Epithelialization of a Synthetic Polymer in the Feline Cornea: a Preliminary Study

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PURPOSE. This study examined the potential of a synthetic polymer to support stable epithelial growth when implanted in the feline cornea.

METHODS. A perfluoropolyether-based polymer was cast into lenticules that were coated with collagen I and implanted in four feline corneas. Epithelial growth onto the lenticules was monitored clinically for 6 weeks, after which time the animals were killed, and three corneas were evaluated histologically. Immunohistochemistry was used to identify proteins associated with the formation of a basement membrane (laminin) and adhesion complexes (bullous pemphigoid antigen and collagen VII). Electron microscopy was used to examine the tissue–polymer interface for evidence of the assembly of these adhesive structures.

RESULTS. Postoperative epithelial growth began on days 2 to 3, and lenticules were fully epithelialized by days 5 to 9. Lenticules were clinically well tolerated and histology showed epithelium consisting of multiple layers adherent to the lenticule's surface. Laminin, bullous pemphigoid antigen and collagen VII were identified at the tissue–polymer interface using immunohistochemistry. Ultrastructural examination showed evidence of assembly of these proteins into a recognizable basement membrane and hemidesmosomal plaques.

CONCLUSIONS. A perfluoropolyether-based polymer coated with collagen I was implanted in the feline cornea and supported epithelial growth that showed signs of persistent adhesion, both clinically and histologically. This polymer shows potential for ophthalmic applications that require sustained epithelialization. (Invest Ophthalmol Vis Sci. 2000;41:1674–1680)

Stable epithelial cover of an implant in the cornea is a shared goal in the design of devices for epikeratoplasty (epikeratophakia) and the optic component of a keratoprosthesis. The requirements for a suitable material are stringent, including absence of toxicity, transparency, permeability, and surface characteristics that permit the migration and persistent adhesion of corneal epithelial tissue.1,2 Ideally, such devices would be incorporated into the cornea during a normal epithelial wound-healing response3,4 that would result in the epithelialization of the device and forming a wettable and self-renewing surface.5 This implies that the anterior surface of the device be covered by a stratified epithelium that is firmly adherent. In intact corneal tissue, the epithelium is anchored to the underlying stroma through the basement membrane by a series of adhesive structures.6,7 Each adhesive complex contains keratin intermediate filaments and hemidesmosomal plaque at the basal aspect of the epithelial cells; anchoring filaments of laminin 5, which traverse the basement membrane; and anchoring fibrils of collagen VII, which interweave with the collagen fibrils of the anterior stroma.8

Previous devices designed for corneal applications that require epithelialization have used polymers of either biologic or synthetic origin. The biologically derived materials have shown some promising results in vivo, although their success has been limited by lenticule remodeling and epithelial tissue abnormalities.9–13 Epikeratophakia lenticules of donor stromal tissue supported epithelial growth and were retained in the primate cornea for 22 to 25 months despite a concomitant build up of cellular debris, pigmented cells, and keratocytes around the grafted tissue.14 Results from a nationwide epikeratophakia study,9 in which human patients received lyophilized donor lenticules to correct aphakia, myopia, and keratoconus, identified persistent epithelial defects as an early problem. Abnormal morphologic characteristics were reported in epithelial tissue covering donor epikeratophakia lenticules in human eyes for up to 16 months after surgery.10 Histologic evaluation of donor human epikeratophakia lenticules that maintained epithelial cover for 8 months in patients revealed fewer hemidesmosomes and basement membrane defects at the epithelium–lenticule interface.11 Epikeratophakia lenti-
cules made from purified human placental type IV collagen (Laboratoires Domilens, St. Just Chaleyssin, France) maintained epithelial cover when tested in primate corneas over a 6-month period. Similar lenticules showed good clinical performance when used in an epikeratoplasty procedure in rhesus monkeys for up to 30 months, although postmortem histology revealed epithelial thinning on the lenticule’s surface and proteolytic degradation of the collagen lenticule. More recently, a two-part full-thickness corneal graft made from solubilized rabbit dermal collagen was tested in rabbit corneas. Although partial epithelialization of the fibrous peripheral zone was achieved in that study, the central optical zone of these grafts failed to support any epithelial growth after 24 days. This response was attributed to the chemistry of the modified collagen. Biologically derived polymers have also been tested in combination with synthetic cross-linkers. Collagen epikeratoplasty lenticules that were polymerized in situ in primates maintained multilayered epithelial cover for more than 1 year with evidence of neovascularization and remodeling of the cross-linked collagen during this time.

