Refractive keratoplasty with intrastromal hydrogel lenticular implants

Bernard E. McCarey and David M. Andrews

The feasibility of using hydrogel lenticular implants of high water content to alter the anterior corneal curvature for purposes of refractive keratoplasty has been investigated in rabbits. Lenticules (6 mm in diameter) of Permalens (Perfilcon-A) were trephined from contact lens and implanted within an intralamellar pocket in the cornea. The in vitro glucose flux across the hydrogel (0.23 mm thick) was measured at 131 ± 7 μg/cm²/hr. For clinical comparison, non-water-permeable disks of Teflon were also implanted. The Teflon implant caused an aseptic ulcer to develop anterior and central to the implant by 9 ± 4 days. The hydrogel lenticular implant did not cause central ulceration during the 7 month postoperative follow-up. There was a thinning and eventual erosion of the stroma anterior to the edge of the hydrogel implant, 16 ± 7 weeks. The glycogen contents of the epithelium anterior to (1) the sham operation, i.e., lamellar pocket dissection, (2) the implanted hydrogel lenticule with or without the presence of an erosion, and (3) the control corneas were statistically from the same population. Yet there was a slight dehydration of the stroma anterior to the hydrogel implant when compared to control tissue. A thin-edged implant lenticule design should overcome the stromal thinning caused by the thick-edged implants. During the short-term follow-up, the hydrogel lenticular implant proved to be successful as a refractive keratoplasty implant material.

Key words: refractive keratoplasty, hydrogel implant, rabbits, epithelium, glycogen, hydration, refraction

The cornea is the most powerful refracting surface of the eye because of the large difference in the index of refraction between air and cornea. Very small alterations in its curvature will create large changes in the eye's refraction. The field of refractive keratoplasty has been nurtured and refined by Barraquer since 1949, yet only recently has it gained the full attention of the ophthalmic community. This might be linked to the layman's acceptance of the idea of performing a surgical procedure such as intraocular lens implantation, to correct major ocular refractive errors.

Refractive keratoplasty procedures can be categorized into three groups: (1) purely surgical, such as wedge resection or radial keratotomy with induced scarring; (2) stromal lenticular additions, such as keratophakia, keratomileusis, and epikeratophakia; and (3) hydrogel lenticular implants (HLIs), which is the procedure investigated in this study.

Intrastromal implants of plastic material have been used by researchers to investigate the mechanisms of corneal hydration control and nutrition and by the clinician as a therapy for endothelial dystrophies. Knowles found that the cornea overlying a water-impermeable membrane thinned and in many cases ulcerated. One of the causative factors was...
investigated by Brown and Mishima who observed within the cornea an aqueous-to-tear movement of water that was created by an increase in the tear tonicity from evaporation during the interval between blinks. A water-impermeable intrastromal implant will prevent the aqueous-to-stroma movement of water and will result in excessive concentration of the tear film, stromal thinning anterior to the implant, and eventually epithelial and stromal breakdown. The stromal resistance to water movement is so great that the water cannot flow around the implanted barrier rapidly enough to prevent this stroma dehydration. Truss et al. found that tissue nutrients such as glucose also had an aqueous-to-anterior cornea movement. Because of the thin shape of the cornea, minimal epithelial permeability, low tear concentrations of glucose, and facilitated transfer of glucose across the endothelium, all of the corneal glucose (except for the peripheral 1 or 2 mm) is supplied by the aqueous. Thus, it is very important that the intrastromal implant be sufficiently permeable to water molecules, glucose, lactate ions, etc., to permit the maintenance of the normal corneal physiology.

A hydrogel is water permeable and thus is a more appropriate plastic material for intrastromal implantation. Dohlman et al. implanted discs of glyceryl methacrylate (GMA) hydrogel (88% water) within the stroma of rabbit and cat corneas to investigate the tolerance of the tissue to the GMA. They found little or no inflammatory reaction in rabbit corneas, but the membranes slowly extruded within 3 months. The authors felt this was related to the water permeability of the GMA and not to toxicity. In the cat cornea the GMA implant was well tolerated for the duration of the follow-up (11 months). Mester et al. investigated intrastromal implants made of hydroxyethylmethacrylate (HEMA), which is 38% water. There were no extrusions in rabbit corneas during the 18 month follow-up. The success of the HEMA implants as compared to the GMA implants is difficult to explain. The GMA implants ranged in thickness from 0.19 to 0.57 mm, whereas the HEMA was 0.2 mm thick. Dohlman et al. did not correlate the GMA thickness to the postoperative time of extrusion. The water permeability, as determined by Refojo, showed the 88% GMA to be 8.7 times more permeable than the 38% HEMA. Since the thickness of the hydrogel has an inverse effect on the water permeability, the implant thickness is very important; an excessively thick implant could have resulted in the implant extrusions encountered by Dohlman et al. Also, the GMA implants were flat disks whereas the HEMA implants had a base curve matching the animal cornea which would indicate less stress and distortion to the lamellar bundles with the HEMA implant.

