Veterinary Ophthalmology (2015) 1–10 DOI:10.1111/vop.12294

Retrospective evaluation of corneal reconstruction using ACell Vet™ alone in dogs and cats: 82 cases

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Abstract

Objectives To retrospectively evaluate the complications, graft clarity, and outcomes associated with the use of commercially available porcine urinary bladder submucosa (ACell Vet™) alone for corneal reconstruction in dogs and cats.

Procedures Dogs or cats receiving an ACell Vet™ graft for corneal reconstruction due to severe ulcerative keratitis or after a keratectomy to remove a corneal sequestrum were included. All received a single layer of ACell Vet™, bandage contact lens, and temporary tarsorrhaphy. Bandage contact lens and temporary tarsorrhaphy were removed after graft vascularization or epithelialization. Topical steroids, cyclosporine, tacrolimus were started after epithelialization. Based on their last examination, outcomes were categorized into five groups based on the presence of corneal vessels, appearance of the scar, and the ability to visualize the posterior and/or the anterior segment through the grafted area.

Results There were 82 eyes included in the study, with 68 eyes with sufficient follow-up time for final assessment. Scarring was minimal in 47 eyes, moderate but not enough to obscure visualization of the posterior segment in 12, and severe in nine. There were five eyes that developed phthisis bulbi, glaucoma or were enucleated and nine that were lost to follow up. Graft dehiscence occurred in 19 eyes. Twelve healed without additional surgical intervention while three required a second graft, two became phthisical, and two were enucleated.

Conclusions and Clinical Relevance Corneal reconstruction with ACell Vet™ alone is a viable alternative and results in minimal scarring and complications in cats. In dogs, scarring is more pronounced than in cats and graft dehiscence rate is higher compared to conventional techniques.

Key Words: corneal perforation, corneal sequestration, corneal ulceration, keratomalacia, porcine urinary bladder submucosa

INTRODUCTION

Corneal ulceration is a common ocular injury in companion animals.[1] Simple, uncomplicated ulcers typically epithelialize rapidly and only minimal medical therapy consisting of topical antibiotics and cycloplegics is indicated. Matrix metalloproteinases, either dysregulated endogenous proteinases or from infectious organisms, can lead to degradation of the corneal stroma.[2] In those cases, aggressive medical therapy consisting of topical antibacterial medications effective against the infecting bacteria, topical or systemic matrix metalloproteinase inhibitors, and cycloplegics are indicated as long as the integrity of the globe is not in jeopardy.[2] If there is risk of perforation, surgery is indicated to avoid possible vision loss associated with this event.[3]

A corneal sequestrum is a necrotic area of corneal stroma. Although reported in dogs[4,5] and horses,[6,7] it is far more common in cats.[8–15] The process that leads to its formation and the reason for its dark coloration is poorly understood.[10,16–19] However, their progression typically consists of a protracted process in which the underlying corneal stroma becomes vascularized and the sequestrum is slowly sloughed. This process can take from several months to over a year. Because many cats often show signs of discomfort and pain due to the sequestrum, surgical removal is recommended to speed recovery. Because a sequestrum can span the entire depth of the

cornea, in many cases removal necessitates reconstruction to restore the tectonic strength of the cornea.

A conjunctival pedicle graft is a versatile surgical technique that can be utilized in many different situations[20–24] and as such is likely the most commonly used method to repair deep corneal defects in veterinary medicine. Conjunctival pedicle grafts provide an immediate source of fibroblasts, blood vessels, epithelial cells, and antimicrobial and antiproteolytic elements to the grafted site.[1,20–24] However, conjunctival grafts provide minimal tectonic support and as conjunctiva is inherently opaque, they impair vision. To avoid these disadvantages, other materials, tissues, or surgical techniques such as cyanoacrylate tissue adhesive,[25–27] conjunctival island patch,[1,20] tarsoconjunctival island graft,[28] corneal–scleral–conjunctival transposition graft,[1,29] lamellar keratoplasty,[14,30] split thickness dermal graft,[31] equine pericardium,[32] bovine pericardium,[33] equine amniotic membrane,[34,35] porcine amniotic membrane,[36] lyophilized acellular porcine corneal stroma,[37] human amniotic membrane,[38] equine renal capsule,[39] and expanded polytetrafluoroethylene (Gore-Tex™)[40,41] have been investigated. A number of these materials can be expensive or difficult to acquire or store. Some, like cyanoacrylate tissue adhesive, can only be used for small defects. Certain techniques require specialized instrumentation, and other techniques, such as a corneal–scleral conjunctival transposition graft, may result in decreased clarity of unaffected cornea.[42]