Synthetic polymers and copolymers offer certain advantages in the making of devices for epikeratoplasty (epikeratophakia) and keratoprosthesis applications. These include the reduced risk of transmission of infectious agents, an unlimited supply, improved optical properties and avoidance of postoperative remodeling. The design of polymers for these applications has been directed primarily at anchorage of the peripheral portion of the device in the corneal stroma. Results from in vivo studies show some success in terms of this aspect of the design, although optimization of a polymer to support epithelial growth over the central optical portion of these devices remains a challenge. A high-water-content contact lens material, lidofilcon A, has been tested as a synthetic epikeratophakia lenticule in rabbit corneas, but the lenses failed to support epithelial growth onto the polymer surface. A plasma-modified poly(vinyl alcohol) material showed only partial epithelial cover when implanted into rabbits as the optical component of a synthetic cornea, even though it supported epithelial growth in vitro during a 21-day period. Recent clinical trials of two different designs for a keratoprosthesis (KPro), the Chirila KPro (Lions Eye Institute, Perth, Australia) and the Biokpro II, (Department of Ophthalmology, Hotel-Dieu de Paris, Paris, France) have demonstrated that a full-thickness synthetic device can be maintained in the corneal stroma for some months. These KPro devices were successfully anchored into the cornea by fibrovascular ingrowth into the peripheral skirt, but the polymers used in the optical core did not epithelialize in either case. Both groups have acknowledged that the long-term stability of such KPros would be improved by the development of a polymer that supported epithelial growth.

We are interested in the design of a synthetic polymer for use as a corneal onlay in the correction of corneal trauma and/or scarring, keratoconus, and high-level refractive errors. Previous work in our group has confirmed the requirement for porosity in a synthetic lenticule implanted in the cornea. In addition to this, collagen I coated on the surface of a microporous synthetic material was found to promote epithelialization in vivo. The stable epithelial growth observed on those lenticules was attributed to the formation of adhesive structures (basement membrane and hemidesmosomes) at the epithelium-lenticule interface. Concurrent with these findings, a perfluoropolyether (PFPE)-based polymer has been tested for use as a synthetic onlay material. The high oxygen permeability of this polymer type led to its original testing in the ophthalmic field as a contact lens material. Other characteristics of this family of polymers that are suited to ophthalmic applications include transparency, low refractive index, and high oxidative and thermal stability. PFPEs are hydrophobic and yet have been shown to support the growth of corneal epithelial cells in vitro. Recently, microporous PFPE-based polymers have been developed that allow for the diffusion of nutrients including glucose and high-molecular-weight proteins such as albumin. In the present study, we report the performance of lenticules made from porous PFPE that were coated with collagen I and implanted in the anterior stroma of a feline model. The performance of these implants was monitored clinically for a 6-week period, at which time the epithelium-lenticule interface was examined histologically for evidence of structures associated with stable epithelial growth.

METHODS

Materials

The porous PFPE lenticules were made by the UV polymerization of PFPE-dimethacrylate.

\[
\text{CH}_3 \quad \text{H} \quad \text{O} \quad \text{O} \\
\text{CH}_2\text{COCH}_2\text{CH}_2\text{NC} \quad \text{OCH}_2\text{CF}_2\text{O}((\text{CF}_2\text{O})_{10}\text{CF}_2\text{O})_4\text{CF}_2\text{CH}_2\text{O} \quad \text{CH}_3 \quad \text{H} \quad \text{O} \quad \text{O} \\
\quad \text{CNCH}_2\text{CH}_2\text{OCC}=\text{CH}_2
\]

Briefly, the PFPE-dimethacrylate was dispersed in a mixture of alcohol, surfactant and 0.3% (wt/wt) of the photoinitiator Darocur 1173 (Ciba Speciality Chemicals, Basel, Switzerland). After mixing, the homogeneous PFPE mixture was dispensed into the polymerization cuvettes and then irradiated with a pair of 117-W twin-lamp UV source. After polymerization, the lenticules were washed with water to remove any unreacted material and after that, the lenticules were stored in a humid environment.

Lenticule Design

The PFPE lenticules used in this study were 4 mm in diameter and were planar, with an overall thickness of 80 µm tapering to 70 µm with square edges. The lenticules had a water content of 52%, representing the relative mass of water required to fill the porous voids within the polymer and providing a measure of the degree of porosity. The permeability of the lenticules to glucose, inulin, and human serum albumin was measured to be 47, 14, and 3.6 × 10⁻⁵ cm/sec, respectively.

Surface Coating

A thin layer of collagen I (approximately 5–10 nm thick) was covalently immobilized on the anterior surface of each lenti-
The animal research in this study was approved by the Animal Care and Ethics Committee of the University of New South Wales. All procedures were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The cats (n = 4) used in this study were 3 to 5 years of age, were of different sexes, and weighed 4 to 5.5 kg.

