

# Surgical repair of deep melting ulcers with porcine small intestinal submucosa (SIS) graft in dogs and cats

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## Abstract

**Purpose** To evaluate the efficacy of using a porcine small intestinal submucosa (SIS) graft for the surgical repair of deep melting ulcers in dogs and cats.

**Methods** Two cats and five dogs presented with deep and large melting ulcers of the cornea. In each case, the necrotic and collagenolytic tissue of the cornea was removed by keratectomy. A SIS graft, 1 mm greater than the corneal defect, was rehydrated in sterile saline and sutured to the edges of the ulcer with a simple interrupted pattern of 9/0 polyglactin 910. A nictitating membrane flap was utilized in two cats and four dogs for 2 weeks. All cases were treated postoperatively with topical and systemic antibiotics, a systemic anti-inflammatory drug and topical atropine. All animals were re-evaluated 15 days, 4 weeks, 35–45 days, 2–3 months and 6 months postsurgery.

**Results** At 15 days postsurgery, a superficial intense corneal neovascularization surrounded the SIS graft. No ocular discomfort was present and fluorescein staining was negative in all cases. At 4 weeks the SIS graft was thick and opaque in all cases, although in one cat the SIS graft had partially detached. Between 35 and 45 days, SIS graft integration was evident in all eyes, and corneal neovascularization had decreased progressively. All eyes healed without complications and retained corneal transparency. This occurred even in the presence of corneal perforation in two cases: one prior to and one during surgery.

**Conclusion** Results of our study suggest the SIS graft may be an effective alternative surgical treatment to the traditional conjunctival grafts commonly used to repair melting ulcers in dogs and cats. The advantages of using a SIS graft include good corneal transparency, preservation of corneal integrity and maintenance of vision.

**Key Words:** cat, dog, melting ulcer, small intestinal submucosa, xenograft

## INTRODUCTION

Corneal ulcers may present with keratomalacia, often termed ‘melting ulcer’. The term ‘melting’ describes the rapid gelatinization and liquefaction of the stroma, which leads in the most extreme cases to stromal ulceration.<sup>1</sup> Microorganisms (bacteria and fungi), inflammatory cells (polymorphonuclear neutrophils), corneal epithelial cells, and fibroblasts can produce and release collagenases and other proteases.<sup>1</sup> Most of the collagenolytic proteases affecting the cornea can be divided into matrix metalloproteinases (MMPs) such as MMP2 and MMP9, and serine proteases such as neutrophil elastase.<sup>2,3</sup> In addition to directly destroying corneal extracellular matrix, some bacterial proteases (e.g. elastase from *P. aeruginosa*) also activate latent corneal MMPs.<sup>4</sup> Although melting ulcers may respond to medical management, a

progression of stromal dissolution is still possible and surgical treatment is necessary to avoid corneal perforation.

Surgical options include a conjunctival flap or graft and corneal transplantation.<sup>5</sup> Due to its antimicrobial and anti-collagenase properties, conjunctival pedicle grafting is a safe and efficient procedure. However, the presence of a permanent scar is a major corneal opacity as it may impair vision.<sup>6</sup>

There is growing interest in the use of SIS grafts in the surgical management of severe corneal disorders. SIS is a biomaterial derived from porcine jejunum and composed of three layers: tunica muscularis mucosa, tunica submucosa and the stratum compactum layer of the tunica mucosa. A combination of mechanical and chemical methods are used to remove the serosa, muscularis and superficial mucosal layers and leave an acellular extracellular matrix that acts as a three-dimensional scaffold for tissue repair and remodeling.<sup>7</sup>

Several studies report the use of the SIS for ocular grafts. These include the removal of a limbal melanoma in a dog,<sup>8</sup> corneal disorders in cats,<sup>9,10</sup> experimental models of corneal SIS transplantation in rabbits,<sup>11</sup> and repair of full-thickness corneal defects in dogs, cats and horses.<sup>12</sup>

The purpose of the present study was to evaluate the use of porcine small intestinal submucosa for the repair of corneal melting ulcers in dogs and cats.

## MATERIALS AND METHODS

Two cats and five dogs with melting ulcers were included in this study. The animals were aged between 2 and 9 years, with a mean age of 3.5 for cats and 5.5 years for dogs.

The clinical observations for these animals are summarized in Table 1.