Porcine small intestinal submucosa (SIS) and porcine urinary bladder matrix (UBM) have been used extensively to repair defects of the esophagus,[43–45] bladder,[46–51] cervicovagina,[52–54] rectum,[55] urethra,[56–59] ureter,[60] trachea,[61] blood vessels,[62–64] orbital floor,[65] thoracic wall,[66] and tendon and ligaments [67, 68] in humans and various animal species. SIS has been investigated extensively and has been used successfully in dogs, cats, and horses to repair various corneal or scleral defects.[21, 69–73] On the other hand, the use of UBM as a sole agent for globe reconstruction has only been reported in 1 dog following resection of a limbal melanoma,[74] in 17 equids for the treatment of keratomalacia,[75] and in four cats for the treatment of corneal sequestrum.[76]

The purpose of this study was to retrospectively evaluate the complications, outcomes, and corneal clarity following corneal reconstruction using commercially available porcine urinary bladder submucosa (ACell Vet™) alone in a large number of dogs and cats.

MATERIALS AND METHODS

Dogs and cats that received a corneal UBM graft using ACell Vet™ (ACell, Inc. Columbia, MD, USA) at our clinic between November 2012 and March 2014 were included in this study. Data collected and reviewed from the medical record included signalment, ocular diseases, clinical presentation, diagnosis, surgical time, time to graft dehiscence, disintegration, or incorporation into the cornea, need for a second graft, and follow-up time. All animals were client owned animals with corneal defects that significantly weakened the cornea or that upon their removal necessitated corneal reconstruction. Informed consent was obtained from all owners prior to surgery.

Potential for vision prior to surgery was considered present if there was at least a positive dazzle reflex in the affected eye and a consensual pupillary light reflex in the unaffected eye. Vision after surgery was considered present if there was a menace response. Cases were allocated to one of five categories (Table 1), based on the presence of corneal blood vessels, the opacity of the corneal scar, and the ability to visualize the posterior and/or the anterior segment through the grafted area at their last examination. The time period between surgery and the final examination was at least 3 months. Visualization of the posterior segment through the grafted area was attempted after pharmacologic dilation of the pupil.

RESULTS

Ophthalmic examination

All animals had a thorough ophthalmic examination including evaluation of menace response, dazzle reflex, pupillary light reflex, palpebral reflex, slit-lamp biomicroscopy (Hawkeye, Dioptrix, Lyon, France or SL-15, Kowa, Kowa Life Science Division, Tokyo, Japan), and indirect ophthalmoscopy (Omega 180, Heine Australia Pty Ltd, Warringah Mall, NSW, Australia). When indicated,

Table 1. Description of the categories used to classify cases based on the appearance of the grafted area at the last examination. The number of dogs and cats assigned to each grade is listed in the rightmost two columns

Grade	Description	Dogs	Cats
0	Ghost stromal vasculature, almost undetectable corneal opacity, and clear visualization of the posterior segment through the graft (Fig. 5)	0	7
1	Minimal stromal opacity and vascularization. Clear visualization of the posterior segment through the graft (Fig. 6)	0	18
2	Mild to moderate stromal opacity and vascularization. Visualization of the posterior segment through the graft is possible, but difficult (Fig. 7)	13	9
3	Marked stromal opacity and vascularization allowing visualization of the anterior chamber through the graft, but not the posterior segment (Fig. 8)	9	3
4	Severe stromal opacity, vascularization, and pigmentation. Anterior chamber cannot be visualized through the graft (Fig. 9)	8	1

fluorescein staining, Schirmer tear test I and applanation tonometry were performed. In most cases, the inciting cause of the corneal injury was not documented in the medical record. However, information such as the depth and size of the corneal defect, signs indicative of infection, fibrin, or iris incarceration within a corneal perforation, the presence of blood or fibrin in the anterior chamber, collapse of the anterior chamber was recorded for all cases.

