Histological and Confocal Changes in Rabbit Cornea Produced by an Intrastromal Inlay Made of Hexafocon A

Ahmad Salamatrad, MD, Mahmoud Jabbarvand, MD, Hesam Hashemian, MD, Mehdi Khodaparast, MD, and Farshad Askarizadeh, MS

Purpose: The aim of this study was to evaluate biocompatibility of a newly proposed intrastromal inlay in rabbit corneas.

Methods: Eighteen eyes of 9 New Zealand rabbits were included in this prospective study. An intrastromal pocket was created in both eyes using Melles instruments. Annular intracorneal inlays made of hexafocon A were implanted randomly into the stromal pocket of one eye of each rabbit. Confocal microscopy was performed at each visit during 6-month follow-up. After 6 months, the rabbits were killed and corneal tissues of both eyes were sent for light microscopic studies.

Results: Mild stromal edema was present during the first few days and disappeared afterward with mild haze around the tunnel site in all cases. Deposits around the lamellar channel developed in 3 implanted eyes and in none of the pocket-only eyes. No neovascularization or epithelial downgrowth was present at the incision site in any case. All inlays remained centered and optically clear. In confocal imaging, we observed no significant difference in keratocyte cell density and inflammatory cells between the control pocket-only group and inlay group. In pathological evaluation, there was no difference in the average epithelial thickness between both groups. Descemet membrane and endothelium appeared normal in both groups.

Conclusions: This study revealed safety and biocompatibility of hexafocon A as an intracorneal inlay in rabbits.

Key Words: intrastromal inlay, hexafocon A, rabbit model

(*Cornea* 2015;34:78–81)

Corneal inlays, first proposed by Barraquer,[1] are reversible intrastromal implants that do not require stromal tissue removal. Instead, synthetic material is added to the stroma to reshape the corneal surface and to correct refractive errors in some cases. Primarily, poor results of implantation were due to low permeability of inlay materials.[2] Over the past decade, more water-, oxygen-, and nutrient-permeable materials such as KAMRA inlays (AcuFocus, Inc, Irvine, CA) and Perma-Vision lenses (Anamed Inc, Lake Forest, CA) were developed, which are commercially available for correction of presbyopia and hyperopia, respectively.[3] Several human and animal studies have proved the safety and efficacy of these inlays. McCarey et al[4] reported favorable results of hydrogel intracorneal inlay implantation into the eyes of rhesus monkey after 2 years of follow-up. Ismail,[5] Sweeney et al,[6] and Xie et al[7] reported on the safety, efficacy, and excellent biocompatibility of intrastromal lens implantation in rabbit eyes. Sweeney et al[6] reported a similar experience with human eyes. Other human studies have reported acceptable visual outcome after corneal inlay implantation for hyperopia correction.[8,9]

Newly proposed techniques for management of keratoconus are increasing, which are stimulated by the demand to avoid keratoplasty. Intracorneal segments and MyoRings (Dioptex GmbH, Linz, Austria) have been helpful in attaining this goal.[10,11] However, each of these new techniques has been associated with some complications, including epithelial abnormalities, especially when implantation is not deep enough.

Recently, with improvement in more nutrient-permeable synthetic materials, nutrient flow through the implant should not be an unmanageable problem any more.[2] We propose an intrastromal inlay to regularize corneal shape in keratoconus. Annular intracorneal inlays (AICI) are corneal inserts made of hexafocon A designed to regularize the corneal surface by flattening the central corneal curvature. Hexafocon A has very high oxygen permeability (Dk 100) and seems to be superior compared with currently available materials used as corneal inlays (polymethyl methacrylate).[12] To the best of our knowledge, this is the first time that hexafocon A has been used as corneal inlay. The purpose of this animal study was to investigate the biocompatibility of the proposed AICI after surgery in the rabbit model. Cellular changes were analyzed at different stromal depths using confocal microscopy. In addition, pathological changes of the rabbit cornea after implantation of this corneal inlay were studied.

MATERIALS AND METHODS

Eighteen eyes of nine 12- to 15-week-old female New Zealand white rabbits weighing 2.3 to 3.2 kg were included in this prospective study. Each rabbit had intrastromal pockets created in both eyes, and the intrastromal inlay was randomly implanted in one of the eyes.

