

Use of a porcine urinary bladder acellular matrix for corneal reconstruction in dogs and cats

Olivier Balland,* Anne-Sophie Poinard,* Frank Famose,† Frédéric Gouille,‡ Pierre-François Isard,§ Iona Mathieson¶ and Thomas Dulaurent§

*Centre Hospitalier Vétérinaire, 95 rue des Mazurots, Ludres 54710, France; †Clinique vétérinaire des Acacias, 42 Avenue Lucien-Servanty, Blagnac 31700, France; ‡Clinique Vétérinaire, Parc d'activité Mermoz, 19 Avenue de la forêt, Eysines 33320, France; §Centre Hospitalier Vétérinaire, 275 route Impériale, Saint-Martin Bellevue 74370, France; and ¶Eyevet Referrals, 41-43 Halton Station Road, Sutton Weaver, Cheshire WA7 3DN, United Kingdom

Address communication to:

O. Balland

Tel.: +33 (0)3 83 26 32 92

e-mail: balland@lorrainevet.fr

Abstract

Objective To describe the use of a porcine urinary bladder acellular matrix for surgical reconstruction of the cornea in cases of canine and feline deep corneal ulcers, and feline corneal sequestra.

Materials and methods Twenty-seven dogs and three cats with deep corneal ulcers and seven cats with corneal sequestra were included in the study with overall 38 eyes. For each patient, the necrotic material (ie corneal sequestrum or collagenolytic tissue) was removed by circular lamellar keratectomy. The collagen graft was then cut and prepared to match the stromal defect and then sutured into the lamellar keratectomy bed using interrupted and continuous patterns of absorbable polyglactin 9–0 sutures. Postoperative medical treatment consisted of topical and systemic administration of antibiotics, combined with topical administration of atropine sulfate. The animals were examined 18, 45, and 90 days after the surgery.

Results Postoperative examination revealed complete integration of the biomaterial in 93.5% of ulceration cases in both species and in 100% of feline corneal sequestrum cases. In two cases of ulceration (1 dog and 1 cat), progression of the collagenolytic process at the graft periphery required an additional conjunctival graft 7 days after the first surgery. At 90 days post-op, 100% of the eyes were sighted.

Conclusion Use of a porcine urinary bladder acellular matrix appears to be effective in the surgical management of deep corneal ulcers and feline corneal sequestra.

Key Words: acell, acellular matrix, cat, dog, sequestrum, ulcer

INTRODUCTION

Feline corneal sequestrum (FCS) is a common disease in the cat, with a breed predisposition reported in Persians and Himalayans.^{1,2} FCS is often associated with chronic mechanical irritation of the cornea by entropion, distichiasis, or diminished lacrimation.^{1–3} Feline herpesvirus 1 (FHV-1) involvement has also been reported in the pathogenesis of FCS.¹ FCS appears as a relatively defined area, ranging from 1 mm in diameter to more than half the corneal surface,¹ with a dark color ranging from brown to black. This part of the cornea is subject to necrosis.^{1,2} FCS can be asymptomatic or can cause moderate to intense pain.² It is sometimes associated with corneal neovascularization.² The surgical treatment of FCS often

requires a deep lamellar keratectomy covering a large area. Corneal reconstruction by grafting is therefore essential for deep or large sequestra.^{2,4–6}

A deep corneal ulcer can be defined as loss of stromal substance that may extend from half of the stromal thickness until perforation of the cornea can occur.⁷ During the scarring phase of the collagen matrix, disequilibrium between the proteases and their inhibitors can result in pathological degradation of the corneal stroma.⁸ This rapid stromal degradation associated with corneal ulcers, caused by proteolytic enzymes acting on the collagen (proteoglycans) and other components of the stroma, is called keratomalacia.⁷ These proteolytic enzymes are produced by the epithelial cells of the cornea and by fibroblasts.⁹ Microorganisms such as *Pseudomonas aeruginosa* also

secrete proteases.¹⁰ These extracellular enzymes of bacterial origin also activate endogenous proteolytic enzymes.¹¹ Additional bacterial infections can lead to the degradation of an existing ulcer or the generation of a deep ulcer, making reconstructive surgery necessary. The aim of surgical treatment of these corneal diseases is to preserve structural integrity and maintain vision by conserving corneal transparency.

A large number of grafting techniques have been described for repairing corneal stromal loss due to FCS or deep ulceration: lamellar keratectomy associated with various conjunctival grafting techniques,^{2,12–16} corneo-conjunctival transposition,^{5,17} homologous and heterologous lamellar and penetrating transplants using fresh or frozen corneal tissue,^{18–21} and the use of autologous and heterologous biomaterials. Many biomaterials have been used to compensate loss of corneal substance, such as amniotic membrane,^{22–26} equine renal capsule,²⁷ equine or bovine pericardium,^{28–30} and porcine small intestinal submucosa (SIS).^{31–36} The combined use of biomaterials and conjunctival flaps has also been described.³⁷

The aim of our study was to enhance the existing literature by determining the efficacy of using a porcine urinary bladder-derived collagen xenograft for the surgical reconstruction of feline corneal sequestra and deep corneal ulcers in dogs and cats.

MATERIALS AND METHODS

Animals included in the study

This study included 27 dogs presenting with deep corneal ulcers (Fig. 1). One of these dogs presented with bilateral corneal ulcers. The breeds represented were as follows: French bulldog (nine), Shih Tzu (five), Pekingese (three), Cavalier King Charles spaniel (two), pug (two), boxer (one), Labrador retriever (one), Chihuahua (one), Jagdterrier (one), American Staffordshire terrier (one), and mixed breed (one). The average age was 6.6 years, ranging from

5 months to 14 years. Gender distribution was 12 males (one neutered) and 15 females (eight neutered). The study also included 10 cats (eight Persians and two domestic shorthair). The average age was 5.6, ranging from 2 to 11 years. Gender distribution was six males and four females, all neutered. Seven cats presented with corneal sequestrum (Fig. 2), while three others presented with a deep corneal ulcer.