Presurgical examination, preparation, and anesthesia

A thorough physical examination, serum biochemistry, electrolyte profile, and complete blood cell count was performed on all animals. A subcutaneous injection of acepromazine maleate (0.03 mg/kg, 10 mg/mL; Vedco Inc., ST Joseph, MO, USA) and butorphanol (0.2 mg/kg, Torbugesic 10 mg/mL; Pfizer Animal Health, Ringaskiddy, Co Cork, Ireland) was given ½ to 1 h prior to induction of general anesthesia. Induction with propofol (1–4 mg/kg to effect, IV 1.0% Vetofol; Norbrook Laboratories Limited, Newry, Co Down, UK) was followed by endotracheal intubation and maintenance of anesthesia with isoflurane (IsoFlo®; Abbott Animal Health, Abbott Park, IL, USA) in oxygen. All animals received perioperative cefazolin (30 mg/kg, IV, at induction and 90 min later. 1 g/5 mL; China Chemical & Pharmaceutical Co. Ltd., Hsinfong, Taiwan, China), and dogs were administered carprofen (2.2 mg/kg, SC; Rimadyl® Pfizer Animal Health, North Ryde, NSW, Australia), while cats were administered meloxicam (0.2 mg/kg, SC; Metacam® Boehringer Ingelheim, North Ryde, NSW, Australia) upon recovery from anesthesia. The corneal surface and conjunctiva were routinely cleansed with 1% povidone iodine solution and sterile 0.9% saline. In cases of corneal perforation, only sterile saline was used. Neuromuscular paralysis was attained with *cis*-atracurium (0.08 mg/kg, IV, Tracrium 25 mg/2.5 mL; Glaxo Smith Kline, Parma, Italy). Intermittent positive pressure ventilation was initiated in the absence of spontaneous ventilation and discontinued once spontaneous ventilation resumed. Crystalloids (10 mL/kg/h, IV) were administered throughout surgery. During the surgical procedure, heart rate, respiratory rate, oxygenation, end tidal carbon dioxide levels, and electrocardiography were monitored and recorded.

Surgery

All surgeries were performed with the aid of an operating microscope (Zeiss OPMI Special, Carl Zeiss, Hong Kong). In cases of corneal ulceration, any loosely adhered corneal epithelium was either removed with a Dacron swab or fine Colibri forceps. Any corneal tissue that appeared nonviable was also resected with corneal scissors. If there was fibrin protruding through the perforation site, it was trimmed to allow visualization of the edges of the ulcer in order to allow for appropriate debridement and suture placement. Viscoelastic (sodium hyaluronate 1.7%; LA Labs, Santa Barbara, CA, USA) was used to re-inflate the anterior chamber whether the anterior chamber was collapsed or there was iris prolapse into the perforation site. An attempt was made to break down any anterior synechia by injecting viscoelastic between the iris and the cornea immediately proximal to the synechia. If the defect was <8 mm in diameter, a dermal biopsy punch or a corneal trephine was used to outline the lamellar keratectomy margins (for sequestra) or outline the edge of viable cornea (for keratomalacia). The depth of the keratectomy extended beyond diseased cornea or to Descemet's membrane. The same size biopsy punch or corneal trephine was used to create a button of Acell Vet™. If the corneal defect was >8 mm or irregular, a freehand lamellar keratectomy was performed and an appropriately shaped graft was prepared to be approximately 1 mm larger than the defect. A single layer of Acell Vet™ was used in all cases, and all grafts were placed with the tunica propria side facing the down. Aligning the triangular flap so that it is on the right with the hypotenuse pointing up assures the tunica propria is facing down (Fig. 1). All grafts were sutured into place with four cardinal sutures of 10-0 polyglycolic acid (PGA, Jestetten, Germany) and additional simple interrupted sutures (if the defect was small) or simple continuous sutures (if the defect was large). Figures 2 and 3 illustrate the range of sizes and geometries of the defects repaired in this study. An appropriately sized bandage contact lens (Acrivet; Veterinary Division, Hennigsdorf, Germany) was placed and a temporary tarsorrhaphy using a single 3–0 nylon (Dermalon, Covidien, Hong Kong) horizontal mattress suture was placed in all cases. The tarsorrhaphy was placed approximately halfway between the nasal and lateral canthus. The mean total surgical time (from debriding the corneal defect to completion of the temporary tarsorrhaphy) was 33 ± 7 min.

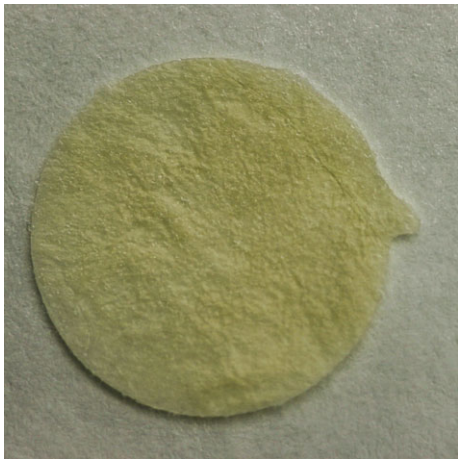

Figure 1. Aligning the triangular flap of the ACell Vet™ to the right of the surgeon, will ensure the tunica propria of the ACell Vet™ faces the ulcer bed. Doing the opposite results in graft extrusion, instead of incorporation.

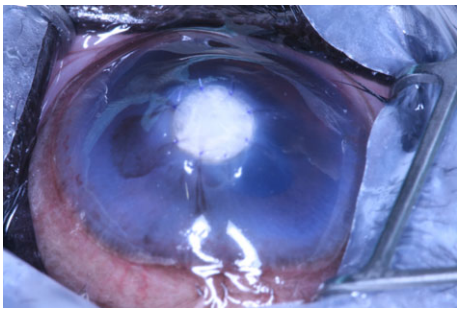

Figure 2. ACell Vet™ placed on a small corneal perforation using simple interrupted sutures of 10-0 polyglycolic acid.

