Progress in the Development of a Synthetic Corneal Onlay

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Purpose. This study evaluated an improved perfluoropolyether polymer formulation designed for use as a corneal onlay to correct refractive error.

Methods. Collagen I coated perfluoropolyether lenticules were implanted in feline corneas exposing a 6-mm diameter area of lenticule surface for epithelial growth. A parallel series of sham-wounded corneas were also studied. All corneas were monitored clinically for 4 or 8 weeks after surgery when animals were terminated and corneas used for histology with light and electron microscopy.

Results. Postoperative epithelial growth began on days 1 and 2. Lenticule surfaces were fully epithelialized by days 5 to 11. Corneas remained clear, and the lenticules maintained epithelial cover until the designated time points. Histology of the implanted corneas showed that the lenticules were well tolerated by the cornea. Each lenticule was fully covered by a multilayered epithelium with microvilli, desmosomes, and a differentiated basal cell layer. Epithelial adhesive structures (basal lamina, hemidesmosomes, and anchoring fibrils) had assembled at the tissue–lenticule interface.

Conclusions. Collagen coated perfluoropolyether lenticules implanted in the feline cornea supported the growth of a stable stratified squamous epithelium. These encouraging results are a step further in the development of a corneal onlay for correction of refractive error. (*Invest Ophthalmol Vis Sci.* 2002; 43:3196–3201)

Current trends in refractive surgery are directed toward the marketplace's demands for permanent correction of refractive error without the need for external devices such as spectacles and conventional contact lenses. The laser procedures of photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) have, to some extent, met this demand. PRK is reportedly safe and effective for patients with mild to moderate myopia, with complications such as regression and haze being more common in patients that have had higher degrees of myopia corrected with this technique.[1] Currently, LASIK is the preferred refractive laser-based technique among surgeons, especially in the treatment of high myopia. Compared with PRK, LASIK treatment results in reduced discomfort, more rapid healing, and the faster return of stable vision for the patient[1]— outcomes that are undoubtedly related to the preservation of the epithelium and Bowman's layer. Complications with LASIK are either laser related or associated with the cutting of the lamellar flap by microkeratome. The latter group includes buttonhole or torn flaps, free or irregular caps, induction of iatrogenic progressive ectasia, epithelial ingrowth, and keratolysis.[1–7] Although the laser procedures have shown some efficacy in the treatment of refractive error, both techniques involve ablation of stromal tissue in the central optical zone of the cornea that is permanent and nonreversible. In addition to which, the correction achieved by laser ablation may not be stable because of regression.

A different approach to the direct laser techniques uses photo-ablative inlay laser in situ keratomileusis (PAI-LASIK). This procedure involves the use of the microkeratome to create a LASIK-style cap and the insertion of a polymer lenticule (inlay) within the corneal stroma before replacement of the cap. It is a potentially adjustable technique, because the laser is used to adjust the polymer and not the stromal tissue; however, similar to LASIK, it is not fully reversible, because a flap has been cut and the cornea has been weakened.[4] Current efforts in PAI-LASIK are directed at the development of a suitable polymer for use as the inlay material[8] and a biocompatible microporous hydrogel material (Nutrapore, PermaVision; Anamed, Inc; Irvine, CA),[9] is being tested for the correction of hyperopia by this procedure.[10]

A synthetic corneal onlay (implantable contact lens) is an alternative approach to refractive correction that offers an adjustable and reversible procedure with minimal surgical intervention of the central optical zone. An onlay involves debridement of the central corneal epithelium (or a LASIK-type removal of the epithelium) and placement of a synthetic lenticule on the exposed stromal surface, leaving Bowman's zone intact. The anterior surface of the lenticule is then covered by the recipient eye's corneal epithelium, incorporating the lenticule to achieve the desired refractive correction by altering the curvature of the anterior corneal surface. Attempts to create lenticules for such an application have largely involved collagen-based materials, and their success has been limited by remodeling of the lenticule and abnormalities of the corneal epithelium covering the lenticule.[11–16] The use of synthetic polymers and copolymers for a corneal onlay is advantageous in that it avoids postoperative lenticule remodeling and offers improved optical properties and unlimited supply with a reduced risk of transmission of infectious agents.[17] The success of a synthetic onlay necessitates that the onlay material be transparent, nontoxic, biocompatible, biostable, and nutrient permeable and have surface characteristics that permit the migration and persistent adhesion of corneal epithelial tissue.[18,19] Aside from our own work,[20,21] there are few published reports of synthetic materials for corneal onlay applications. Lidofilcon A (Allergan, Irvine, CA), a high-water-content

