteroid at two discrete ablation depths, Gartry *et al* found a statistically significant improvement in the induced refraction in the steroid treatment group, but this effect was lost after 3 months when steroids were discontinued. For 6.0D ablations the final effect was statistically insignificant in spite of a 0.78D difference between placebo and control groups, which may be clinically useful.⁸ No effect on haze was found and in view of the known complications the long term use of steroid would seem to be unacceptable.

Antimetabolites such as 5-fluorouracil and mitomycin-C have an established role in preventing fibrosis following drainage surgery for glaucoma, and they enhance the effect of topical steroids following experimental PRK.¹⁰⁰ However, these agents are toxic both to epithelial cells and to fibroblasts, and topical application could delay epithelialisation.¹⁰² Their efficacy and safety therefore need to be validated.

Approaches to the prevention of fibrosis which mimic the natural signals to terminate scarring warrant further investigation. For example, recombinant human EGF stimulates epithelial maturation, while neutralising antibodies applied to the wound could reduce the concentration of TGF- β , the major fibroblastic cytokine of early wound healing.¹⁰³ Similarly, application of α 2-macroglobulin to bind TGF- β may augment natural cytokine clearance.¹⁰⁴ Other agents, such as interferon alfa 2b, which inhibit fibroblast proliferation, migration, and collagenase production are being evaluated.

Any hyperplasia of the corneal epithelium over the bed of a PRK wound will reduce the induced refractive change, and the epithelium tends to be thicker at the edge of an ablation than at the centre.⁸² We are unaware of any method to prevent epithelial hyperplasia in this situation, but we hypothesise that the effect may be reduced by using a larger ablation diameter and blending the transition between the treated and untreated regions of the cornea.

The regenerative response of the cornea is less marked if an intrastromal keratectomy rather than an anterior keratectomy is performed,⁷⁸ suggesting that the fibroblastic stimulus is augmented by epithelial destruction. Laser assisted keratomileusis has therefore been suggested for the correction of higher myopic errors where haze and regression would be greater.¹⁰⁵

Conclusions

At present the predictability of PRK can only be described as fair for myopia up to 4 dioptres. If predictability were increased and haze reduced the procedure would supplant all available refractive surgical procedures but at present enthusiasm is ahead of published data, and the maximum follow up is 3 years. However, it must be said that a high proportion of patients are very pleased with the outcome and objective assessment may not provide a true index of satisfaction. In many instances patients are impressed with the reduction, if not the elimination, of their myopia.

The justification for refractive surgery in an otherwise normal eye must centre on the accuracy of the procedure in the absence of any subsequent functional disability. Stromal regeneration following PRK may produce corneal haze and regression of the induced refraction. Haze is the result of the scattering of light by the deposition of scar tissue.¹⁰⁶ The major options for the development of PRK are thus to limit the surgery to the correction of low myopia where small amounts of scar tissue are produced, to perform intrastromal ablation,¹⁰⁵ or to inhibit scar formation. The development of effective methods to control the scar response will determine the impact that this procedure has on ophthalmic practice.

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