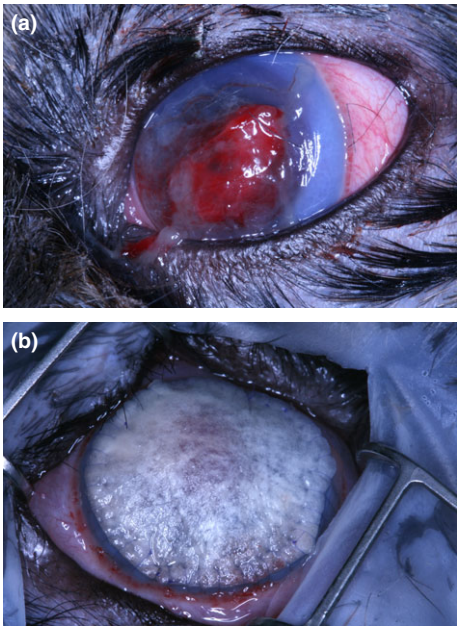

Figure 3. (a) Large corneal perforation with extensive keratomalacia. (b) Same case as in part (a) after placing ACell Vet™ and suturing the graft in place with four cardinal simple interrupted suture and multiple lines of simple continuous suture of 10-0 polyglycolic acid. Figure 8 represents the final outcome of this case.

Postoperative management

Postoperative topical antibiotics consisted of gramicidin/neomycin/polymixin-B ophthalmic solution (QID, Novasporin; Ashford Laboratories LTD, Macau, China) for noninfected lesions while for infected lesions, antibiotics were based on cytology initially and then culture and sensitivity results. Atropine (Atropine sulfate 10 mg/mL ophthalmic solution; Ashford Laboratories LTD.) was used as a cycloplegic and mydriatic when deemed necessary for pain control or to decrease the incidence of synechia. Cephalexin (22 mg/kg, PO BID, for 10 days Apotex Inc., Toronto, ON, Canada) was prescribed in cases of corneal perforation.

Rechecks occurred at 2–3 week intervals until the graft was epithelialized. Fluorescein stain was used to determine epithelialization once the contact lens was removed or after it had fallen out. If the contact lens was still present, fluorescein was not used and examination with a slit-lamp biomicroscope was used to determine extent of epithelialization. The tarsorrhaphy was removed once the graft was epithelialized or completely vascularized. Treatment with topical dexamethasone 0.1% (Maxidex; SA Alcon- Couvruer NV, Puurs, Belgium) once daily, cyclosporine 1%, or tacrolimus 0.05% in olive oil twice daily was commenced after graft epithelialization and stopped after 2–3 months. Topical steroids, cyclosporine, or tacrolimus were used to promote regression of corneal vessels and decrease corneal fibrosis.[70]

Surgical outcome

There were 38 eyes from 37 dogs and 44 eyes from 41 cats included in this study. Dog breeds represented included Shih Tzu (9), pug (9), Pekingese (8), Chihuahua (5), miniature schnauzer (2), Boston terrier (1), miniature pinscher (1), mixed breed (1), and toy poodle (1). Cat breeds represented included exotic shorthair (13), domestic shorthair (7), Persian (7), Scottish fold (4), British shorthair (3), domestic longhair (2), Himalayan (2), Abyssinian (1), American longhair (1), and chinchilla (1).

In dogs, the types of corneal disease requiring surgery included corneal perforation (21), deep corneal ulceration without keratomalacia (6), deep corneal ulceration with keratomalacia (4), and descemetocele (7). In cats, the types of corneal disease requiring surgery included corneal sequestrum (32) corneal perforation (9), descemetocele (2), and deep corneal ulceration (1).

Bilateral grafts were performed in a Pekingese and two exotic shorthair cats. The dog had bilateral corneal perforations, and the cats had bilateral corneal sequestra. Three cases had two grafts placed on the same eye. In two cases, the second graft was required to replace the first graft that disintegrated. This occurred within 2 weeks of surgery in a mixed-breed dog and a domestic shorthair cat. In the third case, the second graft was performed after removing a sequestrum that formed in the middle of the first ACell Vet™ graft that had been placed 14 months earlier after removing a corneal sequestrum. The first graft was not detectable on histopathological examination of the keratectomy sample taken during the removal of the second sequestrum. The only histologic abnormality was histiosuppurative keratitis subtending a sequestrum.

