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Surgical Treatment of Corneal Sequestrum with Porcine Urinary Bladder Submucosa Extracellular Matrix (ACeLL Vet® Corneal Disc) in Two Cats

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Abstract : Two cats were presented to the Dana Animal Hospital Eye Center and were diagnosed with corneal sequestrum through full ophthalmic examination. After lamellar keratectomy using a reusable corneal trephine blade and a crescent microsurgical knife, porcine urinary bladder submucosa extracellular matrix (UBM, ACeLL Vet® corneal disc) was applied to the corneal defects. In both cases, no corneal sequestrum recurrences were observed until 119 days and 253 days after the surgery, respectively. Porcine UBM could be recommended as a surgical scaffold for treatment of corneal sequestrum in cats.

Key words **:** ACeLL Vet® corneal disc, cat, corneal sequestrum, keratectomy, porcine urinary bladder extracellular matrix.

Introduction

Corneal sequestrum, also called corneal necrosis, or keratitis nigrum, is a disease that occurs primarily in medium-aged to older cats (18,20). On the central or paracentral corneal surface, amber to dark black corneal necrotic plaque composed of pigment and iron is formed (14,15). In addition, corneal neovascularization and corneal ulcers occur around the plaque, causing blepharospasm, ocular discharge, ocular discomfort, and epiphora (18,20).

Although the cause of corneal sequestrum is unknown, chronic corneal irritation due to entropion, distichiasis, over exposure of the corneal surface in brachycephalic breeds (Persian, Himalayan), and grid keratotomies for the treatment of corneal ulcer are known as predisposing factors in cats (20). Feline herpes virus type 1 has also been reported to contribute to the development of corneal sequestrum (18,20).

Lamellar keratectomy is recommended as the most useful treatment intervention for corneal sequestrum (17,18,20,22). However, since the recurrence rate after surgery is reported to be 12-38% (16), conjunctival grafts and corneoconjunctival transposition have been performed following lamellar keratectomy to reduce the recurrence of corneal sequestrum (2,17,20). Biomaterials such as amniotic membrane (AM), porcine small intestinal submucosa (SIS), and porcine urinary bladder submucosa extracellular matrix (UBM) have been recently used to reinforce corneal stromal defects (8,11,13).

The advantages of porcine UBM are low antigenicity, stimulus of angiogenesis, cell proliferation and migration, and suppression of bacterial infection (3,5,10,21). It acts as a scaffold that maintains and supports tissue regeneration and wound healing environments in corneal defects (4).

The purpose of this case report is to describe porcine UBM application for treatment of unilateral corneal sequestrum in two cats and discuss the effectiveness and prognosis of porcine UBM in cats.

Case Reports

Case 1 (flap by original uncut ACeLL Vet® corneal disc)

A 10-year-old intact female Persian cat weighing 3.1 kg presented with blepharospasm, epiphora, and dark brown ocular discharge in the left eye (OS). On ophthalmic examinations, including slit-lamp biomicroscope (SL-D7, Topcon Corp, Tokyo, Japan), corneal sequestrum showing oval-shaped, dark brown colored, central corneal necrotic plaque, corneal neovascularization, corneal edema, and ulcer was observed (Fig 2A) and the cat was diagnosed with corneal sequestrum. Surgical intervention was selected to remove the lesion and improve the associated clinical signs. The cat was premedicated with atropine 0.02 mg/kg SC (Atropine®, Daewon Pharm, Korea), butorphanol 0.2 mg/kg IV (Butorphan®, Myungmoon Pharm, Korea), midazolam 0.2 mg/kg IV (Midazolam®, Bukwang Pharm, Korea), cefazolin 20 mg/kg SC (Cefazolin®, Chongkundang, Korea), and meloxicam 0.3 mg/kg SC (Metacam®, Boehringer Ingelheim, Spain). A single drop of 0.5% proparacaine (Alcaine®, Alcon, Korea) was instilled. After induction using 6 mg/kg IV propofol (Provive®, Myungmoon Pharm, Korea), general anesthesia was maintained with isoflurane (Isotroy 100®, Troika Pharm, India) inhalation and oxygen. The eye was disinfected with 0.5% povidone-iodine solution after clipping the periocular region. With the aid of an ophthalmic surgical microscope, keratotomy was performed using a 6.5 mm reusable trephine blade with a universal tre-

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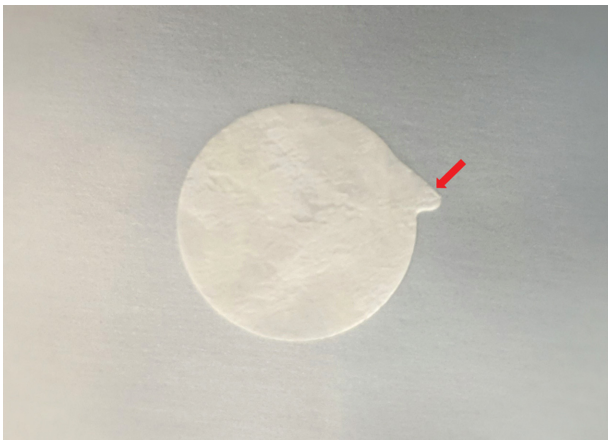

