



DECELLULARIZED PORCINE SMALL INTESTINAL SUBMUCOSA FOR THE REPAIR OF DEEP CORNEAL ULCER IN DOGS

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ABSTRACT

The study investigated the use of decellularized porcine small intestinal submucosa (DPSIS) for the repair of deep corneal ulcer in seven dogs. Following keratectomy decellularized porcine small intestinal submucosa was sutured to the recipient cornea and postoperative care with topical and systemic antibiotics, nonsteroidal anti-inflammatory agents and topical cyclosporine was done. Five eyes healed completely with minimal corneal scarring with a very good vision. Corneal neovascularisation had resolved by 60 days postoperatively as the graft had completely incorporated into the cornea. Thus decellularized porcine small intestinal submucosa appeared to be a suitable biomaterial for repair of corneal ulcer in dogs.

KEYWORDS: Decellularized porcine small intestinals submucosa, SDS, corneal ulcer, dogs.

INTRODUCTION

Corneal ulcer due to trauma is a common complication in companion animals and it may progress to threaten globe integrity and vision (Wilkie and Whittaker, 1997). Although it may respond to medical management, some patients require surgical intervention. Several surgical procedures with grafting techniques to preserve corneal integrity and vision have been reported. Corneal transplantation is one of the most successful forms of tissue transplantation. However, a severe shortage of donor corneas exist worldwide (Golchet *et al.*, 2000). In addition, complications such as infection and immune reactions are possible because of penetrating keratoplasty. Porcine small intestinal submucosa (PSIS) has been used as a natural scaffold for the repair of corneal and scleral defects in various animal species. It acts as a scaffold for repair and provides appreciable tectonic support. The use of decellularized xenograft tissue, in which the donor cells and antigen molecules are completely removed to eliminate the host immune reaction, has become the new strategy for preparing a scaffold (Wilshaw *et al.*, 2006; Oh *et al.*, 2009). The present study describes the use of decellularized porcine small intestinal submucosa in the repair of deep corneal ulcer in dogs.

MATERIALS AND METHODS

The study was conducted on seven dogs presented to Referral Veterinary Polyclinic cum TVCC, IVRI, Izatnagar, Bareilly, Uttar Pradesh with corneal ulcer due to trauma (Fig. 1A). Ophthalmic examination included vision

assessment by blink reflex, pupillary light reflex, transparency of cornea, conjunctival infection and chemosis, corneal neovascularisation, schirmer tear testing (STT), confirmation of corneal integrity using fluorescein staining were performed. Topical ofloxacin and systemic antibiotic were administered 6 to 24 hours prior to surgery. Surgery was performed under general anesthesia and ocular surface of eyes to be operated were sterilized using 0.2% povidone iodine solution prior to surgery.

The standard procedure included removal of loosely adhered corneal epithelium with fine colibri forceps and keratome (3.2mm) and trimming of corneal tissue that appeared nonviable and fibrin protruding through the perforation site with corneal scissors. In cases, where defect was less than 8 mm in diameter, a corneal trephine was used to outline the lamellar keratectomy margins. The same size corneal trephine was used to create a patch of graft. In cases, where corneal defect was more than 8 mm in diameter or irregular, a freehand anterior Lamellar Keratoplasty was performed over the corneal defect and an appropriately shaped acellular PSIS graft decellularized using 1% SDS approximately 1 or 2 mm larger than the defect was prepared. The grafts were secured with simple interrupted sutures using 6/0 polyglactin 910 (Fig. 1B).

A temporary tarsorrhaphy using 2-0 polyamide was placed approximately halfway between the nasal and lateral canthus. Entropion of lower eyelid was present in one dog (Fig. 1C) and it was corrected using Hotz-Celsus technique at the same time as the corneal surgery.



FIGURE 1: Use of decellularized porcine small intestinal submucosa for the repair of deep corneal ulcer in a dog (A-2). A: Preoperative; B: Intraoperative; C: Correction of entropion of lower eyelid; D: Postoperative – 15 days; E: 30 days and F: 60 days.