Received for publication March 10, 2014; revision received September 7, 2014; accepted September 11, 2014. Published online ahead of print October 29, 2014.

From the Ophthalmology Research Center, Ophthalmology Department, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran.

The authors have no funding or conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.corneajrnl.com).

Reprints: Hesam Hashemian, MD, Eye Research Center, Farabi Eye Hospital, South Kargar St, Tehran, Iran 1336616351 (e-mail: h-hashemian@tums.ac.ir).

All animals were cared for in accordance with the Association for Research in Vision and Ophthalmology resolutions regarding the use and ethical treatment of laboratory animals. The protocol was reviewed and approved by the animal care committee of Farabi Eye Hospital.

The AICI made of hexafocon A polymer with a refractive index of 1.415, mimicking normal corneal stroma, was used in this study. This inlay was proposed to regularize corneal shape in patients with keratoconus. By redistributing the stresses on the cornea, this inlay created a hammock effect that reduced the biomechanical force exerted by the intraocular pressure and aqueous, preventing further corneal ectasia. The inlay used in this study had a thickness of 160 μm, an outer diameter of 5 mm, and an inner diameter of 4 mm. This polymer was previously tested and was proved to have good nutrient and oxygen permeability for contact lens application.[12]

Rabbits were anesthetized by intramuscular injection of ketamine hydrochloride and xylazine hydrochloride. In addition, topical tetracaine was applied to each eye before operation.

A 4.5-mm long clear corneal curved wound, approximately 1 to 2 mm anterior to the temporal corneal limbus, was created. A curved Melles stromal dissector was used to create a corneal lamellar pocket to approximately 60% to 70% corneal depth, which was extended to 1 mm from the limbus for 360° with a diameter of 9 mm. The inlay was then inserted into the pocket centering roughly on the geometric center of the pupil. The contralateral control eye had a pocket dissected, but no inlay was placed in it. The wound was closed with 2 radial 10-0 nylon (Ethicon, Somerville, NJ) sutures in both groups (see Video, Supplemental Digital Content 1, http://links.lww.com/ICO/A252).

Postoperatively, topical chloramphenicol 0.5% and topical prednisolone acetate 1% were administered 4 times daily for 1 week to both eyes. Postoperative evaluation was performed at the first and seventh day, and every 4 weeks thereafter. The examination included integrity of the corneal stroma and epithelium above the inlay, assessment of conjunctival redness, position of inlay, and optical clarity and integrity of the inlay.

Postoperative confocal imaging was performed 1 and 3 months after surgery using the HRT3 Rostock Corneal Module (Heidelberg Engineering, Heidelberg, Germany). Confocal microscopy, using the technique described by Mastropasqua et al,[13] was performed by the same operator during all visits. Keratocyte density was calculated as the mean of the numbers of cells of 5 images in each stromal depth, counted manually within a 250- × 250-μm square. Results were given as cells per square millimeter. Refractive outcome was not measured in this animal study.

All animals were killed after 6 months, and the corneoscleral rims were removed using 0.12-mm forceps and Westcott scissors. The animals were killed using intravenous injection of pentobarbital while they were under general anesthesia with ketamine and xylazine before collection of corneoscleral rims. Corneoscleral rims were fixed with glutaraldehyde for light microscopic studies. Epithelial thickness was determined by averaging 4 measurements on 4 different tissue sections above the inlay in both study groups. We evaluated stromal keratocytes and collagen lamellar pattern around the pocket and at the edges of the implant. Descemet membrane and endothelium were also evaluated in both groups.

Normal distribution of data was analyzed by the Kolmogorov–Smirnov test. Student t test was used to compare the 2 study groups. $P < 0.05$ was considered significant.

RESULTS

Clinical Examination

Inlays were successfully implanted in each animal at a depth between 60% and 70% of stromal thickness. Although the exact stromal depth was difficult to assess using slit-lamp biomicroscopy, measurement of pocket depth with confocal microscopy revealed a mean pocket depth of 67% and 65% implantation in the inlay eye and fellow eye, respectively.

Mild stromal edema was present during the first few days and disappeared afterward with slight haze around the tunnel site in all cases. This mild haze seemed to be associated with the wound-healing response in the tunnel site through the wound-healing process. Mild corneal edema resolved between 5 and 10 days postoperatively in all cases. After the first month of operation, no eye had corneal edema or conjunctival injection.

