# Collagen Gel for Ocular Surface

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**A replacement ocular surface requires a substrate that is easily manipulated surgically, does not cause an inflammatory reaction, and is nontoxic to epithelial cells. This work evaluates the usefulness of a collagen gel as a substrate for corneal epithelial cells by determining the ocular toxicity of the gel and the ability of the gel to support and maintain corneal epithelial cells in vitro. Collagen gels, made from Vitrogen®, were easily manipulated and were well-tolerated in rabbit eyes for up to 6 wk (n = 3). Epithelial sheets placed on collagen gels and incubated at 37°C for up to 13 days remained well-apposed to the gels and appeared normal, but thinned, from five to three layers. The basal cells extended cytoplasmic blebs into the gels, but only one sheet of five sheets showed basement membrane deposition by 6–13 days. Thus, the collagen gels appear to meet the criteria defined above and may be a suitable substrate in biofabricated ocular surfaces. Invest Ophthalmol Vis Sci 26:901–905, 1985**

Ocular surface disorders remain a major therapeutic challenge for ophthalmologists. While abnormalities of substrate may cause epithelial adhesion problems in some cases, the success of new surgical procedures such as conjunctival transplantation and keratoepithelioplasty in establishing intact epithelial coverings for diseased corneas suggest that epithelial replacement is also an important therapeutic step. Peripheral placement of the donor epithelium, as in conjunctival transplantation or keratoepithelioplasty, is important since centrally placed donor epithelium will, within a few months, be replaced by host epithelium with possible recurrence of disease.[1–4] In such cases, routine penetrating keratoplasty, even with retained donor epithelium, is not helpful in the long run. In any event, shortages of epithelial donor material either from eye banks or the patient's fellow eyes limits the usefulness of the available peripheral placement techniques in many cases. For these reasons, we have been interested in creating a substitute ocular surface that can be used to replace diseased or damaged corneal and conjunctival epithelial cells.

Transplantation of a sheet of epithelium is technically difficult. Therefore, we have investigated possible substrates to use as a vehicle for cells. A proper carrier should be noninflammatory, surgically manageable, nontoxic to epithelial cells, and epithelial cells should adhere to the surface. Previous attempts to biofabricate such a substitute ocular surface using corneal stroma, sclera, or vitelline membrane as a substrate for cell suspensions of tissue cultured corneal epithelium were unsuccessful because of either minimal thickening of the epithelial layer, or marked inflammatory cell invasion of the substrate.[5]

Based on this concept for biofabrication of ocular surface we have prepared a sheet of artificial collagen gel that potentially could be used as a substrate. It is easily prepared and can be manipulated surgically. In this work we have evaluated: (1) the ocular toxicity of the collagen sheet; and (2) the ability of epithelial cells to adhere to the collagen in vitro. To accomplish this, we applied intact corneal epithelial sheets (obtained using the enzyme dispase II) directly to a fixed collagen gel and studied the epithelial sheet morphology on the gel in vitro with light and electron microscopy. In addition, we implanted the collagen gels onto the bulbar conjunctiva of rabbit eyes in vivo and studied the gels and the reaction of the surrounding tissue clinically and morphologically.

***Materials and Methods.*** *Collagen gel preparation:* Vitrogen-100® (Flow Laboratories, Inc.; McLean VA), prepared from pepsin solubilized bovine dermal collagen, was used to make gels according to established techniques.[6] One milliliter of sodium phosphate dibasic (0.2 M) in 1.3 M sodium chloride was mixed with 1 ml of cold 0.1 M sodium hydroxide and the solution was passed through a Millipore® filter (pore size 0.22 $\mu$m, Millipore Corporation; Bedford, MA). Eight milliliters of Vitrogen-100® was added to this mixture, and the solution was poured into 35-mm sterile culture dishes until the bottom was covered (about 1 ml per dish). The dishes were placed in a tissue culture incubator (37°C, 5%$C0_2$:95% air, water-jacketed) for 15 min, and then were fixed in situ with 0.5% glutaraldehyde at room temperature overnight. The fixed gels were removed with a spatula and were rinsed repeatedly with phosphate buffered saline (0.01 M, pH 7.4) to remove excess glutaraldehyde. The gels were stored in phosphate buffered saline at 4°C. A 5- or 8-mm diameter trephine was used to punch out a circular gel which was placed in a culture dish containing SHEM tissue culture medium[7] and exposed to ultraviolet light for 24 hr prior to each experiment.