In cases 1 and 3 (Fig. 1), the melting ulcer involved the entire surface of the cornea. In the other cases the melting ulcer was localized on the axial cornea (Fig. 2). In most of the cases a melting ulcer developed 1 day after the original ulceration. In case 5 it appeared 3 days after the original ulcer.

In the dogs, the ulceration was due to trauma. One dog (case 7) presented with a bilateral keratoconjunctivitis sicca treated with topical cyclosporine (CsA). The Schirmer tear test (STT) value for the contralateral eye was 9 mm/min. Due to ocular pain and cornea fragility STT was not performed in the affected eye. In the cats, a herpetic origin was suspected in one case (case 2), based on positive polymerase chain reaction (PCR) testing for feline herpesvirus (FHV-1) and the presence of a dendritic ulcer in the other eye. Therefore, we assumed that the melting ulcer was secondary to bacterial complication of a viral ulcer. In the other case (case 1), the etiology of the melting ulcer remained undetermined.

An anti-inflammatory agent, tolfenamic acid, 4 mg/kg (SC) (Tolfedine<sup>®</sup>, Vetoquinol, Lure, France) was administered 2 h before the surgical procedure. All surgical procedures were performed under general anesthesia and with the aid of an operating microscope (Zeiss OPMI 6, Carl Zeiss S.A.S., Le Pecq, France).

The anesthesia protocol was the same for each patient. Following premedication with 0.2 mg/kg IV diazepam (Valium<sup>®</sup>, Roche, Neuilly sur-Seine, France), general anesthesia was induced with 10–15 mg/kg IV sodium thio-pental (Nesdonal<sup>®</sup>, Merial, Lyon, France) and maintained with halothane (Halothane<sup>®</sup>, Vétérinaire Belamont, Neuilly/Seine, France). Systemic cephalixin, 30 mg/kg IV (Rilexine<sup>®</sup>, Virbac, Carros, France), was given before surgery and 3 h later.

Samples for bacterial culture were collected from all dogs. Collection was performed with sterile cotton-tipped swabs moistened with a sterile saline solution. Culture revealed *P. aeruginosa* and *Staphylococcus intermedius* infection in one dog (case 6) and was negative in the other cases. Bacterial culture was not performed on the cats. Both had been treated with topical antibiotics prior to presentation.

The standard procedure for all cases included debridement (keratectomy) of the necrotic and collagenolytic corneal tissue with Castroviejo corneal scissors (Moria S.A., Antony, France) (Fig. 3). Corneal perforation occurred in two cases. In case 3, corneal perforation was already present at the time of examination. In case 2, there was corneal liquefaction deep in the stroma, which led to corneal perforation after intraoperative removal of the collagenolytic tissue.

### Fixation of SIS graft

The SIS (Vet Biosist, Cook Inc., Hertfordshire, UK) was trimmed to produce a disk 1 mm greater in diameter than the corneal defect and rehydrated in sterile saline solution. The graft was placed over the corneal defect and fixed in

**Table 1.** Signalment and outcome of patients with melting ulcers treated with SIS grafts

Case	Breed	Age & gender	OD/OS	Primary lesion	Bacteriology	Complications	Outcomes
1	DSH	3 years MN	OS	Melting ulcer over the whole corneal surface AC non visible	Not preformed	Dehiscence of dorsal edge of SIS at 15 days post surgery	Visual
2	DSH	4 years MN	OD	CP Melting ulcer (10 × 10 mm) AC not visible	Not performed	Anterior synechia at 7 o'clock	Visual
3	Shih Tzu	4 years M	OS	CP Melting ulcer over the whole corneal surface AC not visible	No growth	Axial corneal leukoma Anterior synechia at 11–2 o'clock	Visual
4	Pekinese	5 years M	OS	Melting ulcer (6 × 8 mm)	No growth	None	Visual
5	French Bulldog	7 years F	OD	Hypopion Melting ulcer (4 × 5 mm)	No growth	None	Visual
6	Cavalier King Charles Spaniel	2 years F	OS	Hypopion Melting ulcer (8 × 8 mm)	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus intermedius</i>	None	Visual
7	Shih Tzu	9 years F	OD	KCS Hypopion Melting ulcer (4 × 6 mm)	No growth	None	Visual

DSH: Domestic Short-haired cat; M: male; N: neutered; F: female; OD: right eye; OS: left eye; AC: Anterior chamber; CP: corneal perforation; KCS: Keratoconjunctivitis sicca.