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contact lens material failed to epithelialize when tested in vivo.[22] A plasma-modified poly(vinyl alcohol) material supported epithelial growth when tested in vitro[23] but only partially epithelialized when implanted in vivo.[24] Various synthetic polymers and collagen-synthetic composites, as well as methods of polymerization for corneal onlays have been patented, although any subsequent testing of these materials remains unpublished.[25–28]

We have developed a porous perfluoropolyether (PFPE)-based polymer for use as a corneal onlay.[29–31] PFPE is a transparent, isorefractive polymer that offers high chemical and thermal stability. Previous clinical testing in the feline cornea has demonstrated that PFPE lenticules support the growth of corneal epithelium in vivo when coated with collagen I.[20,21] In the current report, we present results of lenticules made from an improved PFPE formulation that were again coated with collagen I and implanted in the feline cornea by a surgical model that exposed an increased area of the lenticule's surface for epithelialization than in previous trials.[21] The performance of the implants was monitored clinically for up to 8 weeks with animals killed for histologic evaluation at two postoperative time points (4 and 8 weeks). The clinical and histologic data from the implanted corneas in this series has been compared with sham-wounded corneas at equivalent time points and with normal feline cornea.

METHODS

Material, Lenticule Design, and Surface Coating

The PFPE formulation used in this experimental series was made more hydrophilic than that which we have tested previously[21] by the incorporation of a zwitterionic component. Porous PFPE-zwitterion copolymer lenticules were made by the UV copolymerization of the PFPE-dimethacrylate and zwitterionic monomer, (2-(methacryloyloxy)ethyl)-dimethyl(3-sulfopropyl)ammonium hydroxide, inner salt.[32] The porous PFPE-zwitterion copolymer was cast into lenticules for this study by using polypropylene molds that were 8.0 mm in diameter and approximately 90 μm thick at the center with tapering edges. A thin layer of collagen I (Vitrogen; Collagen Corp., Palo Alto, CA) was covalently immobilized on the anterior surface of each lenticule by a two-step process, as described previously.[21] Briefly, a thin layer of collagen I (approximately 5 to 10 nm in thickness) was covalently immobilized on the anterior surface of each lenticule by reductive amination that involved functionalization of the surfaces by exposure to acetaldehyde monomer in a radio frequency glow discharge (RFGD).[33] Immediately after RFGD treatment, lenticules were placed in a solution of 50 μg/mL collagen I in phosphate buffered saline (pH 7.4) on ice and an excess of sodium cyanoborahydride added to act as a reducing agent.[34] Covalent immobilization of collagen was used to prevent its removal or displacement by exchange with other proteins in the eye. Covalent coupling has also been demonstrated to enhance the stability of the collagen coating to enzymatic attack in vitro (unpublished data); however, an assessment of collagen remodeling in vivo is compounded by the deposition of other proteins during the period of implantation.

Lenticule Permeability

The permeability of the collagen-coated PFPE lenticules used in this study was measured with radiolabeled glucose, inulin, and albumin in a two-chamber device, as previously described.[35] The PFPE was clamped between the two chambers, each of which was stirred on the vertical axis to enhance mixing near the PFPE surface. The exposed area of PFPE surface was 0.126 cm^2. The permeabilities to glucose and inulin were measured simultaneously with ^{14}C-glucose and ^{3}H-inulin in PBS containing 0.925 g/L unlabeled glucose and 0.011 g/L unlabeled inulin. The permeability of albumin was measured using ^{125}I-albumin in PBS with 1.0 g/L unlabeled albumin. All measurements were made at room temperature. Using this methodology, the permeability of collagen-coated PFPE lenticules to glucose and inulin and human serum albumin was measured to be 1.95, 0.811, and 0.125 E-04 cm/sec, respectively.