Ophthalmic examination

A complete ophthalmic examination consisting of a menace response, dazzle reflex, direct and indirect pupillary light reflexes, Schirmer Tear Test (STT, MSD Santé Animal, Clermont-Ferrand, France), fluorescein staining (fluorescein 0.5% single-dose eyewash, TVM, Lempdes, France), biomicroscopy (BX900, Haag-Streit, Chambéry, France or Kowa SL 15, Kowa, Düsseldorf, Germany), and indirect ophthalmoscopy (HeineTM Omega 500; Hertsching, Germany; VolkTM 2.2; Panretinal lens, Mentor, USA) was performed in each animal. Indirect ophthalmoscopy was not performed in cases where the cornea was too opaque. Rebound tonometry (Tonovet, Icare, Vantaa, Finland) was performed in each eye except for eyes presenting with anterior chamber collapse or signs of corneal perforation.

Examination with a narrow slit beam of 0.1 mm was performed to evaluate the depth of lesions for 25 eyes. For the 13 others (seven ulcers and six FCS), the depth of corneal degradation was evaluated during surgery following a lamellar keratectomy and trimming of the corneal lesion. All the eyes treated for deep corneal ulcers presented with a loss of substance affecting more than half the stromal thickness.

Graft material

The biomaterial used is marketed by ACell VetTM in the form of lyophilized 15-mm diameter disks (ACell Vet Corneal DiscsTM; Lafayette, USA).

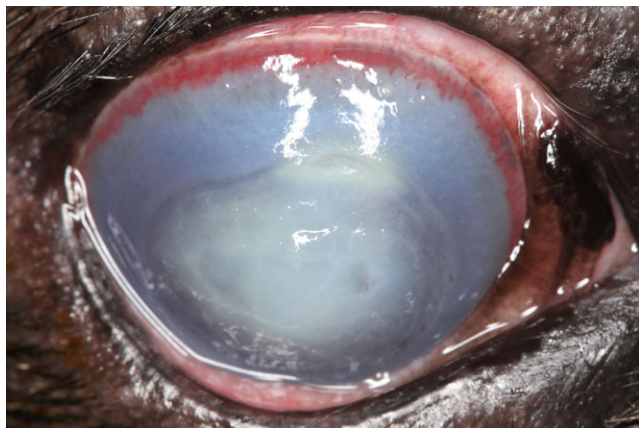


Figure 1. Preoperative photograph of the right eye of Dog 2 showing a deep melting corneal ulcer.



Figure 2. Preoperative photograph of the right eye of Cat 1 showing a feline corneal sequestrum and an entropion of the lower eyelid.

Surgery

Antibiotic prophylaxis was achieved by the intravenous (IV) administration of amoxicillin and clavulanic acid (Augmentin™ 1 g/200 mg; injectable solution, Laboratoire GlaxoSmithKline, Mary le Roi, France) at 20 mg/kg 1 h before surgery. Morphine hydrochloride (Morphine 10 mg, Lavoisier, Paris, France) at 0.05 mg/kg IV and carprofen (Rimadyl™ 50 mg/ml injectable, Zoetis, Paris, France) at a dose of 4 mg/kg were administered subcutaneously (SC) 30 min before induction, to provide analgesia and control inflammation. Premedication was performed 30 min prior to induction by IV administration of medetomidine (Domitor™; Sogeval, Laval, France) at 40 µg/kg. Anesthesia was induced via IV propofol administration (Propovet™; Axience, Pantin, France) at 1 mg/kg and maintained with inhaled 2.5% isoflurane (Isoflo™; Axience, Pantin, France) and oxygen, following endotracheal intubation. The eyelids and conjunctival sacs were then prepared by five successive washes with an iodized polyvidone solution (Vétédine™, Vetoquinol SA, Lures, France) diluted to 1% for the eyelids and to 0.2% for the cornea.

The diameter of the lesions was measured with a Castroviejo caliper; then, the cornea was trephined to the desired depth using a corneal trephine (trephine, and trephine handle open, Beaver-Visitec, Eybens, France) whose diameter corresponded to that of the lesion. In cases of collagenolytic corneal ulcers, all necrotic and collagenolytic tissues were removed with a crescent scalpel (2.5 mm Crescent 55°, Beaver-Visitec, Eybens France). In cases of FCS, the entire pigmented cornea was resected using a crescent scalpel. When the lesions exceeded two-thirds of the corneal stroma 2 layers of ACell Vet were used. In these cases, trephining was performed in two steps: The first trephine was used for the most superficial part to mid-stromal depth; then, a second smaller trephine permitted the excision of the deeper part whose diameter was smaller than that of the more superficial lesion (Fig. 3). In 25/27 dogs and in 7/10 cats, the removed portion of the cornea was processed for bacterial culture.

Following the lamellar keratectomy, the sterile dehydrated collagen disk was trephined with a trephine larger than that used for the lamellar keratectomy. For lesions of 4 to 7.5 mm in diameter, 0.5 mm was added to the diameter of the trephine used for the lamellar keratectomy. For lesions of 8 mm to 10 mm in diameter, 1 mm was added to the diameter of the lesion. The thickness of the dehydrated disk was measured with a micrometer (Exterior Micrometer, Mitutoyo, Roissy en France, France) in 4 cases. The graft was then hydrated using 0.5 ml of saline solution (BSS, Alcon, Rueil-Malmaison, France). The thickness of the rehydrated disk was again measured in the same 4 cases.

The disk was then introduced in the lamellar keratectomy bed. To avoid inverting the two faces, the upper face

was marked with a sterile corneal marker (Dermotrace™ mono pointe, Novatech, La Ciotat, France). The graft was then sutured to the cornea with an absorbable suture material (Vicryl™ Suture absorbable 9-0, polyglactin 910, needle 5.5 mm ½ circle, Ethicon, Issy-les-Moulineaux, France). Four cardinal sutures were placed in the following order: 12, 6, 3, and 9 h in relation to the surgeon. The graft abutted the cornea without overlapping the adjacent healthy epithelium. Then, the spaces located between the four cardinal points were filled by a symmetrical saw tooth suture pattern (Fig. 4). In cases of two-layer lamellar keratectomies, two grafts of different diameters were used, the larger graft being the more superficial. In cases of corneal perforation, a Seidel test was performed at surgery completion (single-dose fluorescein eyewash at 0.5%, TVM, Lempdes, France).

