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Lamellar keratoplasty using decellularised porcine cornea as an acellular scaffold graft for surgical management of deep corneal ulcer in a dog#

A. Aswathy Mohan[1], S. Anoop[2], K. D. John Martin[3], Laiju M. Philip[4], V. N. Vasudevan[5] and Ashlin Michael[1]
Department of Veterinary Surgery and Radiology
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680651
Kerala Veterinary and Animal Sciences University
Kerala, India

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Abstract

Corneal ulcers are one of the most common eye affection in domestic animals, especially dogs. In preclinical studies, porcine corneas were used regularly as an animal model due to its relative similarity to human cornea and its availability in great numbers from slaughter houses. In the present study, decellularised porcine cornea was used for corneal grafting as an acellular scaffold graft material in a case presented with deep corneal ulcer. The efficiency of the graft material was analysed on the day of presentation, days 7, 14, 21 and 60 post grafting, based on corneal clarity, oedema, neovascularisation, pigmentation, visual function and scar tissue formation. Decellularised porcine corneas were found to be very convenient to handle during surgery, provided good tectonic support, had good tissue biocompatibility and produced only mild to moderate graft opacity.

Keywords: *Decellularised porcine cornea, deep corneal ulcers, grafting*

Corneal ulcerations are one among the common ocular disorders and a major cause of blindness in dogs due to excessive scarring or to subsequent corneal perforation (Startup, 1984; Antonia *et al.,* 2014). Corneal ulcerations are characterised by loss of corneal substance, which may be accompanied by oedema, vascularisation, pain and photophobia and are frequently observed in dogs below three years of age (Adarsh *et al.,* 2016). These can be classified as superficial and

1. *MVSc Scholar*
2. *Professor and Head, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Pookode*
3. *Professor and Head*
4. *Assistant Professor*
5. *Associate Professor and Head, Department of Livestock Products Technology*

∗Corresponding author: anoop@kvasu.ac.in, Ph.9446792333

deep, based on the loss of stromal tissue and treatment is instituted based on their depth and rate of progression (Renwick, 1996). General therapeutic methods are applied whether the ulcer is superficial or deep. However, those ulcers which are over half the depth of the cornea or are rapidly progressing requires immediate surgical intervention in order to provide support and to reduce the risk of perforation (Wilkie and Whittaker, 1997; Jhanji *et al.*, 2011; Anoop *et al.,* 2017; Keenan *et al.*, 2020).

A three year old male cane corso dog was presented to University Veterinary Hospital, Mannuthy with history of presence of a reddish protruding mass on the surface of the right eye. On general clinical examination, the animal was in excellent body condition and all the physiological parameters were within normal limits. On ophthalmic examination, the animal had mucoid ocular discharge from the right eye with the presence of a reddish granulation tissue on the surface of the cornea, which appeared cloudy. Schirmer tear test (STT) value was found to be 23mm/min and the affected eye showed positive fluorescein dye test (FDT). Haematological examination was carried out preoperatively and all the parameters were found to be within the normal range. Preoperative medical therapy included combination of antibiotic and anti-inflammatory (moxifloxacin and bromfenac) eye drops and hypertonic saline (hyperosmotic) eye drops (Moore, 2003). Elizabethan collar was advised till the day of surgery. Solid food was withheld for 12 hours and water for six hours prior to surgery. The affected eye was thoroughly lavaged with normal saline and prepared for aseptic surgery.

Fresh porcine corneas procured after pig slaughter from Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University were decellularised using 0.1% Sodium dodecyl sulphate in an orbital shaker for 48 hours and were preserved in deep freezer at -20°C (Isidan *et al.*, 2021). The graft material was kept immersed in sterile normal saline solution for not less than five minutes before the procedure (Thajunnisa, 2020).

The animal was premedicated with atropine sulphate at the rate of 0.045 mg/kg body weight and xylazine hydrochloride at the rate of 1 mg/kg body weight intramuscularly. General anaesthesia was attained by using ketamine hydrochloride at the rate of 5 mg/kg body weight and midazolam at the rate of 0.1 mg/kg body weight intramuscularly. The animal was intubated with cuffed endotracheal tube of size 8 mm internal diameter and the anaesthesia was further maintained using isoflurane two per cent in 100 per cent oxygen. The animal was placed in lateral recumbency, draped with the affected eyes facing upwards and four stay sutures were placed on the bulbar conjunctiva to fix the eyeball. Superficial corneal debridement was performed and granulation tissue was excised. The porcine cornea sized slightly larger than the corneal ulcer was placed on the graft bed and eight sutures were placed around using braided silk size 5/0 in simple interrupted suture pattern between the graft and the bulbar conjunctiva. Temporary tarsorrhaphy was performed and retained for a period of seven days. To prevent self-mutilation and injury to the eye, an Elizabethan collar was advised till complete healing. Use of moxifloxacin eye ointment and D- panthenol ophthalmic gel was advised twice a day topically. Cephalexin at the rate of 20mg/kg bodyweight was given orally twice daily for five days. Steroid eye drops (loteprednol) was advised till the end of the observation period, in a tapering dose (Amon and Busin, 2012). The tarsorrhaphy sutures were removed on day 7 post grafting. Complete epithelialisation was indicated by negative FDT.