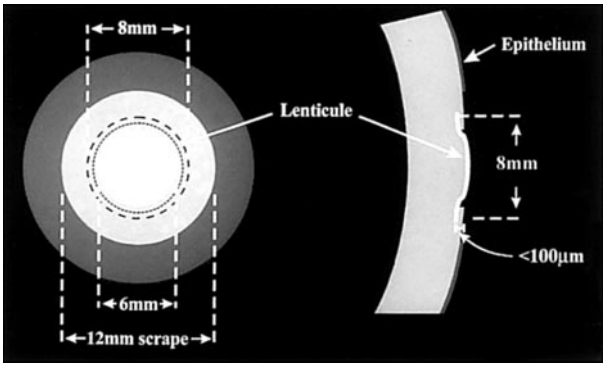

FIGURE 1. Depiction of the surgical wound used to implant a synthetic PFPE lenticule in the feline cornea.

Surgery

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 on the use of animals in ophthalmic and vision research. The cats (n = 11) used in this study were 2 to 4 years of age, not sex matched and weighed 2 to 4.5 kg. Animals were anesthetized with an intramuscular injection of 10 to 15 mg/kg body weight ketamine and 1 mg/kg body weight xylazine to a depth of stage 3, plane 2.[36] The surgical model used in the present study located the implanted lenticule more anteriorly in the cornea and was less invasive of the stroma than that used in a preliminary study by our group.[21] A three-step procedure was used (see Fig. 1). First, the corneal surface was lightly marked in the center with an 8-mm diameter trephine, and the corneal epithelium within this area was debrided with a beaver blade to expose the basement membrane. Second, a vacuum trephine was used to make a superficial keratotomy groove, 6 mm in diameter and approximately 80 to 100 μm deep, centrally within the debrided area. Third, using a freehand technique, the base of the keratotomy groove was expanded approximately 1 mm toward the limbus, creating a circular interlamellar pocket within the stroma. Two series of corneas were prepared. In one series (n = 7), the wounds were implanted immediately with PFPE lenticules that were tucked into the expanded pocket, leaving an area 6 mm in diameter of the lenticule's surface exposed for re-epithelialization (hereafter referred to as implanted corneas). In the second series (n = 4), identical wounds were made, but not implanted with lenticules, creating a series of sham wounds (hereafter referred to as sham-wounded corneas). Clinical examination of the corneas was performed daily by slit lamp biomicroscope and fluorescein to assess the speed and extent of epithelial growth over the lenticules (or wound bed in the case of the shams). Slit lamp biomicrography was also used to check for the formation of a tear film associated with the newly epithelialized lenticules, to check the status (position and integrity) of the lenticule and to check for any signs of inflammation.

Animals were killed at either 4 or 8 weeks with the intention of obtaining a lenticule that had been fully covered with epithelium for either 2 to 3 or 6 to 7 weeks, respectively (depending on how long it took the individual lenticule to be covered). The 4-week time point was selected to check the integrity and thickness of the epithelium on the anterior surface of the lenticule and the deposition of extracellular matrix proteins at the epithelial–lenticular interface. The 8-week time point was selected to evaluate the assembly of cell–matrix junctions known to be responsible for the persistent adhesion of the epithelium to its underlying substratum (basal lamina-basement membrane, hemidesmosomes, anchoring fibrils).[37,38] All corneas were fixed and processed for resin histology immediately after termination. The cor-

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TABLE 1. Summary of Clinical Findings from Sham-Wounded Feline Corneas (Cats 1–4) and Those Implanted with PFPE Lenticules (Cats 6–11)

	Sham				Implanted					
	4 Weeks		8 Weeks		4 Weeks			8 Weeks		
Cat	1	2	3	4	6	7	8	9	10	11
Days to commencement of postoperative epithelial growth inward from wound edge	1	1	1	1	1	2	1	1	2	1
Days for newly grown epithelium to cover surface of wound bed (sham) or lenticule (implanted)	5	3	9	8	5	6	10	7	6	11
Days from surgery to death	28	28	54	54	28	28	31	54	54	64
Days of focal detachment	Nil	Nil	Nil	Nil	Nil	1*	4†	Nil	Nil	Nil
Days of persistent epithelial cover	22	24	44	45	22	20	21	46	46	52

Cat 5 was removed from the study on day 9 due to inflammation.

* A small focal detachment occurred nasally in cat 7 from day 27 to 28 after surgery.

† A small epithelial defect occurred along a short section of the inferior pocket edge in cat 8 from day 27 to 31 after surgery.

neas from two normal, non–surgically treated, age-matched animals were fixed and processed for histology in a manner identical with preparation of the experimental corneas.