**Figure 1.** Case 3: large melting ulcer with axial corneal perforation.

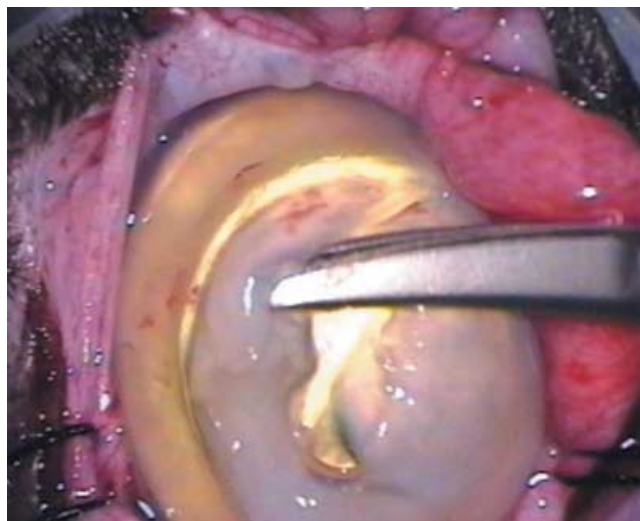


**Figure 2.** Case 4: melting ulcer localized on the axial cornea.

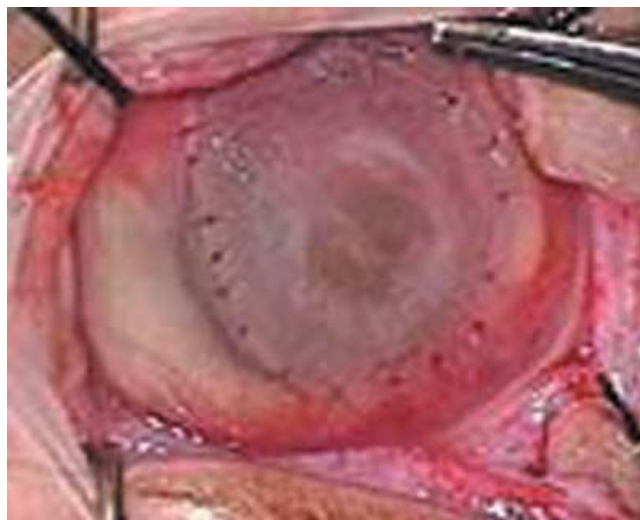
position with simple interrupted sutures of 9/0 polyglactin 910 (Vicryl monofil resorbable suture, Ethicon, Janssen, Noisy-le-grand, France), placed at 12, 3, 6 and 9 o'clock. Further simple interrupted sutures were placed to secure the graft to the healthy cornea (Fig. 4). Case 1 presented with a wide corneal ulcer and the graft was positioned near the limbus in the dorsolateral part of the cornea.

To protect the corneal repair a nictitating membrane flap was placed in all cases except for case 3.

Postoperative treatment consisted of topical application of an antibiotic agent, tobramycin (Tobrex<sup>®</sup>, Alcon, Rueil-Malmaison, France) four times daily for 1 month, and atropine (Atropine Faure 1%<sup>®</sup>, Paris, France) twice daily for 10 days. The FHV-1-positive cat received an adjunctive treatment with



**Figure 3.** Intraoperative photograph of case 1: corneal debridement of the necrotic and collagenolytic corneal tissue with Castroviejo corneal scissors.