Nine cases were not available for follow-up (five dogs and four cats). All died at some time point following surgery from causes unrelated to the ocular disease. One of the dogs that died was the mixed-breed dog that received two grafts over a short period for the same eye. In this study, there were five cases that eventually developed either phthisis bulbi, glaucoma, or were enucleated. Of these, three were dogs and two were cats. They all presented with large corneal perforations with fibrin plugs or iris incarceration and unstable corneal edges. A dazzle was present before surgery in all cases but further examination

of the posterior segment was not possible in all cases due to corneal disease.

The most common complications encountered after surgery were loss of the contact lens and graft dehiscence. A complete list of complications and their incidence is shown in Table 2. Intense 'tea colored' staining of the contact lens was noticed in a number of feline patients, making examination of the graft difficult. However, none of the grafts became discolored, and the graft was clear after contact lens removal (Fig. 4).

Excluding cases that were lost to follow up, a comfortable globe was achieved in 68 of 73 eyes. Excluding those eyes that developed glaucoma, phthisis bulbi, were lost to follow up, or were enucleated, 22 of 30 eyes from dogs, and 37 of 38 eyes from cats remained visual. Table 1 lists the number of cases assigned to each visual outcome category based on examination at the last follow-up (Figs 5–9). There were two eyes that had keratoconjunctivitis sicca before surgery. Both eyes were classified as grade 4 outcomes.

DISCUSSION

Corneal reconstruction using ACell Vet™ alone resulted in a comfortable globe in 93% of cases, with 87% of those cases retaining vision (81% overall). These outcomes are similar to those previously reported for an acellular submucosa (Acell™ or BioSIS™) graft covered by a conjunctival graft[21,22] and for conjunctival pedicle grafts and corneoscleral transposition grafts[1,23,24,42] but lower than for an SIS graft alone.[70] Although there is no objective way to compare the severity of the presenting lesions between the present study and the series using SIS for corneal reconstruction, it is the authors' impression that the higher number of cases seen in the current study with severe complications (resulting in glaucoma, phthisis, or enucleation) can be attributed to the severity of the lesions at presentation. This is supported by the fact that severe complications were only seen in cases with very large perforations with unstable corneal margins.

Table 2. Complications and outcomes associated with each complication encountered after corneal reconstruction using Acell Vet™ alone

Complication	Total (Dogs/Cats)	Other complications	Final outcome
Contact lens loss	10 (6/4)	Graft dehiscence: 8	Uneventful healing: 8 Blind (phthisis): 2
Graft dehiscence or sloughing	19 (8/11)	Contact lens loss: 8	Uneventful healing: 12 Healed after 2nd graft: 3 Blind (phthisis): 2 Enucleated: 2
Glaucoma	1 (1/0)	None	Enucleated

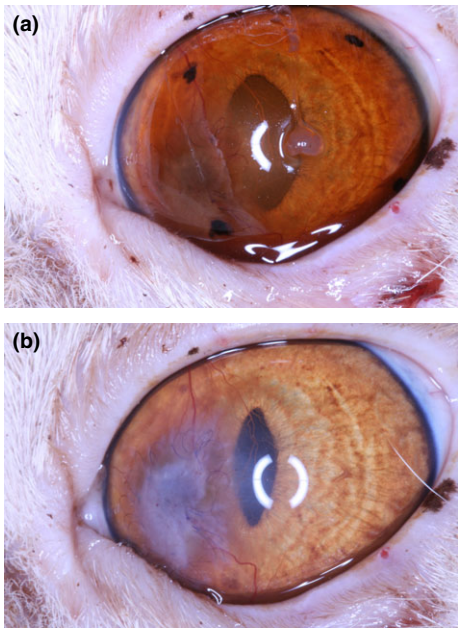

Figure 4. (a) Stained contact lens covering an ACell Vet™ graft in a cat (mucoid discharge present on the surface of the contact lens). (b) Same case as in (a) after removal of the stained contact lens.

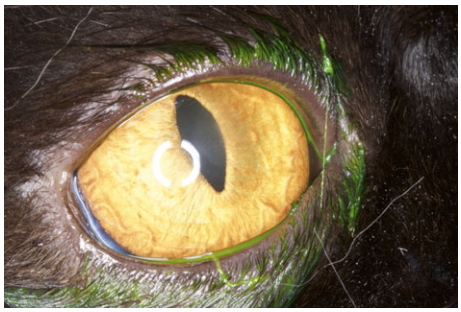

Figure 5. Almost undetectable corneal scarring and no to ghost corneal vessels after removal of a corneal sequestrum and reconstruction with Acell Vet™. Representative of grade 0 outcomes.