Surgery

The surgical procedure used in this study has been previously described.29 A beaver blade was used to remove the epithelium of the central cornea in a 4-mm diameter area. A circular keratotomy 150 μm deep was made in the debrided area using a 2-mm diameter trephine, and the stromal lamella within the trephined area was removed. A sharp corneal dissector was used to make a circular pocket 1 mm wide toward the limbus at the base of the keratotomy. The superior portion of the pocket was further extended until the superior edge of the pocket was approximately 5 mm from the pocket’s mouth. A 5 mm arcuate incision was made along the superior edge of the pocket through which a 4-mm diameter lenticule was delivered by a spatula. The lenticule was tucked into the circular stromal pocket and the superior edge incision secured with sutures.

Ultrastructure

TEM was used to assess the assembly of adhesive structures (basement membrane and hemidesmosomal plaques) at the epithelium-lenticule interface. Tissue was blocked from the central region of one feline cornea implanted with a collagen-coated PFPE lenticule (cat 3). Samples were fixed in 2.5% (vol/vol) glutaraldehyde and 2.5% paraformaldehyde in sodium cacodylate buffer (pH 7.2) with 1% sucrose and 1% calcium chloride for 3 hours and washed overnight in buffer. Samples were postfixed in 1% osmium tetroxide supplemented with 1.5% potassium ferrocyanide and stained en bloc with 2% aqueous uranyl acetate. Samples were dehydrated through a graded series of ethanol to propylene oxide, infiltrated, and embedded in Epon Araldite. Sections 80 to 90 nm thick were stained en grid, using uranyl acetate and Reynolds’s lead citrate, and were examined at 75 kV in a transmission electron microscope (model 7100 Hitachi, Tokyo, Japan).

RESULTS

Clinical

Details of the clinical observations are summarized in Table 1. Growth of corneal epithelial tissue over the collagen-coated PFPE lenticules occurred in each of the four animals studied. Epithelial tissue from the outer edge of the wound began to grow inward over the debrided area on days 2 to 3 after surgery. Newly grown epithelium completely covered the debrided zone and the exposed lenticular surface by days 5 to 9. A sham control for this surgical model demonstrated that the identical wound was completely re-epithelialized on day 3. In the current series, one animal (cat 4) was killed on day 14 because of complications associated with anterior stromal melting and gaping of the superior pocket that was originally made to facilitate delivery of the lenticule. There was no evidence of any stromal melting around the keratectomy edge in the three cats (cats 1, 2, and 3) that maintained epithelial cover in this study. The epithelial tissue covering the lenticules in each of
and not columnar as in intact corneal epithelium. Nevertheless, constituent cells. The basal epithelial cells were squamouslike grown epithelial tissue, despite the unusual morphology of the demonstrated the polarized and stratified nature of the newly microvilli on the apical surface (Fig. 3A). These characteristics lenticule examined using TEM comprised three to four layers Epithelial tissue covering the anterior surface of the PFPE-based Ultrastructure linear pattern at the epithelium–lenticule interface (Fig. 2D). Adhesion complex formation — — Yes — Basement membrane assembly Tight Tight Tight Tight Layers of epithelial tissue 4–5 3–4 3–4 — Tissue apposition to lenticule Tight Tight Tight Tight Laminin deposition at interface Broken linear Broken linear — — BPAG deposition at interface Broken linear Punctate — — Collagen VII deposition at interface Almost linear Almost linear — — Type of histologic evaluation Immunostaining Immunostaining TEM None* BPAG, bullous pemphigoid antigen. * Cat 4 was killed at day 14 due to complications, and no histology was performed.

TABLE 1. Summary of Clinical and Histologic Results

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Cat 1</th>
<th>Cat 2</th>
<th>Cat 3</th>
<th>Cat 4</th>
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<td>Postoperative epithelial growth from</td>
<td>Day 2</td>
<td>Day 2</td>
<td>Day 2</td>
<td>Day 3</td>
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<td>the outer edge of wound onto the</td>
<td>Day 9</td>
<td>Day 5</td>
<td>Day 8</td>
<td>Day 5</td>
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<td>de-brided zone commenced</td>
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<td></td>
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<tr>
<td>Full cover of de-brided zone and</td>
<td>Day 51</td>
<td>Day 43</td>
<td>Day 42</td>
<td>Day 14</td>
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<tr>
<td>exposed lenticule surface occurred</td>
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DISCUSSION

This study examined the performance of a PFPE-based polymer for use in corneal implants that require stable epithelial growth on the anterior surface of the implanted device. Earlier studies indicate that devices made from polymers of biologic origin3,10–12,14,15 have been more successful in supporting epithelial growth in vivo than those made from synthetic materials.3,16–18 The epithelialization of biologically derived polymer surfaces is likely to be due to their collagenous nature, which resembles the physiological surface of a wound bed on which corneal epithelial tissue heals. Histologic evaluation of some of the successfully epithelialized collagen-based lenticules from studies by Thompson et al.12 and McCarey et al.15 has revealed the formation of adhesive structures (basement membrane and adhesion complexes) at the epithelium–lenticule interface. Although encouraging, the long-term success of these lenticules was compromised by degradation of the collagen material and abnormal epithelialization. Together, data from past investigations have identified the need for a synthetic material to be designed specifically to support the growth of corneal epithelial tissue.