*In vivo gel experiments:* All animal investigations described in this manuscript conform to the ARVO Resolution on the Use of Animals in Research.

New Zealand albino rabbits, weighing 2–3 kg, were anesthetized by intramuscular injection of chlorpromazine hydrochloride (25 mg) and ketamine hydrochloride (200 mg) with additional topical proparacaine anesthesia. The eyes were proptosed and a surgical microscope was used during suturing. A 5-

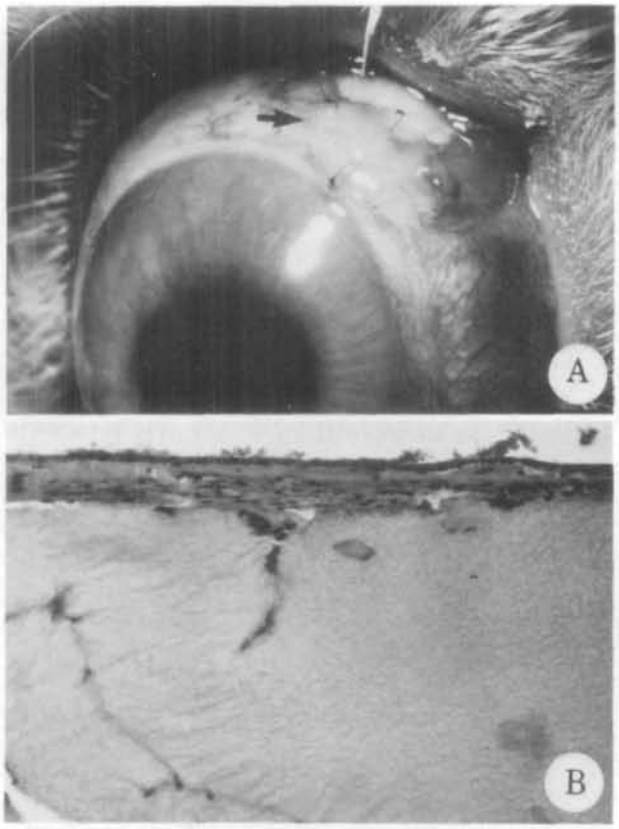


**Fig. 1.** Collagen gels on rabbit eyes in vivo. **A**, A 5-mm diameter collagen gel sutured to the limbus (arrow). **B**, Conjunctival epithelium migrated over the collagen gels in the rabbit eyes and appeared normal at 6 wk. Note minimal infiltration of gel with inflammatory cells (hematoxylin and eosin; original magnification, ×250).

mm diameter gel disk was sutured to the limbus over the conjunctiva with four 10–0 nylon sutures (Fig. 1A). One animal had a donut-shaped gel, 5 mm in width with a central 9-mm diameter hole, sutured to the limbus for 360°. The animals (n = 3) received polysporin antibiotic ointment immediately and were followed for 6 wk, at which time the animals were killed with an overdose of sodium pentobarbital, and the eyes were enucleated and prepared for histologic study.

*In vitro epithelial sheet:gel experiments:* Intact rabbit corneal epithelial sheets were removed with the technique of Gipson and Grill[8] using the enzyme dispase, grade II (Boehringer Mannheim; Indianapolis, IN). The 9-mm diameter sheets were draped over the 8-mm diameter collagen gel disks and cultured in a tissue culture incubator for 1, 3, 6, or 13 days (n = 2 or 3 at each time point) in SHEM tissue culture medium,[7] following which they were prepared for histologic study.

