Morphology of Hydrogel Implants Used for Refractive Keratoplasty

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The pathology of 14 keratophakia procedures in nine nonhuman primates using various hydrogel lenses as donor lenticles is presented. The lenses were treated as if they were human donor corneas using currently practiced keratophakia procedures. This included microkeratome resection of the recipient cornea, staining of the lenses before cryolathing and, in most instances, lathing according to a modified computer program after which the lenses were inserted into the previously dissected recipient lamellar bed. There was no histologic evidence of an inflammatory reaction to the hydrogels. The major pathologic feature was the presence of activated keratocytes lining the dissected intrastromal pockets. Invest Ophthalmol Vis Sci 25:843–850, 1984

We have described previously the successful use of intrastromal hydrophilic lenses to induce steepening of the central cornea.1 Such lenses may offer a way of eliminating many of the negative aspects of the use of human donor corneas for the surgical correction of aphakia, while having theoretic advantages over other surgical techniques for the correction of hyperopic errors.2 These advantages include an unlimited supply, the availability of prelathed lenticles, the reduction of potential risk of opacification, a standardized optical quality, and the capability of correcting large refractive errors.2 Before hydrophilic lenses can be used clinically, the ability of the cornea to tolerate the implanted foreign material must be evaluated.

Materials and Methods

All surgery was performed by one surgeon in nine female baboons (Papio cynocephalus), 15–20 kg in weight. We used six freshly trephined and eight cryolathed aphakic hydrogels.1 Aphakic hydrogels were used because the anterior curvature of the optical zone was similar in curvature to that required for clinical keratophakia lenticles. A 6.0 mm disposable trephine (Edward Weck & Co.; Research Triangle Park, NC) was used to obtain the fresh hydrogels, which measured 0.25–0.59 mm in central thickness. Lenses to be cryolathed were placed in 0.1% light green in M-K medium3 for 1 min and then frozen and lathed (Steinway Instruments; San Diego, CA) using the standard keratophakia program (Queratofaquia 5-E) on a microcomputer1 (Apple Computer Co.; Cupertino, CA). The final lenticles were designed to be 6.0 mm in diameter, 0.2-mm thick centrally, to have an anterior curvature of 5.68 mm, and a posterior curvature of 7 mm. The microkeratome resection and lenticle placement were performed as previously described.1 The animals were evaluated clinically for 5–6 months using an operating microscope, corneal topography, and clinical 35 mm photographs for documentation. At the end of the experiment, the animals were killed with an overdose of intravenous Somethol (Med Tech; Elwood, KS).

The ocular surface was flushed with 1% paraformaldehyde/1% glutaraldehyde fixative (pH 7.4, 608 msm) after which the entire globe was enucleated. The corneas with a 3-mm rim of sclera then were immersed in the fixative for a minimum of 1 hr and then prepared for correlative microscopy.4 Nine control baboon corneas were similarly prepared to compare morphologically with postoperative corneas.

These investigations adhered to the ARVO Resolution on the Use of Animals in Research.

Results

Eight frozen (cryolathed) and six freshly trephined hydrogels were studied. The parameters and types of hydrogels studied have been published previously.1 The eight cryolathed hydrogel implants showed epithelium of normal thickness over the hydrogels, but thickened to seven and eight cell layers in the area of the mi-
crokeratome–wound edge (Fig. 1). Slight tinctorial changes were present in the basal epithelial cells of the anterior cap overlying the implant (Fig. 1) only when thick (>0.3 mm) lenticles were used. The ultrastructure and surface morphology of the epithelium overall eight cryolathed hydrogels appeared normal (Fig. 2).

Ocular surface epithelium appeared to be entrapped at the edges of the freshly trephined hydrogels (Fig. 3). Similar epithelial cysts have been documented in keratophakia and in epikeratophakia. The epithelium over the thick, trephined hydrogels had thinned to three cell layers (Fig. 3).

Numerous bends and folds were found in Bowman’s layer in the anterior cap of both cryolathed and trephined hydrogels (Figs. 1, 3). We suspect these folds represent the expected distortion that occurs when an anterior corneal cap is draped over an intrastromal implant. The posterior lens surface of the one trephined hydrogel which remained in situ appeared relatively smooth compared with the somewhat roughened posterior lens surface of those specimens that were cryolathed. The rough surface did not appear to produce additional morphologic features.

There was no evidence of corneal stromal inflammation. The endothelium appeared normal in all but one specimen (Fig. 4). Activated keratocytes were seen along all interfaces (Figs. 1, 5) in both the cryolathed and trephined hydrogel specimens. The keratocytes tended to bunch up at the edge of the implants. They appeared to be very active with the presence of numerous mitochondria and dilated endoplasmic reticulum with adjacent, newly formed, extracellular collagen (Fig. 5). Only those keratocytes close to the hydrogel interface appeared to be phagocytic and contained primary and secondary lysosomes (Fig. 5).