Histology

Corneas were processed for histology as previously described.[21] Sections for light microscopy (transverse, 3-μm thick) were stained with toluidine blue and examined with a microscope (DMLB; Leica, Cambridge, UK) to assess the overall state of the cornea, the structure and integrity of the epithelium covering the lenticule or wound bed surface, and the morphology of the cells that constituted that epithelium. Ultrathin transverse sections (80–90 nm thick) for electron microscopy were stained en grid with uranyl acetate and Reynold lead citrate and viewed with a transmission electron microscope (model 7100; Hitachi, Tokyo, Japan). We examined several ultrastructural features of the central region of feline corneas implanted with PFPE lenticules at the 4- and 8-week time points. These were compared with normal (nonimplanted) age-matched feline corneas that had been processed in an identical manner. Some of these features (microvilli, desmosomes, hemidesmosomes, and anchoring fibrils) were quantitated in a representative corneal implant at 4 weeks (cat 7), an implant at 8 weeks (cat 10) and a normal feline (nonimplanted) cornea. The number of microvilli on the epithelial cells forming the anterior ocular surface was counted in 40 fields of view at 25,000× magnification. The height of the microvilli on the anterior ocular surface was measured for 20 individual microvilli at 46,000× magnification. The number of desmosomes between the suprabasal cells was counted in 20 fields of view at 25,000× magnification. The occurrence of epithelial adhesive structures (basement membrane, hemidesmosomal plaque, and anchoring fibrils) was examined at the epithelium–PFPE lenticule interface and documented. The percentage of anchoring fibrils associated with hemidesmosomal plaques at the epithelium–PFPE lenticule interface was counted in at least 40 fields of view at 25,000× magnification in sections from an implanted and a normal feline cornea.

RESULTS

Clinical

Details of the clinical observations of sham and implanted corneas appear in Table 1. Daily observations of all corneas were made to monitor the overall health of the cornea and the extent of epithelial growth over the natural wound bed (sham) or the PFPE lenticule's surface (implanted). In both series, epithelium from the outer edge of the wound commenced growth inward over the debrided area on days 1 to 2 after surgery. Epithelium completely covered the wound bed (sham) by days 3 to 9 and the exposed lenticule surface (implanted) by days 5 to 11 in 6 of the 7 implanted corneas (Fig. 2). Ten of the 11 animals in this study were maintained to either week 4 or 8 after surgery. One animal in the implant group was removed from the study on day 9 because of complications related to postsurgical inflammation (cat 5). There was no evidence of an inflammatory or immune response to the presence of the PFPE lenticule that we could detect in the remaining implanted corneas. One animal (cat 7) with an implanted PFPE lenticule showed a small focal epithelial detachment nasally on day 27 that persisted to day 28 when the animal was killed and the cornea processed for histology. One animal (cat 8) showed a small epithelial defect in a short section of the pocket edge from days 27 to 31 when the animal was killed for histology. Overall, the corneas in both series were quiet, remained transparent, maintained multilayered epithelial cover and supported a stable tear film during the observation period. Each of the eyes implanted with a PFPE lenticule showed development of fine crystalline deposits around the lenticule periphery in the region of the stromal pocket at days 16 to 20, and these persisted until termination without clinical effect. Clinically, the fine crystalline deposits in our study appeared to be similar to the lipid deposited in the lamellar channels of human corneas implanted with intracorneal rings[39,40] and the fine deposits associated with intrastromal implants that have been reported by previous investigators.[41–43]

Histology

Transverse sections of epithelial tissue covering the central region of the wound bed (sham) or lenticule (implanted) at

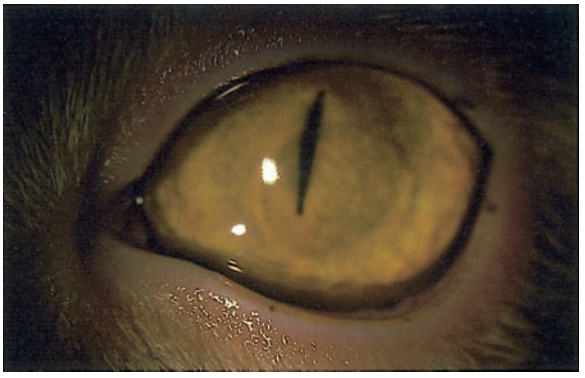

FIGURE 2. Slit lamp biomicrograph of epithelium covering PFPE lenticule in a feline cornea on postoperative day 7 (cat 6).