Deposits around the lamellar channel developed in 3 eyes with AICI implanted between 1 and 3 months postoperatively. There were small, speck-like particles extending along or anterior to the inlay. Underlying structures were clearly visible in all 3 cases. This was not observed in any of the pocket-only eyes. Although edema was more prominent around the tunnel site during the first few days, deposits were mostly present around the implant.

No neovascularization or epithelial downgrowth was present at the incision site in any case. All inlays remained centered and optically clear. An even tear film over the cornea was present in both implanted and control eyes at all visits.

When examined under slit-lamp biomicroscopy, all corneas in implanted and control groups remained clear during consecutive follow-ups. Mild conjunctival injection was observed in the first postoperative days, and it resolved after 1 month. Anterior chambers were clear in all eyes during the follow-up period.

Imaging

Confocal imaging showed no significant difference in keratocyte cell density at 3 stromal depths, and endothelial cell density between the control pocket-only and the inlay groups (Fig. 1; Table 1). There were no differences between both groups in corneal pachymetry and pocket depth ($P = 0.1$ and 0.5, respectively).

Pathology

Hematoxylin–eosin staining was performed for all corneas (Fig. 2). There was no difference in average epithelial thickness between both groups (43.6 ± 6.2 and 47.8 ± 5.9 for implanted and pocket-only groups, respectively; $P = 0.07$). Intrastromal deposits around the implant corresponded to nonhomogeneous

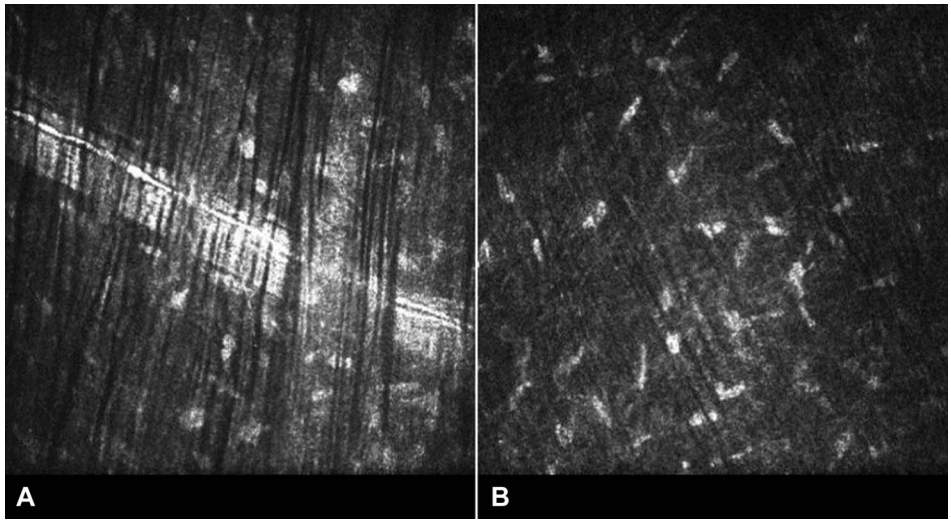

FIGURE 1. Confocal microscopy images of rabbit midstromal cornea. A, Inlay implanted. B, Control. The figures demonstrate no significant difference in keratocyte density and stromal clarity.

cellular formations in pathology sections. Compression of collagen lamellae was evident around the edges of the implant (Fig. 2). Descemet membrane and endothelium appeared to be normal in both groups. The incision site across the cornea was determined by fibrosis. New irregular lamellar collagen formation and hypocellular primitive scar tissue were present around the implant. Keratocyte density was normal around the implant.

DISCUSSION

Barraquer[1] pioneered implanting flint glass and plexiglass into the corneal stroma to reshape the cornea and correct refractive errors. Unfortunately, because of lack of biocompatible materials, most cases had unfavorable outcomes. Sweeney et al[6] summarized studies about implantation of synthetic materials as corneal inlays and provided essential properties for these inlays.

Hexafocon A is a rigid high-Dk material that has been used as a base material for contact lens production worldwide. Hexafocon A has an ISO/Fatt Dk of 100 with proven records of good oxygen permeability.[12] There are no previous studies on nutrient permeability of hexafocon A, but lack of tissue melting and pathological results of this study suggest good biocompatibility and nutrient permeability of the material, which makes it acceptable for intrastromal corneal inlays.