Antibiotic (Ceftriaxone @ 20 mg/ kg body weight) and anti-inflammatory (Meloxicam @ 0.05 mg/kg body weight) drugs were given postoperatively for 5 and 3 days, respectively. Daily antibiotic (Ofloxacin – Zenfox, Mankind Pharma Ltd, New Delhi), anti-inflammatory (Flurbiprofen – Flur, Allergen India Pvt. Ltd.) and topical cyclosporin drops (Hydroeyes 0.05% w/v, Lupin Ltd., Mumbai) were instilled thrice daily for two weeks. Tarsorrhaphy suture was removed on 15th postoperative day.

The efficacy of the bio-engineered PSIS for the reconstruction of corneal defects was evaluated at 15, 30 and 60 days postoperatively (Figs. 1D-1F).

RESULT

The breeds represented were as follows: Mongrel (three), French mastiff (one), Labrador (one), Pomeranian (one) and spitz (one). The age of dogs under this study ranged from 7 months to 6 1/2 years with average age of 3.75 years. There were five males and two females (Table 1).

TABLE 1. Grouping of animals under group A and group B based on signalment, primary lesion and concurrent anomaly

Animal No.	Breed	Age	Gender	OD/ OS	Primary lesion	Concurrent anomaly
A-1	Pomeranian	4 years	Female	OD	Deep stromal ulcer AC not visible	-
A-2	French mastiff	2 ½ years	Male	OD	Deep stromal ulcer AC not visible	Entropion
	Mongrel	7 months	Female	OD	Deep stromal ulcer AC not visible	-
A-3						
A-4	Labrador	6 ½ years	Male	OS	Deep stromal ulcer AC not visible	-
A-5	Mongrel	6 months	Male	OS	Deep stromal ulcer	-
A-6	Mongrel	2 years	Male	OD	Deep stromal ulcer	-
A-7	Spitz	4 years	Male	OD	Melting ulcerative keratitis	-

OD: Oculus Dexter, OS: Oculus Sinister, AC: Anterior chamber

Temporary tarsorrhaphy and Elizabethan collar application following surgery provided protection for the grafted eye. Out of seven cases, six cases appeared comfortable after surgery. After removal of the temporary tarsorrhaphy suture, only the remnant of the graft on the cornea had taken fluorescein dye. Small superficial inflammatory granulomas were found around the sutures as sign of presence of inflammatory reaction in all animals. There was no uptake of fluorescein dye by 30th postoperative day. By day 45 after surgery, complete integration of the

scaffold with the reepithelialisation of corneal tissue was noticed in five eyes along with mild corneal neovascularization. Four of seven cases healed completely without complications and with minimal scarring in the cornea (Fig.1.D, E and F). As the grafted material got resorbed by day 45, cornea started regaining the transparency and the blink reflex became normal in five animals (A-1, A-2, A-3, A-4 and A-5) and sluggish in two animals (A-6 and A-7) due to melanocyte infiltration covering medial half of the cornea (Fig. 2).



FIGURE 2: Melanocyte infiltration in animal A-6

Increase in tear production was observed preoperatively and on day 15 after tarsorrhaphy suture removal which gradually decreased during subsequent intervals reaching near normal value of tear production by the end of day 60. The topical application of ofloxacin and flurbiprofen instilled thrice a day until healing was able to control infection and inflammation in all the cases. The topical administration of cyclosporine drops provided protection against immune response and graft rejection.

Other complication such as increased intra ocular tension was noticed by digital palpation in the opposite eye in animals A-4 and A-7. It was treated with oral administration of acetazolamide (Carbonic anhydrase inhibitor) and topical administration of Timolol maleate (nonselective beta blocker) thrice daily. The drugs were found to be effective in reducing the intraocular tension. Anterior synechia was noticed in one animal (A-3) with a negative pupillary light response in the operated eye (Fig. 3). All the animals were visual postoperatively.