Post-operative evaluations were carried out on day 7, 14, 21 and 60 post grafting. Every recheck consisted of a complete clinical examination and bilateral ophthalmic examination, which include vision assessment via pupillary light reflex, palpebral reflex, cotton ball test and menace test (classified as present or absent) and assessment of corneal clarity, oedema, neovascularisation and pigmentation. Corneal scarring was evaluated on day 60 post grafting. The visual function score was zero on the day of presentation and on day 7 post grafting (Suhas, 2015). The animal became visually active by day 14 post grafting showing

positive response to PLR, palpebral, menace and cotton ball test (Thajunnisa, 2020). On day 7 post grafting, opacity and oedema was noticed around the surgical site (Lavaud *et al.*, 2021) and by day 14 post grafting, the cornea appeared hazy in the centre while the periphery became clear. Oedema was not appreciable on subsequent reviews from day 14 to day 60. Mild superficial vascularisation was observed on the day of presentation, which became profuse by day 7 and 14 post grafting with a prominent dendritic vessel in the superotemporal region (Vanore *et al.*, 2007; Chow and Westermeyer, 2016). However, by day 21, vascularisation became mild which completely resolved by day 60. On examination, mild pigmentation was observed on inferonasal region on day 7 post grafting. It became moderate by day 14 which persisted till day 60 post grafting (Bouhanna *et al.*, 2008; Labelle *et al.*, 2013; Azoulay, 2014). On grading of the scar tissue on day 60 post grafting, only minimal scar tissue formation was observed (Goulle, 2012). Representative images are presented in Fig. 1 (a-f)

Summary

A three-year-old male cane corso dog presented with deep corneal ulceration along with extensive granulation tissue on the right eye was surgically managed by performing lamellar keratoplasty with decellularised porcine corneal graft, which reduced the incidence of vision impairing pigmentation and improved the corneal clarity. Early surgical intervention with proper post-operative care and application of Elizabethan collar was effective in successful management of deep corneal ulcer.

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Conflict of interest

The authors declare that they have no conflict of interest.

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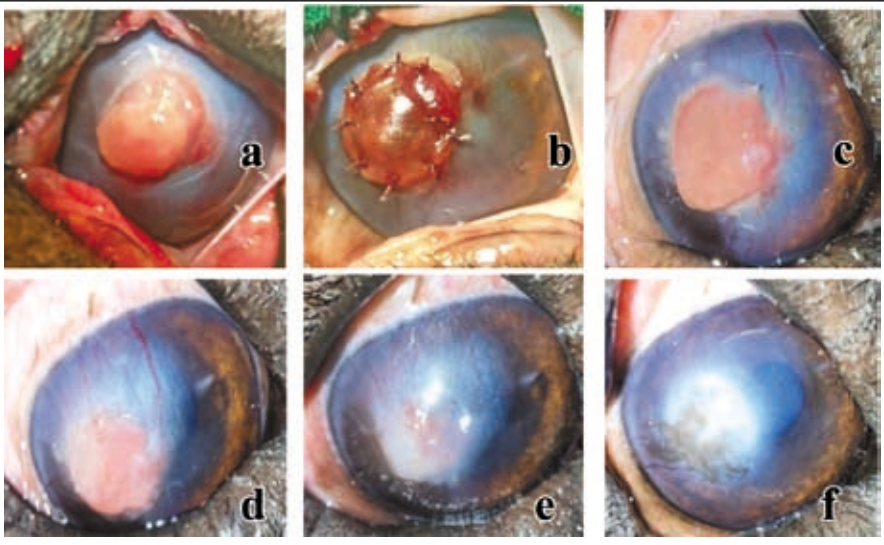

Fig. 1 (a-f) : Use of decellularised porcine cornea for repair of deep corneal ulcer in a dog, (**a**) cornea on the day of presentation showing presence of deep corneal ulcer with extensive granulation tissue, (**b**) cornea on the day of surgery after grafting with decelularised porcine corneal scaffold, (**c**) cornea on day 7 post grafting showing persistence of granulation tissue, (**d**) cornea on day 14 showing minimal granulation tissue with neovascularisation, (**e**) day 21 post grafting with moderate corneal scarring and mild pigmentation, (**f**) day 60 post grafting showing mild scar tissue formation and pigmentation.

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