TABLE 1. Comparison Between the Control Pocket-Only Group and Inlay Group in Confocal Imaging 3 Months After Surgery

	Inlay Eye	Fellow Eye	*P*
Anterior keratocyte density	1821 ± 24	1839 ± 19	0.09
Midstromal keratocyte density	1418 ± 42	1439 ± 48	0.4
Posterior stromal keratocyte density, cells/mm^2	1158 ± 47	1167 ± 50	0.7
Endothelial density, cells/mm^2	4859 ± 97	4809 ± 73	0.06
Pachymetry, μm	356 ± 26	376 ± 26	0.1
Pocket depth, μm	238 ± 29	245 ± 28	0.5

We observed deposits around the lamellar channel in 3 eyes with AICI implanted. Other animal studies on intrastromal inlays[14–16] revealed keratocyte activation, new collagen formation, and lipid deposit around the implants. Similarly, human histopathological findings of electron-dense deposits containing interspersed collagen fibrils, extracellular empty spaces (likely clefts formed from lipid removal during processing), and persistently activated keratocytes have been reported.[17] Extracellular lipid deposits, corresponding to clinically observed crystalline deposits, probably arise from chronic mechanical irritation to keratocytes by the inlay.[17] Roth et al[18] described pathogenesis of experimentally induced lipid keratopathy in rabbits that had similarities to the lamellar channel deposits reported after INTACS implantation. Other investigators described similar findings with both PMMA and hydrogel corneal implants in primates.[16,19]

Several human and animal studies have reported the safety and efficacy of inlay procedures in reshaping the cornea. McCarey et al[4] reported favorable results in implanting hydrogel intracorneal lenses (lidofilcon A and lidofilcon B; Allergan Medical Optics, Irvine, CA) in rhesus monkeys during a 2-year follow-up.

Ismail,[20] Sweeney et al,[6] and Xie et al[7] reported excellent biocompatibility of intracorneal lenses in rabbit eyes. Sweeney et al[6] reported similar favorable results in human eyes. Other human studies reported similar safety and efficacy profiles for hyperopia-correcting corneal inlays.[8,9] Recently, we reported our results of a complete intracorneal ring (MyoRing) to correct keratoconus.[10,11,21] However, some human studies reported complications of intrastromal inlays, including significant visual loss and scarring, intrastromal epithelial opacification, and unacceptable visual outcomes.[8,20,22]

In this animal study, we implanted intracorneal inlays made of hexafocon A in New Zealand white rabbit eyes. No serious epithelial, stromal, and endothelial complications were observed during the 6-month follow-up period.

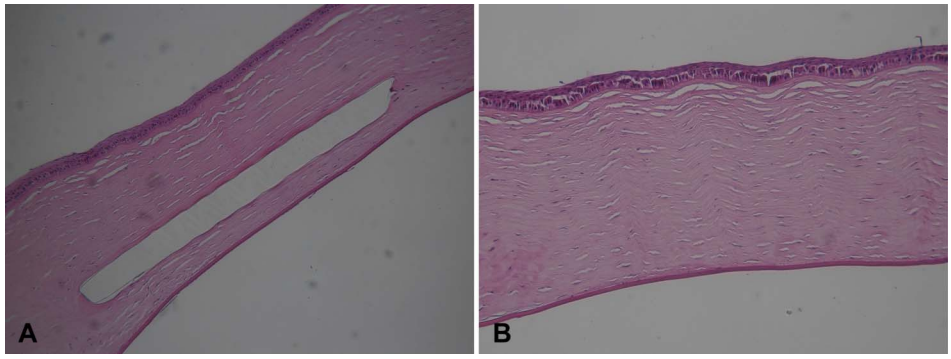

FIGURE 2. Rabbit corneal tissues stained with hematoxylin–eosin. A, Inlay implanted. B, Control tissue demonstrating normal epithelium, stroma, and endothelium. Slight compression of stromal tissue adjacent to the implant is visible.

This study revealed safety and biocompatibility of hexafocon A as an intracorneal inlay in rabbits. The next step is including human corneas of blind eyes in the study to ensure safety and biocompatibility of hexafocon A in human tissue.