Fig 1. Appearance of ACeLL Vet® corneal disc (porcine urinary bladder submucosa extracellular matrix). If the side indicator (red arrow) is in the position shown in the above image, the tunica propria side of the disc faces the corneal stromal defect (17), and the basement membrane side faces away from the corneal stromal defect. The disc should be 1-2 mm larger than the size of the corneal defect.

phine blade holder (A1 medical, Germany) over the range of corneal plaque lesions that had to be removed, and circular lamellar keratectomy was performed to completely remove the corneal sequestrum with a 2.6 mm crescent microsurgical knife (Kai medical, Japan). After debridement of all necrotic tissues, the side indicator of the porcine UBM (ACeLL Vet® corneal disc, ACeLL Inc., MD, USA) was positioned as shown in Fig 1, so that the basement membrane side faced the surgeon and faced away from the corneal stromal defect (12). Placed on the cornea, it was moistened with physiologic saline for two minutes and secured in corneal limbus by cardinal suture to four sites using 8-0 polyglactin 910 (Vicryl®, Johnson & Johnson, Germany) in the following order: 12, 6, 9, and 3 o'clock. In order to keep the biomaterial hydrated and maintained in place, the bulbar surface of the nictitans gland was scarified to cause bleeding with a no. 15 surgical blade, and a third eyelid flap was performed using 6-0 nylon (Blue Nylon®, Ailee, Korea). Topical application of 0.5% levofloxacin q6h (Eyelevo®, Kolmar Pharm, Korea), trifluridine q6h (Ocufridine®, Samil Pharm, Korea), and cyclopentolate q12h (Ocucyclo®, Samil Pharm, Korea) was maintained for 14 days. Doxycycline 10 mg/kg SID (Unidoxy®, Kukje Pharm, Korea) was administered systemically for 19 days, and Elizabethan collar was placed to avoid self-trauma for 19 days. The third eyelid flap sutures were removed 19 days postoperatively (Fig. 2B), and moderate corneal edema and corneal neovascularization were observed. On day 45 after surgery, corneal opacity and corneal neovascularization decreased, and ocular discomfort or epiphora disappeared. Corneal opacity further decreased, and recurrence of corneal sequestrum was not observed until 119 days after surgery (Fig 2C, 2D).

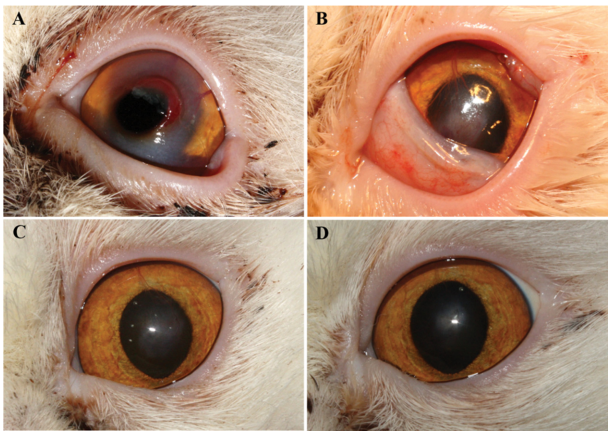

Fig 2. Pre- and postoperative appearances of the left eye in Case 1. (A) Preoperative photograph. An oval, dense, darkly pigmented, central corneal sequestrum was observed with corneal neovascularization and edema surrounding the lesion. (B) Immediately after removal of the third eyelid flap sutures 19 days postoperatively. (C) and (D) 45 and 119 days after surgery, respectively. Corneal neovascularization and corneal opacity decreased (45 days) and almost disappeared (119 days).

Case 2 (graft by ACeLL Vet® corneal disc graft cut to the size of the stromal defect)

A 2-year-old spayed female exotic shorthair cat weighing 4.2 kg was presented to the Dana Animal Hospital Eye Center with ophthalmic signs such as squinting, epiphora, mucoid ocular discharge, and miotic pupil in the OS for 3 weeks. On full ophthalmic examination, there were corneal neovascularization, circular corneal fibrosis surrounded by diffuse faint-brown discoloration of the medial cornea, corneal ulcer, and medial lower eyelid entropion (Fig 3A). Intraocular pressure (IOP) values were 18 mmHg in the right eye (OD) and 7 mmHg in the OS. Aqueous flares were observed in the OS. Corneal sequestrum was diagnosed in the OS, and surgical