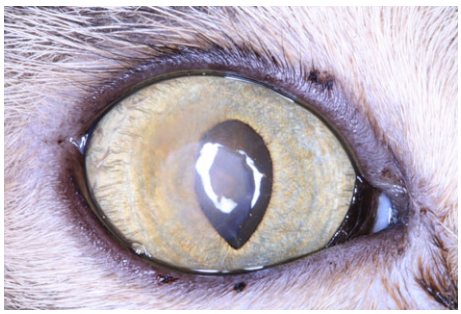

Figure 6. Minimal stromal opacity and vascularization in a cat after keratectomy for a corneal sequestrum and reconstruction with Acell Vet™. Representative of grade 1 outcomes.

In Goulle's[70] study, 70% of cases had minimal corneal scarring and about 30% of cases had marked corneal scarring at the time of final examination using SIS alone

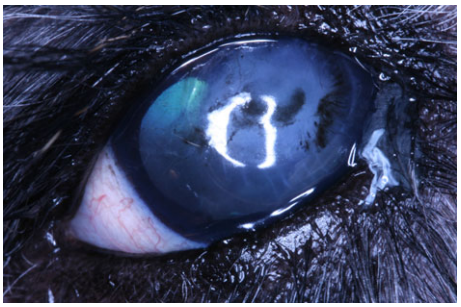

Figure 7. Mild to moderate stromal opacity and vascularization in a miniature Schnauzer after ACell Vet™ grafting for a deep melting corneal ulcer. Representative of grade 2 outcomes.

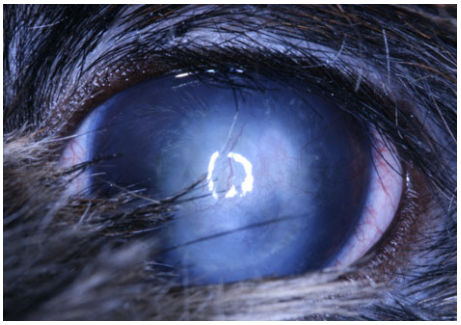

Figure 8. Extensive and marked corneal opacity following corneal reconstruction with Acell Vet™ for a large corneal perforation with keratomalacia. This is the same eye as in Fig. 3 and is representative of grade 3 outcomes.

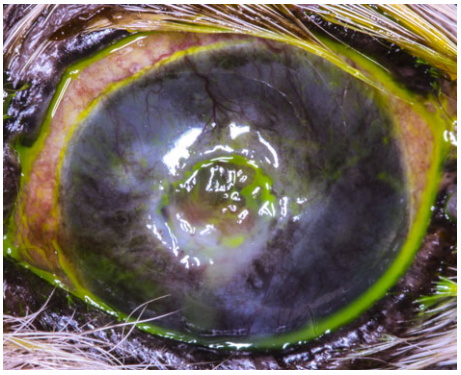

Figure 9. Extensive corneal opacification, vascularization, and pigmentation in a Shih Tzu after ACell Vet™ grafting for a desmetocele. The dog also had keratoconjunctivitis sicca. Representative of grade 4 outcomes.

for corneal reconstruction. Even though the grading system used in the present study is different to Goulle's, the results are comparable. Using Goulle's criterion, 69% of the eyes in this study had no to minimal scarring, 18% had obvious scarring but vision was still present, and 13% scarring to an extent that vision was unlikely. As Goulle[70] also found, dogs that developed severe corneal pigmentation in the current study were brachycephalic breeds with chronic keratoconjunctivitis sicca.

There were marked differences in scarring, incidence of complications, and visual outcome between dogs and cats in the present study. While 34/38 cats had scarring that was categorized as grades 0–2 and only one case had scarring severe enough to prevent visualization of the anterior chamber through the graft, all of the dogs were categorized as grade 2 scarring or worse and 8/30 dogs had scarring that prevented visualization of the anterior chamber through the grafted area. Overall incidence of complications in cats was 38% (15/40), while in dogs, it was 45% (15/33). The incidence of severe complications, resulting in phthisis or enucleation was 5% (2/40) in cats and 9% (3/33) in dogs. Finally, 97% (37/38) of cats had a menace response in the grafted eye, while only 73% (22/30) of dogs retained a menace response in the grafted eye. This difference between species has been previously reported.[33,70] Using SIS alone, Goulle[70] experienced a higher complication rate in dogs (10%, 6/60) than in cats (6%, 3/46) and a higher incidence of vision impairing pigmentation in dogs (14%, 5/36) than in cats (0%). Notably, in both Goulle's and the present report, the predominant disease process was markedly different between cats and dogs. The majority of cats presented with a corneal sequestrum, while the majority of dogs presented with collagenolytic processes typically associated with infection. A small case series evaluating the use of bovine pericardium for corneal reconstruction of melting corneal ulcers in dogs and corneal sequestra in cats,[33] encountered complications that resulted in vision loss in one-third of dogs while none of the cats (0/3) experienced complications. These differences between species may represent interspecies differences in healing or may simply reflect differences attributable to the nature and severity of the underlying disease.