PFPE lenticules that were biostable, transparent, and permeable to nutrient flow were used in the present study. These
lenticules were coated with collagen I, a biologic signal that we have previously shown to allow for rapid and sustained epithelialization in vivo when covalently immobilized onto the surface of a porous synthetic material. The PFPE lenticule was implanted in the anterior stroma of the feline cornea using a surgical model that exposed the anterior surface of the lenticule for epithelial overgrowth. Clinically, the collagen-coated PFPE-based polymer supported a steady rate of epithelial growth over the lenticules, with wound closure occurring between days 5 and 9. This was longer than the 3-day time before closure of an identical wound in the absence of a lenticule. The reason for the longer period is likely to be multifactorial and involve an interplay between the chemistry, permeability, and topography of the PFPE-based polymer and the biologic signal coated on the surface. However, three collagen-coated PFPE lenticules in the present study maintained stable epithelial cover for a period of 34 to 39 days, when the animals were killed for histologic evaluation.

**FIGURE 2.** Immunohistochemistry of the cornea from cat 1 with an implant PFPE lenticule. (A) Immunolocalization of cytokeratin 3 identified epithelial tissue, (B) laminin identified the basement membrane, (C) BPAG identified the hemidesmosomal plaque component of the adhesion complexes, and (D) collagen VII identified the anchoring fibril component of the adhesion complexes. Bar, 10 μm.

**FIGURE 3.** Ultrastructure in cat 3 of the epithelium-lenticule interface that had 34 days of postoperative epithelial cell cover. (A) Stratified epithelial tissue covered the anterior surface of the lenticule. (B) A band of ECM material accumulated between the epithelial tissue and the anterior surface of the lenticule. (C) Fragments of basement membrane (small arrows) and some hemidesmosomal plaques (arrowheads) were observed at the epithelium-ECM interface. Bars, (A) 3.3 μm; (B) 1.0 μm; (C) 200 nm.
corneal epithelium. This may have been a function of time and/or may have been related to an absence of proliferative cells on the lenticule’s surface. Despite this, the epithelial tissue covering the lenticule’s surface had some characteristics of intact tissue. Microvilli were present on the anterior surface of the epithelium and most likely supported the normal tear film observed in the clinical examinations. Desmosomes were present between the constituent cells and would account for the good overall integrity of this epithelial tissue. The cells of the basal layer, which were in direct contact with the lenticule’s surface, could be distinguished by the light staining nature of the cells rather than by their columnar morphology as in normal corneal epithelium. Immunohistochemistry results showed the polarized deposition of ECM proteins associated with the formation of the basement membrane and adhesion complexes at the tissue-lenticule interface. Ultrastructurally, there was evidence of some assembly of this ECM at the interface, as exemplified by the presence of hemidesmosomal plaques along the basal cell membrane and fragments of basement membrane subjacent to this. The substantial accumulation of ECM material between the epithelial tissue and the lenticule’s surface appears to have been the result of continuing deposition of basement membrane and adhesion complex proteins by the epithelial cells. Our immunohistochemistry results showed almost linear staining for collagen VII at the tissue-lenticule interface; although the assembly of this collagen VII into the cross-banded anchoring fibrils of the adhesion complexes was not observed ultrastructurally. This may have been a function of time, because the reformation of anchoring fibrils is known to be a slow process in some species. The accumulation of ECM material in the basement membrane zone observed ultrastructurally in our study was similar to that reported for by other investigators in slightly different circumstances. Massive duplication in the basement membrane zone was reported during the healing of a 7-mm diameter superficial keratectomy wound to the rabbit cornea examined over a 12-month period. Accumulations of basement membrane proteins were also identified in the unwounded corneas of diabetic humans, and this was thought to have decreased the penetration of anchoring fibrils into the anterior stroma and compromised epithelial-stromal adhesion in those patients.

The present study has demonstrated epithelialization of a synthetic material in vivo for the first time. PFPE-based lenticules, with a biologic signal on the anterior surface, supported stable epithelial growth and were clinically well tolerated when implanted in the feline cornea over a 6-week period. The epithelial tissue that covered the lenticules stratified, although at this early stage after implantation, the number of cell layers and the morphology of constituent cells was less than in normal feline corneal epithelium. Despite this, the epithelium showed signs of persistent adhesion, both clinically and histologically. Further refinements in the design of this PFPE-based polymer, the surface coating, and the surgical procedure used to implant the lenticules may optimize the performance of this polymer for ophthalmic applications that require sustained epithelialization.

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References