In all patients in the study, a temporary nictitating membrane flap was placed for 18 days by three points in a U-shape using a single nylon suture (Prolène™ 6/0, Ethicon, Issy-les-Moulineaux, France). In the patient



Figure 3. Intraoperative photograph of the right eye of Cat 1 prior to the second step lamellar keratectomy.

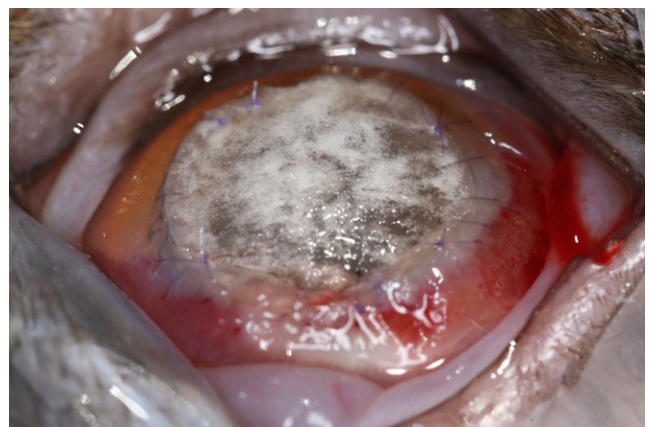


Figure 4. Immediately postoperative photograph of the right eye of Cat 1 showing the graft material sutured into the wound.

presenting with bilateral corneal ulcers, the nictitating membrane fixation was performed in only the more severely affected eye.

Medical treatment

Systemic carprofen (Dolagis™, Sogeval, Laval, France) at 4 mg/kg/day and amoxicillin–clavulanic acid (Kesium™, Sogeval, Laval, France) at 20 mg/kg twice a day was prescribed orally for 10 days. Topical tobramycin (Tobrex 0.3% eyewash, Alcon, Rueil-Malmaison, France) was applied q 4 h directly on the nictitating membrane for 18 days. Topical atropine sulfate 1% (Atropine Alcon™ 1%, Alcon, Rueil-Malmaison, France) was prescribed twice a day for 3 days.

Follow-up

Follow-up examinations were performed at 7 and 18 days after surgery to ensure the nictitating membrane flap remained in place and to evaluate for pain. For the dogs and cats which initially presented with perforations or infected ulcers, the nictitating membrane flap was removed 7 days after surgery, following IV injection of medetomidine (Domitor™, Sogeval, Laval, France) at 40 µg/kg and propofol (Propovet™, Axience, Pantin, France) at 1 mg/kg to check corneal healing and integrity by performing a Seidel test. The nictitating membrane flap was replaced for an additional 11 days in all these patients.

At 18 days postsurgery, the nictitating membrane flap was removed and the corneal graft was evaluated in all animals. All eyes were then treated with topical indometacin (Indocollyre 0.1%™, Chauvin, Montpellier, France) TID.

A complete ophthalmic examination was performed at 45 and 90 days postsurgery. Vision was evaluated by means of menace response, dazzle reflex, and lateralized cotton ball test. Corneal opacity was subjectively classified in three grades: mild opacity, moderate opacity, and severe opacity.

RESULTS

Preoperative examination

Lacrimal gland dysfunction was diagnosed in 5 dogs who presented with a Schirmer tear test (STT) of less than 10 mm, and in 3 dogs and 1 cat with a STT of less than 7 mm. Topical 0.2% cyclosporine (Optimmune, Intervet, Beaucouze, France) and sodium hyaluronate (Viskyl, TVM, Lempdes, France) were administered in these dogs after complete resolution of the corneal ulcer. The cat was treated with topical sodium hyaluronate.

Nasal entropion of the lower eyelid was present in four cats which was corrected at the same time as the corneal surgery. Distichiasis was present in four dogs, one dog had conjunctival ectopic cilia, and two dogs had entropion. Distichiasis and ectopic cilia were treated by

cryotherapy,³⁸ and entropion was corrected using standard Hotz-Celsus technique³⁹ as previously described. All procedures were performed at the same time as the corneal surgery.

Moderate pigmentation of the cornea was present in eight dogs, affecting the inferonasal quadrant with surface pigmentation of less than 25% of the total surface of the cornea. One dog, a French bulldog, presented bilaterally, with a predescemetic ulcer in one eye and a corneal ulcer affecting more than half of the stromal thickness in the other eye.

Seven cats presented with a FCS. One cat presented with a loss of corneal stroma secondary to a chemical burn, one cat presented with a claw scratch, and one cat presented with a deep corneal ulcer of unknown origin.

An overview of the patients included in the present study is presented in Table 1.

Perioperative observations

The mean thickness of the dehydrated disk was 147.50 µm ± 12.5 µm. After rehydration in saline solution, the mean thickness of the graft was 265 µm ± 22.5 µm. Two layers of ACell Vet were used in 23 cases and only one layer in 15 cases.

Postoperative examination

Nine dogs presented with an infected ulcer diagnosed by bacterial culture. Bacterial infection with *Streptococcus* spp was detected in 4 dogs, with *Escherichia coli* in three dogs, and with *Pseudomonas aeruginosa* in two dogs. Bacterial culture was negative in 16 dogs. Bacterial culture revealed bacterial infection with *Escherichia coli* in 1 cat and was negative in 6 cats.

The nictitating membrane flap was removed at 7 days postsurgery in all the patients for whom the bacterial culture was positive and in one additional patient which was subjectively considered at higher risk of complications (Fig. 5). For these 11 cases at 7 days postsurgery, fluorescein staining was positive in 11 and a Seidel test was negative in nine animals. The two animals with aqueous humor leakage needed a complementary surgery consisting of a conjunctival flap to cover the entire biomaterial graft. These animals were one dog and one cat which showed partial suture dehiscence. In the remaining nine animals, no complementary surgery was necessary and the nictitating membrane flap was replaced for 11 additional days.