*Histologic preparations:* Enucleated eyes with attached collagen gels were fixed in 10% buffered formalin. The portion of the eye containing the gel was dissected, sectioned, and stained with hematoxylin and eosin for light microscopic examination.

Gels with attached epithelial sheets were fixed in half-strength Karnovsky's fixative. The fixed gel was bisected through the center. One half was dehydrated, embedded in paraffin, cut into 7-$\mu$m sections, stained with hematoxylin and eosin, and examined by light microscopy. For transmission electron microscopy, the other half of the gel was transferred to 2.5% glutaraldehyde in 0.15 M phosphate buffer, pH 7.4, postfixed for 60 min in 1.0% osmium tetroxide, dehydrated, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined under a Philips 200 electron microscope.

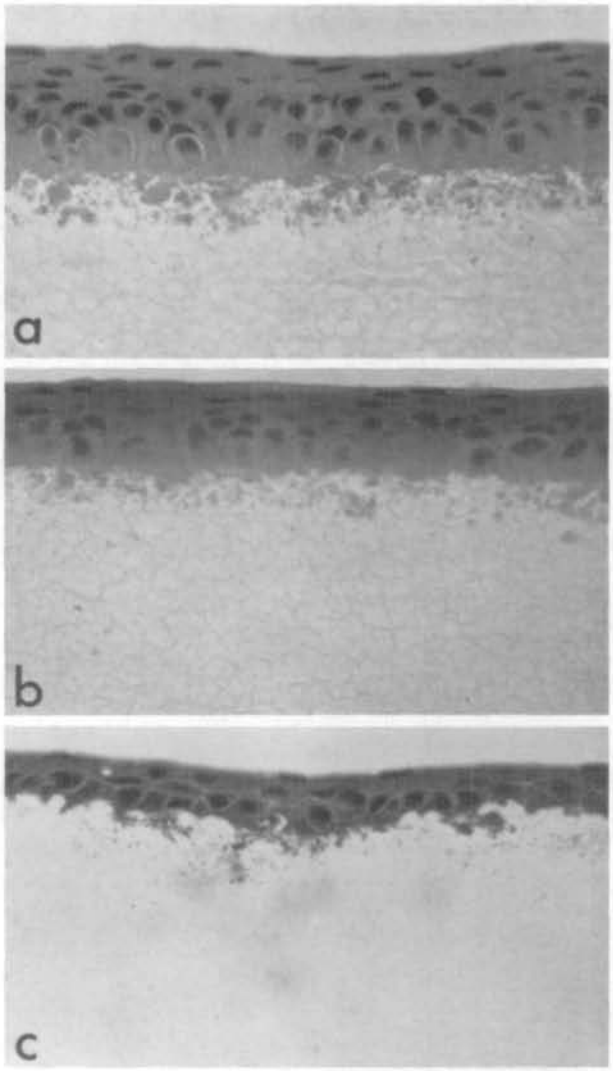


**Fig. 2.** Light microscopy of cultured epithelial sheet:fixed collagen gel combinations. **a**, 3 days on gel. Note blebbing of basal cells into gel. **b**, 6 days on gel. Five cell layered epithelial sheet appears well fixed onto gel. **c**, 13 days on gel. Stratified epithelial layer is thinner than at 6 days, but still intact (hematoxylin and eosin; original magnification, ×400).