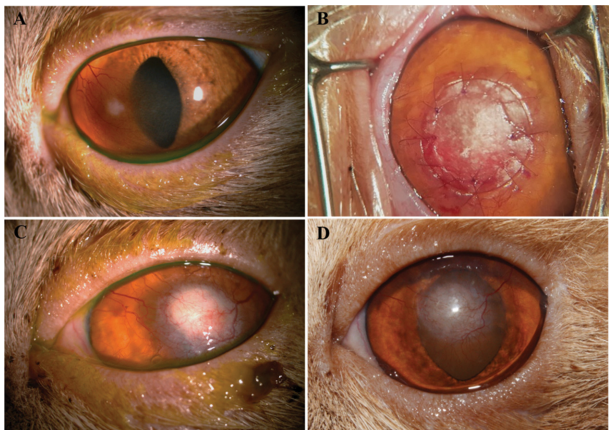

Fig 3. Pre- and postoperative appearances of the left eye in Case 2. (A) Preoperative photograph. Diffuse light-brown discoloration surrounding white fibrotic lesions and corneal neovascularization extending from the dorsomedial limbus were observed on the medial side of the left eye. (B) Final aspect of the ACeLL Vet® corneal disc sutured to the corneal stromal defect. A simple continuous suture pattern using absorbable 9-0 Vicryl® suture was performed after four cardinal sutures. (C) 68 days after surgery. (D) 253 days after surgery. Mild corneal opacity due to scar formation and corneal neovascularization were observed without recurrence of the corneal sequestrum.

treatment was planned. The cat was premedicated with atropine 0.02 mg/kg SC, acepromazine 0.2 mg/kg SC (Sedaject®, Samu median, Korea), tramadol 2 mg/kg SC (Tramadol®, Bukwang Pharm, Korea), cefazolin 20 mg/kg SC, and meloxicam 0.3 mg/kg SC. One drop of 0.5% proparacaine was instilled. Ketamine 5 mg/kg (Ketamine®, Huons, Korea) was administered intravenously for anesthesia induction. General anesthesia was maintained with isoflurane. The preparation of the surgical area was performed in the same manner as in case 1. To correct the entropion of the medial lower eyelid, the Hotz-Celsus technique was performed. After skin incision was made 1.5 mm apart from the eyelid margin at a length of 5 mm, a second incision was made 3 mm apart from the first incision line, and the skin strip and layer of the orbicularis oculi muscle were dissected and removed with Steven's straight tenotomy scissors. The surgical wound apposed with 6-0 nylon (Blue Nylon®, Ailee, Korea) as a simple interrupted suture pattern. Thereafter, the size of the necrotic lesion of the cornea was measured using a Castroviejo ophthalmic caliper, and a corneal incision was made with a 7 mm reusable trephine blade. Lamellar keratectomy was performed so that the necrotic plaque lesion could be completely removed using a 2.6 mm crescent microsurgical knife (Kai medical, Japan). The ACeLL Vet® corneal disc was cut to an 8 mm diameter using a reusable trephine blade, 1 mm larger than the corneal defect, was placed on top of the corneal defect, and then hydrated with physiologic saline for two minutes. After the cardinal suture using 9-0 polyglactin 910 (Vicryl®, Johnson & Johnson, Germany) was applied at 4 sites to anchor the ACeLL Vet® corneal disc to the margin of corneal defect, it was completely fixed to the corneal defect with a simple continuous suture pattern (Fig 3B). As in case 1, a third eyelid flap was performed. Topical eyedrops, such as 0.5% moxifloxacin q6h (Vigamox®, Alcon, Singapore), trifluridine q6h (Ocufridine®, Samil Pharm, Korea), and cyclopentolate q12h (Ocucyclo®, Samil Pharm, Korea) were instilled for 21 days after surgery. Systemic doxycycline 10 mg/kg SID (Unidoxy®, Kukje Pharm, Korea) was administered for 21 days and Elizabethan collar was placed on the cat for prevention of self-trauma for 21 days. On day 21 after surgery, sutures of the third eyelid flap and Hotz-Celsus procedure were removed. The fluorescein dye test was negative in the OS. Follow-up rechecks were performed at 68, 106, and 253 days after surgery (Fig 3C, 3D). Mild corneal opacity due to scar formation and corneal neovascularization remained at 253 days postoperatively, but no recurrence of corneal sequestrum and medial lower eyelid entropion was observed.