**Figure 4.** Intraoperative photograph of case 2 following suturing of the SIS graft into the corneal defect.

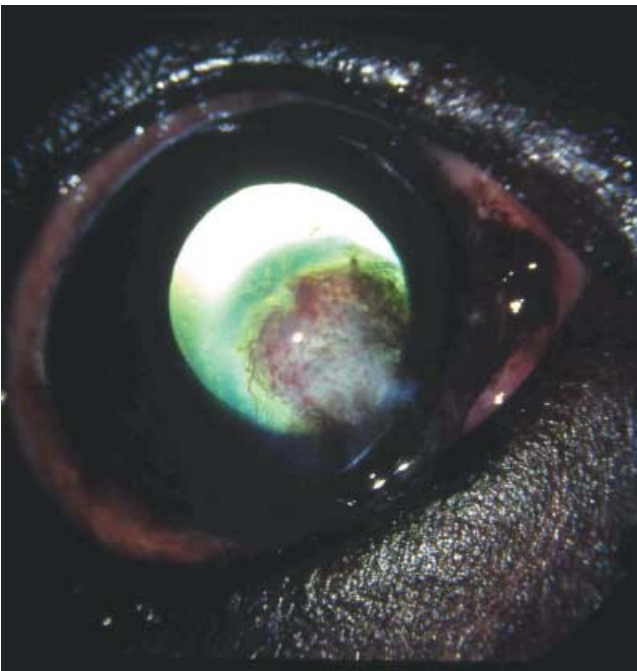
interferon- $\alpha$  (Ropheron<sup>®</sup>, Roche) three times daily for 6 months. Tolfenamic acid (Tolfedine<sup>®</sup>, Vetoquinol) 4 mg/kg once daily for 4 days and cephalexin (Rilexine<sup>®</sup>, Virbac), 15 mg/kg twice daily for 10 days, were administered orally in all patients.

## RESULTS

The nictitating membrane flap was removed 2 weeks postoperatively and all animals were re-evaluated 15 days, 4 weeks, 35–45 days, 2–3 months and 6 months postsurgery. At 15 days there was marked superficial corneal neovascularization surrounding the SIS graft. No ocular discomfort was present, and fluorescein staining was negative. Sutures were still present at the periphery of the SIS graft. Grafts were attached



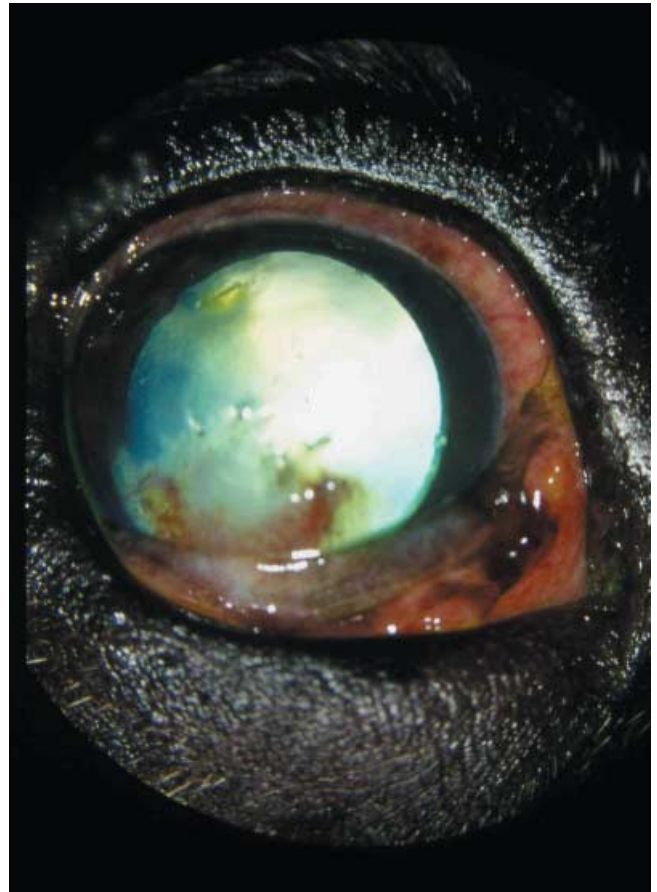
**Figure 5.** Case 1: the edge of the SIS graft is partially deliquesced 15 days postsurgery.



**Figure 6.** Case 5: the SIS graft is opalescent. Corneal neovascularization, inflammatory cellular infiltration and corneal edema are apparent 4 weeks postsurgery.

to the entire surface of the ulcer except in case 1. In this case the dorsolateral edge of the graft was detached, but the corneal shape was preserved (Fig. 5).

At 4 weeks the SIS graft was opalescent in all patients. Significant stromal cell proliferation was present in the SIS graft and corneal neovascularization was increased (Fig. 6). No ocular discomfort was present in any animal. Between 35



**Figure 7.** Case 5: between 35 and 45 days postsurgery, SIS integration is clinically evident. Corneal neovascularization has become less developed and sutures are still present.