A recent report evaluating outcomes associated with corneal reconstruction using a conjunctival pedicle flap with or without acellular submucosa in dogs found a 97% success rate associated with a conjunctival pedicle flap alone and a 93% success rate associated with an acellular submucosa graft covered by a conjunctival pedicle flap.[22] If success in the current study were defined according to Dorbandt *et al.*, only 67% of dog eyes in the current study would be considered to have a successful outcome. As most of the dogs in Dorbandt's and the current report presented with corneal lesions attributable to an infectious process, it is reasonable to conclude that placing a conjunctival pedicle graft is more effective in halting the collagenolytic destruction of the cornea associated with bacterial keratitis. Another factor that may contribute to the marked difference in success rates between these two studies is differences between presenting lesions between the two populations. Corneal perforations comprised 50% of cases in Dorbandt's report, while corneal perforations made up 55% of dog cases in the current report. The

success rate in corneal perforations was 89% compared to 97% for all other lesions in Dorbandt's report. Although this difference was not found to be statistically significant, it is possible that this difference would become significant with additional cases. Dorbandt also did not detect any differences between lesion size and outcome. However, the maximum lesion size recorded in that study was 64 mm^2 (corresponding to a 9-mm diameter lesion). The maximum lesion size recorded in the current study was 177 mm^2 (corresponding to a 15-mm diameter, the maximum diameter that Acell™ can accommodate) (Fig. 3b). It is not unreasonable to suspect that lesions that are that much larger would have a poorer outcome. Nevertheless, considering that the much larger lesions also probably have a much more aggressive infectious component, it also stands to reason that providing a blood supply to the affected area (through the placement of a conjunctival pedicle graft) is advantageous. Despite the higher dehiscence rate when compared to other studies,[21–23] the majority (12/19) of cases healed without further complications or need to additional surgery.

Both SIS and UBM are acellular biosynthetic materials derived from porcine small intestine and porcine bladder, respectively.[43,77] SIS is a trilaminar material composed of tunica muscularis mucosa, tunica submucosa, and stratum compactum of the tunica mucosa[43,69,73] while UBM consists of an epithelial basement membrane and an overlying tunica propria.[43,50] The tunica propria should be facing toward the ulcer bed for the UBM to be incorporated into the site. Placing it with the basement membrane toward the ulcer bed results in extrusion of the graft. Both SIS and UBM induce similar healing processes.[43] Interestingly, there were no histologically detectable signs of a previous UBM being placed in the 1 sample that was evaluated histologically 14 months after surgery. This is consistent with reports of complete incorporation of acellular grafts into target organs[43,50,51,63,64,66,78–80] and the demonstrated capacity of SIS to support normal growth of squamous epithelium, fibroblasts, glandular epithelium, and smooth muscle cells.[81]

The incorporation of the SIS or UBM graft into a host tissue begins with infiltration of the graft with fibroblasts and blood filled capillaries during the first week. This continues through the second week when cellular infiltration begins to decrease.[62,63] By 8 weeks, collagen bundle reorganization is evident and full integration of the graft and host tissue is complete by as early as 4 months.[62,63,82] Based on the appearance of the grafts in this study, the timeline described appears to hold true for the cornea as well. Notably, however, several feline cases in this series had grafts that were incorporated completely with no to minimal blood vessel infiltration (Figs 5 and 10b).

The physical properties during surgical handling of ACell Vet™ are very different to the commercially available veterinary ophthalmic SIS, VetBioSISt™ (Smiths Medical, Dublin, OH, USA) available at the time the surgeries in this study were carried out. Compared to Vet BioSISt® ocular discs, ACell Vet™ is stiffer, thicker and easier to handle even when rehydrated. In our experience when Vet BioSISt® is rehydrated, it looses its rigidity, folding over on itself very easily, and tears easily when sutures are placed through it. This may not be a property of the material itself, but rather the thickness of the Vet BioSISt® (80 μm). ACell Vet™ (50–200 μm) is also available in 15 mm discs, as opposed to Vet BioSISt®, which is available in 10 mm discs, allowing for repair of larger lesions. There were several cases in this study in which the size of the lesion exceeded 10 mm. And for these reasons, the authors chose to use Acell Vet™ over Vet BioSISt® in the cases of this series. The choice to use Acell Vet™ over a conjunctival pedicle graft was based on the possibility of a clearer visual axis with Acell Vet™. In the authors' experience, a conjunctival pedicle graft always results in corneal clarity that would be classified as grade 4 using the categorization scheme used in the current report. In other words, in the authors' experience it is not possible to visualize the anterior segment through a conjunctival pedicle graft even after the pedicle has been resected and the graft has had time to fully incorporate. Additionally, there were some lesions in which it would not have been possible to harvest a large enough