In this investigation, one of the new generation of hydrogels of high water content was used as an intracorneal lenticular implant for the purpose of altering the corneal curvature, i.e., refractive keratoplasty. The Permalens (Perfilcon-A) is an ideal HLI material. It is 71% water when equilibrated with an isotonic solution and thus compares well to the 78% water content of the corneal stroma. The pore diameter of the Permalens hydrogel is 30 Å, which is many times larger than a glucose molecule at 8.6 Å. Thus, a bulk flow of glucose could exist across the Permalens in order to maintain normal epithelial metabolism. The purpose of this investigation was to establish the feasibility of using HLI for refractive keratoplasty.

Material and methods

Implant material. A clear Teflon membrane of 0.025 mm thickness was used as the intrastromal implant material that would be impermeable to water, glucose, and small ions but permeable to oxygen. The membrane was stretched across a 9/16 inch stainless steel ball, sterilized by heat, and cut with a trephine to a diameter of 6 mm. The Teflon was implanted to determine the postoperative time delay until appearance of nutritional necrosis anterior to the implant.

The HLIs were made by trephining out the central 6 mm of a Permalens contact lens (Cooper Laboratories, Inc.), which consists of 26% terpolymer of 2-HEMA vinyl pyrrolidone and other methacrylates. It has 71% water when equilibrated with isotonic saline. The optical zone of the lens is 6 mm. The HLIs had powers of 0.00, +11.00, and +14.00 with central thicknesses of...
0.2, 0.3, and 0.4 respectively, when equilibrated with 308 mOsm NaCl-phosphate solution. The HLI thickness was measured in air with a Haag-Streit Pachometer.

**Glucose flux across hydrogel.** Permalens contact lenses with a base curve of 6.8 mm, 0.00 power, 0.234 mm thickness (measured submerged in isotonic saline with a specular microscope), and 14.00 mm diameter were mounted between two Lucite block chambers (Fig. 1). An air-lift system was used to keep the solution in each half chamber well stirred. The lenses were bathed at 24° C with a sodium chloride-phosphate buffer at pH 7.4 and 308 mOsm on one side and the same buffer plus 5 mM glucose on the other side. The chamber with the glucose was maintained at pressure less than 1 mm Hg greater than that in the contralateral chamber. The rate of appearance of the glucose on the initially glucose-free side was used to calculate the glucose flux. The glucose concentration was measured by a hexokinase assay.

**Surgical technique and follow-up.** Thirty New Zealand albino rabbits, weighing approximately 3.5 kg, were given 1% cyclopentolate hydrochloride eye drops as a cycloplegic, and the preoperative measurements from retinoscopy and pachometry were recorded. The animals were anesthetized with chlorpromazine (Thorazine), 9.0 mg/kg (intramuscularly), followed by pentobarbital, 30 mg/kg (intravenously), and one or two drops of 0.5% proparacaine hydrochloride to the cornea. A blade breaker and razor were used to make a 4 mm incision tangential to and 3 mm from the limbus and approximating midstromal depth. A Martinez corneal dissector with a sharp edge was used to gently form a central lamellar pocket 8 mm diameter. An effort was made to remain in a single lamellar plane. The opening to the pocket was enlarged to 7 mm with corneal scissors. The HLI was slipped into position with the aid of a spatula. The incision was closed with three to five sutures of 10-0 nylon. During the operation, the cornea was periodically moistened with 0.5% chloramphenicol. Neosporin ointment was applied to the cornea once daily for 3 postoperative days. On the seventh postoperative day the sutures were removed. Periodically during the postoperative follow-up, the retinoscopy values and pachometry of the cornea and of the in situ HLI thickness were recorded.