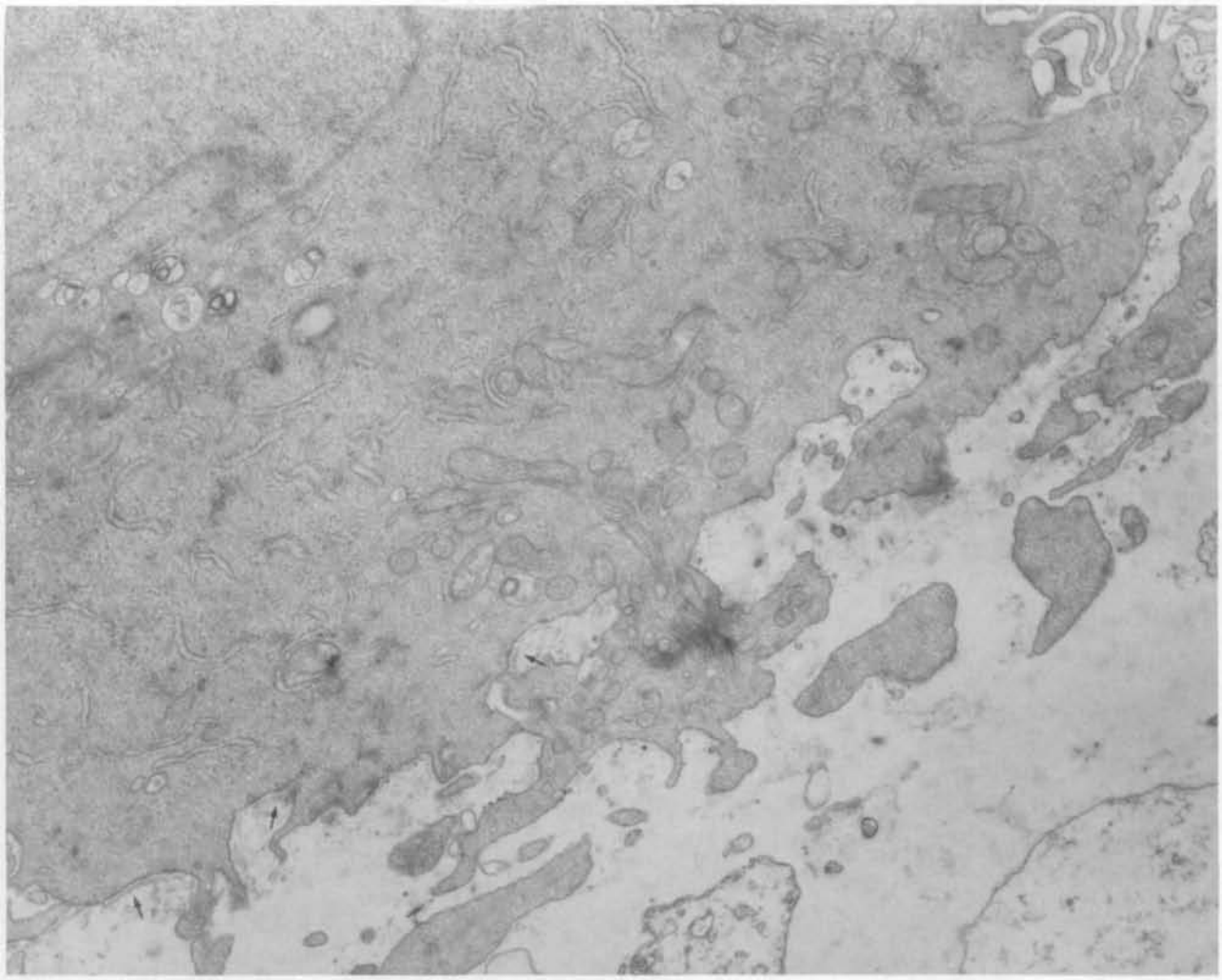

Fig. 3. Electron microscopic picture of cultured epithelial cell sheet:fixed collagen gel combination at 6 days. Basement membrane appears to be present in interrupted segments (arrows). The multilayered epithelium contains normal intracellular organelles. The basal epithelial cells extend blebs into the collagen matrix (original magnification, ×13,400).