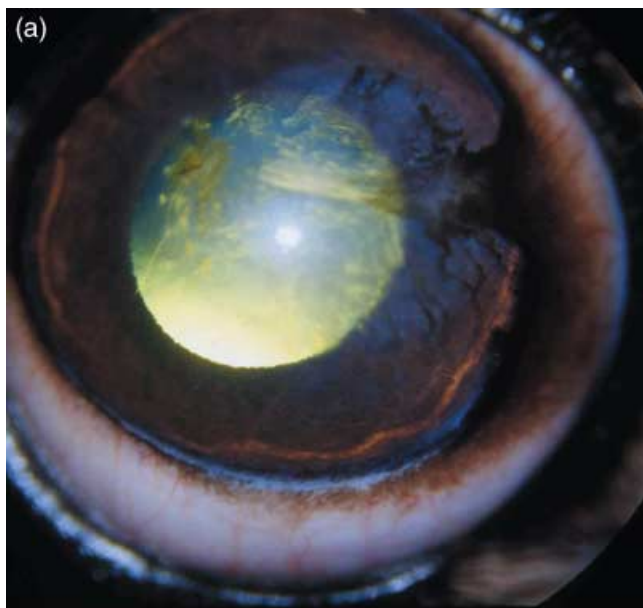
and 45 days SIS graft integration was clinically evident in all eyes. Corneal neovascularization became progressively less developed and the sutures were still present (Fig. 7).

Greater clinical variations between animals were seen at 2–3 months. Corneal neovascularization had disappeared in case 4 (Fig. 8a), while a residual neovascularization was still present in case 1 (Fig. 8b). In case 3, stromal tissue proliferation and a moderate corneal neovascularization were present, their distribution varying according to the original corneal damage (Fig. 8c). In this case a large melting ulcer and corneal perforation had been present, resulting in a more marked stromal tissue proliferation. In all cases, the corneal lesions healed without complications and with good corneal transparency; the final outcome was very good. Two cases (2 and 3) developed an endothelial corneal scar caused by a corneal perforation (Fig. 9).

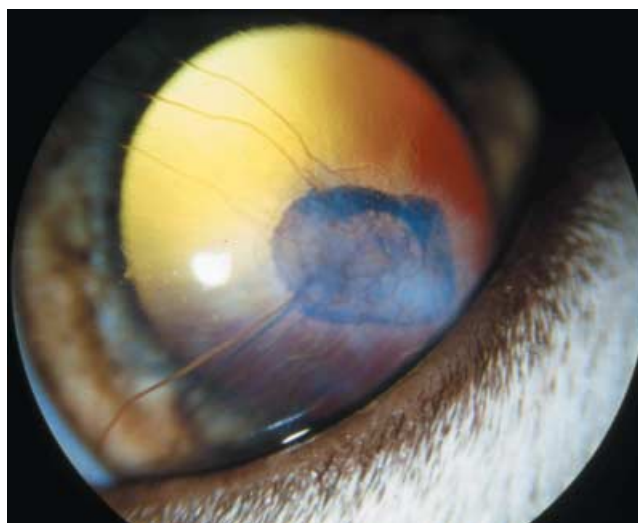
At 6 months, five of the seven animals (four dogs and one cat) had complete corneal transparency. Vision was preserved in all patients.

## DISCUSSION

Melting ulcers are characterized by progressive stromal dissolution secondary to proteolytic activity. Proteolytic



**Figure 8.** Varying SIS integration 2 months after surgery: (a) Disappearance of corneal neovascularization in case 4. (b) Residual corneal neovascularization in case 1. (c) Corneal granulation tissue due to extensive melting ulcer in case 3.



**Figure 9.** Six months postsurgery: in case 2 an endothelial corneal scar is present.

enzymes are important in the slow turnover and remodeling of the normal healthy corneal stroma. These proteolytic enzymes, metalloproteinases (MMPs), are present at all stages of the ulcerative process, from formation of the initiating epithelial defect to ulcer resolution and repair.<sup>13</sup> They play an important role in corneal repair, re-epithelialization and in stromal neovascularization.<sup>1</sup> During the period of repair tissue remodeling, the rate of collagen turnover is much higher than in the normal cornea, suggesting the involvement of MMPs in the remodeling process.<sup>14</sup>