At day 18 postsurgery, the nictitating membrane flap was removed in all animals. At this point, five animals' corneas stained fluorescein positive: two cats with a FCS of 9 mm (Fig. 6), one dog with an infected ulcer of 11 mm in diameter, one dog with a deep corneal ulcer associated with a *keratoconjunctivitis sicca*, and one dog that presented with a stromal keratitis associated with a deep plant foreign body. The corneal integration of the biomaterial was complete at day 18 with re-epithelialization present in the remaining 92% of the cases.

Table 1. Clinical data and outcome of dogs and cats with deep corneal ulcers and cats with corneal sequestra treated with urinary bladder matrix grafts. F: female, NF: neutered female, M: male, NM: neutered male, NP: not performed, Neg: negative, Pos: positive, FCS: feline corneal sequestrum, KCS: *keratoconjunctivitis sicca*, CP: corneal pigmentation. Severe: severe scar. Moderate: moderate scar. Mild: mild scar

Species	Breed	Sex	Age (years)	Corneal Disease	Concurrent anomaly	Number of collagen layers	Bacteriology	Fluorescein staining day 7	Fluorescein staining day 18	Fluorescein staining day 45	Complication	Outcome
Cat 1	Persian	NM	5	FCS	Entropion	1	NP	NP	NEG			Severe
Cat 2	Persian	NM	7	FCS	Entropion, KCS	1	NEG					Moderate
Cat 3	Domestic Shorthair	NM	2	FCS	Entropion	2	NEG		POS	NEG		Severe
Cat 4	Persian	NF	11	FCS		2	NEG		NEG			Moderate
Cat 5	Persian	NF	7	FCS		2	NP					Mild
Cat 6	Persian	NM	4	FCS		2	NEG					Mild
Cat 7	Persian	NM	3	FCS	Entropion	1	NP					Severe
Cat 8	Persian	NF	8	Deep Corneal Wound (chemical burn)		2	NEG					Moderate
Cat 9	Persian	NF	6	Deep Corneal Ulcer		2	NEG					Severe
Cat 10	Domestic shorthair	NM	3	Deep Corneal Wound (claw scratch)		2	Escherichia coli	POS			Suture dehiscence and positive seidel test requiring a complementary conjunctival graft the seventh day after surgery	Severe
Dog 1	French bulldog	M	5	Penetrating corneal wound		2	Escherichia coli	POS				Moderate
Dog 2	Shih-Tzu	NM	8	Deep melting corneal ulcer	KCS	2	Pseudomonas					Moderate
Dog 3	Pug	M	5	Deep Corneal Ulcer	Entropion, CP	1	Streptococcus					Severe
Dog 4	French bulldog	M	6	Deep melting corneal ulcer		1	Streptococcus		POS	NEG		Moderate
Dog 5	Cavalier King Charles spaniel	F	5.5	Deep melting corneal ulcer		2	Escherichia coli		NEG			Moderate
Dog 6	Jagdterrier	NF	8	Penetrating corneal wound		2	Streptococcus					Moderate
Dog 7	French bulldog	NF	3	Deep melting corneal ulcer		2	Escherichia coli					Moderate

Table 1. (Continued)

Species	Breed	Sex	Age (years)	Corneal Disease	Concurrent anomaly	Number of collagen layers	Bacteriology	Fluorescein staining day 7	Fluorescein staining day 18	Fluorescein staining day 45	Complication	Outcome
Dog 8	Shih tzu	M	9	Deep plant foreign body		2	NEG				Suture dehiscence and positive seidel test requiring a complementary conjunctival graft; the seventh day after surgery	Severe
Dog 9	Pekingese	M	2	Deep melting corneal ulcer		1	Pseudomonas		NEG			Moderate
Dog 10	Chihuahua	NF	3.5	Deep Corneal Ulcer		2	Streptococcus					Mild
Dog 11	French Bulldog	F	6	Deep melting corneal ulcer		2	NEG	NP				Moderate
Dog 12	French Bulldog	NF	8	Deep melting corneal ulcer	KCS, Distichiasis, CP	2	NEG					Moderate
Dog 13	Cavalier King Charles spaniel	NF	5	Deep melting corneal ulcer	Distichiasis	1	NEG					Moderate
Dog 14	Shih tzu	NF	9	Deep melting corneal ulcer	Distichiasis, KCS, CP	1	NEG					Moderate
Dog 15	Pug	F	0.4	Deep Corneal Ulcer		2	NEG					Mild
Dog 16	Shih tzu	M	7	Deep Corneal Ulcer	Entropion, KCS, CP	1	NEG					Moderate
Dog 17	French bulldog	M	7	Deep Corneal Ulcer	KCS	1	NEG		POS	NEG		Moderate
Dog 18	French Bulldog	M	6	Deep melting corneal ulcer		2	NEG		NEG			Moderate
Dog 19	Boxer	NF	8	Deep Corneal Ulcer	Conjunctival ectopic cilia	2	NEG					Moderate
Dog 20	Labrador retriever	M	10	Deep Corneal Ulcer	CP	2	NP					Severe
Dog 21	French bulldog	F	2.5	Deep Corneal Ulcer		1	NEG					Mild
Dog 22	Pekingese	F	6	Deep Corneal Ulcer	Distichiasis, KCS, CP	1	NEG					Moderate
Dog 23	Crossbreed	M	11	Deep Corneal Ulcer	KCS, CP	1	NEG					Mild
Dog 24	Shih tzu	M	8	Deep plant foreign body		2	NEG		POS	NEG		Moderate
Dog 25	Pekingese	F	14	Deep Corneal Ulcer	KCS, CP	2	NEG		NEG			Moderate
Dog 26	American Staffordshire terrier	F	10	Deep corneal ulcer		1	NP					Moderate
Dog 27	French bulldog	NF	7	Deep corneal ulcer OU		1 OD 2 OS	NEG					Moderate OU

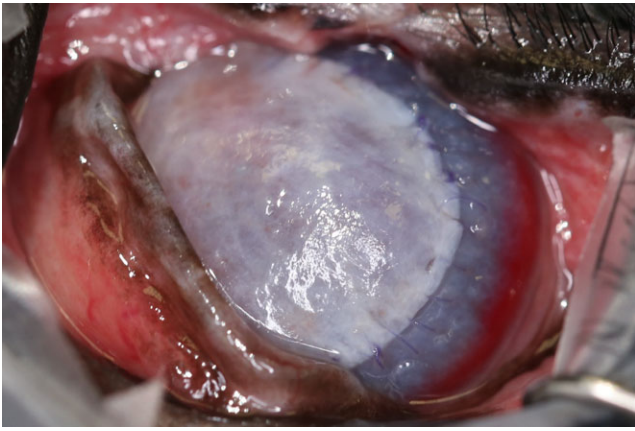


Figure 5. Seven-day postoperative photograph of the left eye of Dog 7 showing the progressive integration of the graft. Corneal edema and neovascularization are also visible.