Few macrophages were present in the optical interfaces and in the adjacent stroma above and below the implanted hydrogels (Fig. 6). These cells were characterized by their large, round shape, numerous pseudopodial extensions, and numerous primary and secondary lysosomes (Fig. 6). This cell type is seen commonly in the normal process of corneal wound

Fig. 1. Low-power, light micrograph of cryopreserved hydrogel 6 months postoperatively. Note the normal structure of the anterior and posterior corneal stroma overlying the hydrogel. Bowman’s layer shows folds in the anterior cap as well as tinctorial changes in the basal epithelium over the hydrogel. Vacuolated keratocytes are seen at the anterior, peripheral, and posterior corneal surfaces (arrows). The underlying Descemet’s membrane and endothelium appear normal (X120).
Fig. 2. Low-power, transmission, electron micrograph of the central, ocular, surface epithelium immediately overlying a cryo-lathed hydrogel. The epithelial cell ultrastructure contains the normal complement of cytoplasmic organelles and normal appearing microvilli (×8,700). Inset: Low power scanning electron micrograph of ocular surface epithelium in the same area. The epithelial surface appears normal with typical light and dark appearing cells (×300).

healing but is not seen commonly in keratoplasty specimens. Since all specimens contained normal appearing keratocytes in the absence of inflammation, these cells possibly may have been stimulated by the presence of the hydrogel lens.

The freshly trephined aphakic hydrogels appeared to be well-tolerated in the early postoperative period, but four of the six extruded with time from the wound site. Of the two remaining, one was intact and the other eroded through the anterior corneal cap (Fig. 7A, B). All the frozen, lathed hydrogels were well-tolerated and did not erode (Fig. 8A, B).

Difficulty was encountered with the complete dehydration of the hydrogels during processing. This was an unusual problem with the development of air bubbles in the specimen that was apparently due to the leaching out of gases from the hydrogels during the process of embedding, which tended to produce numerous vacuoles in our initial sections.

Discussion

Foreign materials, including hydrogels, have been implanted within the cornea in order to study its physiology, to change refractive errors, and to control corneal edema. Knowles\textsuperscript{8} and others previously implanted various low-water content materials in the corneal
Fig. 3. Low-power, light micrograph of a trephined hydrogel keratophakia specimen. Note epithelium entrapped at the edge of the hydrogel. The ocular surface epithelium over the hydrogel is thin, but the epithelium near the wound margin is thickened to eight cell layers. Bowman's layer shows numerous folds. The endothelial cytoplasm appears attenuated with prominent nuclei (X230).

Fig. 4. Transmission electron micrograph of normal appearing endothelium beneath a cryolathed hydrogel implant (X13,000). Inset: Low-power, scanning, electron micrograph of endothelium. The endothelial surface appears normal (X1,000).

stroma and found that the overlying corneal stroma became thin and ulcerated. The main reason for the necrosis of the cornea is a loss of nutrients supplied to the anterior cornea from the aqueous.8

Because hydrogel lenses have a high degree of permeability and do not interfere with the flow of nutrients in the cornea, McCarey and Andrews9,10 used them for implantation in rabbit corneas to produce a change in the anterior corneal curvature. They used a freehand dissection to produce an intrastromal pocket in the recipient cornea to receive an aphakic hydrogel lens (ie, not lathed) designed to induce corneal curvature change. Previous work in our laboratory has demonstrated the advantage of microkeratome techniques to permit an increase in anterior corneal curvature.11

In the rabbit model used by McCarey and Andrews,9 the optical interfaces appeared smooth. They did not document the accumulation of active keratocytes at the interfaces but noted areas of abnormal fibroblast activity at the posterior interface of the hydrogel and stroma. They did not discuss the presence of phagocytic and activated keratocytes in the region of the hydrogel or the presence of macrophages in their specimens. Ultrastructure of the host rabbit cornea was otherwise normal.

In contrast to the work of McCarey and co-workers,9,10 we found activated keratocytes with large and numerous mitochondria, prominent rough endoplasmic reticulum, and newly formed collagen at the edges of the implanted lenticles and on both ocular interfaces (Figs. 1, 5). We also documented the presence of phagocytic keratocytes in the optical interfaces that
Fig. 6. Transmission electron micrograph of a macrophage at anterior stromal-hydrogel interface. Pseudopodia and primary and secondary lysosomes are seen (×9,000).