The activity of these proteolytic enzymes is normally balanced by inhibitors in order to prevent excessive degradation of normal healthy tissue. An imbalance between proteases and protease inhibitor levels due to excessive levels of proteases can cause pathologic degradation of corneal stromal collagen and proteoglycans.<sup>13</sup> Sources of MMPs may be endogenous (tissue) or exogenous (bacteria).<sup>15</sup> *Pseudomonas aeruginosa* is widely recognized as the most virulent corneal pathogen. While trauma is a prerequisite for bacterial adherence and subsequent stromal infection, *P. aeruginosa* adheres to injured corneal epithelial cells more readily than most other bacterial species.<sup>16</sup> Bacterial collagenases have been implicated in the pathogenesis of corneal ulcer in horses, dogs and cats.<sup>15,16</sup>

MMP inhibitors are recommended for treatment of ulcerative keratitis and progressive keratomalacia to reduce the progression of stromal ulceration, speed epithelial healing and minimize corneal scarring.<sup>17</sup> Specific antiproteinases for ophthalmic use include N-acetylcysteine (NAC), disodium ethylene diamine tetraacetate (EDTA), tetracycline antibiotics and autogenous serum. Doxycycline, EDTA and NAC inhibit MMPs by chelation of the zinc and calcium that MMPs require as a cofactor and stabilizing ions, respectively. Autogenous serum contains  $\alpha_2$ -macroglobulin;<sup>17</sup> this is produced in the liver and reduces the activity of proteinases from all major proteinase classes. Autogenous serum is

preferred because of its effects on multiples types of MMPs.<sup>17</sup> A synthetic MMP inhibitor, galardin, appears to be a promising and powerful MMP inhibitor. It is effective against pseudomonas elastase, alkaline protease, MMP<sub>1</sub> and MMP<sub>9</sub>. Galardine was shown to inhibit the synthesis of MMPs produced by pseudomonas in a rabbit model of pseudomonas keratitis.<sup>18</sup> Tetanus antitoxin has been used against MMPs in the horse. A recent study reported that serum, tetanus antitoxin and acetylcysteine prevented cornea digestion at the highest concentration in the horse.<sup>15</sup> Despite repeated treatment with MMP inhibitors, keratomalacia may progress, necessitating surgery to stop corneal breakdown.<sup>5</sup>

Conjunctival grafts have been used successfully in the presence of keratomalacia. To be effective, the graft must extend beyond the keratomalacic area. Faulty debridement or suturing may result in graft dehiscence. In very extensive keratomalacia, a total conjunctival graft may be preferable.<sup>6</sup> The main disadvantage of conjunctival grafts is the residual corneal opacities that can impair vision, especially when the lesion is located in the axial cornea or is very large.<sup>10</sup>

Porcine small intestinal submucosa has been shown to be effective in a range of different situations, including use as a dural substitute,<sup>19</sup> as intra-articular ligamentous graft material,<sup>20</sup> as a large-diameter vascular graft,<sup>21</sup> as a substitute for large fascial defects,<sup>22</sup> for bladder regeneration,<sup>23</sup> and in promoting meniscal regeneration in dogs.<sup>24</sup> Complete resorption of the graft occurred in these cases by 60 days,<sup>19</sup> 26 weeks,<sup>20</sup> 44 weeks,<sup>21</sup> 12 weeks,<sup>22</sup> 48 weeks<sup>23</sup> and 12 weeks,<sup>24</sup> respectively. The SIS extracellular matrix is remodeled into host tissue with the specific structural and functional proprieties of the host tissue.<sup>7,19,20,21,22,23,24</sup>

In veterinary ophthalmology, a report describing a preliminary evaluation of the biocompatibility of SIS in the rabbit cornea suggested that SIS is incorporated into corneal tissue during the healing process. After 15 days, the SIS was partially replaced by a relatively clear corneal stroma and histologically the scar tissue was organized with keratocytes and collagen fibers with an overall sagittal orientation, parallel to the corneal surface.<sup>11</sup>

The purpose of our study was to show the efficacy of a SIS corneal graft following the debridement of necrotic and collagenolytic corneal tissue by keratectomy.

There are three important stages in SIS integration and corneal wound healing: corneal neovascularization, proliferation of epithelial and stromal tissue, and remodeling of the extracellular matrix (ECM) to produce corneal transparency and preserve corneal integrity. Corneal neovascularization is the first step in the integration of SIS into the corneal stroma. Corneal neovascularization is nearly always present at the time of corneal ulceration, is induced by surgery, and is probably amplified during SIS integration because of stimulation by growth factors present within both the SIS and the cornea. When used to promote corneal wound healing, SIS provides a biocompatible protein matrix that is initially invaded by fibroblasts. These are ultimately replaced by corneal stromal cells, resulting in good corneal transparency.