The presence of an inflammatory reaction with small superficial inflammatory granulomas was observed around the sutures in all animals.

At 45 days postsurgery, complete integration of the bio-material graft with re-epithelialization was present in all eyes, as well as moderate corneal neovascularization. The two cases requiring a conjunctival flap healed favorably. In both cases, there was severe corneal scarring adversely affecting corneal transparency.

At 90 days postsurgery, the degree of corneal opacity was scored as severe (Fig. 7) in 8 eyes (21%), moderate (Fig. 8) in 24 eyes (63.2%), and mild (Fig. 9) in six eyes (15.8%). Vision was present in all 38 eyes at the completion of the study. Corneal pigmentation noted prior to surgery in eight animals did not progress over the course of the study.

At 90 days post-op, no recurrence of corneal sequestra was observed in the feline patients. Six animals had a

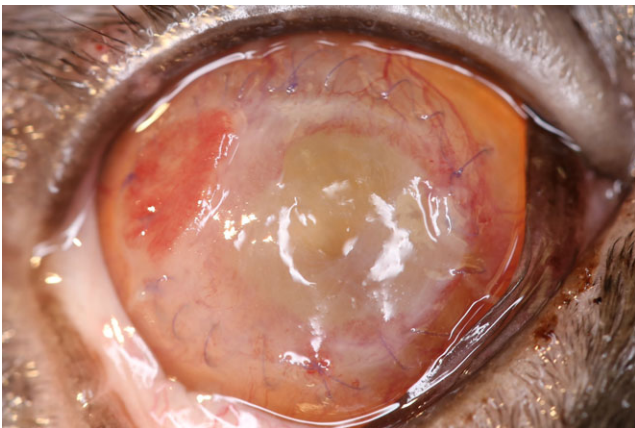


Figure 6. Eighteen-day postoperative photograph of the right eye of Cat 6. The corneal neovascularization is obvious, and the remodeling of the stroma is not complete.

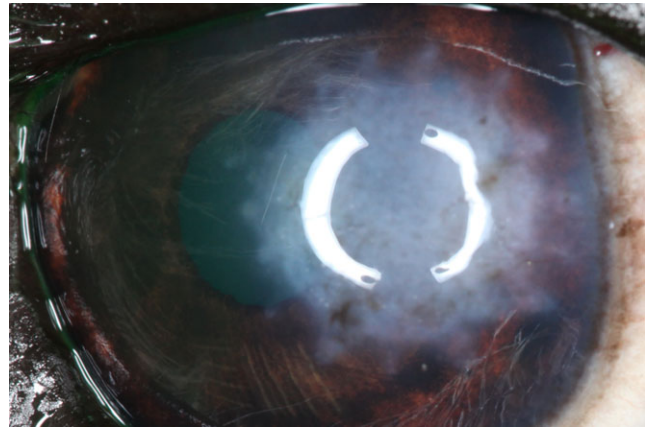


Figure 7. Ninety-day postoperative photograph of the right eye of Dog 20 showing severe corneal scar. Corneal fibrosis is intense in the region of the graft. Some areas of corneal pigmentation are also visible.

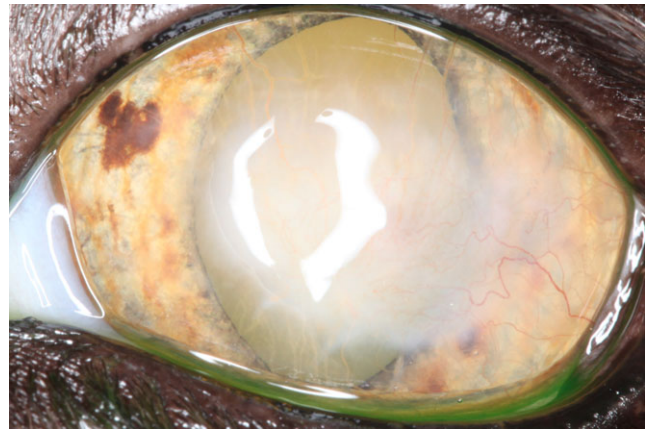


Figure 8. Ninety-day postoperative photograph of the left eye of Cat 4 showing moderate corneal scar. Corneal fibrosis and neovascularization are present, but transillumination of the scar remains possible.

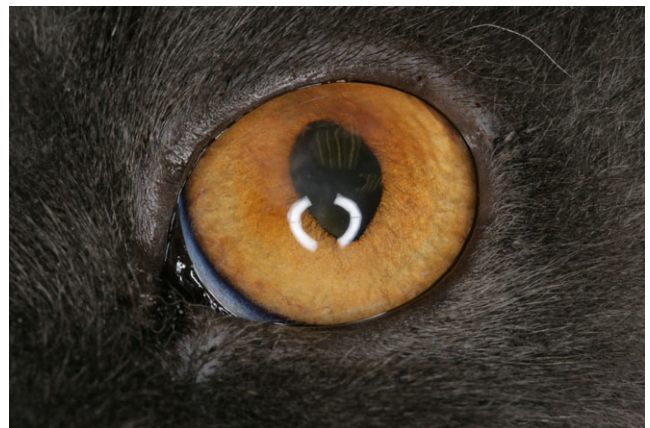


Figure 9. Ninety-day postoperative photograph of the left eye of Cat 5 showing a mild corneal scar.