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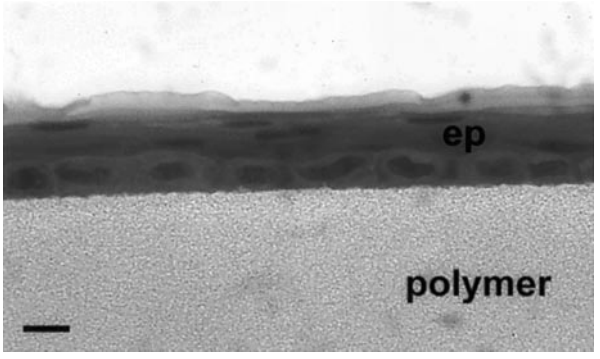

FIGURE 3. Light micrograph of epithelium (ep) covering PFPE lenticule (polymer) implanted in the feline cornea for 8 weeks (cat 10). Toluidine blue–stained transverse section of the central region of the implanted cornea. Bar, 10 μm.

both time points were examined with light and electron microscopy and compared with the central region of normal feline cornea. Light microscopy of the sham-wounded corneas revealed 6 to 7 layers of cells constituting the epithelium on the central portion of the original wound bed at 4 weeks that had increased to 8 to 10 layers by 8 weeks. This was slightly less than the 12 layers of cells in normal feline corneal epithelium. Implanted corneas supported epithelium on the anterior surface of the lenticules that was composed of five to eight cell layers at both time points (Fig. 3). The epithelium that covered the lenticules in each case was distinguished by cuboidal basal cells in direct contact with the lenticule's surface and flattened cells comprising the suprabasal layers. This differed from that covering the wound bed in the sham-wounded corneas at both time points, which, like normal feline cornea, displayed columnar basal cells, a well-defined wing cell layer and several squamous cell layers. Overall examination of the stromal tissue in the implanted corneas at both time points revealed stroma that was relatively normal in appearance with no evidence of thinning (stromal melting). The density of keratocytes in the stroma appeared normal, except for areas immediately adjacent to the implanted lenticules, where an increased number of keratocytes formed a continuous, but single, layer around the implanted lenticule in each of the 4-week corneas examined. The number of keratocytes around the implant had not increased by the 8-week time point and, in one case, it was less than that seen at the earlier time point. There was no evidence to suggest that this was the beginning of formation of a fibrous capsule, which we will seek to demonstrate in future, longer-term studies.

Electron microscopy of the implanted and sham-wounded corneas revealed many similarities to normal feline cornea. Microvilli were present on the anterior epithelial surface of the sham and implanted corneas at both time points. The number of microvilli in the central cornea of eyes implanted with PFPE lenticules was similar at 4 weeks (10.2 ± 2.0) and 8 weeks (8.5 ± 1.9) to that seen in normal feline corneas (8.2 ± 1.6). The height of individual microvilli was also similar in the implanted corneas at 4 weeks (105 ± 18 nm) and 8 weeks (114 ± 28 nm) to that in normal feline cornea (102 ± 15 nm). Desmosomes, structurally similar to those of normal cornea, were present between adjacent epithelial cells in the epithelium of both series (sham and implanted) at 4 and 8 weeks, although the number of desmosomes between suprabasal epithelial cells differed between the implanted and normal feline corneas. Eyes implanted with PFPE lenticules showed fewer desmosomes at both 4 weeks (5.8 ± 1.7) and 8 weeks (4.9 ± 0.9) than seen in normal feline corneas (9.1 ± 1.6).

Ultrastructural examination of the interfacial region between the epithelial tissue and the stroma (sham) at both time points showed the formation of regular hemidesmosomal plaque and a continuous basal lamina that was similar to that observed in normal corneas. Individual hemidesmosomal plaques in the healing sham corneas were pale at the 4-week time point, but grew more electron dense by 8 weeks. In the healing implanted corneas, extracellular matrix (ECM) material had accumulated between the epithelial tissue and the lenticule's surface (Fig. 4A) that was not evident in the sham-wounded or normal corneas. Fragments of basal lamina were observed within the accumulated ECM at regular intervals