**Corneal epithelial glycogen content.** The glycogen content in the corneal epithelium anterior to the 6 mm implant or a central 6 mm zone of a cornea subjected to sham operation, i.e., a lamellar pocket without an implant, was analyzed by the method of Thoft et al. After sacrificing the rabbit with an overdose of pentobarbital, the epithelium was scraped off and immediately frozen with a mixture of Dry Ice and acetone. The frozen epithelium was dried for at least 18 hr in a lyophilizer and then weighed. The epithelium was extracted in 1.0 ml of 20% sodium hydroxide for 15 min at 100° C. The glycogen was precipitated twice in ethanol, followed by a 30 min centrifugation at 10,000 x g. The supernatant was discarded and the precipitate hydrolyzed in 2N sulfuric acid for 90 min at 100° C. After neutralization the glucose content was measured by the hexokinase/glucose-6-phosphate dehydrogenase reaction and the glycogen level was expressed as glucose units per dry weight of epithelium.

**Stromal hydration.** The corneal stroma water content was determined for the stroma anterior to the HLI and anterior to the sham operated dissection plane. These values were compared to normal full-thickness cornea (control). In each case, the sample was harvested and weighed immediately after scraping off the epithelium and reweighed after 48 hr at 100° C in a vacuum oven.

**Results**

**Teflon membrane implant.** The Teflon implant within the corneal stroma did not stimulate vascularization or any generalized tissue reaction during the first postoperative week. By 9 ± 4 days (n = 5), the epithelium and stroma central and anterior to the implant became necrotic and soon afterward sloughed off, revealing the Teflon implant (Fig. 2). During the next week or more, the necrotic area increased in diameter while the eye remained clinically quiet, until one of the...
common ocular microorganisms infected the exposed stroma.

**Hydrogel material.** The glucose flux through the Permalens material (0.234 mm thick) was $131 \pm 7 \mu g/cm^2/hr$, $n = 4$. When comparing the hydrogel implant thickness, as measured by the slit-lamp Pachometer before implantation, to the in situ implant thickness within the stroma, a considerable thinning was observed in situ. The hydrogel thinned by $28\% \pm 2$, $n = 13$, in the stroma. This would cause a shrinking of the hydrogel pore diameter and an expected decrease in the in situ glucose flux across the implant. Considering this factor, one might expect a $94 \mu g/cm^2/hr$ glucose flux in situ for an implant of 0.234 mm thickness.

**Tissue response to hydrogel lenticular implant: clinical observation.** After insertion of the HLI into the rabbit cornea, most of the eyes were clinically quiet with little or no conjunctival vessel dilation and edema, except at the site of the incision. The eyes that did exhibit some reaction quieted down after suture removal (Fig. 3). The HLI did not stimulate vessel formation or stromal scarring as determined by slit-lamp examination. Even after 7½ months the stroma did not attach to the HLI and thus the HLI could be removed after making an incision through the stroma.

Of the 30 corneas with HLIs, eight eyes had slowly progressive thinning of the stroma anterior to the edge of the implant (Fig. 4). At a mean time of $16 \pm 7$ weeks a small zone of stroma, usually about 0.5 mm by 1 or 2 mm, had eroded away anterior to the HLI edge. It is impossible to know whether all the eyes with HLI implants would have developed erosions, since animals were sacrificed periodically for tissue analysis, prior to any signs of erosion. The eyes did not have a reactive response to the stromal thinning/erosion and the implants remained in place. Since the lamellar pocket was opened by the...
stromal erosions, the cornea was prone to infection, yet for up to 9 additional weeks the eyes remained nonreactive. During this period the stroma continued to recede at a very slow rate or remained stable with stromal edema at the margin of the opening. The stromal thinning was unlike that with the Teflon implant since it was always restricted to the stroma anterior to the edge of the implant. The HLI within the stroma was 0.15 to 0.34 mm thick, whereas the contralateral control cornea was 0.35 to 0.45 mm thick. Thus the stromal thinning could be caused by the stress created from an implant that was from 50% to 100% the thickness of the cornea receiving it. Also, the HLI was trephined from a contact lens which resulted in an implant lenticule with an abrupt edge of 0.23 mm thickness, the carrier lens thickness. These factors would indicate the erosion was mechanical in nature and not a nutritional disturbance.

**Epithelial glycogen content.** The corneal epithelial glycogen content had statistically the same (p = 0.8) mean value in the contralateral control eye as it had in the eyes with HLIs in place, Fig. 5. There were four eyes with the HLI in place for 3 months and three eyes with it in place for 5 months. The sham-operated eyes, i.e., a lamellar pocket without an implant, had statistically the same (p = 0.6) epithelial glycogen content as the eye with an HLI. Significantly, the epithelium of the eyes with an ongoing extrusion had the same (p = 0.8) glycogen content as their contralateral control eyes. This is evidence that the stromal thinning was not caused by lack of glucose supply to the corneal layers anterior to the HLI.