***Results.*** The thin gel (300–400 μm) could be sutured carefully to the rabbit globe without being torn. Within 1 to 2 days after the slight inflammation that rabbits typically have, even after minor surgery, the eyes were white and quiet (Fig. 1A). The gel molded to the surface of the eye with no tendency for dellen formation. At 6 wk, there was only a minimal inflammatory reaction visible either on the histology sections or clinically. The conjunctival epithelium underlying the gels had thinned in many areas, but was still present after 6 wk. The rabbits' own conjunctival epithelia migrated over the gel surface and maintained its usual stratified appearance (Fig. 1B).

Light microscopy of the cultured epithelial cell sheet:fixed gel combinations revealed a normal five-layered corneal epithelium with blebbing of the basal cells into the gel substance by 3 days in all cases. By 6 days, the gel was four to five layers thick and appeared well-apposed to the gel surface (n = 3). By 13 days, the epithelium had thinned to three layers (Fig. 2).

Transmission electron microscopy of the cultured epithelial cell sheet:fixed gel combinations showed healthy basal cells with blebs extending into the collagen matrix at 3 days (n = 2). The epithelium appeared unchanged at 6 days (n = 3). In one sample at 6 days, basement membrane was present in interrupted segments (Fig. 3). At 13 days, the three-layered epithelium appeared healthy but attenuated; surface microplicae and desmosomes were still present, but scant basement membrane was visible along the basal cell membrane (n = 2) (Fig. 4).

***Discussion.*** Collagen has been employed in ophthalmology for a variety of uses including scleral buckling bands, suture material for entropion repair,