Table 2. Literature review of the success rate obtained with different graft materials in the surgical treatment of deep corneal ulcers in both dogs and cats, and corneal sequestra in cats

	Deep corneal ulcers in dogs			Deep corneal ulcers in cats			Feline corneal sequestrum		
	Number of cases	Success rate	Reference	Number of cases	Success rate	Reference	Number of cases	Success rate	Reference
Small intestinal submucosa graft	5	100%	Vanore (35)	2	100%	Vanore (35)			
				5	60%	Featherstone (33)	5	100%	Featherstone (33)
							6	100% with one recurrence	Featherstone (2)
	42	88%	Goulle (36)	2	100%	Featherstone (32)			
				7	100%	Goulle (36)	34	100% with four recurrences	Goulle (36)
Pericardium graft	3	66%	Dulaurent (30)				3	100%	Dulaurent (30)
	1	100%	Barros (28)						
Urinary bladder graft							4	100%	Zigler (53)

follow-up time over 1 year, and no progression of the scar appearance was observed.

In the present study, the overall success rate was 96% in canine and 86% in feline patients with deep corneal ulcers and 100% in feline patients with corneal sequestra. A review of reported success rates with the use of different biomaterials in cases of deep corneal ulcers and FCS is reported in Table 2.

DISCUSSION

The use of biomaterials as grafts depends on essential criteria, such as the immune response and minimum inflammation of the recipient,^{40,41} a resorption profile of the tissue inversely proportional to the rate of regeneration,⁴² good cellular attachment, and controlled cellular proliferation, migration, and differentiation.^{42,43} Ideally, biomaterials must be completely degraded and replaced by normal tissue.

A biomaterial derived from porcine urinary bladder called urinary bladder matrix (UBM) is currently marketed by ACell VetTM, in the form of lyophilized disks 15 mm in diameter (Corneal Disc, ACell Vet, Lafayette, USA). UBM is a structure with two faces. The upper face corresponds to the basal membrane and has a dense acellular matrix that serves as a barrier between the epithelium and the other tissues.⁴⁴ The face of the *lamina propria* is a more open acellular collagen matrix, propitious for the integration of conjunctive tissues in the bed of the wound.⁴⁴ The UBM disk has a fail-safe indication on the right side to avoid inversion between the two faces of the graft as it is difficult to distinguish between the two faces with the naked eye. This biomaterial satisfies the requirements of regenerative medicine: It is acellular to reduce immune response, and it resorbs progressively to confer mechanical resistance to the regenerated tissue.

The UBM is mainly employed in human medicine to reconstruct skin tissue,^{45–47} esophagus,^{48–50} tympanic membrane,⁵¹ and myocardium.⁵² In veterinary medicine, the use of this xenograft has been described in the surgical reconstruction of the cornea after removal of sequestra in cats⁵³ and in the surgical management of an epibulbar melanoma in a cat.⁵⁴ More recently, its use has been depicted in association with a conjunctival flap in the surgical management of corneal defects in dogs.³⁷ The grossly similar composition between UBM and other types of acellular matrixes whose use has been validated in veterinary ophthalmology (i.e., SIS and pericardial patch) led us to assess its efficacy in the treatment of deep ulcers in both dogs and cats, and to confirm its usefulness in the corneal reconstruction following deep lamellar keratectomy in cases of feline corneal sequestra.

The surgical technique of UBM grafting closely resembled what has already been described for other grafting procedures. It required the complete ablation of the necrotic, infected, or pigmented corneal tissues before performing the graft.^{34–37} The ablation of these lesions was performed systematically with a trephine to facilitate preparation and positioning of the graft, and to speed and ease the surgery. The size of the graft was larger than the diameter of the corneal defect,³⁶ which ensured a stable position within the defect and minimized the risk of rupture of the biomaterial when tightening the suture. It was rehydrated and then sutured to the healthy cornea as described for SIS^{35,36} and pericardium patches³⁰. In our study, UBM disk thickness was measured prior to and following rehydration in only a small number of cases. This precludes us from drawing definitive conclusion about the disparity in thickness or rehydration characteristics of UBM disks between different lot numbers. However, the relative heterogeneity of the thickness measurements may be the main limitation of the use of this material. Further

investigations are required to confirm this observation. We believe this heterogeneity might be related to the manufacturing process. It would also be interesting to make the same measurements on SIS and bovine pericardial patch (BPP) disks to assess the homogeneity of the materials provided by each manufacturer.

During the follow-up at 7 days, temporary removal of the nictitating membrane flap for 9 eyes was performed to examine graft integration, corneal integrity, and status of corneal infection. The anchoring of the nictitating membrane was left in place for 15 days in the study by Vanore³⁵ and 21 days in that by Gouille³⁶; we chose to compromise with 18 days in the present study. The need to perform general anesthesia to remove the sutures anchoring the nictitating membrane, then replace them, was constraining. However, we considered it was necessary to use this technique to protect the operation site mechanically. Furthermore, anchoring the nictitating membrane prevents the dehydration of the graft during the time required for scarring.³⁶

The results of our clinical study were similar to that obtained by Zigler on cats affected by FCS,⁵³ with an accurate integration of the biomaterial in 100% of the cases, thus confirming that the use of UBM can be considered when managing FCS. The success rate of our procedure was also similar to that obtained with other collagen matrix grafts (i.e., SIS^{33,35,36}, pericardial patch³⁰, amniotic membrane⁴), or lamellar corneal grafts, in the treatment of FCS.^{6,19–21} A successful outcome was also obtained in the management of deep corneal ulcers in both dogs (96%) and cats (86%) with the use of UBM grafts. These results are comparable to those observed with other types of graft (i.e., SIS^{33,35,36}, pericardial patch³⁰, and amniotic membrane²⁴). The two failures of our series (1 of 28 canine eyes and one of seven feline eyes) were secondary to suture dehiscence despite the mechanical protection provided by the anchoring of the nictitating membrane.