FIGURE 3: Anterior synechia in animal A-3

DISCUSSION

Corneal ulceration is a common ocular disease that can lead to impaired vision in humans and animals. Prolonged inflammation of the cornea is detrimental to the corneal stem cells and the epithelial basement membrane resulting in neovascularization, corneal scarring and impaired vision (Thoft *et al.*, 1979; Tsai and Tseng, 1995). In addition, neutrophils, keratocytes and abnormal epithelial cells produce collagenases and other proteolytic enzymes (Kenyon *et al.*, 1979; Gilger *et al.*, 2007) that can cause progressive ulceration of the corneal stroma with the risk of perforation.

Healing of the ulcer can be stimulated surgically by the use of tissue adhesives and soft contact lens, suturing, conjunctival flaps and grafts (Severin, 1996; Crispin, 2005; Gilger *et al.*, 2007; Maggs, 2008).

Porcine small intestinal submucosa has been found to be effective in the repair of full thickness corneal defects in various species of animals (Lewin, 1999; Bussieres *et al.*, 2004; vanore *et al.*, 2007; Sangeetha, 2014). The PSIS was found to be remodelled into host tissue with the specific structural and functional properties of the host tissue (Badylak, 1993).

The porcine cell-containing SIS could trigger a potential adverse immune response elicited by cell membrane epitopes, allogeneic or xenogeneic DNA, and damage-associated molecular pattern molecules (Gilbert *et al.*, 2006). Therefore, decellularization of SIS is considered to be a most important step to eliminate any side effects (Luo *et al.*, 2011).

In the present study, ionic biological detergent (1% SDS) was used for decellularization of PSIS. The dogs were presented with complication of deep corneal ulcer, which were resulted from trauma to the eye by the companion animal or by self mutilation and one animal had severe deep stromal ulcer as a result of chronic irritation of cornea due to entropion. Chinchu (2010) also reported that self mutilation, entropion and trauma are common causes of corneal ulcers in dogs.

Staphyloma and adhesion of iris to the corneal wound were developed in one animal. Refractory corneal ulcers persisting for long period without re-epithelialisation were noticed in almost all the cases. Similar findings about corneal ulcers in dogs were reported by Whitley (2000) and Mandell and Holt (2005).

Corneal ulcers in the present study were properly debrided and made free from any type of necrosed corneal tissues to

promote healing. Debridement of the necrotic tissues and the fibrin clots over the corneal ulcer stimulated corneal wound healing by the proliferation of adjacent epithelium and promoted the attachment of basement membrane. Similarly, Moore (2003) reported that the keratectomy procedure removed the acellular zone of hyaline collagen in the anterior stroma thus eliminating the barrier to epithelial healing and strengthening the adhesions of epithelium to corneal stromal collagen. Granulation, pigmentation and scar formation were the common complications observed in our study. The main complications of superficial keratectomy included infection, granulation, pigmentation, perforation and scar formation (Stanley *et al.*, 1998). Kim (2016) concluded that corneal transplantation using deep anterior lamellar keratoplasty could be performed in dogs that have large corneal defect and vision loss with healthy endothelium. Entropion represents a common problem in brachycephalic dogs and the cornea is often affected by entropion-related changes such as erosion/ulceration, inflammation, neovascularization, and pigmentation. Entropion management is usually surgical and requires general anesthesia (Spadea *et al.*, 2018).

Singh *et al.* (2016) reported the successful use of lab prepared DPSIS to repair corneal defects after extensive debridement of necrotic corneal tissue in five dogs and a cow. They also concluded that ionic biological detergent (1% SDS) produced an effective decellularization of the porcine small intestinal submucosa.

To summarize, decellularized porcine small intestinal submucosa can be used as the sole reconstruction material for the repair of deep corneal ulcers in dogs and our results support the previous reports published by Sangeetha 2014 and Singh *et al.* (2016). The repair of deep corneal ulcers in dogs with DPSIS resulted in vision through the grafted area indicating a good biocompatibility with the corneal tissue.

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