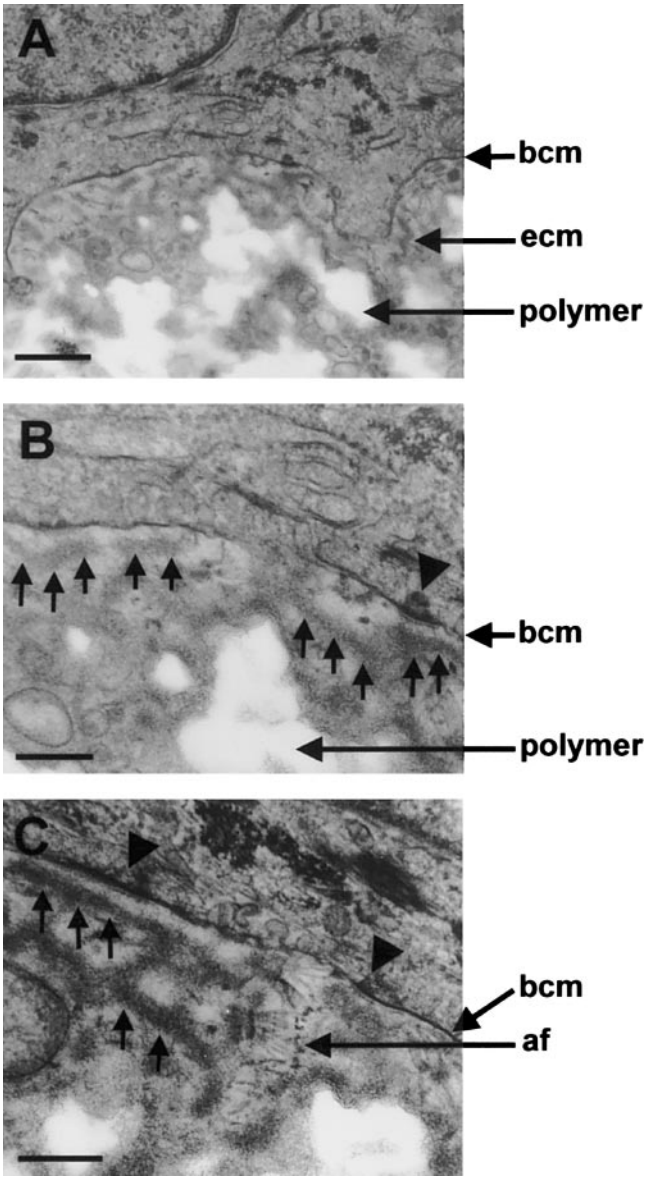

FIGURE 4. Electron micrographs of the epithelium–polymer interface in a feline cornea implanted with PFPE lenticule for 8 weeks (cat 10). (**A**) Extracellular matrix material (ecm) between the basal cell membrane (bcm) of a basal epithelial cell and the lenticule's surface. (**B**) Formation of hemidesmosomal plaque along the basal cell membrane (*large arrowhead*) and fragments of basal lamina subjacent to basal cell membrane (*small arrows*). (**C**) Formation of anchoring fibrils (af) and fragments of basal lamina (*small arrows*) within the extracellular matrix accumulated between the basal cell membrane (bcm) and the polymer. Hemidesmosomal plaque (*large arrowheads*) formed along the basal cell membrane. Bar: (**A**) 1 μm; (**B**, **C**) 200 nm.

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subjacent to the plasma membrane of the basal cells along the tissue–lenticule interface in implanted corneas at both time points (Fig. 4B). Hemidesmosomal plaque had formed along the basal cell membranes of the implanted corneas and, as in the sham corneas, plaques were pale and immature-looking at 4 weeks, but more electron dense and similar to normal corneas by 8 weeks (Figs. 4B, 4C). Fully assembled bundles of anchoring fibrils were noted at various intervals within the ECM accumulated between the epithelial cells and the lenticule surface in the 8-week implanted corneas (Fig. 4C). These anchoring fibrils varied in appearance, size, and orientation and were partly or fully associated with segments of basal lamina and short, randomly distributed collagen fibrils. Not all anchoring fibrils were directly associated with hemidesmosomal plaques. Quantification of this association in a representative cornea showed that 22.6% of the anchoring fibrils at the implanted 8-week interface were subjacent to hemidesmosomal plaques, almost half of the 49.5% association found in normal feline cornea.