REFERENCES

1. Ji Barraquer. Queratoplastia refractiva, estudios e informaciones. *Oftalmologicas.* 1949;2:1–21.
2. Kim E, Ehrmann K, Choo J, et al. The effect of inlay implantation on corneal thickness and radius of curvature in rabbit eyes. *Cornea*. 2013; 32:e106–e112.
3. Kim EK. *In vivo assessment of sub-epithelial vision correction device*. The University of New South Wales; Doctoral dissertation, 2013.
4. McCarey BE, McDonald MB, van Rij G, et al. Refractive results of hyperopic hydrogel intracorneal lenses in primate eyes. *Arch Ophthalmol.* 1989; 107:724–730.
5. Ismail MM. Correction of hyperopia by intracorneal lenses: two-year follow-up. *J Cataract Refract Surg.* 2006;32:1657–1660.
6. Sweeney DF, Vannas A, Hughes TC, et al. Synthetic corneal inlays. *Clin Exp Optom.* 2008;91:56–66.
7. Xie RZ, Evans MD, Bojarski B, et al. Two-year preclinical testing of perfluoropolyether polymer as a corneal inlay. *Invest Ophthalmol Vis Sci.* 2006;47:574–581.
8. Michieletto P, Ligabue E, Balestrazzi A, et al. PermaVision intracorneal lens for the correction of hyperopia. *J Cataract Refract Surg.* 2004;30: 2152–2157.
9. Verity SM, McCulley JP, Bowman RW, et al. Outcomes of PermaVision intracorneal implants for the correction of hyperopia. *Am J Ophthalmol.* 2009;147:973–977.
10. Jabbarvand M, Salamatrad A, Hashemian H, et al. Continuous corneal intrastromal ring implantation for treatment of keratoconus in an Iranian population. *Am J Ophthalmol.* 2013;155:837–842.
11. Jabbarvand M, Salamatrad A, Hashemian H, et al. Continuous intracorneal ring implantation for keratoconus using a femtosecond laser. *J Cataract Refract Surg.* 2013;39:1081–1087.
12. Tsukiyama J, Miyamoto Y, Higaki S, et al. Changes in the anterior and posterior radii of the corneal curvature and anterior chamber depth by orthokeratology. *Eye Contact Lens.* 2008;34:17–20.
13. Mastropasqua L, Nubile M, Lanzini M, et al. Epithelial dendritic cell distribution in normal and inflamed human cornea: in vivo confocal microscopy study. *Am J Ophthalmol.* 2006;142:736–744.
14. Fleming J, Reynold AE, Kilmer L, et al. The intrastromal corneal ring: 2 cases in rabbits. *J Refract Surg.* 1987;3:227–232.
15. Twa MD, Ruckhofer J, Kash RL, et al. Histologic evaluation of corneal stroma in rabbits after intrastromal corneal ring implantation. *Cornea*. 2003;22:146–152.
16. Rodrigues MM, McCarey BE, Waring GO III, et al. Lipid deposits posterior to impermeable intracorneal lenses in rhesus monkeys: clinical, histochemical, and ultrastructural studies. *Refract Corneal Surg.* 1990;6: 32–37.
17. Spirn MJ, Dawson DG, Rubinfeld RS, et al. Histopathological analysis of post-laser-assisted in situ keratomileusis corneal ectasia with intrastromal corneal ring segments. *Arch Ophthalmology.* 2005;123:1604–1607.
18. Roth SI, Stock EL, Siel JM, et al. Pathogenesis of experimental lipid keratopathy. An ultrastructural study of an animal model system. *Invest Ophthalmol Vis Sci.* 1988;29:1544–1551.
19. Parks RA, McCarey BE, Knight PM, et al. Intrastromal crystalline deposits following hydrogel keratophakia in monkeys. *Cornea*. 1993;12: 29–34.
20. Ismail MM. Correction of hyperopia with intracorneal implants. *J Cataract Refract Surg.* 2002;28:527–530.
21. Jabbarvand M, Hashemi H, Mohammadpour M, et al. Implantation of a complete intrastromal corneal ring at 2 different stromal depths in keratoconus. *Cornea*. 2014;33:141–144.
22. Alió JL, Mulet ME, Zapata LF, et al. Intracorneal inlay complicated by intrastromal epithelial opacification. *Arch Ophthalmol.* 2004;122: 1441–1446.