Fig. 7. A, Low-power, scanning, electron micrograph of the surface of the same specimen in Fig. 7B. Note the sharply demarcated edges of the anterior cap over the lenticle that appear to be distorting the cornea (arrows). Note the absence of the central cornea over the hydrogel (white arrows) (×20). B, Low-power, scanning, electron micrograph of freshly trephined aphakic hydrogel that has eroded through the central cap. The edge of the lenticle is very sharp and thick (arrow) due to trephine technique with the anterior cap showing a steep drop to the recipient cornea (×90).

contained secondary lysosomes (Fig. 5). We have seen similar features in keratophakia, epikeratophakia, and in myopic keratomileusis procedures performed in the same animal model in our laboratory. When we used factory-lathed (preground) lenticles, we found similar features in uninflamed eyes, but the number of active keratocytes was significantly reduced compared with this series. The difference in our present series and that of McCarey and co-workers may be due to the animal model, the use of different hydrogel materials, and the use of frozen and/or thick lenticles.

The first reported case of a human hydrogel intracorneal lens implantation by Sendele and co-workers showed few metabolically active-appearing fibroblasts, but fibroblasts and new collagen had partially encapsulated the intracorneal lens. Clinically, there was no refractive change, which was probably due to the intrastromal implantation of the hydrogel without cutting Bowman’s membrane circumferentially. We have shown previously that anterior curvature can be increased significantly with intracorneal implants by using the microkeratome in a fashion similar to keratophakia by cutting Bowman’s layer 360 degrees. Ultrastructurally, our primate model shows findings similar to those described by Sendele and co-workers with newly formed collagen at the interface and active keratocytes (fibroblasts) in the area of the interface (Fig. 5). However, in addition, we found macrophages.

Fig. 8. A, Low-power scanning electron micrograph of the anterior corneal surface overlying the cryolathed hydrogel in Fig. 8B. Note smooth wound apposition of anterior cap to recipient cornea (arrows). The dark appearing cells are more mature than the lighter cells (×20). B, Low-power, scanning, electron micrograph of a cryolathed hydrogel lenticle that was well-tolerated. Note the tapered edge of the lenticle (arrow) (×20).
and phagocytic keratocytes in the area of the implant (Figs. 5, 6) as well as thin epithelium overlying thick, trephined hydrogels (Fig. 3). The phagocytic keratocytes had a slender appearance similar to normal keratocytes, but demonstrated pseudopodial extensions and primary and secondary cytoplasmic lysosomes.

Our findings suggest that the implanted hydrogel lenses of central thickness less than 0.3 mm do not affect the epithelium or endothelium since both appeared to be healthy in all of our cryolathed specimens (Figs. 2, 4). One of our specimens in which the epithelium appears to have been implanted into the wound producing epithelial cysts is of particular interest (Fig. 3). This complication is a known hazard of lamellar refractive surgery.5

An important feature in these experiments was the presence of three cell types at the hydrogel-stromal interface. Active keratocytes noted by extensive and dilated endoplasmic reticulum with newly formed collagen adjacent to its cell membrane is expected in the wound healing process (Fig. 5). The phagocytic keratocyte is of importance since it is usually not seen in normal wound healing (Fig. 5). Its role in the turnover of the extracellular corneal matrix has been supported strongly.15 These two cell types may be involved in a reaction of the corneal stroma to injury, infection, or, in this case, to a foreign material (hydrogel). The third cell type, the macrophage, is distinctly present in this series of hydrogel implants. Although normally seen in wound healing, these cells were still present 6 months postoperatively. The presence of these cells also may represent a reaction to the hydrogel material. Potential toxic effects produced by the hydrogel material do not seem to be a factor due to the absence of inflammatory cells and new vessel ingrowth. Furthermore, we have tested various hydrogels in tissue culture using baboon keratocytes, and no adverse side-effects have been seen (unpublished observation).

The freshly trephined, thick hydrogel specimens in this series had significant, late complications. Four of the six hydrogels were lost through extrusion, however, one remained totally intact, while the other eroded through the anterior cap (Figs. 3; 7A, B). The epithelium over this lenticle was thinned to three cell layers (Fig. 3). Extrusion was due to errors in diameter sizing, the excess thickness of the hydrogels, as well as the nontapered (trephined) edges. When we used cryolathed lenses with minimal central thickness and tapered edges as in the present study or factory lathed (preground) lenticles in a subsequent study13 the morphologic and clinical results were excellent (Figs. 1; 8A, B).

The use of the cryolathed hydrogel lenticles appears to produce the best results. In a more recent series using similarly shaped factory-lathed hydrogel lenticles,13 we have found a significant decrease in the phagocytic keratocyte and macrophage cell types. Encapsulation of the hydrogel lenticle by keratocytes and collagen is of concern since it may cause an opacity in the visual axis, which can appear as a disturbance in the fundus reflex. Our current, ongoing series with hydrogel implants in baboons 23 months postoperatively has crystal clear corneas using implants of 40% and 55% hydration. The elimination of the thick hydrogels or the frozen (cryolathed) hydrogels appears to have reduced significantly the keratocyte reaction. However, further long-term studies are needed to evaluate the interaction of the implanted hydrogel and the host cornea.

Key words: keratophakia, refractive keratoplasty, hydrogel implant, contact lenses, lamellar keratoplasty

References