The clinical evaluation of the healing process after UBM grafting revealed a similar chronology as compared with previous studies utilizing SIS^{35,36,55} and BPP³⁰ grafting under similar conditions. The first phase of healing was led by a centripetal corneal neovascularization which was more obvious in dogs than in cats. The second phase was almost contemporary to the progressive corneal neovascularization and was led by progressive epithelialization of the cornea. This phase was obviously longer in patients with larger corneal lesions. The last phase appeared as a vascular regression and stromal remodeling toward transparency.

CONCLUSION

The results of our study are comparable to those of other studies describing the use of various biomaterials in the surgical management of corneal defects resulting from different pathological insults. The presentation of commer-

cially available collagenic xenografts (UBM, SIS, BPP) is similar, as is the biological behavior of those different biomaterials. Therefore, it appears that UBM, as SIS and BPP, should be considered by veterinary ophthalmologists when surgically reconstructing the cornea following many pathological conditions.

REFERENCES

1. Stiles J. Feline ophthalmology. In: *Veterinary Ophthalmology*, 5th edn. (eds Gelatt KN, Gilger CG, Kern TJ) Wiley-Blackwell, Ames, 2013; 1477–1559.
2. Featherstone HJ, Sansom J. Feline corneal sequestra: a review of 64 cases (80 eyes) from 1993 to 2000. *Veterinary Ophthalmology* 2004; **7**: 213–227.
3. Williams DL, Kim JY. Feline entropion: a case series of 50 affected animals (2003–2008). *Veterinary Ophthalmology* 2009; **12**: 221–226.
4. Barchetti L, Giudice C, Mortellaro CM. Amniotic membrane transplantation for the treatment of feline corneal sequestrum: pilot study. *Veterinary Ophthalmology* 2010; **13**: 326–330.
5. Andrew SE, Tou S, Brooks DE. Corneoconjunctival transposition for the treatment of feline corneal sequestra: a retrospective study of 17 cases (1990–1998). *Veterinary Ophthalmology* 2001; **4**: 107–111.
6. Pena Gimenez MT, Farina IM. Lamellar keratoplasty for the treatment of feline corneal sequestrum. *Veterinary Ophthalmology* 1998; **1**: 163–166.
7. Ledbetter EC, Gilger BC. Diseases and surgery of the canine cornea and sclera. In: *Veterinary Ophthalmology*, 5th edn. (eds Gelatt KN, Gilger CG, Kern TJ) Wiley-Blackwell, Ames, 2013; 976–1049.
8. Geerling G, Jousen AM, Daniels JT *et al.* Matrix metalloproteinases in sterile corneal melts. *Annals of the New York Academy of Sciences* 1999; **878**: 571–574.
9. Fini ME, Girard MT, Matsubara M. Collagenolytic/gelatinolytic enzymes in corneal wound healing. *Acta ophthalmologica Supplement* 1992; **202**: 26–33.
10. Hibbetts K, Hines B, Williams D. An overview of proteinase inhibitors. *Journal of veterinary internal medicine* 1999; **13**: 302–308.
11. Twining SS, Fukuchi T, Yue BY *et al.* Corneal synthesis of alpha 1-proteinase inhibitor (alpha 1 antitrypsin). *Investigative Ophthalmology and Visual Science* 1994; **35**: 458–462.
12. Hakanson NE, Merideth RE. Conjunctival pedicle grafting in the treatment of corneal ulcers in the dog and cat. *Journal of the American Animal Hospital Association* 1987; **23**: 641–648.
13. Gelatt KN. *Handbook of Small Animal Ophthalmic Surgery, Volume 2: Corneal and intraocular procedures*. Pergamon Press, New York, 1995.
14. Hendrix DVH. Diseases and surgery of the canine conjunctiva and nictitating membrane. In: *Veterinary Ophthalmology*, 5th edn. (eds Gelatt KN, Gilger CG, Kern TJ) Wiley-Blackwell, Ames, 2013; 945–975.
15. Peiffer R, Gelatt K, Gwin R. Tarsconjunctival pedicle grafts for deep ulceration in the dog and cat. *Journal of American Animal Hospital Association* 1977; **13**: 387–391.
16. Pumphrey SA, Pizzirani S, Pirie CG. 360-degree conjunctival grafting for management of diffuse keratomalacia in a dog. *Veterinary Ophthalmology* 2011; **14**: 209–213.
17. Parshall C. Lamellar corneo-scleral transposition. *Journal of the American Animal Hospital Association* 1973; **9**: 270–277.