Discussion

The design of a material for use as a corneal implant is challenging, as exemplified by earlier investigators.[12–14,16,22,24,44–46] The potential of PFPE as a synthetic onlay material was demonstrated by our group in preliminary in vivo testing.[21] In that study we used an earlier PFPE formulation to cast lenticules that were implanted into the feline cornea by a surgical procedure that involved a keratectomy wound. Several major improvements were made during the current experimental series. First, the hydrophilicity of the PFPE was increased by the addition of zwitterion to the polymer formulation. Second, the newer surgical model preserved the stroma of central optical zone undamaged. In addition, this surgical procedure was more challenging in terms of polymer testing because the exposed area of the lenticule's surface that needed to be covered by corneal epithelium was increased ninefold (from 3 mm^2 in the case of the previously tested 2-mm diameter lenticules[21] to 28 mm^2 in the current 6-mm diameter series).

The newer PFPE polymer formulation was well tolerated when implanted as lenticules into a less invasive surgical wound in the feline cornea. Epithelial growth began inward from the wound periphery quite quickly after surgery (within 24–48 hours) in a response that was similar to that observed in the sham-wounded corneas. The polymer lenticules were fully epithelialized after 5 to 11 days, which was only slightly slower than the 3- to 9-day time taken for the epithelium to cover the wound bed in the sham-wounded corneas. The steady initial growth of epithelium over the lenticules was undoubtedly associated with the presence of the covalently immobilized collagen I on the anterior surface of the lenticules. Previously, we have shown such a collagen I coating to be the preferred biological signal for epithelial migration[36] and to support persistent epithelial growth in vivo.[21,47] The corneas implanted with PFPE lenticules in the present study maintained stable epithelial cover for up to 48 days, acknowledged to be sufficient time for the formation of adhesive structures (basement membrane and hemidesmosomes) known to be responsible for the persistent adhesion of epithelial tissue.[37,38,48]

Histology confirmed that a stratified squamous epithelium fully covered the anterior surface of the synthetic PFPE lenticules in our study. In the sham-wounded corneas, this newly grown epithelium increased in number of cell layers between 4 and 8 weeks (from 6–7 to 8–10 layers, respectively). The thickness of the epithelium on the anterior surface of the PFPE lenticules in the implanted corneas was equivalent to the shams at 4 weeks but did not increase in thickness with time, and remained at five to seven layers. The basal cell layer in the shams was columnar in shape, similar to normal cornea at both 4 and 8 weeks, whereas those in direct contact with the lenticule surface (implanted) were cuboidal at both time points. These differences may be related to a change in the rate of epithelial turnover and the differentiation of the epithelial cells in the presence of a synthetic material and is a subject for further investigation. Ultrastructural data showed the epithelium covering the implanted corneas in the present study shared many similarities to both the sham-wounded and normal feline cornea. Microvilli, similar in density and size to normal feline cornea, were present on the apical surface of the anterior layer of epithelial cells in implanted corneas and it is likely that this accounted for the stable tear film observed clinically. The epithelial tissue covering the lenticules at both time points had desmosomes between the constituent cells of the suprabasal layer, which provided some integrity to the tissue, although the number of desmosomes in the implanted corneas was found to be less than in normal feline cornea. We observed some formation of adhesive structures at the epithelium–lenticule interface, particularly at the later time point, with a regular distribution of basal lamina fragments, a regular array of hemidesmosomal plaque and some fully assembled anchoring fibrils. Although the density, distribution, and orientation of the anchoring fibrils was not completely normal, we believe that the assembly of these components would increase with time—a hypothesis that we will seek to test in future in vivo work.

Data presented in the present study have shown some progress in the development of a synthetic corneal onlay since our previously published report.[21] The kinetics of epithelialization of the PFPE lenticule was improved, with the migrating epithelium covering a large exposed area of the lenticule surface. The newly grown epithelium on the PFPE lenticule surface was stable for up to 7 weeks. In addition, cell–cell adhesive structures (desmosomes) formed between constituent epithelial cells and cell–matrix adhesive structures (hemidesmosomes, basal lamina, and anchoring fibrils) assembled between the basal epithelial cells and the lenticule surface. Together, these clinical and histologic results suggest that PFPE has great potential as a synthetic lenticule for a corneal onlay application with which to correct refractive error in a reversible procedure that is minimally invasive of corneal tissue.

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