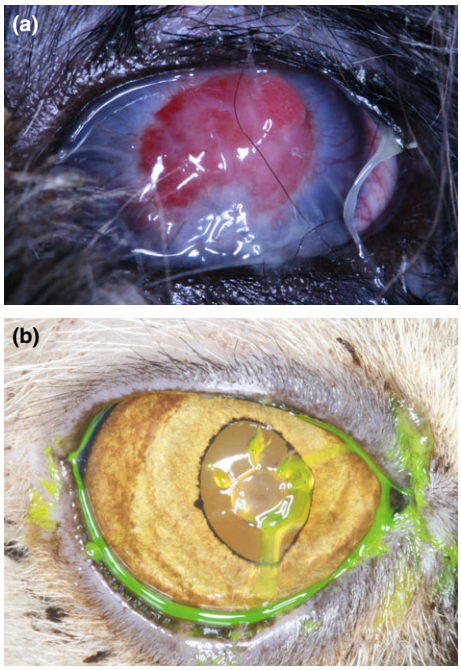

Figure 10. (a) Typical vascularization of the graft approximately 3–4 weeks after graft placement. This is the same eye as Figs 3 and 8. (b) Epithelialized ACell Vet™ graft in a feline cornea which is becoming incorporated into the cornea and becoming clear without the ingrowth of corneal vessels. There is fluorescein stained mucoid discharge adhered to the sutures which are still present 3 weeks after surgery. There is a step defect because the lesion that was removed was nearly full thickness and a single layer of Acell Vet™ did not completely fill the defect. Corneal thickness in the area eventually normalized.

conjunctival graft to cover the entire area (Fig. 3). According to the manufacturer, the thickness of ACell Vet™ is on average 200 μm, but can be as thin as 50 μm in places. This variability is due to the final process in manufacturing being performed manually. All eyes in this study received a single layer of UBM, regardless of the depth of the lesion. In some cases, that led to a small step defect remaining after epithelialization when the original lesion was very deep (Fig. 10b). This defect disappeared over time.

A bandage contact lens and temporary tarsorrhaphy consisting of a single, centrally placed horizontal mattress suture were used instead of a nictitans membrane flap as reported in several other studies.[70,73] The advantages of using a bandage contact lens and a temporary tarsorrhaphy are that the graft is protected and remains hydrated because of the contact lens, but still allows visualization of most of the cornea. Placing a third eyelid flap may interfere with the administration of topical medications while the contact lens may actually act as a depot and slow release device.[83,84] The contact lens also protects the graft from blinking movements and the temporary tarsorrhaphy maintains pressure on the graft, as a third eyelid flap would, which may help in incorporation of the graft.[30,70]

The rationale for placing a contact lens was based on findings by Goulle[57] indicating that grafts tended to dry out and slough if not covered by a third eyelid flap. The temporary tarsorrhaphy was placed in order to increase contact lens retention. There were cases in this study in which the contact lens fell out yet healing and incorporation of the graft continued uneventfully and cases in which the contact lens stayed in place but the graft dehisced, resulting in eventual enucleation. Thus, it is difficult to assess whether the contact lens is truly necessary. It is possible that a temporary tarsorrhaphy would suffice. Graft dehiscence can occur due to inappropriate graft bed preparation, aqueous humor leakage, excessive tension on the graft or sutures, inappropriate suture placement or infection of the graft. Contraction of the graft may also contribute to dehiscence, a phenomenon documented in several studies using SIS or UBM in nonocular tissues.[43,50,64] Finally, the quality of the UBM may have an impact on the graft. The stability of SIS is influenced by how the graft is sterilized, age of the porcine when the graft is harvested, and from which part of the intestine the graft is harvested.[78] Grafts sterilized using ethylene oxide, harvested from pigs >3 weeks of age, and from the distal ileum (i.e., 300 to 400 cm proximal to the ileocecal valve) have higher levels of fibronectin, hyaluronic acid, growth factors, and induce more potent cell proliferation and regeneration.[37,78] This variability in stability has not been documented in grafts constructed from UBM, but it is possible they also exist.

In summary, ACell Vet™ can be used as the sole reconstruction material for corneal defects of virtually any size and depth. In cats, this technique results in excellent corneal clarity and complication rates similar to previously reported techniques. In dogs, this technique may allow for the repair of very large lesions and can result in vision through the grafted area but is associated with dehiscence rates that are greater than reported for conventional techniques.

ACKNOWLEDGMENT

The authors would like to acknowledge Ms Vanessa Au Yeung and Mr Kelvin Yau for their assistance in data analysis.

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