18. Hacker DV. Frozen corneal grafts in dogs and cats: a report on 19 cases. *Journal of American Animal Hospital Association* 1991; **27**: 387–398.
19. Hansen PA, Guandalini A. A retrospective study of 30 cases of frozen lamellar corneal grafts in dogs and cats. *Veterinary Ophthalmology* 1999; **2**: 233–241.
20. Townsend WM, Rankin AJ, Stiles J *et al.* Heterologous penetrating keratoplasty for the treatment of a corneal sequestrum in a cat. *Veterinary Ophthalmology* 2008; **11**: 273–278.
21. Laguna F, Leiva M, Costa D *et al.* Corneal grafting for the treatment of feline corneal sequestrum: a retrospective study of 18 eyes (13 cats). *Veterinary Ophthalmology* 2015; **18**: 291–296.
22. Barros PS, Garcia JA, Laus JL *et al.* The use of xenologous amniotic membrane to repair canine corneal perforation created by penetrating keratectomy. *Veterinary Ophthalmology* 1998; **1**: 119–123.
23. Barros PS, Safatle AMV, Godoy CA *et al.* Amniotic membrane transplantation for the reconstruction of the ocular surface in three cases. *Veterinary Ophthalmology* 2005; **8**: 189–192.
24. Lassaline ME, Brooks DE, Ollivier FJ *et al.* Equine amniotic membrane transplantation for corneal ulceration and keratomalacia in three horses. *Veterinary Ophthalmology* 2005; **8**: 311–317.
25. Ollivier FJ, Kallberg ME, Plummer CE *et al.* Amniotic membrane transplantation for corneal surface reconstruction after excision of corneolimbic squamous cell carcinomas in nine horses. *Veterinary Ophthalmology* 2006; **9**: 404–413.
26. Plummer CE. The use of amniotic membrane transplantation for ocular surface reconstruction: a review and series of 58 equine clinical cases (2002–2008). *Veterinary Ophthalmology* 2009; **12** (Suppl. 1): 17–24.
27. Andrade AL, Laus JL, Figueiredo F *et al.* The use of preserved equine renal capsule to repair lamellar corneal lesions in normal dogs. *Veterinary Ophthalmology* 1999; **2**: 79–82.
28. Barros PSM, Safatle AMV, Malerba TA *et al.* The surgical repair of the cornea of the dog using pericardium as a keratoprosthesis. *Brazilian Journal of Veterinary Research and Animal Science* 1995; **32**: 251–255.
29. Barros PSM, Safatle AMV, Rigueiro M. Use of horse pericardium preserved in glycerin as corneal graft in dogs. Experimental study. *Brazilian Journal of Veterinary Research and Animal Science* 1997; **34**: 138–141.
30. Dulaurent T, Azoulay T, Goulle F *et al.* Use of bovine pericardium (Tutopatch®) graft for surgical repair of deep melting corneal ulcers in dogs and corneal sequestra in cats. *Veterinary Ophthalmology* 2014; **17**: 91–99.
31. Lewin GA. Repair of a full thickness corneoscleral defect in a German shepherd dog using porcine small intestinal submucosa. *Journal of Small Animal Practice* 1999; **40**: 340–342.
32. Featherstone HJ, Sansom J. Intestinal submucosal repair in two cases of feline ulcerative keratitis. *Veterinary Record* 2000; **146**: 136–138.
33. Featherstone HJ, Sansom J, Heinrich CL. The use of porcine small intestinal submucosa in ten cases of feline corneal disease. *Veterinary Ophthalmology* 2001; **4**: 147–153.
34. Bussieres M, Krihne SG, Stiles J *et al.* The use of porcine small intestinal submucosa for the repair of full-thickness corneal defects in dogs, cats and horses. *Veterinary Ophthalmology* 2004; **7**: 352–359.
35. Vanore M, Chahory S, Payen G *et al.* Surgical repair of deep melting ulcers with porcine small intestinal submucosa (SIS) graft in dogs and cats. *Veterinary Ophthalmology* 2007; **10**: 93–99.
36. Goulle F. Use of porcine small intestinal submucosa for corneal reconstruction in dogs and cats: 106 cases. *Journal of Small Animal Practice* 2012; **53**: 34–43.
37. Dorbandt DM, Moore PA, Myrna KE. Outcome of conjunctival flap repair for corneal defects with and without an acellular submucosa implant in 73 canine eyes. *Veterinary Ophthalmology* 2015; **18**: 116–122.
38. Wheeler CA, Severin GA. Cryosurgical epilation for the treatment of distichiasis in the dog and cat. *Journal of the American Animal Hospital Association* 1984; **20**: 877–884.
39. Celsus, cited by Zeis E. *Handbuch der plastischen chirurgie*. Berlin, Germany: G. Reimer 1839.
40. Cooper ML, Spielvogel RL. Artificial skin for wound healing. *Clinical Dermatology* 1994; **12**: 183–191.
41. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Seminars in Immunology* 2008; **20**: 86–100.
42. Mieszawska AJ, Kaplan DL. Smart biomaterials - regulating cell behavior through signaling molecules. *BMC Biology* 2010; **8**: 59.
43. Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. *Nature* 2009; **462**: 433–441.
44. Brown B, Lindberg K, Reing J *et al.* The basement membrane component of biologic scaffolds derived from extracellular matrix. *Tissue Engineering* 2006; **12**: 519–526.
45. Eweida A, Saad M, Gabr E *et al.* Cultured keratinocytes on urinary bladder matrix scaffolds increase angiogenesis and help in rapid healing of wounds. *Advances in Skin and Wound Care* 2011; **24**: 268–273.
46. Mitchell KB, Gallagher JJ. Porcine bladder extracellular matrix for closure of a large defect in a burn contracture release. *Journal of Wound Care* 2012; **21**: 454–456.
47. Kimmel H, Rahn M, Gilbert TW. The clinical effectiveness in wound healing with extracellular matrix derived from porcine urinary bladder matrix: a case series on severe chronic wounds. *Journal of the American College of Certified Wound Specialists* 2010; **2**: 55–59.
48. Nieponice A, Ciotola FF, Nachman F *et al.* Patch esophagoplasty: esophageal reconstruction using biologic scaffolds. *The Annals of Thoracic Surgery* 2014; **1**: 283–288.
49. Nieponice A, Gilbert TW, Badylak SF. Reinforcement of esophageal anastomoses with an extracellular matrix scaffold in a canine model. *The Annals of Thoracic Surgery* 2006; **82**: 2050–2058.
50. Badylak SF, Vorp DA, Spievack AR *et al.* Esophageal reconstruction with ECM and muscle tissue in a dog model. *The Journal of Surgical Research* 2005; **128**: 87–97.
51. Parekh A, Mantle B, Banks J *et al.* Repair of the tympanic membrane with urinary bladder matrix. *Laryngoscope* 2009; **119**: 1206–1213.
52. Badylak SF, Kochupura PV, Cohen IS *et al.* The use of extracellular matrix as an inductive scaffold for the partial replacement of functional myocardium. *Cell Transplantation* 2006; **15**(Suppl 1): 29–40.
53. Zigler MS. Use of Acell Vet xenograft in feline corneal sequestrum. In: Proceedings of the 34th Annual Meeting of the American College of Veterinary Ophthalmologists 2003; 65.
54. Plummer CE, Kallberg ME, Ollivier FJ *et al.* Use of a biosynthetic material to repair the surgical defect following excision of an epibulbar melanoma in a cat. *Veterinary Ophthalmology* 2008; **11**: 250–254.
55. Griguer F, Raymond I, Regnier A. Preliminary evaluation of the bio-compatibility of the small intestinal submucosa (SIS) in rabbit cornea. *Revue de Médecine Vétérinaire* 2001; **152**: 597–604.