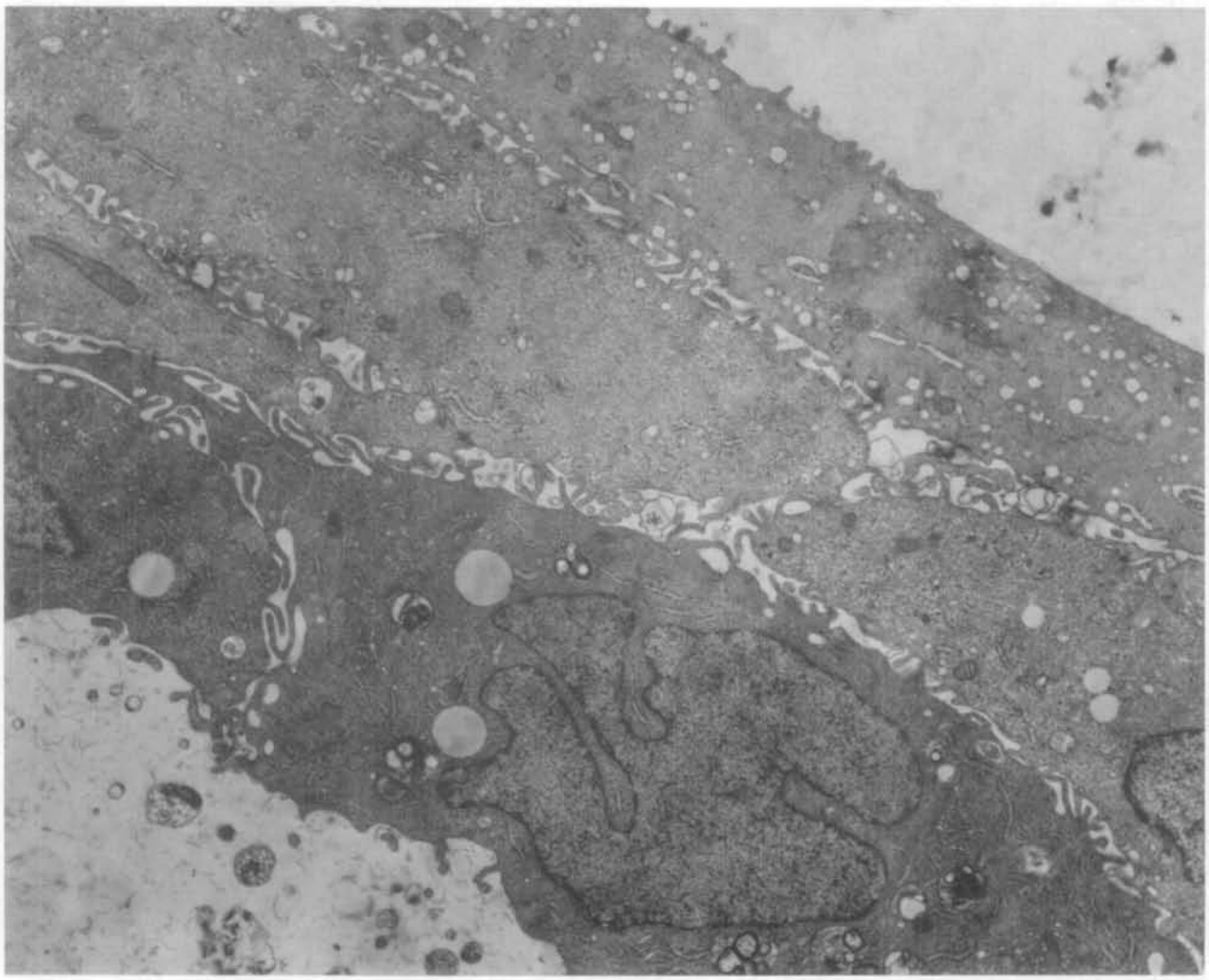

**Fig. 4.** Electron microscopic picture of cultured epithelial cell sheet:fixed collagen gel combination at 13 days. Surface microplicae and desmosomes are present. Scant basement membrane is present along the basal cell membrane (original magnification, ×10,600).

lamellar keratectomy tissue, vitreous substitutes, and glaucoma filter tubes.[9] The eye tolerates various collagen preparations quite well—sometimes partly degrading the collagen over several months[9] or mounting no cellular reaction and leaving the collagen matrix undisturbed.[10] as seemed to be the case for at least 6 wk in these studies.

An ideal ocular surface substrate should meet the following criteria: it should be easily manipulated surgically; induce minimal inflammation; and be nontoxic to epithelial cells. The fixed collagen gel described in this report appears to satisfy these criteria. It is simple to manufacture and is well tolerated in the rabbit eye in vivo for up to 6 wk. Although the gel is quite thin, it can be cut to any desired shape and be sutured to the globe without being damaged. The fixed Vitrogen-100® gel is only minimally infiltrated by inflammatory cells and clinically showed only minimal resorption at 6 wk. The antigenicity of the collagen sheets is, therefore, apparently very low.[10]

It has been demonstrated previously that corneal epithelial cells, as well as other epithelial cells, grow better on collagen than on uncoated tissue culture plates.[11] In the epithelial organ culture model presented here, the epithelial sheets retained their normal morphology for at least 2 wk on the gels in vitro, suggesting that collagen provides a good substrate for the epithelial sheets as well as for cell cultures. The epithelial thickness slowly decreased over the 2-wk period, but the sheet remained well apposed to the gel surface. Although the basement membrane is not apparent even after 2 wk, the cells sheets are sufficiently adherent to the gel to survive the manipulations of the histologic processing. This suggests that basement membrane does not play a vital role in the initial phase of this adhesion. The basal cells may

anchor the sheet to the gel by the blebs they send into the anterior surface of the gels. The absence of basement membrane formation is not surprising, since 2–4 wk are required for the epithelium to lay down basement membrane following keratectomy both in vitro and in vivo.[12,13]

Thus, a system has been developed in which a nontoxic substrate, the collagen gel, can carry an epithelial sheet. Even if the gels degrade over a period of time, they may permit the surgical transfer of viable ocular surface epithelium to a damaged eye. This, in turn, may make epithelial transplantation a more feasible procedure than it currently is.