Discussion

Lamellar keratectomy has been reported as the most recommended surgical method for treatment of feline corneal sequestrum (17,18,20,22). If less than one-third of the corneal thickness is removed by lamellar keratectomy, medical treatment for corneal ulcer is performed after surgery, but when more than half of the corneal thickness is removed, it is recommended to cover the stromal defect with conjunctival grafts (17). Featherstone *et al.* reported that corneal sequestrum recurred in 11 out of 44 cases performed by keratectomy alone for treatment of corneal sequestrum (16). Therefore, lamellar keratectomy for the treatment of feline corneal sequestrum was performed with a conjunctival graft to reduce the recurrent corneal sequestrum after lamellar keratectomy (19). However, conjunctival grafts have the disadvantage that the tectonic support of corneal defects is weak and may obscure visual field if the lesions are large (12,16-18).

In humans, biomaterials other than conjunctival or corneal grafts have been applied for the surgical treatment of corneal diseases. These biomaterials, which serve as scaffolds to compensate for corneal stromal defects, have been reported to ensure transparency of the cornea and promote the regeneration of corneal epithelial cells and have the advantage of high drug permeability and resistance to corneal proteases (3-5,10,21,23).

In veterinary ophthalmology, AM (8), equine renal capsule (1), equine pericardium (9), porcine SIS (11), and porcine UBM (7,12,13,24) have been used to reconstruct corneal defects in various corneal disorders such as deep corneal ulceration, corneal perforation, and descemetocele, to maximize corneal clarity, to maintain vision acuity, and to prevent the recurrence of corneal sequestrum.

The acellular porcine UBM, commercialized by ACeLL Vet® corneal disc with reduced immune response (7) is a biomaterial extracted from the porcine bladder, and is composed of a tunica propria surface and an epithelial basement membrane surface (6). It is thicker than other implants and easier to handle when hydrated (12). The healing process occurs only when the tunica propria surface faces the corneal stromal defect. The side indicator (triangle flap) of the ACeLL Vet® corneal disc should be positioned as shown in Fig 1, so that the basement membrane surface faces away from the corneal stromal defect (12). The merits of the ACeLL Vet® corneal disc are that it is affordable, packaged, and sterilized, so shelf life is long and no special storage facilities are required (13).

In case 1 of this study, an ACeLL Vet® corneal disc was successfully integrated into the corneal defect when the third eyelid flap was removed on the 19th day after surgery. Moderate corneal stromal opacity, corneal neovascularization, and mild superficial inflammatory granuloma remained. In case 2, at the time of third eyelid flap removal on the 21st day after surgery, stable integration was observed without suture dehiscence. At the final reevaluation, both cases showed mild corneal scarring after complete integration of the ACeLL Vet® corneal disc and no recurrence of corneal sequestrum. Balland *et al.* suggested that the successful integration rate was 86% in cats with deep corneal ulceration and 100% in cats with corneal sequestrum when applying ACeLL Vet® corneal disc to cats with lamellar keratectomy (7). In the latter, complete integration was confirmed in 92% on the 18th day and 100% on the 45th day after surgery (7). Zigler also reported that all 4 cats with corneal sequestrum had good prognosis with good corneal clarity when operated with porcine UBM (24).

The most common complication in the application of ACeLL Vet® corneal disc is graft or suture dehiscence (7,12). One study reported that the incidence rate of suture dehis-

cence was 25% (n = 44) when ACeLL Vet® corneal disc was applied to treat corneal diseases such as corneal ulcer, corneal perforation, and corneal sequestrum (12). In another study, suture dehiscence was not observed in the case of ACeLL Vet® corneal disc application to 7 cats with corneal sequestrum (7).

In the above-mentioned studies, the suture materials and suture methods used in surgery varied. Balland *et al.* sutured porcine UBM to corneal stromal defects in a symmetric saw tooth suture pattern using 9-0 Vicryl® suture (7), and Chow *et al.* performed 4 cardinal sutures with 10-0 polyglycolic acid and simple interrupted (small defects) or continuous (large defects) sutures according to the size of corneal defects (12). Zigler applied porcine UBM using 7-0 Vicryl® sutures in four cats with corneal sequestrum (24). In case 1 of our case report, cardinal suture was performed in four sites using 8-0 Vicryl® suture, and in case 2, cardinal suture and simple continuous suture pattern were performed using 9-0 Vicryl® suture. The prognosis after surgery was good in both methods in this study. Additional studies on differences in complications or surgical prognosis depending on the suture materials or suturing methods might be needed.

Limitations of this case report include the relatively short follow-up period after surgery and the small number of surgeries. Since porcine UBM was applied to only two cats, further studies will be necessary to evaluate the effects of the combination of lamellar keratectomy and porcine UBM on larger populations.

Conclusions

Feline corneal sequestrum is one of the most common corneal diseases in cats. In this case report, two cats with corneal sequestrum were treated using a combination of lamellar keratectomy and porcine UBM, and there was no recurrence of corneal sequestrum until 106 days and 253 days after surgery, respectively. The application of porcine UBM could be considered as an effective option of surgical treatment of corneal sequestrum in cats.

Conflict of Interest

The authors declared no conflicts of interest.

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