There is no correlation (r = 0.0001) between the HLI thickness, as measured in situ, and the glycogen content of the epithelium above the implant (Fig. 6). The range of implant thickness was 0.15 to 0.34 mm. This would indicate that for this range of implant thickness, there was a bulk flow of glucose moving across the implant.

**Stromal water.** The water content of the
stroma anterior to the HLI was slightly dehydrated to 65% ± 3, n = 7, as compared to its contralateral control, 77% ± 2, n = 7 (Fig. 7). It was highly significant (p < 0.001) that the mean values were from different populations. It appears that upon initial implantation of the HLI, the water evaporating from the anterior surface of the cornea was greater than the amount flowing through the HLI. This reached an equilibrated state when the imbibition pressure within the anterior stroma increased and in turn increased the posterior-to-anterior gradient for water across the HLI, which also would have caused a decrease in the water gradient from the stroma to the tears. The water content of the anterior stroma in the sham operated cornea (73% ± 2, n = 6) was significantly more hydrated than the corneas with HLIs (65% ± 3, n = 7) and less hydrated than the control corneas (77% ± 2, n = 11).

Refractive alteration. Retinoscopy values show a flattening of the normal rabbit cornea with increasing age. Thus it was necessary to express the data as a net change, i.e., refractive change with the HLI minus refraction of contralateral control eye. The results show a stabilization of the refraction by about the sixth postoperative week. The net spherical equivalent alteration by the HLI was approximately equal to the power of the contact lens from which the HLI was trephined (Table I), in spite of the fact that the HLI has an edge thickness in excess of 0.2 mm.

Discussion

The corneal response to the water-impermeable Teflon membrane was similar to the responses to other water-impermeable membranes reported in the literature. Knowles used various plastics, such as polyethylene, polyvinylidine, and polypropylene, which resulted in the aseptic ulceration of the central cornea anterior to the implant within 9 to 34 days. He also noted the absence of inflammation and the sharp localization of the defect anterior to the implant without alteration to the posterior tissue. Because of the prolonged and variable delay in the appearance of the degenerative process, Knowles believed that something more than simple interference with nutrition was at work. He felt the process might be the result of one or more of the following: (1) lack of vital nutrients from the aqueous, (2) accumulation of a toxic substance, and (3) selective drying of the cornea anterior to the implant.

A permeable implant material that is non-
Fig. 7. The percentage of water in the stroma (grams of water per gram of dry stroma) anterior to the lamellarly directed plane or implant was determined. The presence of the hydrogel implant resulted in stromal dehydration relative to the contralateral control eye.

Table I. Refractive change with hydrogel lenticular implants

<table>
<thead>
<tr>
<th>Base curve (mm)</th>
<th>Power (diopters)</th>
<th>Thickness (mm)</th>
<th>Net spherical equivalent change minus paired control</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8</td>
<td>0.00</td>
<td>0.2</td>
<td>-1.5 ± 0.9* (4)</td>
</tr>
<tr>
<td>6.8</td>
<td>10.56 ± 0.27</td>
<td>0.3</td>
<td>+6.9 ± 2.8 (6)</td>
</tr>
<tr>
<td>8.0</td>
<td>14.00</td>
<td>0.4</td>
<td>+16.6 ± 1.2 (2)</td>
</tr>
</tbody>
</table>

*Mean ± S.D.; numbers of samples in parentheses.

toxic to the corneal tissue should overcome the difficulty which the Teflon membrane failed to overcome. Refojo\(^8\) stated that in hydrogel material of high water content (94% to 74%), the net water flux can be considered to follow primarily viscous flow mechanisms; that is, the molecules move in groups or bulk. In the hydrogel of lower water content (39% to 63%) the molecules are restricted to diffusion coefficient calculations. Since it is desirable to have the least water resistance,\(^*\) the Permalens hydrogel material with 71% water was selected as a hydrogel lenticular implant for refractive keratoplasty. The data showing no correlation between the HLI thickness and epithelial glycogen content (Fig. 6) can be explained either by a bulk flow of glucose across it, as suggested by Refojo's statement,\(^8\) or by the flux of glucose being greater than the anterior stromal and epithelial utilization of glucose. Rabbit corneal glucose utilization has been reported to range from 104 (Riley\(^1\)) to 39 \(\mu g/cm^2/hr\) (Thoft and Friend\(^2\)). The epithelial glucose consumption was estimated at less than 20 \(\mu g/cm^2/hr\) under normal conditions.\(^3\) From these data, a conservative approximation of 50 \(\mu g/cm^2/hr\) for the anterior corneal layers might be expected. The data in this investigation indicated a glucose flux of 94 \(\mu g/cm^2/hr\) for Permalens material, taking into account the in situ dehydration of the hydrogel. Also, there was no correlation between epithelial glycogen content and HLI thicknesses between 0.15 and 0.34 mm (Fig. 6). Thus, it can be assumed that for this range of HLI thickness, the rate of glucose movement across the HLI exceeded the glucose consumption of...
the corneal tissue anterior to the implant.