**Key words:** ocular surface epithelium, biofabricated ocular surface substitute, collagen gels, epithelial sheets

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## References

1. Thoft RA: Conjunctival transplantation. Arch Ophthalmol 95: 1425, 1977.
2. Thoft RA: Keratoepithelioplasty. Am J Ophthalmol 97:1, 1984.
3. Kinoshita S, Friend J, and Thoft RA: Sex chromatin of the donor corneal epithelium in rabbits. Invest Ophthalmol Vis Sci 21:434, 1981.
4. Thoft RA and Friend J: The X, Y, Z hypothesis of corneal epithelial maintenance. Invest Ophthalmol Vis Sci 24:1442, 1983.
5. Friend J, Kinoshita S, Thoft RA, and Eliason JA: Corneal epithelial cell cultures on stromal carriers. Invest Ophthalmol Vis Sci 23:41, 1982.
6. Kleinman H, McGoodwin EB, Rennard SI, and Martin GR: Preparation of collagen substrates for cell attachment: effect of collagen concentration and phosphate buffer. Anal Biochem 94:308, 1979.
7. Jumblatt MM and Neufeld AH: Beta adrenergic and serotonergic responsiveness of rabbit corneal epithelial cells in culture. Invest Ophthalmol Vis Sci 24:1139, 1983.
8. Gipson IK and Grill SM: A technique for obtaining sheets of intact rabbit corneal epithelium. Invest Ophthalmol Vis Sci 23:269, 1982.
9. Chvapil M, Kronenthal RL, and van Winkle W Jr: Medical and surgical applications of collagen. *In* Int. Rev. Connective Tissue Research, Vol 6, Hall D and Jackson DS, editors. New York, Academic Press, 1973, pp. 38–43.
10. Stenzel KH, Branwood AW, and Rubin AL: Collagen-derived membrane: corneal implantation. Science 157:1329, 1967.
11. Gospodarowicz D, Greensburg G, and Budwell CR: Determination of cellular shape by the extracellular matrix and its correlation with the control of cellular growth. Cancer Res 38: 4155, 1978.
12. Gipson IK, Friend J, Spurr SJ, and Brennan SJ: Transplant of corneal epithelium onto abraded or keratectomized rabbit corneas in vivo. ARVO Abstracts. Invest Ophthalmol Vis Sci 24(Suppl):10, 1983.
13. Khodadoust AA, Silverstein AM, Kenyon KR, and Dowling JE: Adhesion of regenerating corneal epithelium: the role of basement membrane. Am J Ophthalmol 65:339, 1968.

# *Activation of the Alternative Complement Pathway by Intraocular Lenses*

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**To determine if posterior chamber polymethylmethacrylate lenses with polypropylene loops activate complement, the authors measured levels of C3a, C4a and C5a by radioimmunoassay in human sera incubated with and without these lenses. Human sera incubated with intraocular lenses showed elevated levels of C3a and C5a but no change in C4a. There were no statistically significant differences in the generation of activated complement by polypropylene loops vs polymethylmethacrylate optics. The authors also compared the ability of intraocular lenses to activate complement with that of zymosan and endotoxin, known activators of the alternative pathway. Our results suggest that polymethylmethacrylate lenses with polypropylene loops generate C3a and C5a by activation of the alternative complement pathway. Invest Ophthalmol Vis Sci 26:905–908, 1985**

Cataract extraction with implantation of an intraocular lens is a frequently performed surgical procedure today. Nearly one million intraocular lenses are now implanted annually with approximately two-thirds of these lenses being of the posterior chamber type.[1] Although posterior chamber intraocular lenses are generally well-tolerated, some cases develop persistent postoperative uveitis.[1]

A previous study suggested that intraocular lenses activate the complement system and that this may be one mechanism by which intraocular lenses cause inflammation.[2] Polymethylmethacrylate lenses with nylon and polypropylene loops were found to be