The SIS also contains growth factors (TGFβs, bFGF), collagen (Type I, III and V), fibronectin, hyaluronic acid, chondroitin sulfate A and heparin sulfate. Three members of the TGFβ family, TGF-β1, -β2 and -β3, have been identified in mammalian cells.<sup>25</sup> TGFβs play an important role in the development and maintenance of homeostasis of the vascular systems by regulating functions of endothelial cells. Although TGFβ has been shown to induce angiogenesis *in vivo* with the chorioallantoic assay, the cellular response modulated by TGFβ can be stimulatory or inhibitory, depending on the cell types and conditions.<sup>26</sup> TGFβs are well-known inhibitors of matrix metalloproteinase synthesis,<sup>27</sup> and TGF-β2 acts as the major inhibitor of collagenase synthesis by corneal stromal cells in culture.<sup>28</sup> TGFβs are produced by many cell types in the human eye, including limbal epithelial cells, conjunctival cells, stromal cells proximal to the ciliary body, and cells of the ciliary body.<sup>25</sup> Generally recognized as a potent growth inhibitor of normal diploid cells, they can also act as a growth stimulator under certain circumstances.<sup>27</sup> TGFβ also appears to be an important regulator of tissue remodeling because of its capacity to stimulate deposition of ECM when injected into tissue *in vivo*.<sup>28</sup> This action has been attributed to the stimulation of ECM component synthesis and inhibition of matrix degradation.<sup>29</sup> The latter effect is thought to be mediated by inhibiting the synthesis of matrix-degrading proteinases such as collagenase, as well as by stimulating the synthesis of proteinase inhibitors.<sup>30</sup> bFGF induces mitosis in epithelial, endothelial and stromal cells. Its presence in the endothelial basement membrane supports its role in promoting endothelial cell migration during wound healing *in vitro*. bFGF also increases proliferation and migration of stromal cells.<sup>31</sup>

A number of growth factors and their associated receptors, including epidermal growth factor (EGF), transforming growth factor (TGF-β), keratinocyte growth factor (KGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF) have been detected in the anterior segment of the eye. They play a vital role in corneal wound healing, by mediating the proliferation of epithelial and stromal tissue and affecting the remodeling of the extracellular matrix (ECM).<sup>31</sup>

Various complications in the use of SIS grafts in dogs, horses and cats have been reported: aqueous leakage, conjunctival graft dehiscence, SIS laceration, chronic uveitis, and hyphema.<sup>9,12</sup> In a series of 10 cases, Featherstone described one cat with a melting ulcer that was managed with a conjunctival pedicle graft in addition to the SIS, which underwent enucleation 48 h postsurgery due to progressive keratomalacia.<sup>9</sup>

Other authors have described covering the SIS graft with a conjunctival graft or flap.<sup>12</sup> In our study, we did not cover the SIS with a conjunctival graft. We placed a nictitating membrane flap to protect the SIS graft for 2 weeks after surgery and no complications occurred. One dog (case 3) had no nictitating membrane flap. At ocular examination 2 months postsurgery, an intense corneal granulation response occurred in this dog (Fig. 8c). At 6 months postsurgery, only

a reduced central leukoma remained and vision was preserved. Due to corneal perforation in cases 2 and 3 another complication, anterior synechiae, was encountered. As before, vision was not affected.

This study reports the successful use of SIS to repair melting ulcers after extensive debridement of necrotic and collagenolytic corneal tissue. Success was defined as complete corneal repair, integrity, and preservation of vision at the last re-evaluation. All corneas healed and all seven patients retained vision. Results of our study support the conclusion that the SIS graft may be used as an alternative to conjunctival graft, which is commonly used to repair melting ulcers in dogs and cats. The advantages of using a SIS graft include good corneal transparency, preservation of corneal integrity and maintenance of vision.

#### ACKNOWLEDGMENTS

The authors sincerely thank J Pink, DVM (Department of Veterinary Surgery, University College Dublin) and A Torriglia, PhD (INSERM U598, France) for reviewing this manuscript.

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