The stroma anterior to the HLI was slightly dehydrated in comparison to control corneal tissue (Fig. 7), presumably because of insufficient hydrogel water permeability relative to the rate of evaporation. This was consistent with slit-lamp observation of the stroma, i.e., grayish light scatter posterior to HLI and golden light anterior to HLI. According to the findings of Hedbys and Mishima the normally hydrated stroma (77% to 79% H2O) has a water flow conductivity value of \(150 \times 10^{-14}\) cm\(^4\)/sec/dyne. The Permalens has a flow conductivity of \(21 \times 10^{-14}\) cm\(^4\)/sec/dyne at a hydration of 71%. As was pointed out, there is a 28% thinning of the hydrogel within the stroma; thus the water flow conductivity of the hydrogel would reduce to \(15 \times 10^{-14}\) cm\(^4\)/sec/dyne. Presumably this in situ thinning of the HLI would be true of any hydrogel. Thus, the Permalens is tenfold and the HEMA is 250-fold less permeable to water than the corneal stroma. The stromal water flow conductivity values measured by Hedbys and Mishima represent the maximum attainable values. They do not reflect the amount of water that is osmotically moved across the cornea (posterior to anterior surface) because of the increased osmolarity of the tear film between blinks. This value is difficult to measure because the water that evaporates from the anterior corneal surface is supplied from both the stroma and the tear film. The disparity between the Permalens water flow conductivity and that of the stroma could be avoided by increasing the water content of the hydrogel to possibly 80% or 90%. Refojo found that a GMA hydrogel with 89% water had the same water flow conductivity as the stroma.

A difficulty encountered with the HLI in rabbit corneas was a thinning of the stroma anterior to the edge of the implant. This thinning was not restricted to a particular quadrant, regardless of the location of the lamellar pocket opening. This problem could be caused by (1) the lamellar dissection not remaining in one plane, (2) the thick-edged implant creating mechanical stress within the stroma, or (3) inadequate nutritional supply. The data rule out the possibility of the problem being nutritional, and since it is an almost universal problem (if enough postoperative time passes) it would seem unlikely to be a surgical problem. It is most likely related to poor implant design, which could be corrected by thin-edged implant lenticules. This would result in an implant of thinner central thickness for a given power, less stress or stretching of the stromal collagen fibrils, and less edge visibility within the stroma.

The preliminary finding that the HLI dioptric power, as determined by the manufacturer, was approximately equal to the postoperative refraction of the animal eye is encouraging, but not surprising. Barraquer has developed the keratophakia and keratomileusis procedure on the basis that he could determine the appropriate stromal lenticular power needed for the patient’s refractive correction. The procedure with the hydrogel implants differs only in the implant’s refractive index. Churms has studied the optical theory of the hydrogel lenticular implant. He concluded that the hydrogel lenticule can induce approximately an additional 15% change in the ocular power over that of a stromal lenticular implant. There are many factors which must be clarified before accurate predictability can be realized. The importance of the depth of the implant bed, the rigidity of the hydrogel material, the alteration of the hydrogel hydration in situ, and diameter of the implant are not well defined.

The findings of this investigation encourage the idea that hydrogel lenticular implants can have an important role in refractive keratoplasty procedures, but the data are restricted to short-term follow-up. A 1 to 3 year postoperative follow-up, in not only rabbits but also cats and primates, is essential before human application can be considered. The possibility of slow stromal alterations by either a remodeling or an encapsulation of the nonbiologic hydrogel material must be investigated carefully by in vitro measurements and histologic ultrastructure studies.

We thank Cooper Laboratories, Inc., for supplying the Permalens contact lenses and Sarah J. Scholl for her technical assistance.
REFERENCES